Available online at www.ijpsdr.com
International Journal of Pharmaceutical Sciences and Drug Research 2014; 6(2): 164-168

Research Article ISSN 0975-248X

Studies on Antimicrobial and Antioxidant Efficacy of *Thevetia neriifolia*, Juss Leaf Extracts against Human Skin Pathogens

Nesy E A[1*], Lizzy Mathew[2]

[1]*Department of Botany, K K T M Govt. College, Pullut, Trichur, Kerala, India*
[2]*Department of Botany, St. Teresa's College, Ernakulam, Kerala, India*

ABSTRACT
To evaluate the significance of *Thevetia neriifolia* in folk medicine for curing skin infections, an efficacy study was conducted using bacterial (*Staphylococcus aureus, Streptococcus pyogenes, Nocardia asteroids, Proteus mirabilis* and *Pseudomonas aeruginosa*) and fungal pathogens (*Candida albicans* and *Trichophyton rubrum*) that cause various dermatological disorders. For antimicrobial properties, different fractions of hot extracts of shade dried leaves were assessed by Agar well diffusion method using concentrations ranging from10 mg/50µl/well and compared with reference antibiotics Gentamicin and Fluconazole (25-50µg/well). Of these, petroleum ether fraction was almost ineffective; chloroform fraction showed better efficiency at very low dozes (0.05-0.0125 mg/well) against *C. albicans* (19.00±1.00) and *N. asteroids* (14.5±0.71), ethyl acetate fraction was sensitive at higher doses against both fungal cultures *T. rubrum* (17.67±0.58), *C. albicans* (15.33±0.58) followed by bacterial strain *N. asteroids* (15.67±1.52), where as methanol division inhibited colony growth moderately against all the tested organisms (10-13mm). Better DPPH free radical scavenging activity was shown by EA and MT fractions of hot extract with IC_{50} values 0.72 and 0.81mg/ml respectively. Similarly, cold methanol extract also acted as powerful free radical scavengers of DPPH, super oxide and nitric oxide with low IC_{50} Values (DPPH-0.46 mg/ml, SOD-0.91 mg/ml, NO-0.88 mg/ml). In conclusion, higher antimicrobial and antioxidant compounds in the leaf add more value to the therapeutic field in drug development for curing many cutaneous bacterial and fungal infections.

Keywords: Antimicrobial, Antioxidant, Human skin pathogens, *Thevetia neriifolia*.

INTRODUCTION

Use of medicinal plants in the treatment of skin diseases was practiced by folk people since many decades. External applications of herbal medicines in crude forms like paste, tincture and infusion play a significant role to nullify the effect of harmful pathogens residing on skin. *Thevetia neriifolia*, Juss belonging to family Apocynaceae was reported to have many curative effects against skin infections, in addition to the healing potential towards various conditions such as edema, insomnia, hemorrhoids, malaria, snake bites, etc. [1] Its root paste was recommended to be applied externally to treat Leprosy. [2] All plant parts, especially the seeds are useful in treating scorpion stings, snake bites, leprosy, ringworm and other skin diseases. [3] According to Kirtikar *et al* [4], the plant is useful in urethral discharges, repelling worms, valuable against skin disorders, leucoderma, wounds and piles and is astringent to bowels. In Guiana, the seeds are used as a purgative in rheumatism and dropsy, it is also considered as a good alexiteric. Studies conducted by various investigators [5-7] revealed the anti-diarrheal, cytotoxic and insecticidal activities of leaves, seeds, stem and roots in addition to antimicrobial activity against some common pathogenic bacteria and fungi.

