Journal of Applied Pharmaceutical Science Vol. 4 (03), pp. 081-085, March, 2014
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2014.40316
ISSN 2231-3354 

# *In-vitro* antidermatophytic and preliminary phytochemical studies of petroleum ether and Inter-polar methanolic leaf extracts of *Thevetia nerrifolia*

Shivakumar Singh P and Vidyasagar G M*

Medicinal Plants and Microbiology Research Laboratory, Department of Post- Graduate Studies and Research in Botany, Gulbarga University, Gulbarga – 585 106, Karnataka, India.

**ARTICLE INFO**

*Article history:*
Received on: 19/08/2013
Revised on: 26/09/2013
Accepted on: 15/10/2013
Available online: 30/03/2014

***Key words:***
Antidermatophytic Activity, 98% Methanolic leaf extract, *Thevetia nerrifolia,* Inter-polar compounds.

**ABSTRACT**

Antidermatophytic activity (Agar well diffusion technique) of petroleum ether and 98% methanolic leaf extracts of *Thevetia nerrifolia* (Apocynaceae) was evaluated against mycotic fungi namely, *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporum gypseum,* dimorphic fungi such as, *Candida albicans* and pathogenic bacteria like, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Brevibacillus spp*. The significant antidermatophytic activity was shown by 98% methanolic extract, which inhibited all the test fungi and bacteria with time and dose dependent activity. The 98% methanolic extract retarded the growth of all the organisms at 40 mg/ml up to three weeks and beyond. The MIC's, MFC's and MBC's were determined against all the test strains. Preliminary Phytochemical tests were carried out using both the crude extracts. This study provides basis for the isolation and purification of anti-dermatophytic inter polar compounds from the leaves of *T. nerrifolia*.

## INTRODUCTION

Nature is a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Cragg and Newman, 2001). According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs.

Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo and Hveem, 1999). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds (Edeoga, 2005). Skin, hair, nail, and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes which cause dermatophytosis (Valeria *et al.*, 1996 and Amer *et al.*, 2006).

Dermatophytoses are one of the most frequent skin diseases of human (Tsang *et al.*, 1996). The disease is widely distributed all over the world with various degrees and more common in men than in women. There are three genera of mould that cause dermatophytosis. These are *Epidermophyton*, *Trichophyton* and *Microsporum*. A few antifungal compounds are available and licensed for use in human being treatment. The use of systemic drugs is limited to treat man due to their high toxicity and problems of residues in products intended for human consumption (Araujo *et al.*, 2009). Different treatments have been recommended to control dermatophytes. In general, pharmacological treatment option includes antifungal agents (Aly, 1997; Agwa *et al.*, 2000), but recently the use of some natural plant products has been emerged to inhibit the causative organisms. In recent years, secondary plant metabolities (Phytochemicals), previously with unknown pharmacological activities, have been extensively

* *Corresponding Author*
*Dr. G. M. Vidyasagar, Professor, Department of Botany,*
*Gulbarga University, Gulbarga, Karnataka, India.*
*Email: - gmvidyasagar@rediffmail.com. Mobile no: 09449258812.*

investigated as a source of medicinal agents (Krishnaraju, *et al.*, 2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Balandrin, *et al.*, 1985). *Thevetia nerrifolia* belongs to the family Apocynaceae. It has been referred with different names as Digoxin, Lucky Nut, Nerium oleander, Yellow Oleander. This plant is native of Central & South America, but now frequently grown throughout the tropical and sub-tropical regions. All parts of the plant contain the milky juice. Many cytotoxic compounds have been investigated in *Thevetia nerrifolia are* Cardiac glycosides, Thevetin A & B, Thevetoxin, Peruvoside, Ruvoside and Nerifolin are found in *T. Peruviana* (Arnold *et al.*, 1935). The present study evaluates the antidermatophytic activity and phytochemical constituents of *T. nerrifolia* leaf of pet-ether and 98% methanolic (inter polar) extracts.

