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Phytochemical and Antibacterial Study of Traditional Medicinal Plants of North East India on *Escherichia coli.*

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Abstract : The study was conducted on various biochemical parameters of some traditional medicinal plants of North East India VIZ., *Leucas aspera, Murraya koengigii, Oxalis corniculata, Alternanthera sessilis, Pagostemon benghalensis, Hydrocotyl rotendifolia, Cyathula prostrata, Piper peepuloides, Potentilla mooniana*. It was observed that *L. lanata, M. koengigi, O. corniculata, A. sessilis, P. peepuloides* and *P. mooniana* shows antibacterial activity against *E. coli*. The nutritive values of these plants varied between 231.59 to 34.59 Cal/ 100 gm and the tannin content ranged from 3.8% and 0.62%. The GC analysis of the fatty acid methyl esters showed the presence of both saturated and unsaturated fatty acid consisting of Palmitic acid, a mixture of oleic, linoleic, linolenic acids and stearic acid in various quantities from 0.06 to 6.33% gm dry leaf powder.

Key words : Medicinal plants, Antibacterial activity, Nutritive value, Fatty acids.

Introduction

Medicinal plants are rich source of metabolites that are potential sources of drugs and essential oils. Some of them are semi domesticated and mostly grow as weeds. The nutritive values of these plants are important as they act as component for human consumption. Nearly all cultures from ancient times until today have used plants as source of medicine. In many developing countries traditional medicine is still the mainstay of health care and most of the drugs and cures come from plants. The wide geographical and climatic diversity of Assam provides a repository of valuable indigenous system of medicine, as their extracts in various forms are being used in traditional system of medicine for the treatment of human ailments particularly those caused by *Escherichia coli* present in the gut of human beings. Therefore an effort has been made to study the phytochemical, nutritive value and antibacterial properties of some medicinal plants *viz Leucas aspera, Murraya koengigii, Oxalis corniculata, Alternanthera sessilis, Pogostemon benghalensis, Hydrocotyl rotendifolia, Cyathula prostrata, Piper peepuloides, Potentilla mooniana* (Fig. 1). The use of these plants for the control of human diseases has certain advantages for instance, the plants are generally readily available and no cultivation cost is involved (Kumar, 2002).

Alternanthera sessilis is a perennial herb and grows up to a height of 1 metre. It is a weed in tropical lands and can grow in all soil types. The shoots and leaves are often eaten as vegetables. *Murraya koengigii* is found almost throughout India up to an altitude of 1500 m. The leaf is used as a natural flavouring agent and the dried leaves are used as

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stomachic. *Oxalis corniculata* is a diffuse herb with procumbent branches. The plant is well known for its medicinal value as a good appetizer and as a remover of Kapa, vata and piles. It is known to cure dysentery, diarrhea and skin diseases (Raghavendra *et al.,* 2005). *Hydrocotyl rotendifolia* is common throughout NE region in damp fallows in association with *Oxalis corniculata.* Its leaf is taken in cough, cold, fever, stomach ache and as antihelmintic. It is also used as a cure for diarrhea and dysentery. The plant is used as a tonic and in bronchitis, asthma, gastric, kidney troubles and dropsy. *Pogostemon benghalensis* is a small herb and reaches up to a height of 1 m and grows in all soil types. It is used in herbal medicine as an aphrodisiac, antidepressant and antiseptic. The oil is used in aromatherapy to treat skin complaints. This oil may also be used for varicose veins and hemorrhoids. *Leucas aspera* is a perennial with woody root stock hairs erect and spreading. The young shoots are used as vegetables. The tender fried leaves are said to be good for cough. The juice of the leaves bruised with lime is used for skin inflammation. *Cyathula prostrata* is a slender herbaceous prostrate plant with branches 1-2 ft. high. A decoction of the roots is given for dysentery in Malaya. The burs are used for the same purpose in certain parts of African countries. *Piper peepuloides* is a small climbing shrub; the fruiting spikes are mostly collected from wild plants in Assam. The fruits exhibit no pungency when chewed but exhibit strong sialogogue action, followed by numbness and tingling sensation on the tongue. *Potentilla mooniana* is a prostrate or erect herb with yellow flowers. *Potentilla mooniana* roots as Trade name *Dori ghas* are used in Ayurveda. The present study is an attempt to analyze the various biochemical parameters and evaluate the nutritive value of the few selected medicinal plants and their antibacterial properties against human pathogen, *E. coli*.