Several types of bacteria have ability to produce skin infections, of these *Staphylococcus aureus* and *Streptococcus pyogenes* are the most common organisms that cause various cutaneous infections such as cellulites, erysipelas, impetigo, folliculitis, furuncle, carbuncle and abscess. [8-9] The genus *Nocardia asteroids* cause nocardiosis, which may be cutaneous [10], sub-cutaneous [11], or lympho-cutaneous. [12] Gram negative bacteria also play a major role in causing superficial cutaneous infections. The conditions of *Pseudomonas aeruginosa* infection included extensive folliculitis, hot tub rash and according to Wu *et al* [13] infectious conditions ranging from localized infections of the skin to life threatening systemic diseases. Similarly, *Proteus mirabilis* was reported to cause acute cellulites with black discoloration [14], skin abscesses in axilla [15], etc. Likewise, common fungal skin infections include athlete's foot and ringworm caused by *Trichophyton rubrum*, and candidiasis by *Candida albicans* that infects skin, mouth (oral), vagina

***Corresponding author: Mrs. Nesy E A,**
Department of Botany, K K T M Govt. College, Pullut, Trichur, Kerala, India; **Tel.:** +91-9847080640;
E-mail: nesyiby@yahoo.in

(vaginal) and digestive tract (gastrointestinal candidiasis). These are some of the common dermatological infections affecting people all around.

Availability of plant derived antioxidants is gaining much importance since past few decades. These are known to protect the body against free radical mediated toxicities and several plants with potent antioxidant activities were reported by many investigators. [16-18] In view of the importance of plant extract's role in curing many infections, present study focused on the analysis of efficacy of leaf extracts against selected pathogenic microbes that cause human skin disorders and evaluation of antioxidant capability for validation of folk uses of this plant.

MATERIALS AND METHODS

Collection of samples: Plant material was collected from various locations of Trichur Dist, Kerala. The specimen was authenticated by Dr. Sunil CN, Associate Professor in Botany, SNM College, Maliankara and the voucher specimen (STHAPC 2458a) is maintained in the Herbarium cabinet of Botany Department, St. Teresa's College, Ernakulam. Fresh twigs were washed thoroughly in running tap water to remove adhering dust particles and other contaminants, and dried in shade for 2-3 weeks at room temperature. Dried leaved were powdered finely in a homogenizer and kept in airtight containers till further assays were done.

Hot extraction: Accurately weighed sample (30 g) was extracted successively [19] with 300 ml of each of petroleum ether (60-80°C, PE), chloroform (CH), ethyl acetate (EA) and methanol (MT) in a Soxhlet extractor for 12-18 h. The solvents were evaporated and the concentrated extracts were kept at 4°C for further studies.

Cold extraction: Similarly, cold extraction was carried out by shaking 5 g of dried powder with 50 ml of 70% methanol in a magnetic stirrer for 24 hours. Supernatant was filtered using Whatman No.1 filter paper, and filtrate was evaporated to dryness in a water bath kept at 45ºC. Dried extract was weighed and stored in labeled air tight bottles for further analyses.

Microbial Collection: Pure cultures of all experimental bacteria and fungi were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh and from Amala Medical Institute of Sciences, Trichur. Antibacterial studies was conducted using three gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Nocardia asteroids*) and two gram negative bacterial strains (*Pseudomonas aeruginosa*, *Proteus mirabilis*). Antifungal activities were evaluated using *Candida albicans* and *Trichophyton rubrum*. All selected strains are mainly associated with skin diseases, in addition to various severe pathological conditions.

Sample preparation: For evaluating antimicrobial properties, a mother solution of 200 mg/ml was prepared in DMSO (Dimethyl sulfoxide) and diluted serially to get varying concentrations of 100, 50, and 25 mg/ml. Further dilutions were made from above samples to obtain very low concentrations in the range of 10 to 0.25 mg/ml. Antioxidant studies were conducted using dilutions ranging from 0.2-1.2 mg/ml.

Agar well diffusion method: Using standard protocol, *in-vitro* antimicrobial assay of all four fractions was carried out by Agar well diffusion method. [20] As per the recommendations of manufacturer (Hi media), nutrient agar medium was prepared and poured into UV sterilized disposable petridishes (Tarson) under aseptic conditions. The labeled plates were inoculated with respective organisms, and loaded with samples of varying concentrations ranging from 0.25-200 mg/ml into 6mm wells. After proper incubation period at 37ºC, zone of inhibition was recorded in millimeters. Gentamicin and Fluconazole (25-50µg/well) were used as positive control for bacteria and fungi respectively and DMSO as negative control.

