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Evaluation of Nutrient Composition and Antibacterial Potential of Leaf and Stem of *Mukia Maderaspatana l.*

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Subject: Biochemistry

Abstract

Mukia maderaspatana L. (called as "musumusukkai" in Tamil) is an important medicinal plant used as a herbal drug in cough and cold and well integrated into South Indian food. The present study was carried out to evaluate the phytochemical constitution, nutrient composition and antibacterial potential of leaf and stem parts of *Mukia maderaspatana.* Phytochemical screening showed the presence of glycoside, flavonoids, phenols, alkaloids, saponin, carbohydrate and steroid in them. Most of the phytochemicals were extracted in ethanol. The moisture control was found to be higher in leaf than in stem (38% Vs 29%). Proximate analysis revealed higher protein content than lipid and carbohydrate in the plant extract. The leaf and stem parts were also analyzed for vitamin (retinol, ascorbic acid, tocopherol, thiamine, riboflavin) and mineral (iron, phosphorus, copper) content. The results obtained suggest that considerable amount of ascorbic acid and phosphorus are present in the plant parts. The antibacterial activity of various extracts of *M.maderasapatana* shows that the acetone fraction of both leaf and stem has pronounced activity at 1000µg/ disc where as the stem hexane extract shows negligible activity. Hence, the findings of the present study suggests that the rich nutrient content and antibacterial activity of *M.maderaspatana* may be responsible for its wide traditional use as food and medicine.

Key words: - *M.maderaspatana, phytochemicals, proximate and antibacterial.*

Introduction

India has one of the world's most sophisticated indigenous medical cultures (accumulated in ayurveda, unani and siddha) since more than four millennia (Sazada Siddiqui *et al.*, 2009). Though this medical heritage is several centuries old, even today people in the rural and remote areas depend upon it for their health care needs as synthetic drug are not only expensive and inadequate for the treatment of diseases but are also often with adulteration and side effects (Pandey, *et al.*, 2006). Even in urban area, some people still depend on medicinal plants at least for the treatment of simple diseases such as cold, cough, fever, headache, poison bite, skin diseases and in tooth infection.

Mukia maderasapatana (Linn.) Cogn. Syn. Melothria maderasapatana, Cucumis maderasapatana or Mukia scabella (family: Cucurbitaceae) is a tendril climber/prostate herb found throughout India. It occurs in wild areas as well as cultivated in kitchen gardens. It is traditionally used as a leafy vegetable and to cure several ailments in South India. It is used to treat cough, cold, constipation,vertigo, burning sensation, dyspepsia, flatulence, dental pain (Boobalan Raja *et al.*,2010). Extensive literature survey has shown that there are no scientific reports available on nutrient composition of *Mukia maderaspatana.L.* Also earlier work focussed on antimicrobial activity of aerial parts in chloroform, hexane, ethyl acetate and methanol. Hence the present study is carried out with the aim to explore phytochemical constitution in water, ethanol, ethyl acetate, acetone and hexane extract of leaf and stem parts, nutrient potential in relation to its ethnomedicinal uses and potential antibacterial activity against few bacterial strains.

Materials and Method

Collection

The plant was collected from Captain Srinivasa Moorthy Drug Research Institute and Ayurveda, Arumbakam, shade dried at 37ºC for 10 days.

Extraction

10g of powdered plant material (leaf and stem) were soaked separately in 30 ml of water, ethanol, ethyl acetate, acetone and hexane for 72 hours. Each extract was stirred for every 24 hours by using sterile glass rods. At the end, each extract was passed through Whattman No.1 filter paper and the filtrate obtained was concentrated using a rotary evaporator.

