A Study of Chemical Composition of some Medicinal Plants Available in the Eastern Ghats and nearby forests

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Antibacterial activity of different crude extracts of *Dodonaea viscosa*

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ABSTRACT

The present study of antimicrobial activities of various crude solvent extracts of Dodonea viscosa were determined against a wide variety of pathogenic bacteria. Crude extracts of Dodonea viscosa shows mild to significant activities for most of the treated bacteria. Crude extracts of n-hexane, dichloromethane, ethyl acetate and methanol showed antibacterial effect against most of the tested organisms. It has been expected that the present work on antimicrobial screening of the plant materials will help researchers who wish to work in designing clinical drugs concerning the killer diseases.

Key words: Antibacterial activity, Mueller-Hinton Agar (MHA), *Dodonea viscosa*

INTRUDUCTION

Many organisms can cause several diseases and now, in this world of modern science, man can face any challenge against any disease. But in spite of the tremendous advancement of medical science and technology, diseases are the leading health problem particularly in the under privileged population in the remote rural areas in the developing countries. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. The wide spread use of herbal remedies and health care preparations, such as those described in ancient texts like Vedas and the Bible, has been traced to the occurrence of natural products with medicinal-properties. In fact, plants produce a diverse range of bioactive-molecules, making them a rich source of different types of medicines. Plants with possible antibacterial activities should be tested against an appropriate microbial model.

The plant *Dodonaea viscosa* belonging to the family Sapindaceae is distributed as a weed from coast to the elevation of more than 2000 meters. The weed is distributed in tropical as well as subtropical regions of the world.

Dodonaea viscosa has many medicinal properties and has been used by native peoples from all regions where it is found. It is a traditional medicine worldwide, administered orally or as poultice to treat a great variety of ailments. Stem or leaf infusions are used to treat sore throats; root infusions to treat colds. The stems and leaves are used to treat fever, and seeds (in combination with those of other plants and coated in honey) to treat malaria. The stems are used as fumigants to treat rheumatism. The leaves are used to relieve itching, fevers swellings, aches and can be used as a antispasmodic agent leaves and roots as a painkiller to soothe toothaches and headaches and a lotion made from unspecified plant parts to treat sprains, bruises, burns and wounds.

EXPERIMENTAL SECTION

PLANT MATERIAL: The plant material *Dodonia viscosa* was obtained from Maredumelli forest area, Andhra Pradesh, India.

PREPARATION OF EXTRACT:
The dried powdered leaf was defatted using petroleum ether later the defatted material was subjected to maceration using distilled water as a solvent. By using Methanol, N-hexane, Di- chloromethane and Ethyl acetate soxhlet extraction had been performed. Each extract was concentrated at 37° C temperature. Those obtained extracts were screened to identify the chemical constituents present; they are stored in Desiccator for further uses.

PREPARATION OF THE TESTED ORGANISMS:
The lyophilized forms of different strains of microorganisms like Escherichia coli [MTCC-2126], Staphylococcus aureus [MTCC-3160], were obtained from the Microbial Type Culture Collection and Gene bank (MTCC), Chandigarh, India and the bacterial Strains Streptococcus faecalis [NCIM-2603] and Streptococcus pyogens , Bacillus subtilis [NCIM-2655] were obtained from National Collection of Industrial Microorganisms (NCIM), Pune India. The bacterial cultures were maintained on Mueller-Hinton Agar (MHA) and were sub cultured periodically. The average number of viable organisms per ml organ stock suspension was about 10^9 colony forming units (CFU) per ml which was maintained by following McFarland Standardization[6] Each time fresh stock suspension had been prepared; constant experimental conditions were maintained to obtain close viable counts.

INOCULATION:
Single loopful of an overnight grown nutrient broth culture of each test organism served as inoculum for the antimicrobial activity determination. The average size of inoculum was about 1×10^6 cells contained in 3mm diameter of standard loop.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION [MIC]:
The solution of nutrient agar medium [250 mL] was prepared and sterilized. 25mL of media was dispersed in each of 5 conical flask, and they were autoclaved after plugged with cotton. Stock solution of Dodonaea viscosa bearing concentration of 4 mg/ml in Dimethyl sulphoxide [DMSO] was prepared. Each Petri dish was equally filled with nutrient agar about 20 mL/petri dish. The petri dishes were marked. One sterile nutrient agar plate without extract but with equal volumes of solvent served as the control plate. All plates were allowed to refrigerate overnight for uniform diffusion of the extract through the media. Those plates were dried at 37° C. A loop of an overnight grown peptone water culture of each test organism was placed in petri dish and they were marked. The spot inoculated plate was also incubated at 37° C, for 24h and the MIC values were obtained [7-8]. The experiment was repeated and the values were given in table number 01.