Antioxidant assays: *In-vitro* biochemical assays used to measure radical scavenging activity of antioxidants against free radicals like 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anion (O^2) and nitric oxide (NO) were analyzed using different concentrations of the cold extract. Successive extracts were subjected to DPPH assay only.

a) DPPH Assay: This assay measures the ability of antioxidants available in the plant extract to reduce DPPH (Sigma Aldrich), a commercially available stable free radical. In its radical form it has purple color and an absorption band at 517nm which become light yellow on reduction by an antioxidant compound. [21] Various concentrations of aliquot (0.2-1.2 mg/ml) of different fractions were added to freshly prepared 1.5mM DPPH solution. The reaction mixture was made up to a final volume of 1 ml using methanol and incubated in dark at room temperature for 20 min. Absorbance was taken at 517nm on UV-visible spectrophotometer (ELICO), using methanol as blank. Percentage of inhibition was calculated using the formula (ab. of blank - ab. of sample) / ab. of blank × 100. All other chemicals and solvents used were of analytical grade.

b) Nitric oxide Assay: Nitric oxide generated from Sodium nitroprusside (SNP) was measured by Griess reagent by the method of Marcocci *et al.* [22] Various concentrations of crude extract (0.2-1.2 mg/ml) and SNP (10mM) in PBS (pH 7.4) was incubated at 25ºC in a final volume of 3 ml for 150 min. After incubation, 0.5 ml of test solution was mixed with 0.5 ml of Griess reagent (1% sulphalinamide, 2% orthophosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). Measured the absorbance of chromophores formed during the reaction at 546nm, immediately after adding Griess reagent. Inhibition of nitric oxide generation was estimated by comparing absorbance value of control with that of the test.

c) Superoxide scavenging Assay: This assay was determined by NBT reduction method of Mc cord and Fridovich. [23] Evaluation was based on the capacity of extract to inhibit the photo reduction of riboflavin, which was detected by nitro blue tetrazolium (NBT). The reaction mixture contained EDTA (6µM) containing 3µg NaCN, riboflavin (0.12µM), NBT (50µM), various concentrations of the extract (0.2-1.2mg/ml) and phosphate buffer (pH-7.8) in a final volume of 3ml. Tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical density was measured at 560nm before and after illumination. Percentage inhibition of superoxide generation was calculated by comparing the absorbance value of control with that of experimental tubes. Experiments were done in triplicate for each substance.

RESULTS

Various fractions obtained after successive soxhlet extraction with different solvents gave following results- PE fraction yielded a dark green sticky paste (4.97% w/w), CH fraction a greenish black solid residue (3.343% w/w), EA and MT

Table 1: Antimicrobial activity of different fractions (PE, CH, EA & MT) of *Thevetia neriifolia* (yellow form) leaf extracts against human skin pathogens (10mg/50µl/6mm well).

Organisms	PE	CH	EA	MT	+ve
Gram-positive bacteria					Gentamicin
Staphylococcus aureus	0.00±0.00	9.00±0.00	11.00±0.00	10.33±0.58	19.67±0.58
Streptococcus pyogenes	0.00±0.00	0.00±0.00	13.00±1.00	12.67±0.58	14.67±0.58
Nocardia asteroids	0.00±0.00	9.67±0.58	15.67±1.52	11.33±1.15	22.00±0.00
Gram negative bacteria					
Pseudomonas aeruginosa	9.00±0.00	0.00±0.00	14.00±0.00	11.33±1.15	29.00±1.00
Proteus mirabilis	0.00±0.00	9.67±0.58	12.33±0.58	12.00±0.58	31.00±1.00
Fungal strains					Fluconazole
Candida albicans	0.00±0.00	0.00±0.00	15.33±0.58	11.50±0.71	11.33±0.58
Trichophyton rubrum	0.00±0.00	9.00±0.00	17.67±0.58	10.50±0.71	13.67±0.58

PE-Petroleum Ether, CH-Chloroform, EA- Ethyl Acetate, MT – Methanol.

