Is the Hype around the Reproductive Health Claims of Maca(*Lepidium meyenii* Walp) justified?

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ABSTRACT

Ethnopharmacological relevance: Maca - *Lepidium meyenii* Walp has been cultivated and used by Andean people for over 1,300 to 2000 years in Peru as food and medicine. Starting in the late 1990's it has developed into an important herbal medicine in China and is now cultivated there widely, too.

Aim of study: This study aims to provide an insight into the emergence of maca on the global market as an alternative remedy to treat reproductive health related problems in both men and women and to critically assess these health claims.

Methodology: A search of electronic databases such as EMBASE and a hand-search was done to acquire peer-reviewed articles and reports about maca.

Results and discussion: Lepidium meyenii is used traditionally as a tonic, fertility enhancer for both humans and cattle, and to treat a variety of ailments such as rheumatism, respiratory disorders and anaemia among others. Maca root is cooked, baked, fermented as a drink and made into porridge. In the last twenty years, maca was introduced onto the global market and demand has dramatically grown over this time with its promotion on the internet, as the 'Peruvian Ginseng' for libido and fertility enhancement. It has also been said to treat menopausal symptoms, erectile dysfunction and benign prostatic hyperplasia. The sky-rocketing demand for the plant has seen a shift from traditional cultivation methods to mass production practices with the use of fertilisers and also pesticides; as maca is now grown in areas other than the Andes such as in the Yunnan province in China. This can potentially affect the phytochemistry and composition of the plant and thus, the quality, safety and efficacy of maca products. Meanwhile, research into maca's medicinal properties has followed the spike in popularity of maca and has been focused mainly on maca's aphrodisiac and fertility enhancing properties. So far, the *in vivo* studies and clinical trials conducted have yielded inconclusive results. Some of the key limitations reside in methodology and sample size. Chemical profiling, led to the discovery of new compounds unique to maca, such as, 'macamides' and also other active metabolites like the glucosinolates; to which the medicinal effects of maca have been ascribed but cannot be confirmed due to lack of data.

Conclusions: To date, the health claims of maca cannot be fully supported from a scientific standpoint and more research is needed. It appears that the indigenous local knowledge about

the health benefits of maca has been dragged out of context to fit the demands of a growing market for herbal remedies. This globalisation (or hype esp. in China) also has had serious consequences for the local producers in Peru. The lack of protocols to regulate the production and marketing of maca during this rapid expansion, poses a threat to both the safety of consumers and the sustainability of supply.

Keywords: Maca, *Lepidium meyenii*, *Lepidium peruvianum*, Pharmacological activity, Phytochemistry, Reproductive health

Compounds studied: Imidazole alkaloids (Lepidiline A, Lepidiline B, Lepidiline C, Lepidiline D and macaridine); Macahydantoins; Macathiohydantoins; Meyeniins; Macamides (alkamides unique to maca); Glucosinolates; tetrahydro-β -carboline

Contents

1. INTRODUCTION

Lepidium meyenii (Walpers 1843; *cf* Chacon 1990), commonly known as maca, is a species indigenous to the Central Andes of Peru. It has been cultivated traditionally as a food crop and for its medicinal properties.

Over the last two decades, the interest and demand for maca has grown worldwide through an aggressive marketing promotion of the plant via the media and internet. This has established maca as one of the flagship products of Peru. Maca is sold as powder, pills, capsules, flour, liquor and extracts (Gonzales et al., 2009) and is available from a variety of retail outlets like health food stores and smoothie shops (Smith, 2014).

Maca has been advertised as a 'superfood' and as the 'Peruvian Viagra' and the 'Peruvian Ginseng' referring to the attributed health claims of increasing vitality and longevity, enhancing fertility and libido and alleviating menopausal symptoms in women (Smith, 2014);(Hermann and Bernet, 2009, Brand, 2016). Different colours of maca hypocotyls are also associated with different health benefits. For instance, red maca is said to promote prostate health support (The Maca Team, 2016).The most notable consequence of this popularity has been the Maca Boom in China which led to a drastic surge in prices, illegal mass cultivation of the plant in China and poses a serious threat to supply and product quality and safety (Hermann and Bernet, 2009, Smith, 2014, Brand, 2016).

From a pharmaceutical and medical perspective, the enthusiasm for maca is understandable. There are currently no approved therapeutic drugs to improve sperm quality in men (Cuya et al., 2016). Drugs available to treat erectile dysfunction like tadalafil and sildenafil and drugs used to treat benign prostatic hyperplasia like finasteride come with side effects, cautions and contraindication (Joint Formulary Committee, 2016) and may not be suitable or acceptable to everyone.

The options available to treat menopausal symptoms are few and an increased incidence of breast cancer and cardiovascular disease has been associated with Hormonal Replacement Therapy (HRT) according to the Women's Health Initiative trial (Rossouw et al., 2002). This has spurred many women to look for non-hormonal alternatives such as herbal remedies to make their transition to menopause easier (Meissneret al., 2006d, Comhaire and Depypere, 2015, Taylor, 2015)

A wide range of studies on maca, both clinical trials and *in vivo* and in vitro assays, have been carried out over the years to examine these health claims. However, the general literature reviews about maca (Gonzales et al., 2014, Gonzales et al., 2009, Gonzales, 2012) report mostly a compilation of findings about the *in vitro* biological properties of maca rather than a critical assessment of all the available evidence investigating these health claims. There is also no report to our knowledge, detailing how and

why maca reached such phenomenal recognition and boomed in China in particular. These gaps are what this study seeks to address.

1.1 AIMS AND OBJECTIVES

The aims and objectives of the present study are:

1) to understand the rise of maca to global prominence
2) to provide an insight into the phytochemistry of maca and discuss factors affecting it
3) to critically evaluate and assess, from a pharmacological perspective, the literature surrounding the reproductive health claims of maca,
    a. as a fertility and libido enhancer and as a treatment for ED and BPH in men
    b. as a fertility enhancer and in managing menopausal symptoms in women

2. METHODOLOGY

An initial search of PubMed database was made using '*Lepidium meyenii*', '*Lepidium peruvianum*' and 'maca' to retrieve articles on the species. Google search was used to find websites of sellers of maca. An online search of MetaLib using the same terms was also conducted. Specifically, the databases, EMBASE, AMED (Allied and Complementary Medicine), BioMed Central, Cochrane Library, PubMed and OVID Journals were searched. Search by hand was also done to retrieve any relevant references. A key word search was used as it brought more results than a construction of search terms, for example, any word=(maca) and any word=(menopause). The abstracts of each paper were read to sort out duplicates, determine their relevance to the topic and triaged accordingly into different folders in the reference database. Hand searches of the bibliographies of important articles were also conducted. Papers with no access via UCL were requested through interlibrary loan. The literature is up to date unitil May 2017.

Papers in Chinese and Spanish were translated and key data extracted [Spanish -MH; Chinese - Ka Yui Kum and Andy Chan (London)]. A database of all references used was constructed using EndNote online.

3. RESULTS AND DISCUSSION

Early interest in the maca root was mainly in its nutritional value and economic botany (Leon, 1964, Johns, 1981). Once maca started gaining in popularity in the 1990s, research focused mainly on the pharmacological actions behind the reproductive health enhancing properties of maca (Wang et al., 2007). Chemical profiling of the plant progressed to identify the active metabolites and key marker compounds of the plant. *(See Supplementary Appendix 1)*

3.1. GENERAL AND ECONOMIC BOTANY

3.1.1. Botanical description

Maca has a rosette of frilly leaves and grows in the harsh climate of Central Andes in Peru, where there are strong winds and the highest temperature is 12^{o}C (Balick and Lee, 2002, Hermann and Heller, 1997). Maca is the only domesticated *Brassicaceae* cultivated for food in the Andes. The edible part, most commonly described in literature as the hypocotyl or root, is made up of the main tap root and the base of the hypocotyls (Leon, 1964). Maca root would be referred to as 'maca root', 'root' or 'hypocotyl', interchangeably throughout the text.

The species is a self-pollinated octoploid (2n=8x=64) (Leon, 1964)and propagated via sexually produced seeds. Up to 13 different colours of hypocotyls have been identified ranging from cream to purple and black, a colouration which depends on the content of carotenoids and anthocyanins in the outer skin (Clement et al., 2010a, Leon, 1964). The three main colours, also most widely studied, are red, yellow and black with yellow maca being the preferred variety for its apparently sweeter taste(Quiros and Cardenas, 1992). Maca is biennial in its natural habitat but has been observed to be annual in improved conditions of warmer temperatures and under the use of fertiliser (Quiros and Cardenas, 1992, Hermann and Bernet, 2009)

3.1.2. Botanical Nomenclature

Maca named as *Lepidiummeyenii* Walp, was first described by German botanist Gerhard Walpers in 1843. Dr. Chacon argued that she discovered a 'new' species in 1990, the domesticated maca, different to the wild maca first described, on the basis of variations in morphological features and proposed to name it *L. peruvianum* Chacon but no consensus was reached (Hermann and Bernet, 2009). More recently, analysis of the two isotypes revealed differences in taxonomy, physical morphology, phytochemistry and DNA sequences. *Lepidpium peruvianum* Chacon was deemed the closest representation of the historically reported maca (Meissner et al., 2015a). While this discrepancy may pose a significant quality control issue in the trade of maca, both names are considered binomial synonymous (The Plant List, 2013) and are still used interchangeably in literature.For the purpose of this paper, the term 'maca' refers to both taxonomic names.

3.1.2.1. Maca ecotypes or cultivars?

Based on the literature, a widespread confusion exists about whether the differently coloured forms of maca hypocotyls should be classified as cultivars, ecotypes or phenotypes, terms that are inherently different. The seeds of maca are said to result from centuries of cumulative selection by farmers (National Research Council, 1989). There are some genetic and phytochemical differences between the different hypocotyl colours (Meissner et al., 2015b, Meissner et al., 2016)but Clement *et al.*indicates that all three colours can grow in the same environment(2010a). It was also reported that growth conditions influence metabolite content more than hypocotyl colour(Zhao et al., 2012, Meissner et al., 2016)From the gathered information, it is likely that the different hypocotyl colours are a phenotypical characteristic of the plant and it would be referred to as such in this text.

3.1.3. Local and traditional uses

The common names of *Lepidium meyenii* include (National Research Council., 1989) maca, Peruvian ginseng) English), *maca, maka, maca-maca, maino, ayak chichira, ayak willku (*Quechua and Spanish):.

For over 1,300 to 2000 years maca has been domesticated in San Blas, Junin, (Ondores) Peru and is an important dietary staple of the indigenous people, used both as food and medicine. It grows at altitudes of between 4500-5000m (Cobo., 1653 in Gonzales et al., 2009).Maca is used as a tonic to overcome stress and fatigue, as a fertility enhancer for humans and cattle, as pain reliever for rheumatism, to cure respiratory disorders, as a laxative and to cure anaemia among others (Leon., 1964, Quiros and Cardenas., 1992). Andean people consume more than 100g of maca daily (Valerio and Gonzales, 2005).

Maca is traditionally baked in underground pits called *pachamanca*, boiled and eaten as porridge, *mazamorra*, fermented into a sweet drink, *macachicha* (Ochoa, 2001, Balick and Lee, 2002) and the cooked roots used in jams, alcoholic cocktails and empanadas (Hermann and Bernet.,2009).

In 1653, Father Cobo was the first to describe the fertility-enhancing properties of maca (Gonzales GF., 2011). Inca warriors were fed maca for strength and resilience in battle. Spanish conquests chroniclers report that maca was taken as tax and fed to livestock to counter their decreasing fertility at high altitudes (National Research Council., 1989, Quiros and Cardenas., 1992, Balick and Lee., 2002, Gonzales et al., 2012). Leon., 1964 reported maca consumption among Indian and white women who want to bear children and as a tonic for postmenopausal women. But one study found that not all Andean farmers believed that maca enhanced fertility (Locher., 2006 in Hermann and Bernet., 2009). Also, contrary to purported belief that maca acts as a libido stimulant (Quiros and Cardenas., 1992, Balick and Lee., 2002), at least one knowledgeable indigenous source indicates that maca was never consumed before as an aphrodisiac but rather for its invigorating effects. More recent claims of indigenous knowledge surrounding in particular, improvement of memory and immune system functions, are suggested to be influenced by the internet hype and maca product promotion arising from the maca boom of the 1990s (Hermann and Bernet., 2009).

3.1.4. Maca's transformation into a global commodity

In 1982, maca was slowly falling into decline and was declared in danger of extinction by the International Board for the Protection of Genetic Resources (National Research Council., 1989). The 1990s sees Maca returning from the verge of extinction pushed by an unprecedented international interest and is now being touted as Nature's Viagra, the Peruvian Ginseng. The table below details maca's journey (Balick and Lee, 2002, Vecchio, 2007, Hermann and Bernet, 2009, Gonzales, 2012, Johns, 1981).

Year	Milestones
1960	First newspaper article on maca's fertility enhancing effects by Pulgar
1961	Chacon's pioneering thesis reporting acceleration of graffian follicle maturation and improvement in sperm production in rats
1964	Leon introduces maca to the international audience with his first article on the economic botany of the plant
1978	Pulgar claims that maca-supplemented heifers have increased fertility as demonstrated through his own studies. The information is spread nationally through national newspaper articles.
1980	As reported by Vilchez in 1999, only 15 hectares of land are under maca cultivation. A maca beverage is promoted in a shop on the road to Lima and Huanuco as a tonic to travellers and truck drivers by the owner Timotea Cordova.
1981	Vilchez starts offering maca roots to several local markets in Central Peru and promotes its health attributes using printed leaflets to a wider audience. First phytochemical studies of maca are published.
1982	US students visit Junin and learn about maca
1986	New law ratified by the Peruvian government to promote maca, among other Andean crops. Maca is introduced to other parts of the Peruvian Andes
1987	Maca gains popularity, especially in the city of Lima. Convenience stores start offering preparations of maca roots that mask its strong flavour to make it more palatable to urban customers
1980s	Efforts to protect maca are stalled as Peru is shaken by the unrest caused by the Shining Path Maoist Guerrilla.
1991	With the subsiding Guerrilla threat, maca promotion continues. The Food and Agricultural Organisation of the United Nations recommend maca along with Andean foods to fight malnutrition
1990s	Growing academic interest in the species and increasing numbers of studies.

1995	Quimica Suiza, first Peruvian pharmaceutical company invests more than 1 million USD in the development and branding of maca nutraceutical Maca Andina®. Several companies follow suit and products are patented
Through the 1990s	Maca is promoted more aggressively abroad, particularly in Japan. Demand and exports of maca to Japan, USA and European markets increase. Cultivation of maca intensified with use of mineral fertilisers and financed by the government, reaches 800 hectares. Maca is exported as pills, capsules, flour, liquor and extracts. Maca promotion as the 'Andean Viagra' or 'Peruvian Ginseng' is also started online.
Between 1998 and 2000	Maca demands in China increases drastically. This pushes prices out of reach of local consumers. Attempts are made to grow maca abroad.
1999	Pure World Botanicals devises a maca extraction procedure to produce a lipid extract, MacaPure, with purported claims of increased libido and aiding erectile dysfunction. The extract is US patented and raises controversy to this day.
2000	Dr. Zheng publishes internationally the first *in vivo* assay using mice and rats investigating the effects of MacaPure on fertility through collaboration with researchers in China
2002	The Peruvian government sets up the National Anti-Biopiracy Commission to fight the stealth of indigenous intellectual property from Peru.
Through 2000s	Maca production and exports continued to expand, reaching exports values of 6,179,011.8 USD in 2010.

Table 1: Timeline of Maca, from 'anonymity to stardom'

The Peruvian government proved to be too slow and meek to act. The Department of Health in China approved the cultivation of maca in 2002 and its use as a food resource for health in 2011 (Shu et al., 2015). In China, a total of 1116.13 hm^2 of maca is cultivated in Yunnan, Xinjiang and Tibet yielding 2500t of maca/year and generating an industrial value of 4,359,198 USD and an additional entrepreneurial value of 14,530,660 USD (Zhou et al., 2015). Chris Kilman also reports a maca boom whereby the very high demand and prices for the species pushed Chinese red dragon triad to smuggle maca from the Andes(Smith, 2014). The popularity of maca in China can be related to the belief in Traditional Chinese Medicine (TCM) system which associates maca, especially black maca (Brand, 2016)with the kidneys; an organ, representing both the storage of energy, Jing and its flow, Chi and is related to sexual problems in both men and women. The function of the kidneys is lost with ageing, not recoverable, and damage can only be mitigated. As such, medicines which are believed to enhance its functions, for example, deer antler base, caterpillar fungus (*Ophiocordycepssinensis*, (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora *syn.: Cordycepssinensis* (Berk.) Sacc.;) and shark fins, are often very expensive and rare. The introduction of maca, as a less expensive and more available alternative with its promotion, though without scientific basis, as a libido enhancer and for the kidneys, helps to explain the maca boom in China (*Conversation* with Ka Yui, 8th January 2017). The demand for maca seems to have reached a plateau during the years 2014 to 2015 (Brand, 2016).

3.2. PHYTOCHEMISTRY OF MACA

3.2.1. Nutrition:

Fresh maca has a high water content of more than 80% which makes it a low energy food source with high nutrient density. It contains 59% hydrolysable carbohydrates, 10 to 16% proteins and 2.2% lipids.

It is also rich in minerals, essential amino acids and contains a significant amount of Iron and Iodine, which tend to be deficient in highlands diets (Dini et al., 1994),(National Research Council 1989).

3.2.2. Secondary metabolites:

Chemical profiling of maca has enabled identification of several secondary metabolites. The most notable ones are: the imidazole, hydantoins and thiohydantoin alkaloids, the alkamides and glucosinolates and the meyeniins (Cui et al., 2003, Jin et al., 2016, Muhammad et al., 2002, Zhao et al., 2005, McCollom et al., 2005, Chain et al., 2014, Zheng et al., 2014, Zheng et al., 2003, Li et al., 2001, Dini et al., 2002, Piacente et al., 2002, Yu et al., 2017b, Zhou et al., 2017; *Supplementary Data SupplementaryAppendix 1.Phytochemicals of maca)*

Also reported are phytosterols, polyphenols, tannins, small amounts of saponins and tetrahydromethyl-β-carboline (Wang et al., 2007, Gonzales and Gonzales-Castaneda, 2009, Leon, 1964, Piacente et al., 2002).

3.2.2.1. Imidazole alkaloids

Four imidazole alkaloids unique to maca have been identified. They are Lepidiline A, Lepidiline B, isolated from a concentrated lipid extract of maca (Cui et al., 2003) and lepidiline C and Lepidiline D, isolated from 95% ethanolic extract of maca (Jin et al., 2016). So far, no information is available about their possible biological properties.

3.2.2.2 Macahydantoins and macathiohydantoins

Macahydantoins A and B are hydantoins unique to maca. Hydantoins are known to have significant biological properties. However, none of macahydantoins A or B showed any cytotoxic activity against five human cancer cells (human promyelocytic leukemia cell HL-60, human hepatocellular carcinoma cell SMMC-7721, adenocarcinomic human alveolar basal epithelial cell A-549, human breast cell MCF-7, and human colon adenocarcinoma cell SW480) (Yu et al., 2017b). Macathiohydantoins B-K are another class of characteristic compounds isolated from maca. They also do not exhibit any cytotoxic or antimicrobial properties against three bacterial strains (*S. aureus, E. coli*, and *P. aeruginosa*) and three fungal strains (A. *fumigatus, C. parapsilosis*, and *C. albicans*) (Yu et al., 2017a). But structure-wise, these compounds can be of interest.

3.2.2.3. Meyeniins

A new sulfur-containing hexahydroimidazo[1,5-c]thiazole derivatives, (+)-meyeniins A−C were isolated as some of the major constituents from the lipidic fraction of maca alongside the macamides, glucosinolates and macaenes. (+)-meyeniin A showed moderate selective cytotoxicities against the HL-60, A549 and MCF-7 human cell lines with IC50 values of 14.41, 32.22, and 33.14 μM, respectively, supporting use of maca as functional and protective food (Zhou et al., 2017).

3.2.2.4Alkamides

Alkamides, which consists of an amine linked to an unsaturated fatty acid by an amide linkage, are usually found in the Rutaceae, Asteracecae, Piperaceae and Aristolochiaceae. However, macamides are alkamides unique to *L. meyenii* within the Brassicaeae. Macamides are secondary amides formed from an unsaturated fatty acid moiety linked through an amide linkage to a benzylamine (Greger, 1984, Esparza et al., 2015). Alkamides can be used as chemotaxonomic or marker compounds to identify maca and also as a means of standardisation. (McCollom et al., 2005, Zhao et al., 2005, Muhammad et

al., 2002). However, it should be noted that macamides are not present in whole, undamaged fresh maca hypocotyls. They occur as a result of traditional Andean practices, which influence the availability of substrates like free fatty acids and benzylamine resulting from the breakdown of glucosinolates (Esparza et al., 2015). In nine different commercial preparations of maca, the marker content was found to vary between 0.15% to 0.84%, indicating a variation in daily intake of these compounds and thus therapeutic effects associated with them (Granzera et al., 2002). McCollom et al., 2005 also reported significant variations of marker compounds in samples of maca ranging between 0.0016 to 0.0123% of dried plant material, from five different vendors. Drying practices during processing may, among other factors, be responsible for the variations observed. Oven-dried whole maca hypocotyls were found to have the highest content of macamides while freeze-drying and steaming reduced macamide content significantly (Pan et al., 2016). This can guide manufacturing protocols for maca-based products requiring specific macamide content for specific health benefits.

3.2.2.5 Glucosinolates

Glucosinolates have been suggested to be responsible for the fertility-enhancing properties of maca (Johns, 1981). They are found in the *Brassicaceae* and confer the pungent aroma and bitter flavour associated with crucifers. The breakdown products of glucosinolates are biologically active compounds like indoles, nitriles, thiocyanates and isothiocyanates, which are known to have anti-cancer effects (Gupta and Prakash, 2014). Of the nine glucosinolates identified in maca, benzyl glucosinolate (glucotropaeolin) and methoxy-benzyl glucosinolate are the most abundant (Li et al., 2001, Dini et al., 2002, Piacente et al., 2002) with glucotropaeolin reported making up 80% of glucosinolate content (Yabar et al., 2011).Fresh maca hypocotyl has also been found to have 10 times more glucosinolate content than other *Brassicaceae* species like broccoli and cauliflower inflorescences. The glucosinolate content decreases significantly after drying and processing of the hypocotyls. For instance, no glucosinolate was found in tonic and liquor of maca. This is believed to be due to hydrolysis of the compounds by myrosinase enzyme liberated upon break down of plant tissues (Li et al., 2001, Esparza et al., 2015).

3.2.2.6Phytosterols

Several phytosterols have been isolated from maca, the main ones being: beta-sitosterol, campesterol, ergosterol, brassicasterol and stigmasterol (Dini et al., 1994, Wang et al., 2007). Beta-sitosterol showed higher concentration in the leaves than in the hypocotyls but did not change with respect to other factors of terrain type and hypocotyls colour (Clement et al., 2010a).

3.2.2.7Fatty acids

20 fatty acids have been isolated as methyl esters (Dini et al., 1994). While, these are commonly referred to as ‘macaenes’ in literature, Wu *et al*. suggest a discontinuation of the term ‘macaene’ because no alkene hydrocarbons have ever been isolated in maca; the only ones being free fatty acids like linolenic acid, linoleic acid (2013).

3.2.3. Factors influencing the phytochemical composition of maca:

3.2.3.1. Maca hypocotyl’s colour:

The link between maca hypocotyls colour and (secondary) metabolites is still debatable. Yabar *et al*. found similar glucosinolate content between yellow, red and black maca hypocotyls (2011). But Clement *et al*. observed that lead-coloured maca had higher glucosinolate content than yellow

hypocotyls, yellow hypocotyls had higher macaenes content and violet maca had higher macamides content (2010a). Another study found that glucosinolate content among three maca phenotypes varied as red > black > yellow (Meissner et al., 2015b).

3.2.3.2. Cultivation practices and area of cultivation:

On the other hand, cultivation practices and terrain type will also influence secondary metabolites content and may have more impact than the maca phenotype itself (Zhao et al., 2012). Maca is known to be a soil depleting plant to grow and the land is traditionally fallowed for 10 years before planting again (National Research Council, 1989). However, with the rapidly growing maca market, most farmers are replanting maca after 4-5 years of fallow only (Hermann and Bernet., 2009). Other studies have shown that the cultivation area can significantly affect the nutritional and metabolite content of maca. For example, yellow maca samples grown in China had a higher mineral and protein content than yellow maca grown in Peru (Zhang et al., 2015, Chen et al., 2017). Additionally, most scientific studies investigated the properties of Peruvian maca but not Chinese maca *(see appendix 2)*. Considering the difference in composition between the two types the results obtained with Peruvian maca may not be correctly extrapolated to Chinese maca.

