Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 6 (6): 441-448, 2012
ISSN 1819-3455 / DOI: 10.3923/rjmp.2012.441.448

Phytoconstituents and Their Influence on Antimicrobial Properties of *Morinda citrifolia* L.

G. Sibi, Parul Chatly, Sayak Adhikari and K.R. Ravikumar
R & D Centre, Robust Materials Technology Pvt. Ltd., Bengaluru, 560072, Karnataka, India

Corresponding Author: G. Sibi, R & D Centre, Robust Materials Technology Pvt. Ltd., Bengaluru, 560072, Karnataka, India

ABSTRACT

Morinda citrifolia L., also known as noni or Indian mulberry is a small evergreen tree having antimicrobial, antitumor, antidepressant and immune enhancing effects. Various parts of *M. citrifolia* have been investigated for its phytochemical and antimicrobial properties. In the present study, methanolic extracts of leaf, stem and roots of *M. citrifolia* has been prepared and analyzed for their phytoconstituents. Qualitative analysis of the extracts revealed the presence of phenols, tannins, saponins, alkaloids, glycosides, flavonoids and steroids at various levels. Further, the extracts were tested against pathogenic bacterial and fungal strains at different concentrations to determine the influence of phytochemicals. The results revealed that root and leaf extracts has significant antimicrobial activities mainly due to phenolics and tannins. *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, major pathogens of nosocomial infections were effectively controlled by the extracts at various concentrations and root extract exhibited significant activity against *Penicillium* sp. and *Fusarium* sp. It has been revealed that, the bioactive substances have influenced the antimicrobial properties of *M. citrifolia* which could be exploited to formulate novel drugs from plant origin against bacterial and fungal infections.

Key words: *Morinda citrifolia*, phytochemicals, antimicrobial, phenolics, tannins

INTRODUCTION

In recent years, research has been focused to find novel compounds from plant, animal and microbial origin. Increasing resistance development against frequently used antimicrobial compounds by the micro organisms is urging to discover new antimicrobial compounds, particularly from plant origin. Many plants synthesize substances that are useful to control the growth of microorganisms and plants are the possible source of antimicrobial agents (Adesina *et al*., 2000). Plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al*., 2007). Though, synthetic and semi synthetic drugs are available in todays' market, there is need for new ones from natural origin to cope up with the increased evolution of multiple resistant strains.

Morinda citrifolia (Rubiaceae), also known as noni or Indian mulberry is a small evergreen tree which is identifiable by its straight trunk, large, bright green elliptical leaves with tubular flowers and its distinctive, ovoid "grenade-like" yellow fruit. Apart from this appellation, there are many local names that are also widely used in their respective countries namely, Noni

apple, Polynesia fruit, Indian mulberry (India), Bumbo (Africa), Mengkudu (Malaysia), Cheeserut (Australia), Painkiller tree (Caribbean Islands), Nhau (Southeast Asia), Morinda (Vietnam), Hai Ba Ji (China).

Various parts of the noni plant extracts have been reported to have significant antimicrobial, antitumor, antidepressant and immune enhancing effects (Wang *et al*., 2002; Gerson, 2002; McClatchey, 2002; Palu *et al*., 2008; Deng and West, 2011). The plant has been studied for its insulinotropic (Hamid *et al*., 2008), antioxidant (Krishnaiah *et al*., 2007; Chanda *et al*., 2011; Krishnaiah *et al*., 2011), wound healing (Nagori and Solanki, 2011), antiosteoporotic (Shirwaikar *et al*., 2011) and antidiabetic (Dompeipen *et al*., 2011) activities. Ridzwan *et al*. (2002) reported that, aqueous extracts of *M. citrifolia* fruit has decreased coronary perfusion pressure and developed tension in isolated rat heart. Due to the pharmaceutical values, *M. citrifolia* has gained a great deal of interest (Hemwimon *et al*., 2007).

The present study was conducted to evaluate the phytochemical properties of various parts of *M. citrifolia* and their influence on antimicrobial properties.

MATERIALS AND METHODS

Plant collection and extraction: Plant materials of *M. citrifolia* such as whole leaves, roots and stem were collected, washed in running tap water and finally with distilled water. The samples were shade dried for 2 days and oven dried at 45°C for 24 h and ground to powder. The ground powder was sieved (200 μm), extracted with methanol for 48 h at room temperature and the solvent was removed by filtration using Whatman No.1 filter paper. Filtrates were concentrated to dryness under reduced pressure for overnight and the final extract was resuspended in methanol to make a stock solution (50 mg mL^{-1}).