Table 2: Antimicrobial activity of different concentrations of CH fraction of *Thevetia neriifolia* (yellow form) leaf extracts against human skin pathogens (inhibition zone in mm)

Organisms	Concentration (mg/well)					+ve
	0.5	0.25	0.05	0.025	0.0125	
Gram positive bacteria						Gentamicin
S. aureus	9.00±0.00	9.00±0.00	9.50±0.71	10.00±0.00	9.50±0.71	19.67±0.58
S. pyogenes	0.00±0.00	8.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	14.67±0.58
N. asteroids	12.00±0.00	11.00±0.00	11.00±0.00	12.50±0.71	14.50±0.71	22.00±0.00
Gram negative bacteria						
P. aeruginosa	9.00±0.00	9.50±0.71	10.00±0.71	11.00±0.71	11.00±0.00	29.00±1.00
P. mirabilis	12.00±0.00	11.00±0.00	11.00±0.00	11.00±0.00	11.00±0.00	31.00±1.00
Fungal strains						Fluconazole
C. albicans	12.00±1.73	15.33±0.58	18.67±1.15	19.00±1.00	14.50±0.71	11.33±0.58
T. rubrum	8.00±0.00	8.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	13.67±0.58

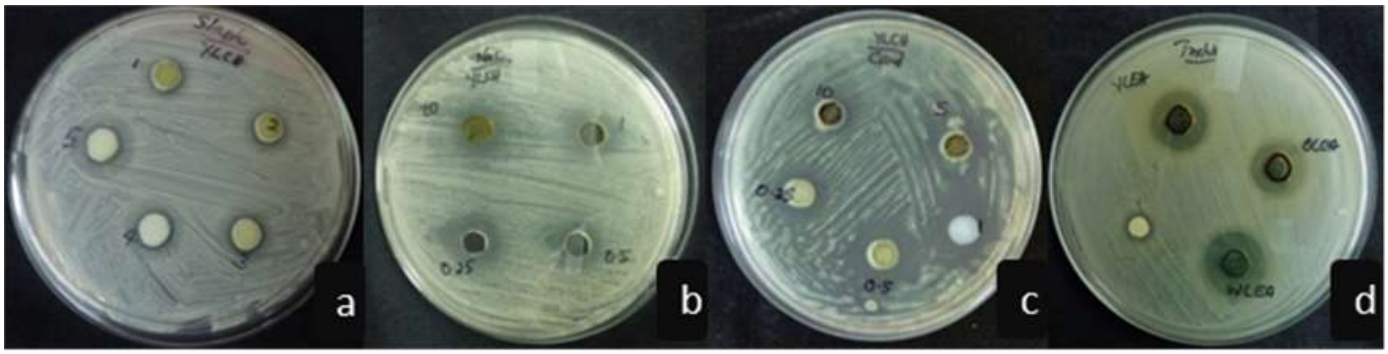

Fig. 1: a-d Antimicrobial activity of CH (0.5-0.0125mg/well) & EA (10mg/well) fractions of *T. neriifolia* leaf extracts. a) *S. aureus* b) *N. asteroids* c) *C. albicans* d) *T. rubrum*

fractions yielded a brownish black sticky matter (1.336% w/w) and brown semisolid residue (0.763% w/w) respectively. Crude methanol residue was solid brown with a yield of 2.58% w/w. Antibacterial drugs either kills bacteria (bactericidal) or prevents the growth of bacteria (bacteriostatic). [24] Efficacy of active compounds was evaluated by measuring the growth inhibition zone produced by the drug, usually due to bacteriostatic activity. Studies conducted with four fractions of leaf extract was evaluated on seven human skin pathogenic microbes using agar well diffusion method revealed that PE fraction was inactive at all dozes against each pathogen. At higher dozes CH fraction was totally inactive, but EA fraction was good enough to fight against all bacteria and fungi. Zone of inhibition at upper concentration (10 mg/well) gave better impact ranging from 11-18mm against all microbes (Table 1) to EA fraction. Among tested bacteria, *N. asteroids* and *P. aeruginosa* showed better activity. Good susceptibility was recorded by both fungal species C. *albicans* and *T. rubrum* to this fraction with a maximum inhibition zone of 15.33±0.58 and 17.67±0.58mm respectively. Methanol fraction responded moderately against all pathogens with inhibition zones ranging from 10-13 mm. A remarkable sensitivity showed by most of the organisms towards CH fraction at very low concentrations (10-0.25 mg/ml or 0.5-0.0125 mg /50µl/well) was presented in Table 2. At a concentration of 0.025-0.0125 mg/well, sensitivity of pathogen was higher especially for *S. aureus*, *N. asteroids* and *C. albicans* (Fig. 1 a-c) than the preceding (0.5-0.25 mg/well) dose.

