Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

# *PHYLLANTHUS WIGHTIANUS* MÜLL. ARG: A POTENTIAL SOURCE FOR ANTIBACTERIAL AND PHYTOCHEMICAL ANALYSIS

**D. NATARAJAN[1]* R. SRINIVASAN[1] AND M. S. SHIVAKUMAR[2]**

**[1]Natural Drug Research Laboratory, [2]Molecular Entomology Laboratory, Department of Biotechnology, Periyar University, Salem 636011, Tamil Nadu, India.**

*Received: 16 July 2012, Revised and Accepted: 29 Aug 2012*

**ABSTRACT**

*Phyllanthus wightianus* Müll. Arg. is belongs to Euphorbiaceae family used in folklore medicines. Different solvent (methanol, chloroform, dichloromethane, acetone, ethyl acetate, petroleum ether and hexane) leaf extracts of *P. wightianus* were evaluated for antibacterial activity against 13 human bacterial pathogens (*Bacillus subtilis*, *Corynebacterium diphtheriae, Staphylococcus aureus*, *Enterococcus faecalis, Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli, Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei, Vibrio alginolyticus* and *V. vulnificus*) by agar well and disc diffusion methods. The preliminary phytochemical analysis of the extracts was carried out by standard methods. The results showed all these plant extracts exhibited various levels of antibacterial activity on different test organisms. Remarkable antibacterial activity was noticed in acetone extract. Phytochemical screening of different extracts of *P. wightianus* shows that the presence of saponin, phenols, tannin, glycoside, flavonoid, steroid, alkaloid and oils. HPLC analysis revealed that the identity of bioactive constituents present in plant extracts. FT-IR spectral data reflects functional groups of chemical components present in the methanolic extract of *P. wightianus*. The results from these investigation encourages that the plant extracts may be used as anti-infective agents.

**Keywords**: *Phyllanthus wightianus*, Agar well diffusion, Disc diffusion, Phytochemical, HPLC, FT-IR.

## INTRODUCTION

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in developing countries[1]. Therefore there is an urgent need to discover an alternative new, more active, broad spectrum, and safer antimicrobial agents[2,3]. Plant materials remain an important resource to combat serious diseases in the world. Pharmacognostic investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents[4] because of the side effects and the resistance against many synthetic antibiotics to the indiscriminate use of synthetic antimicrobial drugs[5]. Recent attention has been paid to extract and isolate the biologically active compounds from plant species used in herbal medicines[6].

*Phyllanthus wightianus* is a monoecious subshrub, branchlets in close spirals, leaves are alternate, distichous, elliptic to oblong, dark green above, glaucous below. It occurs in the hills (750 to 1000 m) of peninsular India, on the floor and border of shoals7. It possesses a wide spectrum of biological activities *viz.* antimicrobial8,9 analgesic10 wound healing11 and antioxidant properties12. The present study was focused on the antibacterial properties of different solvents extracts from the pulverized leaves of *P. wightianus.*

## MATERIALS AND METHODS

### Plant material

Leaves of *P. wightianus* were collected from the higher altitudes of Kolli-hills, a part of Eastern Ghats, Tamil Nadu, India. The nomenclature of plant material was identified by Dr. D. Natarajan, Assistant professor, Department of Biotechnology, Periyar University, Salem, Tamil Nadu. The voucher specimen has been deposited in the Natural Drug Research Laboratory (NDRL), Department of Biotechnology, Periyar University, Salem, India.

### Preparation of the extracts

Freshly collected leaves were washed, shade-dried and powdered. The powdered leaves were extracted separately with methanol, chloroform, dichloromethane, acetone, ethyl acetate, petroleum ether and hexane in a soxhlet apparatus and the extract was evaporated in vacuum at 40°C to yield a dark greenish mass extracts. This paste was stored at 4ºC for further study.

### Microorganisms used

Four Gram positive (*Bacillus subtilis*, *Corynebacterium diphtheriae, Staphylococcus aureus*, *Enterococcus faecalis*) and nine Gram negative bacterial strains (*Klebsiella pneumoniae*, *Salmonella typhi*, *Escherichia coli, Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei, Vibrio alginolyticus* and *V. vulnificus*) were used in the entire investigation. All the bacterial cultures were obtained from clinical laboratories of Salem District, Tamilnadu. The broth cultures of each test organism was prepared by inoculating a loop-full of culture in a 5 ml of nutrient broth and incubated at 37°C for 14 to 16 hours.

