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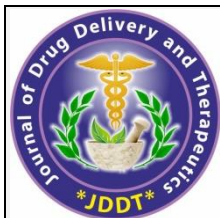
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Phytochemical profile of *Azadirachta indica* A. Juss. and *Melia azedarach* Linn.

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ABSTRACT

The seasonal variation of phenol, tannin and ascorbic acid content has been investigated from leaves, stem and bark of *Azadirachta indica* and *Melia azedarach*. Comparative account of phenol contents of *Azadirachta indica* showed higher level in leaves (range 3.273 to 3.476 mg/g dry wt.) than stem (range 2.855 to 3.022 mg/g dry wt.) and bark (range 1.370 to 1.596 mg/g dry wt.). Comparative account of tannin contents of *Azadirachta indica* higher level in leaves (range 1.645 to 1.815 mg/g dry wt.) than stem and bark. Comparative account of ascorbic acid contents of *Azadirachta indica* showed higher level in leaves (range 1.152 to 1.374 mg/g dry wt.) than stem (range 0.756 to 0.953 mg/g dry wt.) and bark (range 0.542 to 0.725 mg/g dry wt.). Comparative account of phenol contents of *Melia azedarach* showed higher level in leaves (range 2.615 to 2.859 mg/g dry wt.) than stem (range 2.145 to 2.306 mg/g dry wt.) and bark (range 1.737 to 1.897 mg/g dry wt.). Comparative account of tannin contents of *Melia azedarach* higher level in leaves (range 2.121 to 2.316 mg/g dry wt.) than stem and bark. Comparative account of ascorbic acid contents of *Melia azedarach* showed higher level in leaves (range 1.581 to 1.712 mg/g dry wt.) than stem (range 1.130 to 1.268 mg/g dry wt.) and bark (range 0.734 to 0.840 mg/g dry wt.).

Keywords: Phenol, tannin, ascorbic acid, *Azadirachta indica* and *Melia azedarach*

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INTRODUCTION

Since the ancient times, nature has been a huge source of medicinal agents. All over the world, plants have served as the richest source of raw materials for traditional as well as modern medicine^[1,2]. The medicinal value of plants is mainly due to the presence of some chemical substances known as photochemical. Secondary metabolites are organic compounds produced by bacteria, fungi, or plants which are not directly involved in the normal growth, development, or reproduction of the organism. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. Secondary metabolites often play an important role in plant defense against herbivores and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings, pigments, and recreational drugs^[3]. They are basically plant

metabolites, are synthesized in all parts of plant body itself and have some definite physiological action on animals^[4].

Azadirachta indica (Neem) is an evergreen tree, cultivated in various parts of subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various ailments from antiquity. Neem has been extensively used as Ayurveda, Urani and Homeopathic medicine. The Neem Tree is an incredible plant that has been declared the Tree of the 21st century by the United Nations^[5]. Extracts from the Neem tree (*A.indica*) are most consistently recommended in ancient medical texts for gastrointestinal upsets, diarrhoea and intestinal infections, skin ulcers and malaria^[6]. Its leave can be used as drug for diabetes, eczema and reduce fever. Barks of Neem can be used to make toothbrush and the roots has an ability to heal diseases and against insects. The seed of Neem tree has a high concentration of oil. Neem oil is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis^[5,7,8]. The Chemical constituents contain many biologically active compounds that can be extracted from

neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, Carotenoids, steroids and ketones. Azadirachtin is actually a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective^[9]. Other compounds that have a biological activity are salannin, volatile oils, meliantriol and nimbin^[10].

The plant (*Melia azedarach* Linn) is traditionally used for the treatment of leprosy, inflammations, and cardiac disorders. Its fruits extracts possess ovicidal^[11] and larvicidal activity^[12]. The leaf extracts also possess antiviral^[13] and antifertility activity^[14] of *Melia azedarach* L. In Ayurveda *Melia azedarach* Linn. has been well documented for its therapeutic potentials. It has been used in Ayurvedic medicine for more than 4000 years due to its medicinal and a wide range of economic properties. This plant has long been recognized as an insecticidal and medicinal plant all over the world^[15,16]. *Melia azedarach* L., a close relative of neem, from the family Meliaceae which occurs in India and other tropical and subtropical countries. It contains a chemical constituent which makes it a candidate in pest control. It has been reported to possess antimicrobial, insecticidal and nematocidal properties. It is also known for its antioxidant, antiviral, antiparasitic, anti lithiasis, analgesic, hematological, antimalarial, anti-inflammatory and anti-fertility activity.

MATERIALS AND METHODS

The leaves, stem and bark of *Azadirachta indica* and *Melia azedarach*, were collected during different season's viz. summer, monsoon and winter continuous two years for estimation of phenol, tannin and ascorbic acid.

