JPR INFO
Journal of Pharma Research

Journal of Pharma Research
Available online through
www.jprinfo.com

Research Article
ISSN: 2319-5622

Identification of Secondary Metabolite and Screening for Antibacterial and Antioxidant Activity in Leaf Extracts of *Derris trifoliata* L.

Nymathullah. Sharief Md[1], A. Srinivasulu[1] P. Satya Veni[1] and Uma Maheswara Rao V[2]
[1]*Department of Biochemistry, V.S.Lakshmi Women's Degree & P.G. College, Kakinada*
[2]*Department of Microbiology, Acharya Nagarjuna University, Guntur, Andhra Pradesh, INDIA.*

Received on: 29-03-2014; Revised and Accepted on: 17-04-2014

ABSTRACT

Leaf extracts of Derris trifoliata L. were prepared in ethyl acetate, acetone, methanol and ethanol. The crude extract were qualitatively considered for the presence of alkaloid, flavonoids, saponins, steroids and tannins and quantitatively determined for antioxidant and antibacterial activity. The antioxidant activity was screened through ABTS method. The leaf extracts in methanol demonstrated the higher antioxidant activity. The antibacterial activity were evaluated against Escherichia coli MTCC 7410, Enterobacter aerogenes MTCC 7324, Enterobacter cloacae MTCC 7408, Klebsiella pneumonia MTCC 7028, Proteus vulgaris, MTCC 426, Pseudomonas aeruginosa MTCC 7083, Bacillus subtilis MTCC 736, Bacillus cereus MTCC 430, Enterococcus faecalis MTCC 9845, Lactobacillus delbrueckii MTCC 911, Staphylococcus aureus MTCC 737 and Streptococcus pyogenes MTCC 1928 by agar well diffusion method and compared with standard antibiotic gentamicin and ciprofloxacin. The extent of inhibition by the leaf extracts diverge from one solvent system to the other, among them acetone and ethanol extracts exhibited higher level of inhibition against the gram positive test cultures compared to gram negative test cultures employed. The extracts that possessed antibacterial activity were further subjected to the determination of the Minimum Inhibitory Concentration (MIC) using different concentrations viz., 1.25, 2.5 and 5.0mg /100µl. The value of MIC was found to be 1.25mg/100µl to 5mg/100µl. Further, the extracts that demonstrated the antibacterial activity were checked for their bactericidal or bacteriostatic nature. The phytochemical results are in correlation with antioxidant and antibacterial activity. The present study reveals the potential of leaf extracts of Derris trifoliata L as antioxidant and antibacterial agent.

Keywords: *Derris trifoliata L, Leaf extracts, Secondary metabolites, Antioxidant activity, Antibacterial activity.*

INTRODUCTION

The plant kingdom represents an enormous reservoir of biologically active compounds with protective, disease preventive properties. Plants produce two types of metabolites. Primary metabolites are involved directly in growth and metabolism, viz. carbohydrates, lipids and proteins. Secondary metabolites are considered products of primary metabolism and are generally not involved in metabolic activity viz. alkaloids, flavonoids, lignins, phenols, sterols, tannins, terpenes etc. These secondary metabolites are the major sources of antibacterial, antioxidant and anticancer agents [1]. The use of plants as a source for remedy from illness can be tracked since ancient times. The plants used in traditional medicine are still a large source of natural antioxidants. Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease [2]. Plants serve as a reservoir of effective chemotheraputants and provide valuable sources of natural products in the control of several bacterial and fungal diseases [3, 4]. Antibiotics are the most important weapons in fighting against bacterial infections. Many pathogenic organisms are developing plasmid-mediated resistance to the popular drugs. Hence, there is a need for unique natural compounds that can be obtained from the nature either from microorganisms or from plants. The potential of mangrove plants as a source of new principles is still unexplored. Only a small percentage of mangrove plants have been investigated phytochemically and the fraction submitted to biological activity is even smaller. The present study is paying attention on the exploitation of leaves of *Derris trifoliata* L for extraction in different organic solvents to evaluate their phytochemical constituents and screen for their antibacterial activity against selected MTCC bacterial cultures and antioxidant activity by ABTS method.