### Antibacterial assay

### Agar well diffusion method

Agar well diffusion method was employed as per the modified method of Natarajan *et al*[13]. A suspension of test organisms (0.1ml) was swabbed on the Muller Hinton Agar (MHA) by using sterile cotton swab. Thereafter, a sterile cork borer (5 mm diameter) was used to made wells in the seeded Müller Hinton agar. Then, 50µl of each extract was separately delivered into wells and allowed to diffuse at room temperature. Equal volumes of DMSO and 25µl of ciprofloxacin (0.1µg/µl) were served as negative and positive control. The plates were incubated at 37°C for 24 hours and the zone of growth inhibition was measured in mm**.**

### Disc diffusion method

The disc diffusion test was performed by using the standard procedure of NCCLS with some modifications. About 0.1ml of test microbial suspensions was spread on the MHA. Sterile discs (5 mm diameter) were loaded with 50µl of each extracts and allowed to dry. The discs were placed on the inoculated MHA plates. The plates were incubated for 24 hours at 37° C and the diameter (in mm) of clear zone of growth inhibition were recorded.

### Phytochemical screening

The extracts were further subjected to determination of phytochemical constituents *i.e.* alkaloids, flavonoids, tannins, saponins, steroid, glycoside, reducing sugar, phenolic compound and fixed oils using the methods of Evans *et al*[14].

### HPLC Analysis

HPLC analysis of methanolic extract of *P. wightianus* was performed using the methodology of Murali *et al*[15]. About 1mg of concentrated

sample was dissolved in 1ml of methanol and 20µl was injected and the UV absorbance was recorded at 254nm to determine the phyto-constituents. The experiment was performed in Waters Pump Model No: 1515, an instrument equipped with a Waters Model No: 2487 UV detector in order to determine the peak purity. LCGC C18 column (25cm×4.6mm) was used for isocratic resolution using methanol:water (50:50 v/v) as the mobile phase at a flow rate of 1.0Ml min-1. Using the detector waters model No: 2487, UV detector absorbance was recorded at 254nm.

**FT-IR Analysis**

FT-IR analysis of methanolic extract of *P. wightianus* was performed using the modified methodology of Natarajan *et al*[16]. ART model FT-IR Spectrophotometer was used for the analysis of methanolic extract of *P. wightianus*. The spectrum was focused in the mid IR region of 400-4000cm$^{-1}$ by the KBr pellet technique. The spectrum was recorded using Attenuated Total Reflectance technique beach measurement.

**RESULTS AND DISCUSSION**

The results of antimicrobial activity of leaf extracts of *P. wightianus* were done by agar well diffusion and disc diffusion methods (Table 1 and 2). The results showed agar well diffusion method is an ideal for assay of antimicrobial tests than disc diffusion method. Acetone extract of *P. wightianus* contributed significant activity against all tested pathogens followed by methanol extract. Ethyl acetate and chloroform extracts expressed moderate activity. Hexane and water extracts showed least activity against all tested pathogens. The results of preliminary phytochemical analysis of *P. wightianus* showed the presence of saponin, phenols, tannin, glycoside, flavonoid, steroid, alkaloid and oils (Table 3). HPLC spectrum of methanolic leaf extract of *P. wightianus* expresses the highest peak value was observed as 2.653 and 2.860 respectively. Two values having smaller peaks (6.628, 7.685) and rest of the peaks which falls in between the low to highest peak values (Fig. 1). The results of FT-IR analysis to identify the functional group of the active components, based on peak value of the infrared radiation (Fig. 2; Table 4 and 5).