3.2.3.3. Adulterants and contaminants:

There is also, at least one report from Japan of maca stock heavily contaminated with pesticides (Hermann and Bernet., 2009) and a health warning issue by the French Food Safety Agency in 2007 concerning the presence of tetrahydromethyl-β-carbolines in maca. However, this was refuted by Gonzales and Gonzales-Castenadas, 2008, as naturally occurring and non-toxic on consumption. There are also anecdotal reports of maca adulteration with yam powder (USP Safety Review of Maca, 2012) and one study also detected a sildenafil analogue, desethylcarbodenafil in a maca-containing herbal supplement (Huang et al., 2016).

The shift from traditional methods of cultivation and manufacturing of maca to modern ones in new regions, inevitably affects the composition of the plant. The lack of standard quality control protocols in place (Li et al., 2001, Shu et al., 2015) renders the quality, efficacy and safety of available maca products questionable and poses a threat to consumers worldwide. Suggestion about loss of secondary metabolites from grazing insects as suggested by reviewer number 2?

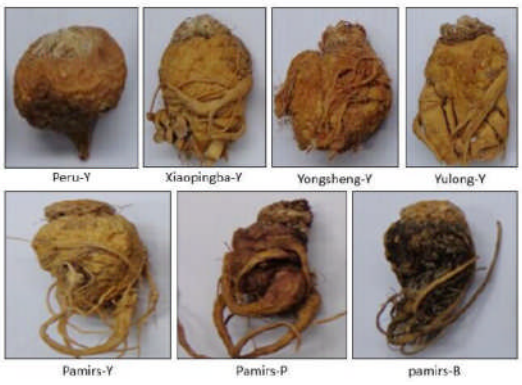

Fig 2: Peruvian maca and Chinese grown maca studied by Chen et al., 2017(Y – Yellow, P – Purple, B – Black).

3.3. PHARMACOLOGY

3.3.1. Maca in male reproductive health: *in vivo* studies

In some studies, the preparation of aqueous extract of maca for *in vivo* study purposes was claimed to reflect a traditional method of preparing maca done by boiling and then filtrating (Gonzales et al., 2001b, Gonzales et al., 2004, Chung et al., 2005, Bustos-Obregon et al., 2005, Rubio et al., 2006, Gonzales et al., 2006a, Gonzales et al., 2005).Other extract types have also been investigated and may help give an indication as to which bio-active compounds are responsible for the observed effects of maca on male fertility (Zheng et al., 2000, Cicero et al., 2002, Gonzales et al., 2003b, Gonzales et al., 2007, Gonzales et al., 2008, Yucra et al., 2008, Inoue et al., 2016, Ohta et al., 2016, Zhang et al., 2016).Maca powder was also tested (Cicero et al., 2001, Clement et al., 2010b, Lavana et al., 2013).

The effects of different maca phenotypes have been investigated(Gonzales et al., 2005, Gonzales et al., 2006a, Gasco et al., 2007a, Noratto et al., 2013, Inoue et al., 2016). Experimental evidence seems to suggest that three phenotypes of maca have different physiological effect. For instance, black maca and to a lesser extent, yellow maca was found to influence sperm production but not red maca. Red maca and to a lesser extent yellow maca reduced prostate size in rats with testosterone-induced prostatic hyperplasia but not black maca (Gonzales et al., 2009, Gonzales, 2012).

A number of studies use very high doses of maca extract such as 2.2g/Kg (Rubio et al., 2006), 666mg/Kg BW (Gonzales et al., 2004)and 4g/Kg BW (Zheng et al., 2000)daily. While these may seem not practical clinically, it has been argued that Andean people consume an average of more than 100g of maca daily (Valerio and Gonzales, 2005).

3.3.1.1. Spermatogenesis

Several studies have investigated the effects of maca and its extracts on spermatogenesis in rodents and other animals. Spermatogenesis is the process of formation of sperm cells that occurs in the seminiferous tubules of the testes (Ray et al., 2015).Stage VIII of the seminiferous tubules epithelium cycle is the restructuring and release of mature spermatids from the sertoli cells into the seminiferous tubule lumen. Since, it determines the number of sperm entering the epididymis and thus the sperm content of the ejaculate, it is viewed as critical for male fertility(O'Donnell et al., 2011; s*ee Appendix 1:in vivo Studies of Maca on Spermatogenesis.*

3.3.1.1.1. Influence of aqueous extracts of different maca phenotypes

There is limited evidence to support that aqueous extracts of different maca phenotypes have different effects on spermatogenesis in rodents. The overarching evidence suggests that aqueous maca extract increases the frequency of Stage VIII of spermatogenesis often with an accompanying epididymis weight increase but that the overall sperm count may not necessarily be affected (Gonzales et al., 2001b, Gonzales et al., 2004, Chung et al., 2005, Gonzales et al., 2006a, Gasco et al., 2007a, Gonzales et al., 2006b).

Gonzales *et al*. reported that 666.6mg/day of aqueous extracts from black and yellow maca phenotypes increased the length of Stage VIII and Epididymal Sperm Count (ESC) in rats at both 7 and 42 days of treatment but that the red maca aqueous extract did not(2006a). Black maca extract was the only one to increase Daily Sperm Production (DSP). Likewise, Gasco *et al*. report that ESC was increased with the black and yellow maca extracts but not with the red maca extract at a dose of aqueous maca extract equivalent to 1g of maca/Kg BW. But it is also reported that all three maca phenotypes increased the sperm count in the vas deferens without affecting DSP after treatment over 84 days(2007a). Additionally, a dose-related effect on increasing Stage VIII in rats with a yellow maca aqueous extract

was observed. The smallest effective dose reported was 0.01g/Kg BW and a plateau effect was observed at 1g/Kg BW of extract (Chung et al., 2005). There is also report of an aqueous extract of black maca at the dose of 666.6mg/day over 12 days changing the pattern of spermatogenesis but not that of overall sperm count in rats (Gonzales et al., 2006b).

The lengths of treatment employed and the amounts of extract used vary between studies as does the methodology. This makes comparison and drawing an accurate conclusion difficult. The limited available evidence suggests that an aqueous extract of black and yellow maca phenotypes may increase the pattern of spermatogenesis to some extent but not an aqueous extract of red maca. There is also not enough evidence to support that black maca or yellow maca aqueous extract increases daily sperm production.

3.3.1.1.2. Influence of other types of maca extract preparation

Several extracts of maca have also been assessed and the very limited evidence suggests that different types of maca extracts may have varying effects on spermatogenesis in rodents. A key limitation to these studies is that the maca treatment is given and assessed over a length of time less than a full cycle of spermatogenesis in rats, which is 12 days (Aslam et al., 1999 in Gonzales et al., 2006a) or 8.6 days in mice (Ray et al., 2015). An ethanolic extract of maca, given at 96mg/day showed a decrease in length of Stage VIII but an increase in lengths of Stage VII and IX-XI with an increase in sperm count (Gonzales et al., 2003b).

Other extracts investigated, showed that ethyl acetate and chloroform fractions of black maca had a higher potency in increasing DSP and epididymis sperm count than petroleum ether, n-butanol and hydroalcoholic fractions when given over 7 days (Yucra et al., 2008). The ethyl acetate fraction and chloroform fractions are relatively less polar than the n-butanol and hydroalcoholic fractions but relatively more polar than the petroleum ether fraction (Murov, 2010), this indicates that potential compounds responsible for the effects observed are likely found in a less polar extract. However, another study found that the methanolic extractsof yellow and black maca and their respective aqueous extracts were more effective than their butanolic fractions in improving spermatogenesis in mice at a dose equivalent to 1g raw material/Kg BW over 3 days(Inoue et al., 2016), indicating the efficacy of a more polar extract on spermatogenesis. Composition-wise, the methanolic extract and its butanolic fraction but not its aqueous fraction contained alkaloids. The aqueous fraction also had higher phenolic content than the butanolic fraction (Inoue et al., 2016). This may mean that polyphenols and not alkaloids, are responsible for the effects observed on spermatogenesis. While the scarce evidence available suggests that extract composition will influence the observed effects of maca on spermatogenesis, it cannot be determined what specific extract fraction is the most conducive to spermatogenesis. Further research into the extract type, composition and corresponding effect on spermatogenesis with standardised length of treatment and methodology is required to draw an accurate conclusion.

3.3.1.1.3. Influence of maca on semen parameters.

The limited evidence available on whether or not maca enhances fertility by improving semen parameters is inconclusive. In rats treated with yellow maca aqueous extract, no effect on sperm motility (Chung et al., 2005)was noted but black maca aqueous extract in rats, showed a significant improvement in sperm motility after 42 days of treatment (Gonzales et al., 2006a). In pre-pubertal breeding bulls fed a mix of all three dried maca phenotypes at 233mg/Kg BW, an increase in sperm motility was noted with an increase in ejaculation volume. The effect was noticeable in animals with an initially poorer

sperm quality and only in those that had been fed for a longer length of time of 10 weeks (Clement et al., 2010b). However, no increase in ejaculation volume or sperm concentration after an 8 week long supplementation with milled maca dried hypocotyls in rams at the same dose was noted (Lavana et al., 2013).

3.3.1.1.4. Protective effects of maca on spermatogenesis

Maca has also been shown to have some protective effects against external noxious factors that can affect spermatogenesis. For instance, the multi-factorial negative effects of high-altitude exposure on spermatogenesis in rats were seen to be reversed by administration of 666.6mg/day of aqueous maca filtrate (Gonzales et al., 2004). Moreover, administration of 66.6mg/day of aqueous maca extract over 21 days was shown to protect the Stages of VII, VIII and IX of spermatogenesis from the inhibiting effects of a single dose of 80mg/Kg of malathion in mice (Bustos-Obregon et al., 2005). Aqueous macaextract at a dose of 2.2g/Kg daily in rats was also found to reverse the damaging effect of lead acetate on Stages VII, VIII and IX-XI of seminiferous epithelium lengths and maintained corpus and cauda epididymis sperm count, daily sperm count and spermatid count (Rubio et al., 2006).

A study by Cuya *et al*. of maca on chemically and physically induced sub-fertility in mice also gave concurring results with 666mg/Kg BW daily of aqueous extract (2016). The inhibiting effect of Ketoconazole on CYP450 enzyme in the gonadal androgen synthesis pathway seems to have been reversed with aqueous maca supplementation, preserving sperm count and sperm motility. While in ELF-MF (extremely low frequency magnetic waves) induced sub-fertility where mitochondrial function is affected, maca supplementation did not protect sperm parameters but seemed to decrease occurrence of DNA fragmentation, suggesting anti-oxidant properties(Cuya et al., 2016).

It is plausible to say that the perceived indigenous fertility enhancing properties of maca may stem from the protective and nutritional properties of maca rather than from a direct effect on sperm production.

3.3.1.2. Prostatic hyperplasia

Benign prostatic hyperplasia entails the enlargement of the stromal and epilthelial cells in the prostate and an increase in 5-alpha reductase enzyme activity (Diaz et al., 2016). There is some evidence demonstrating the prostrate-reducing effect of red maca in rodents treated with testosterone enanthate (TE) to induce prostatic hyperplasia (Gonzales et al., 2005, Noratto et al., 2013, Gasco et al., 2007b, Gonzales et al., 2007, Gonzales et al., 2008). A reduction in prostate size was also found in the absence of TE-induced prostatic hyperplasia after 42 days of treatment with red maca (Gonzales et al., 2006a). This property was more pronounced with the red maca phenotype than in the yellow or black maca phenotypes (Gonzales et al., 2005, Gonzales et al., 2006a, Noratto et al., 2013). The red maca extract appeared to be prostrate-specific, unlike finasteride, and did not affect other androgen-dependent organs like the seminal vesicle(Gasco et al., 2007b, Gonzales et al., 2007, Gonzales et al., 2005, Gonzales et al., 2012). Doses of 0.1g/Kg BW and 0.5g/Kg BW daily extract was also found to be more effective than 0.6mg/Kg BW of finasteride in reducing prostate size (Gasco et al., 2007b; see*Appendix 3*).

The smallest effective dose of red maca aqueous extract reported is 0.1g/Kg BW. The reduction of prostate size appeared to be inversely proportional with the dose of extract given (Gasco et al., 2007b). A similar effect is reported with increasing doses of benzyl-glucosinolate ranging from 0.02 to 0.08mg/ml in red maca extracts (Gonzales et al., 2007). This suggests that benzyl –glucosinolate may be responsible for the effects on prostrate.

Boiling time in the preparation of aqueous red maca extract did not significantly affect the effectiveness of the extract. There was also no difference between the prostate reducing effect of an aqueous and a hydroalcoholic extract of red maca in rats after 14 days at 1ml of extract (Gonzales et al., 2007). However, in mice, a 60% hydroalcoholic extract of red maca administered at 140mg/Kg BW did not produce a reduction in prostate until day 21 but effects on stromal cells were observed from day 7 (Gonzales et al., 2008). This might be due to an inter-species difference or a variation in hydroalcoholic extract preparation and thus phytochemical composition.

While the limited evidence seems to support that red maca extract can reduce prostate size and may potentially be used to treat BPH, a key limitation is that the maca extract is administered at the same time as the TE in the majority of these studies. This means that prostatic hyperplasia would potentially not have developed before treatment was given, contrary to clinical practice. Indeed, an aqueous extract of red maca did not reverse the effect of TE on prostate size after day 7 and day 14 of treatment if it was not administered at the same time TE was first given on day 1 (Gonzales et al., 2012). This suggests that red maca might have more of a preventative than curative effect in the treatment of BPH but more research is needed to prove that.

3.3.1.3. Sexual performance

There is some evidence to support that maca can enhance sexual performance in rodents and rams(Zheng et al., 2000, Cicero et al., 2001, Cicero et al., 2002, Zhang et al., 2016, Lavana et al., 2013). For instance, the lipid extract of maca standardised to 0.6% macamides and macaenes, increased the number of complete intromissions at a smallest effective dose of more than 45mg/kg BW. The number of sperm-positive females also increased significantly when male mice were given a dose of 4g/Kg BW of the extract (Zheng et al., 2000). Chemical analysis of the extract determined benzylglucosinolate as a major compound, appearing to concord with the suggestion that glucosinolate may be responsible for the fertility-enhancing effect of maca (Johns, 1981) and that these are concentrated in that fraction of maca. However, an investigation to determine which of methanolic, chloroform and hexanic extracts of maca was more effective in improving sexual performance, delivered debatable results. The hexanic extract improved only one extra parameter of mount Latency (ML) relative to the methanolic fraction and both had displayed similar effects (Cicero et al., 2002). Conversely, an ether extract of pulverised dried maca root was found to be the most effective at improving sexual performance in castrated mice and more effective than the aqueous or ethanolic extracts. It was suggested that the non-polar ether fraction may possibly contain alkaloids and aromatic isothiocyanates which may be responsible for the effects observed (Zhang et al., 2016; see*Appendix 4*).

The effects of maca on sexual performance appeared to change with length of treatment and dose administered. Acutely administered 15mg/kg BW and 75mg/kg BW of pulverised maca root to mice decreased mount latency (ML), intromission latency (IL) and inter-copulatory interval (ICI). Only 75mg/kg dose decreased post-ejaculatory latency (PEL). Chronically administered both 15mg/kg and 75mg/kg decreased ML, IL, ejaculation latency (EL) and post-ejaculatory latency (PEL) and appeared to be dose-related while only 75mg/kg decreased ICI (Cicero et al., 2001). On the other hand, an aqueous solution of maca was found to increase the time to first ejaculation and PEL to a small extent in rats when administered over seven but not 21 days. The copulatory efficiency and ICI were not affected. As commented by Dr. Francois Guilliano, the results of the experiment indicate that maca could potentially be used to treat premature ejaculation in men (Lentz et al., 2007) In contrast, in a study in rams using dried black maca, Lavana *et al*. reported an increase in libido measured through increased number of mounts and ejaculations (2013). The effects of maca were also observed to last 8 weeks after

maca treatment had stopped (Lavana et al., 2013).Another study found that maca increased sexual capacity in low performance but not in high performance rams (Avelar et al., 2016)

While the limited evidence suggests that maca extracts can enhance sexual performance, the premise is still arguable. It is unclear which parameters of sexual performance are increased since all the studies do not employ the same methodology and do not assess the same parameters or the same number of parameters (Zheng et al., 2000, Cicero et al., 2001, Cicero et al., 2002, Zhang et al., 2016, Lentz et al., 2007). It is also uncertain which type of extract, dose or duration of treatment is needed to elicit the desired effects of enhanced libido.

3.3.1.4. Erectile dysfunction

MacaPure extract also showed an increase in electronically stimulated latent penile erection (LPE) time in rats fed a dose of 45mg/Kg BW and above with no more increase at higher doses of 180mg/Kg BW and 1800mg/Kg BW (Zheng et al., 2000). Kimura *et al* 2016 reports a significant increase in intra-cavernous pressure to mean arterial pressure ratio in maca treated rats with streptozotocin-induced diabetes mellitus (SID) relative to untreated rats with SID (2016). These two studies suggest potential use of maca in treating erectile dysfunction related to hypogonadism and microvascular damage in diabetes. More research is needed to ascertain the results of these studies (see *Appendix 4)*.

3.3.2. Maca in male reproductive health: Clinical studies

3.3.2.1. Sexual desire

A clinical trial of 56 healthy men using 1.5 to 3.0g daily of gelatinised maca showed a significant improvement in sexual desire after 8 week but not 4 weeks independently of Hamilton depression and anxiety scores(Gonzales et al., 2002). Maca also improved subjective sexual desire in men suffering from Hypoactive Sexual Disorder after 3 months treatment at a lower dose of 960mg/day. Maca combined with testosterone had a better effect in improving HSD in men with hypogonadism relative to the use of testosterone only (Poyato et al., 2009). However, with roughly twenty subjects per treatment group and no regression analysis provided in relation to the Rosenberg self-esteem test score and PSS-10 test, larger trials taking the psychological element of the condition into consideration, are needed to confirm the results. In a small pilot study, dyadic sexual desire increased in cyclists after 14 days treatment at 2000mg/day of an aqueous maca extract (Stone et al., 2009).This may suggest that the aphrodisiac properties are found in the aqueous extract of maca, explaining why the effect was noticed much earlier than with gelatinised maca but the sample was also very small, which decreases accuracy of results. Another larger cohort study of 175 participants treated with 3g of spray-dried maca over a period of 12 weeks showed only a modest increase in sexual desire with red maca relative to placebo (Gonzales-Arimborgo et al., 2016; See Appendix 4)

3.3.2.2. Erectile dysfunction

Zenico *et al*. reports a small improvement in erectile dysfunction (ED) and social and physical performance of STA-P score with maca treatment as measured by International Index of Erectile Function (IIEF -5) and Satisfaction Profile (SAT-P) (2009). It is postulated by authors, while considering the limits of the study of mild ED patient within a short period of time, that larger scale trial run for a longer time and with patients with more serious ED should be done to confirm effect of maca on ED. The multi-factorial nature of ED involving both a psychological and a clinical component makes it difficult to identify whether maca improves ED by acting centrally or specifically on the

reproductive tract(Zenico et al., 2009). Nonetheless, the pilot results look promising and warrant further investigation in larger scale trials.

3.3.2.3. Semen parameters

Most trials investigating the effects of maca on semen parameters involve relatively small numbers of subjects and report an improvement in sperm motility after 3 months of maca treatment (Gonzales et al., 2001a, Tancara et al., 2010, Poveda et al., 2013, Melnikovova et al., 2014). Gonzales *et al.* reports an increase in sperm count related to an increase in ejaculation volume (2001a) and Melnikovova et al. reports an increase in sperm motility and sperm count relative to placebo (2014).Tancara *et al.* attributes two pregnancies among the test subjects' couples to subjects with the highest increase in sperm motility after 3 hours following the intervention (2010). While, on the other hand, only an increasing trend with no statistically significant change in semen parameters was observed in a trial of 18 healthy subjects over 12 weeks (Melnikovova et al., 2015). Similar to the results seen in *in vivo* studies, the evidence for improving fertility in men remains inconclusive.

3.3.3.Is there any evidence for androgen-mediated action of maca?

In males, androgens play an essential role in the reproductive and sexual function. For instance, deficiency of androgen production, specifically testosterone, in the leydig cells in the testes will impair spermatogenesis, leading to infertility. Testosterone is also important in the development of male sexual organs (Dohle et al., 2003). Hence, the potential for maca to exhibit androgen-mediated pharmacological action, which could support its claim as a fertility and libido enhancer in men, is discussed.

Despite evidence of activity on libido and fertility parameters in men, maca supplementation did not change serum hormone levels of prolactin, 17-hydroxyprogesterone, testosterone, 17-β estradiol, Luteinising Hormone (LH) and Follicle Stimulating Hormone (FSH), suggesting a non-androgen mediated mechanism of action(Gonzales et al., 2001a, Gonzales et al., 2002, Gonzales et al., 2003a, Tancara et al., 2010, Melnikovova et al., 2015, Melnikovova et al., 2014).This reflects what was found in the vast majority of experimental studies on rodents. Apart from a size-reducing effect on prostate by red maca only, there was no weight change in androgen-dependent organs like seminal vesicles and testes and no change in serum testosterone levels, with any phenotypes at any doses of aqueous extract or dry powder of maca tested(Gonzales et al., 2001b, Gonzales et al., 2005, Rubio et al., 2006, Gonzales et al., 2006a, Gasco et al., 2007b, Gonzales et al., 2007). Furthermore, methanol, ethanol, hexane and chloroform extracts of maca were unable to regulate glucocorticoid response element activation *in vitro*(Bogani et al., 2006). Red maca extract did not affect the viability of human prostate cancer cell line LNCaP (Diaz et al., 2016).

However, Ohta *et al*. reported an increase in testosterone level and size of seminal vesicles in pubertal rats fed with a 60% hydroalcoholic extract of maca (2016). While the developmental stage of the rats might have influenced the results, a higher level of testosterone production in leydig cells taken from maca-fed rats and cultivated with 22R-hydroxyprogesterone and pregnenolone *in vitro*, was also observed. Feeding hydroalcoholic extract powder of maca to rats was also found to enhance testicular gene expression of 3β-hydroxysteroid dehydrogenase, the enzyme that catalyses the biosynthesis of progesterone from pregnenolone (Ohta et al., 2017). Long-term treatment with hydroalcoholic maca extract to male rats enhanced steroidogenic ability of Leydig cells and appeared to alleviate the decline in testosterone production associated with ageing in mature rats (Yoshida et al., 2017). An increase in seminal vesicle weight and testosterone levels was also noted in mice treated with an ether extract of

maca (Zhang et al., 2016). Interestingly, a clinical case report was made of maca consumption causing testosterone assay interference, suggesting that components of maca may have an androgen-like moiety (Srikugan et al., 2011).

While the presence of an androgen-mediated pharmacological action *in vivo* remains unclear, other possible mechanisms of action and responsible bio-actives can be inferred. Gasco *et al*., who showed a decrease in prostatic size similar to 5-alpha reductase inhibitor, finasteride, without affecting seminal vesicle weight(2007b), suggests that maca may potentially be acting at the post-dihydrotestosterone conversion stage in the testes. Along with reducing prostate size, red maca extract was also found to reduce intra-testicular zinc levels; increased zinc levels are correlated with increased incidence of benign prostatic hyperplasia (Gonzales et al., 2012). This suggests that bio-actives in maca likely modulates some aspect of metabolism or cellular transport in reproductive organs as was speculated before regarding the effect of black maca on the pattern of spermatogenesis and sperm distribution (Gonzales et al., 2006b).The synergistic effect of maca with phosphodiesterase inhibitor, taladafil, in improving erectile dysfunction in rats with streptozotocin-induced diabetes mellitus(Kimura et al., 2016), and a reversal of CYP450 mediated ketoconazole induced sub-fertility in rats (Cuya et al., 2016), suggest a possibly enzymatic modulation of maca extract. The pharmacological action of metabolites in maca remains open to debate.

3.3.4. Maca in female reproductive health: *in vivo* studies

3.3.4.1. Fertility

The effect of maca consumption on improving the female fertility has been studied mainly through *in vivo* studies in intact mice and rats.