***Corresponding author:**
Mr. Nymathullah. Sharief Mohammad
Department of Biochemistry
V S Lakshmi Women's Degree & PG College, NFCL Road,
Kakinada, East Godavari District, Andhra Pradesh, India.
Contact No. - 919346440238
**E-Mail: nshariefmd@gmail.com*

MATERIALS AND METHODS

Collection of plant material:

The leaves of *Derris trifoliata* L. were collected from East Godavari mangroves at Corangi Reserved Forest, (Geographically located between 16° 39' N longitude-17° N longitude and 82° 14' E latitude -82° 23'E lattitude) Kakinada, Andhra Pradesh, India. The leaves were collected in new polythene bags and surface sterilized with 1% mercuric chloride solution. The leaves were chopped separately into small pieces and shade dried at room temperature for seven days.

Extraction:

The extraction of leaves was carried out with different solvents in their increasing order of polarity viz., ethyl acetate, acetone, methanol and ethanol by soaking the plant material in the respective solvents overnight at room temperature one after the other [5]. The contents of each flask were subjected to reflux below the boiling point of the respective solvents viz., ethyl acetate (77°C), acetone (55°C), methanol (65°C) and ethanol (78°C) for 6-8h in order to extract the active compounds into the solvent. The extracts were filtered through whattman No.1 filter paper and the residual material was re-extracted with fresh solvent. After 24h the process was repeated. Pooled extracts were individually concentrated by removing the solvent under reduced temperatures using vacuum rotator evaporator. These extracts were further concentrated by solvent evaporation using thin film method. Dried leaf extract of 100mg each was dissolved in 10ml of 1:10 diluted DMSO in sterile distilled water so as to obtain the final concentration of 10mg/ml [6]. All the extracts thus prepared were stored in a refrigerator at 4°C.

Identification of Secondary Metabolites:

The crude extracts were subjected to the qualitative determination of secondary metabolites viz., alkaloids, flavonoids,

saponins, steroids and tannins by the standard protocols of phytochemistry [7, 8].

Test for Alkaloids:

To 5 ml of the crude extracts were stirred with 10 ml of 1% aqueous HCl on water bath and then filtered. To 2ml filtrate 4-6 drops drops of Dragendroff's reagent was added. Formation of orange –red precipitate was considered as positive to alkaloids.

To another 2 ml filtrate few drops of Mayer's reagent was added and appearance of buff-coloured precipitate was taken as existence of alkaloids.

Test for Flavonoids:

About 5ml of the test solution was boiled with 10 ml of distilled water and then filtered. Then, 2 ml of lead acetate solution was added to 2 ml of the filtrate. Appearance of buff coloured precipitates considered as presence of flavonoids.

To 2 ml of the filterate, 5 ml of dilute ammonia solution was added followed by 4-6 drops of concentrated sulphuric acid. Appearance of yellow color indicates the presence of flavonoids.

Test for Saponins:

About 5 ml of crude extract was shaken with 5 ml of water in a test tube and it was warmed in a water bath. The persistent froth indicates the presence of saponins.

Test for steroids:

About 5 ml of each extract was dissolved in 2ml of chloroform, followed by the addition of conc. H_2SO_4 to form reddish brown colour at interphase indicates the presence of steroids.

To 5ml of each extract, 2ml of 3.5% $FeCl_3$, 4-6 drops of glacial acetic acid and 2ml of conc. H_2SO_4 were added carefully. Appearance of reddish brown ring at the junction is a positive indication for the existence of steroids.

Test for tannins:

About 5ml of each extract was stirred with about 10ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2ml of the filtrate. Occurence of a blue-black, green or blue-green precipitate indicates the presence of tannins.

About 5 ml of each extract was added with 1 ml of 1% HCl solution. Formation of red precipitate indicates the presence of tannins.

Determination of antioxidant activity by ABTS method:

Total antioxidant capacity of each extract was measured using 2,2′-azinobis[3-ethylbenzthiazoline]-6-sulfonic acid (ABTS) assay. ABTS and potassium per-sulfate were separately dissolved in deionized distilled water to a final concentration of 7mM and 2.45mM respectively. The two solutions were mixed and allowed to stand in dark at room temperature for 16h before use in order to produce ABTS radical (ABTS•+). The resultant intensely-coloured ABTS•+ radical cation was diluted with 0.01M PBS (phosphate buffered saline), pH 7.4, to give an absorbance value of ~0.70 at 734 nm. The test compound was diluted 100X with the ABTS solution to a total volume of 1ml. Absorbance was measured spectrophotometrically at time intervals of 3min after addition of each extract. The assay was performed at least in triplicate. Controls were run using PBS in place of the extract. The assay relies on the antioxidant capability of the samples to inhibit the oxidation of ABTS to ABTS•+ radical cation. Percent inhibition was calculated using the following formula [9].