**Table 1: Antibacterial activity of different solvent extracts of *P. wightianus***

| S. No. | Organisms | Agar well diffusion method Diameter of zone of inhibition (in mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Acetone | Methanol | Water | Petroleum ether | Chloroform | Hexane | Ethyl acetate | Dichloro methane | Control |
| 1 | *C. diphtheriae* | 7 | 11 | - | 8 | 8 | 6 | 8 | 8 | 21 |
| 2 | *V. vulnificus* | 12 | 12 | - | 8 | 12 | 7 | 11 | 10 | 35 |
| 3 | *B. subtilis* | 10 | 13 | - | 9 | 10 | 9 | 10 | 8 | 21 |
| 4 | *V. alginolyticus* | 11 | 14 | - | 9 | 7 | 8 | 8 | 8 | 30 |
| 5 | *E. faecalis* | 20 | 16 | 14 | 8 | 12 | 10 | 10 | 11 | 30 |
| 6 | *S. flexneri* | 18 | 22 | 23 | 12 | 18 | 14 | 14 | 12 | 27 |
| 7 | *E. coli* | 7 | 8 | - | - | - | - | - | - | - |
| 8 | *S. typhi* | - | 7 | - | 9 | - | 7 | - | 8 | 24 |
| 9 | *K. pneumoniae* | - | - | - | - | - | - | - | - | - |
| 10 | *S. sonnei* | 12 | 11 | - | 7 | 9 | 8 | 7 | 8 | 19 |
| 11 | *S. boydii* | 10 | 13 | - | 11 | 10 | 7 | 8 | 10 | 24 |
| 12 | *S. dysenteriae* | - | - | - | 11 | 8 | 7 | 9 | 10 | 29 |
| 13 | *S. aureus* | 10 | 12 | - | 8 | 8 | 7 | 10 | 7 | 21 |

Control – Ciprofloxacin (0.1 µg/µl) - = No activity

**Table 2: Antibacterial activity of different solvent extracts of *P. wightianus***

| S. No. | Disc Diffusion method Diameter of zone of inhibition (in mm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Organisms | Acetone | Methanol | Water | Petroleum ether | Chloroform | Hexane | Ethyl acetate | Dichloro methane | Control |
| 1 | *C. diphtheriae* | 10 | - | - | 8 | 6 | - | 7 | - | 7 |
| 2 | *V. vulnificus* | 8 | 7 | - | - | 9 | - | - | - | 22 |
| 3 | *B. subtilis* | 9 | - | - | 7 | - | - | 8 | - | 12 |
| 4 | *V. alginolyticus* | 15 | - | - | - | - | - | - | - | 31 |
| 5 | *E. faecalis* | 9 | 8 | 6 | 6 | 7 | 6 | 7 | 8 | - |
| 6 | *S. flexneri* | 12 | 8 | 8 | 6 | 6 | 7 | 7 | 7 | - |
| 7 | *E. coli* | - | - | - | - | - | - | - | - | - |
| 8 | *S. typhi* | 10 | 7 | - | 9 | - | 7 | - | 8 | 12 |
| 9 | *K. pneumoniae* | 8 | - | - | - | - | - | - | - | 14 |
| 10 | *S. sonnei* | 8 | - | - | - | - | - | - | - | - |
| 11 | *S. boydii* | - | - | - | - | 11 | - | 6 | - | 25 |
| 12 | *S. dysenteriae* | - | - | - | - | - | - | - | 7 | 11 |
| 13 | *S. aureus* | 10 | - | - | 9 | - | - | 6 | - | 12 |

Control – Ampicillin 10 mcg disc - = No activity

**Table 3: Preliminary Phytochemical analysis of *P. wightianus***

| S. No. | Extracts | Saponin | Carbohydrate | Phenolic compound | Tannin | Glycoside | Flavonoid | Steroid | Alkaloid | Fixed oils |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Water | + | + | + | + | - | + | - | + | - |
| 2 | Acetone | - | + | + | + | + | + | + | + | + |
| 3 | Dichloro methane | - | - | - | - | + | + | + | + | + |
| 4 | Ethyl acetate | - | - | - | - | + | - | + | - | + |
| 5 | Methanol | + | + | + | + | + | + | + | + | + |
| 6 | Hexane | - | - | - | - | + | - | + | - | - |
| 7 | Petroleum ether | - | - | - | - | + | + | + | + | - |
| 8 | Chloroform | - | + | + | + | + | + | + | + | + |

