RESEARCH PAPER | Biochemistry | Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Estimation of Alkaloids and Total phenol In Roots of *Derris Trifoliata* L and Evaluation For Antibacterial And Antioxidant Activity

KEYWORDS: Derris trifoliata, Alkaloid, Total phenol, Antibacterial activity, Antioxidant activity, Minimum Inhibitory concentration

N. SHARIEF MD
Department of Biochemistry, V.S.Lakshmi Women's Degree & P.G. College, Kakinada.

A. SRINIVASULU
Department of Biochemistry, V.S.Lakshmi Women's Degree & P.G. College, Kakinada.

UMA MAHESWARA RAO V
Department of Microbiology, Acharya Nagarjuna University, Guntur

ABSTRACT *This study was designed to extract and estimate the amount of alkaloids and total phenolics as well as their antibacterial and antioxidant potential in ethyl aceate, acetone, methanol and ethanol with the roots of Derris trifoliata L (DL). The antibacterial activity was done by agar well diffusion method alongside Escherichia coli, Klebsiella pneumonia, Proteus vulgaris,, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus, Enterococcus faecalis and Lactobacillus delbrueckii. The inhibitory effect of the crude extracts was compared with standard antibiotics gentamicin, tetracycline and ciprofloxacin. The Minimum Inhibitory Concentration [MIC] was determined by serial dilution method and the antioxidant activity is done by ABTS method. The ethanol fraction is showing high amount of alkaloid, where as the total phenolics is rich in methanol. However, the efficacy of the inhibitory effect is high for acetone and ethanol extracts. The MIC value was found to be between 1.25 to 5mg/100µl. This study provides the necessary information for extraction and characterization of bioactive principles that enjoy the antioxidant and antibacterial action in root extracts of DL*

INTRODUCTION

Asian countries have been using crude plants as a source of medicine for relief from illness can be traced back since Vedic periodThe potentail of higher plants as source for new lead molecules is still largely unexplored. Plants are important source of potentially useful structure for the development of new chemotherapeutic agents. Medicinal plants would be the best source for obtaining a variety of drugs. About 80% population of the developed countries use traditional medicines. Therefore, plants should be investigated thoroughly to determine their structural and functional properties[1]. The systematic screening of antibacterial principle in plant extracts represents a continuous effort to find new compounds with the potential to fight against multi resistant bacteria and antibiotic resistance has become a global concern [2] . Many studies point out that plants hold many bio-active compounds such as alkaloids, flavonoids, glycosides, saponins, terpenoids, etc with antimicrobial, antioxidant, antidiuretic activity and this is the driving force for the scientific community to search for novel bioactive entities to combat the infectious diseases. Globally, mangroves are distributed across the tropical and subtropical forests and are predominantly found in tropical region. Mangroves are a diverse group of plants predominantly shrubs and trees growing in the marine intertidal zone and these plants produce wide variety of bioactive compounds to withstand the hard environmental conditions [3]. Derris trifoliata L belongs to the family fabaceae. It is perennial climber grows up to 8 meters . Aerial parts of the plant traditionally used against diarrhea and dysentery [4]. In the present study, we examined extracts prepared in ethyl acetate, acetone, methanol and ethanol from the roots of Derris trifoliata L for the alkaloid and total phenol content as well as their antioxidant and antibacterial activity.

MATERIALS AND METHODS

Collection of Plant material

This plant grows in the areas where soil is sandy and less saline. It is a large, woody, climbing shrub, branches are wiry. The roots were collected from Corangi Reserved Forest, Kakinada, East Godavari, Andhra Pradesh, India. Geographic location - between 16° 39′ N longitude - 17° N longitude and 82° 14′ E latitude - 82° 23′E latitude. All the roots were surface sterilized with 1% mercuric chloride solution and thoroughly washed with filter sterilized distilled water[5]. The washed root, were then chopped to small pieces and shade dried until they were suitable for extraction in the selected solvents.

EXTRACTION

Root extracts in ethyl acetate, acetone, methanol and ethanol were prepared according to the standard protocols [6]. The chopped root material (100g) was initially soaked in 500ml of the respective solvent at room temperature for 24h. Subsequently, the soaked material was refluxed for 6h below the boiling point of the respective solvent. Infusions 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.

QUANTIFICATION OF TOTAL ALKALOID CONTENT

Total alkaloid content was determined by the Fazel *et al.*, 2008 method [7]. The plant extract (10mg/ml) was dissolved in 4 ml of the 2 N HCl and then filtered. One ml of this solution was transferred to a separating funnel and then 5 ml of Bromo cresol green (BCG) solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted up to the volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The results are expressed as atropine equivalents (mg of A/ g dry extract).

ATROPINE STANDARD CURVE.

Atropine standard solution was taken by dissolving 1mg pure atropine in 10ml distilled water.

Pipetter out different aliquots (0.4, 0.6, 0.8 and 1) of atropine standard solution and transfer each to different separating funnels. Then, add 5 ml pH 4.7 phosphate buffer and 5 ml BCG solution and shake a mixture with 4 ml of chloroform. The extracts were collected in a 10-ml volumetric flask and them with make up to the mark with chloroform. The absorbance of the complex in chloroform was measured at 470 nm

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

against blank prepared as above but without atropine.

DETERMINATION TOTAL PHENOLS

Estimation of Total Phenols: TP in the examined plant extracts was determined by Folin-Ciocalteu method [8,9]. 1ml of crude extract (100µg/ml) was mixed with 5ml Folin-Ciocalteu reagent and 4ml of 7.5% Sodium carbonate. Tubes were vortexed and incubated for 30min at room temperature for colour development. Absorbance was measured at 765nm using UV-VIS spectrophotometer. The samples were analysed in triplicates and the mean absorbance value was recorded. The standard curve was plotted using 250µg/ml of gallic acid in ethanol. TPC in each extract was expressed in terms of gallic acid equivalent (mg of GA/g dry extract).

DETERMINATION OF ANTIOXIDANT ACTIVITY BY FRAP METHOD

The FRAP assay was carried out by Benzie and Strain method with some modifications [10]. The FRAP method (Ferric Reducing Antioxidant Power) is based on the reduction of complexes of 2,4,6-tripyridyl-*s*-triazine (TPTZ) with ferric chloride,

Reagent preparation: FRAP reagent prepared freshly by mixing 25ml of acetate buffer (Ph 3.6) with 2.5 ml of 10 mM 2,4,6 tripyridyl triazine (TPTZ) and 2.5 ml of 20mM Ferric chloride solution. The reagent was kept at 37 °C before use.

Procedure : 300 µL of the stem extract(100mg/1000 µL) were dispensed into 2700 µL of the freshly prepared FRAP reagent and incubated at 37 °C for 30 minutes. The absorbance was recorded at 593 nm against a blank. Standard curve was prepared with 100 µM $FeSo_4$ solution. All determinations were done in triplicates and expressed as µM Fe^{2+} equivalents per gram of the sample.

DETERMINATION OF ANTIBACTERIAL ACTIVITY

Antibacterial activity of all the extracts prepared in different solvents from roots of DL was determined using standard agar well diffusion method [11]. The bacterial strains used in our study were *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris,, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus cereus, Enterococcus faecalis* and *Lactobacillus delbrueckii*. Each experiment was performed in triplicates and the average value for zone of inhibition was calculated. The zones were compared with that of broad spectrum antibiotics Gentamicin, Tetracyclin and ciprofloxacin 30µg/disc [12].

DETERMINATION OF MIC

Minimum Inhibitory Concentration [MIC] was determined by broth dilution assay method [13]. Plant extracts were serially diluted in Mueller Hinton broth to get the concentrations of 1.25, 2.5, 5.0 and 10mg/100µl. Each experiment was repeated thrice and the mean values were tabulated.

RESULTS AND DISCUSSION

The Alkaloids are naturally occurring nitrogen containing pharmacologically active organic compounds present in plant kingdom. These have made major impact on plant medicine because of its vast application. Around 12,000 alkaloids of various types have been know to occur in all plants including more than 150 families, such as apocynaceae , fabaceae .papaveraceae, rubiaceae, solanaceae etc. Alkaloids may be present systematically in whole plants, or they may be accumulated in large quantities is specific organs like roots, stem barks and seeds. The present study was carried out in quantification of alkaloid, total phenolics their antibacterial and antioxidant activites with specified organic solvents such as ethyl acetate, acetone, methanol and ethanol. Many methods are reported for the determination of alkaloids which include colorimetry, fluorimetry, HPLC, gas chromatography and electro chromatography. Spectrophotometric method is known for its simplicity, sensitivity, and rapid determination. This method is based on the reaction of alkaloid with bromocresol green (BCG), forming a yellow colored product. The result of alkaloid total phenols and antioxidant activity were given in table 1. All the solvents used in our study are finding the infusion of DT, but the extent of alkaloids and total phenols varies amid the solvent used.

The alkaloid content is more in ethanol extract (58 mg of AE) and less in ethyl acetate (15 mg of AE). This may be due to more polar nature of ethanol our results are in agreement with the study of Rao ganga *et. al* [14]. Total phenols are high in methanol extract followed by ethanol, acetone and ethyl acetate. The outcome of the present study indicates that ethanol is the ideal solvent for the extraction of alkaloids and methanol for phenols. The antioxidant activity is done by FRAP method and the trend in antioxidant activity among the solvents used is different from that of the alkaloid and total phenol. The highest antioxidant activity is seen in methanol extracts followed by ethyl acetate, acetone and ethanol. This observation is strange compare to that of the alkaloid and phenol content. This suggest that the antioxidant activity of ethyl acetate fraction is not only due to alkaloid and phenols but also due to some other compounds are present in it.

Table. 1. Alkaloid, Total phenolic and Antioxidant activity in root extracts of *Derris trifoliata*

	Ethyl acetate	Acetone	Methanol	Ethanol
Alkaloid (mg of AE/g dry extract)	15	27	42	58
Total phenolics (mg of GAE/g dry extract)	36	48	69	53
Antioxidant activity (FRAP Units)	886	658	974	714

ANTIBACTERIAL ACTIVITY

Mangroves and mangrove associates are rich source of secondary metabolites namely, steroids, triterpenes, saponins, flavonoids, alkaloids, glycosides, phenolics, limonoids, lignins, and tannins [15]. It is a well established fact that these secondary metabolites are used as classical antibacterial compounds. So, the chemistry of mangrove plants is of growing importance because of their great potential as a source of novel agrochemicals and compounds of medicinal value. They may also provide a new source for many already known biologically active compounds. All these secondary metabolites are not soluble in one particular solvent and their solubility differs from one metabolite to other and depends on the chemical nature and functional group present in them. In vitro antibacterial activity of root extracts of DL with gram positive and gram negative cultures was determined by agar well diffusion method and results are displayed in Figure-1and 2 respectively. The inhibitory effect of DL root extracts were found to possess various degrees of antibacterial activity against the test cultures used. Acetone, methanol and ethanol extracts inhibited the growth of all the gram positive test cultures with a zone of inhibition extend from 10.33 mm to 18.66 mm. The difference in rate of inhibition activities appear to be directly related to the quantitative diversity of the compounds that are present in the extract. The infusions of ethyl acetate were active only against *Bacillus subtilis* and *Lactobacillus delbrueckii* with same zone size of 14.66 mm. our results are in agreement of Khan *et al* work [16]. They screened various parts of *Derris elliptica, Derris indica* and *Derris trifoliata* in different organic solvents and demonstrated different degrees of antibacterial activity. The degree of inhibitory effect is high for ciprofloxacin than gentamicin and tetracycline. None of the extracts were having higher zone of inhibition than ciprofloxacin. However, the inhibitory effect of acetone, methanol and ethanol extracts were higher than tetracycline to *Bacillus subtilis* and *Bacillus cereus*. The acetone and ethanol infusion were active with all the gram negative test cultures employed. Where as, the ethyl acetate extract is active against *Escherichia coli* and *Klebsiella pneu-*

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

monia with same zone size 12.66 mm. Ethyl acetate extract did not inhibit *Proteus vulgaris* and *Pseudomonas aeruginosa* negative results did not mean absence of bioactive principles in the ethyl acetate extract. The bioactive principle may be insufficient to cross the membrane, or the microorganism may have mechanism to over come the effect of the bioactive present in the extract However, methanol extracts also exhibited a zone size of 12.66 mm with *Escherichia coli* and *Proteus vulgaris*. The effectiveness of the acetone extracts is overwhelming than that of gentamicin and tetracycline with all the gram negative test cultures. The present work brings additional information of the antibacterial activities in roots of DT against 8 bacterial species with 4 solvent systems.

Fig- 1 Antibacterial activity of *Derris trifoliata* against gram positive cultures.

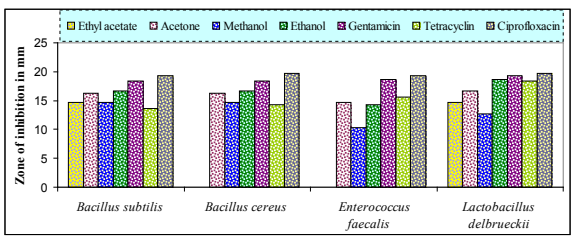
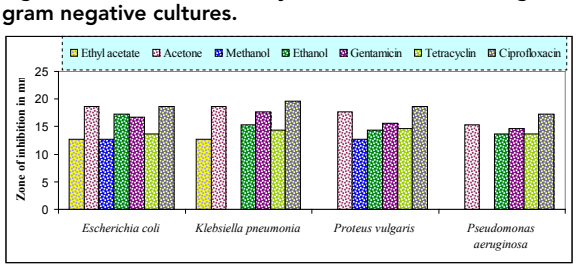


Fig- 2 Antibacterial activity of *Derris trifoliata* against gram negative cultures.



Minimum Inhibitory Concentration (MIC)

Further, these extracts were analyzed to determine the minimum inhibitory concentration. The MIC values ranged from 1.25 -5mg/100µl and varied from one extract to the other and the results are displayed in table-2. The MIC of acetone extracts towards gram negative cultures is 1.25mg/100µl and for gram positive test culture it is 2.5mg/100µl . This difference may be explained by susceptibility testing condition, physico chemical characters of the bioactive principle present in the extract and even strain to strain difference. However, the infusion of ethanol are having similar MIC values 2.5 mg/100µl to gram negative gram and positive microorganism used.

Table-3: MIC of *Derris trifoliata* root extracts (mg/100µl)

Micoorganisms	Ethyl cetate	Ac-etone	Metha-nol	Etha-nol
Escherichia coli	5.0	1.25	5.0	2.5
Klebsiella pneumonia	5.0	1.25	00	2.5
Proteus vulgaris	00	1.25	5.0	2.5
Pseudomonas aeruginosa	00	1.25	00	2.5
Bacillus subtilis	5.0	2.5	5.0	2.5
Bacillus cereus	00	2.5	5.0	2.5
Enterococcus faecalis	00	2.5	5.0	2.5
Lactobacillus del-brueckii	5.0	2.5	5.0	2.5

In comparison to some of the earlier reports on MIC values of pure compounds, our MIC values may be higher[17]. 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.

Fai-Chu Wong [18] studied the MIC on *S. aureus, M.luteus, E.coli* and *P.aeruginosa* with ampicillin and selected medicinal plant extracts and reported the MIC value of Amphicillin is between 0.02 - 1mg/1000µl and that of the plant extracts are in the range of 6.3 – 50 mg/1000µl. Our results are far superior compare to that of the plant extracts but inferior to that of the standard antibiotic.

CONCLUSION

Our results show that the secondary metabolites present in the root extracts of *Derris trifoliate* are more of polar in nature as their content is observed in ethyl acetate, acetone, methanol and ethanol solvents . The antioxidant activity and antibacterial activity is observed in all the solvent extracts. Hence *Derris trifoliate* is strongly recommended for considering a valuable source for isolation, identification and characterization of bioactive principle responsible for antioxidant and antibacterial activity. However, further work in this direction could lead to the discovery of powerful bioactive principle from the *Derris trifoliata*

ACKNOWLEDGEMENTS

The first author Mr. N.Sharief Mohammad, would like to express heart full thanks to the Secretary and Correspondent Dr. V. Sitarama Raju, 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 and Associate Professor of Biochemistry Department, Dr. A. Srinuvasulu, V.S. Lakshmi Women's P.G. College, Kakinada.

REFERENCE

1.Ellof J N (1998). Which extractant should be used for the screening and isolation of | antimicrobial components from plants. Journal of Ethanophamacalogy; 60: 1-6. | | 2. Westh, H. Zinn, C.S. Rosdahl, V.T. Sarisa Study Group (2004): An international multicenter | study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from | 15 hospitals in 14 countries. Microbial Drug Resistance 10: 169-176. | | 3. Bandaranayake,W .M.(2002). Bioactivities, bioactive compounds and chemical | constituents of mangrove plants. Wetlands Ecology and Management 10: 421-52. | | 4. . Kambaska Kb, Purandra M and Dyanidhi M (2006). Green leaves for Diarrhoeal | diseases used by the tribals of kenojhar and mayurbhanj district of Orissa, | India. Ethanobotanical Leaflets, 10: 305-328. | | 5. N. Sharief Md and UmaMaheswara Rao v.: Antibacterial activity of stem and | root extracts of Avicennia officinalis L . International Journal of Pharmaceutical | Application. 2011; Vol2, Issue 4: 231-236. | | 6. P.Satya Veni, S.Sunitha and A. Srinivasulu (2013). Evaluation of antibacterial | activity on selected bacteria and screening of secondary metabolites of Avicennia | Alba stem. International journal of Advanced Biotechnology and Research, 4: 511-517. | | 7. Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi ,Mohammadreza Verdian-rizi. | (2008). Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. | Thai J. Pharm. Sci. 32: 17-20. | | 8.Djeridane B Yousfi M. Vidal N, Nadjemi, N, Lesgards J.F. and Stocker P. Screening of | some Algerian medicinal plants for the phenolic compounds and theirantioxidant activity | European food research and technology, 224 (2007) 801-809. | | 8. Kahkonen M.P. Hopia A.I. Ucorela H.J. Rauha J.P. Pihlaja K and Kujala T.S. | Antioxidant activity of plant extrcuts containing phenolic compound. Journal of Agricultural | and food Chemistry 47 (1999) 3954 – 3962. | | 10. Benzie,I .F.F. & Strain J.J (1999). Ferric reducing/antioxidant power assay:direct | measure of total antioxidant activity of biological fluids and modified version | of simultaneous measurement of antioxidant power and ascorbic acid | concentration. Methods Enzymology, 299: 15-27. | | | 11. S. Ravikumar, M. Gnanadesigan, P. Suganthi A. Ramalakshmi(2010): Antibacterial | potential of chosen mangrove plants against isolated urinary tract infections bacterial | pathogens. International Journal of Medicine and Medical Sciences, 2: | 12. Jigna parekh and Sumitra Chanda (2007) : Antibacterial and phytochemical studies on | twelve species of Indian medicinal plants, Africal Journal of Biomidecal Research, | Vol.10: 175-181. | | 13. 14. Okoli, S. and Iroegbu, CU (2005). In vitro antibacterial activity of Synclisa scabrida | whole root extracts, African journal of biotechnology, 4(9): 946-52. | | 14. B. Rao Ganga1, P. Rao Umamaheswara, E. Rao Sambasiva, T. Rao Mallikarjuna and | V. S. Praneeth D.(2011). Studies on phyto chemical constituents, quantification | of total phenol, alkaloid content and In-vitro anti-oxidant activity of Coccinia | cordifolia International journal of Pharmacy and Life sciences. Vol. 2; 10: 1177-1182. | | 15. Bandaranayake, W. M.(1995) Survey of mangrove plants from Northern Australia for phytochemical constituents and uv-absorbing compounds, Current topics in phytochemistry, 14: 69-78. | | 16. 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. | | 17. Maria DC, Torrado, T., Maria, H., Sarragiotto,, Benicio, Alves., de Abreu, Filho, Celso | VN,Benedito P, Dias F (2003): In Vitro Antibacterial activity of a 7-O- ß-D- | glucopyranosyl- nutanocoumarin from Chaptalia nutans (Asteraceae). Mem inst | Oswaldo Cruz, Riode Janerio; 98: 283-86. | | 18. Fai-Chu Wong, Anni-Li Yong, Hean-Chooi Ong and Tsun-Thai Chai (2013). | Evaluation of the antibacterial activities of selected medicinal plants and determination | of their phenolic constituents. Science Asia; 39: 591-595. |