1) Total Phenols - The concentration of total phenols in the plant extract was determined by using Folin method^[17]. Catechol was used as standard. 0.2 ml ethanolic (80%) extract (4 mg/ml) of plants and 0.2 ml Folin reagent were mixed thoroughly. After 4 min, 1 ml of 15 % sodium carbonate was added and the mixture was allowed to stand for 2 hours at room temperature. The absorbance was measured at 760 nm. The concentration of total phenols was measured equivalent to catechol (as a standard drug) by using standard calibration curve of catechol.

2) Total Tannin - Total tannin in plant extract was determined by Folin-Denis method^[18]. 0.5 g of powdered drug was boiled for 30 min with 75 ml of double distilled water. It was cooled, centrifuged at 2000 rpm for 20 min and supernatant was collected in 100 ml volumetric flask and the volume was made up with double distilled water. 1 ml of this solution was transferred to a 100 ml volumetric flask containing 75 ml water and 5 ml of Folin-Denis reagent + 10 ml of sodium carbonate solution were added and diluted up to 100 ml with water. After shaking, the absorbance was read at 700 nm after 30 min. Blank solution was prepared with water instead of the sample. Standard graph was prepared by using 0-100 µg of tannic acid. Total tannin content of the sample was measured equivalent to tannic acid by standard graph.

3) Ascorbic Acid - Total ascorbic acid content in plant extract was determined by Sadasivam method^[19]. 2 g dried powdered sample was extracted with 4 % oxalic acid and the volume was made up to 100 ml. It was centrifuged at 1000 rpm for 10 min. 5 ml supernatant liquid was transferred to a conical flask and 10 ml of 4 % oxalic acid was added. It was titrated against standard dye solution (2, 6-dichlorophenolindophenol) to a pink end point. The

procedure was repeated with a blank solution (without adding sample). 5 ml ascorbic acid of 100 ppm was used as standard. Ascorbic acid content was calculated using the formula.

RESULTS AND DISCUSSION

The phenol content of leaves of *Azadirachta indica* was higher (3.476 mg/g dry wt.) in summer over than winter (3.408 mg/g dry wt.) and monsoon (3.273 mg/g dry wt.). The range of phenol content of stem was from (2.855mg/g dry wt. to 3.022 mg/g dry wt.). The range of phenol content in bark was from 1.370 mg/g dry wt. to 1.596 mg/g dry wt. and show higher in summer. The phenol content of bark of *Azadirachta indica* was very low in all season. The phenol content showed increasing order of bark < stem < leaves (Table No. 1 and Graph No.1). The phenol content of leaves of *Melia azedarach* was higher (2.859 mg/g dry wt.) in summer over than winter (2.671 mg/g dry wt.) and monsoon (2.615 mg/g dry wt.). The range of phenol content of stem was from (2.145 mg/g dry wt. to 2.306 mg/g dry wt.). The range of phenol content in bark was from 1.737 mg/g dry wt. to 1.897 mg/g dry wt. and show higher in summer. The phenol content of bark of *Melia azedarach* was very low in all season. The phenol content showed increasing order of bark < stem < leaves (Table No. 2 and Graph No.2).

The Tannin content of leaves *Azadirachta indica* of was 1.815 mg/g dry wt. in summer, 1.750 mg/g dry wt. in winter and 1.645 mg/g dry wt. in monsoon ,higher being observed during summer i.e. (1.815 mg/g dry wt.). The range of tannin content in stem of *Azadirachta indica* (1.190 mg/g dry wt. to 1.312 mg/g dry wt.). Maximum concentration of tannin was noted during summer (1.312 mg/g dry wt.). The range of tannin content of bark was low (range 0.907 to 1.128 mg/g dry wt.). Generally, the concentration of tannin of *Azadirachta indica* were found to be in increasing order of bark < stem < leaves (Table No. 1 and Graph No.1). The Tannin content of leaves *Melia azedarach* of was 2.316 mg/g dry wt. in summer, 2.224 mg/g dry wt. in winter and 2.121 mg/g dry wt. in monsoon ,higher being observed during summer i.e. (2.121 mg/g dry wt.). The range of tannin content in stem (1.546 mg/g dry wt. to 1.760 mg/g dry wt.). Maximum concentration of tannin was noted during summer (1.760 mg/g dry wt.). The range of tannin content of bark of *Melia azedarach* was low from (1.158 to 1.381 mg/g dry wt.). Generally, the concentration of tannin were found to be in increasing order of bark < stem < leaves (Table No. 2 and Graph No.2).