Phytochemical assay

Determination of total phenolics: Total phenol content in the extracts was determined using a modified Folin-Ciocalteu method (McDonald *et al*., 2001). One milliliter of the extract was added to 10 mL deionized water and mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of 1 M sodium carbonate. The tubes were vortexed and allowed to stand for 30 min at room temperature. The resulting blue complex was then measured at 765 nm using UV-VS spectrophotometer.

Determination of total flavonoids: Total flavonoids were estimated by mixing 0.5 mL of extract with 0.5 mL of 2% $AlCl_3$ ethanol solution (Ordonez *et al*., 2006). The tubes were allowed to stand for 1 h at room temperature and the resulting yellow colour was measured at 420 nm.

Determination of tannins, alkaloids and saponins: The presence of tannins in the extract was identified by mixing 0.5 mL of extract with 1 mL of water and 1-2 drops of ferric chloride solution (Iyengar, 1995). For the presence of alkaloids, about 0.5 mL of the extract was stirred with 5 mL of 1% aqueous hydrochloric acid on a steam bath for 5 min and filtered. A few drops of Dragendorff's reagent were used to treat 1 mL of the filtrate and turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids. To detect saponins in the extract, 0.5 mL of the extract was dissolved in distilled water in a test tube and frothing which persisted on warming was taken as preliminary evidence for saponins.

Determination of terpenoid and steroid: 0.5 mL each of acetic anhydride and chloroform was added to 4 mL of extract. The mixture was then added with concentrated sulphuric acid for colour formation (Siddiqui and Ali, 1997).

Determination of glycosides: To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added and observed for the coloration (Siddiqui and Ali, 1997).

Antibacterial assay: The disc diffusion method was used to study the antibacterial activity (Bauer *et al*., 1966) of *M. citrifolia* extracts. Bacterial isolates viz., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Escherichia coli*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were tested against the extracts. Each bacterial culture was diluted as 1:100 with fresh sterile nutrient broth and inoculated (2.5×10^8 CFU mL^{-1}) on sterile Mueller Hinton Agar (MHA) plates by swabbing. Different concentrations (100, 200, 300, 400 and 500 μL) of extract were loaded in sterile filter paper disc and placed on the MHA plate. The plates were incubated at 37°C for 18-24 h and after incubation; the diameter of the zone of inhibition was measured by using HiMedia antibiotic scale.

Antifungal assay: The disc diffusion method was used to study the antifungal activity by mixing different concentrations (100, 200, 300, 400 and 500 μL) of various parts of plant extracts into potato dextrose agar in triplicates with dimethyl sulfoxide (DMSO) as control. The plates were inoculated with 5 days old fungal cultures viz., *Penicillium*, *Cladosporium*, *Fusarium*, *Alternaria* and *Aspergillus* at the center as point inoculation and incubated at 25°C for 5 days. After incubation, the diameter of the colonies were measured for the inhibitory activity of the extracts and compared with control. Complete suppression of growth by a specific concentration was considered as significant.

Statistical analysis: The data were subjected to one way Analysis of Variance (ANOVA) and differences between samples were determined by Duncan's Multiple Range test using the Statistical Analysis System (SAS, 1999) program. p-values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Information on chemical constituents of plants helps for the discovery of novel drugs. Qualitative phytochemical investigation revealed that the extracts of *M. citrifolia* contained phytoconstituents viz., phenols, alkaloids, flavonoids, glycosides, tannins, saponins and steroids at various levels (Table 1). These bioactive components are naturally occurring in most plant materials, known to be bactericidal and fungicidal, thus conferring the antimicrobial property to plants (Van der Watt and Pretorius, 2001; El-Astal *et al*., 2005).

Table 1: Phytochemical analysis of methanol extracts of *M. citrifolia* (mg g^{-1})

Extract	Phenols	Flavonoids	Alkaloids	Tannins	Saponins	Terpenoids	Steroids	Glycosides
Leaf	+	+	++	++	+	++	-	++
Stem	-	-	+	+	-	+	-	+
Root	++	++	-	++	+	-	++	+

++: Quantitatively present, +: Present, -: Absent

Phenolic compounds have been found to be the major group of functional micronutrients in noni plant (Morton, 1992; Dixon *et al*., 1999; Wang and Su, 2001). The present study revealed that significant levels of phenolic compounds were present in leaves and roots followed by stem extracts of *M. citrifolia*. Phytochemical analysis of root extract of *M. citrifolia* as determined by the total phenol, flavonoids, tannins and steroids were higher than that of the leaf and stem extracts.

On the other hand, the leaf extract of the plant has higher level of total alkaloids and glycosides. Saponins were present in leaf and root extracts and quantitative amount of terpenoids were present in leaf extract. Glycosides were present in all the extracts.

Quercetin, isoflavones, epigallocatechin represents a group of flavonoids and their antimicrobial properties has been well documented (Cushnie and Lamb, 2005). Antibacterial and antifungal activity of saponins isolated from plant materials have been reported by previous studies (Campbell, 1993; Soetan *et al*., 2006; Barile *et al*., 2007). However, saponins have ineffectiveness on Gram negative bacteria due to poor penetration on cell membranes (Soetan, 2003).

The last two decades has witnessed increased investigations on plants as a source of human disease management (Aiyelaagbe, 2001; Prashanth *et al*., 2001) due to the genetic variability of microorganisms against the antibiotics. The antibacterial activity of *M. citrifolia* has been evidenced by many reports and is due to the presence of phenolic compounds (Atkinson, 1956).

The antibacterial activities of *M. citrifolia* extracts were tested by the presence or absence of inhibition zones and zone diameter (Fig. 1). The effect of plant extract was different with different bacterial strains. Among the different extracts tested, roots extract exhibited significant antibacterial activity with larger zones of inhibition followed by the leaf extract and the most susceptible microbes to all extracts were *S. epidermidis* and *P. aeruginosa*. Leaf extract has significant levels of alkaloids which attributed in control the growth of bacteria. Antibacterial activity of alkaloids particularly against gram positive bacteria was reported by Karou *et al*. (2006). *S. epidermidis* and *P. aeruginosa* are known pathogens of biomaterial infections and their inhibition by the *M. citrifolia* root extracts might suggest their possible use in the treatment of

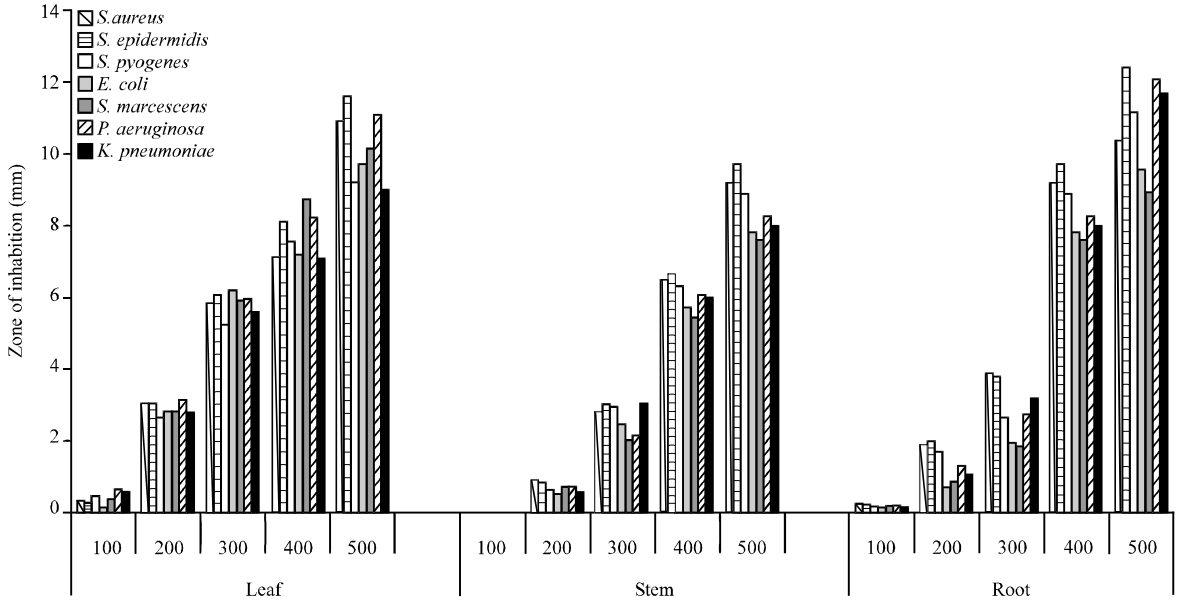

Fig. 1: Antibacterial activity of *M. citrifolia* extract

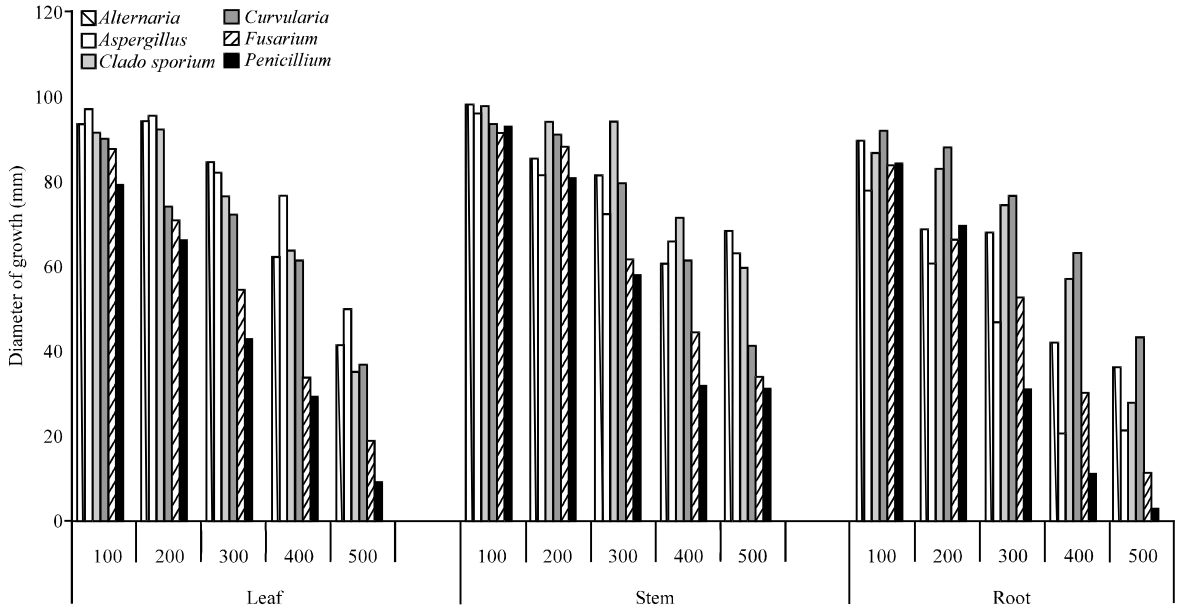

Fig. 2: Fungal growth at different concentration of *M. citrifolia* extract

nosocomial infections. The overall result suggests that *S. epidermidis* is the most susceptible strain and the resistant was *S. marcescens* in the presence of root extract and *S. pyogenes* with leaf extract. Sharma and Smita (2010) reported that, ethyl acetate extract of *M. citrifolia* exhibited broad spectrum of inhibition against gram negative bacteria, whereas alcoholic extract of *M. citrifolia* has exhibited potent antimicrobial activity (Kumar *et al.*, 2010). Although, the antibacterial properties of stem extracts of *M. citrifolia* are not as effective as the leaf and root extracts, it still possesses some activity against bacterial strains used in this study. Apart from Gram positive and Gram negative bacteria, antibacterial activity of *M. citrifolia* against mycoplasma and plant pathogen was revealed by previous studies (Rivera *et al.*, 2011; Sunder *et al.*, 2011).

All the concentrations of the extract inhibited the fungal species with varying degree of sensitivity (Fig. 2). The stronger and broader spectrum of antifungal activity was observed in root extract of *M. citrifolia* against *Penicillium* and *Fusarium* followed by leaf extract. In higher concentrations, prominent antifungal activity against was observed which supports the earlier investigations (Banso and Adeyemo, 2007; Chung *et al.*, 1993; Bele *et al.*, 2010) that the tannins isolated from the medicinal plants possess remarkable toxic activity against bacteria and fungi. However, *Aspergillus* was able to tolerate higher concentrations (500 µL) of leaf extract and *Curvularia* in the presence of root extract. Weak antifungal activity of stem extract was seen throughout the study.

CONCLUSION

In general, the order of antimicrobial activity follows the sequence: root extract>leaf extract>stem extract. Thus, the methanolic root extract of *M. citrifolia* can be used as the active constituent of antimicrobial agents. Bioactive substances from *M. citrifolia* plant could be employed in the formulation of antimicrobial agents for the treatment of various bacterial and mycotic infections. The presence of antimicrobial activity in *M. citrifolia* plant extracts give support to their traditional use for treating conditions associated with microorganisms in humans and consequently seems to fight against multi-resistant microbes.

REFERENCES

Adesina, S.K., O. Idowu, A.O. Ogundaini, H. Oladimeji, T.A. Olugbade, G.O. Onawunmi and M. Pais, 2000. Antimicrobial constituents of the leaves of *Acalypha wilkesiana* and *Acalypha hispida*. Phytother. Res., 14: 371-374.

Aiyelaagbe, O.O., 2001. Antibacterial activity of *Jatropha multifida* roots. Fitoterapia, 72: 544-546.

Atkinson, N., 1956. Antibacterial substances from flowering plants. 3. Antibacterial activity of dried Australian plants by rapid direct plate test. Aust. J. Exp. Biol. Med. Sci., 34: 17-26.

Banso, A. and S.O. Adeyemo, 2007. Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea*. Afr. J. Biotechnol., 6: 1785-1787.

Barile, E., G. Bonanomi, V. Antignam, B. Zolfaghari, S.E. Sajjadi, F. Scala and V. Lanzotti, 2007. Saponins from *Allium minutiflorum* with antifungal activity. Phytochemistry, 68: 596-603.

Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.

Bele, A.A., V.M. Jadhav and V.J. Kadam, 2010. Potential of tannins: A review. Asian J. Plant Sci., 9: 209-214.

Campbell, J.B., 1993. Saponins. In: The Theory and Practical Application of Adjuvants, Stewart-Tull, D.E.S. (Ed.). Butterworth-Heinemann Inc., Toronto, London, New York.

Chanda, S., R. Dave and M. Kaneria, 2011. *In vitro* antioxidant property of some Indian medicinal plants. Res. J. Med. Plant, 5: 169-179.

Chung, K.T., S.E. Jr. Stevens, W.F. Lin and C.I. Wei, 1993. Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. Lett. Applied Microbiol., 17: 29-32.

Cushnie, T.P.T. and A.J. Lamb, 2005. Review antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 26: 343-356.

Deng, S. and B.J. West, 2011. Antidepressant effects of noni fruit and its active principles. Asian J. Med. Sci., 3: 79-83.

Dixon, A.R., H. McMillen and N.L. Etkin, 1999. Ferment this: The transformation of Noni, a traditional polynesian medicine (*Morinda Citrifolia*, Rubiaceae). Ecol. Bot. 53: 51-68.

Dompeipen, E.J., Y. Srikandace, W.P. Suharso, H. Cahyana and P. Simanjuntak, 2011. Potential endophytic microbes selection for antidiabetic bioactive compounds production. Asian J. Biochem., 6: 465-471.

El-Astal, Z.Y., A.E.A. Ashour and A.A.M. Kerrit, 2005. Antimicrobial activity of some medicinal palnt extracts in Palestine. Pak. J. Med. Sci., 21: 187-193.

Gerson, S., 2002. The Foundation for Holistic Medical Research. Brewster Publisher, New York, USA.

Hamid, M., S.P.M. Bohari, M.S. Bastami, A.M. Ali, N.M. Mustapha and K. Shari, 2008. Evaluation of the insulinotrohic activity of Malaysian traditional plants extract. J. Boil. Sci., 8: 201-204.

Hemwimon, S., P. Pavasant and A. Shotipruk, 2007. Microwave-assisted extraction of antioxidative anthraquinones from roots of *Morinda citrifolia*. Sep. Purif. Technol., 54: 44-50.

Iyengar, M.A., 1995. Study of Crude Drugs. 8th Edn., Manipal Power Press, Manipal, India, pp: 2.

Karou, D., A. Savadogo, A. Canini, S. Yameogo and C. Montesano *et al.*, 2006. Antibacterial activity of alkaloids from *Sida acuta*. Afr. J. Biotechnol., 5: 195-200.

Krishnaiah, D., R. Sarbatly and N.L. Nah, 2007. Recovery of phytochemical components from various parts of *Morinda citrifolia* extracts by using membrane separator. J. Applied Sci., 7: 2093-2098.

Krishnaiah, D., R. Sarbatly and R. Nithyanandam 2011. Optimization of spray for drying *Morinda citrifolia* L. fruit extract. J. Applied Sci., 11: 2276-2283.