With maca powder, an increase in progesterone level in mice and rats was observed but no change in implantation rate in mice (Oshima et al., 2003, Meissner et al., 2006a). There was also a significant increase in LH and FSH level but no change in pattern of LH surge (Uchiyama et al., 2014) or estrus cycles (Gasco et al., 2008). Aqueous extract of red, yellow or black maca did not affect the number of oocytes, uterine weight (Gasco et al., 2008)or implantation site, gestation length or sex ratio of pups (Ruiz-Luna et al., 2005). However, maca-treated group had a larger litter size by day 4 post-birth (Ruiz-Luna et al., 2005)and an improvement in pregnancy rate with increased post-birth survival of pups was noted with methanol and pentane maca extract (Pino-Figueroa and Maher, 2009). Some of the doses used in these studies, like 30g extract/Kg BW (Uchiyama et al., 2014)are very unrealistic if translated to humans (see *Appendix 7)*

3.3.4.2. Menopause

Ovariectomised rats have been used as a model of menopause and sham operated animals as control to investigate the claims that maca can help stabilise hormones imbalance during menopause and prevent associated bone loss. Measurement of organs affected by estrogen and progesterone like the uterus and hormone levels were made.

Overall, the evidence gathered from *in vivo* studies is too incongruent to support claims regarding hormone balance but though scarce, look promising for protection against bone loss *(see Appendix 7)*.

3.3.4.2.1. Hormone levels:

Maca powder administration at a dose of 250mg/Kg BW appeared to decrease Estradiol and Progesterone levels at 4 weeks (Meissner et al., 2006b) but seemed to increase E2 level at a dose of

500mg/Kg BW ethanol extract (Zhang et al., 2008) at 7 months and at 1.8g/Kg BW of yellow maca powder at 7 weeks (Wang et al., 2009). An increase in E2 levels at 28 weeks with 0.096g/Kg of a 0.6% macamides standardised ethanol extract of maca but not with the higher dose of 0.24g/Kg (Zhang et al., 2014).

Results for uterine weight also appear inconsistent. While an increase in uterine weight is noted with 1g of aqueous extract/Kg BW in mice (Ruiz-Luna et al., 2005)and a reduction in uterine weight loss observed with 1.8g/Kg BW pulverized maca in rats (Wang et al., 2009, Barraza et al., 2015), no restoration of uterine weight following ovariectomisation and treatment with maca was observed with an ethanol extract at either 0.096g/Kg or 0.24g/Kg (Zhang et al., 2014).

Furthermore no increase in uterine weight was found with yellow, red or black maca when given at a standardised dose of 4.3mg polyphenol (Gonzales et al., 2010). It is possible that the doses used were too low in the second instance or that the actives affecting uterine weight not present in the standardised ethanolic maca extract. While the doses investigated in these studies are very high compared to those employed in humans in clinical trials, the safety of taking maca as an HRT that does not cause endometrial hyperplasia is not ascertained. As Dr. Muller emphasized, different patients may have differing levels of sensitivity to maca administration and the dose should be adjusted accordingly (Walker, 1998).

On the other hand, maca administration seemed to improve blood lipid profile to some extent without affecting body weight (Meissner et al., 2006a, Meissner et al., 2006b, Zhang et al., 2008, Wang et al., 2009, Barraza et al., 2015). ACTH and Cortisol levels were also lowered with an associated anti-depressant effect (Meissner et al., 2006a, Meissner et al., 2006b).

It is not known whether ovariectomised rats can be used as a model for pre-menopausal women. The studies also do not report at what stage of the oestrus cycle the control rats or mice are at the time of the readings of hormone levels. Follicle Stimulating Hormone (FSH), Luteinising Hormone (LH), Estradiol and Progesterone (PGR) levels would naturally fluctuate (Hubscher et al., 2005) hindering accurate comparison between the ovariectomised rats and sham operated rats and contributing to discrepancy of results.

3.3.4.2.2. Osteoporosis

Effects of maca on bone metabolism seemed positive. An increase in bone mineral density, absolute femur weight, increase in Blood gla protein, a bone formation marker, and a reversal of bone loss after ovariectomy which was due to increase in trabecular area have been reported. The effects were also found to be more pronounced with red and black maca over yellow maca (Zhang et al., 2006, Wang et al., 2009, Gonzales et al., 2010). The positive effect of maca on osteoporosis was also demonstrated *in vitro* using MC3-T3 E1 osteoblast-like cell. Macamide *N*-benzylpalmitamide , promoted cell-proliferation and a dose-response effect in increasing extracelluar matrix mineralisation at a concentration of 10^{-8} M. Importantly, ethanolic extract with same content of *N*-benzylpalmitamide, had better effect than the compound alone (Liu et al., 2015), suggesting a synergistic effect of the metabolites in maca in the protection against bone-loss.

3.3.5. Maca in female reproductive health: Clinical studies

3.3.5.1. Menopause

There exist several accounts from alternative medicine practitioners and doctors describing how maca use alleviates menopausal symptoms of hot flashes, night sweats, depression and vaginal dryness and improves sex life in peri-menopausal and post-menopausal women and in women who had undergone hysterectomy. This is associated with the belief that maca can restore hormonal balance in menopause during which, there is a sharp decline in estrogen which causes the onset of menopausal symptoms(Walker, 1998, Hudson, 2008). Physiologically, the secretion of Luteinising Hormone (LH) and Follicle Stimulating Hormone (FSH) by the pituitary gland increases significantly as the levels of Estrogen (E2) and Progesterone (PG) fall in menopause (Fox, 2011; see *Appendix 9*).

In recent years, several clinical trials have been run to investigate these potential claims. Most suggest that maca may have some positive effects on menopausal symptoms and hormone levels. However, a significant positive placebo effect is also reported in all studies, a relatively small sample size is assessed and no blinding results are provided (Meissner et al., 2005, Meissner et al., 2006e, Meissner et al., 2006c, Meissner et al., 2006d, Brooks et al., 2008, Stojanovska et al., 2011, Stojanovska et al., 2015). This undermines the drawing of firm conclusions from results. The fact that four of these studies come from the same research group (Meissner et al., 2005, Meissner et al., 2006c, Meissner et al., 2006d, Meissner et al., 2006e) increases the chance of bias.

3.3.5.1.1.Hormone balancing effect of maca

From available evidence, the hormone balancing property attributed to maca is inconclusive. The earliest studies that assessed the effects of maca over a period of 2 months or more, reported a change in hormone levels but the results were inconsistent.

For instance, treatment with 2g of maca powder over two months in a group of 20 Caucasian post-menopausal women appeared to lower FSH levels in one trial (Meissner et al., 2005), increase it in another (Meissner et al., 2006e) or was not changed relative to placebo in a third trial (Meissner et al., 2006d). Reports about LH levels were similarly inconsistent with an increase reported by Meissner et al., 2005 but a decrease reported by Meissner et al., 2006d. On the other hand, an increase in serum E2 levels was reported at the same dose and duration of 2g of maca powder daily over two months (Meissner et al., 2006e, Meissner et al., 2006d) while Meissner et al., 2005 reported such an increase only after 8 months of treatment at the same dose.

The difference might be due to the high placebo effect noted and the relatively small sample size used. Alternating treatment in different sequences of placebo and maca in cross-over design trial, did not completely mitigate the placebo effects but seemed to support that maca was responsible for the improvement noted (Meissner et al., 2006c, Meissner et al., 2006d).

Nevertheless, more recent studies, which assessed the effects of maca over a period of 6 weeks and 12 weeks at a higher dose of maca powder at 3.5g daily and 3.3g daily, showed no significant change in hormone levels relative to placebo and baseline (Brooks et al., 2008, Stojanovska et al., 2011, Stojanovska et al., 2015).

Interestingly, over 4 weeks at a dose of 3g/day, maca was also found to improve sexual experience with a significant correlation with change in testosterone levels in a group of 42 pre- and post-menopausal women suffering from SSRI/SNRI–induced sexual dysfunction (Dording et al., 2015).

3.3.5.1.2. Menopausal symptoms

The impact of maca treatment on menopausal symptoms has been assessed mainly through administration of questionnaires. Generally, an improvement in some aspect of menopausal symptoms is reported by all studies. Menopausal discomfort from hot flushes and night sweating was reduced along with anxiety, irritability and depression according to both Kupperman Menopausal Index (KMI) and Greene's Climacteric Scale (GSC) scores after two months treatment of 2g of maca daily (Meissner et al., 2005, Meissner et al., 2006c, Meissner et al., 2006d). It is also reported that the improvement relative to placebo is mainly in psychological symptoms of anxiety, depression and somatic symptoms (Brooks et al., 2008, Stojanovska et al., 2011, Stojanovska et al., 2015). Improvement in the anxiety level of a Japanese woman undergoing menopause was also reported (Jikyo et al., 2017)However, both the GCS scores (Stojanovska et al., 2011, Stojanovska et al., 2015) and KMI scores (Meissner et al., 2006c) reported a reduction in symptoms with both maca and placebo. Other questionnaires used such as the Quality of Life SF36-v2 Scale and theWomen's Health Questionnaire Scale revealed significantly increased scores in general and mental health in both treatment groups (Stojanovska et al., 2011, Stojanovska et al., 2015). This highlights the limitations of such methods in assessing menopausal symptoms which are subjective.

3.3.5.1.3. Bone Health

A small scale pilot study investigating the effect of maca on bone density in menopausal women showed an increase in bone density measures with maca treatment over 4 months but not with placebo (Meissner et al., 2006d). Considering the small number of participants, six per treatment group, the evidence is weak and a larger scale trial would be needed to confirm the results.

3.3.6. Is there any evidence for estrogen or progesterone–mediated action of maca?

It seems that when used for periods of at least two months, maca affects female hormones levels of estrogen and FSH (Meissner et al., 2005, Meissner et al., 2006e, Meissner et al., 2006c, Meissner et al., 2006d). This suggests that maca would be acting on some hormone-mediated pathway. However, Brooks et al., 2008 reports that even at physiologically high doses of maca (4mg/ml methanolic extract), no estrogenic or androgenic action was detected in yeast-based hormone dependent reporter assay. In-silico estrogen receptor type ER-alpha and ER-beta docking assay revealed no activity from the phytochemicals of maca (Powers and Setzer, 2015). However, another study found that the macamide *N*-benzylpalmitamide, virtually docked to estrogen receptor and increased expression of ER-alpha and ER-beta genes at 10^{-7} M in MC3T3-E1 osteoblast-like cell (Liu et al., 2015).

3.4. TOXICOLOGY

Fresh maca is believed to be deleterious to health and natives advise consuming dehydrated and boiled maca root only. Scientific investigations have found maca to be very safe. No adverse events were reported in any of the clinical trials *(see Appendix 2).in vivo* studies demonstrated an LD50 value of more than 15g/Kg body weight in mice and more than 7.5g/Kg BW in rats (Meissner et al., 2005, Meissner et al., 2006b, Meissner et al., 2006e). There is one report of maca use causing vaginal bleeding in a young woman of 24 years old (Srikugan et al., 2011) and one report of a manic episode related to maca consumption in a 27-years-old male without any psychiatric history (Quandt and Puga, 2016). None of the adverse events reported according to a 2012 USP Safety Review of Maca, were fatal. This reflects the use of maca as a food for centuries by the Andean people, who consume on average over 100g of maca root per day (Valerio and Gonzales.,2005).However, an important consideration is the potential adulteration of maca products arriving on the market with previously unknown and untested

substances (Huang et al., 2016) which may have deleterious consequences on the health of consumers.*(See 3.2.3.3 Adulterants and Contaminants)*

4. CONCLUSION

The hype surrounding the claims of maca is not completely misleading but the overall evidence is too limited or lacking to draw any firm conclusions.

Some of the main limitations are that there is not enough information to determine an appropriate effective dose, frequency of dosing or length of treatment needed to achieve the therapeutic effects desired. Another is that while Andean people are reported to consume over 100g of maca per day, the doses used in the clinical trials are comparatively very low and may not bring about the traditionally believed effects if these are true. There is also a lack of data regarding the type of extract most likely responsible for the observed effects, indicating that use of whole maca could be preferable. Furthermore, there is not enough information about the composition of the material under investigation reported in the literature of pharmacological and clinical studies. Additionally, the extraction solvents used for chemical profiling are not always similar to the ones used in pharmacological studies. An absence of any pharmacokinetic data is noted, which renders a guess at the compounds responsible for maca's health benefits even harder. These limitations prevent drawing a link between secondary metabolites of maca, the maca phenotype and the observed therapeutic effect.

Still, the value of maca as a plant of medicinal value cannot be entirely dismissed. Fertility-wise, maca's effects appear to stem more from a protective effect against noxious factors rather than from a direct effect on increasing sperm production and semen parameters. Supporting this hypothesis is the fact that maca was found to reverse the damage to spermatogenesis from high altitude exposure, malathion and lead acetate administration in rats. Along the same line, red maca extract was also found to reduce prostate size in rodents *in vivo* with TE-induced prostatic hyperplasia if it was administered at the same time. Benzyl glucosinolate, found in maca, was linked to this prostate reducing effect (Gasco et al., 2007). Hence, the claim that maca can support prostate health is also not completely invalid.

In women, to our knowledge, there has been no clinical trial conducted to demonstrate the effects of maca on fertility. But *in vivo* studies showed an increase in pregnancy rate and survival rate of pups in rodents. Interestingly, maca was also shown to promote bone health in menopausal women and ovariectomised rats. Maca extract showed chondro-protective effect in human cartilage samples (Miller et al., 2006). One *in vitro* assay demonstrated that the macamide *N*-benzylpalmitamide, promoted proliferation of osteoblast cell model MC3T3-E1(Liu et al., 2015). Again, this highlights most likely, a protective effect of maca. Other studies have indicated significant anti-oxidant and neuroprotective effects of maca (Sandoval et al., 2002, Valentova and Ulrichova, 2003, Valentova et al., 2006, Pino-Figueroa, 2009, He et al., 2017), which might be co-responsible for the observed effects.

The libido-enhancing effect of maca seem so far to be positive according to results of *in vivo* studies in male rodents but the evidence available is still limited by the methodology employed and the number of test-subjects. Similarly, though positive, the results of clinical trials are limited by how few studies have been done, the subjectivity of the experience of libido itself and the small sample size. The potential of maca to improve sexual dysfunction, however, cannot be completely ignored. In men, the two clinical trials conducted, showed some improvement in patients with erectile dysfunction. Maca can be a potential candidate for supportive health supplements to aid with sexual dysfunction resulting from the treatment of testicular cancer. A single study showed an improvement in sexual experience in pre- and post-menopausal women with antidepressant-induced sexual dysfunction. It is possible that

the perceived libido-enhancing of maca is due to its energising properties. The energising and anti-fatigue properties of maca have been investigated with promising results (Li et al., 2017, Gonzales-Arimborgo et al., 2016, Yang et al., 2015, Miao, 2015, Tang et al., 2017, Choi et al., 2012). Further investigation in this area is warranted.

In female menopause, a similar situation arises. Most studies reveal an improvement in psychological symptoms along with some amelioration of hot flushes according to KMI and GCS scores, following treatment with maca but they also report a significant positive placebo effect. There was also not enough evidence to support the hormone balancing of maca in menopause. In both men and women, maca did not appear to have any hormone-mediated mechanism of action considering the absent and inconsistent reports of changes in hormone levels in clinical trials and the absence of hormone-mediated activity *in vitro*.

From the available evidence, it is more likely that the observed effects of maca are mostly invigorating in nature and arises from the nutritional properties of the plant which seem to confer some increased resilience against the stresses of highland living. Chemical profiling has also revealed that maca flaunts a wide nutritional portfolio including most of the essential amino acids and compounds like glucosinolates that have previously established anti-oxidant properties. Indeed, in 1991, the Food and Agricultural Organization of the United Nations had recommended maca among other Andean plants to prevent nutritional deficits in the Peruvian population.

In light of these findings, it seems that the re-discovery of maca, has led to the distortion of the original local health claims by dragging it out of context to fit the demands of an emerging global market for alternative remedies. The financial incentive tied with meeting this international demand has spurred a rapid, unregulated and unsustainable production and supply of maca. The extent of this problem is such that the composition of maca products available on the market cannot be accurately gauged and not much can be confidently said about their safety or health benefits. Importantly, it is not clear how the commercial production in maca under very different ecological conditions using very different production systems impacts on the composition of maca. Anecdotal evidence from markets in China points to American maca being considered to be better and a systematic abalytical/ metabolomics comparison of the various proveniences and qualities is one of the key needs in order to ascertain best practice in the supply of maca products. Despite of a lack of data, there can be no doubt that poor quality is one of the main limitations of maca's current uses.

While we did not address the multiple questions of sustainable use and benefit sharing, there clearly is a need to investigate these questions in great detail and to develop strategies for preventing the problems caused in the boom in Peru (see Smith, 2014; Brand. 2016). In 2017, 25 years after the Convention on Biological Diversity was signed, the example of maca highlights the need to ascertain not only that benefit sharing agreements are agreed upon prior to commercialisation, but also that there are much better regulations of the supply of the *materia prima* and its processing into a supplement or herbal medicine. The benefits to the original keepers of knowledge and producers has been minimal at best and the huge demand has in fact impacted very negatively on the regions of origin. Now that maca is on the worldwide scene, the need for regulatory measures such as best practice in manufacturing protocols and honest marketing practices remain. This is necessary to ensure the safety of these products, protect consumers and enable an educated decision when choosing between treatment options or when looking for a lifestyle drug.

In conclusion, while there is some evidence for relevant pharmacological effects, the current main risks and problems associated with the use of maca are linked to poor and unsustainable products and poorly managed value chains.

AUTHORS CONTRIBUTIONS

This paper is based on an MPharm dissertation of BeSh, who conducted the research and wrote the drafts. M suggested the topic, supervised the project and edited the MS

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APPENDICES

Appendix S1 (Supplementary data). Important secondary metabolites in Maca

Appendix 1: In-vivo Studies of Maca on Spermatogenesis

***in vivo* studies of maca on spermatogenesis**									
Properties investigated	**Source of Maca**	**Extract and formulation**	**Model**	**Dose range tested**	**Control used**	**Duration of study**	**Outcomes**	**Comments**	**Reference (Author., Year)**
Spermatogenesis in rats	*L. meyenii* root obtained from Carhuamayo (4000m altitude)	maca root was prepared traditionally by boiling for 30minmin and filtered to produce a filtrate of 67mg/ml of root. 67mg/ml aqueous root extract	3 months old Holtzman male rats	1 ml of 67mg/ml administered twice daily for 14 days	Untreated control used	14 days administration of the root extract. Rats were sacrificed one day after last dosing.	increased frequency of the Stages IX-XVI of spermatogenesis and a decreased frequency of the Stages I- VI of spermatogenesis was observed by day 14. By day 7, increase Stage VIII (spermiation) was increased. Transillumination technique was employed to do so. Frequency measured relative to control which was assigned a value of 1.Weight of the testicles and the epidiymis were increased but not that of seminal vesicles discarding the possibility of an androgenic-like action of maca extract.	Weight of rats in control and treated groups differed. It is not clear whether the increased weight of the epididymis was due to an increase in sperm production, considering that it is the frequency of Stages IX - XIV, which occurs in the seminiferous tubules, that increased or because the release of sperm at Stage VIII which also increased.	Gonzales et al., 2001b
Effect of high altitude on spermatogenesis in rats	*L. meyenii* from Carhuamayo at4000m altitude	500g of dried maca root, pulverized and boiled in 1500ml water and filtered. Filtrate administered. Filtrate used of boiled maca ratio 1:3 for 30minmin used. 333.3mg/ml filtrate	Holtzman strain three-months old adult male rats	666.6mg/day for altitude studies; dose-response study employed doses of 0,6.6,66.6 and 666.6 mg/day	Untreated control used	Altitude study: days 7, 14 and 21. Dose-response study administered over 7 days at the same time as first 7 days of altitude study	Statistical analysis done. Dose-response study: weight seemed to increase more rapidly with higher maca doses of 66.6 and 666.6mg/day but mean weight at day 7 were the same between doses. Epididymal sperm count was significantly higher in maca-treated rats with the highest count observed with 666.6mg/day maca. Stage VIII (spermiation) of seminiferous epithelium cycle increased with increased maca concentration but no difference observed at Stages IX-XI. For altitude studies: maca supplementation at 666.6mg/day prevented weight loss and decrease in sperm count observed in non-maca treated group and these were similar to maca-treated mice at sea-level.	A rise in testosterone, previously unseen in prior studies on rats and men treated maca, was noted in untreated rats at sea-level which was chalked down to stress from intubation with vehicle. No such rise was noted in maca-treated group even though they were exposed to intubation as well. This points towards the potential of maca as an adaptogen in dealing with stress. As the reasons for decrease in spermatogeneis at high altitude is multi-factorial - increase in serotonergic action, spurring weight loss and decreasing appetite leading to nutritional deficiency that could contribute towards decrease in spermatogeneis - the exact mechanism of action of maca is as yet undetermined. It is potentially due to be, as the authors point out, to the high nutritional value of maca, which also contains most of the essential amino acids, including arginine.	Gonzales et al., 2004

dose-response effect of maca on weight of testes, epididymis, seminal vesicles, liver, kidney, spleen and lungs, stages of seminiferous tubules, epididymal sperm count and motility, and serum testosterone and estradiol levels in rats	*L. meyenii* from Carhuamayo at4000m altitude, yellow ecotype	500g of dried maca root, pulverized and boiled in 1500ml water for 120min, cooled, filtered and lyophilized. 1g dry maca hypocotyl yielding 0.46g. lyophilized aqueous extract diluted further for different concentrations	Holtzman strain three-months old adult male rats	0.0, 0.01, 0.1, 1 and 5g/kg daily of lyophilised aqueous maca corresponding to 0.0, 0.022, 0.22, 2.2 and 11.0g daily of dry maca hypocotyl.	group administered only vehicle for 0.0 dose - untreated control	7 days duration	Statistical analysis done. Stage VII-VIII and sperm count increased with increasing dose of maca administered. Highest effect observed with dose of 1g/Kg and plateau-d with higher doses. But no increase in sperm motility observed. Decrease in seminal vesicle weight observed and related with drop in serum testosterone levels with 0.01g/kg and 0.1g/kg dose of maca but not with other doses of maca. Effect not observed in previous studies with rats and no decrease in testosterone levels in both men and rats. No significant increase in body weight or organ weight observed.	1.0g/Kg seems to be optimum dose for effect on sperm production. Not necessary to take higher doses. It seems that increase spermiation and initial spermatozoa migration to epididymis for storage is triggered by maca administration. Why does maca increase spermiation without seemingly affecting serum testosterone levels is undetermined	Chung et al., 2005
effect of maca on spermatogenesis in mice treated with a single dose of malathion 80mg/Kg	*L. meyenii* from Carhuamayo, Junin	maca root was prepared traditionally by boiling for 30min and filtered to produce a filtrate of 333mg/ml of root. aqueous extract	48 Asnesll male mice 10-12 weeks old	66.6mg aqueous maca extract daily	none; group treated with malathion only and group treated with maca only	7,14 and 21 days observations post-treatment	Statistical analysis done. Maca was found to protect Stages VII, VIII, and IX (spermiation) despite inhibiting effect of malathion.	Fourth group should have been trial-ed in the same time frame to decrease possible confounding variables of time, mice generation and malathion batch. Mechanism of action is still unknown.	Bustos-Obregon et al., 2005
Effect of alcoholic extract of maca on spermatogenesis in rats	*L. meyenii* from Carhuamayo, Junin	dry ethanolic extract; hexane maceration for 3 days at rtp to remove fat, ethanolic filtrate evaporated to give dry extract. Liquid 5% ethanolic formulation.	Holtzman strain three-months old adult male rats -	28mg/ml and 48mg/ml	Untreated control used	7,14 and 21 days observations post-treatment	Statistical analysis done. Treatment with the ethanolic extract of Maca for 7 days resulted in increase of the lengths of stages VII and IX-XI and a relative reduction in stages XIII-XIV and II-III of the seminiferous tubules. At the higher dose of 96 mg/day, a reduction in stage VIII length was also observed but increase in sperm count was still noted at only day 7. With lower dose, Stage VIII was no longer increased after 7days but sperm count was increased relative to control at all three time points. No change in serum testosterone.	The authors believe that the aqueous extract has a factor that increases the length of stage VIII, which is not present in the ethanolic extract. The ethanolic extract has a factor that favors spermiation and as a result, the sperm count is constantly elevated after treatment at 7 days, 14 days or 21 days with 48 mg/day. This extract has another factor that favours the onset and progression of spermatogenesis, as stages IX-XI	Gonzales et al., 2003b