Antioxidant activity of the leaf extracts was evaluated on the basis of radical scavenging effect of stable DPPH free radical, superoxide radical and nitric oxide radical. Among successive fractions, MT and EA was found to be the most effective solvents in extracting antioxidant compounds more efficiently (IC_{50}-0.72 & 0.81 mg/ml) than the other two. More than 1mg of CH and PE fractions were required to reduce 50% DPPH free radical available in the reaction mixture (Fig. 2). Likewise, in crude MT extract, active principles needed to neutralize 50% free DPPH radicals were low (IC_{50}-0.46 mg/ml) when compared to super oxide and nitric oxide radicals (IC_{50} values of O^2-0.91 mg/ml, NO-0.88 mg/ml). These two assays showed almost similar trends in scavenging free radicals. Cold methanol extract exhibited better NO scavenging activity *in-vitro* in a dose dependent manner and reached a peak of 80% at a concentration of 1.2 mg/ml. A comparative evaluation of antioxidant assays of crude MT extract was presented in Fig. 3 indicated that it has highest scavenging activity of DPPH radical than superoxide and nitric oxide radicals.

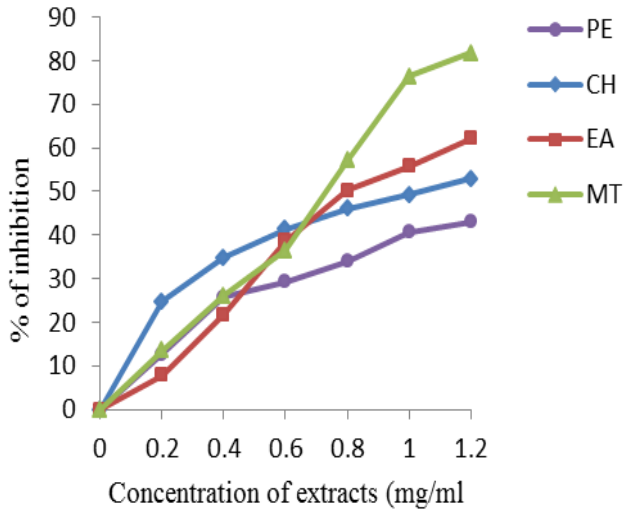

Fig. 2: DPPH Assay of *T. neriifolia* leaf PE, CH, EA & MT fractions with IC_{50} values (PE >1.2, CH- 1.04, EA-0.81, MT-0.72 mg/ml)

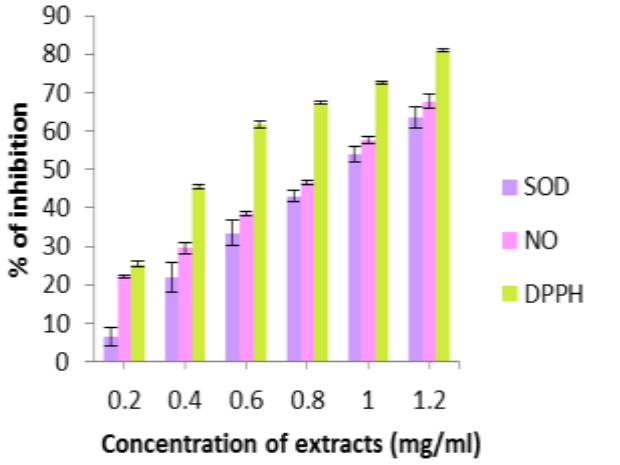

Fig. 3: Antioxidant assays of *T. neriifolia* 70% cold methanol leaf extract (IC_{50} Values of SOD – 0.91 mg/ml, NO-0.88 mg/ml, DPPH – 0.46 mg/ml)