% inhibition of oxidation of ABTS to ABTS•+ =

$$\frac{\text{Initial absorbance - Final absorbance X 100}}{\text{Initial absorbance}}$$

Determination of antibacterial activity:

The bacterial strains employed in this study are viz., *Escherichia coli* MTCC *7410, Enterobacter aerogenes* MTCC *7324, Enterobacter cloacae* MTCC *7408, Klebsiella pneumonia* MTCC *7028, Proteus vulgaris,* MTCC *426, Pseudomonas aeruginosa* MTCC *7083, Bacillus subtilis* MTCC *736, Bacillus cereus* MTCC *430, Enterococcus faecalis* MTCC *9845, Lactobacillus delbrueckii* MTCC *911, Staphylococcus aureus* MTCC *737* and *Streptococcus pyogenes* MTCC *1928* are done by agar well diffusion method [10], and zones of inhibition were measured. Each experiment was performed in triplicate and the average value of inhibition and standard deviation were calculated. The zone of inhibition was compared with that of standard antibiotics Gentamicin 30 µg disc as well as Ciprofloxacin 5 µg disc.

Determination of MIC:

Minimum Inhibitory Concentration (MIC) as well as bactericidal or bacteriostatic activity was determined by broth dilution assay method. For the determination of MIC, the reconstituted extract in DMSO was serially diluted in Mueller Hinton broth medium to get the concentrations of 1.25, 2.5and 5.0mg/100µl [11].

Determination of Bactericidal or Bacteriostatic activity:

For the determination of bactericidal or bacteriostatic activity, 0.1ml of culture medium from each broth tube showing no apparent growth was picked upon and sub-cultured on fresh Mueller Hinton agar medium. After incubation at 37° C for 24 hrs, plates showing no visible growth of bacteria were considered for Bactericidal effect and plates with visible growth of bacteria as Bacteriostatic [12].

RESULTS

Phytochemical Analysis of Secondary Metabolites:

Plants are important source of potentially useful principles for the development of new bioactive entities. The knowledge of secondary metabolites and their biological activities is desirable, not only for discovery of novel active principles, but also useful in disclosing new sources of already known biologically active compounds. *Derris trifoliata* L is the member of Fabaceae and this family includes many plants that are rich source of wide-range of chemical constituents. The leaves of *Derris trifoliate* were proved to have many therapeutic uses including stimulant, antispasmodic, counter-irritant and rheumatism [13]. The leaves contain the chemical compound rotenone, a poison that kill a wide range of creatures from insects, earthworms and fishes by inhibiting the complex I of the oxidative phosphorylation [14].

Table No. 1: Phytochemical analysis of Leaf extracts of Derris trifoliata

	Ethyl acetate	Acetone	Methanol	Ethanol
Alkaloid	+	+	-	-
Flavonoid	+	+	+	+
Saponin	-	+	+	+
Tannins	+	+	+	+
Steroids	+	+	+	

The data of phytochemical studies is given in **Table 1**. From this data it is very clear that flavonoids and tannins are there in all the solvents used for the extraction of secondary metabolites present in leaves. Alkaloids are in attendance only in ethyl acetate and acetone extracts. Whereas, saponins are seen in all the solvents used except ethyl acetate. However, Steroids are detected in ethyl acetate acetone and methanol only. These secondary metabolites are incredible group of compounds present in all the plants.

Antioxidant Activity:

Several techniques have been used to determine the antioxidant activity. Free radicals are known to play a definite role in a wide variety of pathological symptoms. Antioxidants fight against free radicals and protect us from various diseases. The non enzymatic antioxidant activity of plants is authorized to the secondary metabolite present the antioxidant activity of the fractions was estimated spectrophotmetrically by ABTS method and the data is given in **Fig. 1**. The outcome of antioxidant activity by ABTS method is high for methanol extract followed by ethanol,

acetone and ethyl acetate. These results correlate with the amount of secondary metabolites present the leaf extracts of *Derris trifoliate* in ethyl acetate, acetone, methanol and ethanol.