		ethanolic extract						and thereafter XIII-XIV are increased.	
can maca reverse the deleterious effect on spermatogenesis of i.p. administered lead-acetate in rats; Parameters investigated: length of seminiferous stages, weight of testes, epidiymis, seminal vesicles and ventral prostate, testicular and epididymal sperm count, sperm motility, daily sperm production (DSP), sperm transit state and serum testosterone level	*L. meyenii*	Maca root was prepared traditional method - 500g of dry and pulverised maca root boiled fro 120min, cooled and filtered. aqueous extract	Holtzman strain three-months old adult male rats - 7 per groups assessed	Lead-acetate administered: 0,8,16,24mg/kg; Maca extract: 2.2g/Kg	Untreated control used - no lead acetate and no maca	lead acetate administered i.p from day 1 to 35. And maca administered from day 18 to 35.	Statistical analysis done. Maca extract administration reversed the damaging effect of lead acetate administration on Stages VII, VIII and IX-XI of seminiferous epithelium lengths and on the decrease in corpus and cauda epididymis sperm count. (no decrease in sperm count was observed with lead acetate 24mg/kg). Seminnal vesicle weight was not changed with or without maca. Spermatid count and DSP decrease with LA was reversed with maca. No change in sperm transit rate observed. Serum testosterone decreased with LA and was correlated with decrease in Stages VII- VIII and IX-XI. However, maca did not reverse decrease in serum testosterone levels.	Results concord with previous studies that demonstrated the promoting effect of maca aqueous extract on Stage VIII of the seminiferous epithelium cycle, the increase in sperm count and the apparent nil effect on serum testosterone in rats. The protective maca against lead acetate poisoning was also clear. No explanation given as to why the 24mg/Kg LA group did not have any effect on sperm count. 7 rats per group might be too few.	Rubio et al ., 2006

effects of short-term and long-term treatments of maca ecotypes red, yellow and black on spermatogenesis in rats	*L. meyenii* - Red Maca, Yellow Maca and Black Maca from Carhuamayo, Junin	maca root was prepared traditional method - 500g of dry and pulverised maca root boiled fro 120min, cooled and filtered. aqueous extract	Holtzman strain four months old male rats - 6 per group assessed	666.6mg of aqueous extract daily or an average of 1.66mg/kg BW daily	Untreated control	7 days and 42 days duration	Statistical analysis done. After 7days, no change in body weight and organ weight of testes, epidiymis, seminal vesicle, kidney, liver, spleen, lungs and heart. But significant weight decrease of prostate noted with red maca after 42 days. Stage VIII length increased with all 3 maca ecotypes after 7days. Black maca also increased length of Stage II-VI. But after 42 days, red maca showed no difference in Stage VIII length relative to control while both yellow and Black maca continued to show a significant increase. Yellow and Black Maca significantly increased epididymal sperm count but not Red Maca after both 7 and 42 days of treatment. Only Black Maca increased DSP. No effect observed in spermatid transit rate with any ecotype. Only Black Maca showed a significant increase in sperm motility after 42 days of treatment. No effect observed in serum testosterone, estradiol and testosterone:estradiol ratio levels with any ecotypes for any length of treatment.	Black Maca seems to be the most effective maca ecotype for promoting male fertility. Yellow Maca also shows some similar effect to a lesser extent than black maca but more than red maca. Red Maca is least effective if not at all, in enhancing male fertility. However, an interesting observation is in the decrease in prostate size noted after treatment with red maca extract after 42 days ,indicating potential use in the treatment of benign prostatic hyperplasia.	Gonzales et al., 2006a
Chronic effect of Red, Yelllow and Black Maca on Daily Sperm Production (DSP), Epididymal Sperm Count (ESC), Sperm Count in the Vas Deferens (SCVD) and quantity of testis DNA.	*L. meyenii* - Red Maca (RM), Yellow Maca (YM) and Black Maca (BM) varieties from Carhuamayo, Junin	freeze-dried aqueous extract obtained after traditional boiling process of 100g of raw dried maca in 600ml of water for 60min. - (1g RM=0.40g extract), (1g YM=0.36g extract) and (1g BM = 0.56g extract)	3 months old Holtzman strain male rats -8 animals per dosage group investigated	Dose equivalent to 1g of raw material/Kg body weight daily	Untreated control - administered only vehicle (water) by gavage	84 days treatment	Statistical analysis done. Yellow and Black maca increased ESC but not Red maca. All three RM, YM and BM increased SCVD without affecting DSP, testis DNA levels and reproductive organ weight or body weight. No toxicity was observed through histology of the spleen and liver.	ESC has been shown before to depend on antioxidant-oxidant balance in the reproductive tract with and increase in antioxidant activity increasing ESC. The results may point towards the anti-oxidant activity of maca as shown in previous studies. The difference in activity of Black Maca and Yellow relative to Red maca in increasing ESC, points towards a difference in active secondary metabolites. It is still to be found out whether the fertility of sperm after maca treatment is affected or whether maca affects apoptosis in the testicles. The effect of maca on sperm count appears to modulatory rather than proliferative.	Gasco et al., 2007a

Effect one black maca on Daily Sperm Production (DSP), epididymal sperm and vas deferens sperm count in male rats at days 1,3,5,7 and 12.	*L. meyenii*- Black hypocotyl variety, from Carhuamayo Junin	aqueous extract, traditional boiling process of 500g in 1500ml of water for 60min - 333mg equivalent to dry hypocotyl per 1ml of filtrate	4 months old Holtzmann strain male rats -60 total - 10 rats per group.	corresponding to dry maca hypocotyl weight of 2g/Kg BW daily	Untreated control administered only vehicle (water) by gavage	Treatment groups at days 1,3,5,7 and 12 days	Statistical analysis done. Black maca administration appears to change the pattern of daily sperm production without affecting the sperm count overall in 12 days of treatment relative to control. Black Maca showed an increase in epidymal sperm count at days 1,3 and 7 relative to control but not at days 5 and 12 and an increase in vas deferens sperm count at day 3,5 and 7 but not at days 1 and 12. Seminiferous tubular area shows a similar pattern of decreasing on days 7 and no difference on day 12 relative to control. Epididymal tubule lumen area also increased on day 7 relative to control and as epididymal sperm count increased	The results shows that Black maca interferes with the spermaotogenic cycle in rats only at the level of sperm distribution along the reproductive tract i.e. It has a modulatory effect. Moreover, neither intratesticular testosterone nor serum testosterone levels were found to change.	Gonzales et al., 2006b
Elucidation of which hydroalcoholic extract fraction has most effect on male fertility through parameters of Daily Sperm Production (DSP), Epididymal Sperm Count (ESC) and Vas Deferens Sperm Count	*L. meyenii* - Black Maca from Carhuamayo, Junin	Hydroalcoholic extract prepared with 60% ethanol - 100g of dried black maca hypocotyl produced 7.6g of hydroalcoholic black maca. Successive extraction with solvents petroleum ether (23.7mg), chloroform (176.8mg), ethyl acetate (207.7mg), n-butanol (2.46g), water fraction (remaining aqueous solution) (25.93g)	42 adult male rats of Holtzman strain 3 months old - 7 per group	1g of raw material/kg body weight of maca hydroalcoholic extract.	untreated control	7 days	Statistical analysis done. No significant change in body weight and weight of reproductive organs cross all groups. Rats treated with ethyl acetate fraction had higher DSP levels after 7 days while in the other groups there was no significant change. Ethyl acetate and chloroform fractions treated rats showed a higher sperm number in epididymis than rats treated with petroleum ether, n-butanol, aqueous fraction and hydroalcoholic extract. No statistically significant change of hydroalcoholic extract relative to control. Sperm count in vas deferens was higher in rats treated with chloroform fraction than those treated with petroleum ether or ethyl acetate fractions.	It is postulated that the observed effect of superior potency in increasing DSP and epididymis sperm count of ethyl acetate fraction might be due to the presence of polyphenols, which are primarily concentrated in that fraction and possess anti-oxidant activity that has been implicated in increasing sperm number.	Yucra et al., 2008

Effect of maca supplementation on bovine sperm quantity and quality	*L. meyenii* from Oca, Junin and provided by Agronaturales, Lima , Peru - mix of yellow, red and black colours hypocotyls in ration of 2:1:1	traditionally dried hypocotyl milled to a powder of 0.8mm particle size and mixed with oral feed. No extract. But dried hypocotyl	78 peri-pubertal breeding bulls aged between 55 to 84 weeks with borderline sperm quality or ; 23 control group, 29 maca early and 26 maca late groups	233mg/Kg	untreated control	23weeks including 3 weeks adaptation period (2 X 10 weeks). So maca treatment given over 10 weeks at a time; maca early (10 wk treatment then 10wk placebo) and maca late (10 wk placebo then 10 wk treatment), control (20 wk no treatment)	Statistical analysis. No significant change in body weight, testes circumference and rectal temperature was observed with maca treatment. Sperm motility and % frequency of sperm with damaged chromatin was significantly improved to a large extent with early maca supplementation (first 10 weeks), more than in bulls with already adequate sperm quality. Only bulls in the early maca supplementation group showed a significant increase of 26% in sperm count (also related to increased ejaculation volume) in the second 10week window., indicating delayed cary-over effects of maca supplementation.	This finding suggests that maca supplementation is beneficial only when applied sufficiently long and might be even more efficient when supplemented longer than for 10 wk.	Clement et al., 2010
determine the effect of black maca and camu camu alone or in combination on spermatogenic cycle in the seminiferous epithelium in adult male rats using the transillumination technique	black maca (*L. meyenii)* from Junin, Peru; camu camu (Myrciaria dubia) from Iquitos Loreto, Peruvian Amazon	aqueous extraction prepared by 24hr maceration in distilled water at 4oC	three months old Holtzmann strain rats - 6 rats per group	black maca 50mg/day; camu camu 50mg/d; black maca+camu camu at (25mg + 25mg)/day	untreated control (with vehicle - distilled water)	7 days	Statistical analysis done. Camu camu had higher total polyphenol and flavonoid content and higher antioxidant activity than black maca. Weight of testis, epididymis, seminal vesicles and prostate did not change significantly across all groups. DSP and epididymal sperm count was increased significantly with black maca, camu camu and the combination of both. Extract of black maca increased lengths of Stages VII and VIII significantly but not camu camu unless co-administered with black maca. Black maca alone does not increase length of Stage XII but co-administration with camu camu does. Black maca, camu camu and mixture increase Stages IX-XI w.r.t control. Mixture of black maca and camu camu was able to increase Stages VII, VIII, IX-XI and XII of spermatogenesis in rats.	Mitosis occurs at Stages IX-XI; Meiosis at Stage XII; Spermiation (release of sperms to the lumen of the seminiferous epithelium) at Stage VII-VIII. Therefore, black maca was acting on mitosis and spermiation and camu camu was acting on meiosis. This points towards different mechanisms of actions. Changes in spermatogenic cycle also concords with observed increase in sperm count in the epididymis and testis.	Gonzales GF et al., 2013b

evaluate the effect of maca on chemically and physically induced sub-fertility in male mice models	*L. meyenii* - yellow ecotype of maca from Junin, Peru	methanolic extract-fractionated to butanol fraction and aqueous fraction. Methanolic and butanolic fraction but not aqueous fraction were confirmed to have alkaloids from creamy and orange precipitate using Mayer and Dragendorff's reagent respectively	BALB/c male mice aged 5-6 weeks old; chemical sub-fertility induced by administration of Ketoconazole 400mg/Kg daily fro 30 days before study; physical sub-fertility induced by exposure to extremely low frequency-magnetic field (ELF-MF) of 0.55mT and 60Hz (length of exposure not specified), field was monitored every 2 weeks	666mg/Kg body weight per day orally	untreated control; ELF-MF sham exposure		Statistical analysis done. The effect on sperm motility of chemically induced sub-fertility was apparently reversed with maca administration to values similar to control group but not in ELF-MF exposed mice where it improved only by a little with maca treatment was not significant. The same trend was observed with the sperm count parameter. Maca also seemed to have a protective effect against the damaging effects on sperm DNA fragmentation observed in mice exposed to ELF-MF, potentially due to antioxidant properties.	Length of treatment of maca not given. Length of ELF-MF exposure not mentioned. No report of testosterone levels if assessed but increase in testosterone levels discussed in the discussion section of paper. But results of study seems to concord with previous study of maca in terms of the sperm motility and sperm count enhancing properties. Protective antioxidant effects also observed. Maca apparently reversed effects of ketoconazole on cytochrome P450, restoring the gonadal androgen synthesis pathway. It seems maca does not affect mitochondrial activity as it could not reverse the effects of ELF-MF exposure which affects this parameter.	Cuya et al., 2016

effect of butanolic fraction of maca on daily sperm count, epididymis sperm count and vas deferens sperm count of adult mice	*L. meyenii* - black maca and yellow maca from Carhuamayo Junin	no extract. But milled powder of black hypocotyl	90 days old Swiss strain mice - 6 per group	equivalent of 1g of raw material/Kg body weight daily	untreated control - 4 mice	3 days	Statistical analysis done. Black maca had higher phenolic content than yellow maca and aqueous fractions of either had higher TPC than their butanolic fraction. Yellow maca had more antioxidant capacity than black maca. Methanolic extract of black maca was more effective than methanolic extract of yellow maca in increasing sperm count in the epididymis and vas deferens whereas the methanolic extract of yellow maca increased DSP more. Butanolic extract values of DSP and epididymal sperm count were similar to control indicating no effect.	Study should have confirmed whether the butanol fraction did indeed contain MCTA before making the claim that MCTA in maca does not affect sperm count. The fact that the same process of extraction was used does not guarantee the same compounds will be obtained. While macamides are the compounds characterising maca, these are primarily concentrated in the non-polar fraction. The study and previous studies have demonstrated the activity of the polar fraction in increasing fertility. Since TPC was higher in aqueous fraction which also showed significant activity, it is possible that phenols are responsible for the activities observed.	Inoue et al., 2016
effect of maca on libido and semen characteristics in hair sheep rams	*L. meyenii* - black maca from Macandina		thirty 15 months old proven male Saint croix rams	233mg of dried maca hypocotyl/Kg body weight /day	untreated control	16 weeks - maca supplied for first 8 weeks and normal feed for next 8 weeks to assess the residual effect of supplementation. 16 weeks covered 2 spermatogenic cycle	Maca supplementation significantly increased the number of mounts and ejaculations, decreasing the time between ejaculations by week 8. no difference was found in reaction time, time until 1st ejaculation, testes circumference, ejaculation volume, sperm concentration and ram efficiency (mounts/ejaculations). These effects persisted 8 weeks later (residual effect) after no supplementation in the treated animals.	Authors point out the difference in physiology between species as a possible reason for the difference in results observed. Mechanism of action of maca is till unknown. Residual effect of maca suggests a lingering effect of accumulated or with longer elimination rate of specific secondary metabolites or a combination of these exerting the effects seen on specific mating behaviours of increased mounts and ejaculations after 8 weeks since treatment was stopped.	Lavana et al., 2013

Appendix 2: In-vivo Studies of Maca on Prostatic Hyperplasia

Table of*in vivo* studies of maca on Prostatic Hyperplasia									
Properties investigated	**Type of Maca studied**	**Extract and formulation**	**Model**	**Dose range studied**	**Control used**	**Duration of study**	**Outcomes**	**Comments**	**Reference (Author., Year)**
effects of three maca ecotypes on testosterone induced prostatic hyperplasia and prostate size in rats	*L. meyenii* - Red Maca, Yellow Maca and Black Maca from Carhuamayo, Junin	aqueous extract. maca root was prepared traditional method - 500g of dry and pulverised maca root boiled fro 120min, cooled and filtered	Holtzman rats - prostate size study:12 rats per maca ecotype + 35 rats for control; TE-induced PH study: no mention of number of rats per group - control, red maca short term, red maca long term	Prostate size study: equivalent of 2g/Kg BW red maca; TE-induced PH study: 2g/Kg BW red maca+ 25mg TE i.m.	Untreated control	prostate size study: 7 days; TE-induced PH: red maca treated for 14 days and then 42 days	Statistical analysis done. Red maca significantly reduced prostate weight but not Yellow or Black maca. No significant difference observed in body weight, seminal vesicle weight among rats treated with different maca ecotypes. No significant change or difference between ecotype groups in serum testosterone and estradiol level either. Red maca significantly reduce prostate weight of rats treated with TE after 14 days and decreased by more than 50% prostate size of rats treated with TE after 42 days treatment. Red maca reduced the androgen-dependent prostate epithelium height and luminal areas in rats treated with TE with apparent membrane blebbing and nuclear distortion (suggesting pro-apoptotic effect). Phytochemistry of reconstituted lyophilized aqueous extract of red maca with ethanol showed the presence of alkaloids, steroids, saponins and cardiotonic glycosides, with absence of flavonoids, tannins, sesquiterpene lactone and coumarins.	Red maca aqueous extract shows promising ability to treat benign prostatic hyperplasia in men. Possibility of glucosinolate metabolite form red maca interfering with androgen action on prostate. The effects observed might also be due to anti-proliferative of maca which can in part be supported from phytochemical analysis of the extract which was found to contain a higher content of glucosinolates than other ecotypes.	Gonzales et al., 2005

Effect of length of boiling time on biological activity of red maca; compare activity of an aqueous extract and a hydroalcoholic extract of red maca after controlling for glucosinolate content. Biological activity investigated is effect on TE-induced prostatic hyperplasia (PH) in rats.	*L. meyenii* - Red Maca from Carhuamayo, Junin	aqueous extract - one boiled for 2 hours and another for 3hours; hydroalcoholic extract	3 months old adult Holtzman male rats; 1) control (six rats treated with vehicle); 2) TE control (15 rats treated with only TE); 3) TE + red maca for 2-h boiling (five rats); 4) TE + red maca for 3-h boiling (five rats); 5) TE + hydroalcoholic extract of red maca (10 rats); and 6) positive control treated with TE + 0.1 mg finasteride (six rats). red maca extracts used containted 0.1mg/ml benzylglucosinolate ; For Dose-response study - rats with TE induced PH, received red maca extract with benzyl glucosinolate contents of 0.02, 0.04, 0.06 and 0.08mg/ml benzylglucosinolate analysed by HPLC	1 mL maca (freeze dried aqueous extract or spray dried hydroalcoholic extract) given daily to the rats contained 0.1 mg benzyl glucosinolates.	Both negative (untreated control and TE only treated control) and positive control (TE and finasteride control) used	14 days administration of extract. Rats were sacrificed one day after last dosing.	Statistical analysis. Spray-dried hydroalcoholic extract of red maca had a higher glucosinolate content than aqueous freeze-dried extract produced from longer boiling time (3hours). Not much difference in IR spectra between extracts. Length of boiling time did not affect the size-reducing' effect of the two aqueous extracts of red maca on the size of prostate. Over 14 days administration, finasteride 0.1mg, aqueous freeze-dried extract 0.1mg/ml benzylglucosinolate and spray-dried hydroalcoholic extract 0.1mg/ml benzylglucosinolate, all significantly reduced prostate TE-induced PH in rats. Finateride also decreased seminal vesicle weight but not any of the red maca extracts. Dose dependent reduction in prostate weight with increasing dose of benzylglucosinolate content (0.02-0.08mg/ml) observed with no decrease in seminal vesicle weight.	Both the aqueous and hydoralcoholic extract of maca containing each 0.1mg/ml benzylglucosinolate, reduced TE-induced PH to the same degree. The dose-response effect observed with varying doses of benzylglucosinolate also indicated the importance of the biological activity of benzylglucosinolate in decreasing PH. Aqueous extract results in very polar compounds being extracted like glucosionlates. Hydroalcoholic extract can give both very polar to compounds with lower polarity. Another factor, aside from the benzylglucosinolate content (which varies with heat applied and in this case no difference was observed in the final outcome), could be responsible for observed effect. Mechanism of action of red maca in reducing PH is still unclear. It might be a post-adrogenic receptor interference since seminal vesical weight was not changed with red maca extract, unlike with finasteride treatment. This would also explain the absence of changes observed in serum testosterone and estradiol levels observed in other studies.	Gonzales et al., 2007

Dose-response effect of red maca TE-induced prostatic hyperplasia and whether effect is comparable to effect of finasteride.	*L. meyenii* - Red Maca from Carhuamayo, Junin	freeze-dried aqueous extract of maca root suspended in 2ml water.	3 months old Holtzman strain male rats -6 animals per dosage group investigated	0,0.01,0.005,0.1,0.5g/Kg BW of freeze-died maca in 2 ml water suspension.	untreated control (negative), control treated with finasteride but not red maca (positive control)	21 days. TE was given on day 1 and 7. red maca extract given from day 1 to 21.	Statistical analysis done. Red maca was found to reduce TE-induced prostatic hyperplasia in rats significantly in dose-response fashion. The red maca extract doses of 0.1mg/kg BW and 0.5mg/kg BW were found to be more effective than the dose of 0.6mg/Kg BW of Finasteride administered in reducing the prostate size in rats treated with TE. Finasteride but neither of the red maca doses decreased weight of seminal vesicle. Serum testosterone level with or without maca treatment was similar. Red maca extract used was found by HPLC to have a content of 0.693g/100g.	As effect of red maca was found to be specific to prostate weight, unlike Finasteride, a 5-alpha reductase inhibitor, it was postulated that red maca extract actives might be acting at a post-Dihydrotestosterone conversion level. The presence of benzylglucosinolate was also found in the extract used and the anti-proliferative properties of such compounds understood to potentially contribute to the observed effect.	Gasco et al., 2007b
Effect of Red Maca on prostatic stroma in mice with BPH	*L. meyenii* from Carhuamayo, Junin, Red Maca	Hydroalcoholic extract prepared with 60% ethanol	10 weeks old adult mice	Red Maca at 140mg/Kg BW OD; Finasteride 3.6mg/kg BW OD	Positive control: TE i.m. + Finasteride oral and a Negative control: vehicle oral + oil i.m.	TE+Finasteride: 21 days; TE+RM, 3 groups assessed at 7, 14 and 21 days respectively.	Statistical analysis done. No significant difference between the testis weight and epididymis weight in mice treated with TE, TE + RM, TE + Finasteride and vehicle. The weight of the seminal vesicle was not different in mice treated with TE, TE + RM and TE + Finasteride. Red maca only produced a significant reduction in prostate weight induced by TE after 21 days of administration - a possibility that the dose administered of 140mg/Kg might have been too low. The reduction in prostatic acinar area (which was increased by TE) by red maca did not occur until after 14 and 21 days of administration of extract. Prostatic epithelial height measurements were not significantly different between groups. There were no significant differences between the groups treated with RM or Finasteride. RM administration did not modify the serum testosterone and oestradiol levels in mice treated with TE.	The mice model of TE-induced BPH seems to be a better model for BPH in humans because similar to humans, BPH was observed to present primarily as an increase or hyper-proliferation of acinar and stroma areas with TE. Specificity of red maca for prostate over androgen-dependent organs like the seminal vesicles, testes or epididymis was demonstrated. The effect on stromal area was observed as early as 7 days of treatment with RM, suggesting that RM might interfere with the androgen action mainly at the prostatic stromal cells, exerting its effects at a stromal and acini level rather than on epithelial cells. Authors postulate, following phytochemical study, that polyphenols present in maca aqueous extract and thus most polar fraction, could be responsible for reducing prostatic hyperplasia in mice.	Gonzales et al., 2008

Effect of red maca on prostate zinc levels in rats with TE-induced BPH and to determine best marker for effect of RM on sex accessory glands - prostate, seminal vesicles and preputial glands.	*L. meyenii* - Red Maca, from Carhuamayo, Junin	aqueous extract prepared traditional method of 100g dry hypocotyl boiled with 600ml of water for 60min, cooled, filtered and freeze-dried	thirty-six 3 months old Holtzman strain adult male rats; 6 rats per group; BPH was modelled by administration of 25mg of TE at day 1 and day 7 in rats	Red Maca: 2g of raw material /kg BW per day; Finasteride: 0.1mg/day	untreated control with and without TE	Treatment from day 1 to 14 and treatment from day 7 to 14 with TE administered only on day 1 and day 7.	Statistical analysis done. No difference between testes and epididymis weights between groups. RM started at day 7 to day 14 did not reduce seminal vesicles glands, preputial gland and prostate gland relative to TE treated-only group but Finasteride at day 7 did reduce prostate size in TE treated-only group. RM did not reverse the effect of TE-only treated group. RM at day 1 reversed effect of TE on preputial gland weight, reduced prostate gland weight but no that of seminal vesicles unlike Finasteride at day 1 which reduced all parameters. RM at day 1 reduced zinc levels in prostate relative to TE-treated group but Finasteride only partially reduced it. There was a correlation between higher zinc levels and BPH.	The results suggest that RM and Finasteride have different mechanisms of action in reducing BPH. RM may potentially decrease BPH by regulating Zinc levels in prostate. Also, effect of RM on BPH may not be seen until at least 7 days of treatment have been provided. Whether it is the glucosinolates or polyphenols in RM that decrease BPH is not yet known. Prostate weight and Zinc levels in prostate were deemed as suitable markers when using the proposed model to test the effect of RM on BPH under same conditions.	Gonzales et al., 2012
Whether consumption of maca prevented BPH in TE-induced mice	*L. meyenii* - Red Maca and Black Maca	No mention	adult male mice	Finasteride (0.6mg/Kg/day); Red maca (167mg/Kg/day); Black Maca (167mg/Kg/day)	negative control - normal mice administered vehicle and mice with TE-induced BPH without treatment	21 days. TE was given on day 1 and 7. red maca, black maca extract and finasteride given from day 1 to 21.	Treatment with red maca, black maca and finasteride prevented effects of Testosterone on prostate. Red maca had a more pronounced effect in decreasing prostate weight relative to finasteride and black maca.	Results are concordant with previous studies that have demonstrated that red maca has most effect against BPH induced by TE (Gonzales GF et al., 2005, Gonzales C et al., 2006, Gonzales GF et al., 2007b and Gonzales GF et al., 2008) However, the mechanism of action red maca is still undetermined.	Noratto G et al., 2013