## MATERIALS AND METHODS

### Collection of plant material

The leaves of *T. neriifolia* were collected in fresh bags from around Gulbarga University and identified with help of Herbariums deposited in the Department of Botany, Gulbarga University, Gulbarga, Karnataka ( Voucher No. HGUG-890). The collected plant materials were initially rinsed with distilled water to remove soil and other contaminants and dried on paper towel in laboratory at 37 ± 2ºC for a week.

### Extraction of plant material by soxhlet apparatus

The leaf material after drying were ground in a grinding machine in the laboratory. 25g of shade dried powder was weighed and extracted successively with petroleum ether and 98% methanol in soxhlet extractor for 48h. The extracts were concentrated under reduced pressure and preserved in refrigerator in airtight bottles for further use.

### Test microorganisms

Five fungal cultures strains such as, *Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes, Microsporum gypseum, Candida albicans* and five bacterial strains, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Brovibacillus* Spp. were obtained from M.R.M.C medical college, Gulbarga, Karnataka, India and used in the present study. Bacterial cultures were grown in nutrient broth (Himedia, M002) at 37ºC and maintained on nutrient agar slants at 4ºC, while fungal cultures were grown in potato dextrose broth at 28 ºC and maintained on potato dextrose agar slants at 4ºC.

### Agar-well diffusion method

The assay was conducted by agar well diffusion method. About 15 to 20 ml of potato dextrose agar medium was poured in the sterilized petri dishes and allowed to solidify. Fungal lawn was prepared using 5 days old culture strain. The fungal strains were suspended in a saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (108 CFU/ml). 1 ml of fungal strain was spread over the medium using a sterilized glass spreader. Using flamed sterile borer, wells of 4 mm diameter were punctured in the culture medium and required concentrations of serially diluted extracts (0.62, 1.25, 2.5, 5, 10, 20 and 40mg/ml) were added to the wells. The plates thus prepared were left for diffusion of extracts into media for one hour in the refrigerator and then incubated at 37$^{o}$C. After incubation for 48h, the plates were observed for zones of inhibition. The diameter zone of inhibition was measured and expressed in millimetres. Dimethyl formamide (DMF) was used as a negative control. The experiments were conducted in triplicates. The same method was followed for testing antibacterial activity using nutrient agar medium incubated at 37$^{o}$C for 18h.

### Minimum Inhibitory Concentration

One ml of sterile liquid Sabouraud medium was added to 11 sterile capped tubes, 1 ml of each solvent extract suspension was added to tube 1. The contents were mixed and 1 ml was transferred to tube 2. This serial dilution was repeated through to tube 9. 1 ml was discarded from tube 9. Fifty µl of inoculum was added to tubes 1-10 and the contents were mixed. Medium control (without inoculum and drug) and inoculum control (no drug) tubes were prepared. The tubes were incubated at 30$^{o}$ C for 96 h. The fungal growth in each tube was evaluated visually depending upon the turbidity in the tubes.

MIC was defined as the drug concentration at which the turbidity of the medium remains same as the medium control. 10µl aliquot of cell suspension from the tube without observed growth of dermatophytes was inoculated on to Sabouraud dextrose agar, and MFC, MBC of test compound were determined as the lowest concentration of the agent at which no colonies were seen after 4 days at 30$^{o}$C. Triplicate sets were maintained for each experiment.

### Preliminary Phytochemical screening

Preliminary tests, for the detection of secondary metabolites like Alkaloids, Flavonoids, Phenols, Saponins, Sterols, Glycosides, Triterpenoids and Tannins were carried out for the 98% methanolic leaf extract of *T. nerrifolia* by adopting standard methods (Gibbs, 1974; Dey and Herborn, 1989; Gokhale *et al.*, 1993; Harborne, 1998).