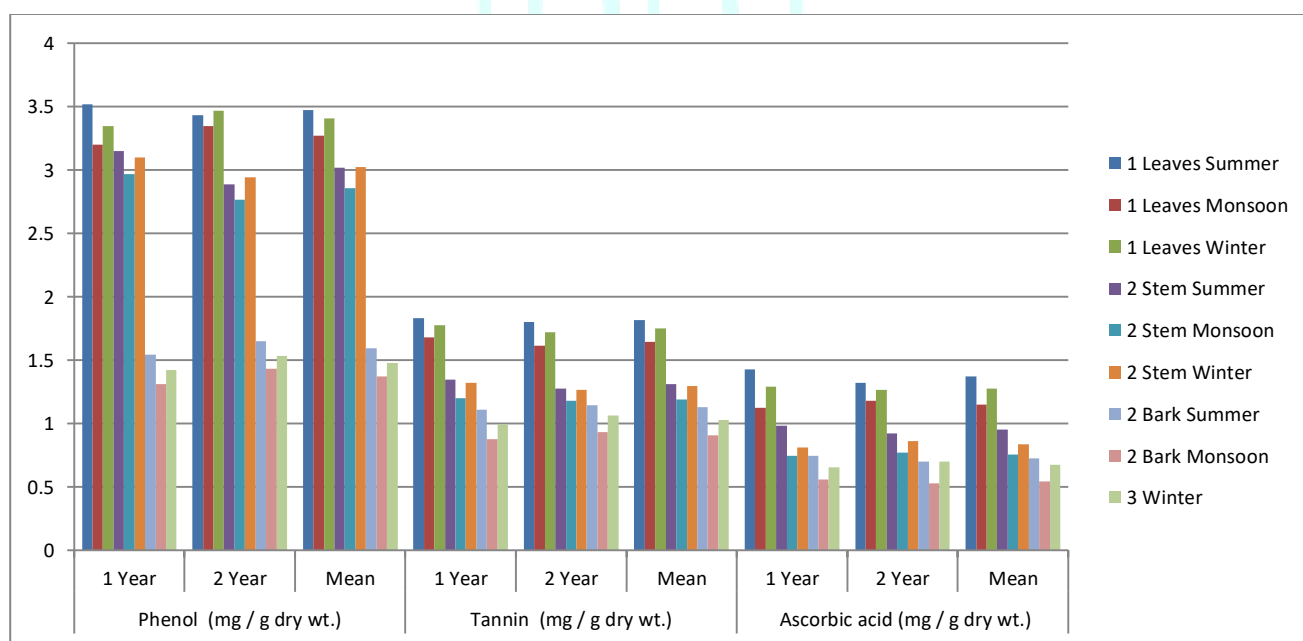
The ascorbic acid concentration of leaves of *Azadirachta indica* was higher in summer (1.374 mg/gm dry wt.) over that of monsoon (1.152 mg/gm dry wt.) and winter seasons (1.277 mg/gm dry wt.). The stem of ascorbic acid concentration was ranging from (0.756 mg/gm dry wt. to 0.953 mg/gm dry wt.) and significantly higher in summer (0.953 mg/gm dry wt.). The ascorbic acid content of bark of *Azadirachta indica* was comparatively low (0.542 mg/gm to 0.725 mg/gm) (Table No. 1 and Graph No.1). The ascorbic acid concentration of leaves was higher in summer (1.712 mg/gm dry wt.) over that of monsoon (1.581 mg/gm dry wt.) and winter seasons (1.634 mg/gm dry wt.). The stem of ascorbic acid concentration was ranging from (1.130 mg/gm dry wt. to 1.268 mg/gm dry wt.) and significantly higher in summer (1.268 mg/gm dry wt.). The ascorbic acid content of bark was comparatively low (0.734 mg/gm to 0.840 mg/gm) (Table No. 2 and Graph No.2).

Table No. 1 Seasonal variation of phenol, tannin and ascorbic acid levels of different plant parts of *Azadirachta indica*