Appendix 3:in vivo Studies of Maca on Sexual Performance

Table 3*in vivo* studies of maca on sexual performance

Properties investigated	Type of Maca studied	Extract and Formulation	Model	Dose range tested	Control used	Duration of study	Outcomes	Comments	Reference (Author., Year)
Sexual performance, Erectile dysfunction	*L. meyenii*	MacaPure- M-01 (contains more polysaccharide and less macaene and macamides than M-02) and MacaPure M-02. Extracts made from dried maca roots from Peru through a process patented by Pure World Botanicals. Lipid-extract- 0.6% macaene and macamides - administered in 10% ethanol as suspension. M-01 and M-02 was also found to contain benzyl isothiocyanate as the major compound and p-methoxy benzyl isothiocyanate in smaller amounts.	Mice for sexual behaviour and rats for erectile dysfunction with castrated male rats as models of hypogonadism.	45mg/Kg, 180mg/Kg, 1800mg/Kg of body weight	Yes. Positive control used - testosterone treated normal rats. negative control also used for ED study (surgically removed testes and untreated with maca).	22days fed 40mg/Kg extract study with mice , also once-only bolus administration of 4g/kg extract study with mice mated after 1 hour of feeding; 20 days fed ED study with rats	MacaPure M-01 MEC >45mg/Kg body weight for ED as measured through LPE; MacaPure M-02 effective at 45mg/Kg for ED. Similar LPE times of two higher doses of M-01 and all three doses for M-02 suggest plateau effect - maximum possible intake by rat models.; Both effective at 45mg/Kg onwards for sexual performance.	It is assumed that effect on Latent Penile Erection (LPE) can be considered as an indication for effectiveness in ED occurring due to hypogonadism and that increased rate of intromissions is indicative of improved sexual performance. ;No statistical test used.; Given the composition of the MacaPure extract used, the effects might be due to synergy. Results are indicative of effectiveness of maca in treating ED and improving sexual performance. There were quite a lot of parameters and properties investigated in this one study which made it difficult to follow.	Zheng et al., 2000

Sexual performance and locomotor activity. Sexual performance measured as: first mount (ML), intromission latency (IL), ejaculation (EL), post-ejaculatory latency (PEL), Intercopulatory interval (ICI) and Copulatory efficacy (CE)	*L. meyenii*	Pulverised *L.meyenii* root standardised at 0.6% macaenes and macamides obtained from Santiveri (Barcelona, Spain) . powder diluted in saline	normal sexually trained male rats.	15mg/Kg, 75mg/Kg or 0.5ml/Kg saline body weight	Yes. Untreated controls. Negative control - maca effect on locomotor activity tested to remove association with increased sexual performance	15days administration of maca extract. Results measured on first (acute effect) and last day (chronic effect)	acutely administered 15mg/kg and 75mg/kg extract decreased ML, IL and ICI. Only 75mg/kg dose decreased PEL. Chronically administered both 15mg/kg and 75mg/kg decreased ML, IL, EL and PEL. Effect on IL, EL and PEL seems to be dose-related (increased dose, decrease parameter). Only 75mg/kg decreased ICI. most part of tested sexual behaviour occurs within first 10 minutes of observation.; In spontaneous locomotor activity study, no increased activity was noted until 2nd and 3rd 10 minutes of observation.	No explanation as to why acute administration decreased ICI with both doses but after 15 days of chronic administration, only 75mg/kg dose had this effect. Chronic dosing with lower dose also contributed to PEL decrease when it did not at acute dose - suggesting a build-up of actives in the body. In acute dosing, no mention of EL parameter results given. However, results do confirm like Zheng et al., 2000 that maca extract (standardised 0.6% macaenes and macamides) improve sexual performance that is not related to increase in spontaneous locomotor activity as the latter only increased later indicating maca takes longer to affect general activity.	Cicero et al., 2001

Extract type on rat sexual performance	*L. meyenii* from Naturalfa Quimica Suiza, collected from surroundings of Lake Chinchaycocha	Hexanic extract; methanolic extract; chloroformic extract of maca root. 52mg of hexanic extracted diluted in saline administered 0.5ml/Kg; 870mg of methanolic extract diluted in saline administered 0.5ml/Kg and 24mg chloroformic extract diluted in saline administered 0.5ml/Kg	one hundred 60 days old Sprague-Dawley rats	Administration over 5 days as per diluted extract used	Untreated control used	5 days administration	Hexanic extract was found to improve more parameters of sexual performance than the other extracts - methanolic and chloroform fractions suggesting that the bioactives of maca thought to improve sexual performance is found in this hexanic fraction, Tentative compounds are postulated to be glucosinolates. However benzylglucosinolate has a Log P value of -0.2 indicating a greater affinity for the polar fraction of an extraction solvent.	Also sexually inexperienced (naive) mice were used and only 10% of the group treated with methanolic extract could complete the testing procedure. Such a low rate of participation would affect results obtained and furthermore, hexanic extract increase only one extra parameter of ML. Hence, overall statement that the hexanic fraction is potentially the most effective one at improving sexual function is questionable. Also methanol extraction gave the highest amount of residue (103.3g). Hexane extraction gave 6.5g and Chloroform extraction gave only 3.0g suggesting that the majority of bioactives are in methanol fraction. Also, hexanic and methanol fraction were found to have almost similar effects. It is possible macaenes and some more lipophilic macamides are in the hexanic fraction while the less lipophilic macamides (shorter fatty acid chains) and the glucosinolates are in the methanolic fraction. It is desirable to repeat the experiment this time with sexually trained rats instead.	Cicero et al., 2002

effect of maca on libido and semen characteristics in hair sheep rams	*L. meyenii* -black maca from Macandina	no extract. But milled powder of black hypocotyl	thirty 15 months old proven male Saint croix rams	233mg of dried maca hypocotyl/Kg body weight /day	untreated control	16 weeks - maca supplied for first 8 weeks and normal feed for next 8 weeks to assess the residual effect of supplementation. 16 weeks covered 2 spermatogenic cycle	Maca supplementation significantly increased the number of mounts and ejaculations, decreasing the time between ejaculations by week 8. No difference was found in reaction time, time until 1st ejaculation, testes circumference, ejaculation volume, sperm concentration and ram efficiency (mounts/ejaculations). These effects persisted 8 weeks later (residual effect) after no supplementation in the treated animals.	Authors point out the difference in physiology between species (rams in this study and bulls in previous study) as a possible reason for the difference in results observed. Mechanism of action of maca is till unknown. Residual effect of maca suggests a lingering effect of accumulated or with longer elimination rate of specific secondary metabolites or a combination of these exerting the effects seen on specific mating behaviours of increased mounts and ejaculations after 8 weeks since treatment was stopped.	Lavana et al., 2013
effect of different fractions of maca on sexual performance in mice, seminal vesicle weight and serum testosterone levels in castrated mice	*L. meyenii* - pulverised dried maca root standardised at 0.6% macamides and macaenes offered by MAYA bio-engineering Ltd.Co, Huei, China, collected from Peru	ether, alcoholic and aqueous extracts of maca	140 male mice and 210 female mice of the Kunming grade II strain - 10 mice per group; rats to test weight of seminal vesicles were emasculated.	all maca extracts were administered at either 4mg/g body weight or 0.8mg/g body weight daily	positive control treated with methyltestosterone and negative control treated with 0.9% saline	6 days	Statistical analysis done. Maca significantly increased the number of complete intromission with all three extracts, irrespective of dose and with ether extract being most effective. Intromission latency was also decreased but not with ethanolic extract. Weight of seminal vesicles of emasculated mice were increased significantly when treated with maca extract at the higher dose or with methyltestosterone 10mg/g relative to emasculated control where seminal vesicles weight had decreased significantly. There no significant changes in body weight, thymus, spleen weight or serum testosterone levels between maca-treated group and control.	Ether extract being most effective in increasing the number of intromission suggests that the non-polar fraction (alkaloids and aromatic isothiocyanates) may be responsible for improving sexual function in man. This matches what Cicero et al., found about hexanic fraction (most non-polar) increasing copulatory parameters. Increase in weight of seminal vesicles was not found in most previous studies except in a recent study by Ohta et al., 2016. Since no increase in testosterone levels was observed in either group, this may suggest a non-adrenergic action of maca, similar to previous studies. It is also notable that the increase in seminal vesicle weight occurred in	Zhang et al., 2016

								castrated mice with maca supplementation. It would have been interesting to assess the sexual performance of such mice.	
Acute and Chronic Dosing of L. Meyenii (Maca) on male rat sexual behaviour; also effect of chronic administration of maca on anxiety	*L. meyenii*	aqueous solution of maca	male Sprague-Dawley rats -6-9 animals per group	25mg/Kg or 100mg/Kg daily	Untreated control	acute testing on day 1 after 25-30min of first oral administration; chronic testing on day 7 and day 21 after 4-7hours of oral administration	Statistical analysis done. Acute and daily administration of maca over 7 days increased time to first ejaculation and post-ejaculatory latency in rats without affecting copulatory efficiency and intercopulatory interval. But the changes were small. No effect was observed at day 21. No significant increase in anxiety or locomotion observed though the lower dose of maca group showed decreased locomotion and the higher dose maca showed behaviour akin to less anxiety.	Results do not reflect the effects observed in previous studies by Cicero et al., 2001 where maca appeared to decrease intromission and ejaculation latency. The difference may be due to the different doses used, source and preparation of maca used or experimental protocol. The study is not explicit about the source of maca or its preparation. It mentions aqueous solution of maca. However as noted by Dr. Francois Guilliano, the results of the experiment indicates that maca could potentially be used to treat premature ejaculation in men.	Lentz et al., 2007

Table 4:*in vivo* studies of maca on erectile dysfunction									
Properties investigated	**Type of Maca studied**	**Extract**	**Model**	**Dose range studied**	**Control used**	**Duration of study**	**Outcomes**	**Comments**	**Reference (Author., Year)**
Sexual performance, Erectile dysfunction	*L. meyenii*	MacaPure- M-01 (contains more polysaccharide and less macaene and macamides than M-02) and MacaPure M-02. Lipid-extract- 0.6% macaene and macamides - administered in 10% ethanol as suspension. M-01 and M-02 was also found to contain benzyl isothiocyanate as the major compound and p-methoxy benzyl isothiocyanate in smaller amounts.	mice for sexual behaviour and rats for erectile dysfunction with castacted male rats as models of hypogonadism.	45mg/Kg, 180mg/Kg, 1800mg/Kg of body weight	yes. Positive control used - testeosterone treated normal rats. negative control also used for ED study (surgically removed testes and untreated with maca).	22days fed 40mg/Kg extract study with mice , also once-only bolus administration of 4g/kg extract study with mice mated after 1 hour of feeding; 20 days fed ED study with rats	MacaPure M-01 MEC >45mg/Kg body weight for ED as measured through LPE; MacaPure M-02 effective at 45mg/Kg for ED. Similar LPE times of two higher doses of M-01 and all three doses for M-02 suggest plateau effect - maximum possible intake by rat models.; Both effective at 45mg/Kg onwards for sexual performance.	It is assumed that effect on Latent Penile Erection (LPE) can be considered as an indication for effectiveness in ED occurring due to hypogonadism and that increased rate of intromissions is indicative of improved sexual performance. ;No statistical test used.; Given the composition of the MacaPure extract used, the effects might be due to synergy. Results indicative of effectiveness of maca in treating ED and improving sexual performance. There were quite a lot of parameters and properties investigated in this one study which made it difficult to follow.	Zheng et al., 2000
effect of maca on glucose metabolism and erectile dysfunction in streptozotocin (STZ)-induced diabetic rats	*L. meyenii*	no mention	40 adult male rats (8 weeks old) - 8 per groups. Diabetes Type 1 was induced with single dose administration of intra-peritoenal Streptozotocin at 0.1 citrate buffer and 60mg/kg body weight and rats with blood glucose levels of 200mg/dL were considered diabetic.	Maca at 100mg/Kg body weight daily; Taladafil at 1mg/Kg daily	negative control - untreated; positive control of rats with taladafil	8 weeks	Statistical analysis done. Maca administered group with STZ, even in combination with Taladafil, significantly decreased body weight and blood glucose levels while increasing significantly the intracavernous pressure/mean arterial pressure (ICP/MAP) relative to STZ diabetic group untreated. STZ Taladafil treated group also showed an increase in ICP/MAP.	Maca shows potential for treating erectile dysfunction in diabetic rats. The fall of blood glucose levels in also promising. Authors also report synergistic effect of maca with Taladafil which is a phosphodiesterase inhibitor. Exact mechanism of action maca still needs to resolved.	Kimura et al., 2016

Appendix 4:in vivo Studies of Maca on Erectile Dysfunction

Appendix 5: Clinical Studies of Maca in Men

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Table 5: Clinical studies of maca in men									
Properties investigated	**Type of Maca**	**Maca form used**	**Model**	**Dose range tested**	**Control used**	**Duration of study**	**Outcomes**	**Comments**	**Reference (Author., Year)**
Semen parameters - ejaculate volume, sperm motility, sperm consistency, sperm morphology and sperm concentration; blood hormone levels of: luteinising hormone, follicle stimulating hormone, prolactin levels, testosterone and Estradiol E2.	*L. meyenii*	Not an extract but gently cooked at low heat maca - 'gelatinised' to digest the tougher fibres and ease digestion.	Nine 24-44 years old normal healthy man who did not have any medical intervention during the three months prior to the study	6 subjects on 1500mg/day and 3 subjects on 3000mg/day	None	4 months oral administration; fasting blood test between 08:00 to 09:00 were taken along with semen by masturbation following 3 days abstinence at the beginning and end of treatment.	No significant difference noted in serum hormone levels; Significant increase noted in ejaculation volume, total sperm count, motile sperm count and sperm motility grade a+b.; No statistical test used.	The seminal vesicles (60%), the prostrate(30%) and the epididymis (10%) contribute to the semen volume. All three are androgen dependent and so is sperm motility. But no increase in serum testosterone was found. Mechanism of action of maca in unknown. It may potentiate binding of androgens to receptors or may not have any androgenic effects at all. The sample size investigated is also small. No data regarding the maca tablets used are given. The participants may not have been honest in following the guidelines of the study. Other confounding factors such as a change in diet or exercise during the study are not accounted for. No declaration of interest made while the product from a specific company (Hersil Laboratories) was used in the study.	Gonzales et al., 2001a
Effect on sexual desire and whether it is influenced by an improvement in mental health scores and hormone levels because of maca administration	*L. meyenii*	Tablets contained 500mg each of dehydrated maca root.	57 healthy male adults ages 21-56 years old.	1500mg/day and 3000mg/day	parallel, double-blind, randomised, placebo controlled study	Treatment assessment at points 4, 8 and 12 weeks over 12 weeks period.	No significant difference in serum hormone levels in man treated with maca and placebo.; No significant difference in self-reported sexual desire in man treated with maca and placebo at week 4. But significant improvement in sexual desire noted at week 8 and week 12. Maca was found to improve sexual desire in men without increasing serum hormone levels and independently of Hamilton depression and anxiety scores when analysed with logistic regression. No dose-response effect observed. Statistical analysis used: Chi-square test, logistic regression (multi-variate analysis),	Interesting take on maca's effect on sexual desire in men. It would have been interesting to see whether and how maca affected Hamilton anxiety and depression scores independently. It is ambiguous whether participants enrolled in the study are healthy though it is mentioned that most of them did not smoke or used drugs in the three months prior to the study. Hamilton rating scale should also not be used as a diagnostic test. Mechanism of action of maca still undetermined. No declaration of interests but acknowledgement to Hersil Laboratories - where was funding taken from?	Gonzales et al., 2002

Effect on serum hormone levels - LH, FSH, prolactin, 17-OHP, 17-β-estradiol, testosterone	*L. meyenii*	Maca Gelatinizada La Molina tablet 500mg provided from hersil Laboratories in Lima, Perum - 500mg each of dehydrated maca root.	56 healthy male adults aged 21-56 years old	1500mg - 3 tablets OD; 1500mg - 1 tablet TDS; 3000mg - 2 tablets TDS	parallel, double-blind, randomised, placebo controlled study	Treatment assessment at points 2,4, 8 and 12 weeks over 12 weeks period.	No significant difference in serum levels of any of the hormones assessed were noted. Maca has no effect on serum hormone levels of LH, FSH, Prolactin, 17-OHP, 17-β-estradiol and testosterone. Time of administration had no effect either and neither did the dose administered. Logarithm applied and two-way analysis of variance was done. A multiple regression analysis was also performed to determine the independent effect of treatment, time of treatment, and serum hydroxyprogesterone and estradiol levels on serum testosterone levels (dependent variable)	Study was thorough and well designed. No side effect was noted relative to placebo group.	Gonzales et al., 2003a
Effect of maca on hypoactive sexual desire in men on its own and relative to testosterone	*L. meyenii*	no mention	1st group - 31 healthy males aged 50-62 years old treated with maca only; 2nd group -23 males with Hypogonadism and no other sexual dysfunction treated with i.m. depot of testosterone and maca ; 3rd group - 21 males with Hypogonadism and no other sexual dysfunction, treated with i.m depot of testosterone only	480mg every 12 hrs daily	None	3 months	Maca improved subjective sexual desire in men suffering from HSD after 3 months treatment. Maca had a better effect in improving HSD in men with hypogonadism relative to use of testosterone only.	Maca shows potential for improving sexual desire. No comment made on Rosenberg test results for self-esteem or PSS-10 test for perceived stress.	Poyato et al., 2009

Effect of maca on endurance performance and sexual desire in trained cyclists. Parameters: 40km cycling time trial performance and self-reporting questionnaire of Sexual Desire Inventory (SDI)	*L. meyenii* Walp from Cerro de Pasco	**400mg capsules taken orally - aqueous extract obtained from maceration with water**; maca root: extract powder ratio of 5:1	8 male cyclists aged between 23-37 years old and were healthy	2000mg/day	placebo - untreated control	14 days	Statistical analysis done; Maca supplementation was found to significantly increase time trial of 40km in cyclist when compared to pre-supplementation time with no significant change in Heart Rate or Rating of Perceived Exertion. Maca was also found to improve rating of dyadic sexual desire but not that of solitary sexual desire. No changes were observed with placebo supplementation	Maca apparently increased endurance performance but it was not related to increased effort since there was no change in HR and RPE. However, it should be noted that the difference between placebo supplementation and maca supplementation on time trial improvement was not significant. Maca was also showed as in previous studies to improve sexual desire in men. However, in this study it was observed at 2 weeks whereas in others it was shown at 8 weeks (Gonzales et al., 2002). The number of 8 cyclists might be too few to confirm finding and larger trials are needed. mechanism of action of maca on the investigated parameters are still unresolved.	Stone et al., 2009
Subjective effect of maca on well-being and sexual performance of patients with mild erectile dysfunction	*L. meyenii,* imported from Peru and provided by Ibersan, Srl, Forli, Italy.	pulverized and dehydrated maca root - no extract used; oral tablet	50 caucasian31-41 years old men with mild erectile dysfunction as measured by International Index of Erectile Function (IIEF - 5) and Satisfaction Profile (SAT-P)	2400mg/day i.e. 1200mg twice daily	placebo controlled	12 weeks trial	Statistical analysis done; Maca treated group experienced a higher significant increase in IIEF score than placebo and a higher significant improvement in psychological performance SAT-P scores, though placebo effect was also significant. But only maca treated group reported a significant improvement in social and physical performance of STA-P score. No significant change in hormone levels and no report in adverse events were made during the study. Maca improving effect on IIEF-5 scores appeared to be inversely related to baseline scores.	It is postulated by authors, while considering the limits of the study of mild ED patient within a short period of time, that maca produced a small improvement in erectile dysfunction relative to placebo and that larger scale trial run for a longer time and with patients with more serious ED should be done to confirm effect of maca on ED. The multi-factorial nature of ED involving both a psychological and a clinical component makes it difficult to identify whether maca improves ED by acting centrally or specifically on the reproductive tract. Nonetheless, the pilot results look promising and warrant further investigation on the matter.	Zenico et al., 2009
The effect of oral supplementation with Spermotrend, Maca extract (L. meyenii) or L-Carnothine in semen parameters of infertile men	*L. meyenii*	oral 1g pill Maca extract from Nature's Way Product, Inc - (found online standardised to 3mg macaenes and macamides)	60 infertile but healthy men with no previous treatment, non-alcoholics, non drug-users and non-smokers split into four groups for each treatment	1g twice daily or 2g/day	placebo controlled	Sperm parameters evaluated at 0, 30, 60 and 90 days.	Group I (L-carnitine group) showed a statistically significant increase in sperm sample concentration. Groups II (Spermotrend) and III (Maca) improve the sperm motility (grade a+b) in the sample more than the groups I and IV. No difference in the percentage of normal sperm (morphology) was found across the study in any groups.	Maca was shown to improve sperm motility. This matches the results found by Gonzales GF et al., 2001 in a pilot clinical trial on 9 healthy men.	Povedo et al., 2013

effect of maca administration on semen quality and serum hormones levels	*L. meyenii*	Dried milled maca hypocotyl	20 healthy male volunteers aged between 20 to 40 years old	1.75g per day	placebo controlled	sperm parameters evaluated at 6 and 12 weeks of treatment and blood hormone levels evaluated at 12 weeks of treatment	Sperm volume and blood hormones levels did not change significantly after 3 months of maca treatment. But there was an apparent increase in sperm motility and sperm count relative to placebo.	Quality of sperm was improved independently of blood hormones levels with maca treatment.	Melnikovova et al., 2014
effect of maca administration on semen quality and serum hormones levels in healthy adult men	*L. meyenii* - yellow ecotype from Cerro de Pasco, Peru provided by Peruvian company Andean Roots SRL.	oral entero solvent capsule containing 350mg gelatinised maca powder each	18 healthy male volunteers aged between 20 to 40 years old, non smokers who did not use any substances that could change their hormones levels 3 months before treatment; placebo: 7 and maca: 11	1.75g per day or 5 capsules per day	placebo controlled	12 weeks trial - semen collected before trial, on week 6 and week 12; blood was collected before and after trial	Statistical analysis done. No statistically significant changes in semen parameters were observed, possibly due to significant within group variations. However, all semen parameters showed a rising trend in the maca group after 12 weeks of treatment. No statistically significant changes in serum hormones levels were observed. Macamides n-benzylhexadecanamide, n-benzyl-(9Z.12Z)-octadecadienamide, and n-benzyl-(9Z.12Z.15Z)-octadecatrienamide were found to be the most abundant	It is postulated by authors, while considering the positive results in previous trials in men for longer length of time by Gonzales et al., 2001, that length of maca treatment positively influences semen parameters and that maca's effects warrants further study as a fertility enhancing agent in men with sub-fertility. Macamides content, the chemotaxonomic compounds in lipid maca fraction showed variations and should ideally be determined prior to conducting any study.	Melnikovova et al., 2015
effect of Maca on spermatogenesis and spermatic quality in subjects diagnosed with infertility.	*L. meyenii*	pulverized and dehydrated maca root in capsule	10 males with alteration in semen parameters classified as sub-fertility	3000mg/ day	None	3 months but spermatogram before and after 3 hours of treatment	Importantly, no change to sperm count per ml. Motility and vitality (only after 3 h) were increased significantly as was the %-age of normal sperm cells (from 40 to 45%) while there was a significant decrease of immature / twin cells.	It seems that 2 pregnancies (20%) resulted from the intervention and this is linked to the main finding, the increased motility 3 h post intervention. Those subjects with he highest increase in motility fathered these children	Tancara et al., 2010
Compare the effect of maca supplementation on the libido of low v/S high sexual capacity hair sheep rams-investigated are genital sniffs,	*L. meyenii* – black maca hypocotyls-maca meal MacaAndina	Milled powder suspended in 250ml water	Animals were 40 sexually naive Saint croix rams, 11-15 months old, weighing 48.6 ± 8.5 kg at the	233 mg of dry maca/kg of body weight/day during four weeks	10 rams of each category: control diet with commercial concentrate diet	10 rams in each control group: control diet for 8 weeks or a maca supplemented diet for four	In LP rams, genital sniffs, nudgings and ejaculations increased ($P < 0.05$) during maca supplementation up to frequencies observed in HP rams, while mounts increased even at higher levels. During the residual phase, all sexual behaviours in LP rams decreased ($P < 0.05$; genital	Results suggests that maca supplementation is not only necessary to improve sexual capacity, but also to keep it at high levels. It was concluded that maca supplementation affects males differently, according to their sexual capacity. Maca consumption improves some mating behaviours in rams. But above all, it	Avelar et al., 2016

nudging, mounts and ejaculations			beginning of the experiment; 20 rams high performers and 20 rams low performers		formulated for breeding rams, with 16% protein (Nutres®	weeks, followed by four weeks of a control diet (residual phase)	sniffs, mounts and ejaculations) or tended to decrease ($P > 0.10$; nudging), not to baseline levels, but to controls. In HP rams, genital sniff and nudging frequencies increased ($P < 0.05$) during maca supplementation, remaining at high levels during the residual phase, while mounts and ejaculations remained unaffected during the whole experiment. The number of ejaculations in HP rams remained similar to their pre-treatment levels and controls during the supplementation and residual phases. These findings suggests that in HP rams, maca supplementation might act only as a trigger, favouring a response in some sexual behaviours that will remain high, even four weeks after maca supplementation ceased.	demonstrates that this effect is achieved particularly by low sexual capacity rams that will reach high sexual capacity levels, and that maca supplementation has a short residual effect.	

