Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 3, 2012

Research Article

COMPARATIVE PHYTOCHEMICAL AND ANTIMICROBIAL STUDY OF *MORINDA PUBESCENS* SM. AND *MORINDA CITRIFOLIA* L.

KALPANA SAHOO*, NABIN K DHAL, SOUNDARYA L SAHOO AND SASWATI S. LENKA

Institute of Mineral and Materials Technology (IMMT), Council of Scientific and Industrial Research (CSIR), Bhubaneswar 751013, Odisha, India. Email: kalpanactc@gmail.com

Received: 23 Jan 2012, Revised and Accepted: 23 Mar 2012

ABSTRACT

The phytochemical and antimicrobial activity of crude methanol and chloroform extract of two different plant species such as *Morinda tinctoria* Roxb. and *Morinda citrifolia* L. belonging to the genus Morinda were analysed. In phytochemical analysis, the methanol extract of leaf and stem was found to contain all the phytoconstituents such as alkaloid, glycoside, steroid, triterpenoid, tannin, carbohydrate, protein and flavonoids etc. Whereas in antimicrobial study, *Morinda citrifolia* L. was found to be active against most of the test pathogens such as *Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli, Salmonella typhii, Staphylococcus aureus, Salmonella paratyphii, Pseudomonas aeruginosa, Chromobacterium violecum, Micrococcus leuteus, Pseudomonas aquatum, Aspergellus niger, Aspergillus fumigatus, Candida albicans, Rhizopus oryzae, Sclerotium* sp., *Helminthosporium* sp., and *Curvularia* sp. Maximum antibacterial activity was found against *K. pneumoniae* (20mm), in the leaf extract of *M. citrifolia* whereas maximum antifungal activity was found against *R. oryzae* (18mm) in both plant parts. The MIC values are less than 5-6mg/ml for each extract against pathogens. The result highlights that the studied plants are potential sources of phytochemical constituents and antimicrobial agents which needs further pharmacological screening for drug development programme.

Keywords: Morinda, Phytochemical, Antimicrobial, Traditional medicine, Astaranga

INTRODUCTION

Plants are the source of inspiration of novel drug compounds as they provide key chemical structure for the development of new antimicrobial drugs as well as phytomedicine[1] to be used for the treatment of various diseases. Ayurveda is the most ancient health care system and is practiced widely in India, Srilanka and other countries[2]. In India the Ayurvedic system has been in use for over 3000 years. Considering worldwide comparison of patronization of modern and alternative medicine, it is depicted that 75% of the population world over is compelled to use the alternative system of medicine, especially the herbal medicine indigenous to that part of the world. According to World health Organization (WHO), it has been estimated that 80% people of the developing countries relies mainly on traditional medicine and 85% of the traditional medicine use of plant extracts[3]. Several plants and herb species used traditionally have potential antimicrobial and antiviral properties[4] and this has raised the optimism of scientists about the future of phyto-antimicrobial agents[5]. Natural products of higher plants may give a new source of antimicrobial agents[6,7,8]. Screening of medicinal plants for antimicrobial activities and phytochemical is important for finding potential new compounds for therapeutic use. The present study mainly includes the phytochemical and antimicrobial screening of two different species of one genus such as *M. tinctoria* Roxb. and *Morinda citrifolia* L.

MATERIALS AND METHODS

Study area and Collection of samples

The plant material has been collected from Astaranga which is located at Lat. 19^0 58' N, and Long. 86^0 16' E situated near the mouth of the Devi River and about 60 kilometres east of Puri which lies on the Bay of Bengal coast. The collected plant parts were separated and kept for air drying at room temperature. The identification was done by referring "The Flora of Orissa"[9] and consulting herbarium (RRL-B) bearing voucher specimen number 12734 for *M. tinctoria* Roxb. and 12735 for *M. citrifolia* L.

Preparation of extract

The dried leaves were powdered by using grinder to powder and stored in zipped polythenes for further use. Powdered samples of stem and leaves of *M. citrifolia* and *M. tinctoria* were packed into Soxhlet extracter and were extracted separately with different solvents such as methanol and chloroform at 30-40^{o}C. The extractions were evaporated using rotary evaporator at 40-60^{o}C and then the obtained extracts were kept in desscicator. The obtained crude extract was stored in airtight container in refrigerator at 4^0 C for further studies.