DETERMINATION OF ZONE OF INHIBITION:
Agar Well Assay Method: In Agar Well Assay Method 20 ml nutrient agar medium was poured in sterilized Petri plates (100 X15 mm) and allowed to solidify at room temperature. 24 h broth culture of test bacteria was used as inoculums under sterile conditions. The freshly prepared 100µl or 0.1ml (1×10^9 cells/ml) of organisms was set to 0.5 optical density spread with a sterile L shaped. Using cork borer several wells of 6mm in diameter were punched. To each well 100µl extract three sets of two dilutions (2mg/ml, 4mg/ml) of C. *Dodonaea viscosa* extracts of methanol, n-hexane, dichloro methane and ethyl acetate extracts prepared in double distilled water ,were poured into wells. The Petri dishes were incubated at 37 ºC for 24hrs and the diameter of the zone of inhibition were measured in mm. Similar procedure was adopted for the pure ciprofloxacin and the corresponding zone diameter were compared accordingly. The experiment was repeated in triplicate and average values were written in the Table no 01.

RESULTS AND DISCUSSION

Results for the antibacterial activity of *Dodonaea viscosa* extracts of n-.hexane Di- chloromethane and ethyl acetate extracts are shown in table given before. MIC and zone of inhibition of both extracts are carried out by using five bacterial strains the MIC of test compound have been compared with standard drug. From the results zone of inhibition values all the extracts have their activity on Gram positive bacteria when compared with Gram negative bacteria. According to the zone of inhibition Bacillus subtilis showed very less sensitivity to the aqueous extracts. The remaining four bacterial strains effectively inhibited by the ethanolic and aqueous extracts at various

concentration levels. From the above observations various extracts of *Dodonaea viscosa* can be selected for the further antibacterial studies against gram positive bacteria and eve pathogenic strains can be studied to know the potency of these extracts. Fascinatingly E.coli and Bacillus subtilis shown slight resistant to the standard drug ciprofloxacin. Streptococcus faecalis [NCIM-2603] shows similar sensitivity to all extracts extracts of *Dodonaea viscosa* at the same concentration levels.

Table number 01: Determination of MIC of various extracts of *Dodonaea viscosa*

S.No.	Name of the bacteria	Conc. of methanolic		Conc.of n-hexane		Conc. of Dichloro methane		Conc.of ethyl acetate	
		2mg/mL	4mg/mL	2mg/mL	4mg/mL	2mg/mL	4mg/mL	2mg/mL	4mg/mL
1.	*Staphylococcus aureus*	+	+	+	+	+	+	+	+
2.	*Bacilli subtilis*	+	+	+	+	+	+	+	+
3.	*Staphylococcus faecalis*	+	+	+	+	+	+	+	+
4.	*Streptococcus pyogens*	+	+	+	+	+	+	+	+
5.	*E.coli*	-	-	-	-	-	-	-	-

Table number 02: Determination of zone of inhibition of methanolic and n-hexane extracts of *Dodonaea viscosa*

S.No.	Name of the bacteria	Concentration of methanolic extract		Concentration of n-Hexan extract		Ciprofloxacin (µg/mL)			
		2mg/mL	4mg/mL	2mg/mL	4mg/mL	10	20	30	40
1.	*Staphylococcus aureus*	10±0.11	13±0.15	13±1.23	16±1.2	20±0.14	23±0.24	25±0.35	28±0.25
2.	*Bacilli subtilis*	07±0.34	10±0.16	10±1.25	13±1.43	-	-	-	-
3.	*Staphylococcus faecalis*	10±0.26	13±0.24	12±1.26	13±1.24	10±0.41	15±0.25	18±0.34	20±0.35
4.	*Streptococcus pyogens*	15±0.31	20±0.31	10±1.24	15±1.24	13±0.26	15±0.24	20±0.24	23±0.42
5.	*E.coli*	1±0.12	1±0.24	1±0.24	1±0.38	09±0.24	11±0.26	13±0.34	15±0.41

**All values are mean of triplicate readings; - Absent; values ± Standard deviation*

Table number 03: Determination of zone of inhibition of Dichloro methane and ethyl acetate of *Dodonaea viscosa*

S.No.	Name of the bacteria	Concentration of di chloro methane extract		Concentration of ethyl acetate extract		Ciprofloxacin (µg/mL)			
		2mg/mL	4mg/mL	2mg/mL	4mg/mL	10	20	30	40
1.	*Staphylococcus aureus*	10±0.11	13±0.15	14±1.23	18±1.2	20±0.14	23±0.24	25±0.35	28±0.25
2.	*Bacilli subtilis*	09±0.34	11±0.16	10±1.25	13±1.43	-	-	-	-
3.	*Staphylococcus faecalis*	11±0.26	13±0.24	13±1.26	14±1.24	10±0.41	15±0.25	18±0.34	20±0.35
4.	*Streptococcus pyogens*	16±0.31	22±0.31	10±1.24	15±1.24	13±0.26	15±0.24	20±0.24	23±0.42
5.	*E.coli*	1±0.12	1±0.24	1±0.24	1±0.38	09±0.24	11±0.26	13±0.34	15±0.41