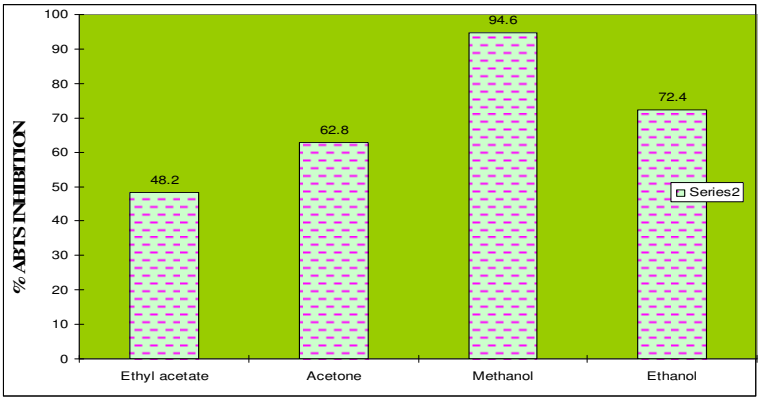

Fig. 1: Antioxidant activity by ABTS Method

Antibacterial Activity:

The efficacy of the leaf extract of *Derris trifoliata* L against the gram negative test cultures used are given in **Fig. 2**. The effect of ethyl acetate extracts on the entire gram negative cultures used are moderately active than the two standard antibiotics used. methanol and ethanol extracts show higher zone of inhibition on all the gram negative test organisms used compare to that of the ethyl acetate and acetone extracts. Whereas, the acetone extracts are effective only on *Escherichia coli*, *Enterobacter aerogenes* and *Proteus vulgaris* with same zone size of 13.66 mm. Amid the antibiotics engaged ciprofloxacin displayed higher zone of inhibition on all the cultures used in this study. *Escherichia coli*, *Enterobacter aerogenes* and *Proteus vulgaris* are the cultures which are inhibited by all the extracts. The efficacy of the methanol extracts is extreme on the gram negative test cultures than gentamicin and moderate than ciprofloxacin. None of the extracts exhibited higher zone of inhibition than ciprofloxacin. The effect of the extracts and standard antibiotics followed a trend with *Escherichia coli* in which the zone of inhibition is in increasing order from ethyl acetate extract (12.66mm) to ciprofloxacin (20.66mm). Among all the extracts the maximum zone of inhbition is demonstrated by methanol extract to *Escherichia coli* (19.66 mm).

The antibacterial activity of *Derris trifoliate* leaf extracts with gram positive cultures was summarized in **Fig. 3**. Acetone, methanol and ethanol extracts were active beside all the gram positive cultures used. Ethyl acetate infusions were active only against, *Bacillus subtilis, Bacillus cereus,* (15.33mm) *Staphylococcus aureus* and *Streptococcus pyogenes* (13.66mm).