DISCUSSION

In many countries, incidence of high frequency of certain skin infections is not regarded as a significant health problem in the development of public health strategies because of low level of severity and lethality. Skin infections mentioned here, include common disorders of exclusively the superficial layers of skin that are affected by bacteria and fungi. Various herbal drugs were effectively used in the treatment of many skin diseases like wounds, impetigo, carbuncle, cellulites and candidiasis caused by *S. aureus, E. coli, P. aeruginosa* and *C. albicans.* [25] The 95% alcohol extract of *Thevetia* leaf proved to be active against various pathological strains including *Klebsiella pneumonia, P. aeruginosa, E. coli, S. aureus* and *C. albicans.* [26] Phytochemical analysis of methanol crude extract of *Thevetia* leaf showed the presence of secondary metabolites like alkaloids, flavonoids, saponins, glycosides, tannins and phenolic compounds [27], which may be responsible for the potent antimicrobial activities shown by these extracts. Sensitivity shown by all strains especially gram positive *N. asteroids* and gram negative *P. aeruginosa* towards EA fraction proved the efficacy of plant in removing cutaneous infections such as folliculitis, carbuncle and other inflammations. Kareru *et al* [28] prepared a skin care herbal lotion using *Thevetia* seed oil against bacteria *S. aureus* and *E coli*. Other therapeutic uses like antihemorrhoidal, antiarthritic, antirheumatic [29] and insecticidal properties of *T. peruviana* leaf, stem and roots were also reported. Flavanone and flavanol glycosides from the leaves showed HIV-1 reverse transcriptase and HIV-1 integrase inhibitory activities [30], proved that the plant is a vast repository of many powerful drugs.

Fungal infections of the skin, also known as 'mycoses' are generally mild. Superficial fungal infections affect outer layers of the skin, nails and hair and the main group that causes superficial fungal infections are dermatophytes (tinea), Candida and moulds. The highest sensitivity shown by *T. rubrum* towards EA fraction confirmed the use of leaf extract to eradicate ring worm by traditional practitioners in the form of paste or any other external applications. Both CH and EA fractions were equally effective in wiping out the most common infectious fungus, *C. albicans* at lower and higher concentrations, as observed by the sensitivity shown in the assays. Results supports the finding of Hammuel *et al*, that the methanol fraction of seed oil showed strong activity against *C. albicans*, an organism that affects the mucous membrane of mouth and vagina, also indicated the efficiency of plant against infections caused by this microbe. Previous studies revealed the notable efficacy of flower and fruit rind extracts [31-32] in retarding the growth of these microbial strains more effectively.

Antioxidant evaluation of four successive extracts and one crude extract of *Thevetia* leaves was conducted using DPPH, NO and super oxide free radical scavenging assays. In living systems continuously generating free radicals often cause extensive damage to living tissues and biomolecules leading to various disease conditions like cancer, aging, cataract, cardiovascular and neurodegenerative diseases. [33] All fractions of hot extract showed moderate DPPH scavenging activity, revealed that isolation of each soluble compound having radical scavenging property was not completed through a single extraction method. Hence, extraction efficiency is an important factor in quantification of antioxidant activity. Higher temperature facilitates extraction of more compounds from samples, as observed from the better activity of antioxidants in each successive sample.

The NO generated from SNP reacts with oxygen to form nitrite. Molecules in the extract compete with oxygen to inhibit the formation of nitrite, revealed that the extract has potent scavenging power of NO. The present results revealed that crude extract was a direct scavenger of NO, so can inhibit the pathological conditions caused by the excessive generation of NO, and its oxidation product, peroxinitrite in reducing the NO induced damage to macromolecules. An excess NO was known to damage the immune system and deteriorate health. [34] Similarly, antioxidants present in cold extract can scavenge harmful super oxide anions that affect cellular components, thus protecting the system from cellular and tissue damage. These *in-vitro* assays indicate that this plant extract is a significant source of natural antioxidants, which might be helpful in preventing various diseases related to oxidative stresses.