Kumar, K.T., D.S. Panda, U.N. Nanda and S. Khuntia, 2010. Evaluation of antibacterial, antifungal and anthelmintic activity of *Morinda citrifolia* L. (Noni). Int. J. PharmaTech Res., 2: 1030-1032.

McClatchey, W., 2002. From polynesian healers to health food stores: Changing perspectives of *Morinda citrifolia* (Rubiaceae). Integr. Cancer Therapies, 1: 110-120.

McDonald, S., P.D. Prenzler, M. Antolovich and K. Robards, 2001. Phenolic content and antioxidant activity of olive extracts. Food Chem., 73: 73-84.

Morton, J.F., 1992. The ocean-going noni, or Indian mulberry (*Morinda citrifolia*, Rubiaceae) and some of its colorful relatives. Ecol. Bot., 46: 241-256.

Nagori, B.P. and R. Solanki, 2011. Role of medicinal plants in wound healing. Res. J. Med. Plant, 5: 392-405.

Ordonez, A.A.L., V. Gomez, M.A. Vattuone and M.I. lsla, 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem., 97: 452-458.

Owolabi, O.J., E.K.I. Omogbai and O. Obasuyi, 2007. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigella africana* (Bignoniaceae) stem bark. Afr. J. Biotechnol., 6: 1677-1680.

Palu, A.K., A.H. Kim, B.J. West, S. Deng, J. Jensen and L. White, 2008. The effects of *Morinda citrifolia* L. (Noni) on the immune system: Its molecular mechanisms of action. J. Ethnopharmacol., 115: 502-506.

Prashanth, D., M.K. Asha and A. Amit, 2001. Antibacterial activity of *Punica granatum*. Fitoterapia, 72: 171-173.

Ridzwan, B.H., Y.S. Fong, M.H. Hasniwati, B. Shaari and K.C.A. Jalal, 2002. The effect of *Morinda citrifolia* on isolated rat hearts. Pak. J. Biol. Sci., 5: 1094-1097.

Rivera, A., S. Giono, M. Gonzalez, N. Rodriguez and L. Cedillo, 2011. Antibacterial effect of *Morinda citrifolia* fruit juice against mycoplasmas. Ann. Biol. Res., 2: 491-497.

SAS, 1999. SAS User's Guide. SAS Institute Inc., Cary, North California, USA.

Sharma, M.C. and S. Smita, 2010. Phytochemical screening of methanolic extract and antibacterial activity of *Eclipta alba* and *Morinda citrifolia* L. Middle-East J. Sci. Res., 6: 445-449.

Shirwaikar, A., Y. Kamariya, B. Patel, S. Nanda, V. Parmar and S. Khan, 2011. Methanol extract of the fruits of *Morinda citrifolia* Linn., restores bone loss in ovariectomized rats. Int. J. Pharmacol., 7: 446-454.

Siddiqui, A.A. and M. Ali, 1997. Practical Pharmaceutical Chemistry. 1st Edn., CBS Publishers and Distributors, New Delhi, pp: 126-131.

Soetan, K., M. Cyekunle, O. Aiyelaagbe and M.A. Fafunso, 2006. Evaluation of the antimicrobial activity of saponins extract of *Sorgham bicolour* L. Monech. Afr. J. Biotechnol., 5: 2405-2407.

Soetan, K.O., 2003. Evaluation of some pharmaceutical and haematological activities of saponins in guinea corn (*Sorghum bicolor* L. Moench). M.Sc. Thesis, Department of Biochemistry, College of Medicine, University of Ibadan, Nigeria.

Sunder, J., S. Jeyakumar, A. Kundu, R.C. Srivastava and D.A. Kumar, 2011. Effect of *Morinda citrifolia* extracts on *in vitro* growth of *Ralstonia solanacearum*. Arch. Applied Sci. Res., 3: 394-402.

Van der Watt, E. and J.C. Pretorius, 2001. Purification and identification of active antibacterial components in *Carpobrotus edulis* L. J. Ethnopharmacol., 76: 87-91.

Wang, M.Y. and C. Su, 2001. Cancer preventive effect of *Morinda citrifolia* (Noni). Ann. N.Y. Acad. Sci., 952: 161-168.

Wang, M.Y., B.J. West, C.J. Jensen, D. Nowicki, C. Su, A.K. Palu and G. Anderson, 2002. *Morinda citrifolia* (Noni): A literature review and recent advances in noni research. Acta Pharmacol. Sin., 12: 1127-1141.