Screening

1. Preliminary Phytochemical Screening

Phytochemical screening of the different solvent extracts was carried out by using the standard protocols as described by Harbone[10].

2. Antimicrobial screening

Test organisms

The antibacterial activity was carried out against some selected pathogens viz. *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella paratyphii B, Chromobacterium violaceum, Salmonella typhii*, *Proteus mirabilis, Micrococcus leuteus, Pseudomonas aquatum* ,*Pseudomonas aeruginosa* and antifungal activity against some human pathogenic fungi viz. *Aspergillus niger, Candida albicans, Aspergillus fumigatus and Rhizopus oryzae, Helminthosporium* sp., *Curvularia* sp., *Sclerotium* sp. by disc diffusion assay method and MIC determination at different dilution. The strains used for the present study obtained from Microbiology laboratory O.U.A.T and Hi-tech medical college, Bhubaneswar.

Preparation of extract

The semi solid extracts dissolved with DMSO for complete dissolution and were stored at 4^{0}C for further study.

Inoculum preparation

The starter culture broth was prepared in tubes with pathogens inoculated into NB and PDB for bioassay and incubated for 24 and 48 hrs at 37^{0}C and 28^{0}C for bacteria and fungi respectively. The turbidity of the medium indicates the growth of organisms.

Antimicrobial study

Antimicrobial activity was assayed by disc diffusion method[11]. Inoculation of the fungal culture into the Petri dish containing medium was carried out uniformly using sterile cotton swab. The crude extracts of methanol and chloroform were dissolved in 5% DMSO prepared as individual stocks in sterile vials. Different solvent extracts were loaded in sterile Whatmann filter paper

discs and air dried thoroughly for 1hr during assay. The plates were incubated overnight at 37^{0}C in BOD incubator to allow the maximum growth of microorganisms and inhibition of bacterial and fungal growth was determined by measuring the diameter of the clear zone in mm around each disc. The experiment was repeated thrice and the results were the mean of the three replicates[11]. A control set of experiment has been carried out with different solvents such as methanol, chloroform and standard set of experiment was carried out using different synthetic discs such as ampicillin (10µg), amikacin (30µg), gentamycin (10µg), streptomycin (10µg) and penicillin G (10µg). The inhibition zone diameter was measured and recorded for each organism. Minimal Inhibitory Concentration (MIC) was determined using different dilutions of extracts following the same procedure as antimicrobial screening against various test pathogens.

RESULTS

From the qualitative phytochemical screening study it has been observed that the methanol extract of leaf and stem of *M. citrifolia* L. contains all the necessary secondary metabolites such as alkaloid, glycoside, steroid, triterpenoid, tannin, carbohydrate, protein and flavonoids except saponins whereas chloroform extract of leaf lacks tannin, saponin, protein and stem extract lacks glycoside, tannin and saponin. Like that methanol leaf extract of *M. tinctoria* Roxb. contains all the metabolites except saponin but the stem extract contains all the secondary metabolites along with saponin. But chloroform extract of leaf lacks glycoside, tannin, saponin, flavonoid and stem extract lacks steroid, saponin and flavonoid. So although these two plants belong to same genus, they still have some difference in the production of secondary metabolites (Table. 1-2).

Table 1: Phytochemical screening of leaf and Stem extracts of *Morinda citrifolia*

Metabolites	Plant extract	Methanol	Chloroform
Alkaloid	Leaf	+	+
	Stem	+	+
Glycoside	Leaf	+	+
	Stem	+	-
Steroid&triterpenoid	Leaf	+	+
	Stem	+	+
Tannin	Leaf	+	-
	Stem	+	-
Saponin	Leaf	-	-
	Stem	-	-
Flavonoid	Leaf	+	+
	Stem	+	+
Carbohydrate	Leaf	+	+
	Stem	+	+
Protein	Leaf	+	+
	Stem	+	+