Present study was undertaken to assess the *in-vitro* antimicrobial activity of seven human pathogenic microbes that cause common skin diseases. Our observations support folklore use of this plant in treating various skin related infections. These crude extracts contain potent antimicrobial

agents which can be exploited for better economic and therapeutic utilization in developing new drugs against skin pathogens. Plant based skin care products offer a natural alternative against synthetic chemicals. Hence, active principles can perform as an ingredient in cosmetics and therapeutics for skin care. Of course, the antibacterial and antioxidant principles in this plant might have helped people in curing majority of skin diseases, if formulations were applied in proper dosages.

ACKNOWLEDGEMENT

The authors are thankful to the Director, Amala Ayurvedic Hospital & Research Centre, Trichur for providing necessary facilities for the completion of this project.

REFERENCES

1. Nellis DW. Poisonous Plants and Animals of Florida and the Caribbean, Pineapple press, Florida, 1997.
2. Dabur R, Gupta A, Mandal TK, Singh DD, Bajpai V, Gurav AM, Lavekar GS. Antimicrobial Activity of Some Indian Medicinal Plants. Afr. J. Trad. CAM. 2007; 4 (3): 313-318.
3. Udayan PS, Balachandran I. Medicinal plants of Arya Vaidya Sala, Department of Publications, Arya Vaidya Sala, Kottakkal, Kerala, 2009.
4. Kirtikar KR, Basu BD, An CIC. Indian Medicinal Plants, Vol II, Bishen Singh Mahenrda Pal Singh, Dehra Dun, 1980.
5. Hassan MM, Saha AK, Khan SA, Islam A, Zaman MMU, 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.
6. Hammuel C, Abdullahi MS, Mankilik M, Anyim BP, Adesina OB, Inekwe UV, Udiba UU, Batari ML. The phytochemical and antimicrobial activities of oil from the seed of *Thevetia peruviana* plant. J. Appl. Environ. Biol. Sci. 2011; 1(12): 597-601.
7. Mollah JU, Islam W. Toxicity of *Thevetia peruviana* (Pers) Schum Extract to Adults of *Callosobruchus maculates* F (Coleoptera: Bruchidea). J Agric. Rural Dev. 2007; 5(1&2): 105-109.
8. Stulberg DL, Penrod MA, Blatny RA. Common bacterial skin infections. Am. Fam. Physician 2002; 66 (1): 119-124.
9. Rhoads J. Advanced Health Assessment and Diagnostic Reasoning, Lippincott Williams & Wilkins, Walnut Street, Philadelphia. Chapter 5. Integumentary Disorders. 2006; pp 95.
10. Folgaresi M, Ferdani G, Coppini M, Pincelli C. Primary cutaneous nocardiosis. European journal of Dermatology. 1998; 8(6): 430-431.
11. Lyngdoh WV, Mawlong MG, Sailo SL, Khyreim AB. Sub-cutaneous abscess caused by *Nocardia asteroids* in renal transplant recipient. Indian J Med. Sci. 2011; 65 (4): 172-174.
12. Tsuboi R, Takamori K, Ogawa H, Mikami Y, Arai T. Lymphocutaneous nocardiosis caused by *Nocardia asteroids*. Case report and literature review. Arch. Dermatol. 1986; 122 (10): 1183-1185.
13. Wu DC, Chan WW, Metelitsa AI, Fiorillo L, Lin AN. *Pseudomonas* skin infection Clinical features, Epidemiology and Management. Am. J. Clin. Dermatol. 2011; 12(3):157-169.
14. Krajden S, Deitel M, Fuksa M. Blackened toes caused by *Proteus mirabilis* infection. CMA Journal 1982; 127: 869-870.
15. Mistry RD, Scott HF, Alpern ER, Zaoutis TE. Prevalence of *Proteus mirabilis* in skin abscesses of the axilla. J Pediatr. 2010; 156(5):850-851.