Phytochemical analysis

Qualitative analysis for Alkaloids, Saponins, Glycosides, Phenolic compounds Tannins, Flavonoids, Steroids and Terpenoids were carried out by Harbone method, 1973. Quantitative analysis were carried for phenol (Singleton *et al*.,1999), Tannin (Schanderl SH, 1970), Flavonoids (Middleton E, 1996.Other estimation carried out were- Protein (Lowry *et al*.,1951), Carbohydrate (Anthrone method, Roe, 1955), Lipids (Dryer method), Vitamin A (Neeld Pearson method,), Vitamin C, Vitamin E (Tsen CC, 1961), Thiamine, Riboflavin, Iron (Ramsay's method), Copper, Phosphorous (Fiske-Subbarao method).

Antibacterial activity

Antibacterial activity of all the plant extracts was determined by disc diffusion method using Muller Hinton agar (MHA) medium. The Muller Hinton Agar medium was poured on the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA plate and 20 µl of [sample concentration : 1000µg] each samples were placed in the disc. Pure DMSO was taken as control. The plates were incubated for 24 hrs, at 37ºC .Then the microbial growth was determined by measuring the diameter of zone of inhibition. The experiment was performed 3 times. Each extract was diluted in dimethyl sulphoxide (DMSO)

Results

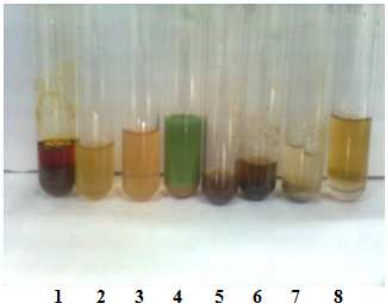


Fig. A: Leaf water extract

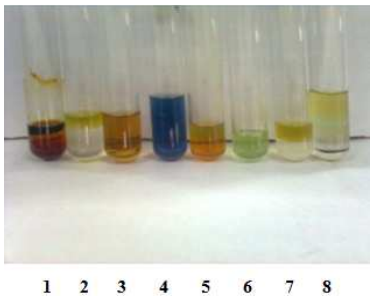


Fig. C: Leaf ethyl acetate extract

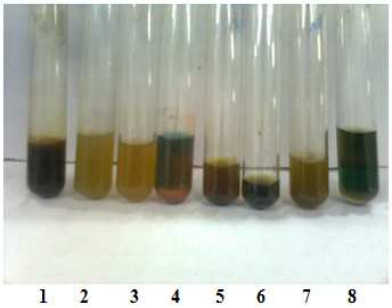


Fig. B: Leaf ethanol extract

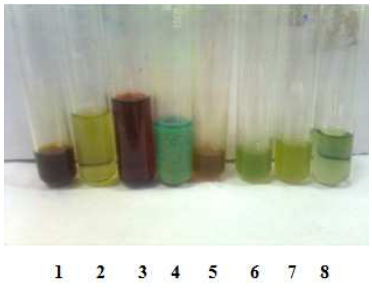
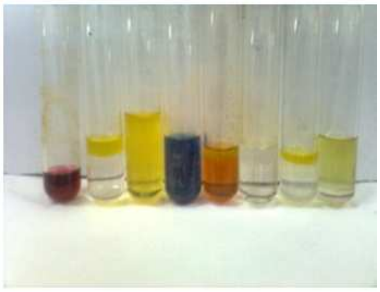


Fig. D: Leaf acetone extract



1 2 3 4 5 6 7 8

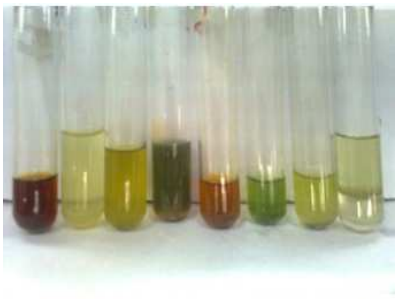
Fig.E: Leaf hexane extract

1-Alkaloids 2-Saponin 3-Glycosides 4-Carbohydrates 5-Phenols & Tannin 6-Protein 7-Flavonoids 8-Steroids &Terpenoids

Figure-A-F: Results of phytochemical analysis of various Leaf extract of *M.maderasapatana*