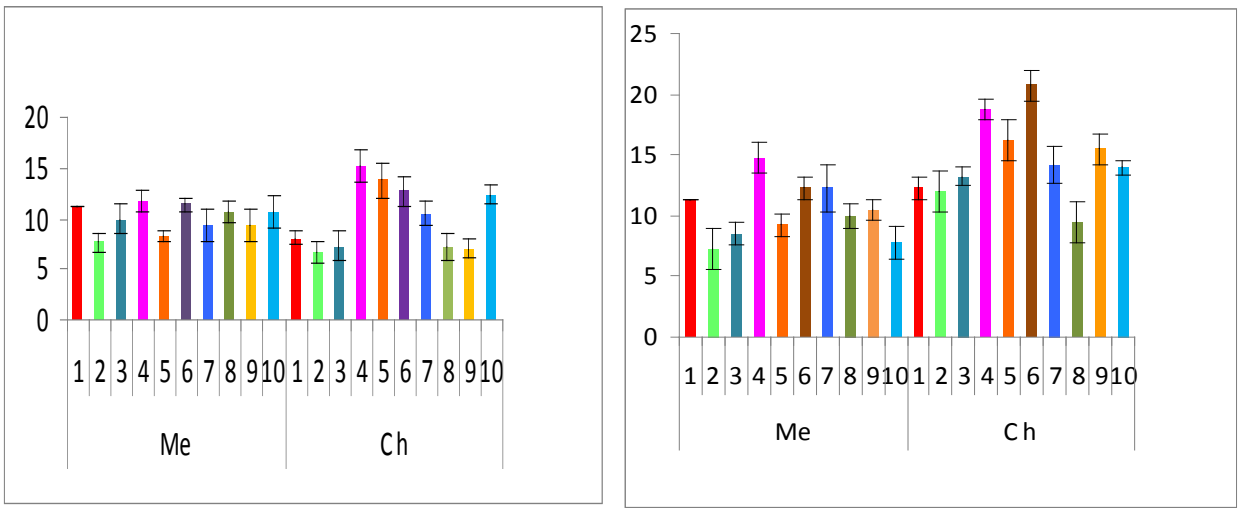
Table 2: Phytochemical screening of leaf and stem extracts of *Morinda tinctoria*

Metabolites	Plant extract	Methanol	Chloroform
Alkaloid	Leaf	+	+
	Stem	+	+
Glycoside	Leaf	+	-
	Stem	+	+
Steroid & triterpenoid	Leaf	+	+
	Stem	+	-
Tannin	Leaf	+	-
	Stem	+	+
Saponin	Leaf	-	-
	Stem	+	-
Flavonoid	Leaf	+	-
	Stem	+	-
Carbohydrate	Leaf	+	+
	Stem	+	+
Protein	Leaf	+	+
	Stem	+	+

The methanol extracts of both plant parts of *M. citrifolia* L. showed maximum activity against *K. pneumoniae (*15mm, 11mm) where the least zone of inhibition was observed against *S. paratyphii* B (8mm) and *P. aquatum* (7mm) respectively. The chloroform extract of *M. citrifolia* showed maximum zone of inhibition against *E. coli* (15mm) and *K. pneumoniae* (20mm) respectively whereas least effect was observed for chloroform extract against *S. typhii* (8mm) (Fig.1). The methanol leaf and stem extract of *M. tinctoria* Roxb. showed maximum zone of inhibition against *K. pneumoniae* (11mm) and *P. aeruginosa* (15mm) respectively. Leaf and stem extract showed minimum inhibition against *P. mirabilis* (8mm) and least inhibition against both *S. paratyphii* B (7mm) and *P. aeruginosa* (7mm). Chloroform extract of leaf and stem showed maximum zone of inhibition against both *K. pneumoniae* (18mm) (Fig. 2&5) and the leaf extract has the minimum inhibition against *P. aquatum* (7mm) and stem extract against both *S. paratyphii* B and *M. leuteus* (7mm).

