

## PHYTOCHEMICAL AND MICROBIOLOGICAL OBSERVATIONS ON

PHYLA NODIFLORA [*LIPPIA NODIFLORA* (L)]V.R. Ravikumar<sup>1\*</sup> and T. Sudha<sup>2</sup>

<sup>1</sup>Department of Pharmacognosy, The Erode College of Pharmacy and Research, Erode, Tamil Nadu, India.

<sup>2</sup>Department of Pharmaceutical Analysis, The Erode College of Pharmacy and Research, Erode, Tamil Nadu, India.

\*Corresponding Author: [ravisrkumar@yahoo.com](mailto:ravisrkumar@yahoo.com)

**ABSTRACT**

*Phyla nodiflora* or *Lippia nodiflora* (L) (verbenaceae) is a perennial herb. In the present study evaluated by the phytochemical analysis for the various secondary metabolites and antimicrobial activity of the stem and leaves extracts of *phylanodiflora* against pathogenic bacteria like gram positive (*Staphylococcus aureus* and *Micrococcus luteus*) and gram negative (*Proteus micrococcus luteus* and *Shigella boydii*). Gentamycin used as the standard drug. In antifungal studies were performing fungus like (*Aspergillus niger* and *Candida albicans*) and Amphotericin-B used as the standard drug. Paper disc method was followed for both studies. It was found that when compared with standard and among the extracts, the ethanol extracts showed a significant antibacterial and antifungal activity.

**Keywords:** Phytochemical, *Micrococcus aureus*, Amphotericin-B, Gentamycin, *Phyla nodiflora*.

**INTRODUCTION**

Man used plants to treat common infections diseases and some of the traditional medicines are still included on part of the habitual treatment of various maladies<sup>1,2</sup>. Scientific interest in medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs and raising concerns about the side effects of modern medicine. The continuing emergence of drug resistant organisms and increasing evolutionary adaptations by pathogenic organisms to commonly used anti microbial have reduced the efficiency of antimicrobial agents currently in use. Therefore the search for new drugs plants continues to be major sources of commercially consumed drugs have their origin from natural plant products<sup>3</sup>. *Phyla nodiflora* is a perennial herb grows in maritime area near rivers through out the subcontinent Africa and other most tropical and sub tropical regions. Areal parts of this plant contain number of medicinally

important compounds such as triterpinoid lippiaein and a benzofuranone rengylone<sup>4</sup>, steroid<sup>4, 5</sup>, dimethoxy benzoxyl stigmesterol<sup>5</sup>. The plant is used as gastro protective effect<sup>6</sup>, anti inflammatory, antineoplastic<sup>7</sup>, antioxidant<sup>8</sup> and diuretic<sup>9</sup>. The present investigation is carried out to find out the antimicrobial activity and anti fungal activities of the stem and leaves extracts were estimated by paper disk method.

**MATERIALS AND METHODS**

The plant *Phyla nodiflora* was collected from the lands of Anaikatti hills in Coimbatore district. Tamil nadu and identified by Dr.G.V.S Murthy, Joint director, Botanical survey of India, Southern regional center, TNAU campus, Coimbatore Tamilnadu. The stem and leaves of the plant were removed carefully. Shaken to remove unwanted particles like sand and soil drying was done under shade by spreading the stem and leaves

over the paper on a wooden table for one week. The powdered stem and leaves powder was extracted with nonpolar to polar solvents like petroleum ether, ethanol and water. The yield of the different extracts is 1.79%w/w, 12.52%w/w, 11.15%w/w respectively. The extracts were used to determine the phytochemical constituents and anti microbial activity.

#### Phytochemical Screenings

Preliminary phytochemical screening was performed as per standardized procedure<sup>10, 11</sup> the various phyto constituents in petroleum ether; ethanol and aqueous extracts were identified.