## RESULTS AND DISCUSSION

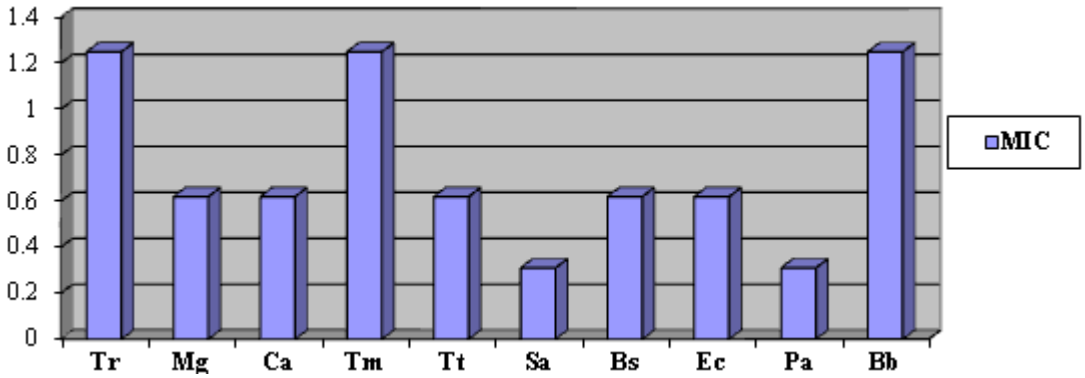
In the present investigation five fungal and five bacterial strains were tested to determine the antimycotic activity of petroleum ether and 98% methanolic leaf extracts of *T. neriifolia*. The zone of inhibition values given in tables -1 and 2 are the mean of the three observations. The 98% Methanolic extract exhibited a higher degree of antimicrobial activity as compared with petroleum ether extract. The 98% methanolic leaf extract of *T. nerrifolia* exhibits significant antidermatophytic activity at 40mg/ml conc. against *M gypseum* (20mm) followed by *T. rubrum*(16mm), *C. albicans (*12 mm), *T. mentagrophytes (*12 mm) and *T. tonsurans* (11mm). Whereas in antibacterial studies, the

maximum of 20 mm inhibition was observed in *S. aureus* at 40 mg /ml followed by *B. subtilis* 18 mm, *E coli* 17 mm, *P. aeruginosa* 17 mm and 14mm in *Brevibacillus* Spp. The zone of inhibition increased on increasing the concentration of extract. This showed the concentration dependent activity. The petroleum ether leaf extract shows very less inhibition zones at 40 mg/ml conc. against test fungi and bacteria. The negative control used DMF could not show inhibition against all the tested fungal and bacterial strains. Ketoconazole used as standard at conc.5mg/ml showed 26.00mm inhibition zone, whereas streptomycin used standard against bacteria showed 25.00mm. The effective 98% methanolic leaf extract was selected and performed MIC, MFC and MBC, the results are shown in figure 1 & 2. The antimicrobial activities observed with the 98% methanolic extract suggest the presence of intra-polar bioactive compound(s) which can serve as antimicrobial agent or lead compound for the synthesis of an effective and less toxic antimicrobial agent. The phytochemical analysis of the two extracts of *T. nerrifolia* leaf were performed (Table 3), the 98% methanolic extract revealed the presence of flavonoids, glycosides, phenols and tannins strongly. Flavonoids and tannins have been reported to possess antimicrobial activity (Yebpella, G.G.*et al.*, 2011 and Hammuel, C., 2011). The antimicrobial activity of flavonoid is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall, while that of tannins may be related to their ability to inactivate microbial adhesion enzymes and cell envelop proteins [Dahot, M.U,1999]. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavanoids (Tsuchiya *et al.*, 1996), phenolics and polyphenols (Mason and Wasserman, 1987), tannins (Ya *et al.*, 1988), terpenoids (Scortichini and Pia Rossi, 1991), sesquiterpenes (Goren, 1996) etc., are effective antimicrobial substances against a wide range of microorganisms. The results imply that, the 98% methanolic leaf extract exhibited more pronounced antimycotic and antibacterial potencies affecting fungi and bacteria used in the present study. *T. nerrifolia* is used in the Indian system of medicine in various human ailments also contains hallucinogenic properties in leaves (Shukla and Jain, 1997). The plant is a potential source of cardiac glycosides and is considered to be a promising drug for congestive heart failure (Arora *et al.*, 1967). Several earlier reports also supporting the present research study. Alcoholic extract of *Thevetia peruviana* seeds was effective against the fungus *Cladosporium cucumerinum* (Llgia Gata *et al.*, 2003). The alcoholic extract of *T. nerrifolia* showed antimicrobial activity in a range of 75-1200µg/ml (Rajesh Dabur et al., 2007). Whereas, the present study determine, 0.3-0.15 µg/ml conc. for MFC and 0.3-0.03 µg/ml conc. for MBC. There were reports on separate aqueous (K. Buvaneswari *et al.*, 2011) and ethanol (M.M. Hassan, 2011) extracts. However, no studies on antimycotic and antibacterial activity of 98% methanolic leaf extract reported.