Appendix 6: In-vivo Studies of Maca on Female Fertility

***in vivo* studies for female fertility**								
Properties investigated	**Form of maca**	**Model**	**Dose range studied**	**Control used**	**Duration of study**	**Outcomes**	**Comments**	**Reference (Authour., Year)**
steroid hormone levels - 17-β estradiol in female mice and testosterone in male mice and embryo implantation	maca powder dissolved in drinking water (5.0g in 100ml water) with ad libitum access	10 three week old ICR mice(not sexually mature)	not measureable as ad libitum access	10 three week old ICR mice on plain water	30 days	No difference in 17-β estradiol serum levels between treated and untreated groups. Statistically significant increased in progesterone levels in female mice in maca treated group relative to placebo. Statistically significant difference in testosterone levels in male mice in maca treated group. No significant difference in implantation rate between maca treated groups and untreated groups.	The fact that not sexually mature mice were used might explain the increase in hormone levels as these mice reach/grow through puberty during the study. Ad libitum access to maca powder does not give control over dosage which makes drawing a final conclusion elusive. Authors purport that presence of saponins in maca acting as an adaptogen might be responsible from observed effects (Arletti R et al., 1999, Kelly GS., 2001, Kropotov et al., 2001)	Oshima et al., 2003
Effects of short and long term administration of maca powder and how hormones are related to observations; Blood morphology, biochemistry (lipids, hormones and minerals), histology of internal organs were determined, homogenates of skeletal muscles and bones of rats were analysed.	Maca-GO, pregelatinized maca powder suspended in water	Sprague-Dawley rats: 45, nine week old females weighing 240 g to 250 g and 45, twelve weeks males weighting 340 to 350g at the beginning of the experiment. Trial I (short-term -28days): 10 animals/group (5male +5 female), Trial II (long-term - 90 days): 20 animals/group (10 male + 10 female)	0.75g/Kg BW and 7.5g/Kg BW by gavage	Yes, given vehicle.	28 and 90 days	Maca-Go has low toxicity (LD=7.5g/Kg). There were different responses of male and female rats to different levels of Maca-GO administered during short- and longer-term periods. When administered at higher dose for extended period of time (90 days), Maca-GO acted as a toner of hormonal processes in adult female rats at increased progesterone and a steady estradiol level, without affecting levels of blood FSH, LH and TSH. Substantial decrease in blood cortisol levels in a short- and longer-term trial and simultaneous tendency to lower blood ACTH was observed. After short-term treatment, maca reduced weight in male rats but did not reduce weight gain relative to control in female rats until after long-term treatment. The weight loss and slowing of weight gain was associated with a lowering in serum of TSH levels. Triglycerides in blood plasma were lowered and there was increasing calcium and phosphorus deposition in bone and muscle tissues.	Authors advise use of the whole root to achieve therapeutic effect due to complexity of maca phytochemistry. Maca showed potential for further investigation in humans and use in sportsmen.	Meissner et al., 2006a Short and Long-Term Physiological Responses of Male and Female Rats to Two Dietary Levels of Pre Gelatinized Maca (L. Peruvianum Chacon)
reproductive index - fertility index, gestation index, post-natal viability index, weaning viability index, sex ratio index	Yellow maca - aqueous extract lyophilised	11 three months old virgin female mice Balb/C strain	1g extract /Kg body weight daily	12 three months old virgin female mice 0.5ml water by gavage	15 days prior to mating, the whole gestation period and 21 days after birth (lactation period)	no significant difference in fertility indexes measured between groups. No difference in body weight. No difference in implantation sites, gestation length, sex ratio or vaginal opening day of female pups between maca-treated group and control. Maca-treated group had larger litter size by day 4 post-birth.	It is suggested that maca may have a protective effect against resorption during pregnancy, explaining the higher number of pups. Uterine weight was also increased in ovariectomised mice. Postulated mechanisms of action include increased uterine receptivity, altered immune function and an effect on the vascular system. Maca is suggested to have a progestin-like effect.	Ruiz-Luna et al ., 2005

varieties of maca on female reproductive parameters in adult intact rats	Red, Yellow and Black maca - aqueous lyophilised extract	8 three months old Holtzman female rats	1g dried hypocotyl /Kg body weight daily	8 three months old female rats	28 days (7 estrus cycles)	No difference in phases of estrous cycles, body weight, uterine wet weight and number of oocytes recovered from oviduct between control group and maca treated groups. No difference in serum estradiol level in yellow maca treated group and control. Black and Red maca-treated groups not assessed for estradiol level. Total polyphenols content found in black, yellow and red maca freeze-dried extracts was 0.56, 0.57 and 0.58 g of pyrogallol/100g, respectively.	Maca did not show any estrogen-like activity.	Gasco et al., 2008
effect of methanol and pentane extract of maca on female rat fertility	*L. meyenii* methanol and pentane extracts	6 female breeders rats per group	3mg/Kg BW of pentane extract, 30mg/Kg BW of crude extract	6 female breeders rats treated with vehicle only	21 days prior to mating	Maca-treated animals had 79 pups and 100% pregnancy rates compared to 56 pups and 67% pregnancy rate with control. Mortality of pups at post-natal day 6 was 10.7% for control group and 7.6% and 2.5% for methanol and pentane maca extract-treated groups respectively. No difference between groups of weight at birth of pups and rate of increase in BW through post-natal day 6.	The doses used are very high (30mg extract/Kg BW) and not clinically useful.	Pino-Figueroa and Maher, 2009
effect of maca on serum LH during the pro-estrus LH surge in female rats	*L. meyenii* - powder from Yamano del Peru SAC,	2 weeks old Sprague-Dawley female rats t beginning of experiment; 10 in 5% maca group, 10 in 25% maca group, 20 in 50% maca group 20 in control group	maca powder mixed to CE2 laboratory chow feed at 5%, 25% and 50% and provided ad libitum corresponding to 3.0, 15 or 30g/Kg BW as feed intake was constant.	20 two weeks old female Sprague-Dawley rats in control group only with CE2 laboratory chow feed	7 weeks	Serum levels of LH and FSH increased significantly relative to control. LH serum levels increases 4.5 times in the pro-oestrus of feeding with 50% maca powder in female rats. Initiation time of LH surge, LH surge pattern, elevation of FSH time and ovulation were not affected with respect to control group. LH serum levels in the pulsate LH phase (associated with menopause and not with an improvement in triggering of ovulation) did not change either.; There was a dose-dependent relationship between LH serum level and amount of maca intake with the enhancement of LH levels sustained at an intake of greater than 15g maca/Kg BW.	The dose is very high (30g maca/Kg BW) but authors explain that it is plausible because Andean people have traditionally consumed large quantities of maca and postulate that a high maca dose might be necessary for fertility in humans or rats.	Uchiyama et al., 2014

***in vivo* studies of menopause**									
Properties investigated	**Type of maca studied**	**Model**	**Dose range studied**	**Control used**	**Control used**	**Duration of study**	**Outcomes**	**Comments**	**Reference (Author., Year)**
B.1: Hormonal activity of maca , B.2: blood morphology, blood plasma and lipid levels, B.3: behavioural and cognitive function - anti-depressive, anti-anxiety, motoric function and long term cognitive function	pre-gelatinised organic maca powder (Maca-GO)	12 OVX and 12 sham-operated Wistar female sexually-experienced rats weighing between 330 - 370g	250mg/Kg BW maca-GO suspension twice daily by gavage	12 OVX and 12 sham-operated Wistar female sexually-experienced rats weighing between 330 - 370g given vehicle (distilled water) by gavage	12 OVX and 12 sham-operated Wistar female sexually-experienced rats weighing between 330 - 370g given vehicle (distilled water) by gavage	28 days	In sham-operated rats, maca-GO decreased Estradiol (E2) and increased progesterone (PGR) levels. In OVX rats, maca-GO decreased both E2 and PGR. Maca-Go lowered ACTH and Cortisol after OVX but did not normalise Cortisol levels. Maca administration appeared to restore TSH balance after OVX. After 4 weeks of treatment, there was a slight reduction in blood cholesterol and triglycerides but no significant change in blood morphology parameters of haemoglobin and granulocytes. Total Fe serum levels did increase as did RBC. Maca-GO showed anti-depressant like and sedative effects but not anxiolytic effects, which were associated with lowered Cortisol and ACTH levels. Maca-Go showed impairment in spontaneous activity in OVX rats but not in sham-operated rats. Maca-GO also improved long-term cognitive function at 48 hours test.	There is no indication at what point of the estrus cycle were the rats (esp. the sham-operated ones) when the measurements were taken. This would inevitably have influenced the results of the study. It is suggested that maca may have some value in protecting depressive effects in menopausal women.	Meissner et al., 2006b (B)
uterine weight in ovariectomised rats	Yellow maca - aqueous extract lyophilised	7 three months old virgin female mice Balb/C strain were ovariectomised and treatment started3 months post-surgery	1g extract /Kg body weight daily	eight ovariectomised mice 0.5ml water by gavage	eight ovariectomised mice 0.5ml water by gavage	42 day period	maca-treated significantly increased uterine wet weight relative to control group		Ruiz-Luna., 2005

effect of ethanol extract of maca on osteoporosis in ovariectomised rats	*L. meyenii* - 95% ethanol extract	90 day old female Sprague-Dawley rats ovariectomised (OVX) - 10 per group	0.096g extract/Kg BW or 0.24g extract/Kg BW equivalent to 0.5g and 1.25g of dried maca root/Kg BW respectively	10 sham operated female rats and 10 ovariectomised female rats given vehicle (distilled water) only	10 sham operated female rats and 10 ovariectomised female rats given vehicle (distilled water) only	28 weeks	There was no significant difference in body weight between groups. Uterine weight in OVX and maca treated group all decreased relative to sham group. Only femur diameter in maca treated group at 0.24g/Kg BW increased significantly relative to OVX. The Bone Mineral Density (BMD) of Lumbar vertebrae LV-6 and calcium content increased significantly with 0.24g/Kg BW maca treatment relative to OVX group but there was no change in mid-shaft femur BMD and ash weight. No significant difference were observed in femur biomechanical strength properties of max-load, max-stress, elastic energy in either sham operated, OVX and OVX-maca treated groups. No difference in serum calcium and inorganic phosphorus levels in any groups. Administration of Maca had no influence on serum ALP and osteocalcin. Maca administration appeared to restore architecture in trabecular bone that was disrupted in OVX rats at both maca doses.	The lack of femur midshaft BMD decrease is probably due to higher Ca diet that suppressed cortical bone loss in OVX rats. The results suggest that ovariectomy combined with normal Ca supplementation has less influence on cortical bone loss. BMD-increasing effect of Maca is more obvious in cancellous bone-rich regions than in cortical bone-rich ones. Since Maca did not increase bone metabolic markers in serum while protecting against bone loss and not increasing uterine weight, this suggests that maca does not act directly on the mechanism of bone metabolism. Maca has a fair amount of calcium, magnesium and silica that are easily absorbed and protect against bone loss. Biological activity of maca on bone may be due to one or more phytochemical present.	Zhang et al., 2006
effect on serum hormone and blood lipid levels in ovariectomised rats	*L. meyenii* ethanol extract	ovariectomised rats as a model for menopause	0.5g of maca powder/Kg BW and 1.25g of maca powder/Kg BW once daily	ovariectomised rats and sham-operated rats on 0.1% tween-80	ovariectomised rats and sham-operated rats on 0.1% tween-80	7 months	Compared with the ovariectomized control rats, the serum FSH level decreased remarkably (P <0. 01) after treated with 1. 25 and 0.5 g·kg-1 of Maca ethanol extract, the serum E2 level increased (P<0. 01) with 0. 5 g·kg -1, but the serum T level did not change at the end of 7 th month after administration. Also, Maca extract definitely decreased serum cholesterol, while increased serum triglyceride level in 3rd month but decreased the level at the end of the administration.	Maca extract definitely improves endocrine disturbance after ovariectomy in rats.	Zhang et al., 2008
effect on body fat, sexual hormone, bone metabolism in post-ovariectomised rats	*L. meyenii* (MACA) - yellow maca pulverised powder	Sprague-Dawley rats (5 groups of 11)	low dose maca (0.3g/Kg), middle-dose maca (0.6g/Kg), high dose (1.8g/Kg) suspended in water given by gavage	sham-operated and ovariectomised rats untreated	sham-operated and ovariectomised rats untreated	7 weeks	OVX rats given high dose maca showed an increase in blood estrogen and Blood gla protein (BGP) level and had less uterine weight loss and body weight gain. The triglyceride, LDL-C and ALP level were similar to sham operated group while these were increased in untreated OVX group.	Authors suggest dietary supplementation with maca may help prevent abnormal lipid levels and help bone metabolism at post-menopause by acting through a different mechanism to estrogen.	Wang et al., 2009

effect of different varieties of maca on bone structure in ovariectomised rats	*L. meyenii* - Yellow, Red and Black phenotype - hydroalcoholic extract standaradised to polyphenol content (polyphenol content of pulverized dried hypocotyl varied as red > yellow > black); (polyphenol content of hydroalcoholic extract varied as yellow > red > black)	6 three-months old female ovariectomised (OVX) rats per group (36 total)	maca dose equivalent to 4.3mg polyphenol/Kg BW	sham operated female mice and ovariectomised female mice treated with vehicle; positive control - OVX mice treated with 40microgram/Kg estradiol valerate IM daily	sham operated female mice and ovariectomised female mice treated with vehicle; positive control - OVX mice treated with 40microgram/Kg estradiol valerate IM daily	4 weeks	Black and red maca reduced effect of ovariectomy on absolute weight of femur but not yellow maca. Red maca also increased dried femur weight relative to untreated OVX rats' values. No difference in dried femur weight in esradiol, black, yellow and sham groups. Treatment with red, yellow, black and estradiol reversed the effects of ovariectomy on femur diameter. Black and red maca showed higher values in increasing femur width that yellow maca but there were no significant difference between groups. Treatment with red or black maca had a protective effective against bone loss in increasing trabeculaer bone area and decreasing intra-trabecular bone space with significant difference with estradiol treated group. Yellow maca had similar effects but to a lesser extent. Neither yellow, black or red maca increased uterine weight in OVX rats	Maca, especially the red and black variety, showed protective effects against bone loss in ovariectomised rats without increasing uterine weight. The polyphenols, polyunsaturated fatty acids and phytosterols found in maca are thought to be potentially responsible fro the observed effect. It is postulated that red and black maca might be acting as a selective estrogen receptor modulator (SORM) or a gonadal steroidogenic acute regulatory (StAR) protein but further investigation into the mechanism of action is needed. The fact that the study dose was standardised according to polyphenol indicates that other metabolites might be responsible for the effects shown.	Gonzales et al., 2010
effect of ethanol extract of maca on the serum hormone levels in ovariectomised rats and compare these effects with diethylstilbestrol (DES)	*L. meyenii* - standardised at 0.6% macaenes and macamides, 95% ethanol extract	90-days old female Sprague-Dawley rats - 10 rats per group	0.096g/Kg BW and 0.24g/Kg BW maca ethanol extract daily	10 sham operated female sprague-dawley rats untreated , 10 ovariectomised female rats untreated and 10 ovariectomised female rats treated with diethylstilbestrol at 0.05mg/Kg daily	10 sham operated female sprague-dawley rats untreated , 10 ovariectomised female rats untreated and 10 ovariectomised female rats treated with diethylstilbestrol at 0.05mg/Kg daily	28 weeks	maca treated group showed an increase in BW similar to sham and OVX control groups while DES group showed a significant decrease. Weight of uterine was significantly lower in maca and OVX treated groups relative to sham operated and DES group showed a significant increase in uterine weight relative to OVX. No change in Estradiol (E2) and FSH levels at 12 weeks. Lower dose of maca showed a significant decrease in testosterone levels relative to OVX control group. A significant increase in E2 levels was observed with lower dose maca extract at 28 weeks relative to OVX control. Both maca doses showed a significant decrease in FSH levels. Conversely, DES group showed a statistically significant increase in E2 levels and a statistically significant decrease in Testosterone and FSH levels at both week 12 and week 28.	Higher dose of maca did not have an effect. Maca appear to have some modulatory effect on hormone balance in menopausal women but the mechanism of action is unknown and the properties of maca in this regard needs further attention.	Zhang et al., 2014
to assess effects of natural supplement maca and Warmi on body and uterine weight and serum biochemical parameters in ovariectomised female mice	*L. meyenii* supplement - 0.5625mg daily equivalent to 1560mg/60kg human body weight	Swiss type female mice - 4 per group (twenty total), ovariectomised (OVX)	0.5625mg daily	sham operated female mice and ovariectomised untreated female mice	sham operated female mice and ovariectomised untreated female mice	10 weeks	OVX group had higher weight than treated group. Uterine weights in treated group were significantly higher relative to OVX control group. Triglyceride and cholesterol levels were lower at the end of the study in treated group as compared to OVX control group	Maca shows potential in the prevention of body weight gain, lipid rise and uterine atrophy after ovariectomy and warranted further research. Warmi is a blend of maca and hesperidin	Barraza et al., 2015 (focus on maca only treatment group)

Appendix 8: Clinical Studies of Maca in Women

Clinical Studies in women								
Study Type	**Title of study**	**Sample size, condition, age(years)**	**Intervention (regimen)**	**Control intervention (regimen)**	**Measurement of menopausal symptoms**	**Outcome**	**Comment**	**Reference (Authour., Year)**
double-blind, placebo-controlled clinical pilot	Use of gelatinized maca (*L. peruvianum*) in early post-menopausal women	20 Caucasian healthy early-menopausal women for three months (Trial I), 8 quit, left with 12 participants eventually	two placebo (cellulose and sorbitol) 500mg hard gel capsules twice daily for 1 month followed by two pre-gelatinized Maca-GO 500mg hard gel capsules twice daily for two months	cross-over study; placebo given at two 500mg hard capsules twice daily	Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH), Estrogen (E2), Progesterone (PG), Greene's Menopausal Index	LH level significantly increased with non-statistically significant decrease in FSH after 2 months Maca-GO treatment. Progesterone level increased during 1st month of placebo but decreased after two months of Maca-GO.A positive placebo effect was observed. Placebo treatment was reported to decrease nervousness, fatigue, difficulty falling asleep and night sweats. Maca-GO treatment for 2 months, correcting for placebo effect, showed an improvement in energy, less nervous tension, return of interest in sex life and reduction in hot flushes. Night sweating, feeling of anxiety and depression, excessive crying and irritability were inconclusive.	It is suggested that the properties of maca may be attributed to the peculiar composition of the plant, especially the sterols which may have a multifunctional effect on endocrine function contributing to the observed effect. It is postulated that maca may be acting as a toner of hormonal processes. The distinctive positive placebo effect was noted.	Meissner et al., 2005 (Trial I)
double-blind, placebo-controlled clinical pilot	same as above	8 women for nine months (Trial II)	two placebo (cellulose and sorbitol) 500mg hard gel capsules twice daily for 1 month followed by two pre-gelatinized Maca-GO 500mg hard gel capsules twice daily for eight months	cross-over study; placebo given at two 500mg hard capsules twice daily	Luteinising Hormone (LH), Follicle Stimulating Hormone (FSH), Estrogen (E2), Progesterone (PG), Greene's Menopausal Index	Level of FHS significantly decreased after two months with further reduction after 8 months of maca-GO administration. LH was significantly increased with slight further increased after 8 months treatment. Levels of progesterone increased as trial II progressed but was significant only at month 8. Estradiol level was also significantly increased after 8 months treatment. After correcting for positive placebo effect, maca-Go treatment showed a significant improvement in menopausal symptoms.		Meissner et al., 2005 (Trial II)

double-blind, cross-over, randomized, pilot study	Therapeutic Effects of Pre-Gelatinized Maca (*L. Peruvianum* Chacon) used as a Non-Hormonal Alternative to HRT in Perimenopausal Women - Clinical Pilot Study	20 Caucasian women (10 per group) aged 41-50 years old in perimenopausal stage (E2 above 40pg/ml and FSH below 30IU/ml); women were healthy, regularly menstruating and had not used hormonal treatment before	Two Maca-Go (dried pre-gelatinized and pulverized maca hypocotyl) 500mg hard capsules twice daily for two months. Maca root in distribution of 16% black, 48%yellow and 9% red/purple.	cross-over study; placebo given at two 500mg hard capsules twice daily	Estrogen (E2), Follicle Stimulating Hormone (FSH), Luteinising Hormone (LH), Progesterone (PGS), Cortisol (CT), Adrenocorticotrophic Hormone (ACTH), Thyroid Hormones (TSH, T4, T3), minerals (Ca, K, Fe) and lipid profiles (Triglycerides, Total Cholesterol, LDL, HDL), body weight, blood pressure, Kupperman's Menopausal Index	There was a distinctive placebo effect. Significantly alleviated symptoms of menopausal discomfort (hot flushes, night sweats, interrupted sleep patterns, nervousness, depression and heart palpitations) in 74-87% of women according to Kupperman's Index. There was a significant increase in E2, FSH, PGS, ACTH, HDL and Iron and reduction in blood pressure, body weight, Triglycerides and cholesterol levels.	A possible hormone balancing and toner effect of maca acting on the hypothalamus and pituitary is postulated.	Meissner et al., 2006e
double-blind, randomised, placebo-controlled, multi-centre clinical study	Hormone-Balancing Effect of Pre-Gelatinized Organic Maca (*L. peruvianum* Chacon): (II) Physiological and Symptomatic Responses of Early-Postmenopausal Women to Standardized doses of Maca in Double Blind, Randomized, Placebo-Controlled, Multi-Centre Clinical Study	102 healthy Caucasian women aged 49- 58 years old and with follicle stimulating hormone (FSH) >30 IU/ml and estrogen (E2) <40 pg/ml levels at admission.	two 500mg vegetable hard gel capsules of maca-GO powder twice daily for three months; 62 women given (placebo-maca-maca) and 40 women given (maca-maca-placebo)	two 500mg placebo capsules twice daily for three months	blood levels of FSH, E2, progesterone (PRG) and luteinising hormone (LH), serum cholesterol (CHOL), triglycerides (TRG), high- and low density lipoproteins (HDL and LDL); Greene's Menopause Score (GMS) and Kupperman's Menopause Index (KMI)	There was no statistically significant difference between the two sequence groups (APMM and AMMP) in the levels of the four hormones profile recorded on a monthly basis in any of the four blood sampling points. It seems at least two months of maca-GO treatment are needed to bring down TGL, LDL and increase HDL. Placebo treatment for one month caused a decrease in 11 of KMI symptoms and after following two months with maca-GO, there were no change in KMI symptoms relative to that recorded with placebo but a significant improvement in hot flushes and nervousness were reported. GMS scores with maca-GO treatment relative to placebo showed a significant improvement in hot flushes, excessive sweating, nervousness, excessive alertness, lack of energy/feeling of tiredness and to a lesser degree irritability and headaches. Effects were more pronounced after 2 months treatment with maca-GO.	Failure to comply to treatment protocol and drop-outs were not counted in the final statistical analysis (no Intent-to-treat analysis). It seems that placebo effect is significant in bringing desired increase in E2 levels. KMI and GMS Scores were consistently improved.	Meissner et al., 2006c (Trial I)