#### Antimicrobial Screening

The extracts were subjected to antibacterial (*S.aureus*, *M.luteus*, *P.mirabilis*, *Shigella boydii*) and antifungal (*Aspergillus niger*, *Candida albicans*). The anti microbial screening was performed by agar diffusion method using a paper disc<sup>12, 13</sup>. The sterilized (autoclaved at 120 °C for 30 min) medium was inoculated with the suspension of the micro organisms. The paper impregnated with the extracts (1000µg/ml) was placed on the solidified medium. The petri dishes were pre incubated for one hour at room temperature and incubated at 37°C for 24hrs and 48 hrs for antibacterial and antifungal activity respectively. Gentamycin (1000µg/ml) used as a standard for anti bacterial and anti fungal activities respectively.

#### Separation of the Compound

The compound present in *Phyla nodiflora* were quantitatively analyzed by using TLC which is commercially available. The aluminum sheets with silica gel -60F254 were used. The isolation and separation of steroids, carbohydrates and alkaloids was done<sup>14</sup>. The result was reported in Table 1.

## RESULTS

#### Phytochemical Screening

The results of the phytochemical screening revealed the presence of alkaloids, fats, fixed oils, proteins and amino acids. In petroleum

ether, ethanol and aqueous fractions while glycosides, carbohydrates, gums, mucilage, flavors and flavanoids presence in ethanol and aqueous fractions. Steroids presence only in petroleum ether fractions (Table 2).

#### Anti-Microbial Activity

Anti microbial activities of the petroleum ether, ethanol and aqueous fractions are summarized in tab3&4. The ethanol extracts showed zone of inhibition ranging from 3-12mm against all the test organisms. The zone of inhibition produced by petroleum ether fraction ranged from 6-10mm produced by all the test organisms. The zone of inhibition produced by aqueous fraction ranged from 10-12mm against all the test organisms except *Staphylococcus aureus*, *Micrococcus luteus*.

## DISCUSSION

The results of phytochemical screening of the ethanol extracts, petroleum ether and aqueous fraction revealed the presence of alkaloids, carbohydrates, phenolic compounds, proteins amino acids and flavonoids. These metabolites have been reported to possess antimicrobial activity<sup>15</sup>.

The ethanol extracts showed significant anti bacterial activity against the entire organism. Petroleum ether fractions show moderate activity against all the organisms. Aqueous fractions did not active against positive bacterial species. It is important to note that the strong activity of ethanol extract and the petroleum ether and aqueous fractions against all the organisms that indicate that the plant serves as a source of an anti fungal agent. The anti microbial activity of the ethanol and petroleum ether fraction suggest the presence of bio active compounds which serve as anti microbial agent or lead compound for the synthesis of an effective and less toxic antimicrobial agent.

## CONCLUSION

The study showed that the ethanol extract and petroleum ether from the leaves and stems of *Phyla nodiflora* have anti microbial properties.

**Table 1: TLC of petroleum ether, ethanolic and aqueous extracts of *Phyla nodiflora***

S. No.	Extracts	Color of spots observed				Rf value		
		Day light	UV		Iodine chamber	Spot1	Spot2	Spot3
			Short $\lambda$	Long $\lambda$				
1	Petroleum ether	Green	Green	Yellowish Brown	Yellowish Brown	0.99	0.99	0.98
2	Ethanol	Green	Green	Brown	Brown	0.99	0.98	0.97
3	Aqueous	Green	Green	Brown	Brown	0.99	0.99	0.98

**Table 2: Preliminary Phytochemical analysis of *phyla nodiflora*(L) with different solvents**

S. No.	Name of the compound	PE	E	W
1	Alkaloids	+	+	+
2	Carbohydrates	-	+	+
3	Glycosides	-	+	+
4	Steroids	+	-	-
5	Fixed oil	+	+	+
6	Fats	+	+	+
7	Tannins&Phenolic compounds	-	+	+
8	Proteins	+	+	+
9	Amino acids	+	+	+
10	Gums	-	+	+
11	Mucilages	-	+	+
12	Flavours	-	+	+
13	Flavonoids	-	+	+

+ -present, (-) absent, PE- petroleum ether, E- ethanol, W- water.

**Table 3: Anti bacterial activity profile of three extracts of three extracts from the stem and leaves of *Phyla nodiflora***

Test bacteria	Zone of inhibition(mm)					
	Ethanol 1000 $\mu$ g/ml	Standard gentamycin 1000 $\mu$ g/ml	Petroleum ether 1000 $\mu$ g/ml	Standard gentamycin 1000 $\mu$ g/ml	Aqueous 1000 $\mu$ g/ml	Standard gentamycin 1000 $\mu$ g/ml
<i>Staphylococcus aureus</i>	6	20	6	22	0	25
<i>Micrococcus luteus</i>	10	27	12	24	0	24
<i>Proteus mirabilis</i>	8	25	4	23	12	24
<i>Shigella boydii</i>	7	26	3	22	10	23

**Table 4: Anti fungal activity profile of this extracts from the stem and leaves of *phyla nodiflora***

Test Fungus	Zone of inhibition(mm)					
	Petroleum ether 1000g/ml	Standard Gentamycin 1000 $\mu$ g/ml	ethanol 1000 $\mu$ g/ml	Standard Gentamycin 1000 $\mu$ g/ml	Aqueous 1000 $\mu$ g/ml	Standard Gentamycin 1000 $\mu$ g/ml
<i>Aspergillus niger</i>	12	18	13	18	12	18
<i>Candida albicans</i>	10	17	14	17	11	19

**REFERENCES**

1. Heinrich and Simon Gibbons S. Fundamental of pharmacognosy and phototherapy, Churchill Livingstone, Edinburgh, 2004:243-247.
2. Rios and Recio. Medicinal plants and antimicrobial activity. Journal of Ethno pharmacology. 2005;100:80-84.
3. Sofawara EA. Medicinal plants and traditional medicines in Africa, John Wiley &sons Ltd Nigeria, 1982:64-79.
4. Bina S. Siddiqui, Fayeze Ahmed, Fouzia A. Sattar and Sabira Begum. Chemical constituents from the aerial parts of *Lippia nodiflora* linn. Archives of pharmacol Research. 2007;30:1507- 1510.
5. Bina S. Siddiqui, Fayeze Ahmed, Kashif Ali, Sobiya perwaiz and Sabira Begum. Steroidal constituents from the aerial parts of *Lippia nodiflora* Linn. Natural product research. 2009:436-441.
6. Khalil. H, Ismail H, Taye A and Kamel M. Gastro protective effect of *Lippia nodiflora* Linn extracts in ethanol induced gastric lesions. Pharmacognosy magazine. 2007;3(12):258-261.
7. Ahmed F, Selim MST, Das AK and Chouduri MSK. Anti-inflammatory and antineoceptive activities methanolic extract of *Lippia nodiflora* Linn. Die pharmazie. 2004;59(4):329-30.
8. Durairaj Ashokkumar, vaiyapuri Tamilselvan, Upal kanti Mazumder and Malaya Gupta. Antioxidant and free Radical Scavenging Effects of *Lippia nodiflora*. Pharmaceutical biology. 2008;46(10-11):762-771.
9. Sangita Shukala, Rashmika patel and Rajiv Kukkar. Study of phytochemical and diuretic potential of methanol and aqueous extracts of aerial parts of *Phyla nodiflora* linn. International journal of pharmacy and pharmaceutical sciences. 2009;1(1):85-91.
10. Kokate CK. Text book of Pharmacognosy. Nirali prakasan, Pune, 2004; 29th Edn: 108-109.
11. Kokate CK. Practical Pharmacognosy. Vallabh prakasan, New Delhi, 2005; 4th Edn: 107- 109.
12. The Indian Pharmacopoeia. The controller of publication, Government of India, New Delhi, Appendix A, 1996; A100-108.
13. Balamurugan G and Muthusamy P. Antimicrobial studies on *Trianthema portulacastrum* linn extracts. Indian drugs. 2009;46(13):250-252.
14. Khandelwal KR. Practical pharmacognosy. Nirali prakashan publication, NewDelhi. 2004;11th Edn: 184.
15. Cowan MM. Plants products as antimicrobial agents. Clinical microbiology Review.1999;12(4):564-582.