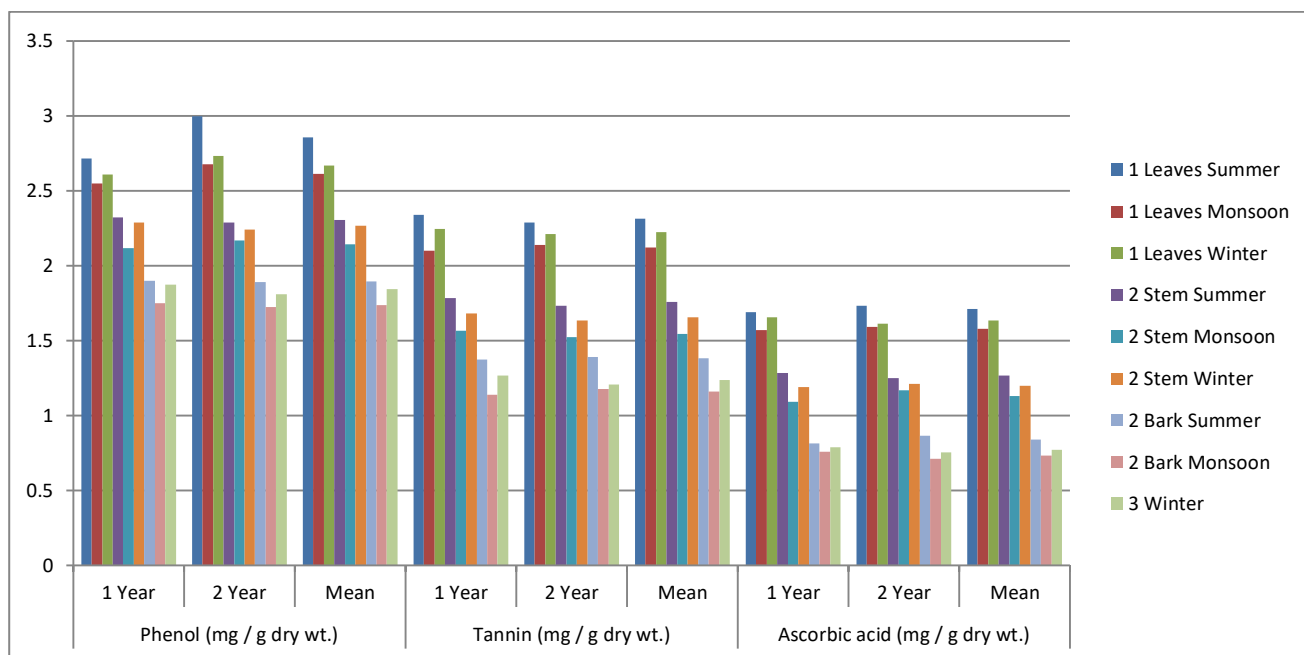
Sr. No.	Plant Parts	Season	Phenol (mg / g dry wt.)			Tannin (mg / g dry wt.)			Ascorbic acid (mg/g dry wt.)		
			1 Year	2 Year	Mean	1 Year	2 Year	Mean	1 Year	2 Year	Mean
1	Leaves	Summer	3.519	3.432	3.476	1.830	1.800	1.815	1.426	1.322	1.374
		Monsoon	3.200	3.346	3.273	1.678	1.612	1.645	1.125	1.178	1.152
		Winter	3.348	3.467	3.408	1.777	1.722	1.750	1.289	1.265	1.277
2	Stem	Summer	3.150	2.890	3.020	1.345	1.278	1.312	0.983	0.922	0.953
		Monsoon	2.966	2.764	2.855	1.201	1.179	1.190	0.744	0.769	0.756
		Winter	3.100	2.943	3.022	1.321	1.267	1.294	0.811	0.863	0.837
3	Bark	Summer	1.542	1.649	1.596	1.109	1.146	1.128	0.747	0.702	0.725
		Monsoon	1.309	1.431	1.370	0.879	0.935	0.907	0.557	0.526	0.542
		Winter	1.421	1.532	1.476	0.995	1.065	1.030	0.653	0.699	0.676

Table No. 1 Seasonal variation of phenol, tannin and ascorbic acid levels of different plant parts of *Melia azedarach*

Sr. No.	Plant Parts	Season	Phenol (mg / g dry wt.)			Tannin (mg / g dry wt.)			Ascorbic acid (mg/g dry wt.)		
			1 Year	2 Year	Mean	1 Year	2 Year	Mean	1 Year	2 Year	Mean
1	Leaves	Summer	2.718	2.999	2.859	2.342	2.289	2.316	1.689	1.734	1.712
		Monsoon	2.552	2.678	2.615	2.100	2.141	2.121	1.569	1.592	1.581
		Winter	2.610	2.732	2.671	2.247	2.211	2.224	1.655	1.612	1.634
2	Stem	Summer	2.323	2.289	2.306	1.786	1.734	1.760	1.286	1.250	1.268
		Monsoon	2.119	2.170	2.145	1.568	1.523	1.546	1.093	1.167	1.130
		Winter	2.290	2.242	2.266	1.680	1.636	1.658	1.189	1.211	1.200
3	Bark	Summer	1.900	1.893	1.897	1.372	1.390	1.381	0.812	0.867	0.840
		Monsoon	1.750	1.723	1.737	1.137	1.178	1.158	0.756	0.711	0.734
		Winter	1.875	1.811	1.843	1.267	1.209	1.238	0.789	0.754	0.772



Graph No. 1- Seasonal variation of phenol, tannin and ascorbic acid levels of different plant parts of *Azadirachta indica*



Graph No. 1- Seasonal variation of phenol, tannin and ascorbic acid levels of different plant parts of *Melia azedarach*

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