16. Ayoola GA, Folawewo AD, Adesegun SA, Abioro OO, Adepoju-Bello AA, Coker HAB. Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria, African journal of Plant Science. 2008; 2 (9) 124-128.
17. Badami S, Prakash O, Dongre SH, Suresh B. *In-vitro* antioxidant properties of *Solanum pseudocapsicum* leaf extracts. Indian J Pharmacol. 2005; 37(4):251-252.
18. Dey TK, Emran TB, Saha D, Rahman MA, Zahid Hosen SM, Chowdhury N. Antioxidant Activity of Ethanolic Extract of *Cassia hirsuta* (L.) Leaves. Bull. Pharm. Res. 2012; 2(2):78-82.
19. Harborne JB. Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis, 3rd Edition, Springer (India) private Ltd, New Delhi, 1973.
20. Navarro V, Villarreal ML, Rogas G, Lozoya X. Antimicrobial evaluation of some plants used in Mexican traditional medicine for the treatment of infectious diseases. J Ethnopharmacol. 1996; 53: 143-147.
21. Aquino R, Morelli S, Lauro MR, Abdo S, Saija A, Tomaino A. Phenolic constituents and antioxidant activity of an extract of *Anthurium versicolor* leaves. Journal of Natural Products 2001; 64 (8): 1019-1023.
22. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. Biochem. Biophys. Res. Commun. 1994a; 15: 748-755.
23. McCord JM, Fridovich I. Superoxide dismutase: An Enzymic Function for Erythrocuprein (hemocuprein). J. Biol. Chem. 1969; 244: 6049-6055.
24. Parkavi V, Vignesh M, Selvakumar K, Mohamed JM, Ruby JJ. Antibacterial Activity of Aerial Parts of *Imperata cylindrica* (L) Beauv. Int. J. Pharm. Sci. Drug Res. 2012; 4(3): 209-212.
25. Chanda S, Baravalia Y. Novel leads from herbal drugs for infectious skin diseases. Current Research, Technology and Education Topics in Applied Microbiology and Microbial Technology, a Mendez-Vilas Ed. 2010; 451-456.
26. Reddy U. Antimicrobial Activity of *Thevetia peruviana* (Pers.) K. Schum, and *Nerium indicum* Linn. The internet journal of Pharmacology. 2010; 8(2): 2.
27. Kumar A, Singh S, Mahout K, Vihan VS, Gururaj K. Phytochemical Analysis of Some Indigenous Plants Potent Against Ectoparasite. Asian J. Exp. Biol. Sci. 2011; 2(3): 506-509.
28. Kareru PG, Keriko JM, Kenji GM, Thiongo GT, Gachanja AN, Mukiira HN. Antimicrobial Activities of Skincare Preparations from Plant Extracts. Afr. J Trad. Complement Alt. Med. 2010; 7(3): 214-218.
29. Zavaleta AN, Zavaleta AN. Medicinal Plants of the Borderlands: A Bilingual Resource Guide, Author House Publications, USA. 2012.
30. Tewtrakul S, Nakamura N, Hattori M, Fugiwara T, Supavita T. Flavanone and Flavonol Glycosides from the Leaves of *Thevetia peruviana* and their HIV-1 Reverse Transcriptase and HIV-1 Integrase Inhibitory Activities. Chem. Pharm. Bull. 2002; 50(5):630-635.
31. Nesy EA, Jolly CI, Mathew L. Assessment of *In-vitro* Antimicrobial and Antioxidant Potential of *Thevetia neriifolia*,Juss Flower Morphoforms. IOSR J Pharm. Biol. Scien. 2014; 9(2): 11-16.
32. Nesy EA, Mathew L. A comparative Evaluation of Antimicrobial and Antioxidant Properties of *Thevetia neriifolia*, Juss Fruit Rind Extracts. Int. J. Curr. Pharm. Res. 2014; 6(2): 47-50.
33. Phar-Huy LA, Hua H, Pham-Huy C. Free Radicals, Antioxidants in Disease and Health. Int. J Biomed. Sci. 2008; 4 (2): 89-96.
34. Jagetia GC, Rao SK, Baliga MS, Babu KS. The Evaluation of Nitric Oxide Scavenging Activity of Certain Herbal Formulations *in-vitro*: A Preliminary Study. Phytother. Res. 2004; 18: 561-565.