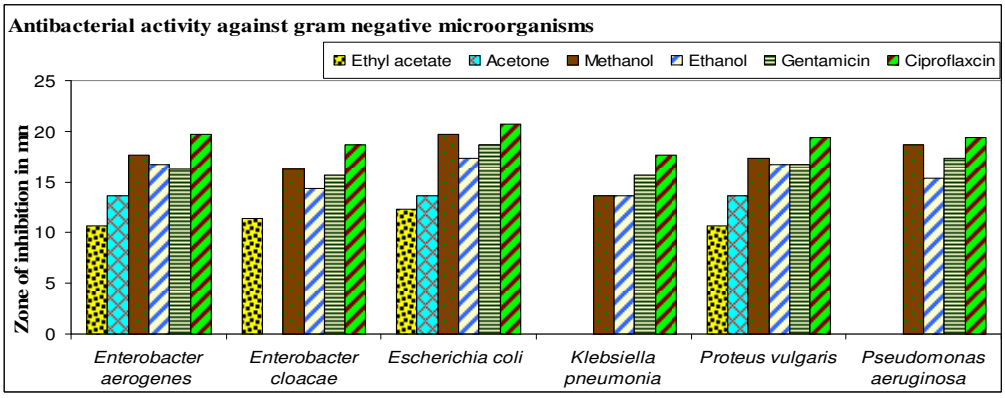

Fig. 2: Antibacterial Activity by the Extracts with Gram Negative Test Organisms

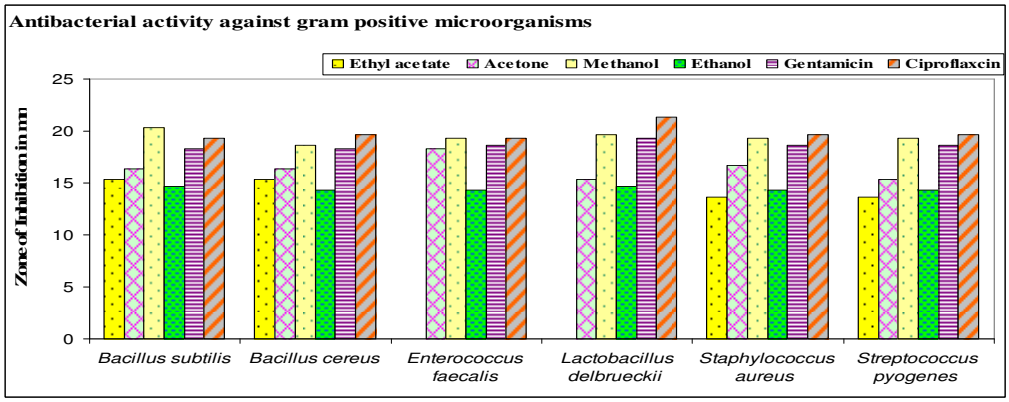

Fig. 3: Antibacterial Activity by the Extracts with Gram Positive Test Organisms

The efficacy of methanol extracts is more than gentamicin and less than ciprofloxacin for gram positive test cultures. Where as, the zone of inhibition given away by the acetone and ethanol extracts are inferior than gentamicin and ciprofloxacin. Among all the extracts the maximum zone of inhbition is demonstrated by methanol extract to *Bacillus subtilis* (20.33 mm) amid antibiotic it is ciprofloxacin to *Lactobacillus delbrueckii* (21.33mm).

The positive extracts for antibacterial activity, were further tested to determine the Minimum Inhibitory Concentration (MIC) at different concentrations viz., 1.25, 2.5 and 5.0 mg /100µl and the data is given in **Table 2**. The value of MIC was found to be in the range of 1.25 to 5.0 mg /100µl for leaf, extracts of *Derris trifoliata* L. against all the bacteria tested.

Table No. 2: MIC of the *Derristrfoliata* leaf extracts in mg/100µl

Micoorganisms	Ethyl Acetate	Acetone	Methanol	Ethanol
Escherichia coli	5	5	2.5	2.5
Enterobacter aerogenes	5	-	2.5	2.5
Enterobacter cloacae	5	5	2.5	2.5
Klebsiella pneumonia	-	-	5	2.5
Proteus vulgaris	5	5	2.5	2.5
Pseudomonas aeruginosa	-	-	2.5	2.5
Bacillus subtilis	2.5	2.5	1.25	5
Bacillus cereus	2.5	2.5	1.25	5
Enterococcus faecalis	-	2.5	1.25	5
Lactobacillus delbrueckii	-	2.5	1.25	5
Staphylococcus aureus	5	2.5	1.25	5
Streptococcus pyogenes	5	2.5	1.25	5

The MIC of leaf extracts in Ethyl acetate was found to be 5mg/100µl for all the bacteria tested except *Bacillus subtilis*, *Bacillus cereus* (2.5mg/100µl). Whereas, the acetone extract exhibited 2.5mg/100µl MIC value for the gram positive test cultures and 5mg/100µl for the gram negative microorganisms. However, the methanol infusion given away 1.25mg/100µl to gram positive microorganisms and 2.5mg/100µl to the entire gram negative cultures except *Klebsiella pneumonia* (5mg/100µl). The MIC value of ethanol extracts will be as 2.5mg/100µl and 5mg/100µl for gram negative and gram positive microorganisms respectively.

Bactericidal or Bacteriostatic Nature:

The bactericidal or bacteriostatic nature of the stem and root extracts that are positive for antibacterial activity are given in **Table 3**. The leaf extracts of acetone is bactericidal in nature to gram negative cultures employed and bacteriostatic for the gram positive organisms used. Whereas, ethyl acetate, methanol and ethanol extracts of leaf are bacteriostatic to all the cultures

Table No. 3: Bactericidal or Bacteriostatic Nature of Derris trifoliata Extracts

Organism	Ethyl acetate	Acetone	Methanol	Ethanol
Escherichia coli	BS	BC	BS	**BS**
Enterobacter aerogenes	BS	-	BS	**BS**
Enterobacter cloacae	BS	BC	BS	**BS**
Klebsiella pneumoniae	-	-	BS	**BS**
Proteus vulgaris	BS	BC	BS	**BS**
Pseudomonas aeruginosa	-	-	BS	**BS**
Bacillus subtilis	BS	BS	BS	**BS**
Bacillus cereus	BS	BS	BS	**BS**
Enterococcus faecalis	-	BS	BS	**BS**
Lactobacillus delbrueckii	-	BS	BS	**BS**
Staphylococcus aureus	BS	BS	BS	**BS**
Streptococcus pyogenes	**BS**	**BS**	**BS**	**BS**

