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DETERMINATION OF ALKALOIDS, AMINO ACIDS AND PROTEIN CONTENT IN LEAVES OF TEN MEDICINAL PLANTS OF LALING FOREST, DHULE DISTRICT (MAHARASHTRA)

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ABSTRACT

The seasonal variation of alkaloids, amino acids and protein have been investigated in leaves of *Asparagus racemosus* wild ,*Boswellia serrate* Roxb., *Dolichandrone falcata* Seem, *Grewia tiliaefolia* Vahl., *Lannea coromandelica* (Houtt.)Merr., *Mytenus emarginata* (Wilid)D.Hou., *Rhus mysurensis* Heune., *Securinega virosa* Roxb.,*Wrightia tinctoria* R.Br. and *Zizyphus glabrata* Heyne, which are medicinally important plants of Laling forest. The leaves of *Asparagus racemosus* showed high level of alkaloid content (range 0.641 to 0.772 mg/g dry wt.) and lowest amount of alkaloid content in the leaves of *Securinega virosa* (0.129 to0.208 mg/g dry wt.) comparatively as compared to other investigated taxa. Comparative account of protein content of leaves of *Wrightia tinctoria* showed higher level (range 2.422 to 2.824 mg/g dry wt.) and lowest amount of protein content in the leaves of *Rhus mysurensis* (range 1.224 to 1.526 mg/g dry/wt.). The leaves of *Mytenus emarginata* showed high level of amino acid content (range 3.752 to 4.476mg/g/dry wt.) and lower amino acid content of *Dolichandrone falcata* (range 1.226 to 1.426 mg/g dry wt.) comparatively other investigated taxa.

KEYWORDS: Alkaloid, amino acids, protein, medicinal plant and Laling forest.

INTRODUCTION

All human beings require a number of complex organic/inorganic compounds in diet to meet the need for their activities. The important constituents of diet are carbohydrates, fats, proteins, vitamins, minerals and water (Indrayan et al., 2005). Every constituent plays an important role and deficiency of any one constituent may lead to abnormal developments in

the body. Plants are the rich source of all the elements essential for human beings. There is a relationship between the element content of the plant and its nutritional status. Some elements are essential for growth, for structure formation, reproduction or as components of biologically active molecules (New Wall*et.al*., 1996). Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants grow wild in different parts of our country. In India, the use of different parts of several medicinal plants to cure specific ailments has been vogue from ancient times. Though at present Indian health care delivery consists of both traditional and modem systems of medicines, both organized traditional systems of medicine like Ayurveda, Siddha and Unani and unorganized systems like folk medicine have been flourishing well. These two systems of medicine use plants, minerals, metals and animals as source of drugs, plants being the major source. It is estimated that roughly 1500 plant species in Ayurveda and 1200 plant species in Siddha have been used for drug preparation (Jain, 1987). In Indian folk medicine use, about 7500 plant species are recorded as medicinal plants. A plant species grown in different geographical localities also show quantitative variation in their chemical constituent (Mallavarapuet, *et.al.,* 1995).

The root of *Asparagus racemosus* is employed in diarrhoea and dysentery. Fresh root juice is given with honey as a demulcent. The fleshy tubers are eaten and also used as refrigerant, demulcent, diuretic, antiseptic, antidiarrhoea and in veterinary medicine. *Boswellia serrate* gum resin is widely used in ayurvedic formulation for treating asthama and arthritis (Anonymous, 1976). The bark of *Dolichandrone falcata* used as fish poison and fruits are used in preparation of various medicines. The bark of *Grewia tiliaefolia* is astringent, sweet, refrigerant, expectorant, vulnenary, aphrodisiac and tonic. It is useful in burning sensation, cough, skin diseases, ulcer, diarrhoea. The plant parts of *Lannea coromandelica* is useful in cuts, wounds, ulcers, diarrhoea and dysentery (Kirtikar and Basu,1975).

The fruits of *Rhus mysurensis* are eaten cooked and roasted. Leaves are eaten and also used as fodder. The bark of *Securinega virosa* used in tannin industry. Leaves are used in fever and bark in diarrhoea, fever and induce sleep in children. The leaves of *Wrightia tinctoria* are usefull in odontalgia, dyspepsia and hypertension. The roots of *Zizyphus glabrata* are bitter, sweet, cooling, nervine and tonic. The roots are useful in nervous disorders, dyspepsia, tumour and throat infections. The leaves are used in veneral diseases. The root is useful as a decoction in fever. Roots used in decoction of fever and powder applied to old wounds and ulcers (Anonymous, 1989).