1 2 3 4 5 6 7 8

Fig.1A: Stem water extract



1 2 3 4 5 6 7 8

Fig.1B: Stem ethanol extract

1 2 3 4 5 6 7 8

Fig.1C: Stem ethyl acetate extract

1 2 3 4 5 6 7 8

Fig.1D: Stem acetone extract

1 2 3 4 5 6 7 8

Fig.1F: Stem hexane extract

1-Alkaloids 2-Saponin 3-Glycosides 4-Carbohydrates 5-Phenols & Tannin 6-Protein 7-Flavonoids 8-Steroids &Terpenoids

Figure-1A-1F: Results of phytochemical analysis of various stem extract of *M.maderasapatana*

Table 1: Phytochemicals in various leaf extracts of *Mukia maderaspatana*

S. N	Phytochemicals	Extract				
		W	E	EA	A	H
1.	Alkaloids	+	+	-	+	-
2.	Saponins	-	+	-	+	-
3.	Glycosides	+	+	+	+	-
4.	Carbohydrates	+	+	-	+	-
5.	Phenols /Tannins	-	+	-	-	-
6.	Protein	-	-	-	-	-
7.	Flavonoids	+	-	-	-	-
8.	Steroids/terpenoids	-	+	+	+	+

Table 2: Phytochemicals in various stem extracts of *Mukia maderaspatana*

S.No	Phytochemicals	Extract				
		W	E	EA	A	H
1.	Alkaloids	+	-	-	+	-
2.	Saponins	-	+	-	+	-
3.	Glycosides	-	-	-	+	-
4.	Carbohydrates	-	+	+	+	+
5.	Phenols /Tannins	-	-	-	-	-
6.	Protein	-	-	-	-	-
7.	Flavonoids	+	-	-	-	-
8.	Steroids/terpenoids	-	+	+	+	+

+ Presence, - Absence,
W-water, E-ethanol, EA-ethyl acetate, A-acetone, H-hexane

Table 3: Levels of crude phenol, tannins and flavonoids in leaf and stem extracts of *M.maderaspatana* (mg/g of extract)

Extract	Phytochemicals		
Leaf	Phenol	Tannin	Flavonoids
W	0.01±0.001	0.00	1.91±0.01
E	0.01±0.001	7.7±0.001	1.39±0.03
EA	2.29±0.001	0.53±0.003	2.80±0.04
A	0.07±0.001	0.40±0.001	0.40±0.05
H	0.007±0.001	0.28±0.001	-
	Stem		
W	5.63±0.02	-	-
E	1.87±0.001	2.1±0.1	0.16±0.001
EA	1.405±0.001	0.70±0.003	0.0007±0.001
A	4.41±0.03	0.40±0.001	-
H	3.19±0.02	-	-

+ Presence, - Absence,
W-water, E-ethanol, EA-ethyl acetate, A-acetone, H-hexane

Table 4: Proximate composition of *M. maderasapatana* parts (mg/g of extract)

Extract	Proximate contents		
Leaf	Protein	Carbohydrate	Lipid
W	4.75±0.001	0.092±0.001	10±0.2
E	98.4±2.4	0.009±0.001	
EA	14.06±0.1	0.009±0.001	
A	0.048±0.001	0.028±0.001	
H	0.937±0.001	0.14±0.002	
	Stem		
W	1.5±0.002	0.009±0.01	168±3.2
E	-	0.09±0.01	
EA	-	-	
A	1.5±0.002	0.06±0.01	
H	9.92±0.02	-	

W-water, E-ethanol, EA-ethyl acetate A-acetone H-hexane
Mean value of 3 determinants (mean ± S.D)

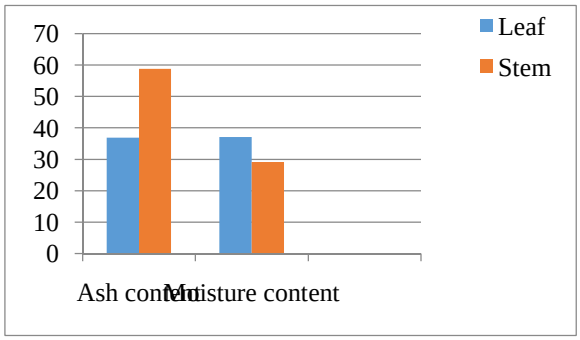


Figure 3: Percentage of physicochemical characteristics in different parts of *M.maderasapatana* Values are expressed in percentage i.e. g/100g of extract