DISCUSSION

Abiy Yenesew et al. [15] reported that the acetone extract of roots of *Derris trifoliata* have larvicidal activity mainly due to Rotenone and Deguelin. Khan et al. [16] reported the inhibition of growth of 24 bacterial cultures tested by the leaf and root extracts of *Derris trifoliata* in petroleum ether, dimethyl chloride, ethyl acetate, butanol, and methanol fractions. The phytochemical studies revealed that the leaf extract of ethyl acetate contains alkaloids, flavonoids, sterols, tannins and triterpenoids, where as the methanol extracts have flavonoids and tannins. The present experimental data is in accordance with that of Govindasamy *et.al* [17] who studied the chemical constituents of different mangroves in India. Sugunya and Thangaraj 2014 [13] commented that the Leaf extracts of *Derris trifoliata* in methanol were controlled the growth of *Escherichia coli* and *Staphylococcus aureus* but failed to inhibit the growth of *Klebsiella pneumonia*. Anil kumar et.al (2011) studied the effect of *Derris trifoliate* leaf extract in Chloroform, Petroleum ether, Methanol and Ethanol [18]. According to them all the extracts were having antimicrobial activity, our results are in agreement with their study.

According to Pushpa Damayanthi Abeysinghe (2012) the leaf extracts of *Derris trifoliate* in Ethanol failed to control the growth of *Proteus* species [19] but our result are not in accord with them. The Ethanol extracts in our study inhibited all the cultures used, one amid them is *Proteus vulgaris*. The zone of inhibition of the extracts are more on gram positive cultures compare to that of the gram negative cultures, This result are in consensus with the report of Pushpa Damayanthi Abeysinghe. Plant extracts generally have more inhibitory effect with gram positive microorganisms than gram negative microorganisms. This may be due to composition of the outer membrane layer of their cell wall. Gram negative bacterial cell wall is very rich with lipopolysaccharide and it may restrict the passage of the active principles present in the extracts

In 2011 Saifullah studied the phytochemical screening of *Derris trifoliate* plant in 80% Ethanol and concluded that it contains more amount of flavonoids and tanins and moderated amount of alkaloid [20]. The outcome of our result is in agreement with their study. Shamsuddin et.al (2013) [21] exposed the antibacterial activity of *Derris trifoliate* along with some selected mangrove leaves extracts in Methanol and Aqueous solvents against *Vibrio* species and reported that inhibitory effect of *Derris trifoliate* is very less compare to that of the other tested mangrove leaves. The Methanol infusion in our study will have higher zone of inhibition against the gram negative microorganisms compare to that of the other extracts. This difference may be due to the bioactive principle present in the extract, or the *Vibrio* species may have some resistance capacity against the bioactive principle present in the extracts.

In our study also, the leaf extracts of *Derris trifoliata* in Methanol and Ethanol were active against all the test cultures used. This may be due to the presence of bioactive principles like alkaloids, flavonoids, saponins, sterols, tannins and triterpenoids. This observation holds in good concurrence with the above mentioned earlier reports. The present phytochemical studies and also the antioxidant activity studies justifies the parameter. Leaf extracts in different solvents used in our study exhibited variation in effectiveness towards antioxidant activity and the antibacterial property against the tested bacteria. These properties may be due to the bioactive principles that are present in the plant extracts.

However, some extracts were unable to exhibit antibacterial activity against tested bacterial strains. This may be due to masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extract, or these bacterial strains may have some kind of resistance mechanisms, for example, enzymatic inactivation, target site modification and decreased intracellular drug accumulation. The variation of antibacterial activity of our extracts might be due to availability of bioactive principle, which assorted from fraction to fraction. The leaf extracts that showed antibacterial activity were compared with broad spectrum antibiotic gentamicin 30 µg disc as well as ciprofloxacin 5 µg disc.

From this comparison, it was observed none of the extract was more effective than ciprofloxacin, but some of the extracts in crude form itself were more effective than gentamicin. With the case of the positive extracts exhibiting equal or less effectiveness in comparison to gentamicin, there is every possibility for having more antibacterial activity than gentamicin when the bioactive compounds of these extracts were purified and tested.