**Table 1: Antimycotic activity of petroleum ether and 98% methanolic leaf extracts of *Thevetia nerrifolia*.**

| Extracts | Conc. mg/ml | Test dermatophytes & zone of inhibition in mm | | | | |
|---|---|---|---|---|---|---|
| | | *T. rubrum* | *M .gypseum* | *C .albicans* | *T. tonsurans* | *T. mentagrophytes* |
| PE | 40 | 06.00 | 05.00 | 05.00 | - | - |
| | 20 | 04.00 | - | - | - | - |
| | 10 | - | - | - | - | - |
| | 05 | - | - | - | - | - |
| | 2.5 | - | - | - | - | - |
| | 1.25 | - | - | - | - | - |
| | 0.62 | - | - | - | - | - |
| 98% ME | 40 | 16.00 | 18.00 | 12.00 | 11.00 | 12.00 |
| | 20 | 14.00 | 15.00 | 11.00 | 10.00 | 11.00 |
| | 10 | 09.00 | 13.00 | 08.00 | 08.00 | 09.00 |
| | 5 | 08.00 | 11.00 | 07.00 | 06.00 | 08.00 |
| | 2.5 | 06.00 | 09.00 | 05.00 | 05.00 | 06.00 |
| | 1.25 | - | 07.00 | 04.00 | 04.00 | 05.00 |
| | 0.62 | - | 05.00 | - | - | - |
| | Control | - | - | - | - | - |
| | Ketoconazole | 26.00 | 24.00 | 22.00 | 18.00 | 21.00 |

PE- Petroleum ether extract, 98 % ME- 98% methanolic extract, *T. rubrum - Trichophyton rubrum, M .gypseum- Microsporum gypseum, C. albicans - Candida albicans, T. tonsurans -Trichophyton tonsurans, T. mentagrophytes -Trichophyton mentagrophytes*, - No zone inhibition.




*Tr - Trichophyton rubrum, Mg- Microsporum gypseum, Ca - Candida albicans, Tt -Trichophyton tonsurans, Tm -Trichophyton mentagrophytes, Sa- Staphylococcus aureus, Bs- Bacillus subtilis, Ec- Escherichia coli, Pa- Pseudomonas aeruginosa, Bb -Brevibacillus.*

**Fig. 1:** Minimum Inhibition Concentration (mg/ml) of 98% methanolic leaf extract of *Thevetia nerrifolia* against tested strains.

**Table. 2:** Antibacterial activity of petroleum ether and 98% methanolic leaf extracts of *Thevetia nerrifolia* leaves.

