Plant Archives Volume 20 No. 1, 2020 pp. 2505-2508 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STUDIES ON PHYTOCHEMICAL CONSTITUENTS OF *AZADIRACHTA INDICA A. JUSS.* AND *MELIA AZEDARACH LINN.*

Vilas C. Bhagwat[1] and Vasant B. Kadam[2]*

[1]Department of Botany, Swami Muktanand College of Science, Yeola, Nashik (M.S.), India.
[2]*P.G. Department of Botany and Research Centre, K. R. T. Arts,
B.H. Comm. and A.M. Science (K.T.H.M.) College, Nashik- 2 (M.S.), India.

Abstract

The aim of the study was to investigate the seasonal variations of phytochemical compounds (proteins and amino acids) in the leaves, stem and bark of *Azadirachta indica* and *Melia azedarach.* Comparative account of protein contents of leaves, stem and bark of *Azadirachta indica* showed higher (range1.563 to 3.878 mg/g dry wt.) than *Melia azedarach* (range 1.880 to 3.326 mg/g dry wt.). Comparative account of amino acid contents of leaves, stem and bark of *Azadirachta indica* showed higher (range 0.856 to 2.611 mg/g dry wt.) than *Melia azedarach* (range 1.231 to 2.496 mg/g dry wt.).

Key words: Proteins, amino acids, *Azadirachta indica* and *Melia azedarach.*

Introduction

The world is rich with natural and unique medicinal plants. Medicinal plants are now getting more attention than ever because they have potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacological. The medicinal value of these plants lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al.,* 2007). Some of the most important bioactive phytochemical constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, phenolic compounds and many more (Edeoga *et al.,* 2005). These natural compounds formed the foundations of modern prescription drugs as we know today (Goh *et al.,* 1995). Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common su- gars, amino acids, proteins and chlorophyll while seconddary constituents consists of alkaloids, terpenoids and phenolic compounds (Krisahnaiah *et al.,* 2007).

The *Azadirachta indica* (Neem) Tree is an incredible plant that has been declared the Tree of the 21st century by the United Nations (Puri, 1999). In India, it is commonly known as 'Divine Tree', 'Life giving tree', 'Nature's Drugstore', 'Village Pharmacy' and 'Panacea for all diseases' (Shoforowa, 1993). Neem has extensive utilization in Ayurveda, Unani and Homeopathic medicine (Kausik et al, 2002 and Girish & Shankara, 2008). The Chemical constituents of Neem contain many bioactive compounds including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Azadirachtin is a mixture of seven isomeric compounds (Verkerk & Wright, 1993). To clean wounds, soothes, swellings and erases skin problems, boiled Neem is used. Neem leaves have been demonstrated to have vast properties like as immunomodulatory, antiinflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic (Hoque et al, 2007).

Melia azedarach L. (Family: Meliaceae) is a deciduous tree that is native to northeastern India. It has several common names such as, White cedar, Persian lilac, Tulip cedar and Chinaberry. The exuded gum

***Author for correspondence :** E-mail: drvbkadam@yahoo.com

obtained from *Melia azedarach* trunk is considered useful in spleen enlargement, wood extract given in asthma (Dhiman, 2003). Decoction of bark is prescribed in paroxysmal fever to relive thirst, nausea, vomiting and general debility, and loss of appetite and skin disease (Dhiman, 2003). Poultice of the leaves are applied to relieve nerve headache and to cure eruption on the scalp. Leaf juice is act as anthelmintic, diuretic, emmenagouge, expectorant, vermifuge and their decoction is astringent, stomachic, used in hysteria, leprosy, scrofula (Dhiman, 2003; Sharma *et al.*, 2001). Flowers have astringent, anodyne, refrigerant, emmenagouge, diuretic, resolvent, deobsturent properties (Warrier *et al.*, 1995). Fruits are considered anthelmintic, diuretic, emollient and purgative also, prescribed internally in indigestion, colic and intestinal catarrah (Rani *et al.*, 1999) Seeds are considered anthelmintic, expectorant, aphrodisiac and are useful in typhoid fever, helminthiasis, pain in the pelvic region and scrofula, also prescribed in rheumatism. Seed oil is used in skin diseases. Roots are astringent, emmenagouge, anodyne, febrifuge, expectorant, constipating. These are useful in sciatica, lumbago, piles, cough, asthma, ulcers, wounds, diabetes, intermittent fever, post labor pain in uterus, amenorrhea and in leuoderma (Warrier *et al.*, 1995).