Table 5: Concentration of vitamins present in *M.maderaspatana* (µg/mg of extract)

Extract	Vitamins				
Leaf	**Vitamin A**	**Vitamin E**	**Vitamin C**	**Thiamine**	**Riboflavin**
W	-	0.002±0.001	7.69±0.01	-	-
E	9±0.001	0.0009±0.002	75.38±0.2	-	-
EA	5±0.002	0.0011±0.003	44.61±1.3	0.05±0.02	0.05±0.001
A	1±0.001	0.03±0.001	75±2.4	-	0.03±0.001
H	-	0.0005±0.002	44.61±2.07	-	0.04±0.001
Stem					
W	-	0.001±0.003	44.61±3.1	-	-
E	-	0.001±0.001	63.0±2.0	-	-
EA	7±0.003	0.002±0.001	56.92±3.2	0.03±0.03	0.03±0.001
A	-	0.001±0.002	60±2.9	-	0.05±0.02
H	-	0.001±0.003	66.1±3.6	-	-

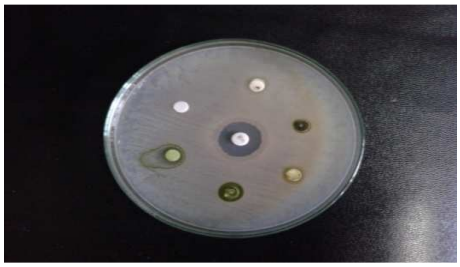
Mean value of 3 determinants (mean ± S.D) W-water, E-ethanol, EA-ethyl acetate A-acetone H-hexane

Table 6: Concentration of minerals detected in *M.maderasapatana* (µg/mg of extract)

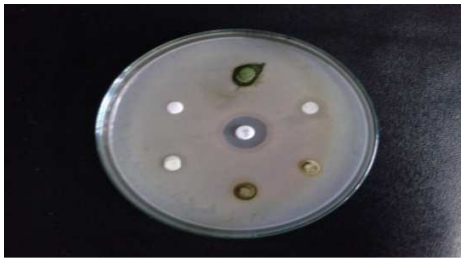
Extract	Minerals		
Leaf	**Iron**	**Copper**	**Phosphorous**
W	-	66±0.02	10±0.02
E	-	-	100±0.02
EA	2±=0.01	12±0.02	120±0.03
A	-	15±0.02	200±0.01
H	4±0.02	30±0.02	30±0.02
Stem			
W	34±0.03	-	-
E	4±0.02	-	200±0.02
EA	4±0.02	9±0.02	40±0.02
A	-	45±0.02	-
H	-	-	-

Mean value of 3 determinants (mean ± S.D)
W-water, E-ethanol, EA-ethyl acetate A-acetone H-hexane