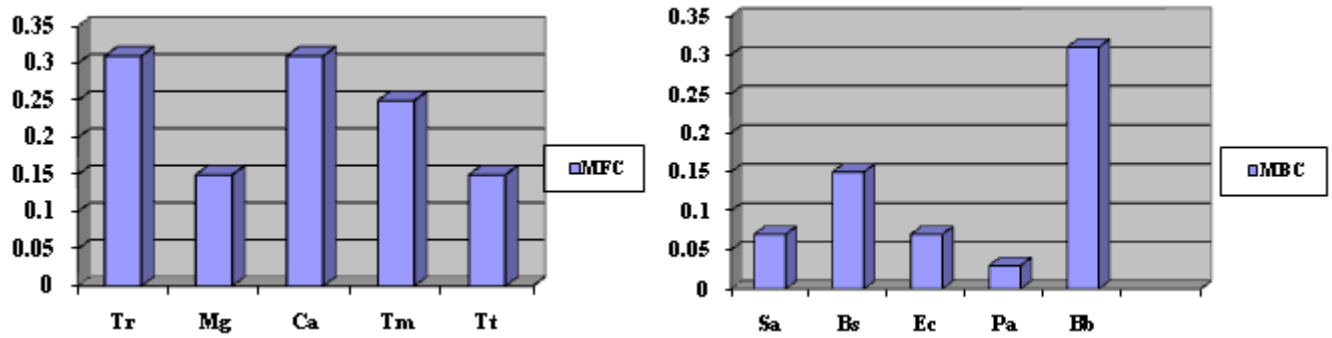
| Extracts | Conc. mg/ml | Test bacteria & zone of inhibition in mm | | | | |
|---|---|---|---|---|---|---|
| | | ***S. aureus*** | ***B. subtilis*** | ***E. coli*** | ***P. aeruginosa*** | ***B. bacillus*** |
| **PE** | 40 | 07.00 | 05.00 | 07.00 | 04.00 | - |
| | 20 | 04.00 | 04.00 | 06.00 | - | - |
| | 10 | - | - | 04.00 | - | - |
| | 05 | - | - | - | - | - |
| | 2.5 | - | - | - | - | - |
| | 1.25 | - | - | - | - | - |
| | 0.62 | - | - | - | - | - |
| **98 % ME** | 40 | 20.00 | 18.00 | 17.00 | 17.00 | 14.00 |
| | 20 | 16.00 | 16.00 | 15.00 | 16.00 | 11.00 |
| | 10 | 14.00 | 14.00 | 13.00 | 15.00 | 08.00 |
| | 05 | 13.00 | 13.00 | 12.00 | 13.00 | 06.00 |
| | 2.5 | 12.00 | 08.00 | 10.00 | 12.00 | 05.00 |
| | 1.25 | 10.00 | 06.00 | 09.00 | 11.00 | - |
| | 0.62 | 08.00 | 05.00 | 08.00 | 10.00 | - |
| Control | | - | - | - | - | - |
| Streptomycin sulphate | | 24.00 | 25.00 | 22.00 | 24.00 | 24.00 |

PE- Petroleum ether extract, 98 % ME- 98% methanolic extract, *S. aureus- Staphylococcus aureus, B. subtilis- Bacillus subtilis, E. coli- Escherichia coli, P. aeruginosa- Pseudomonas aeruginosa, B. bacillus -Brovibacillus.*

**Table. 3:** Preliminary Phytochemical screening for secondary metabolites in *Thevetia nerrifolia* leaf extracts.

| Secondary metabolites | Name of the test | PE crude extract | 98% ME crude extract |
|---|---|---|---|
| **Alkaloids** | Mayers test | - | - |
| | Dragendoff's test | + | - |
| | Wagner's test | - | - |
| **Phenol** | Hot water test | - | + |
| | Ferric chloride test | - | + |
| | Ellagic acid test | + | + |
| **Flavonoids** | Ferric chloride test | - | + |
| | Leadacetate test | - | + |
| | Shinoda test | + | + |
| | Zinc/HCl test | + | + |
| **Tannins** | Gelatin test | - | + |
| **Triterpenoids** | Salkowski's test | - | - |
| | Libermann-Burchard test | + | - |
| **Steroids** | Salkowski's test | - | - |
| | Libermann-Burchard test | + | - |
| **Saponins** | Foam test | - | + |
| **Glycosides** | Keller-Killiani test | + | + |
| | Conc. H2So4 test | - | + |
| | Molisch's test | - | + |
| | Glycoside test | + | + |