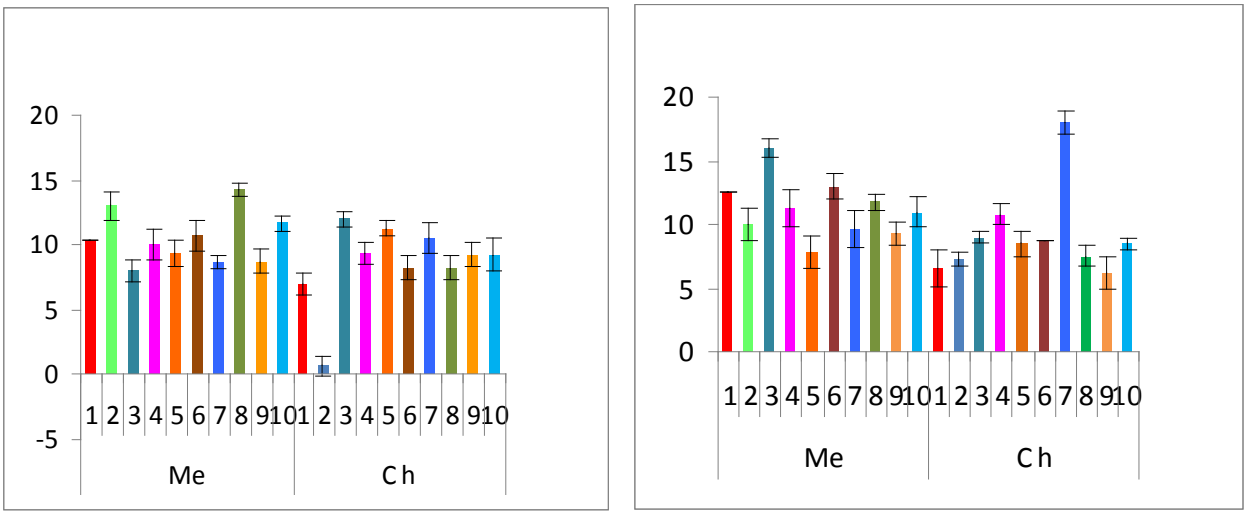
In case of antifungal activity plant parts of *M. citrifolia,* the methanol extract was found to have maximum zone of inhibition against *Helminthosporium* sp. (12mm), *R. oryzae* (10mm) and least activity against *Sclerotium* sp. (7mm) (Fig. 3). Similarly the chloroform extract of both stem and leaf was found to have maximum inhibition against *R. oryza*e i.e. 17mm for stem and 18mm for leaf whereas less activity against *Sclerotium* sp. (8mm). The methanol extract of *M. tinctoria* showed maximum activity against *A. niger* (12mm, 13mm) and minimum effect against *Sclerotium* sp. (8mm) and *C. albicans* (7mm) respectively. Similarly chloroform extract showed maximum inhibition against *Curvularia* sp.(14mm, 11mm) and minimum inhibition was shown against *A. fumigatus* (6mm) (Fig. 4&6). MIC value of *M. citrifolia* and *M. tinctoria* against different microorganisms also varied significantly.

The MIC of methanol extract of *M. citrifolia* was found to be 7mm against *R. oryzae* whereas chloroform extract has inhibition of 7mm against *R. oryz*ae and *Helminthosporium* sp. In case of *M. tinctoria,* methanol extract has inhibition of 7mm, 8mm and 7mm against *K. pneumoniae*, *P. aeruginosa* and *A. niger* respectively. Chloroform extract of leaf and stem of *M. citrifolia* has 8mm and 7mm of inhibition against *R. oryzae* and *Helminthosporium* sp. whereas in case of *M. tinctoria* has inhibition of 7mm and 6mm against *K. pneumoniae* and *P. aeruginosa* respectively.



(a) **(b)**

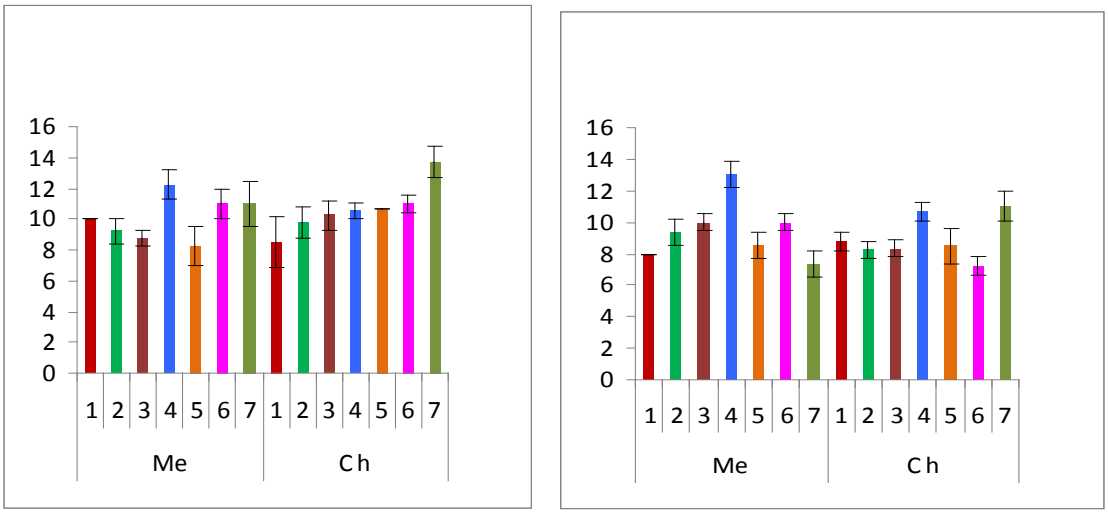
Fig. 1: Antibacterial activity of Leaf (a) and Stem (b) extracts of *M. citrifolia*



