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Journal of
CURRENT PHARMA
Research
www.jcpronline.in/

Journal of Current Pharma Research 6 (1), 2015, 1723-1729.

Research Article

Isolation, Characterization and Antimicrobial Activity of Bioactive Compound from *Ficus Sp*

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Received 06 Dec. 2015; received in revised form 22 Dec. 2015; accepted 22 Dec. 2015
Available online 27 December 2015.

Abstract

Nature has provided with potential things for the survival of humankind over the years, including the tools for therapeutic intervention. It is loaded with medicinally valuable phytochemicals, where its screening is an important step in the detection of bioactive compounds present in medicinal plants which will lead to novel environmentally friendly bio herbicides and drug discovery. The study focuses on to isolate and characterizes the bioactive compounds from the leaves of *Ficus sp* by cold extraction method. . The crude extracts of ethanol, petroleum ether and aqueous extracts were spotted in TLC plate to figure out the number of probable compounds in each extract. Column chromatography was performed to collect purified fractions. Phytochemical screening of crude samples were tested, the presence of flavonoids, steroids, tannins, alkaloids, glycosides, phenols, reducing sugars were identified. Antimicrobial activity of ethanol, petroleum ether fractions were tested using agar-well diffusion method, maximum activity was recorded in a concentration-dependent manner against *Escherichia coli*, *Staphylococcus sp, Streptococcus sp, Pseudomonas sp, Proteus sp, Bacillus sp, Aspergillus sp and Candida sp*. Among the total fractions obtained, few active fractions inhibited growth of organisms which would significantly serve as a potential source for new antimicrobial compounds. GCMS was performed to identify the potential compound to be Sitosterol exhibiting antimicrobial activity and further the structure elucidation was determined by using NMR studies.

Keywords: *Ficus sp*, Phytochemical Screening, Antimicrobial activity and Sitosterol.

1. Introduction

Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. Bioactive molecules occur in plants as secondary metabolites and as a defense mechanism against predation, herbivores, fungal attack, microbial invasion and viral infection[1]. Antimicrobial medicines can be grouped according to the microorganisms they primarily react.

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Ficus is genus of about 850 species of wood trees, shrub, vines, epiphytes and hemi epiphytes in family Moraceae. It is known as fig or fig tree. *Ficus* species are characterized by their unique inflorescence. The wood of *Ficus* trees is often soft and the latex precludes which is used to make mummy caskets in ancient Egypt. *Ficus* were also a common food source for the romans. It was cultivated from Afghanistan to Portugal, also grown in Pithoragarh in the Kumaon hills of India. The fruit of most other species are also edible though they are usually of only local economic importance or eaten as

bush food. However, they are extremely important food resources for wildlife.

The fruits of the tree attract much importance as both food and traditional medicine, as it contains laxative substances, flavonoids, sugars, vitamin A and C, acids, enzymes etc., The crude extract of *Ficus* was able to prevent the growth of selected microorganisms. Extracts of these herbs have many reports on their antimicrobial activity[3, 4] and also to treat female infertility[14]

Materials and Methods

Plant material

Fresh leaves of *Ficus sp.* were collected from different locations of Thiruvandrum, Kerala (Figure 1). The plants were identified taxonomically and authenticated at the Herbarium, Department of Botany, Women's Christian College, Chennai. Fresh leaves were washed thoroughly 2 - 3 times with running tap water and then with sterile water followed by shade-drying, powdered and used for extraction.

Test microorganisms

Human bacteria such as *Escherichia coli* , *Enterobacter sp, Bacillus sp, Proteus sp Pseudomanas sp. , Candida sp and Aspergillus sp* were collected from MTCC, Chandigarh. India. All the test bacterial species were maintained on nutrient agar media and aseptically subcultured in periodic intervals.

Preparation of Plant Extract:

The leaf sample of *Ficus sp* was shade dried, powdered and stored in a clean container[2]. The extract was prepared by adding aqueous, ethanol and petroleum ether solvent in the ratio of 1: 10. i.e., 5ml of the powder sample were soaked in 50ml of ethanol, petroleum ether, aqueous in glass beaker for 24 hours at room temperature. The samples were filtered using whatmann filter paper No. 1. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4°C in airtight bottles until further use. 1 g of each solvent residue was dissolved in 10 ml of respective solvents were used for further studies.

Chromatography Techniques

Thin Layer Chromatography

10ml solvent was prepared using a mixture of chloroform: methanol: acetone at a ratio of 8:1:1 which was used as a mobile phase for ethanolic crude extract. The samples were spotted in TLC plates at the bottom with the help of a capillary tube and based on the number of spots obtained the approximate number of compounds present in the sample were identified.

10ml solvent was prepared using a mixture of Petroleum ether: ethyl Acetate in the ratio of 7:3, which was used, a mobile phase for Petroleum ether crude extract. Both crude extracts were allowed to run in TLC plate and the spots identified indicate the approximate number of compounds present in the sample.

Column Chromatography

Packing of Column

The column was packed with silica (100mesh) dissolved in the solvent, shaked continuously to set the column and the silica mixture was loaded onto the column apparatus. The cotton was plugged at the end of the apparatus and tapped continuously to set the pack.

Running of Column

The filtered petroleum ether extract and ethanol extract were mixed with silica . Extracts were dried by keeping it in the water bath. The dried ethanolic and petroleum ether extracts were poured on the top of two separate silica columns, cottons were plugged at the top of the pack. The sample was allowed to run, the separated fractions were collected and mixed with silica.

Phytochemical Screening

The preliminary tests, for the detection of secondary metabolites, were carried out with 5ml of sample and the crude extract of samples were tested to identify the compounds[5,6]

1. Test for Alkaloids (Mayer's test): The sample was taken and potassium mercuric iodide was added to obtain the yellow colour precipitate, which indicates the presence of alkaloids.

2. Test for Carbohydrates (Benedicts test): 5gm of sample was taken; Benedict's reagent was added and heated gently to obtain the orange red precipitate, which indicates a positive result.

3. Test for Saponins (Foam's test): In this test, 0.5g of the extract was shaken with 2mL of water. The result was observed for the presence of foam which indicated the presence of saponis.
4. Test for Phytosterol (Salkowski's test): The sample was mixed with chloroform and filtered; the filtrate obtained was added with few drops of Con. H_2S0_4. Appearance of golden yellow colour indicates the presence of phytosterols.
5. Test for Phenol (Ferric chloride test): 3-4 drops of ferric chloride was added to the sample, formation of bluish black colour indicates the presence of phenols.
6. Test for Flavanoids (Lead acetate test): The sample was mixed with lead acetate solution. Yellow precipitate indicates the presence of flavanoids.
7. Test for proteins (Xanthoproteic test): Concentrated Nitric acid was added to the sample and yellow colour indicates the presence of proteins in the sample.
8. Test for Amino acids (Ninhydrin test): In this test, 0.25% of Ninhydrin reagent was added to the sample and heated for few minutes, amino acid presence was indicated by the appearance of blue colour.
9. Test for Diterpenes (Copper acetate test): The sample was dissolved in water and 3-4 drops of copper acetate solution was added, appearance of emerald green colour indicates the presence of diterpenes.

Antimicrobial activity:

Antimicrobial activity of Aqueous, Ethanol and Petroleum ether extracts were tested against *Escherichia coli*, *Staphylococcus sp, Streptococcus sp, Pseudomonas sp, Proteus sp, Bacillus sp, Aspergillus sp and Candida sp* [7,8]. The organisms were inoculated into sterile Nutrient Broth and a bacterial organism was swabbed on Muller Hinton Agar and fungal organisms on Sabouraud Dextrose Agar aseptically. Wells were punctured in each plate and samples were loaded. The plates were incubated at 37˚C and observed for the zone of inhibition after 24hrs for bacteria and fungi results were observed after 3-4 days of incubation. The results were tabulated.

Results and Discussion

Preparation of Extract

The overnight extracts were collected and filtered using whatmann filter paper no.1. The filtered aqueous extracts, ethanol [9] and petroleum ether were dried up and the dry weight was recorded.

Thin Layer Chromatography

The samples of ethanol and petroleum ether crude extracts were spotted in TLC plates at the bottom with the help of capillary tube. The extracts were allowed to run in TLC plate with the above mentioned mobile phase and the approximate number of compounds present in the sample were identified by the spots obtained (Figure 2).

Column Chromatography

The column was packed with silica gel and dried ethanol extract were added on top of the pack and the column were run with the standardized mobile phase mentioned above. The column chromatography was done and four purified ethanolic fractions were obtained (Figure 3). Similarly two purified fractions of petroleum ether were obtained (Figure 4). The extracts were collected in screw cap tube. The dry weight of ethanol and petroleum the fractions were tabulated (Table 3 & 4)

Phytochemical Screening

The phytochemical screening results of the crude extracts[10] showed the presence of Carbohydrate, Phenol, Saponins, Tannins and Diterpenes in Ethanolic extract. Alkaloids. The presence of Phytosterols, Tannins and Diterpenes in Petroleum Ether fractions was reported and presence of Phenol, Protein, Saponins, Flavanoids, Phytosterols and Tannins in Aqueous extract. These compounds known to support bioactivity. Qualitative analysis of metabolites from *Ficus sp* leaf extract is tabulated in Table 2.

GCMS Analysis

Gas chromatography mass spectroscopy analysis was carried out in the purified fractions of *Ficus sp*. The thin layer chromatography (TLC) of ethanol extract, petroleum ether extract was compared with the GC-MS profile to identify the probable bioactive compound. The peaks in the chromatogram were integrated and were compared with the database of spectrum of known components already stored in the GC-

MS library. The GCMS analysis in correlation with the TLC results the bioactive compound was identified as Sitosterol[11] , which exhibited an good antimicrobial activity. The Figure- 6 shows the GCMS result of Ethanol crude extract where the probable compounds are enlisted below. Beta- Sitoterol [12], Tarasterol, 12- Oleanen-3 YL Acetate, Lup- 20(29)- EN-3-OL acetate, Lupan -3 –OL acetate**.** The Figure- 7 shows the GCMS result of Petroleum Ether crude extract where the probable compounds are enlisted below.

1. Monolinoleooyl Glycerol Trimethylsilyl Ether, Alpha Amyrin, URS-12-EN-3- OL acetate, Phenol 3-5 BIS (1, 1 Dimethylethyl).

Antimicrobial Activity

The crude extracts and purified fractions were subjected to antimicrobial activity against pathogenic organism like *Escherisia coli .Pseudomonas sp, Proteus sp, Bacillus sp, Aspergillus sp, Candida sp* using MHA medium and the zone of inhibitions were recorded after 24hours for bacteria , 3-4 days for fungal organisms and the results are tabulated below (Table 3; Figure 8).

The petroleum ether fractions F1 and F2 had a significant antibacterial especially against *E.coli (13mm & 12mm)* and *Enterobacter sp.(10mm & 9mm)* than other bacterial pathogens. Similarly a good antifungal activity was exhibited against *Aspergillus sp* (8mm for both fractions) when compared to *Candida sp.* Diverse varieties of plant show antimicrobial activity which also varies depending on the concentration[13]. Indeed *Ficus* sp is specifically used in the treatment of female infertility [14].

Conclusion

The *Ficus sp* crude extract samples was subjected to phytochemical screening and antimicrobial activity. The phytochemicals such as Carbohydrate, Phenol, Saponins, Tannins, Alkaloids, Phytosterols, Diterpenes, Protein and Flavanoids were present. Among the purified extract fractions, Petroleum Ether fractions showed high antimicrobial activity than the purified ethanol fractions. Correlating the column fractions with the GCMS library, Sitosterol was identified as a potential bioactive compound. Further the structural elucidation of Sitosterol needs to be determined by NMR studies. This study offers a valuable source for the discovery of drugs.

Acknowledgements

The authors sincerely thank the University Grant Commission for the financial support to carry out the study.

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Figure 1: Leaf of *Ficus* plant.

Figure 2: Thin layer chromatography of Petroleum ether and Ethanol crude extracts

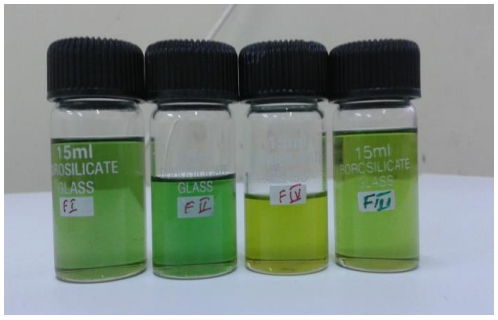


Figure 3: Ethanol fractions.

Figure 4: Petroleum ether fractions.

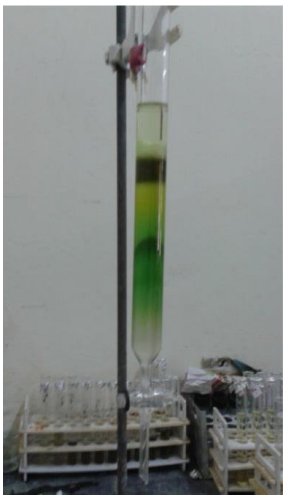
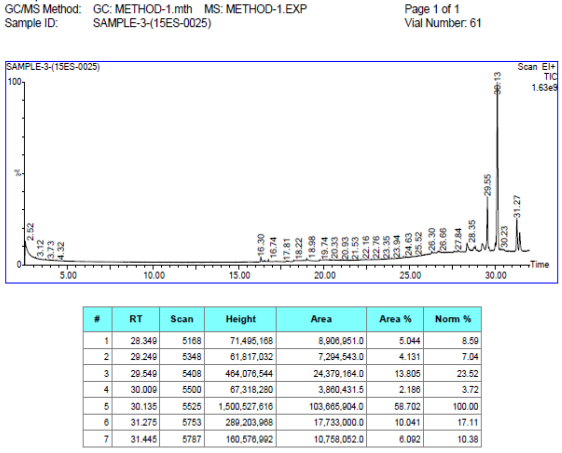


Figure 5: Column chromatography Ethanol and Petroleum ether crude extracts



#	RT	Scan	Height	Area	Area %	Norm %
1	28.349	5168	71,495,168	8,906,951.0	5.044	8.59
2	29.249	5348	61,817,032	7,294,543.0	4.131	7.04
3	29.549	5408	464,076,544	24,379,164.0	13.805	23.52
4	30.009	5500	67,318,280	3,860,431.5	2.186	3.72
5	30.135	5525	1,500,527,616	103,665,904.0	58.702	100.00
6	31.275	5753	289,203,968	17,733,000.0	10.041	17.11
7	31.445	5787	160,576,992	10,758,052.0	6.092	10.38

Figure 6: GCMS of Ethanol crude extract.

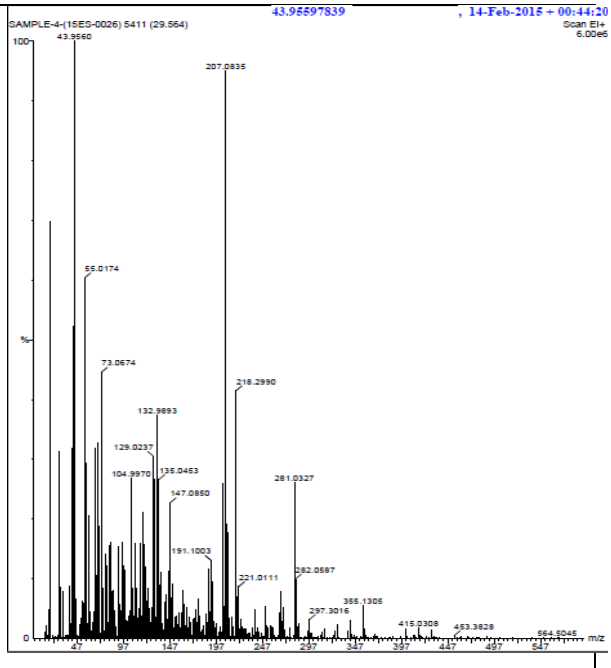
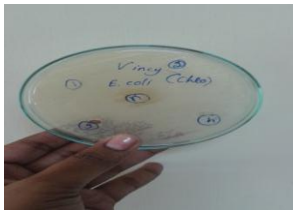


Figure 7: GCMS of Petroleum Ether crude extract.

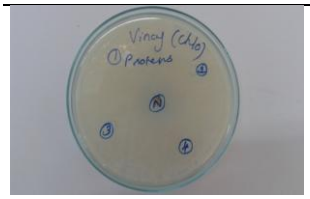
Ethanol Extract



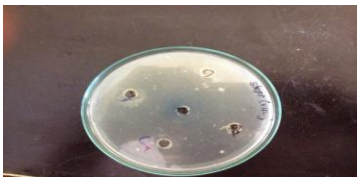
Eschericia coli

Enterobacter sp

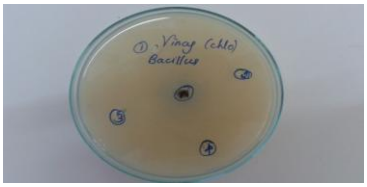
Pseudomonas sp



Proteus sp

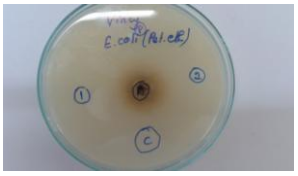


Bacillus sp



Streptococcus sp

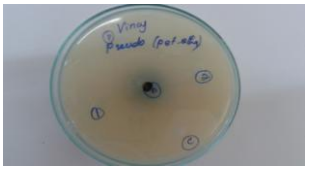
Petroleum Ether



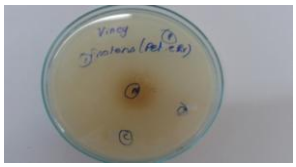
Escherisia coli

Enterobacter sp

Streptococcus sp



Pseudomonas sp



Proteus sp

Bacillus sp

Source of Support: Nil.

Conflict of Interest: None declared

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