Fig. no. 1: Graphical representation for the obtained results of methanolic extract of *Dodonaea viscosa*

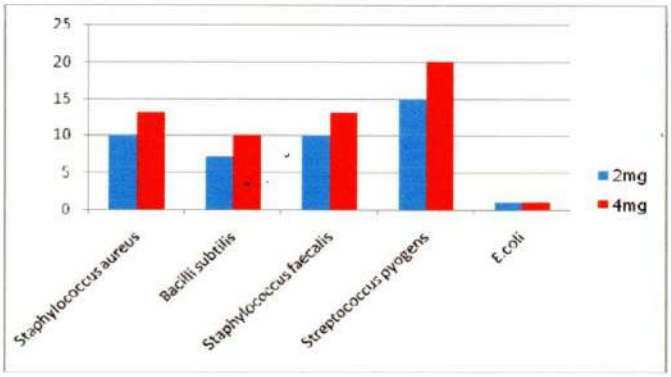


Fig. no. 2: Graphical representation for the obtained results of n-hexane extract of *Dodonaea viscosa*

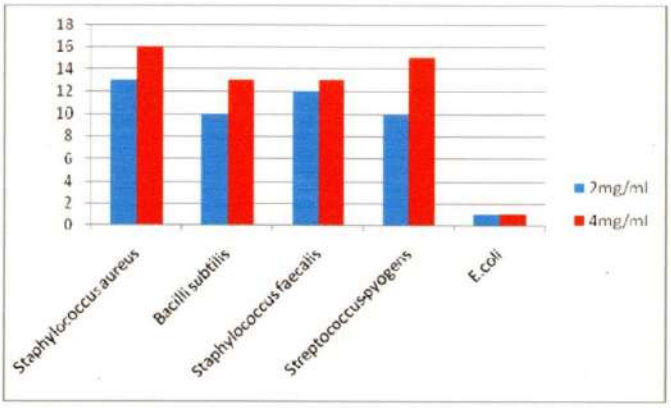


Fig. no. 3: Graphical representation for the obtained results of dichloro methane extract of *Dodonaea viscosa*

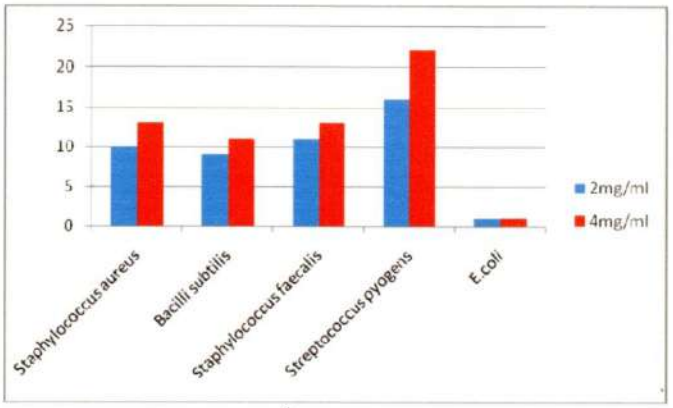


Fig. no. 4: Graphical representation for the obtained results of Ethyl acetate extract of *Dodonaea viscosa*

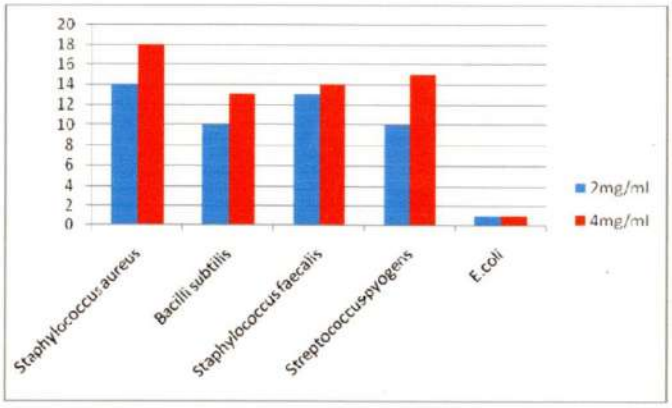
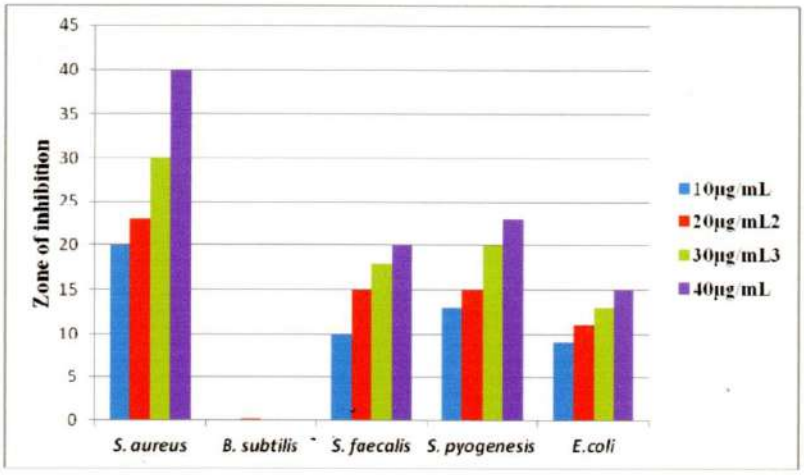