(a) **(b)**

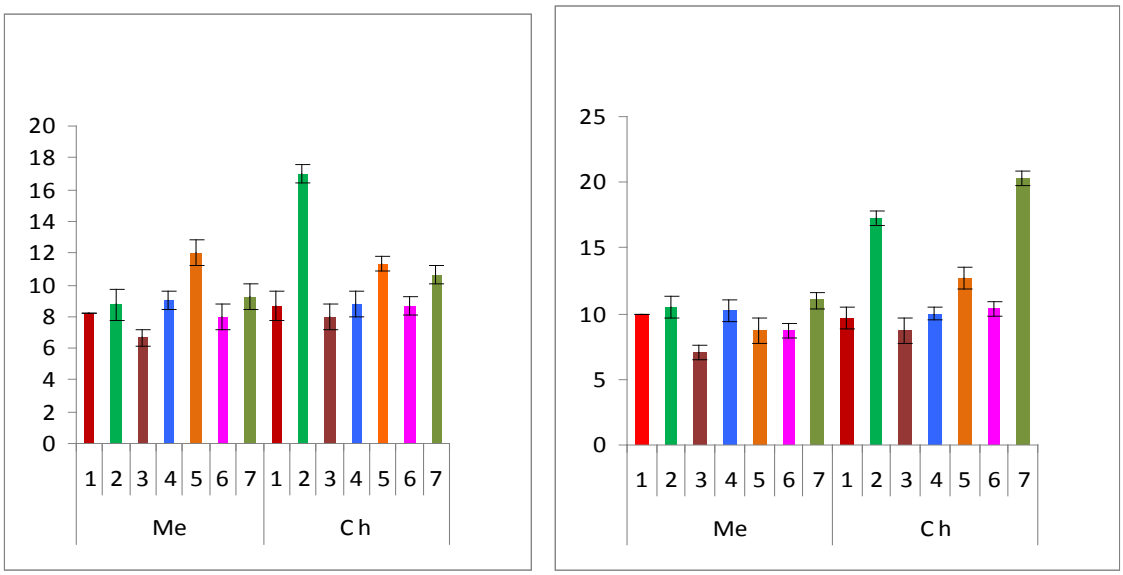
Fig. 2: Antibacterial activity of Leaf (a) and Stem (b) extracts of *M. tinctoria*

*All values are mean ± standard deviation of the four determinants. 1. *P. aquatum* 2. *P .aeruginosa* 3. *M. leuteus*, 4. *S. typhii*, 5. *P. mirabilis*, 6. *E .coli*, 7. *S. paratyphii*, 8. *S.aureus*, 9. *K. pneumoniae*, 10. *C. violaceum*. Me, Ch stands for methanol & chloroform respectively



(a) **(b)**

Fig. 3: Antifungal activity of Leaf (a) and Stem (b) extracts of *M. citrifolia*



(a) **(b)**

Fig. 4: Antifungal activity of Leaf (a) and Stem (b) extracts of *M. tinctoria*

*All values are mean±standard deviation of three determinations. 1. *C.albicans;* 2. *R. oryzae* 3.*Sclerotium sp.*; 4.*A.niger* 5. *Helminthosporium* 6.*A. fumigatus* 7.*Curvularia.* Me and ch stand for methanol and chloroform respectively.

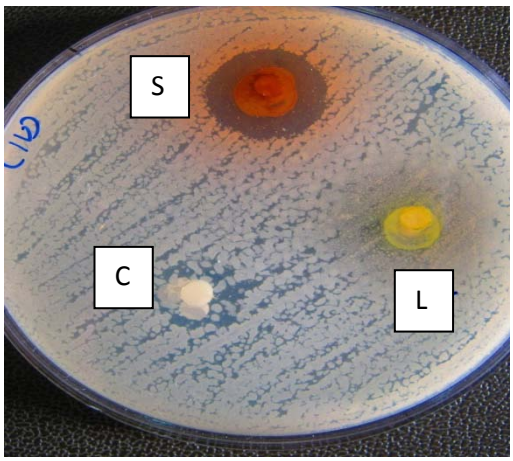


Fig. 5: Antibacterial activity against *K. pneumoniae*

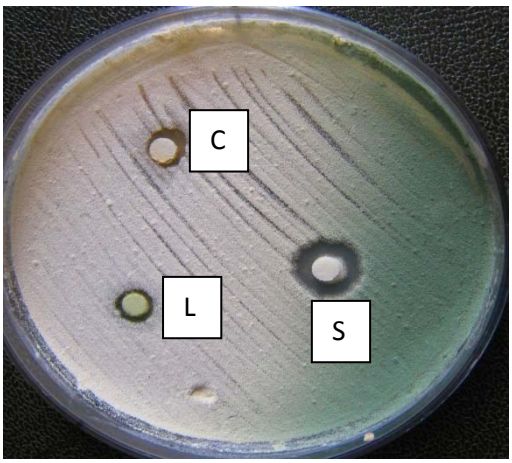


Fig. 6 Antifungal activity against *R. oryzae*

DISCUSSION

From the above study, the chloroform extracts of both the plants showed better inhibition than methanol as it contains all the phytoconstituents. The antimicrobial activity have been screened because of its great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem[12,13]. Knowledge of the phytochemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils precursors for the synthesis of complex chemical substances[14]. Many plants have limitless ability to synthesize secondary metabolites of which at least 12,000 have been isolated and these substances serve as plant defense mechanism against predation by microorganisms, insects and herbivores[15]. Many plants and their extracts used against microbial infections due to the presence of secondary metabolites such as phenols, essential oils, terpenoids, alkaloids and flavanoids[16,17]. Several phenolic compounds like tannin present in the cells of plants are potent inhibitors of many hydrolytic enzymes such as proteolytic enzymes used by plant pathogens. Other preformed compounds like saponins also have antifungal properties[18]. Many plants contain non-toxic glycosides that can get hydrolyzed to release phenolics that are toxic to microbial pathogens[18]. Natural products either extract or pure compounds provide unlimited opportunities for the development of new drugs due to the availability of chemical diversity[19]. To overcome the problem of antibiotic resistance ethnic medicinal plants have been extensively studied as an alternative treatment for diseases due to their ability to produce a variety of compounds of known therapeutic properties[20,21] and much attention has been paid to plant extracts and their biologically active compounds[22]. In this study, it has been observed that *M. citrifolia* extract was found to be more effective than extracts of *M. tinctoria* because *M. citrifolia* has been reported to have a broad range of health benefits for cancer, infection, arthritis, asthma, hypertension and pain[23].

CONCLUSION

From the present investigation, it has been found that both chloroform extract of stem of *M. citrifolia* and *M. tinctoria* was more active in inhibiting most of the bacteria and fungi which are responsible for causing various disease in human. The results of the

present study is highly promising and highlights the new ways for doing studies for further antibacterial and antifungal bioassay to ascertain the maximum potential of plant extract as drugs with minimum effective dose. Literature studies have shown that *M. citrifolia* has been largely exploited for its enhanced antimicrobial activity but work performed on both the species of the genus Morinda such as *M. citrifolia* and *M. pubescens* has revealed that *M. tinctoria* has slightly less antimicrobial potentiality than the other species and hence recommended for further study.

ACKNOWLEDGEMENT

The authors are thankful to Director IMMT, Bhubaneswar for providing necessary facilities to carry out the work and Dr. Sudhi Ranjan Mishra (Asst. Prof.) Hi-tech Medical College for bacterial pathogens and OUAT, Plant Pathogen Division for fungal pathogens.