Thus, as the experimental plant species possess immense medicinal properties, therefore the aim of the present study is to estimate the biochemical compounds of *Azadirachta indica* and *Melia azedarach.*

Materials and Methods

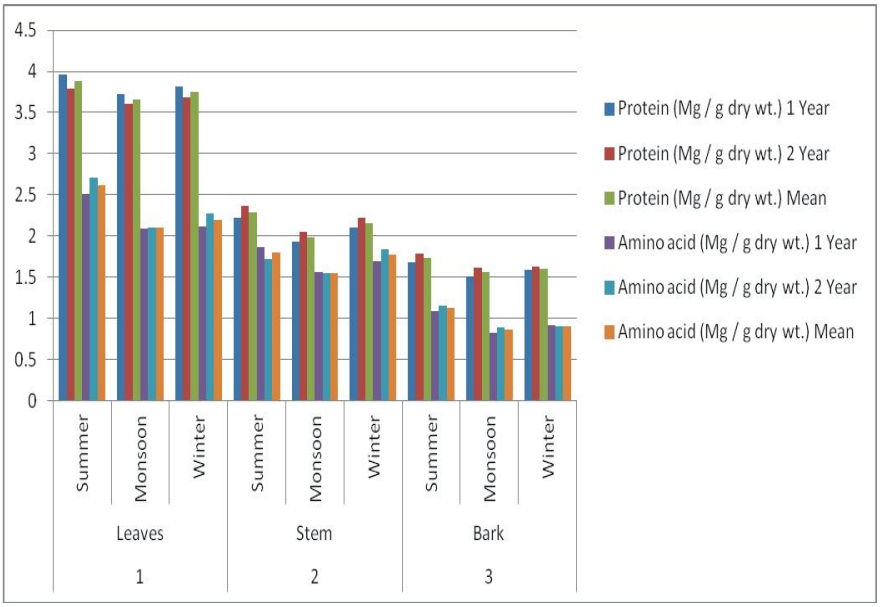
1) The protein was quantitatively estimated by the Lowey *et. al.* (1951) method.Chemicals: 0.1% N NaOH - (4gm in 1000ml), 2% Na_2CO_3 - (2gm in 100ml distilled water), 0.5% $CuSO_4$- (0.5gm in 100ml distilled water), 1 % Na-K-tartarate and 5 % Trichloro acetic acid/per chloric acid.

Reagents: Lowry A-2% Na_2CO_3in 0.1% N NaOH, Lowry B-5% CuSO4 in 1% Na-K-tartarate and Lowry C-98ml A and 2 ml B, Lowry D - Folin phenol reagent.