double-blind, randomised, placebo-controlled, multi-centre clinical study	same as above; Trial II aims to study the placebo effect when introduced in different sequence alongside maca treatment	66 healthy Caucasian women aged 49- 58 years old and with follicle stimulating hormone (FSH) >30 IU/ml and estrogen (E2) <40 pg/ml levels at admission.	two 500mg vegetable hard gel capsules of maca-GO powder (pre-gelatinised organic maca powder) twice daily for four months; six individual treatment sequences were used (A-P-M-M-P; A-P-P M-M; A-M-M-P-P; A-M-M-P-M; A-P-P-P-M; A-M-M-M-P). Eleven women were assigned to each treatment sequence.	two 500mg placebo capsules twice daily for four months	blood levels of FSH, E2, progesterone (PRG) and luteinising hormone (LH), serum cholesterol (CHOL), triglycerides (TRG), high- and low density lipoproteins (HDL and LDL); Greene's Menopause Score (GMS) and Kupperman's Menopause Index (KMI)	Maca did not seem to have any residual effect while it did tend to lower FSH whilst on treatment. E2 levels increased significantly in APPMM sequence and almost significantly in AMMPM sequence. Two months of maca treatment showed a more pronounced effect on decreasing FSH levels and increasing E2 levels than one month treatment only. No change in progesterone levels. Significant improvement in KMI and GMS scores were more pronounced after two months treatment with maca-GO.	Authors state that it is reasonable to assume that Maca-GO, although it contains no plant hormones, stimulated and/or contributed to the regulatory mechanism responsible for optimizing ovarian function and secretion of the quantity of estrogen – in some cases– well above the 30pg/ ml considered as desired minimum. Irrespective of the sequence in which Placebo was introduced during the four month trial, two months of Maca-GO application magnified the therapeutic effects of the treatment.	Meissner et al., 2006c (Trial II)

double-blind, randomised, placebo-controlled, cross-over, out-patient study	Hormone-Balancing Effect of Pre-Gelatinized Organic Maca (*L. peruvianum* Chacon): (III) Clinical responses of early postmenopausal women to Maca in double blind, randomized, Placebo-controlled, crossover configuration, outpatient study	22 healthy early post-menopausal Caucasian women aged 49-58 years old with blood Estrogen (E2<40 pg/ml) and Follicle Stimulating Hormone (FSH>30 IU/ml) at admission (A) (11 participants per group - Maca (M) and Placebo (P) over 4 months A-P-P-M-M and A-M-M-P-P (2 X 2))	Two 500mg vegetable hard capsules twice a day (total 2g/day)	cross-over study: placebo 2g/day	Hormone levels of: E2, PGR, LH, FSH, ACTH, Cortisol (C), TSH, T4, T3, TGR, CHOL, HDL, LDL, blood pressure, body weight, serum mineral content, Menopausal symptoms and associated overall discomfort experienced measured through Greene's Score (MGS) and Menopausal Kupperman's Index (MKI)	Maca-Go significantly decreased BMI after 4 month in A-P-P-M-M treatment group. No significant difference in decreasing systolic or diastolic blood pressures. No significant differences in FSH and PGR levels in either treatment sequence but Maca-Go significantly increased E2 levels while decreasing simultaneously LH levels relative to placebo. Changes due to placebo introduced prior to maca treatment were more pronounced than those with placebo after maca treatment suggesting that treatment with Maca-Go had residual effect. No statistically significant difference in concentrations of the TSH, T4 but T3 and ACTH and Cortisol showed significant reduction. No statistically significant change in plasma cholesterol, HDL and triglycerides though a significant reduction in LDL level was noted with Maca-Go treatment. In A-M-M-P-P group there was a significant increase in serum Iron and calcium levels. Both treatment sequence showed a significant reduction in GS and KMI scores relative to placebo. The positive effect with placebo observed after 1 month in the A-P-P-M-M treatment sequence decreased after the second month placebo treatment, indicating that Maca-GO is indeed responsible for observed improvement in GS and KMI scores. Effect of maca-GO relative to placebo was more noticeable with KMI score than GS score.	in addition to a significant increase in E2, lowered LH, T3, CT, ACTH and steady FSH and PRG levels, there was a highly significantly reduction in BMI and both frequency and intensity of menopausal symptoms such as hot flushes, profound perspiration (night sweating), as well as reduced depression, irritability, difficulty in falling asleep and other as demonstrated by the KMI and GMS, which may indicate similar effect as the one induced by the HRT treatment. Maca-GO applied to early-postmenopausal women (i) acted as a toner of hormonal processes along the Hypothalamus-Pituitary-Ovarian axis, (ii) balanced hormone levels and (iii) relieved symptoms of menopausal discomfort, (hot flushes and night sweating in particular), thus, (iv) exhibited a distinctive function peculiar to adaptogen, providing an alternative non-hormonal plant option to reduce dependence on hormone therapy programs (HRT). Length of treatment with maca influences the positive effect observed with two months treatment magnifying the effects relative to one month treatment.	Meissner et al., 2006d (Trial III) (A) Hormone levels

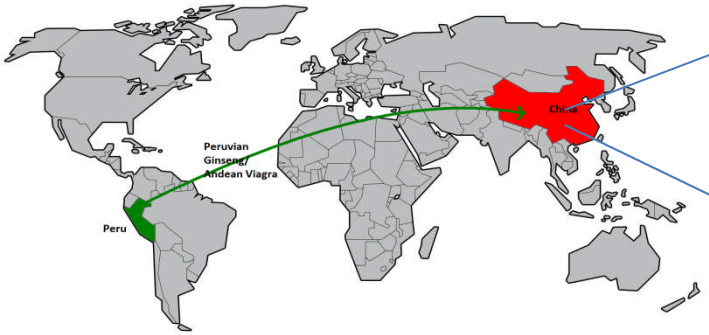
double-blind, randomised, placebo-controlled, out-patient study	Hormone-Balancing Effect of Pre-Gelatinized Organic Maca (*L. peruvianum* Chacon): (III) Clinical responses of early postmenopausal women to Maca in double blind, randomized, Placebo-controlled, crossover configuration, outpatient study	12 healthy early post-menopausal Caucasian women aged 49-58 years old with blood Estrogen (E2<40 pg/ml) and Follicle Stimulating Hormone (FSH>30 IU/ml) at admission (6 per group M and P both assessed after 4 months)	Two 500mg vegetable hard capsules twice a day (total 2g/day)	2g/day placebo	bone density markers: bone density scan	Bone density measures were found to increase with maca-Go treatment with an associated decrease in serum FSH(55%) and increase in E2 (135%) levels but not with placebo.	see above	Meissner et al., 2006d (Trial III) (B) Pilot Bone Density Observation
randomised, double-blind, placebo-controlled, crossover trial	Beneficial effects of *L. meyenii* (Maca) on psychological symptoms and measures of sexual dysfunction in postmenopausal women are not related to estrogen or androgen content	14 postmenopausal women aged 50-60 years old who were amenorrheic for 12 months or more and reported experiencing fatigue, lack of energy, difficulty in sleeping, and hot flushes of moderate severity and not taking any treatment	3.5g/day of maca powder provided in sachets consumed in breakfast cereal, milkshakes or soup for 6 weeks	Cross-over study; placebo of refined white rice flour for 6 weeks	Estradiol, Follicle Stimulating Hormone, Luteinising Hormone, sex-hormone biding globulin, Greene's Climacteric Scale	Body weight and body mass index did not change significantly during the study. There was no difference between groups for serum parameters but test reported as not sensitive enough. Greene's Climacteric test showed significant reduction of scores for psychological symptoms of anxiety, depression and sexual dysfunction. Total score reduction of 18% relative to baseline and 17.3% relative to placebo treatment. No significant changes between somatic and vasomotor score.	The absence of change in serum hormone levels, indicate that maca has no estrogenic effect unlike Meissner at al., 2006. *In-vitro* assay also confirms absence of physiologically significant estrogenic activity in maca	Brooks et al., 2008

randomised, double-blind, placebo-controlled, crossover trial	The effect of *L. meyenii* (MACA) on physiological and psychological parameters in postmenopausal Hong Kong Chinese women.	31 healthy Hong Kong Chinese postmenopausal women.	3.3g/day of maca powder encapsulated for 6 weeks and placebo for 6 weeks and vice-versa (total 12 weeks)	placebo for 6 weeks	Blood sampled at baseline, after 6 weeks and 12 weeks: Estradiol, FSH, Sex hormone binding globulin, thyroid stimulating hormone, lipid profile and plasma cytokines, Greene Climacteric scale, SF-36 Version 2 scale, Women's Health Questionnaire, Utian Quality of Life Scale.	Significant decrease in systolic and diastolic pressure while no differences were found in serum concentrations of estradiol, FSH, LH, TSH, SHBG, glucose, lipid profile and plasma cytokines (IL-2, IL-4, IL-5, IL-10, IL-12 (p70), IL-13, GM-CSF, IFN-g and TNF-a) between baseline, Maca treatment and placebo. GCS showed a significant reduction in both maca and placebo. Women's Health Questionnaire Scale showed significant reduction in anxiety scores in both Maca and placebo ($p=0.01$), while significant reduction in depression score was seen only with Maca administration ($p=0.04$). Utian Quality of Life Scale showed no significant increase in QoL in the emotional and sexual domain. The Quality of Life SF36-v2 Scale revealed significantly increased scores in general ($p=0.02$) and mental health ($p=0.03$) in both Maca and placebo groups, while placebo significantly increased the scores ($p<0.05$) in social functioning.	Maca does not exert estrogenic action, however, despite its high placebo effect it appears to decrease blood pressure, reduces psychological symptoms including anxiety, depression and improves general health and well being in Chinese postmenopausal women.	Stojanovska et al., 2011

randomised, double-blind, placebo-controlled, crossover trial	Maca reduces blood pressure and depression, in a pilot study in postmenopausal women	29 postmenopausal Hong Kong Chinese women aged 46-59 years.	3.3g/day maca capsules (seven 426mg maca capsules; 4 at breakfast and 3 at dinner) over 6 weeks (15 women started maca treatment first)	placebo (refined white rice flour) over 6 weeks (14 women started placebo treatment first)	Estradiol, Follicle Stimulating Hormone (FSH), sex-hormone biding globulin (SHBG), Thyroid Stimulating Hormone (TSH), full lipid profiles, glucose and serum cytokines, Greene's Climacteric Scale (GCS), SF-36 Version 2, Women's Health Questionnaire (WHQ), Utian Quality of Life Scales (UQLS)	No difference between maca treated group and placebo group in serum estradiol, FSH, SHBG, TSH (granted test used not sensitive enough), lipid profiles, glucose and serum cytokines. With GCS, Anxiety and depression subgroup score showed significant reduction relative to both baseline and placebo. Somatic symptoms were also decreased significantly by 27% relative to baseline. A significant positive placebo effect was present on the overall score. With SF-36 V2, no significant difference was observed between maca and placebo groups for mental and overall well-being. No difference in physical functioning, vitality, emotional well-being, body pain. With WHQ study, both placebo and maca showed and improvement in fear/anxiety. Placebo even improved sleeping patterns and somatic symptoms. GCS and WHQ showed a non-significant improvement in sexual function. UQLS study showed no statistical difference in any domains of occupational health, emotional health and sexual quality of life.	Maca did not show estrogenic effect in Chinese Hong Kong women, nor was there an effect on immunological function as tested through biomarkers and cytokines, BMI, blood glucose and triglycerides, HDL, LDL and cholesterol. The decreasei n diastolic blood pressure with maca treatment suggests a potential cardiovascular effect of maca. Placebo effect was e high. All the tests did not have the same level of sensitivity.	Stojanovska et al., 2015

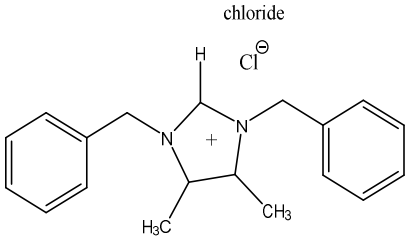
double-blind, placebo-controlled clinical trial	A Double-Blind Placebo-Controlled Trial of Maca Root as Treatment for Antidepressant-Induced Sexual Dysfunction in Women	42 female outpatients (aged 18-65; mean age of 41.5 ± 12.5 years) with SSRI/SNRI-induced sexual dysfunction with remitted depression (30 premenopausal and 12 post-menopausal); patients should have been taking SSRI, venlafaxine, or a tri/hetero cyclic antidepressant for the treatment of depression at a stable dose for at least 4 weeks.	1500mg maca root twice daily	placebo twice daily	Arizona Sexual Experience Scale (ASEX), Massachusetts General Hospital Sexual Function Questionnaire (MGH-SFQ), Clinical Global Impression-Severity (CGI-S) and Clinical Global Impression-Improvement (CGI-I) scales; For anxiety and depression, 28 item HAM-D, 14 item HAM-A and Keller's Symptoms Questionnaire; serum assay of estradiol, progesterone, prolactin and testosterone	ITT analysis. Remission rate for attainment of ASEX score < or= 8 and MGH-SFQ score < or = 12, were higher for the maca group than the placebo group. Higher remission rates were observed in the postmenopausal group with maca than placebo. Maca improved orgasm in post-menopausal women but not in premenopausal women where only arousal was improved. Change in testosterone from baseline to endpoint in placebo group did not correlate significantly but correlated significantly in maca group. (This indicates possible androgenic action of maca). Maca was well-tolerated overall but Liver Function Tests were not performed.	Authors postulate that changes in FSH and LH via negative feedback loop result in an increase production of androgens that could explain the improvement in sexual functioning. However, it is stated that the assays employed to detect testosterone levels in women may not have been sensitive enough and that self-reporting of orgasm is subjective. Maca has more positive effect in menopausal women than premenopausal women with AISD.	Dording et al., 2015

The Journey of Lepidium Meyenii (Maca)

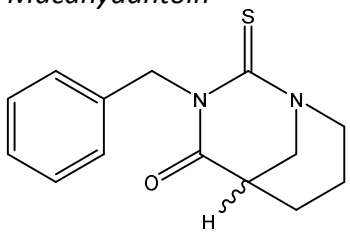


Phytochemistry

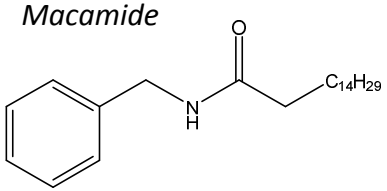
Lepidiline



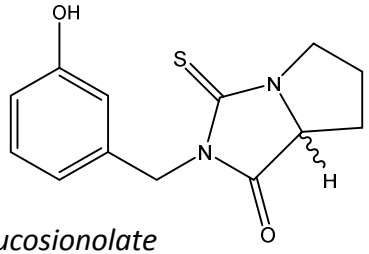
Macahydantoin



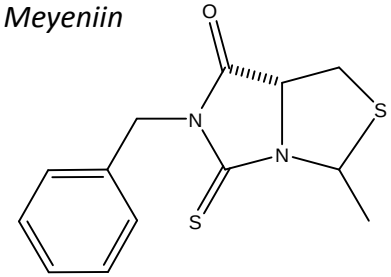
Macamide



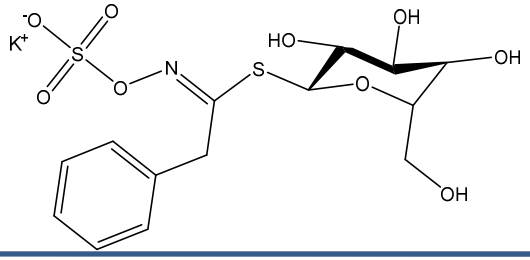
Macathiohydantoin



Meyeniin



Glucosionolate



Pharmacology – an evaluation of purported benefits

♂

Male Reproductive Health

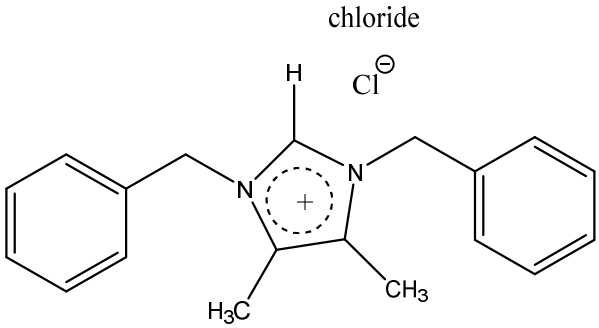
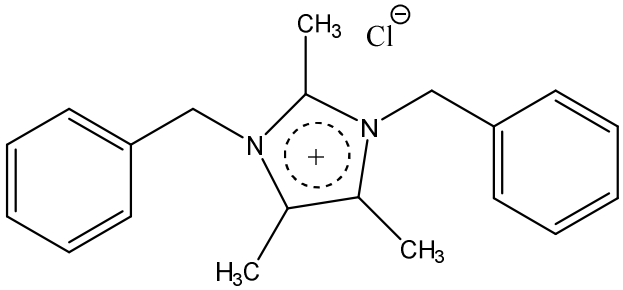
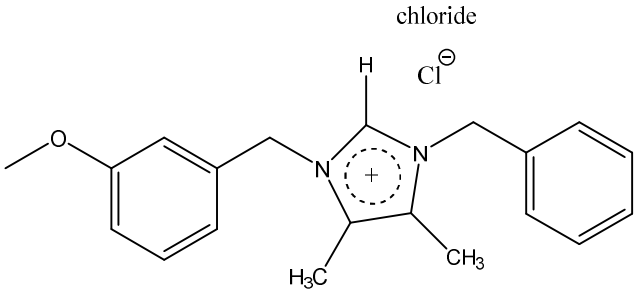
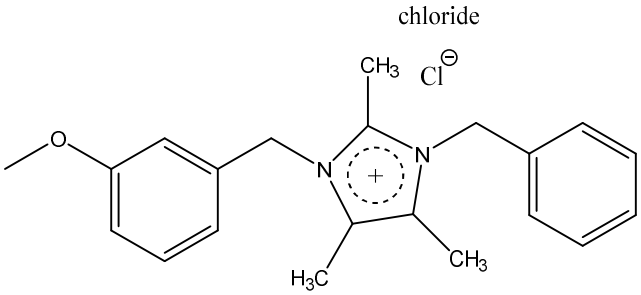
- Spermato-genesis
- Prostatic Hyperplasia
- Sexual Performanc e
- Erectile Dysfunction

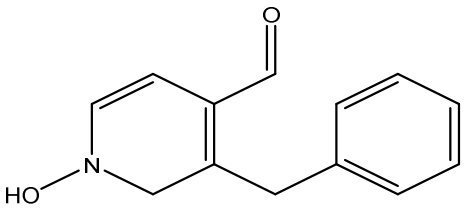
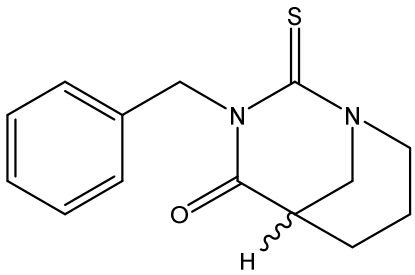
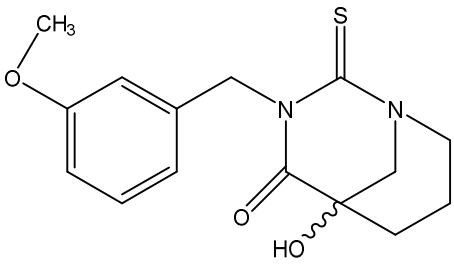
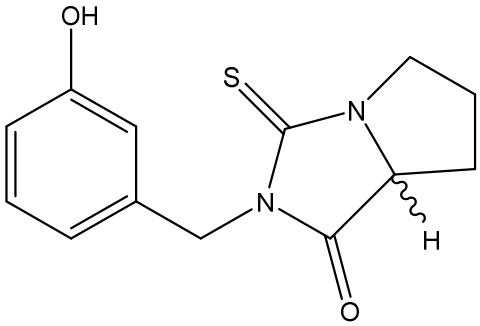
♀

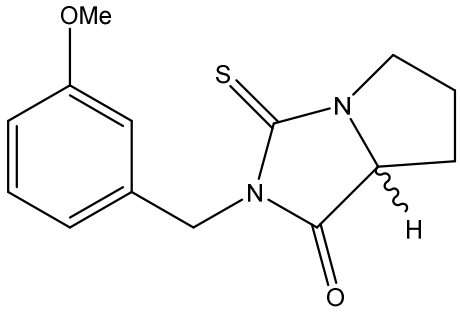
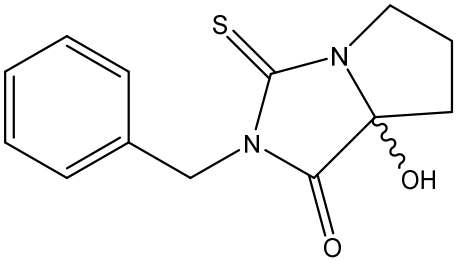
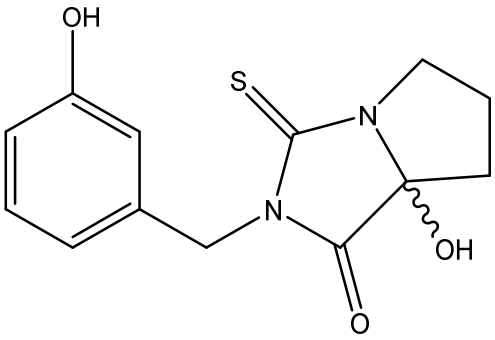
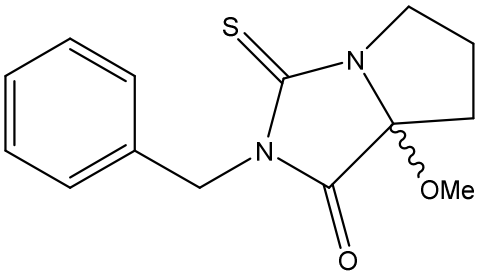
Female Reproductive Health

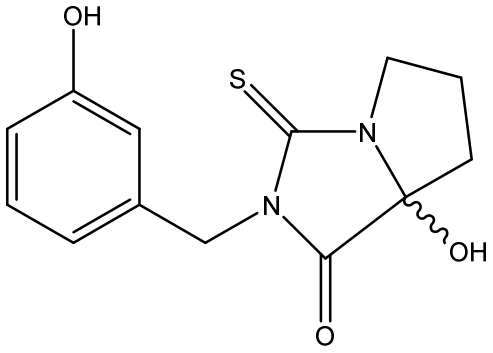
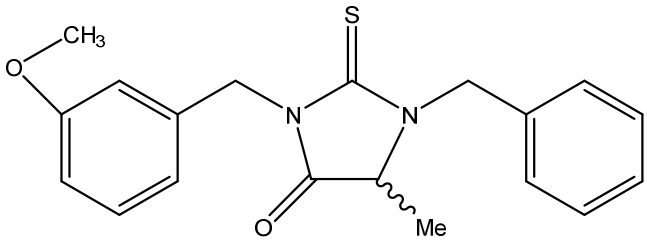
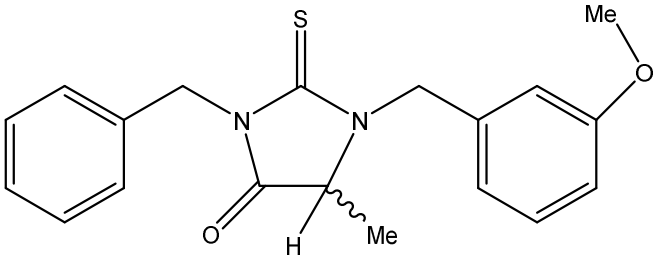
- Fertility
- Menopause
  - Hormone imbalanc e
  - Bone health

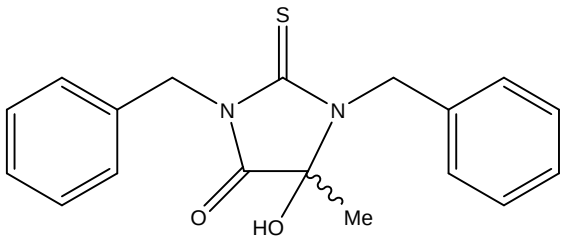
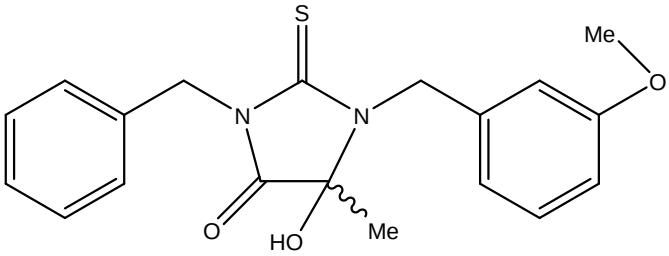
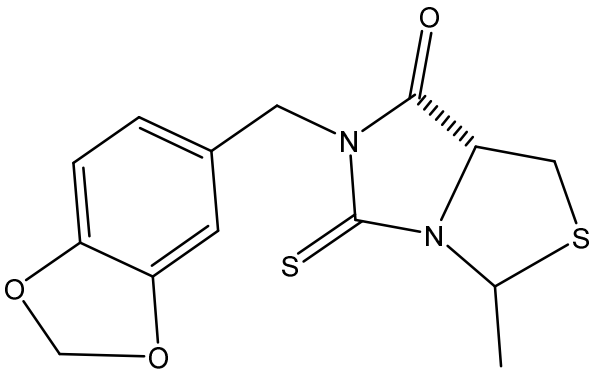
(Supplementary data) Appendix S1: Secondary metabolites found in Maca1