Staphylococcus aureus

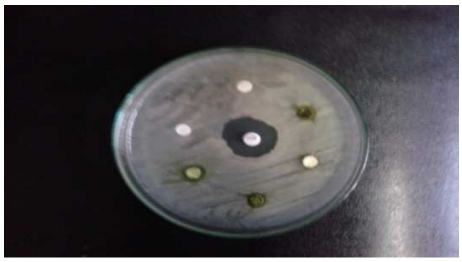


Leaf



Stem

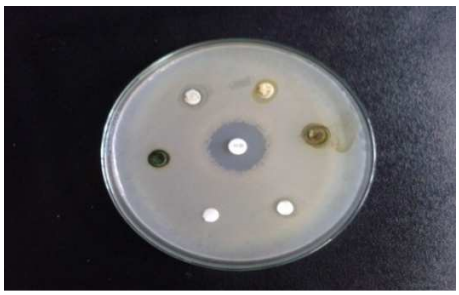
Bacillus subtilis



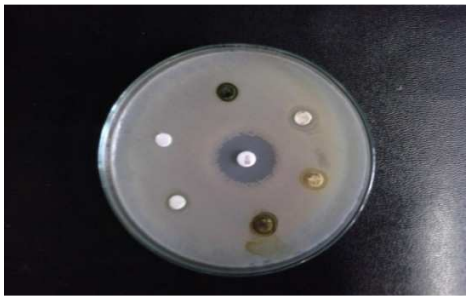
Leaf

Stem

E.coli



Leaf



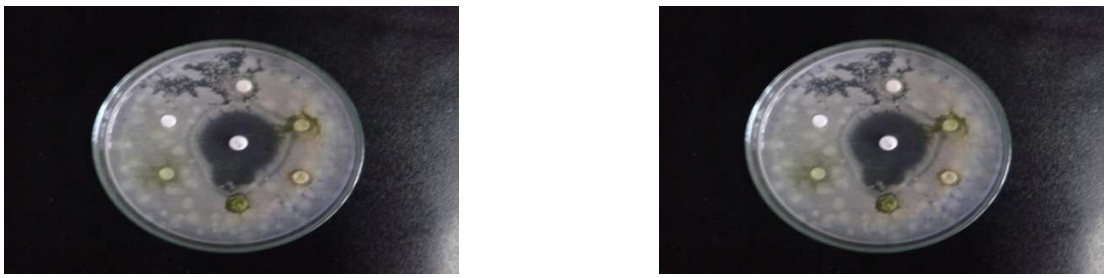
Stem

Klebsella pnuemoniae

Leaf

Stem

Pseudomonas aerugenosa



Leaf **Stem**

Figure 4: Antibacterial activity of leaf and stem extracts of *Mukia maderaspatana* on micro-organisms

Discussion

Qualitative analysis is very essential for identifying the compounds present in the medicinal plants (Chitravadivu *et al*., 2009). Hence in our present study phytochemical screening was performed on different extracts of *Mukia maderasapatana* parts as a preliminary step. Ethanolic extract was found to contain more number of phytochemical such as carbohydrate, protein, alkaloid, tannin, saponins, steroids and glycosides followed by acetone extract. The results of present study also showed the presence of flavonoids in the aqueous extract of both stem and leaf of *M.maderasapatana.* Alkaloids, saponins, phenols and flavonoids were absent from the fractions of hexane and ethyl acetate although steroids and glycosides were found to be present in them. Aqueous extract of both leaf and stem of the plant showed the presence of alkaloids and flavonoids.

Quantitative estimation of the crude phenol, tannin and flavonoids were also carried out in the plant extract. The plant contained the highest % crude yield of flavonoids in ethyl acetate leaf extract (2.8mg/g) and phenols in aqueous stem extract (5.63mg/g). Tannin were obtained in maximum in ethanolic leaf extract (7.7mg/g) but the yield recorded in all other extracts were minimal. Phenolic content is moderate in both leaf and stem extracts.

The results of th e present study reveal that the leaf of *M.maderasapatana* contain appreciable amount of tannin, alkaloids and flavonoids when compared to stem. These phytoconstituents may act as a source of pharmacologically active agents and also natural antioxidants. The observed beneficial activity of *M.maderasapatana* among the public may be due to the presence of these phytochemicals in them as it is proved in various studies. For instance, flavonoids have been shown to have antibacterial, anti inflammatory, anti allergic, anti viral activity (Alan and Miller, 1996). Many of these alleged effects have been linked to their known functions as strong antioxidant, free radical scavenger and metal chellators. Steroidal compounds are of importance in pharmacy because of their relationship with compounds used as sex hormones.