\+ present, - absent




*Tr - Trichophyton rubrum, Mg- Microsporum gypseum, Ca - Candida albicans, Tt -Trichophyton tonsurans, Tm -Trichophyton mentagrophytes, Sa- Staphylococcus aureus, Bs- Bacillus subtilis, Ec- Escherichia coli, Pa- Pseudomonas aeruginosa, Bb -Brevibacillus.*

**Fig. 2:** Minimum Fungicidal & Bactericidal Concentrations (mg/ml) against tested strains.

## CONCLUSION

The antidermatophytic activity of *T. nerrifolia* may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even more potency with respect to inhibition of microbes. The work carried out was a basic approach to find out the antimicrobial activity in *T. nerrifolia*. Further studies on the types of phytoconstituents and purification of individual group of bioactive inter-polar components can reveal the exact potential of the plant to inhibit skin pathogenic microbes.

## ACKNOWLEDGEMENT

Authors are thankful to the UGC for extending financial support and Gulbarga University for providing research facility.

## REFERENCES

Cragg GM, Newman DJ. Medicinals for the millennia. Ann NY Acad Sci.2001; 953:3-25.

Arnold, Ayuso, M.J. Cytotoxic activity of *Retama Spaerocarpa*. Fitoterapia. 1935; XVI: 495-498.

Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillinresistant *Staphylococcus aureus*. Journal of Ethnopharmacology, 1996;50: 27-34.

Scortichini M, Pia Rossi M. Preliminary *in vitro* evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill) Winslow *et al*. Journal of Applied Bacteriology, 1991; 71: 109-112.

Mason TL, Wasserman BP. Inactivation of red beet betaglucan synthase by native and oxidized phenolic compounds. Phytochemistry, *1987;* 26: 2197-2202.

Goren N, Woerdenbag H, Bozok-Johansson C. Cytotoxic and antibacterial activities of sesquiterpene lactones isolated from *Tanacetum praeteritum* subsp. *praeteritum*. Planta Medica, 1996; 62: 419-422.

Ya C, Gaffney SH, Lilley TH, Haslam E. 1988. Carbohydrate-polyphenol complexation. p. 553. In: Hemingway, R.W. and Karchesy, J.J. (ed.), Chemistry and significance of condensed tannins. Plenum Press; New York.

Valeria FM, Preve L, Tullio V. Fungi responsible for skin mycoses in Turin (Italy). Mycoses, 1996; 39: 141-150.

Amer S, Aly MM, Sabbagh S. Biocontrol of dermatophytes using some plant extracts and actinomycetes filtrates. Egyptian Journal of Biotechnology, 2006; 330-315.

Tsang P, Hopkins T, Jimenesz-Lucho V. Deep dermatophytosis caused by *Trichophyton rubrum* in a patient with AIDS, 1996; 34(6): 1090-1091.

Araujo CR, Miranda KC, Fernandes OFL, Soares AJ, Silva MRR. In vitro susceptibility testing of dermatophytes isolated in Goiania, Brazil, against five antifungal agents by broth microdilution method. Revista do Instituto de Medicina Tropical de São Paulo, 2009; 51: 9-12.

Aly MM. 1997. Potancy of selected actinomycete for certain antifungal production. PhD Thesis, Tanta University, Cooperation system between Egypt and France, 375.

Agwa A, Aly MM, Bonaly R. 2000. Isolation and characterization of two *Streptomyces* species produced non polyenic antifungal agents. Journal of Union Arab Biology, 2000; 7: 62-84.