Fig. no. 5: Graphical representation for the obtained results of Ciprofloxacin



CONCLUSION

Plants are the natural sources to promote health, from the present study it can be concluded that the antibacterial activity of *Dodonaea viscosa* leaf extracts of methanolic, n-hexane, dichloro methane and ethyl acetate are effective against various gram positive organisms. And extensive studies are to be carried out to find out which of the chemical constituent might be responsible for antibacterial activity. Finally Dodonaea viscosa may be useful to various other diseases also; further investigation need to be done for this.

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Antibacterial and Antifungal Actuvity of Organic Solvent Extracts Of Knoxia Corymbosa

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Abstract
Objective: *In this study the potency of antibacterial activities of crude organic solvent extracts ethyl acetate, dichloromethane, and methanol extracts of Knoxia corymbosa were tested on bacterial pathogens Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Streptococcus pneumonia, Bacillus subtilis and the potency of antifungal activities of crude organic solvent extracts ethyl acetate and methanol extracts were tested on fungal pathogens Aspergillus niger and Candida albicans.*
Key words: *Anti-bacterial activity, Anti-fungal activity, Potato dextrose agar (PDA), Streptomycin sulphate.*

I. Introduction

The ethno medical and ethno botanical uses of the plants are completely crude uses of plants parts like leaves, roots stem, bark etc. But the chemical analysis intended to find out to the chemical substances, which is responsible for the medicinal effect. So this is an interdisciplinary subject. A lot of work has been going on this way throughout the globe. In Andhra University a significant work has been going on. Ayurveda, the ancient healing system of India, flourished in the Vedic Era in India. According to historical facts, the classical texts of Ayurveda, Charaka Samhita and Sushruta Samhita were written around 1000B.C.The Ayurvedic Materia Medica includes 600 medicinal plants along with therapeutics. Herbs like turmeric, fenugreek, ginger, garlic and holy basil are integral part of Ayurvedic formulations. The formulations incorporate single herb or more than two herbs (poly-herbal formulations). Knoxia corymbosa is an erect perennial herb Family Rubeacea mostly 40-90 cm tall. Stems are little-branched, velvety. Leaves are narrow-lance shaped to ovate, mostly 2.5-8 cm long, 0.8-3.5 cm wide, tip pointed to long-pointed, base wedge-shaped to flat, margins with fine hairs. Both surfaces of the leaves are velvety, and the leaf-stalk is 3-10 mm long. Flowers are borne in a dense, cyme up to 4 cm long, carried on a 0.2-2.5 cm long stalk, at the end of branches. Flowers stalks are 0.5-1 mm long. Flowers are usually 1.5-2 mm long, purplish blue or rarely white. Anthers protrude out. Fruit is ellipsoid about 1.5-2 mm long, crowned by sepals, usually falling entire. Knoxia corymbosa is found in Indo-Himalaya region, peninsular India and Sri Lanka. Flowering: September-October. Knoxia corymbosa is assumed to containing some medicinal values because some authors reported that they isolated some chromone glycosides. Knoxia valerianoides is a Knoxia species from which an herbal medicine and β-sitosterol is one of its main components was isolated by Je-Chuan Ye etal. B-sitosterol is known to control cholesterol levels, reduce the activity of cancer cell, promote prostate gland health enhance immunity in the human body.

II. Materials And Methods:

Collection Of Plant Material:
Fresh plants of Knoxia corymbosa free from disease were collected from Y.Ramavaram of Addatheegala, virgin forests of Bison hills of East Godavari District Andhra Pradesh India. Location coordinates of Y.Ramavaram are 17.48330N and 82.01670c. Plant identification was verified by M.Ravikumar of Department of Botany V.S.M college Ramachandrapuram.The plants were washed thoroughly 2-3 times with running water and finally once with sterile distilled water. The plant material was then air dried on sterile blotter under shade for 5 days and then powdered with the help of a blender.