REFERENCES

1. Abukakar MG, Ukwuani AN and Shehu RA. Phytochemical screening and antibacterial activity of *Tamarindus indica* Pulpextract. Asi J Biochem 2008; 3(2): 134-138.
2. Rout SP, Choudary KA, Kar DM, Das L, Jain A. Plants in Traditional Medicinal System - Future Source of New Drugs. Intern. Jol. of Pharm. and Pharmac Sci 2009; 1 (1): 1-23.
3. Fransworth NR. Screening Plants for New Medicines. E.O.Wilson (editor), Washington, D.C., National Academic Press. 1998; 346-347.
4. Zaika LL. Spices and herbs: Their antimicrobial activity and its determination. J Food Safety 1988; 9: 97-118.
5. Das S, Pal S, Mujib A and Dey S. Biotechnology of medicinal plant Recent advances and potential. 1st Edition, (UK992 Publications, Hyderabad), 1999; 2: 126-139.
6. Hamil FA, Apio S, Mubiru NK, Bukenya-Ziruba R, Mosanyo M and Magangi Ow. Traditional herbal drugs of southern Uganda, II. Literature analysis and antimicrobial assays. J Ethnopharmacol 2003; 84: 57-78.
7. Motsei ML, Lindsey KL, Vanstaden J and Jaeger AK. Screening of traditionally used South African pantsfor antifungal activity against *Candida albicans*. J Ethnopharmacol 2003; 86: 235-41.
8. Kadamban D, Ramanujam MP and Balachandran N. Plants used for improving fertility and curing gynecological diseases by the traditional medicinal practitioners of Pondicherry region. J Swamy Bot 2003; 20: 111-114.
9. Saxena HO and Brahmam M. The Flora of Orissa. 1994-96; Vol-II.
10. Harbone JB. Phytochemical methods: A guide to modern techniques on plant analysis, third edition. Kluwer Academic Publishers, United Kingdom. 1998.
11. Bauer AW, Kirby WMM, Sherris JC and Tarek M. Antibiotic susceptibility testing by a standardized single disc method, Am J Clin Pathol 1966; 45: 493-496.
12. Austin DJ, Kristinsson KG and Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci, USA 1999; 96: 1152-6.18.
13. Venkatesan D and Karrunakaran CM. Antimicrobial activity of selected Indian medicinal plants. J of Phytology 2010; 2(2): 44–48.
14. Akrout EI, Jani H, Zammouri T, Mighri H and Neffati M. Phytochemical screening and mineralcontents of annual plants growing wildin the southern of Tunisia, J of Phytology 2010; 2(1): 034–040.
15. Wink M. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theor and Appl Gen 1998; 75: 225-233.
16. Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E and Palmas F. *In vitro* antimicrobial activity and chemical composition of *Sardinian thymus* essential oils. Lett in Appl Microbil 1999; 29: 130-135.
17. Daferera DJ, Ziogas BN and Polissiou MG. The effectiveness of plant essential oils in the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. *michiganensis*. Crop Protection, 2003; 22: 39-44.
18. Aboaba OO, Efuwape BM. Antibacterial properties of some Nigerian species. Bio Res Comm 2001; 13: 183-188.
19. Cos P, Vilietinck AJ, Vanden Berghe D, and Maes L. Anti-infective potential of natural products: how to develop a stronger *in vitro* proof-of concept. J of Ethanopharmacol 2006; 106: 290-302.
20. Kumar B, Vijaykumar M, Govindarajan R and Pushpangadan P. Ethnopharmacological approachesto wound healing-Exploring medicinalplants of India. J of Ethnopharmacol 2007; 114 (2): 103-113.
21. Ramappa R. and Mahadevan, G. D. *In Vitro* antimicrobial activity of various plant latex against resistant human pathogens. Intern J of Phar and Pharmac Sci 2011; 3(4): 70-72
22. Suresh G, Ramesh B, Kavitha K, Ravichandran N, Suresh R, Gopalakrishnaan A and Vijaiyan Siva G. Preliminary screening of antibacterial compounds from Pal arriver basin flora. J of Phytol 2010; 2(2): 24-29.
23. Whistler, W. Tongan herbal medicine. *Isle Botanica,* Honolulu Hawaii. 1992; 89-90.