MATERIALS AND METHODS

The plant material of *Asparagus racemosus* wild, *Boswellia serrate* Roxb., *Dolichandrone falcata* Seem, *Grewia tiliaefolia* Vahl., *Lannea coromandelica* (Houtt.)Merr., *Mytenus emarginata* (Wilid) D.Hou., *Rhus mysurensis* Heune. ,*Securinega virosa* Roxb.ex.,*Wrightia tinctoria* R.Br. and *Zizyphus glabrata* Heyne. were collected from Laling forest, Dhule district (Maharashtra), during different season's viz. summer, monsoon and winter continuous two years for estimation of alkaloids, amino acids and protein. Quantitative estimation of alkaloids was carried out by the method of Sairam and Khanna (1971). Each sample was ground to fine powder. To each one gram powder 0.75ml 25 % ammonium hydroxide, 1ml 95% ethyl alcohol and 2 ml ethyl ether were added. The material was allowed to macerate for 12 hours and dried. The dried material was extracted with chloroform for 24 hours in a soxhlet apparatus and the extract obtained was evaporated to dryness, and the residue was mixed with 2.5 ml 0.1 Methanol (90 %) HCL. The extract, thus obtained was centrifuged to take supernatant and discard pellet. The solution was evaporated and the total alkaloids were weight after drying at 100 ^{0}C.

The protein was quantitatively estimated by the Lowry *et.al* (1951). 1gm plant material was homogenized with 10 ml 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 80c for 20 minutes. The pallete was centrifuged and the supernatant was discarded. Residue was washed with 10 ml distilled water and again centrifuged. The supernatant was discarded. 2% 10 ml Na_2CO_3 in 0.1 N NaoH was add to the residue and incubated for an hour at 30 ^{0} C. 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 and mixed thoroughly. The sample was incubated for 10 minutes and 1ml of reagent D was added to it. The color intensity was read at 660 nm using spectrophotometer. The protein concentration of an unknown sample was calculated using standard graph.

The estimation of total amino acid was adapted by Krishnamurthy *et al* (1989) method. 500 mg plant material was grounded in mortar and pestle with few drops of cold 80% ethanol. Then 2.5 ml of distilled water and 10 ml of boiling 80% ethanol were added to it. The extract was centrifuged for 15 minutes at 10,000 rpm. Residue was discarded. The supernatant was collected and total volume was made 15 ml with distilled water. 1ml of sample was taken in a

test tube and 3ml alcoholic ninhydrin was added to it. Test tube was kept at 60^0C for 20 minutes. The test tubes were cooled and 1ml 50% ethanol was added. Read at 420 nm in spectrophotometer. Glycine was used as standard.

RESULTS AND DISCUSSION

The total alkaloid content of leaves of *Asperagus racemosus* was ranging from 0.641 to 0.772 mg/g. The seasonal variation did not play a significant role in the leaves of *Grewia tiliaefolia* in summer, monsoon and winter 0.431, 0.440 and 0.410 mg/g dry wt. resepctively. The *Boswellia serrata* has stored more total alkaloids in its leaves in summer (0.310 mg/g dry wt.) season than monsoon (0.262 mg/g dry wt.) and winter (0.139 mg/g dry wt.) The total alkaloid content of leaves of *Dolichandrine falcata* was higher in winter (0.497 mg/g dry wt.), than summer (0.449 mg/g dry wt.) and monsoon (0.413 mg/g dry wt.) *Lannea coromandelica* accumulated highest level of total alkaloids (0.510 mg/g dry wt.) in its leaves in winter season. The total alkaloid content in the leaves of *Maytenus emarginata* was (0.381 mg/g dry wt.) in summer, (0.307 mg/g dry wt.) in monsoon and (0.404 mg/g dry wt.) in winter season (table 1) The *Rhus mysurensis* had stored more total alkaloids (0.312 to 0.392 mg/g dry wt.) in its leaves. The lowest amount of alkaloid content in the leaves of *Securinega virosa* (0.129 to 0.208 mg/g dry wt.) compared to other investigated taxa. The total alkaloid noted in the leaves *Wrightia tinctoria* was (0.258 mg/g dry wt.) in summer, (0.221 mg/g dry wt.) in monsoon and (0.301 mg/g dry wt.) in winter. The range of alkaloid content in the leaves of *Ziziphus glabrata* was (0.346 to 0.427 mg/g dry wt.) in summer the highest amount of alkaloids content (0.427 mg/g dry wt.) in the leaves of *Ziziphus glabrata*.

The amino acid content of leaves of *Asparagus racemosus* was (0.062 mg/g dry wt.) in summer, (3.978 mg/g dry wt.) in mansoon and (3.726mg/g dry wt.)in winter. Highest being during mansoon (Table 1). The ranges of amino acid content of leaves of *Boswellia serrate* were from (3.120 to 3.755 mg/g dry wt.). Maximum amount of amino acid was noted during mansoon (3.755mg/g dry wt.).

The range of amino acid content of leaves of *Dolichandrone falcate* was noted is summer (1.426 mg/g dry wt.) monsoon (1.226 mg/g dry wt.) and winter (1.290 mg/g dry wt.). It was comparatively lower than that of *Asparagus racemosus* and *Boswellia serrate* in all seasons. The amino acid content of leaves of *Grewia tiliaefolia* ranged from (1.226 to 1.582 mg/g dry wt.). The summer sample of leaves exhibited the elevated levels of amino acid in the investigation. The amino acid content of leaves of *Lannea coromandelica* was (3.767 mg/g

dry wt.) in summer, (4.962 mg/g dry wt.) in monsoon and (3.220 mg/g dry wt.) in winter. The amino acid content of leaves of *Lannea coromandelica* was higher in monsoon (4.962 mg/g dry wt.) season.

The amino acid content of leaves of *Mytenus emarginata* was (4.192 mg/g dry wt.) in summer, (4.476 mg/g dry wt.) in monsoon and (3.752 mg/g dry wt.) in winter and attained it's maximum in monsoon. The lowest amount of amino acid content in the leaves of *Rhus mysurensis* (0.922 to 1.262 mg/g dry wt.) compared to other taxa. The amino acid content of the leaves of *Securinega virosa* was significantly raised from (3.436 to 4.261 mg/g dry wt.) the summer (4.022 mg/g dry wt.) in all seasons. In *Wrightia tinctoria* the amino acid content of leaves was higher in monsoon (4.162 mg/g dry wt.) than summer (4.022 mg/g dry wt.) and winter (3.726 mg/g dry wt.). The amino acid content of the leaves of *Ziziphus glabrata* was noted in summer (3.621 mg/g dry wt.) monsoon (3.826 mg/g dry wt.) and winter (3.241 mg/g dry wt.). The amino acid content of leaves of *Mytenus emarginata* was higher in all seasons than other investigated taxa.The lowest amount of amino acid content of leaves of *Rhus mysurensis* in winter (0.922 mg/g dry wt.) season than all other taxa.

The protein content of leaves of *Wrightia tinctoria* was highest (2.824 mg/g dry wt.) in summer than all other plants. The protein content of leaves of *Rhus mysurensis* and *Dolichandrone falcate* were very low in all seasons (1.224 to 1.862 mg/g dry wt.) than all the taxa investigated. *Asparagus racemosus, Grewia tiliaefolia* and *Ziziphus glabrata* were not much different (1.890 to 2.096 mg/g dry wt.), (1.867 to 1.466 mg/g dry wt.) and (1.840 to 1.473 mg/g dry wt.) resepectively in all seasons.

The protein content of the leaves of *Boswellia serrate*, *Lannea coromandelica*, *Maytenus emarginata* and *Securine gavirosa* are also not more different (1.724 to 2.494 mg/g dry wt.) (1.820 to 2.382 mg/g dry wt.), (1.784 to 2.461 mg/g dry wt.) and (2.20 to 2.416 mg/g dry wt.) resepectively(Table 1). The protein contents of the leaves of *Boswellia serrate* in summer was (2.494 mg/g dry wt.), monsoon (1.916 mg/g dry wt.) and winter (1.724 mg/g dry wt.). The protein content of leaves of *Securinega virosa* was not noted more different in summer (2.200 mg/g dry wt.), Monsoon (2.304 mg/g dry wt.) and winter (2.416 mg/g dry wt.) resepectively. It was interesting to note that the protein content remained almost same in few plants in all seasons (Table 1).

Table .1 - Estimation of alkaloids, amino acids and protein content in leaves of ten medicinal plants.

Sr. No.	Name of Plant	Alkaloids mg / gm. fresh wt.			Amino acids mg / gm. fresh wt.			Protein mg / gm. fresh wt.		
		Season			Season			Season		
		Sum.	Mon.	Win.	Sum.	Mon.	Win.	Sum.	Mon.	Win.
1	*Asparagus racemosus*	0.734	0.641	0.772	3.062	3.978	3.726	1.890	1.466	2.096
2	*Boswellia serrate*	0.310	0.262	0.139	3.120	3.755	3.682	2.494	1.916	1.724
3	*Dolichandrone falcate*	0.449	0.413	0.497	1.426	1.226	1.290	1.592	1.326	1.862
4	*Grewiatiliae folia*	0.431	0.440	0.410	1.582	1.326	1.462	1.867	1.466	1.667
5	*Lannea coromandelica*	0.478	0.460	0.510	3.767	4.962	3.220	2.382	1.962	1.820
6	*Mytenus emarginata*	0.381	0.307	0.404	4.192	4.476	3.752	2.461	1.826	1.784
7	*Rhus mysurensis*	0.350	0.312	0.392	1.262	1.122	0.922	1.526	1.224	1.396
8	*Securinega virosa*	0.182	0.129	0.208	3.976	4.261	3.436	2.200	2.304	2.416
9	*Wrightia tinctoria*	0.258	0.221	0.301	4.022	4.162	3.726	2.824	2.422	2.721
10	*Ziziphus glabrata*	0.409	0.346	0.427	3.621	3.826	3.241	1.840	1.473	1.696

CONCLUSION

The present paper incorporate the results of the biochemical investigations of ten medicinally important taxa of Laling forest , Dhule district are *Asparagus racemosus* wild ,*Boswellia serrate* Roxb.,*Dolichandrone falcata* Seem, *Grewia tiliaefolia*Vahl., *Lannea coromandelica* (Houtt.) Merr.,*Mytenus emarginata* (Wilid) D.Hou., *Rhus mysurensis* Heune., *Securinega virosa* Roxb., *Wrightia tinctoria* R.Br. and *Zizyphus glabrata* Heyne. The range of alkaloid , amino acid and protein content of all investigated plants are (0.129 to 0.772 mg / g fresh wt.), (1.122 to 4.962 mg / g fresh wt.) and (1.224 to 2.824 mg / g fresh wt.) respectively.

REFERENCES

1. Anonymous, 1976: Medicinal plants of India. ICMR, Cambridge Printing Works. Kashmere Gate, Delhi.
2. Anonymous, 1989: Useful plants of India, edited by Ambasta, CSIR, New Delhi.
3. Indrayan, A.K., Sharma, S., Durgapal, D., Kumar, N., and Kumar, M., (2005), Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Science.*, 89: 1252-1255.
4. Jain S.K, (1987), Endangered species of medicinal herbs in India.*Vivekananda Kendra Patrika*, 16(1).
5. Lowry, O.H., Rosebrough, N. J., Farr, A. L. and Randall, R.J., (1951), Protein measurement with the Folin –phenol reagent, *J. Biol. Chem*., 193: 265 -275.

6. Kirtikar, K.R and Basu. B.D., (1988), Indian Medicinal Plants, 2: 1038-1063. *International Book Distributer*, Dehradun.
7. Krishnamurthy, R., Ambazhagan M and Bhagwat, K. A., (1989), Testing salt tolerant variability in the nutritional quality of seed produced by rice cultivars subjected to salinity. *Seed Science and Technology*., 17: 269 - 275.
8. Lowry, O.H., Rosebrough, N. J., Farr, A. L. and Randall, R.J., (1951), Protein measurement with the Folin –phenol reagent, *J. Biol. Chem*., 193: 265 -275.
9. Mallavarapu,G.R.,Ramesh,S., Chandrashekharan,R.S., Rao, R. B. R., Kaul, P.N. and Bhattacharya, A.K.,(1995), Investigation of the essential oil of *Cinamomum* leaf grown at Bangalore and Hyderabad flavour, *Fragrance Journal*, 10(4): 231-249.
10. New Wall,C.A.,Anderson,L.A.and Phillips,J.D., (1996), Herbal Medicines-A guide for healthcare professionals.The Pharmaceutical Press,London,*Clinical Pathology*, 45: 493-496.
11. Sairam T. V. and Khanna, P., 1971: Effect of tyrosine and phenylalanine on growth and production of alkaloids in *Dathura tubula*, *Tissue Culture*. Lloydia, 34.