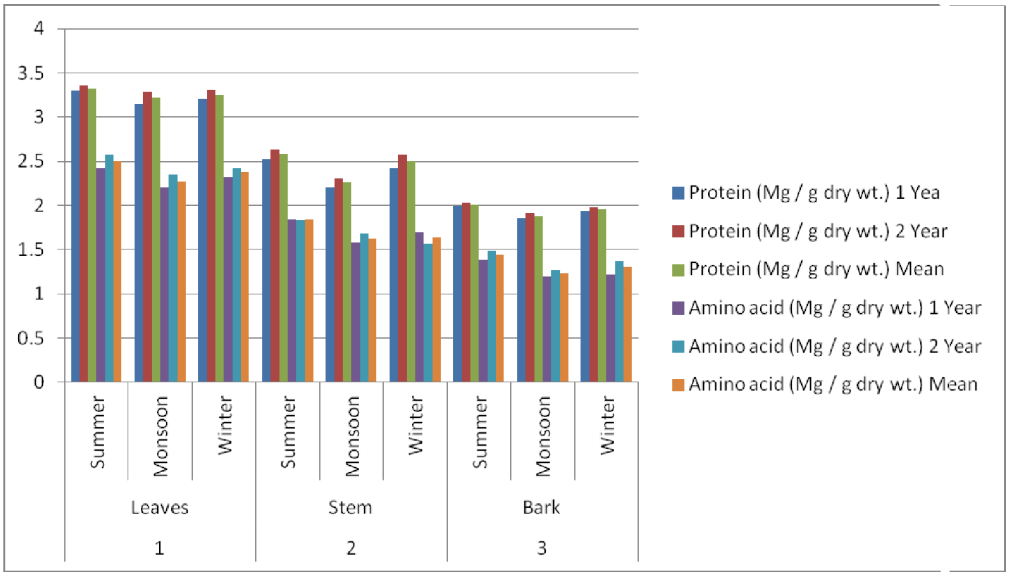
Procedure: 1gm of plant material was homogenized with 10ml, 80% ethanol. The extract was centrifuged at 5000 rpm. for 5 min. and the supernatant was discarded. 5%, 10 ml Trichloro acetic acid (TCA) or Per chloric acid (PCA) was add to residue and incubated at 80^0C for 20 minutes. The pellet was centrifuged and the supernatant was discarded. Residue was washed with 10 ml distilled water and again recentrifuged. The supernatant was discarded. 2%, 10ml Na_2CO_3 in 0.1 N NaOH was added to the residue and incubated for an hour at 30 °C and again centrifuged and residue was discarded. The final volume of supernatant was measured and it was used as a sample for protein. 1ml of aliquot of sample was taken and 5ml reagent C was added to it mixed it thoroughly. The sample was incubated for 10 minutes and 1ml of reagent D was added to it. The colour intensity was read at 660 nm. using Spectrophotometer. The protein concentration of an unknown sample was calculated byusing standard graph.

2) The estimation of total amino acid was carried out by Krishnamurthy *et al.,* (1989) method.

Reagents: Alcoholic ninhydrin. (100 ml alcohol + 400 mg ninhydrin) and Glycine (Std.) (10mg glycine +



Graph 1: Seasonal variation of proteins and amino acid content of different plant parts of Azadirachta indica A. Juss.



Graph 2: Seasonal variation of proteins and amino acid content of different plant parts of Melia azedarach Linn.

Table 1: Seasonal variation of proteins and amino acid content of different plant parts of Azadirachta indica A. Juss.

Sr.No.	Plant part	Season	Protein (Mg / g dry wt.)			Amino acid (Mg / g dry wt.)		
			1 Year	2 Year	Mean	1 Year	2 Year	Mean
1	Leaves	Summer	3.966	3.789	3.878	2.511	2.710	2.611
		Monsoon	3.720	3.599	3.660	2.095	2.103	2.099
		Winter	3.812	3.685	3.749	2.109	2.278	2.194
2	Stem	Summer	2.216	2.367	2.292	1.865	1.722	1.794
		Monsoon	1.924	2.052	1.988	1.556	1.546	1.551
		Winter	2.100	2.219	2.160	1.698	1.834	1.766
3	Bark	Summer	1.680	1.783	1.732	1.092	1.156	1.124
		Monsoon	1.512	1.614	1.563	0.819	0.893	0.856
		Winter	1.581	1.622	1.602	0.912	0.900	0.906

100ml distilled water)

Procedure: 500 mg plant material was ground in mortar and pestle with few drops of cold 80% ethanol. Then 2.5ml of distilled water and 10ml of boiling 80% ethanol were added to it. The extract was centrifuged for 15 minutes at 10,000rpm. Residue was discarded the supernatant was collected and total volume was made 15ml with distilled water. Test tube was kept at 60 °C for 20 minutes. The test tube was cooled and 1ml 50% ethanol was added. Read at 420 nm in spectrometer. Glycine was used as stand rand.