Diallo D, Hveem B, Mahmoud MA, Betge G, Paulsen BS, Maiga A. An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biology,1999;37:80-91.

Edeoga HO, Okwu DE, Mbaebie BO, Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 2005;4:685-688.

Balandrin MF, Kjocke AJ, Wurtele et al. Natural Plant chemicals sources of Industrial and mechanical materials, Science, 1985; 228:1154-160.

Krisharaju AV, Rao TVN, Sundararaju. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Altenaria *salania*) lethality assay. Int. J. Appl. Sci Engg, 2005; 2: 125-134.

Shukla Rashmiand Jain SK. Some potential psychoactive plants of India, Sci. Letter, 1997;20: (3&a): 29.

Arora RB, Sharma JN, Bhatiya MT. Pharmacological evolution of peruveside a new cardiac glycoside from *Thevetia neriifolia* with a notes on its clinical trials in patients with congestive heart failure. Indion J.Exp. Biol, 1967; 9(21):31-38.

Yebpella GG, Adeyemi Hassan MM, Hammuel C, Magomya AM, Agbaji AS, Okonkwo EM. Phtyochemical screening and comparative study of antimicrobial activity of *Aloe vera* extracts African Journal of Microbiology Research, 2011;5(10):1182-1187.

Dahot MU.Antibacterial and antifungal activity of small protein of indigofera oblongifere denchorides leaves. J. Ethnophavmacol, 1999; 64(3):77-282.

Hammuel C, Yebpella GG, Shallangwa GA, Magomya AM, Agbaji AS. Phytochemical and antimicrobial screening of methanol and aqueous extracts of *Agave sisilana*. Acta Poloniae Pharmaceutica - Drug Research, 2011;68 (4): 535 – 539.

Gibbs RD. 1974. Chemotaxonomy of flowering plants. MGill Queen's University Press, Monttreal and London. Vol. I.

Dey PM, Harborne, JB. 1989. Methods in Plants Biochemistry, Vol. I, Plants Phenolics. Academics Press. London. Pp.180-250.

Gokhale SB, Kokate CK, Purohit AP. 1993. A Text Book of Pharmacognosy. Nirali Prakashan Pune, India.1-50.

Horborne JB. 1998. Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis 3rd Eds. Chapman and Hall. London.

Trease and Evans. 1989. Pharmacognocy,13th Edn. Bailliere Tindall. London. 51:95-102.

Llgia Gata-Goncalves, Nogueirab JMF, Olfvia Matos C, Raill Brono de Sousad. Photo active extracts from *Thevetia peruviana* with antifungal properties against Cladosporium cucumerinum, Journal of Photochemistry and Photobiology B: Biology, 2003;70 (1): 51-54.

Rajesh Dabur, Amita Gupta, Mandal TK, Desh Deepak Singh, Vivek Bajpai, Gurav AM, Lavekar GS. Antimicrobial activity of some indian medicinal plants, Afr. J. Tradition. Cam, 2007; 4 (3): 313 – 318.

Buvaneswari K, Ramamoorthy D, Velanganni J. Preliminary Phytochemical and Antimicrobial Activity Studies on the Leaves of the Indian Plant *Thevetia neriifolia* Juss. World Journal of Agricultural Sciences, 2011; 7 (6): 659-666.

Hassan MM, Saha AK, Khan SA, Islam A, Mahabub-Uz-Zaman M, Ahmed SSU. Studies on the antidiarrhoeal, antimicrobial and cytotoxic activities of ethanol-extracted leaves of yellow oleander (*Thevetia peruviana)* Open Veterinary Journal, 2011; 1: 28-31.

**How to cite this article:**

Shivakumar Singh P and Vidyasagar G M. In-vitro antidermatophytic and preliminary phytochemical studies of petroleum ether and Inter-polar methanolic leaf extracts of *Thevetia nerrifolia*. J App Pharm Sci, 2014; 4 (03): 081-085.