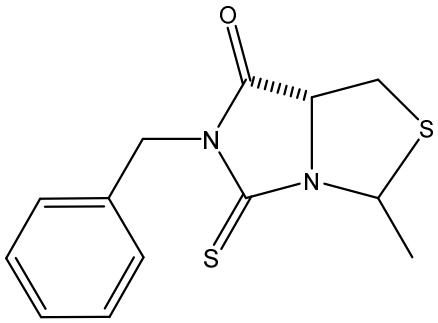
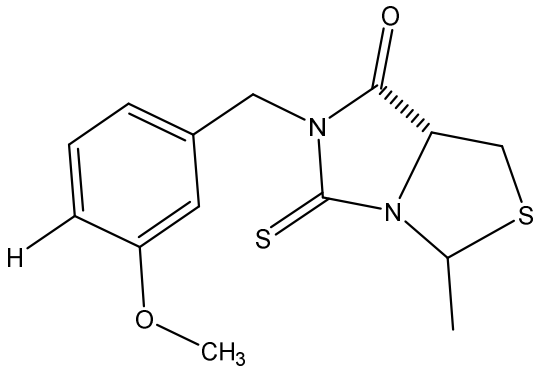
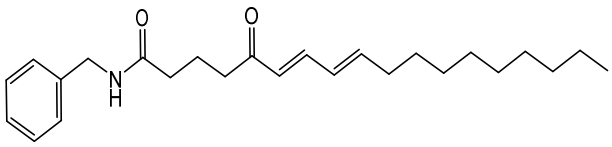
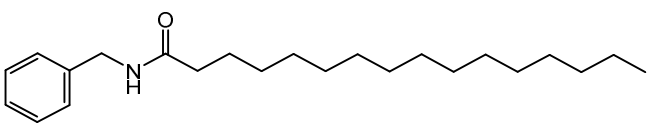
Num-ber	Compound	Structure	Source	Refer-ence
ALKALOIDS				
1	Lepidiline A.1,3-dibenzyl-4,5-dimethylimidazolium chloride		Concentrated lipid extract of maca root	Cui et al., 2003
2	Lepidiline B. 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride		Concentrated lipid extract of maca root	Cui et al., 2003
3	Lepidiline C. 1-benzyl-3-(3-methoxybenzyl)-4,5-dimethylimidazolium chloride		Ethanolic 95% of dried maca root extract	Jin et al.,2016
4	Lepidiline D. 1-benzyl-3-(3-methoxybenzyl)-2,4,5-trimethylimidazolium chloride		Ethanolic 95% dried maca root extract	Jin et al.,2016

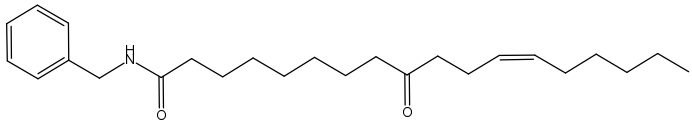
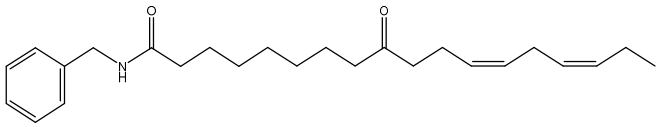
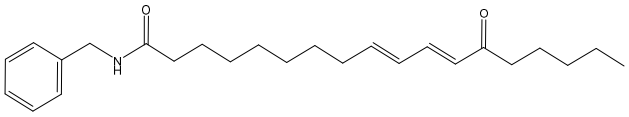
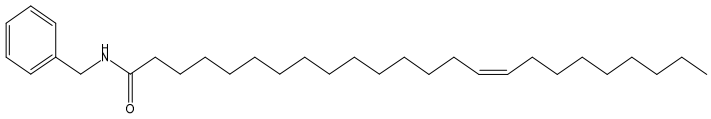
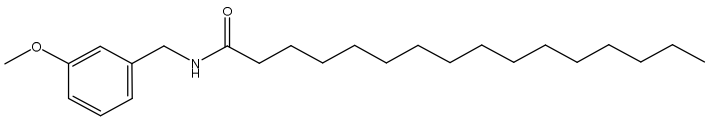
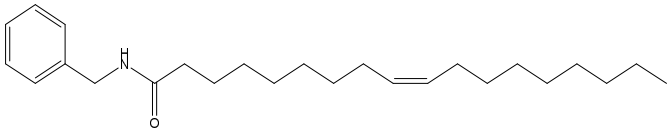
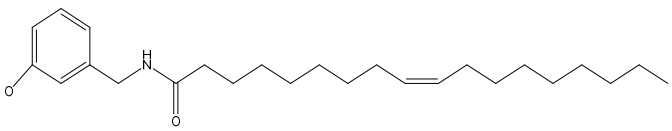
5	Macaridine: 3-Benzyl-1,2-dihydro-N-hydroxypyridine-4-carbaldehyde		petroleum ether extract of tubers of L.meyenii	Muhammad et al., 2002
MACAHYDANTOINS				
1	3-benzyl-2-thioxo-1,3-diazabicyclo[3.3.1]nonan-4-one (Macahydantoin A)		No mention of extract. L. meyenii from regions of Lijiang and Dali in China	Yu et al., 2017a
2	5-hydroxy-3-(3-methoxybenzyl)-2-thioxo-1,3-diazabicyclo[3.3.1]nonan-4-one (Macahydantoin B)		No mention of extract. *L. meyenii*from regions of Lijiang and Dali in China	Yu et al., 2017a
MACATHIOHYDANTOINS				
1	2-(3-hydroxybenzyl)-3-thioxohexahydro-1H-pyrrolo[1,2-c]imidazol-1-one (Macathiohydantoin B)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b

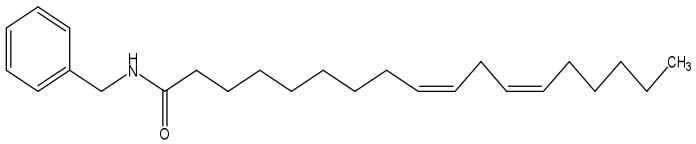
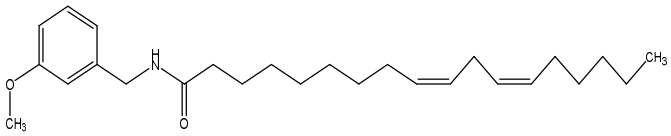
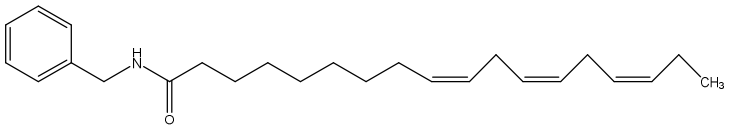
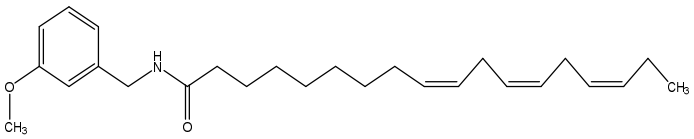
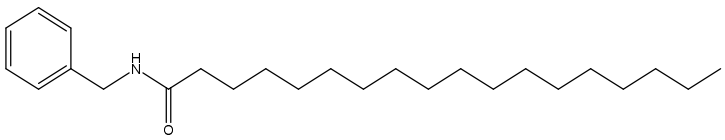
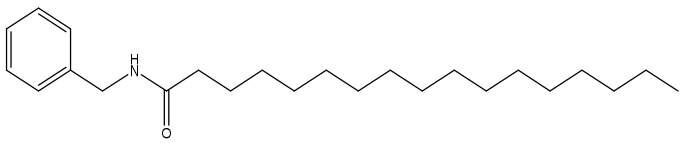
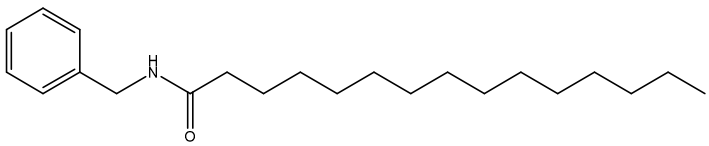
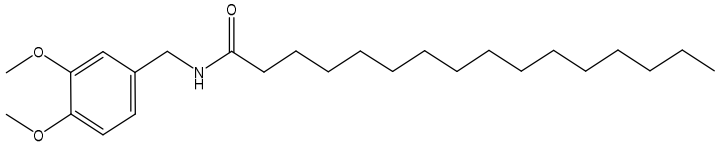
2	2-(3-methoxybenzyl)-3-thioxohexahydro-1H-pyrrolo[1,2-c]imidazol-1-one (Macathiohydantoin C)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b
3	2-benzyl-7a-hydroxy-3-thioxohexahydro-1H-pyrrolo[1,2-c]imidazol-1-one (Macathiohydantoin D)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b
4	7a-hydroxy-2-(3-hydroxybenzyl)-3-thioxohexahydro-1H-pyrrolo[1,2-c]imidazol-1-one (Macathiohydantoin E)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b
5	2-benzyl-7a-methoxy-3-thioxohexahydro-1H-pyrrolo[1,2-c]imidazol-1-one (Macathiohydantoin F)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem	Yu et al., 2017b

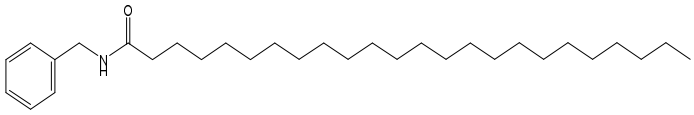
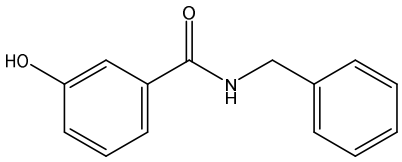
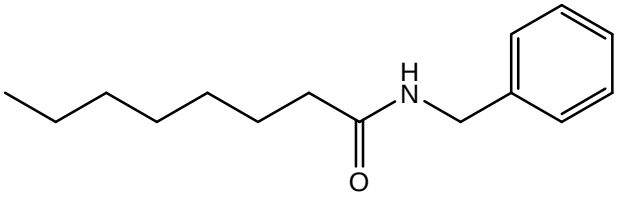
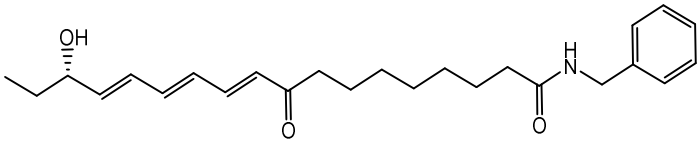
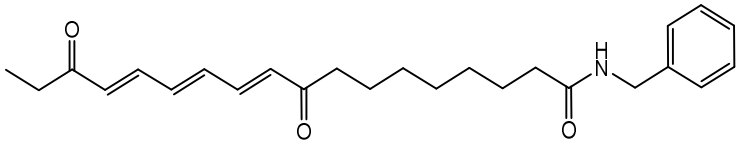
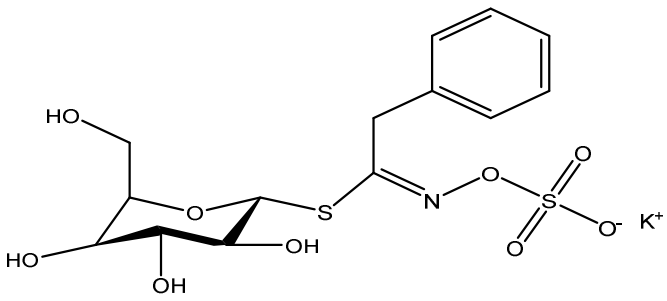
			extracted with 95% methanol	
6	7a-hydroxy-2-(3-hydroxybenzyl)-3-thioxohexahydro-1H-pyrrolo[1,2-c]imidazol-1-one (Macathiohydantoin G)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b
7	1-benzyl-3-(3-methoxybenzyl)-5-methyl-2-thioxoimidazolidin-4-one (Macathiohydantoin H)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b
8	3-benzyl-1-(3-methoxybenzyl)-5-methyl-2-thioxoimidazolidin-4-one (Macahtiohydantoin I)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b

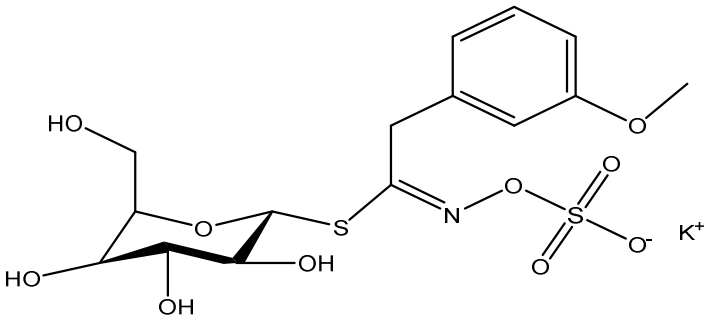
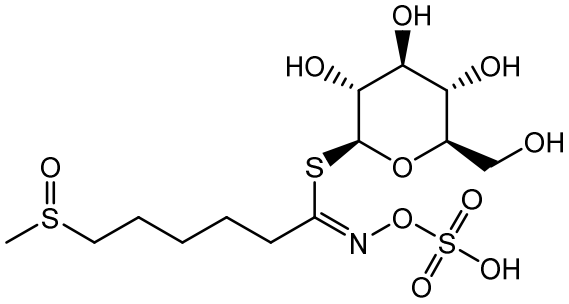
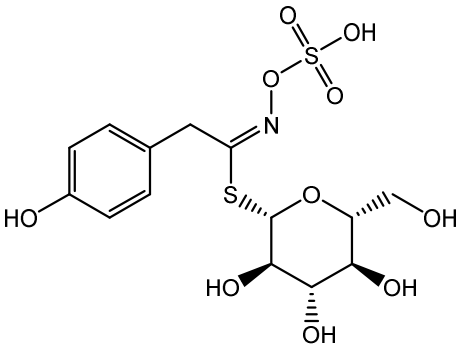
9	1,3-dibenzyl-5-hydroxy-5-methyl-2-thioxoimidazolidin-4-one (Macahydantoin J)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b
10	3-benzyl-5-hydroxy-1-(3-methoxybenzyl)-5-methyl-2-thioxoimidazolidin-4-one (Macathiohydantoin K)		Rhizomes of *L.meyenii*collected from Li Jiang of Yunnan Province of China in 2014. Extract: air-dried powdered maca stem extracted with 95% methanol	Yu et al., 2017b
MEYENIINS				
1	(7aR)-6-(benzo[d][1,3]dioxol-5-ylmethyl)-3-methyl-5-thioxotetrahydro-3H,7H-imidazo[1,5-c]thiazol-7-one (Meyeniin A)		*L. meyenii* were collected from Lijiang, Yunnan province, China, in September 2014. Extract: air-dried tubers extractedeat 80% aqueous acetone at room temperature	Zhou et al., 2017

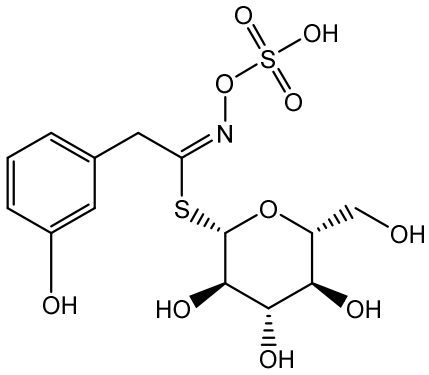
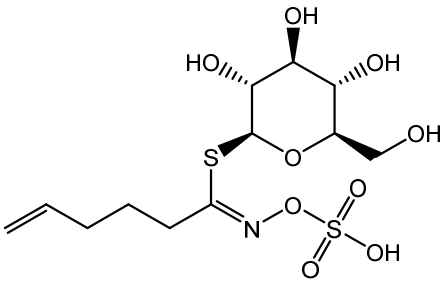
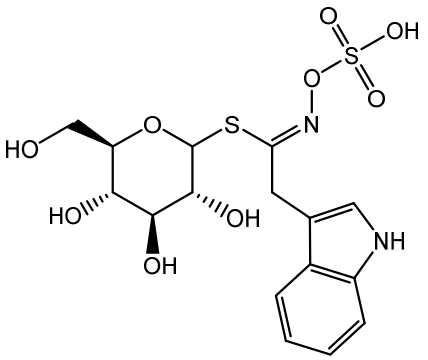
2	(7aR)-6-benzyl-3-methyl-5-thioxotetrahydro-3H,7H-imidazo[1,5-c]thiazol-7-one (Meyeniin B)		*L. meyenii* were collected from Lijiang, Yunnan province, China, in September 2014. Extract: air-dried tubers extractedeat 80% aqueous acetone at room temperature	Zhou et al., 2017
3	(7aR)-6-(3-methoxybenzyl)-3-methyl-5-thioxotetrahydro-3H,7H-imidazo[1,5-c]thiazol-7-one (meyeniin C)		*L. meyenii* were collected from Lijiang, Yunnan province, China, in September 2014. Extract: air-dried tubers extractedeat 80% aqueous acetone at room temperature	Zhou et al., 2017
MACA ALKAMIDES - MACAMIDES				
1	N-benzyl-5-oxo-6E,8E-octadecadienamide		petroleum ether extract of dried ground tubers of L.meyenii	Muhammad et al., 2002
2	N-benzylhexadaecanamide (N-benzyl palmitamide)		petroleum ether extract of tubers of L.meyenii	Muhammad et al., 2002; McCollom et al., 2005

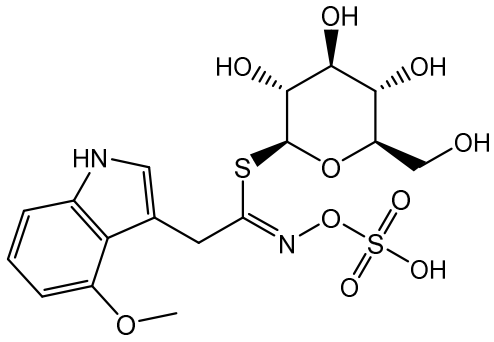
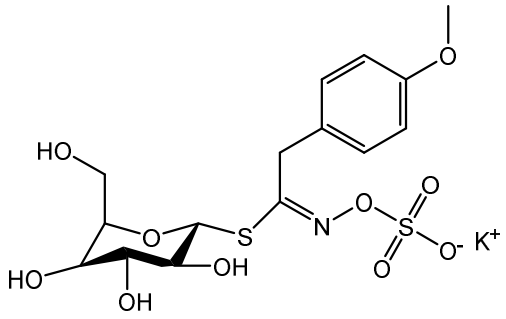
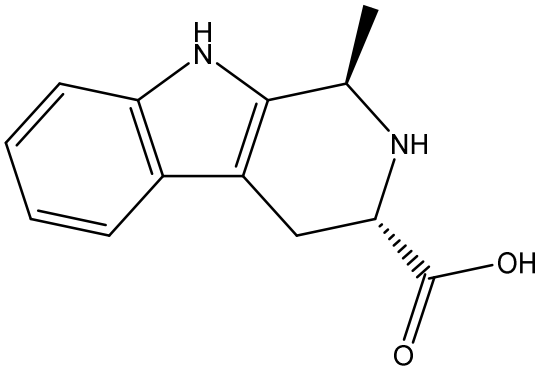
3	(6E,8E)-5-oxooctadeca-6,8-dienoic acid (acyclic keto acid)		petroleum ether extract of tubers of L.meyenii	Muhammad et al., 2002
4	N-benzyl-9-oxo-12Z-octadecenamide		95% EtOH extract of dried ground tuber	Zhao et al., 2005
5	N-benzyl-9-oxo-12Z,15Z-octadecadienamide		95% EtOH extract of dried ground tuber	Zhao et al., 2005
6	N-benzyl-13-oxo-9E,11E-octadecadienamide		95% EtOH extract of dried ground tuber	Zhao et al., 2005
7	N-benzyl-15Z-tetracosenamide [n-benzyllnervonamide]		95% EtOH extract of dried ground tuber	Zhao et al., 2005
8	N-(m-methoxybenzyl)hexadecanamide		95% EtOH extract of dried ground tuber	Zhao et al., 2005
9	N-benzyloleamide		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005
10	N-(3-methoxybenzyl)oleamide		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005

11	(9Z,12Z)-N-benzyloctadeca-9,12-dienamide [n-benzyllinoleamide]		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005
12	(9Z,12Z)-N-(3-methoxybenzyl)octadeca-9,12-dienamide		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005
13	(9Z,12Z,15Z)-N-benzyloctadeca-9,12,15-trienamide [n-benzyllinolenamide]		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005
14	(9Z,12Z,15Z)-N-(3-methoxybenzyl)octadeca-9,12,15-trienamide		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005
15	N-benzylstearamide		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005
16	N-benzylheptadecanamide		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005
17	N-benzylpentadecanamide		petroleum ether extract of dried ground tubers of L.meyenii	McCollom et al., 2005
18	N-(3,4-dimethoxybenzyl)-hexadecanamide		hexane extract of dried ground tubers of L.Meyenii	Chain et al., 2014

19	N-benzyltetracosanamide		hexane extract of dried ground tubers of L.Meyenii	Chain et al., 2014
20	N-benzyl-3-hydroxy-benzeneamide		waterextrract of L.Meyenii roots cultivated in Jilin, China	Zheng et al., 2014
21	N-benzyl octanamide		extraction from L.meyenii root using aqueous solvent comprising of 90% by vol. or more water	Zheng et al., 2003 US Patent. US 6,552,206 B1
22	N-benZyl-16(R,S)-hydroXy-9-oXo-10E,12E,14E-octadecatrieneamide		extraction from L.meyenii root using aqueous solvent comprising of 90% by vol. or more water	Zheng et al., 2003 US Patent. US 6,552,206 B2
23	N-benzyl-9,16-dioxo-10E,12E,14E-octadecatrieneamide		extraction from L.meyenii root using aqueous solvent comprising of 90% by vol. or more water	Zheng et al., 2003 US Patent. US 6,552,206 B3
Glucosinolates				
1	benzylglucosinolate		Methanol extract of dry maca tubers; 70% methanol extract of fresh hypocotyl, fresh leaf, fresh seed, sprout, dry hypocotyl, flour,	Dini et al., 2002, Li et al., 2001; Piacente et al., 2002

			capsule, pill and mayonnaise.	
2	m-methoxybenzyl-glucosinolate (m-methoxy-glucotropaeolin)		Methanol extract of dry maca tubers	Dini et al., 2002; Piacent eelat., 2002)
3	5-methylsulfinylpentyl glucosinolate (glucoalyssin)		70% methanol extract of fresh maca hypocotyl, leaf, seed, sprout, dry maca hypocotyl, flour, capsule, pill and mayonnaise	Li et al., 2001
4	p-hydroxybenzylglucosinolate (glucosinalbin)		70% methanol extract of fresh maca hypocotyl, leaf, seed, sprout, dry maca hypocotyl, flour, capsule, pill and mayonnaise	Li et al., 2001

5	m-hydroxybenzylglucosinolate		70% methanol extract of fresh maca hypocotyl, leaf, seed	Li et al., 2001
6	4-pentenyl glucosinolate (glucobrassicanapin)		70% methanol extract of fresh maca hypocotyl and leaf	Li et al., 2001
7	Indolyl-3-methyl glucosinolate (glucobrassicin)		70% methanol extract of fresh maca hypocotyl, fresh leaf and dry hypocotyl	Li et al., 2001

8	4-methoxyindolyl-3-methyl glucosinolate (4-methoxyglucobrassicin)		70% methanol extract of fresh hypocot, fresh leaf, seed and capsule	Li et al., 2001
9	p-methoxybenzylglucosinolate (glucolimnanthin)		70% methanol extract of fresh hypocotyl, fresh leaf, sprout, dry hypocotyl, flour, capsule, pill, mayonnaise	Li et al., 2001
Other compounds				
1	Tetrahydromethyl-b-carboline		Methanol extract of dry maca tubers	Piancente et al., 2002