+ = presence,- = absence

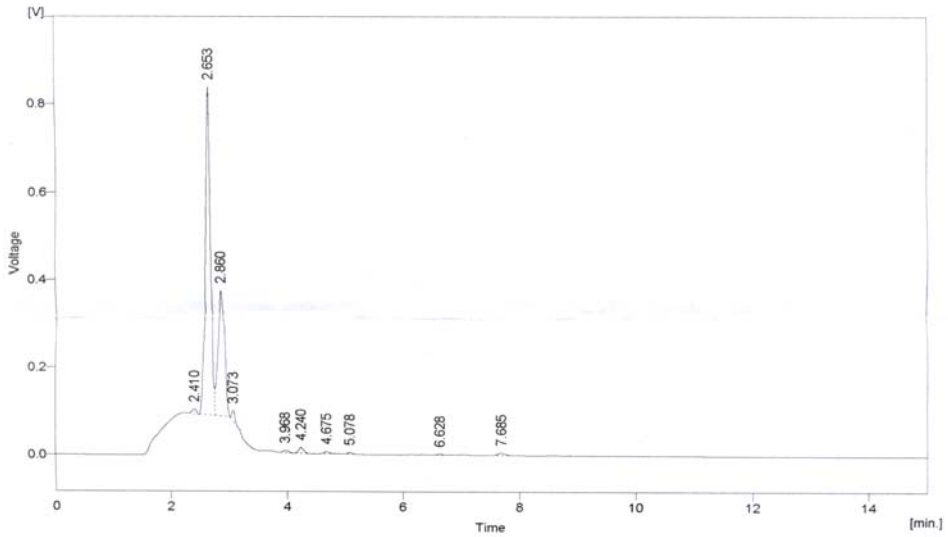


**Fig. 1: HPLC Chromatogram of methanolic extract of *P. wightianus***

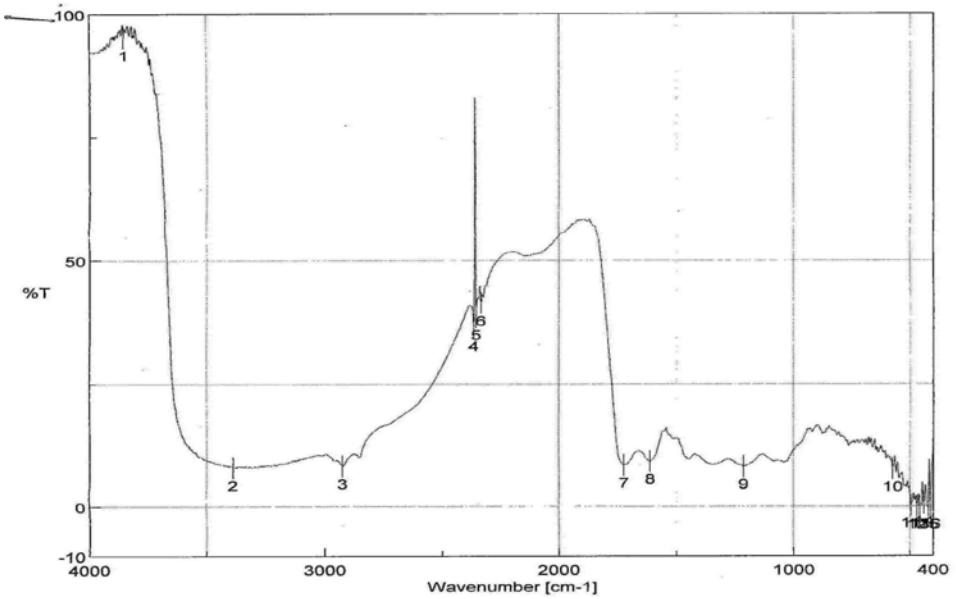


**Fig. 2: FT-IR analysis of methanolic extract of *P. wightianus***

**Table 4: FT-IR analysis of peak position and intensity of plant sample**

| Number | Position | Intensity |
|---|---|---|
| 1 | 3852.11 | 95.205 |
| 2 | 3388.32 | 7.9115 |
| 3 | 2925.48 | 8.24364 |
| 4 | 2364.3 | 36.254 |
| 5 | 2354.66 | 38.5036 |
| 6 | 2334.41 | 41.3765 |
| 7 | 1723.09 | 8.41528 |
| 8 | 1611.23 | 9.21404 |
| 9 | 1213.97 | 8.14104 |
| 10 | 575.647 | 7.63217 |
| 11 | 496.58 | 0.302884 |