Materials and Methods

The fresh and disease free plant leaf specimens collected from Jorhat and other NE region were washed in running tap water, dried under the sun then crushed in a mortar and pestle and were subjected to various biochemical analysis. The moisture content was determined by taking the fresh plant samples in petri dishes and kept overnight in an air oven at 100-110°C till they attained a constant weight. The loss in weight was regarded as a measure of moisture content (Indrayan *et al*., 2005). The total carbohydrate content was estimated by anthrone method (Sadasivam and Manickam, 1996). Total protein was estimated by Lowry's method (Lowry *et al.*, 1951). The lipid was determined by extracting the sample with chloroform: methanol (2:1) following the method of Folch (Folch *et al*., 1957; Unni *et al.,* 1996). For determination of crude fibre, 2 g of moisture and fat-free material was treated with 200 ml of 1.25% H_2SO_4 and then with 1.25% NaOH. It was the filtered, washed with hot water and then again1.25% H_2SO_4 and with hot water. The residue was ignited at 130 ± 2° and the ash weighed. Again it was ignited at 600 ± 15°. Loss in weight gave the weight of crude fibre (Sadasivam and Manickam, 1996). Crude fibre was calculated by the following formula:

$$\text{Crude fiber (\%)} = \frac{\text{Loss in weight on ignitation } (W_2 - W_1) - (W_3 - W_1)}{\text{Weight of the sample}} \times 100$$

Where,

W_1 = weight of ashing dish

W_2 = weight of sample after drying at 139 ± 15°C

W_3 = weight of sample after drying at 600 ± 15°C

The total tannin content was estimated by treating the sample with Folin-Denis reagent and sodium carbonate solution (Sadasivam and Manickam, 1996). The nutritive value was finally determined by the formula:

Nutritive Value = 4 × percentage of protein + 9 × percentage of fat + 4 × percentage of carbohydrate (Indrayan *et al.*, 2005).

The fatty acid composition was determined through GC analysis after converting the lipids into methyl esters (Sadasivam and Manickam, 1996). The GC analysis was done using a Hewlett-Packard 5890 Series II gas chromatograph equipped with a FID detector and a HP-1 fused silica column (24 m × 0.32 mm, 0.25 mm film thickness) was used.

To study the antibacterial activity plants extract was prepared. Different plants were homogenized (3g/5 ml) in sterile water at room temperature. After centrifugation (6200 rev/min) the clear supernatant was used for testing antibacterial activity against *E.coli* by agar cup assay method (Unni *et al.*, 2004). The solvent (water) used for extraction was kept as control. Antibacterial activity of antibiotics was studied by using Himedia octodisc placed over the inoculated bacterial plates. All the plates were incubated at 30 ± 2°C for 24 hour and the zone of inhibition formed was measured by using HiAntibiotic zone scale.

Statistical analysis was done by the analysis of variance (ANOVA); mean difference of the treatment significance was calculated at $p<0.05$. Results are means of three replicates each.

Results and Discussion

The results of the percentage of various biochemical constituents are presented in Table 1. The medicinal plants were nutritionally rich (Handique and Handique, 2005), though the percentage of carbohydrate was quite low in all the plants. The fatty acid content showed considerable variability and ranged from 19.83% in *H. rotendifolia* to 0.86% in *L. lanata*. The leaves of *O. corniculata* also exhibited higher percentage of protein (12.5%). The leaves of *H. rotendifolia* also showed similar value for protein percentage, however in *P. mooniana* it was found to be low. High variation was also observed in fiber percentage where *P. nooniana* exhibited highest fiber percentage (51.6%) and *H. rotendifolia* showed the lowest value of 3.10%. The crude fiber itself is not a nutrient component since is not digested but it is considered to be nutritionally important and in fact a daily intake of 40 gm dietary fiber is recommended (Handique *et al.*, 2005). The tannin component was highest in *M. koengigii, P. mooniana* and lowest in *O. corniculata.*

The composition of fatty acids is given in Table 2. The Linoleic acid and linolenic are two important fatty acids, which are essential for human beings. It was found that among these plants *H. rotendifolia* contained the highest percentage of C_{18}, which is a mixture of oleic, linoleic and linolenic acid where as *M. koengigii* contained highest percentage of C_{16} i.e, palmitic acid. Apart from that, stearic acid was detected in *C. prostrata* and *P. mooniana*.

The results of the antibacterial activity of plants sample and antibiotics are shown in Table 3 and Table 4 respectively. Among the nine traditional medicinal plants, *L. lanata, M. koengigii, O. corniculata, A. sessilis,* and *P. peepuloides* showed good inhibition zones. The results indicated that *H. rotendifolia* had a high nutritive value of 231.59 cal/100 gm but its water extract did not show any antibacterial activity in this study. This is followed by *O. corniculata,* which had a nutritive value of

Table 1 : Phytochemical composition of some medicinal plants (% dry weight basis)

Plants	Carbohydrate	Fatty acids	Protein	Fiber	Tannin	Moisture %	Nutritive value
Pogostemon benghalensis	0.27±0.1	6.175±0.2	4.59±0.1	7.10±0.1	1.6±0.2	84.77±2.4♣	75.01±1.6♣
Leucas lanata.	0.37±0.2♣	0.86±0.32♣	6.35±0.2♣	14.90±0.6♣	2.6±0.1	88.59±3.0♣	34.59±0.9
Murraya koengigi	0.75±0.2♣	7±0.6♣	2.1±0.3♣	3.70±0.2	3.8±0.5♣	62.54±1.1	74.4±1.2
Hydrocotyl rotendifolia	0.78±0.4♣	19.83±0.7♣	12.5±0.7♣	3.10±0.3♣	1.6±0.2	93.60±3.2♣	231.59±8.4♣
Oxalis corniculata	1.36±0.3♣	13.2±0.7♣	12.5±0.5♣	6.20±0.3♣	0.62±0.3♣	92.30±2.8♣	174.24±4.6♣
Alternanthera sessilis	1.01±0.2♣	5.8±0.2	3.1±0.4♣	3.50±0.2	1.2±0.2	81.00±1.1	68.652±1.7♣
Cyathula prostrata	0.43±0.1	8.7±0.4♣	2.48±0.1	21.4±0.7♣	3.5±0.6♣	35.40±1.6♣	89.94±3.2♣
Piper peepuloides	0.66±0.2♣	2.8±0.1♣	2.37±0.2♣	22.4±0.3♣	3.68±0.2	24.92±0.3	38.64±3.2♣
Potentilla mooniana	0.19±0.1	9.9±1.0	1.8±0.3♣	51.6±3.5♣	3.8±0.3♣	27.60±1.3	97.06±0.3

[a] *Values are mean of three replicates of 30 plants each ± SE*

♣*Values are statistically significant at ($p<0.05$)*

Table 2 : Fatty acid composition of medicinal plants (% dry weight basis)

Plants name	Methyl esters		
	Palmitic acid	Mixture of oleic, Linoleic and Lenolenic	Stearic
Pogostemon benghalensis	0.75	3.75	nd
Leucas lantana	nd	0.088	nd
Murraya koengigi	5.83	0.75	nd
Hydrocotyl rotendifolia	5.49	6.33	nd
Oxalis corniculata	1.8	3.8	nd
Alternanthera sessilis	0.8	3.6	nd
Cyathula prostrata	1.05	1.1	0.197
Piper peepuloides	2.44	1.99	nd
Potentilla mooniana	0.64	1.11	0.06

nd, Not detected (<0.005 mg/g dry weight)

Table 3 : Antibacterial activity of water extract of different medicinal plants against *E. coli.*

Plants	Inhibition zone (mm)
Pogostemon benghalensis	-
Leucas lanata.	30
Murraya koengigi	21
Hydrocotyl rotendifolia	-
Oxalis corniculata	60
Alternanthera sessilis	42
Cyathula prostrata	-
Piper peepuloides	30
Potentilla mooniana	-

-, Absent

Table 4 : Antibacterial activity of different antibiotic against *E.coli.*

Antibiotics(mcg)	Results
Gentamicin (10mcg)	S
Ampicillin (10mcg)	R
Norfloxacin (10mcg)	S
Streptomicin (10mcg)	S
Chloramphenenicol (30mcg)	S
Tetracycline (30mcg)	S
Cephalexin (30mcg)	S
Mecillinam (33mcg)	R

S-sensitive; R-resistant

174.24cal/100 gm with a positive antibacterial activity in water extract.

Conclusion

The uses of medicinal plants are well known to the people of North East India. In our study we tried to find out the biochemical constituents and nutritive value of some folk medicinal plants so that the nutritive supplements could be made from these plants. We also made an attempt to analyze the antibacterial property of these plants against human pathogen *E. coli*. Results are encouraging but isolation of bioactive molecule and its antibacterial activity against *E. coli* needs further ethnochemical studies, which might be helpful in developing drugs from natural product.

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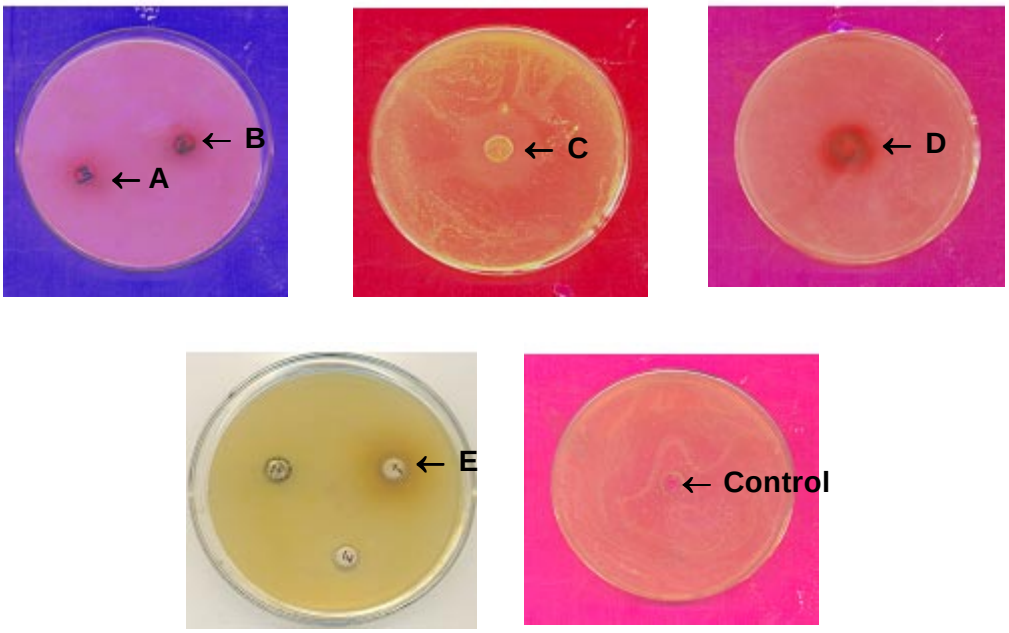

Fig. 1 : Antibacterial activity of (A): *Leucas aspera*, (B): *Murraya koengigii*, (C): *Oxalis corniculata*, (D): *Alternanthera sessilis*, (E): *Piper peepuloides* against *E. coli.*

References

Folch J., Lees M. and Stanley G. H. S. (1957): A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem*., **266,** 497-509.

Handique G.K. and Handique A.K. (2005): Nutritive value of some non-conventional leafty vegetables. *Indian J Agric Biochem.*, **18**(1), 47-49.

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 Uttraranchal. *Current Science*, **89**(7), 1252-1255.

Kumar S. (2002): *The Medicinal Plants of North East India*, Scientific Publishers (India), Jodhpur. 1-35

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.

Raghavendra M.P., Satish S. and Raveesha K.A. (2005): Phytochemical analysis and antibacterial activity of *Oxalis corniculata*; a known medicinal plant. *My Science.*, **1**(1), 72-78.

Sadasivam S. and Manickam A. (1996): *Biochemical Methods for Agricultural Sciences*, New Age International (P) Ltd., New Delhi. 1-97

Unni B.G., Kakoty Ajit C., Khanikor D., Bhattacharya P.R., Pathak M.G., Pillai K., Pillai R., Chouhury A., Saikia P. Ch. and Ghosh A.C. (1996): Lipid and fatty acid composition of muga silkworm, *Antheraea assama*, host plants in relation to silkworm growth. *J. Lipid Mediators and Cell Signalling*,**13**, 295-300.

Unni B.G., Yadav A., Chaudhary A., Kumari J., Wann S.B. and Sarma R. (2004): *Chebulic myrobalan* for controlling bacterial disease in muga silkworm *Antherae assama*. A preliminary report. *Natural Product Radiance.*, **3(5)**, 347-348.