Solvent Extraction:
25gm of shade dried powder was filled in the thimble and extracted successively with Methanol, Ethyl acetate, Dichloromethane solvents in soxhlet extractor for 48 hours. The solvent extracts were concentrated under pressure and preserved at 4^{0} C in an airtight bottle for further use.

Growth and Maintenance of Bacterial and Fungal Cultures for Antimicrobial studies:

The lyophilized forms of different strains of microorganisms like Escherichia coli [MTCC-2126], Staphylococcus aureus [MTCC-3160], were obtained from the Microbial Type Culture Collection and Gene bank (MTCC),Chandigarh, India and the bacterial Strains Streptococcus faecalis [NCIM-2603] and Streptococcus pyogens ,Bacillus subtilis [NCIM-2655] were obtained from National Collection of Industrial Microorganisms (NCIM), Pune,India.

Preparation Of Inoculum For Anti Microbial Studies:

The Gram positive (Staphylococcus aureus, Staphylococcus pneumonia and Bacillus subtilis)and Gram negative(Escherichae coli and Klebsiella pneumonia) bacteria were precultured in Nutrient Broth over night in a Rotary shaker Incubator at 370c, the culture broth was centrifuged at 10,000rpm for 5minutes, pellet was suspended in double distilled water and the cell density was standardized Spectrophotometrically(A610nm). The fungal inoculum for Aspergillus Niger was prepared from 5-10day old culture grown on Potato Dextrose Agar medium the petri dishes were flooded with 10ml of distilled water and the conidia were scroped with a sterial spatuala. The spore density of each fungus was adjusted with spectrophotometer (A 595nm) to obtain final concentration of approximately 105 spores per ml

Method: Antibacterial activity of isolated crude organic solvent extracts ethyl acetate, dichloromethane and methanol extracts of Knoxia corymbosa was checked by agar well diffusion method.

Anti Bacterial Activity Assay:

The Gram positive bacteria Staphylococcus aureus, Staphylococcus pneumonia and Bacillus subtilis and Gram negative bacteria Escherichia coli and Klebsiella pneumonia were tested for their susceptibility to extracts of Knoxia corymbosa by disc diffusion method. Crude organic solvent extracts of Knoxia corymbosa were prepared for antimicrobial assays by reconstituting with the respective organic solvents. The test bacteria were seeded into Mueller Hinton agar medium Spread plate method10µl (106cells/ml) with overnight grown cultures of Bacteria in Nutrient broth, The Filter paper discs 5mm in diameter impregnated with 5µg/ml-1 of the crude organic solvent extracts were placed on test organism seeded plates were used for the antibacterial tests .Streptomycin sulphate (10µg /ml-1)was used as positive control and the organic solvents were used as negative control. The antibacterial assay plates in triplicates were incubated at 370C for 24 hrs.The diameters of the inhibition Zones were measured in mm.

Anti Fungal Activityassay:

Aspergillus niger and Candida albicans were tested for their susceptibility to crude organic solvent extract of Knoxia corymbosa .The antifungal activity was tested by disc diffusion method .The Potato Dextrose Agar plates were inoculated with each fungal culture (10 days old)by point inoculation.The filter paper discs (5mm in diameter)impregnated with 5µg/ml-1 concentrations of the crude organic solvent extracts were placed on test organism seeded plates .The respective organic solvents were used to dissolve the extract and was completely evaporated before application on test organism seeded plates.A blank disc impregnated with the respective organic solvent followed by drying off was used as negative control and the fungicideNystatin (10µg/ml-1) was used as positive control.The activity was determined after 72 hrs of incubation at 280C .The diameters of the clear inhibition zones were measured in mm.

III. Results And Discussion:

Ethyl acetate extract showed antibacterial activity against a total of five bacterial strains *Escherichia coli, Klebsiella pneumonia ,Staphylococcus aureus ,Streptococcus pneumonia, Bacillus subtilis* and and antifungal activity against a fungal species *Aspergillus niger*. dichloromethane extract doesn't show any antimicrobial and antifungal activity. Methanol extract showed antibacterial activity against three bacterila strains *Staphylococcus aureus ,Streptococcus pneumonia, Escherichia coli* and antifungal activity againt *Aspergillus niger.*

Table 1: Antibacterial activity of crude organic solvent extracts of knoxia corymbosa -5 µl/50 µl solvent concentration and antibiotic streptomycin sulphate (10 µg/ml) against bacterial species tested by Disc Diffusion assay

Test organism	Diameter of zone of inhibition(mm)			
Gram positive	EAE	DCME	MEE	SMS
Bacillus subtilis	4	0.00	0.00	
Streptococcus	9	0.00	2	
Staphylococcus aureus	23	0.00	4	15
Gram Negative				
Escherichia coli	20	0.00	2	18
Klebsiella pneumonia	18	0.00	0.00	0.

EAE = Ethyl acetate extract
DCME = Dichloromethane extract
MEE=Methanol extract; SMS = Streptomycin sulphate

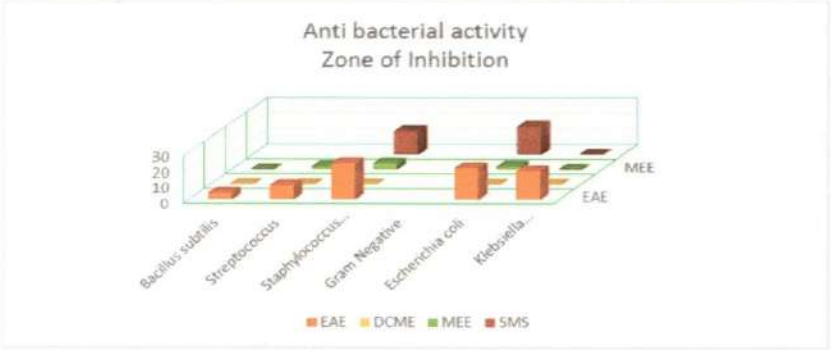
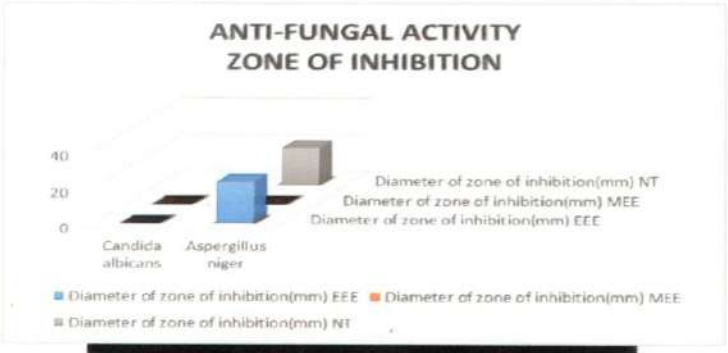
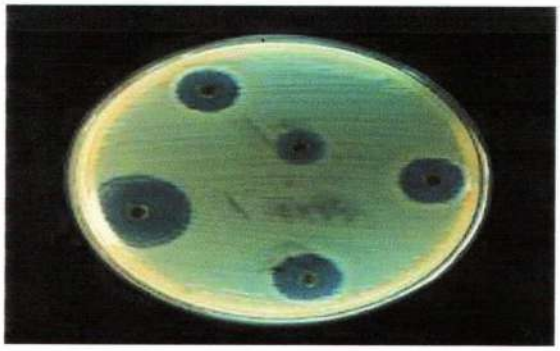


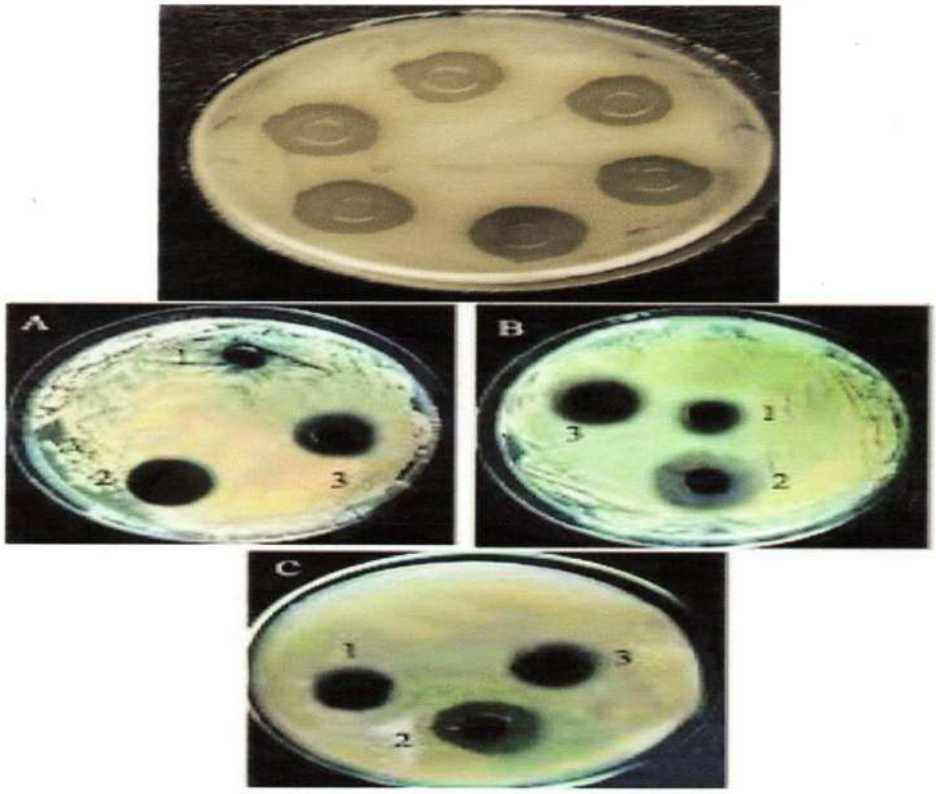
TABLE 2: Antifungal activity of crude organic solvent extracts of knoxia corymbosa -5µl/50µl solvent concentration and fungicide Nystatin against fungal species tested by Disc Diffusion assay

Test organism	Diameter of zone of inhibition(mm)		
	EEE	MEE	NT
Candida albicans	0.00	0.00	
Aspergillus niger	23	0.00	20

EAE = Ethyl acetate extract
MEE = Methane extract
NT = Fungicide Nystatin







IV. Conclusion

Plants are the natural sources to promote health, from the present study it can be concluded that the antibacterial activity of Knoxia corymbosa leaf extracts of Ethyl acetate, dichloromethane, and methanol extracts are effective against various gram positive organisms and gram negative organisms and antifungal activity against fungal organism. Extensive studies are to be carried out to find out which of the chemical constituent might be responsible for antibacterial activity and antifungal activity. Finally Knoxia corymbosa may be useful to various other diseases also; further investigation need to be done for this.

Acknowledgments

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Isolation of Urosilic Acid from *Knoxia corymbosa*

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Abstract

Air-dried, milled leaves of *Knoxia corymbosa* (220 gm) were extracted repeatedly with dichloromethane. After removal of solvent in vacuo, the CH_2Cl_2-solvent residue was fractionated by using hexane and ethyl acetate solvents. After fractionation, all fractions are submitted for NMR spectroscopy. Out of all fractions interesting fractions are subjected to column chromatography, so that we isolated one pure compound as Ursolic acid which is a known compound as it is characterized by using reference data. This is the first time to be isolated the above compound from this species. Ursolic acid is a five-membered cyclic triterpenoid compound. A structure of the isolated compound has been assigned on the basis of their analytical data. By surveying the literature, we came to understand that the compound ursolic acid is a cyclic five-member triterpenoid first to be isolated from *Knoxia corymbosa*. And also, it is first time to isolate the above compound from this species *Knoxia corymbosa*.

Keywords: *Knoxia corymbosa*; CH_2Cl_2; Ursolic acid; Column chromatography; Spectroscopy

Introduction

Pharmacological activities of plants and plant derived drugs necessitate for the search of new and useful drugs globally. India is the largest producer of medicinal herbs. These values are shown vast and tremendous biodiversity potential in India, which can be utilized in drug industry. *Knoxia corymbosa* is assumed to containing some medicinal values [1-6] because girijans of the forest area are using for fevers and skin diseases. Some authors reported that they isolated some chromone glycosides [7,8] from *Knoxia corymbosa*. Knoxia species reported to contain herbal medicine, β-sitosterol which is one of its main components was isolated. β-sitosterol is known to control cholesterol levels, reduce the activity of cancer cell, promote prostate gland health enhance immunity in the human body. The plants of family Rubiacea is an important source of medicinal natural products, particularly alkaloids and triterpenes, quinovic acid glycosides, flavonoids and coumarins have been isolated from this family. Pharmacological studies are described according to cytotoxicity, anti-inflammatory, antiviral, immune stimulation, antioxidant, CNS-related response, vascular, hypertensive, mutagenicity and antibacterial properties. The compounds obtained from this family are used as immunomodulatory, anti-inflammatory and vascular-related conditions. The information summarized here is intended to serve as a reference tool to practitioners in the fields of ethno pharmacology and natural products chemistry.

Various natural products occur in Rubiaceae plants. Extensive phytochemical investigation has been realized regarding the natural occurrence of triterpenoids [10-16], anthraquinones and indole alkaloids [17-20] in the family. Rubiaceae family plants exhibited antimalarial, antimicrobial, antihypertension, antidiabetic, antioxidant, and anti-inflammatory activities. Bioactive compounds including indole alkaloids, terpenoids, anthraquinones and anti-tumors have been isolated from these plants.

Materials and Methods

220 gms of shade dried powder leaves of *Knoxia corymbosa* were filled in the thimble and extracted successively with n-hexane, dichloromethane, ethyl acetate and methanol solvents in soxhlet extractor for 48 hours intervals. The solvent extracts were concentrated under pressure and preserved at 40°C in an airtight bottle for further use. After fractionation, all fractions are submitted to NMR Spectroscopy. Out of all fractions dichloromethane fraction is subjected to column chromatography. By thin layer chromatography (TLC) method Dichloromethane extract seems to be containing more compounds. So, the extract from dichloromethane is subjected to column chromatography with n-hexane and ethyl acetate solvents. The purity of fractions was tested with the help of TLC.

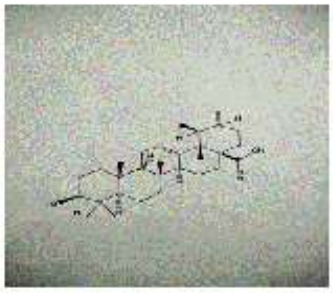

Figure 1: Structure of Ursolic acid. IUPAC Name of ursolic acid is: 3-beta-3-hydroxy-urs-12-ene-28-oic-acid, or 3-β-hydroxy-urs-12-en-28-oic acid, urson, prunol or malol.

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Out of all fractions, the fractions having similar R_f values were combined together and isolated three samples. The obtained samples were submitted to UV, IR, NMR and Mass Spectroscopy. The obtained data is compared with reference data and confirmed that, out of three samples one sample i.e., sample-3 (sample-1 and sample-2 are not pure so rejected). seems to be pure one and confirmed the structure. The obtained data is compared with reference data and confirmed the structures and the compound is identified and confirmed as ursolic acid (Figure 1).

Spectral data of *Knoxia corymbosa*

UV spectral data is at 474, 442 and 422.

FT-IR KB Absorption bands assigned to the compound are C-O stretching is (1036.04 cm^{-1}) olefinic system (C=C) (1458.48 cm^{-1} and 2860.52 cm^{-1}), carboxylic acid stretching is (2928.24 cm^{-1}), carbonyl system (1690.38 cm^{-1}), and hydroxyl group (3444.99 cm^{-1}).

^{1}H NMR: (400 MHz, CD3OD): δH=0.728 (3H, s, CH_3, H-27), 0.768 (3H, s, CH_3, 25), 0.884 (3H, CH_3 H-30), 1.05 (3H, S, CH_3, H-26), 0.965(3H, d, CH_3, H-29), 1.28 (3H, S, CH_3, H-23), 1.106 (3H, s, CH_3, H-24), 1.352-2.025 (13H, m, [1, 2, 5-7, 9, 11, 15, 16,19-22]), 2.178 (1H, d, H-18), 3.303 (1H, d, H-3) and 5.217(1H, m, olelifinic proton, H-12).

^{13}C NMR: (400 MHz, CD3OD): δC=38.14 (C-1), 27.88 (C-2), 79.69 (C-3), 38.14 (C-4), 54.40 (C-5), 17.83 (C-6), 31.8 (C-7), 39.83 (C-8), 48.14 (C-9), 34.33 (C-10), 21.60 (C-11), 126.80 (C-12), 139.71 (C-13), 40.76 (C-14), 28.7 (C-15), 24.08 (C-16), 43.24 (C-17), 54.40 (C-18), 39.83 (C-19), 39.99 (C-20), 30.76 (C-21), 34.33 (C-22), 27.88 (C-23), 16.06 (C-24), 16.38 (C-25), 17.66 (C-26), 24.08 (C-27), 181.98 (C-28), 17.83 (C-29), 21.60 (C-30).

Mass spectrum: $C_{30}H_{48}O_3$; Molecular weight-456.5 (m/z 457 (M^+), 389, 300, 248, 207, 203, 189, 147, 133, 119, 105, 44).

Results and Discussion

Dichloromethane extract to column chromatography with n-hexane and ethyl acetate as eluting solvent mixture and obtained 198 fractions. One pure compound obtained from fractions of 159 to 176. The fractions are subject to thin layer chromatography TLC. The fractions having same R_f values are combined together [23]. The sample sent to IICT Hyderabad for spectral data. The data obtained is compared with the existing data and concluded the results and confirmed the structure and name of the is confirmed.

Modern man is confronted with increasing incidences of cancer and cancer deaths annually. Statistics indicate that men are largely plagued by lung, colon, rectal and prostate cancer, while women increasingly suffer from breast, colon, rectal, and stomach cancer [24]. The literature indicates that many natural products are available as chemo protective agents against commonly occurring cancer types [25]. Species of Rubiaceae as well as their isolated compounds possess diverse biological activities, including anti-inflammatory, antitumor, antimicrobial, larvicidal, antioxidant, gastrointestinal, anti-ulcer, and hepato protective, with alkaloids and iridoids as the major active principles. Crude leaf extracts of *Knoxia corymbosa* is proved to having antibacterial and antifungal activity [26].

Conclusion

Ursolic acid is the important constituent of leaves of *Knoxia corymbosa* which are proved to be having very effective medicinal value. Researches proved that ursolic acid having potency of curing tumors and killing cancer cells, induces eryptosis, reduces muscle atrophy, shows potential cardio protection, induces neural regeneration after sciatic nerve injury, liver disorders etc., So, it is necessary to do much more work on the above plant as the above mentioned diseases are challenging to the health sciences. Utilization of natural products as drugs is not only good for human health but also no side effects. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Plants with possible antibacterial activities should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it.

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