Results and Discussion

The protein content of leaves of *Azadirachta indica* was higher (3.878 mg/g dry wt.) in summer over than winter (3.749 mg/g dry wt.) and monsoon (3.660 mg/g dry wt.). The range of protein content of bark of *Azadirachta indica* was noted from (1.563 mg/g dry wt. to 1.732 mg/g dry wt.). The range of protein content in stem was from (1.988 mg/g dry wt. to 2.292 mg/g dry wt.) and shows higher in summer. The protein content of *Azadirachta indica* showed increasing order of bark < stem < leaves (Table 1 and Graph 1). The protein content of leaves of *Melia azedarach* was higher (3.326 mg/g dry wt.) in summer over than winter (3.256 mg/g dry wt.) and monsoon (3.212 mg/g dry wt.). The range of protein content of stem was noted from (2.257 mg/g dry wt. to 2.579 mg/g dry wt.). The range of protein content in bark of *Melia azedarach* was from (1.880 mg/g dry wt. to 2.011 mg/g dry wt.) and shows higher in summer. The protein content of *Melia azedarach* showed increasing order of root < wood <leaves<bark (Table 2 and Graph 2).

The amino acids content of leaves of *Azadirachta indica* was 2.611 mg/g dry wt. in summer, 2.194 mg/g dry wt. in winter and 2.099 mg/g dry wt. in monsoon. Higher being observed during summer *i.e.* 2.611 mg/g dry wt. The range of amino acids content in stem of

Table 2: Seasonal variation of proteins and amino acid content of different plant parts of Melia azedarach Linn.

Sr.No.	Plant part	Season	Protein (Mg / g dry wt.)			Amino acid (Mg / g dry wt.)		
			1 Year	2 Year	Mean	1 Year	2 Year	Mean
1	Leaves	Summer	3.296	3.356	3.326	2.426	2.566	2.496
		Monsoon	3.151	3.281	3.216	2.206	2.346	2.276
		Winter	3.200	3.312	3.256	2.319	2.423	2.371
2	Stem	Summer	2.529	2.629	2.579	1.849	1.834	1.842
		Monsoon	2.204	2.310	2.257	1.573	1.678	1.626
		Winter	2.426	2.568	2.497	1.699	1.569	1.634
3	Bark	Summer	1.992	2.029	2.011	1.382	1.489	1.436
		Monsoon	1.850	1.910	1.880	1.198	1.263	1.231
		Winter	1.938	1.986	1.962	1.226	1.371	1.299

Azadirachta indica from 1.551 mg/g dry wt. to 1.794 mg/g dry wt. Maximum concentration of amino acids was noted during summer 1.794 mg/g dry wt. The range of amino acid content of bark of *Azadirachta indica* from 0.856 mg/g dry wt. to 1.124 mg/g dry wt. Generally, the concentration of amino acids were found to be in increasing order of bark < stem < leaves (Table 1 and Graph 1). The amino acids content of leaves of *Melia azedarach* was 2.496 mg/g dry wt. in summer, 2.371 mg/g dry wt. in winter and 2.276 mg/g dry wt. in monsoon. Higher being observed during summer *i.e*. 2.496 mg/g dry wt. The range of amino acids content in stem of *Melia azedarach* from 1.626 mg/g dry wt. to 1.842 mg/g dry wt. Maximum concentration of amino acids was noted during summer 1.842 mg/g dry wt. The range of amino acid content of bark of *Melia azedarach* from 1.231 mg/g dry wt. to 1.436 mg/g dry wt. Generally, the concentration of amino acids were found to be in increasing order of bark < stem < leaves (Table 2 and Graph 2).

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