Several reports are documented in literature on determination of MIC values of several plant extracts. Different workers reported different ranges of MIC values with respect to the solvents, plants and plant parts. Anil *et.al* (2011) studied the MIC values of the acetone, methanol and ethanol extracts of stem bark of *Bridelia retusa* Spreng and reported the MIC values ranging from 2.5 to 5mg/ml [22]. Okoli and Iroegbu (2005) [23], from their studies reported the MIC range value of 3.125 to 12.50 mg/ml for the ethanol root extracts of *Synclisa scabrida*.

In our study, the MIC value for all the positive extracts against the tested bacteria were between 1.25mg/100µl to 5mg/100µl. Gram positive test cultures have lower MIC values than Gram negative test cultures to the leaf extracts in ethyl acetate and acetone. However, leaf extracts in methanol will have lower MIC value for gram positive cultures used than gram negative cultures. These differences may be explained by susceptibility testing condition, physico chemical characters of the bioactive principle present in the extract and even strain to strain difference.

In comparison to some of the earlier reports [24] Maria *et al.* 2003 and Celso *et al.*1999 [25] on MIC values of pure compounds, our MIC values may be higher. But this can be substantiated by the argument that this value is for the crude extract. However, the purified form of bioactive compound of the crude extract responsible for antibacterial activity may exhibit the inhibitory effect at a lower concentration. So, the leaves of *Derris trifoliata* L is strongly recommended for consideration as a valuable source for identification, isolation and characterization of potential bioactive compounds with antibacterial property. Finally, there is a need to explore this area further to understand the potentiality of the mangrove plants towards the development of new era medicines with different solvents against many pathogenic microorganisms, so as to get the lead molecule to combat the diseases caused by the microorganisms.

CONCLUSION

This study has exposed the evidence of antioxidant and antibacterial ability of *Derris trifoliata* leaf extracts in specific solvents. Alkaloids, flavonoids, steroids and tannins are known for the antibacterial activity and the phytochemical analysis of different solvent extracts indicates that flavonoids and tannins are common in all the extracts with antibacterial properties. These studies also validate that the *Derris trifoliata* leaf extracts are used for the treatment bacterial disorder. The *Derris trifoliata* leaf extracts can be used to discover new bioactive natural products and can be used a potential source that may control microbial disorders. Hence, there is need for further studies on the plants parts in order to isolate, identify, characterize and elucidate the structure of bioactive compounds which possess antioxidant and antimicrobial activity.

ACKNOWLEDGEMENTS

The first author Mr. Nymathullah .Sharief Mohammad, would like to express heart full thanks to the Secretary and Correspondent Dr. V. Sitaramaraju of V.S.Lakshmi Women's Degree and P.G. College, Kakinada, for providing the necessary facilities to pursue the work. Thanks are also due to the Director, P.G. Courses Dr. A. Srinivasulu of V.S.Lakshmi Women's P.G. College, Kakinada.

REFERENCES:

1. De Boer, HJ. Kool, A., & Broberg, A. Antifungal and antibacterial activity some herbal remedies from Tanzania. J. Ethnopharmacol., **2005**; 96: 461-469.
2. Padma R, Parvathy N.G, Renjith V, Kalpana P. Rahate. Quantative estimation of tannins, phenols and antioxidant activity of Methanolic extract of Imperata cylindrical, International Journal of Research in Pharmaceutical Sciences, **2013**; 4(1): 73-77.
3. Khan, M.R., Kihara, M., Omoloso, A.D. Broad spectrum antibacterial activity of the leaves, stem and root barks of Myristica subabulata, Natural product sciences, **2001**; 7(1): 9-12.
4. Perez, R.M. Antiviral activity of compounds isolated from plants, Pharmaceutical Biology, **2003**; 41: 105-57.
5. Choudhury S A. Sree, S.C. Mukherjee, P.Patnaik and M. Bapuji. In Vitro Antibacterial Activity of Extracts of Selected Marine Algae and Mangroves against Fish Pathogens, Asian Fisheries Science, **2005**; 18: 285-294.
6. Nkere,C.K., Iroegbu, C.U. Antibacterial screening of the root, seed and stem bark extracts of Picralima nitida, African journal of biotechnology, **2005**; 4(6): 522-26.
7. P.Satya Veni, S. Sunita and A.Srinivasulu. Evaluation of antibacterial activity on selected bacteria and screening of secondary metabolites of Avicennia alba stem. International Journal of Advanced Biotechnology and Research, **2013**; 4(4): 511-517.
8. Rahmi Nurdiani, Muhamad Firduas, Asep Awaludin Prihanto. Phytochemical screening and antibacterial activity of methanol extract of mangrove plant Rhizophora mucronata from Porong River Estuary. Journal of Basic Science and Technology, **2012**; 1(2): 27-29.
9. N.Sharief Md, A.Srinivasulu, P.Satya Veni and Uma Maheswara Rao V. Quantification of Tannins, Antioxidant Capacity and Antibacterial Activities of Stem extracts of Avicennia marina (L). International Journal of Biological & Medical Research, **2014**; 5(1): 3869-3873.
10. Okeke MI, Iroegbu CU, Eze EN, Okoli AS and Esimone CO. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity, Journal of Ethanopharmacology, **2001**; 78:119-127.
11. Damintoti K, Mamoudou HD, Jacques S, Alfred ST. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina faso, African Journal of Biotechnology, **2005**; 4: 823-828.
12. R Suganya and M Thangaraj. Mangrove plant Derris trifoliate evaluation of antibacterial property Asian Journal of Pharmaceutical and Clinical Research, **2014**; 7(l): 1230-232.
13. Peter K L N and N Sivasothi. "A Guide to the Mangroves of Singapore I: The Ecosystem and Plant Diversity", Singapore Science Centre, **1999** (p. 108: description, habit, photo).
14. Abiy Yenesew, E.K., Mushibemartha, I., Solomon, D., Midiwo, J.O., Jacques, M., Kabaru, M., Heyedenreich, A. K.,Martin, G. Peter. 7a-O-methyldeguelol, a modified rotenoid with an open ring-C, from the roots of Derris trifoliata, Phytochemistry, **2005**; 66: 653-657.
15. Khan, M. R., Omoloso, A. D., Barewai Y. Antimicrobial activity of the Derris elliptica, Derris indica and Derris trifoliata extractives, Fitoterapia, **2006**; 77: 327-330.
16. Govindasamy Acoramoorthy, Fu-an Chen, Venugopalan Venkatesalu, Daih-Huang Kuo and Po-Chuen Shea. Evaluation of antioxidant polyphenols from selected mangrove plants of India. Asian Joournal of Chemistry, **2008**; 20(2): 1311-1322.
17. Vunduru Anil Kumar, Kanduru Ammani and Subi Siddhardha. In vitro antibacterial activity of leaf extracts of certain mangrove plants collected from Godavari estuarine of Konaseema delta, India. International Journal of Medicinal Aromatic plants, **2011**; 2: 132-136.
18. Pushpa Damayanthi Abeysinghe. Antibacterial activity of aqueous and ethanol extracts of mangrove species collected from Southern Sri Lanka. Asian Journal of Pharmaceutical and Biological Research, **2012**; 2(1): 79-83.
19. Saifullah Al Mamoon, Mohammad gulam Azam. Preliminary phytochemical screening and antidiarrhoeal activity of Derris trifoliate Lour. International Journal of Pharmaceutical Sciences and Research, **2012**; 3(1): 97-100.

20. A.A. Shamsuddin, M. Najiah, A. Suvik, M.N. Azariyah, B.Y. Kamaruzzaman, A.W. Effency and B. Akbar John. World Applied Sciences Journal, **2013**; 25(2): 333-340.
21. Anil U Tatiya, Ganesh G Tapadiya, Sneha Kotecha and Sanjay j Surana. Effect of solvents on tatal phenolics, antioxidant and antimicrobial properties of Bridelia retusa Spreng. Stem bark. Indian Journal of Natural Products and Resources, **2011**; 2(4): 442-447.
22. Okoli, S., and Iroegbu, C.U. In vitro antibacterial activity of Synclisa scabrida whole root extracts, African journal of biotechnology, **2005**; 4(9): 946-52.
23. Celso VN, Tania UB, Erika B, Abraho FNM, Diogenes AGC, Benedito PDF. Antibacterial activity of Ocimum gratissimum L. Essential oil. Mem. Inst. Oswaldo. Cruz, Riode Janeiro., **1999**; 94: 675-8.
24. Maria DC, Torrado, Truiti., Maria H, Sarragiotto,, Benicio, Alves., de Abreu, Filho, Celso VN, Benedito P, Dias F. In Vitro Antibacterial activity of a 7-O- β-D-glucopyranosyl-nutanocoumarin from Chaptalia nutans (Asteraceae). Mem inst Oswaldo Cruz, Riode Janerio., **2003**; 98: 283-6.

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil