Research Article

Phytochemical Finger Printing and Antimicrobial Activity of *Phyllanthus niruri*

Rehana Anjum Shah*, Shahana Khan, Pooja D. Sonawane, Wahida Rehman
Department of Botany, The Institute of Science, 15, Madame Cama Road Mumbai, India.
***Corresponding author's E-mail:** shahrehanaa@yahoo.com

Received: 29-11-2016; **Revised:** 09-02-2017; **Accepted:** 22-05-2017.

ABSTRACT

The medicinal plants represent an enormous reservoir of potential phytochemical compounds that could be useful as an alternative to allopathic drugs and are being used to develop Pharma drugs. This study was carried out to evaluate the phytochemical finger printing and antimicrobial analysis of *Phyllanthus niruri.* The whole plant was analyzed for proximate and mineral element compositions. The results indicate that phytochemical analysis of *Phyllanthus niruri* with three different solvent extracts revealed the presence of Alkaloids, Flavonoids, Glycosides and Saponins. In the present study *P.niruri* showed antimicrobial potent activity against bacterial as compared to fungal strains. The methanolic extracts exhibited antimicrobial activity of *Phyllanthus niruri* on *E.coil* with increase in the concentration of compound there is an increases in the zone of inhibition. *Aspergillus niger and Aspergillus flavus* showed moderate inhibition zone. *Bacillus subtilis* were not inhibited by the extract of the plant.

Keywords: Phytochemical fingerprinting, antimicrobial, *Phyllanthyus niruri.*

INTRODUCTION

The plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. *Phyllanthus niruri* originated in India, usually occurring as a winter weed throughout the hotter parts. The *Phyllanthus* genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas. *Phyllanthus niruri* has medicinal properties for the effective management of several aliments including Hepatitis. *Phyllanthus niruri* is an herb of Euphorbiaceae family that grows up to 60 cm. *Phyllanthus* means "leaf and flower" because the flowers, as well as the fruit, seem to become one with the leaf. *Phyllanthus nirui* is a common kharif (rainy season) weed found in both cultivated fields and wastelands. Traditionally this plant is used as an antipyretic, antidiarrhoeal, for healing of cuts and wounds, to relieve irritable bowels, anticancerous and antioxidant. It is also used for treatment of hepatitis B infection and malaria[1]. Phytochemical screening is one of the necessary steps to find out the chemical constituents which lead the isolation of bioactive compounds. Since the middle of the 19th century different bioactive phytoconstituents have been isolated and characterized. They were known to show the medicinal activity as well as physiological activity. Bio molecules of plant origin appear as alternatives for the control of even resistant species of bacteria and human pathogens and their uses have been shown to have a scientific basis [2, 3]. In the last few decades bacterial resistance to antibiotics has become a serious therapeutic problem and the rate at which new antibiotics are being produced is slowing[4]. Thus, the search for novel antimicrobial agents is of the utmost importance[5]. Worldwide attention has been shifted towards finding new herbal chemicals for the development of new drugs. These natural products can provide unique elements of molecular diversity and biological functionality, which is indispensable for novel drug discovery[6, 7].

MATERIALS AND METHODS

Collection of plant sample

Fresh *Phyllanthus niruri* were collected from Palghar district (Maharashtra) and were botanically identified at The Institute of Science, Mumbai.

Solvent extraction of plant material

10gm of dried and powdered plant material was packed in a muslin cloth and placed in separately to a Soxhlet apparatus for extraction in 200ml methanol, chloroform and petroleum ether for 8 and 48 hours[8]. The filtrates were evaporated by using a rotary evaporator to get a viscous mass which was dried further to get accurate mass of the three solvents viz. methanol, petroleum ether and chloroform. The extracts were stored separately at 4^{o}C in labeled amber colored airtight bottles. This extract was used for phytochemical analysis and antimicrobial activity.

Phytochemical fingerprinting

Crude methanol, chloroform, petroleum ether extracts of *Phyllanthus niruri* were analyzed for presence of various phytochemicals using HPTLC method. The analysis was carried out on LINOMAT-V supplied by CAMAG. The scanning and detection were performed at Institute of science, Mumbai. Standard procedure was used for fingerprinting which involved separation and detection saponins, Total glycosides, alkaloids and flavonoids[9, 10]. Observations were recorded at wavelengths ranging from

254 to 366 nm at regular interval of 50 nm, peaks were counted for various range of end Rf, viz.0.01 - 0.10, 0.91-1.0. Various phytochemicals were detected using a specific solvent system (Table 1).

Table 1: Specific solvent systems

Sr. no.	Phytochemicals	Solvent system
1	Alkaloids	Toluene: Ethyl acetate : Diethyl amine (7 : 2 : 1)
2	Total glycosides	Chloroform : Methanol (9 : 1)
3	Flavonoids	Ethyl acetate :Formic acid :Glacial acetic acid 1 : 1.1 :1.1 : 2.6)
4	Saponins	$CHCl_3$: Glacial acetic acid : MeOH : water (6.4 : 3.2 :1.2 : 0.8)

Antimicrobial Activity

Preparation of disc for antibacterial and antifungal activity

5mm diameter discs were prepared using sterile Whatman No. 1 filter paper. The discs was impregnated with 20 ul of methanolic extracts *Phyllanthus niruri* at four different concentrations of 2mg/ml, 4mg/ml, 6mg/ml and 8mg/ml to check their antibacterial and antifungal activity.

Collection of Microbial Cultures

Bacterial cultures of *Bacillus subtilis, Escherichia coli* and the fungal cultures of *Aspergillus niger*, *Aspergillus flavus were* obtained Department of Microbiology and Department of Botany, The Institute of Science, Mumbai respectively.

Determination of antibacterial activity of *Phyllanthus niruri*

Preparation of Bacterial inoculum

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of nutrient broth and incubated at 37^0C for 3-5 hours till the moderate turbidity was developed.

Disc diffusion method

The antibacterial activity of the methanolic plant extract of *Phyllanthus niruri* was determined by disc diffusion method [11]. Petri plates were prepared by pouring 20 ml of Nutrient agar and allowed to solidify. 1ml of the inoculum as prepared above were poured and uniformly spread on the surface of agar. The excess inoculum was drained. After drying the discs with the extracts were place on the surface of the plates with sterile forceps and pressed gently to ensure contact with the agar. The plates were incubated at 37^0Cfor 24 hours. The zone of inhibition was observed and measured in millimeter.

Determination of Antifungal activity

Preparation of Fungal inoculum

Fungal inoculums was prepared by inoculating a loopful of test organisms in 5 ml of sterile saline and incubated at 37^0C for 3-5 hours till the moderate turbidity was developed. The medium was autoclaved at 120lbs for 20 min.

Disc diffusion method

The antifungal activity of methanolic plant extract of *Phyllanthus niruri* was determined by disc diffusion method [11]. Petri plates were prepared by pouring 20 ml of Sabouraudand Dextrose agar and allowed to solidify.1 ml of the inoculum as prepared above were poured and uniformly spread on the surface of agar. The excess inoculum was drained. After drying the disc with the extract were placed on the surface of the plates with sterile forceps and pressed gently to ensure contact with the agar. The plates were incubated at 37^0Cfor 48-72 hours. The zone of inhibition was observed and measured in millimeter.

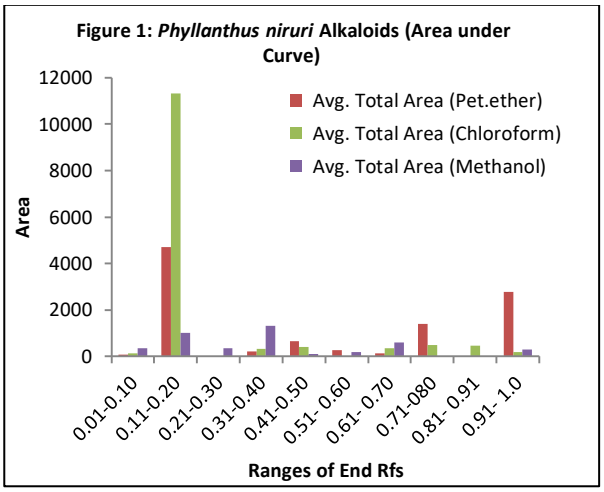
RESULTS AND DISCUSSION

Phytochemical finger printing of *Phyllanthus niruri*

Phytochemical finger printing of methanol, chloroform and petroleum ether plant extracts of *Phyllanthus niruri was* done using HPTLC. The results obtained were analyzed using ANOVA. Phytochemical analysis of *Phyllanthus niruri* showed presence of Alkaloids, Flavonoids, Glycosides and Saponins.

Alkaloids

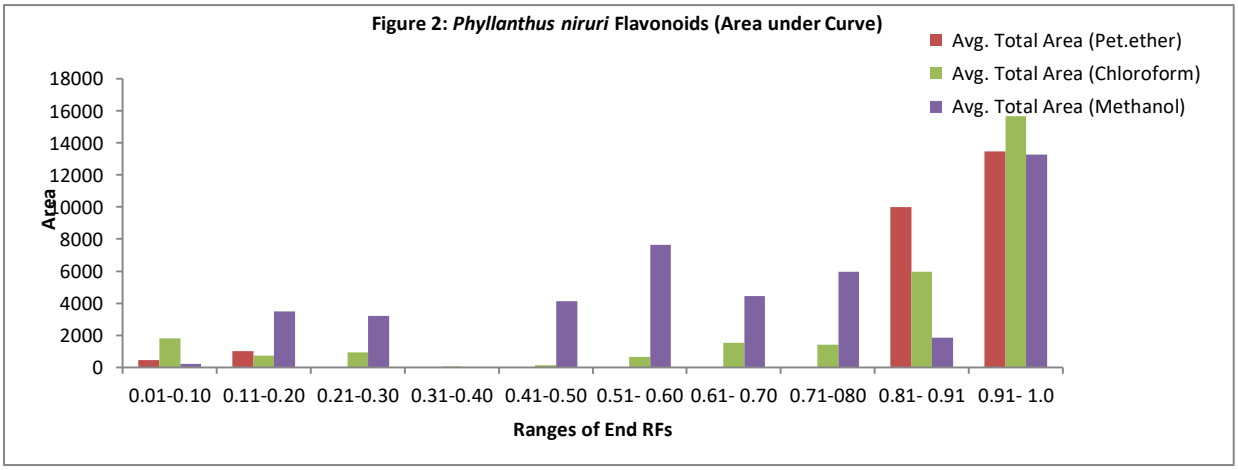
Separation of alkaloids was performed using Toluene: Ethyl acetate: Diethyl amine (7: 2: 1) as solvent system. Maximum number of peaks (5) for petroleum ether was obtained in the Rf range of 0.11 - 0.20 and 0.61-0.70 and the area cover under the peak for 0.11-0.20 was highest.



Maximum number of peaks (4) for chloroform was obtained in the Rf range of 0.11 - 0.20 and 0.91 - 1.0 and the corresponding area under the peak was highest and lowest for the same. Maximum number of peaks (10) for methanol was obtained in the Rf range of 0.01 - 0.10. The maximum area under the peak for methanol obtained for Rf range 0.11 - 0.20 for 3 peaks (Fig. 1).

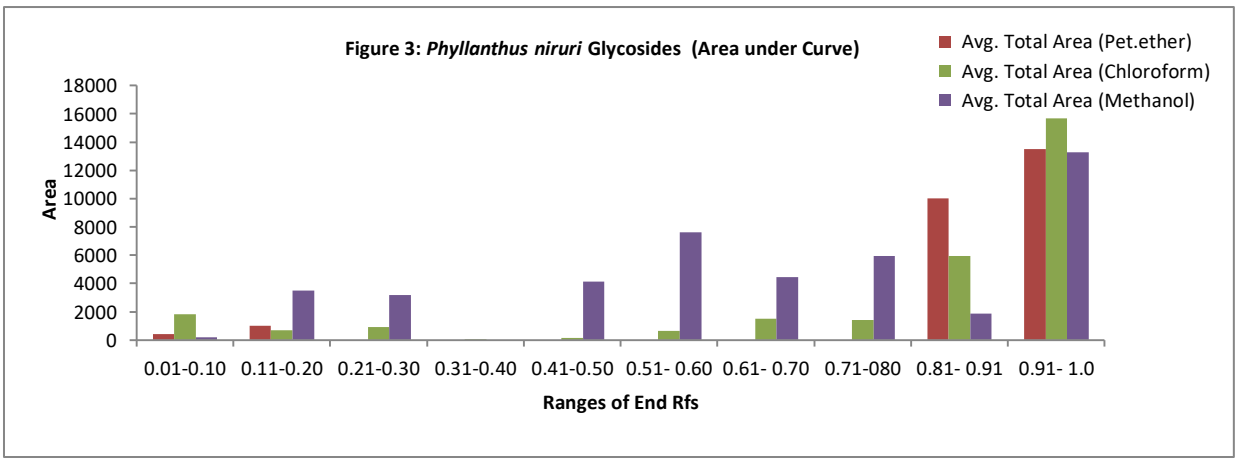
Flavonoids

Separation of flavonoids was achieved using Ethyl acetate: Formic acid: Glacial acetic acid (1:1.1:1.1:2.6) as a solvent. Maximum number of peaks (4) for pet ether was obtained in the Rf range of 0.01 - 0.10, 0.11 - 0.20 and 0.91-1.0 and the area cover under the peak for 0.91 - 1.0 was highest. Maximum number of peaks (4) for chloroform was obtained in the Rf range of 0.01 - 0.10, 0.41- 0.50 and 0.91-1.0 the area cover under the peak for 0.11 - 0.20 was highest. Maximum number of peaks (4) for methanol was obtained in the Rf range of 0.11 - 0.20, 0.21 - 0.30, 0.41- 0.50, 0.51-0.60, 0.71-0.80 and0.91-1.0 and the area cover under the peak for 0.91 - 1.0 was highest (Fig. 2).



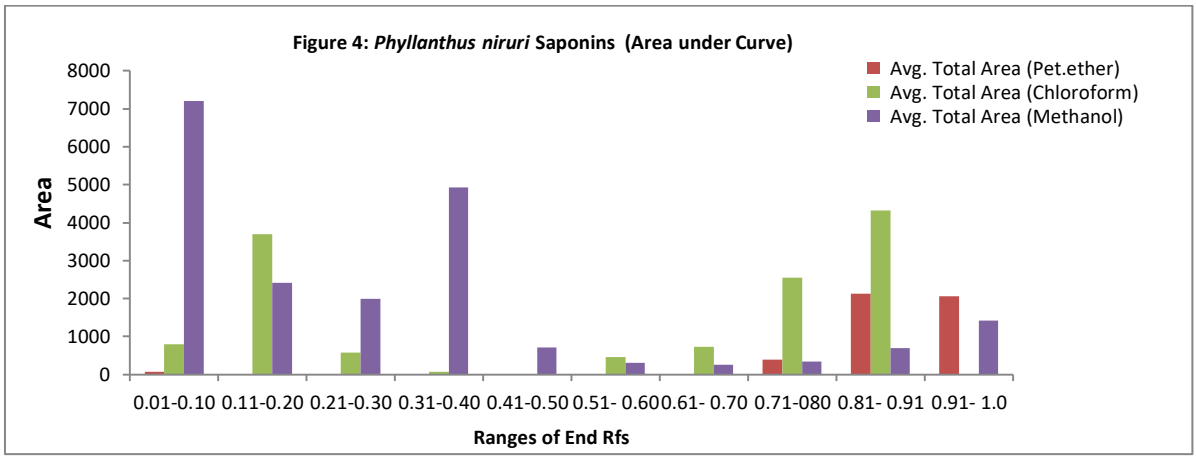
Glycosides

For total glycosides the solvent system used was chloroform: Methanol (9:1). The results in petroleum ether obtained are maximum number of peak (4) even though qualitatively average area showed that maximum area in the range (3) 0.81-0.91. Maximum number of peaks (7) for chloroform was obtained in the RF range of 0.61-0.70 and the area cover under the peak for 0.81-0.91 was highest. Maximum number of peaks (5) for methanol was obtained in the Rf range of 0.11-0.20 and the area cover under the peak for 0.91-1.0 was highest (Fig. 3).



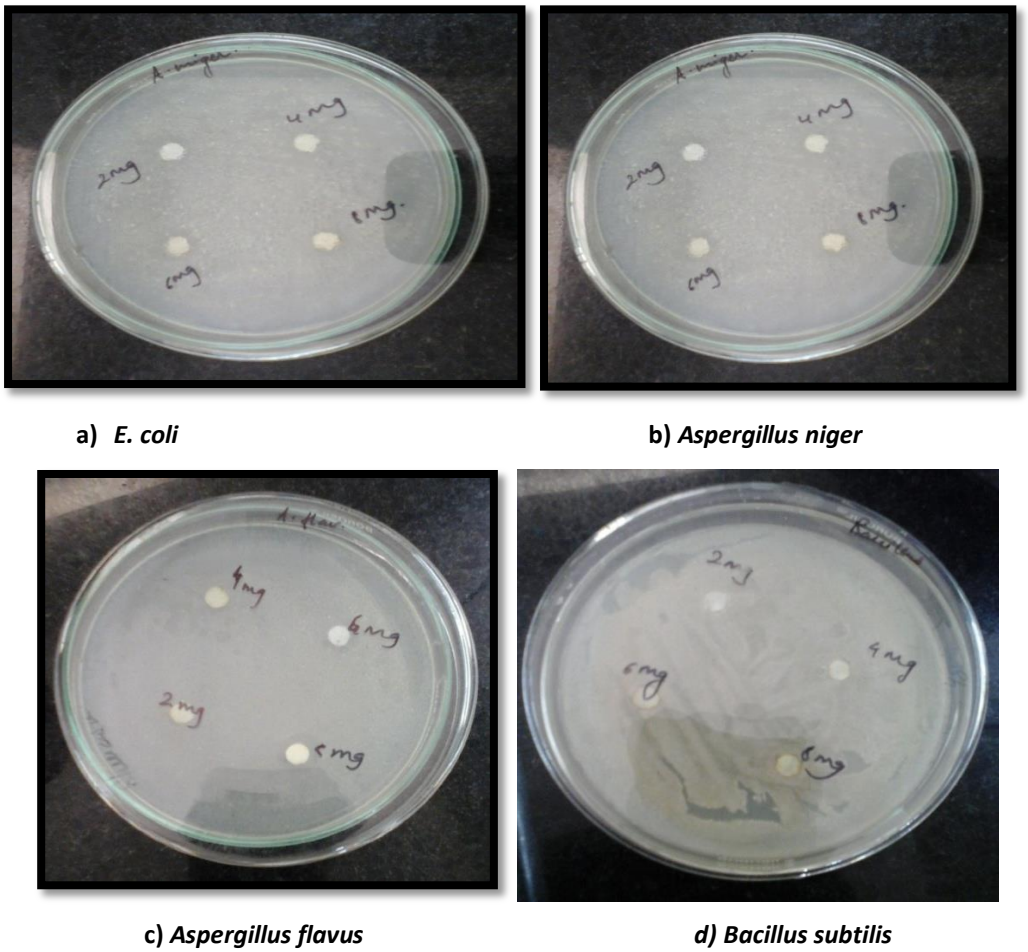
Saponins

Separation of saponins was achieved using chloroform: Glacial acetic acid: methanol: water (6.4: 3.2: 1.2:0.8) as solvent. Maximum number of peaks (6) for pet ether was obtained in the Rf range of 0.01 - 0.10 and the area cover under the peak for 0.81 to 0.91 was highest. Maximum number of peaks (6) for chloroform was obtained in the Rfrange of 0.01 - 0.10, 0.61 - 0.70 and 0.71-0.80 and the area cover under the peak for 0.81 - 0.91 was highest. Maximum no. of peaks (5) for methanol was obtained in the Rf range of 0.11 - 0.20, 0.61 - 0.70 and the area cover under the peak for 0.01 - 0.10 was highest (Fig. 4).



Antimicrobial activity of crude extracts

Antimicrobial activity of *Phyllanthyus niruri* on *E. coil* with increase in the concentration of compound there is an increase in the zone of inhibition. *Aspergillus niger and Aspergillus flavus* showed moderate inhibition zone. *Bacillus subtilis* were not inhibited by the extract of the plant (Fig. 5).



a) *E. coli* **b) *Aspergillus niger***

c) *Aspergillus flavus* ***d) Bacillus subtilis***

Figure 5: Antimicrobial activity of methanolic leaf extract of *Phyllanthus niruri*

DISCUSSION

Phytochemicals screening of *Phyllanthus niruri* was performed by using methanolic extracts of various plant parts [12]. The qualitative and quantitative phytochemical screenings of different plant parts of P. amarus with water, methanol, ethyl acetate and petroleum ether have been reported [2]. Similar studies have been reported in *Phyllanthusfraternus*[13] and different species of *Phyllanthus* leaf extracts for preliminary phytochemical evaluation[14]. *Withania somnifera* Phytochemical fingerprinting of leaf and root extracts by HPTLC shows presence of secondary metabolites [15]. This led to the new search for drugs and dietary supplements derived from plants. During the last 10 years pace of development of new antimicrobial drugs has slowed down, while prevalence of resistance has increased multifold [16]. The

problem of microbial resistance of growing and outlook for the use of antimicrobial drugs in future is still uncertain therefore, action must be taken to reduce this problem, such as controlling the use of antibiotics and carrying out research for better understanding of genetic mechanism of resistance. This prompted to evaluate plants as source of potential chemotherapeutic and antimicrobial agent along with their ethno medicinal use[17]. Earlier attempts on antimicrobial activity on other species of *Phyllanthus* [18,19] have shown promising results against variety of microbial flora. In the present investigation initial screenings of the experimental plant for possible antimicrobial activities was done using crude methanolic extracts. Nearly all of the identified components from plants that are active against microorganisms are aromatic or saturated organic compounds.

CONCLUSION

The maximum quantity of Alkaloids and Saponins in plant extract is present in methanolic extract. The maximum quantity of Flavonoids and Glycosides was found in chloroform extract. Antimicrobial activity of *Phyllanthus niruri* showed *Bacillus* proved to be resistance to the plant extract. *E.coli* was most sensitive to methanolic extract of *Phyllanthus niruri*. *Aspergillus niger* was showed antimicrobial activity against the *Phyllanthus niruri. Aspergillus flavus* was showed moderated activity against the *Phyllanthus niruri.* There is still a lot of scope for further research, especially towards the mechanism of biological activity of phytochemicals and antimicrobial activity from this plant.

Acknowledgements: The authors are thankful to the Department of Microbiology, The Institute of Science, and Mumbai for providing cultures.

REFERENCES

1. Bharat gami L, Kothari IL, Antioxidant and Antimicrobial activity of *in vivo* and *in vitro* grown plants of *Phyllanthus niruri,* International Journal of Pharma and Bio Sciences, 2(B), 2011, 78-89.
2. Awasthi A, Singh R, Agrawal MK, Qualitative and quantitative phytochemical screening of different plant parts of *Phyllanthu samaras* Schum. And Thonn. Collected from central India with respect to the traditional claims for their medicinal uses. International Journal of Pharmaceutical Sciences and Research, 6, 2015, 393-398.
3. Mathias AJ, Somashekar RK, Sumithra S, Subramanya S, Antimicrobial Agents, Indian Journal of Microbiology, 40, 2000, 183–190.
4. Russell AD, Antibiotic and biocide resistance in bacteria: Introduction Journal of Applied Microbiology Symposium Supplement, 92, 2002, 1S–3S.
5. Gootz TD, Discovery and development of new antimicrobial agents. Clinical Microbiology Reviews, 2(7), 1990, 176-181.
6. Nisbet LJ, Moore M, Will natural products remain an important source of drug research for the future? Current Opinion in Biotechnology, 8, 1997, 706-712.
7. Ummey N, Zakaria A, Antimicrobial Activity of *Phyllanthus emblica* and *Allium sativum*: Comparative Analysis of Antimicrobial Action of Crude and Ethanolic Extract of These Natural Plant Products, IOSR Journal of Pharmacy and Biological Sciences, 4, 2014, 21-26.
8. Duke JA, Wain KK, The Medicinal Plants of the World, Computer index with more than 85,000 entries, 3, 1981, 1654.
9. Sofowora A. Medicinal Plants and Traditional Medicine in Africa, University of Ife Press, Nigeria, 1984, 1-23.
10. Kanchanapoom T, Kasai R, Yamasaki K, Iridoid and phenolic diglycosides from *Canthiumber beridifolium*, Phytochemistry, 61, 2002, 461–464.
11. Bauer AW, Kirby WMM, Serris JC, Turk M, Antibiotic susceptibility testing by a standard method, American Journal of Clinical Pathology, 45, 1966, 493-496
12. Mathur M, Sharma R, Sharma J, Pareek R, Kamal R, Phytochemical screening and antimicrobial activity of *Phyllanthus niruri* Linn, 2012, 8487-8489.
13. Kavit M, Patel BN, Jain BK. Phytochemical analysis of leaf extract of *Phyllanthusfraternus*,Research Journal of Recent Sciences, 2, 2013, 12-15.
14. Gopinath SM, Rakesh CK, Murthy TP, Dayananda KS, Preliminary phytochemical evaluation of leaf extracts of *Gymnema sylvestre, Phyllanthus amarus, Phyllanthus reticulates* of Siddarabetta, Tumkur district, Karnataka, International Journal Pharmacognosy and Phytochemical Research, 3, 2012, 109-111.
15. Shah RA, Khan S, Evaluation of Withaferin-A in Ashwagandha's Leaves from Different Climatic Regions of India, Advances in Plant Sciences. 25(II), 2012, 709-711.
16. Akinpelu DA, Onakaya TM, Antimicrobial activities of medicinal plants used in folklore remedies in South-Western, African Journal of Biotechnology, 5(11), 2006, 1078-1081.
17. Prashanth KN, Neelam S, Chauhan S, Harishpadhi B, Ranjani M, Search for antibacterial and agents from selected Indian medicinal plants, Journal of Ethno pharmacology, 107, 2006, 182-188.
18. Akinjogunla OJ, Eghafona NO, Enabulele IO, Mboto CI,Ogbemudia FO, Antibacterial activity of ethanolic extracts *of Phyllanthus amarus* against extended spectrum β-lactamase producing *Escherichia coli* isolated from stool samples of HIV sero-positive patients with or without diarrhea, African Journal ofPharmacy& Pharmacology, 4(6), 2010, 402-407.
19. Rajeshwar Y, Ahmad R, Sunder AS, Devilal J, Gupta M, Mazumdar UK, In vitro lipid peroxidation inhibitory and antimicrobial activity of *Phyllanthus niruri* extract, Iranian journal of pharmacology & therapeutics. 7(1), 2008, 67-70.

Source of Support: Nil, **Conflict of Interest:** None.