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CHEMICAL SCREENING AND ANTIBACTERIAL STUDIES OF *CITRULLUS COLOCYNTHIS* (LINN.) SCHRADER

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ABSTRACT

C. colocynthis contained carbohydrate, protein, separated amino acid, tannins, phenolics, flavanoids, glucosides, terpenoids, alkaloids, anthranol, steroids, cucurbitacins, saponin, cardic glycoloids, trace elements and many other chemical groups. It possessed antioxidant, Antidiabetic, antimicrobial, anticancer, anti-inflammatory, analgesic, gastrointestinal, reproductive and protective and many other pharmacological effects. This paper will highlight the phytochemical screening, antibacterial studies, GCMS and FT-IR studies of *C. colocynthis*.

Key words: *C. colocynthis*, preliminary phytochemical, antibacterial, GCMS and FT-IR.

INTRODUCTION

World Health Organization survey indicated that about nearly 80% of the world's populations rely on nonconventional medicine, mainly of herbal sources, in their primary healthcare. This is especially the case in developing countries where the cost of consulting a western style doctor and the price of medication are beyond the means of most people [1-2]. There are hundreds of significant drugs and biologically active compounds developed from the traditional medicinal plants. Plant showed wide range of pharmacological activities including antimicrobial, antioxidant, anticancer, hypolipidemic, cardio-vascular, central nervous, respiratory, immunological, anti-inflammatory, analgesic antipyretic and many other pharmacological effects [3-40]. *C. colocynthis* contained carbohydrate, protein, separated amino acid, tannins, saponins, phenolics, flavanoids, flavone glucosides, terpenoids, alkaloids, anthranol, steroids, cucurbitacins, saponarin, cardic glycoloids, trace elements and many other chemical groups. It possessed antioxidant, Antidiabetic, antimicrobial, anticancer, anti-inflammatory, analgesic, gastrointestinal, reproductive and protective and many other pharmacological effects.

Plant profile

Common name: Bitter Apple, Colocynth, Bitter cucumber, Egusi, Vine of Sodom

Bengali : ndrayan, panjot, indrabaruni
Hindi : badi indrayan, ghorumba, indarayan
Kannada: hamekkae, hara-mekki-kayi
Malayalam : kattuvellari
Marathi : kadu-indravani
Sanskrit : atmaraksha, brihadvaruni, brihatphala
Tamil : kumatti, pey-komatti
Telugu : chitti-papara
Urdu : hanzal, indyaran, shahme-hinzal.

Botanical name: *C. colcoynthis*

Family: *Cucurbitaceae* (Pumpkin family)

Synonyms : *Cucumis colocynthis*, *Colocynthis vulgaris*.

Distribution

It was native to dry areas of North Africa and it has been known in the Mediterranean region since Biblical times. The plant now is found in Northern Africa: Algeria, Egypt; Libya, Morocco, Tunisia; Northeast Tropical Africa: Chad, Ethiopia, Somalia; East Tropical Africa: Kenya; West Tropical Africa: Mali; Asia: Kuwait, Saudi Arabia, Iraq, Jordan, Lebanon, Syria, Yemen, Afghanistan, Iran, Turkey, India, Pakistan, Sri Lanka; Europe: Greece, Italy, Spain; and Australia [41-43].

Description

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Bitter Apple is an annual plant resembling the common watermelon. The stems are herbaceous and beset with rough hairs. Leaves, on long stalks, are alternately arranged. They are triangular, many time cut, variously undulating, blunt, hairy, a fine green on upper surface, rough and pale on the underside. Flowers are yellow, appearing singly at axils of leaves. Fruit is round, size of an orange, yellow and smooth, when ripe contains within a hard leathery rind, a white spongy pulp enclosing numerous ovate compressed white or brownish seeds. This species is globally distributed from Africa, Mediterranean, except Spain, to Indo-Malaysia. Within India, it is found wild in the warm, arid and sandy parts throughout, up to an altitude of 1500 m.

Traditional uses

The root was used in inflammation of the breasts, joints pain; externally it was used in ophthalmia and in uterine pains. The fruit and root were rubbed with water and applied to boils and pimples. A paste of the root is applied to the enlarged abdomen of children [44].

Medicinal uses

It is a powerful drastic hydragogue cathartic producing, when given in large doses, violent griping with, sometimes, bloody discharges and dangerous inflammation of the bowels. Death has resulted from a dose of 1 1⁄2 teaspoonful of the powder. It is seldom prescribed alone. It is of such irritant nature that severe pain is caused if the powdered drug be applied to the nostrils; it has a nauseous, bitter taste and is usually given in mixture form with the tinctures of podophylum and belladonna. Colocynth fruits broken small are useful for keeping moth away from furs, woolens, etc [45].

MATERIALS AND METHODS

Collection of plant material

C. colcoynthis seeds were collected from foot hills of Kodaikanal, Dindigul District, Tamil Nadu, India, identified and authenticated at The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Trichy, Tamil Nadu. The leaves were shade-dried and coarsely powdered. Taxonomic identification of this plant was carried out by Dr. S. John Britto, Director at the Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. Voucher specimens (*C. colocynthis* RHT67104) have been deposited at the Rapinat Herbarium.

Preparation of plant extracts

20gms of leaf powder was taken in an aspirator bottle; 150 ml of Acetone, Ethanol, Methanol and Aqueous were used and the mixture was shaken occasionally for 72 hours. Then the extract was filtered. This procedure was repeated three times and all extracts were decanted and pooled.

GC-MS analysis

For the GC-MS analysis, the concentrated ethanolic extracts of the selected plant materials were used, and was carried out at Department of Applied Chemistry, Cochin University, Kerala. The conditions and specifications of were adopted with a small modification in temperature. GC-MS analysis was carried out on an Agilent-7890 GC-MS System comprising an AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing given conditions. The oven temperature was programmed from 100°C (isothermal for 2 min), with an increase of 10°C/min up to 200°C, then 5°C/min up to 250°C, ending with a 9 min isothermal at 250°C. The samples were diluted to 1/10 with ethanol and 10µl of the diluted sample was injected using automatic injector (Agilent). Mass spectra of the samples were taken with GC/MSD ChemStation Software at 70eV with a scan interval of 0.5 seconds and fragments from 40 to 550 Da. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) which consists of more than 62,000 patterns. The spectrum of the unknown compound was compared with the spectrum of the known compound stored in the NIST library. The names, molecular formula, molecular weight and molecular structures of the compounds of the test extracts were ascertained from the databank of PubChem and ChemSpider [7].

FT-IR analysis

The powdered materials of the selected plants (section 4.2) were used for the FT-IR analysis, which was carried out at Archbishop Casmir Instrumentation Centre (ACIC) of St. Joseph's College, Trichy. The Potassium Bromide (KBr) technique and procedure of was adopted. The powdered samples were ground in an agate mortar and pestle in order to obtain fine powder. Each powdered plant material was mixed with completely dried potassium bromide (ration of 1/100), and the mixture was subjected to a pressure of 5x106 pa in an evacuated die to produce a KBr pellet for FT-IR spectrometric analysis [8].

The FT-IR spectrum of each sample was recorded with Perkin Elmer FT-IR Spectrum RX1. The pellets of the sampled plants were scanned at room temperature (25±2 ºC) at spectral range of 4000-400cm^{-1}. For the noise reduction of each spectrum, the spectral resolution was set to 4.0cm^{-1}. The number of scans was adjusted to 15 times to obtain optimum results. The spectrum of each sample was recorded with the software Spectrum version 5.0.2. Background spectra collected under identical conditions were subtracted from the sample spectrum. Interpretations of the peaks obtained in the spectrum were done by referring to standard FT-IR tables for assigning corresponding functional groups [9].

Antibacterial screening

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Antimicrobial activity of the leaf extracts of *C. colcoynthis* was tested using the disc diffusion method. Nutrient agar plates were prepared and inoculated test organisms namely *S. typhi, P. aeruginosa, E. coli, P. mirabilis, B. Subtilis, P. vulgaris, S. aureus, Vibrio cholerae, K. pneumoniae* and *B. cereus* by a streak plate method. The sterilized filter paper disc of 5 mm diameter (Whatmann's No. 1 filter paper) was used and the leaf extracts of concentration of 100µl were added to each disc. The sterile impregnated disc with plant extracts were dried and placed on the agar surface with forceps and pressed gently down to ensure complete contact of the disc on the agar surface. All the plates were incubated at 37°C for 24 hours. The antimicrobial activity of plant extract was assessed by the presence or absence of inhibition zone and the diameter of the zone were measured.

RESULTS AND DISCUSSION

The result of GC-MS analysis leads to the identification of five (Fig.1) phytochemical compounds from ethanol extract namely Sulfurous acid, butyl tridecyl ester, 2-Methyl-1H-benzimidazol-5-amine (Table-1). These compounds are in use as Catalyst, anti-inflammatory and rheumatic symptoms, antidote, pesticide, pharmacology and toxicology.

The FT-IR spectrum was used to identify the functional group of the active components. The outcome of FT-IR functional groups are represented (Table-2). The FT-IR spectrum profile is illustrated (Fig-2). The FT-IR spectrum confirmed the presence of Alcohol, Amine and Amide, Alkane, Acid, Alkene, Aromatic, Nitro, Ether, Alkyl Halide and Ester.

The results of antimicrobial activity of acetone, ethanol, methanol and ethyl acetate extracts of *C. colocynthis* against nine different bacterial strains are presented (Table-3). The antibacterial activity is illustrated (Fig-3). The MIC of antibacterial activity is illustrated (Fig-4). MIC were determined for plant organs at different maturation stages. Aqueous and diluted acetone extracts (from the plant's leaves) were screened for activity against Gram negative bacteria such as *Salmonella typhi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens, Proteus mirabilis and P. vulgaris* and Gram positive bacteria such as *Bacillus cereus, B. subtilis* and *Staphylococcus aureus*. All extracts showed activity against all strains. The highest MICs were obtained from the ethyl acetate and methanolic extracts (MIC 24.67±0.5 against *Pseudomonas aeruginosa*, MIC 24.33±1.15 against *K. pneumoniae* and *Staphylococcus aureus*), the lowest MICs were obtained from the methanolic extract (MIC10.67±0.58 against *P. mirabilis*) [46].

The antimicrobial activity of all extracts from *C. colocynthis* were examined against nine local bacterial strains isolates (*Serratia marcescens, P. vulgaris, B. subtilis, B. cereus, K. pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, P. mirabilis, Salmonella typhi*) using agar disc diffusion method. The most active antibacterial activity of extracted ethyl acetate were shown against *Pseudomonas aeruginosa*. Broth dilution methods were used to determine the minimum inhibitory concentration (MIC) for the extracted ethyl acetate. The study showed that MIC values of 600 µg/ ml, 3000 µg/ ml, were recorded against *Staphylococcus aureus*, and *E. coli* isolates respectively [47].

The crude extracts, exhibiting wide range of bactericidal properties, indicate that the plants can be potential sources of antibiotics with a wide spectrum of properties. The results of the investigation confirm the traditional claim of the plants being used against infectious diseases.

Table 1. GC-MS analysis

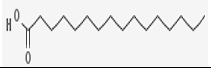
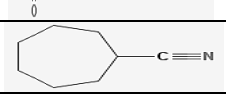
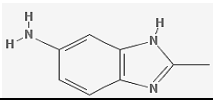
S.N0	Name	Molecular Formula	Molecular Structure	Molecular Weight	Retention Time
1	Sulfurous acid, butyl tridecyl ester	$C_{17}H_{36}O_3S$		320.53094 g/mol	3.991
2	1,10-Dichlorodecane	$C_{10}H_{20}Cl_2$		211.1718 g/mol	20.659
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$		256.42408 g/mol	23.097
4	Cycloheptyl cyanide	$C_8H_{13}N$		123.1955 g/mol	26.398
5	2-Methyl-1H-benzimidazol-5-amine	$C_{14}H_{13}N_3$		223.27 g/mol	26.782

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Table 2. FT-IR peak values and Functional groups.

S. No	Name of the Bond	Functional Group	Stretching Frequency (cm^{-1})
1	(Stretch, H-Bonded), N-H (Stretch) and N-H (Stretch)	Alcohol, Amine and Amide	3382.07
2	C-H (Stretch) and O-H (Stretch)	Alkane and Acid	2922.42
3	C-H (Stretch) and O-H (Stretch)	Alkane and Acid	2854.55
4	C=C (Stretch) and C=O (Stretch)	Alkene and Amide	1641.44
5	C=C (Stretch) and N-O (Stretch)	Aromatic and Nitro	1516.62
6	-C-H (Bending) and C=C (Stretch)	Alkane and Aromatic	1418.45
7	C-N (Stretch) and C-O (Stretch)	Amine and Ether	1237.25
8	C-F (Stretch), C-O (Stretch) and C-O (Stretch)	Alkyl Halide, Ester and Ether	1072.61
9	=C-H (Bending)	Alkene	832.18
10	C-Cl (Stretch)	Alkyl Halide	668.06

Table 3. Antibacterial activity using Disc diffusion method

S.No	Name	Acetone	Ethanol	Methanol	Ethyl Acetate	Antibiotic
1	*Serratia marcescens*	19.7±0.58	18±1	17.67±0.58	22.67±0.58	14.67±0.58
2	*Proteus vulgaris*	19±1	14.7±0.58	17.67±0.58	22±1	10.67±0.58
3	*Bacillus subtilis*	19.3±0.58	11.67±0.58	14.67±0.58	23.67±0.58	12.33±0.58
4	*Bacillus cereus*	16±1	14.67±0.58	12.67±0.58	16.33±0.58	10.33±0.58
5	*Klebsiella pneumoniae*	23.6±0.58	22±1	20.33±0.58	24.33±1.15	15.33±1.15
6	*Staphylococcus aureus*	22.3±1.15	20±1	23.67±0.58	24.33±1.15	18.33±0.58
7	*Pseudomonas aeruginosa*	20.3±0.58	21.67±0.58	24.67±0.58	24.67±0.58	16.67±0.58
8	*Proteus mirabilis*	11±1	11.3±0.58	10.67±0.58	15.67±0.58	9.33±0.58
9	*Salmonella typhi*	23±1	22.67±0.58	22.67±0.58	21±1	20.67±0.58

Fig 1. GC-MS analysis

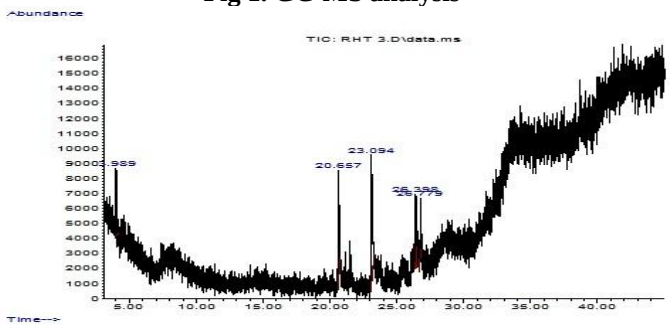

Fig 2. FT-IR Spectrum

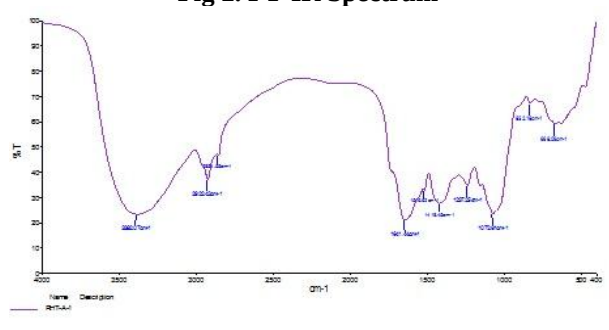

Fig 3. Antibacterial Activity

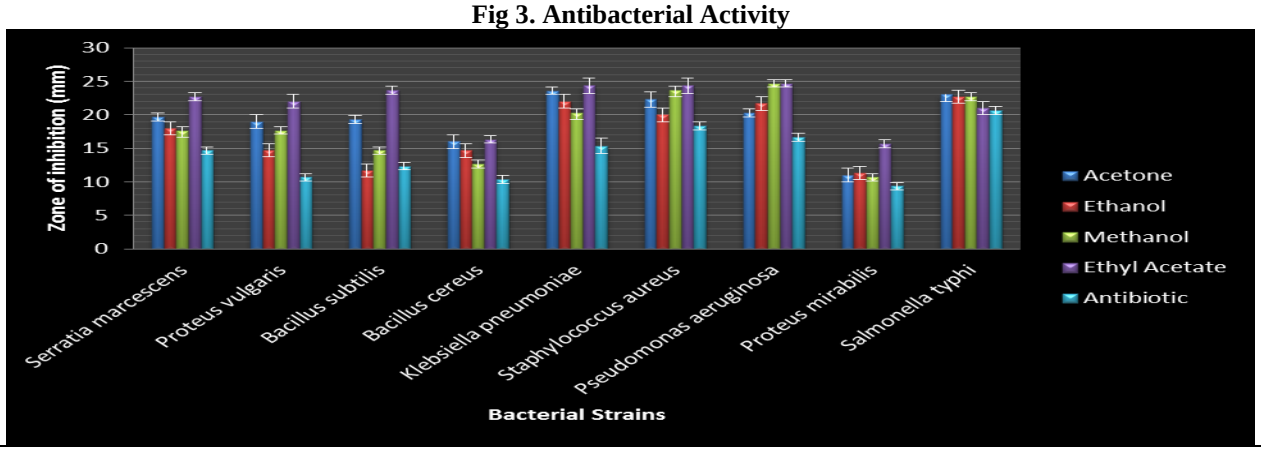

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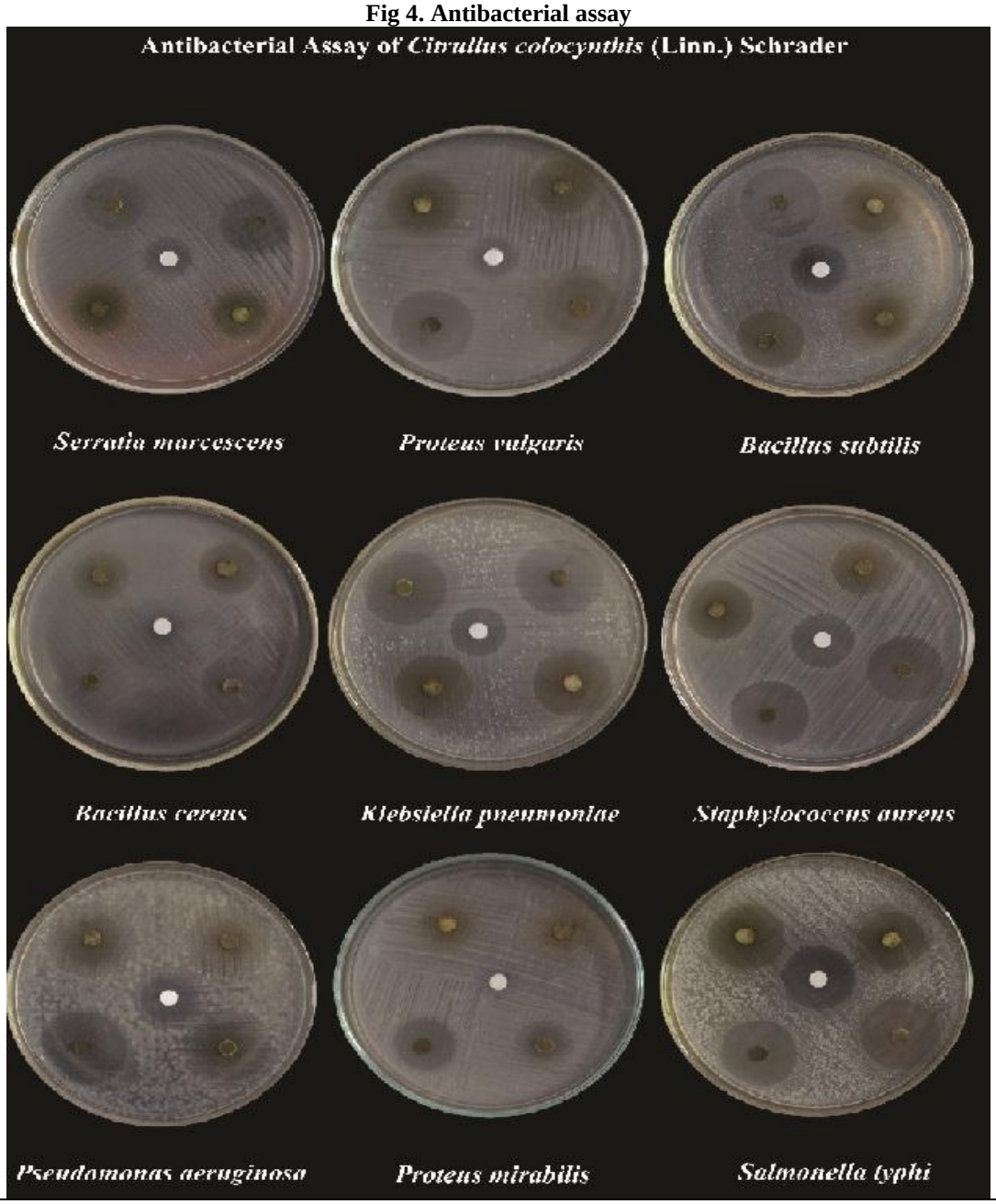
Fig 4. Antibacterial assay



CONCLUSION

As a result of preliminary and advanced phytochemical analysis of *C. colocynthis* through GCMS and FTIR. The presence of functional groups of compounds was as Alcohol, Amine and Amide, Alkane, Acid, Alkene, Aromatic, Nitro, Ether, Alkyl Halide and Ester were identified. The study has established that the ethyl acetate and methanolic extracts exhibited significant antibacterial activities in a concentration dependent manner. This study revealed the presence of four phytochemical compounds. Therefore, the plant extracts can be used for the treatment of infections caused by the strains of the test bacterial organisms.

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CONFLICT OF INTREST

No conflict of Interest

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