Saponins have been reported to show hypocholesterolemic and tumour inhibiting activity on experimental animals (Johns T 1996). Saponins may also enhance nutrient absorption and in animal digestion. Alkaloids often have pharmacological effects and are used as medication and recreational drugs (Roger and Wink, 1998).Tannins and phenols , which together constitute the polyphenolic group are known to have antioxidant, anticancer and antimicrobial activities (Rice *et al*,1996). The occurrence of tannins in M.maderasapatana shows that the plants may be useful in various industries like food, pharmaceutical and leather (Nguji, 1988). Presence of glycosides are reported to influence insulin secretion in vitro (Sumana and Suryawashi SA, 2001).

The results of physicochemical characteristics of *Mukia maderasapatana* reveal that the moisture content was considerably greater in leaf than in the stem (39% Vs 29%) whereas ash content is found to be high in stem than in leaves of *M.maderasapatana* (39% Vs 59%).The crude protein values are considerably significant in leaf ethanolic extract (98.4mg/g) when compared to carbohydrate and other solvent extract. This suggests that the plant is a good

dietary source of the proximate principles. Vitamin C cannot be synthesized by human system and are thus entirely dependent upon dietary sources to meet the needs. Ethanolic and acetone leaf extract of *M.maderasapatana* is found to contain maximum of 75.38 µg of ascorbic acid/mg of extract. The other potentially beneficial antioxidant vitamin i.e. Vitamin E has been determined to the extent of 0.03µg/mg of leaf acetone extract. In addition to its antioxidant activities, vitamin E might be involved in anti inflammatory processes, inhibition of platelet aggregation, enhanced immune function and help prevent or delay coronary heart disease, cancer, age related macular degeneration and neurodegenerative diseases (Ahamed F *et al.*,2010)

Vitamin A is also powerful antioxidants and are considered as preventive factors against cardiovascular diseases, carcinogenesis, rheumatism, Parkinson disease and infertility. In ethanolic leaf extract, the amount of retinol present is found to be 9µg/mg extract. Significant amount of iron, phosphorous and copper is found to be present in leaf and stem of *Mukia maderasapatana*. Maximum amount of iron (34µg/mg extract) is found to be present in aqueous leaf extract. Among the three minerals iron, copper and phosphorous, phosphorous is found to be in higher concentration (200µg/mg leaf extract). Thus the results of the present study provide a basis for the traditional use of this plant as a leafy vegetable.

The antibacterial activity of various extracts of *M.maderasapatana* shows that the acetone fraction of both leaf and stem has pronounced activity at 1000µg/ disc where as the stem hexane extract shows negligible activity. Both the leaf and stem extract showed negligible activity against *Klebsiella pneumoniae*. This means that the extract have no effect on this organism. The ethanolic extract of leaf shows pronounced activity against *E.coli*. The aqueous extract of leaf shows maximum inhibition zone in *S.aureus.* The ethyl acetate extract of leaf shows maximum antibacterial activity against *Klebsiella.*

Thus it shows that the leaf possesses significant antibacterial activity when compared to stem and among the various solvent extract, the ethanolic extract is most active against all studied microorganisms.

The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity. The differential activity might be due to the presence of some oils, wax, rennin or fatty acids in the plant material which may block the plant extract from entering the bacterial cell wall (Parekh *et al.*, 2006). The results of the present study indicate that acetone and ethanol extract of *M.maderasapatana* leaf and stem are high in phenolic content and so exhibit strong antibacterial activity. In addition, such results justify the traditional use of *M.maderasapatana* in various diseases.

Conclusion

The drug derived from the herb *Mukia Maderaspatana* L. may have the possibility of its use in medicine because of its good antibacterial activity. Also this plant could be well integrated into Indian food considering its nutritional potential as observed in the present investigation. However, further research in this pursuit focusing on the isolation of individual compounds from the plant, its characterization and finally subjecting it to clinical trials would confirm it.

"Cite this article"

M.Kavitha,.P.T.Srinivasan, S.L. Priya L.V. Jayakumar "Evaluation of Nutrient Composition and Antibacterial Potential of Leaf and Stem of Mukia Maderaspatana l" Int. J. of Pharm. Res. & All. Sci.2013; Volume 2, Issue 3,68-76

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