**Table 5: Peak value and its functional groups of methanolic extract of *P. wightianus***

| S. No. | Peak value | Functional Groups |
|---|---|---|
| 1. | 3388.32 | N-H Stretching |
| 2. | 2925.48 | C-H Stretching in CH2, CH3 |
| 3. | 1723.09 | C=O Stretching (may be aldehyde or ketone or lactones) |
| 4. | 1611.23 | C=C Stretching (or) N-H bending (in amide) |
| 5. | 1213.97 | ☰CH Bending (or) C-O stretching |
| 6. | 575.647 | C-I stretching |

Since the ancient times plants has been a veritable source of drugs. The present work deals with the aqueous and organic solvent extracts of *P. wightianus* were tested against 13 human bacterial pathogens. The results highlighted that significant antibacterial activity was noticed in *E.faecalis, S.flexneri, V.vulnificus* and *C.diphtheriae.* Similar conclusion was drawn by Mohanasundari *et al*[8] using the same plant extracts. Somachit *et al*[17] reported that *in vitro* antimicrobial and phytochemical assay of *Acalypha indica* was exhibited better activity against *S.aureus* and *B.subtilis*. The results of preliminary phytochemical analysis showed the presence of phenols and tannins may contribute the antimicrobial effect. Likewise, other findings focused on antimicrobial activities of *Phyllanthus acidus* was tested against *S.aureus, E.coli* and *Candida albicans* by four different methods i.e. well diffusion, stokes disc diffusion, pour plate and streak plate (Jagessar *et al*[18]). The results of phytochemical analysis highlighted that the presence of glycoside and steroid in all extracts except aqueous. Flavonoid and alkaloid are also present in many of the extracts except hexane and ethyl acetate extracts. Similar type of investigation was done by Rajeshwari Sivaraj *et al*[19] analysed the preliminary phytochemicals of some Euphorbiaceae members (*Euphorbia hirta, Jatropha gossypiifolia* and *Phyllanthus niruri)* revealed the presence and absence of alkaloids and saponins. Other phytochemicals *i.e.* steroids, tannins, phenols, cardio glycosides, alkaloids, amino acids and protein were present in the most of the plant extracts.

Methanolic extract of *P. wightianus* was analyzed by HPLC and the result reflects two highest peak values are 2.653 and 2.860. This study was correlated with Annamalai and Lakshmi[20] performed to isolate the bioactive compounds (Phyllanthin) from *Phyllanthus amarus* under HPTLC and HPLC. The highest peak was observed in the leaves which quantified to 0.8335% (w/w) while the smaller peak resolution quantified to 0.0016% in roots. Other report focused on the identification and quantification of phenolic antioxidants (ferulic acid, caffeic acid), present in methanolic extracts of some medicinal plants by RP-HPLC with UV detection (Proestos *et al*[21]). The results of FT-IR analysis from the methanolic extract of *P. wightianus* highlighted the highest peak value 3388.82 which has N-H stretching as the functional group. The similar type of work was made by Devmurari *et al*[22] and Natarajan *et al*[16] analysed the functional groups of bioactive compounds from the solvent extracts of *Triumfetta rhomboidea* and *Gymnema kollimalayanum* using FT-IR spectrum.

## CONCLUSION

Based on the findings, the present investigation was revealed that *P. wightianus* may be used as alternative drugs for the treatment of many infectious diseases. The antimicrobial principles from the bioactive extracts may be needed further purifications to have its synthetic analogues which will be carry out in future.

## ACKNOWLEDGEMENT

The authors are gratefully thanks to the University Grants Commission (UGC), New Delhi (Ref. No. 37-296 / 2009 (SR)) for providing financial assistance and Department of Biotechnology, Periyar University, Salem for necessary facilities.

## REFERENCES

1. Das S, Sarkar A, Seth A, Gupta N, Agrawal RC. Evaluation of *in - vitro* antibacterial potential of ripe fruits of *Aegle marmelos.* Int J Pharm Pharm Sci 2012; 4(3): 179-181.
2. Doudach L, Meddah B, Alnamer R, Chibani F, Cherrah Y. *In vitro* antibacterial activity of the methanolic and aqueous extracts of *Anacyclus pyrethrum* used in Moroccan traditional medicine. Int J Pharm Pharm Sci 2012; 4(3): 402-405.
3. Vermani K, Garg S. Herbal Medicines for Sexually Transmitted diseases and AIDS. J Ethnopharmacol 2002; 80: 49-66.
4. König GM, *Meeresorganismen als Quelle pharmazeutisch bedeutsamer Naturstoffe.* Deutsche Apotheker Zeitung 1992; 132(14): 673–683.
5. Ahmad I, Mehmood Z, Mohmmad F. Screening of some Indian medicinal Plants for their antimicrobial properties. J Ethnopharmacol 1998; 62(2): 183-93.
6. Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharmacol 2000; 70(3): 343-9.
7. Mathew KM. *An excursion flora of central Tamilnadu, India*, Oxford and IBH publishing Co. Pvt. Ltd, 1995. p. 469.
8. Mohanasundari C, Natarajan D, Srinivasan K, Anbuganapathi G, Gowrishankar J, Perumal G. Antibacterial efficacy of leaf extracts of *Phyllanthus wightianus* Müll. Arg. J Phytological Res 2005; 18(2): 171-173.
9. Sengottuvel R, Srinivasan K, Mohanasundari C, Natarajan D, Perumal G. Screening of antimicrobial properties of leaf extracts of *Smilax zeylanica* and *Phyllanthus wightianus*. Adv Plant Sci 2007; 20(1): 273-275.
10. Valarmathi R, Scnthamarai R, Akilandeswari S. Analgesic and antimicrobial activity of the dried root extracts of *Reidia floribunda* Wight. Hamdard Medicus 2009; 52(2): 18-23.
11. Siva Priya O. Wound healing activity of *Phyllanthus wightianus Inter Herbal Conference* 2009; Bangalore.
12. Priya OS, Viswanathan MBG, Balakrishna K, Venkatesan M. Chemical constituents and *in vitro* antioxidant activity of *Phyllanthus wightianus.* Nat Pro Res 2011; 25(10): 949-958.

13. Natarajan D, John Britto S, Srinivasan K, Nagamurugan N, Mohanasundari C, Perumal G. Anti-bacterial activity of *Euphorbia fusiformis* – A rare medicinal herb. J Ethnopharmacol 2005; 102(1): 123-126.
14. Evans WC, Trease, Evans. *Pharmacognosy*, 15th edition, W.B.Saunders company Ltd, London, 2002. p. 191-393.
15. Murali B, Amiy A, Anand MS, Dinesh TK, Samiulla DS. An improved methodology for estimation of phyllanthin and hypophyllanthin in *phyllanthus amarus.* J Nat Rem 2001; 1: 55-59.
16. Natarajan D, Yuvarajan R, Srinivasan R, Gomathi M. Antibacterial potential and FT-IR analysis of *Gymnema kollimalayanum* A. Ramachandran and M B Viswan: A new record plant India. Inter J Pharmaceutical Sci 2011; 7(2): 167-170.
17. Somchit MN, Abdul Rashid R, Abdullah A, Zuraini A, Zakaria ZAM, Sulaiman R, et al. In vitro antimicrobial activity of leaves of *Acalypha indica* Linn. (Euphorbiaceae). Afri J Microbiol Res 2010; 4(20): 2133-2136.
18. Jagessar RC, Mars A, Gomes G. Selective Antimicrobial properties of *Phyllanthus acidus* leaf extract against *Candida albicans, Escherichia coli* and *Staphylococcus aureus* using Stokes Disc diffusion, Well diffusion, Streak plate and a dilution method. Nat Sci 2008; 6(2): 24-38.
19. Rajeshwari Sivaraj, Balakrishnan A, Thenmozhi M, Venckatesh R. Preliminary phytochemical analysis of *Aegle marmelos, Ruta graveolens, Opuntia dellini, Euphorbia royleana* and *Euphorbia antiquorum.* Inter J Pharma Sci Res 2011; 2(1): 146-150.
20. Annamalai A, Lakshmi PTV. HPTLC and HPLC analysis of Bioactive Phyllanthin from different organs of *Phyllanthus amarus.* Asian J Biotech 2009; 1: 154-162.
21. Proestos C, Chorianopoulos N, Nychas E, Komaitis M. RP-HPLC Analysis of the Phenolic Compounds of Plant Extracts. Investigation of their antioxidant capacity and antimicrobial activity. J Agric Food Chem 2005; 53(4): 1190–1195.
22. Devmurari VP, Ghodasara TJ, Jivani NP, Antibacterial Activity and Phytochemical Study of Ethanolic Extract of *Triumfetta rhomboidea* Jacq. Inter J Pharm Tech Res 2010; 2(2): 1182-186.