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Anti-bacterial activity of coumarin enriched extract from Cyperus rotundus rhizomes against Urinary Tract Infection

Sivadhanu M, Sakthi Priyadarsini S*
Department of Pharmacognosy, SRM College of Pharmacy, Faculty of Medicine and Health Sciences, SRM Institute of Science and Technology, SRM Nagar, Kattankulathur - 603203, Kanchipuram, Chennai TN, India.

Abstract

Rhizomes of Cyperus rotundus have been traditionally used for several ailments, particularly in urinary tract infection and inflammation. This study aims to determine the antibacterial activity of coumarin enriched Cyperus rotundus extract in inhibiting the growth of Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and Escherichia coli. The FT-IR spectra of root extracts revealed the presence of functional groups characteristic of alcohols, carboxylic acids, aromatic compounds, alkanes, alkenes, and amines. The GC-MS investigation led to the identification of diverse phytoconstituents. Further, various concentrations of C. rotundus extract at 500 µg/ml, 250 µg/ml, 100 µg/ml and 50 µg/ml was studied and compared with Gentamycin as standard. The results showed that the extract of C. rotundus could inhibit the growth of microorganisms with the high inhibition zone as indicated by a concentration of 500 µg/ml. Thus, the results of the present study will create a way for the invention of plant-based medicines for various microbial infections using C. rotundus which may lead to the development of novel drugs against drug-resistant microbial infections.

KEYWORDS: antibacterial activity, coumarin enriched fraction, Cyperus rotundus, FT-IR, GC-MS

Introduction

Urinary tract infection (UTI) is one of the most common human diseases, affecting people throughout the lifespan range, from newborns to geriatric[1]. Worldwide, about 150 million people are diagnosed with UTIs per year[2]. It is the most often acquired bacterial infection, accounting for 25% of all infections. Gram-negative bacteria produce 80-85% of infections, whereas Gram-positive bacteria cause 15-20% [3,4]. Members of the Enterobacteriaceae family including *Escherichia coli*, *and Pseudomonas* species are the primary causative agents of UTIs [5,6,7]. Medicinal plants for healthcare have traditionally served a vital sociocultural and spiritual role in the lives of many rural and tribal cultures around the world. Natural products from medicinal plants, either pure compounds or standardized plant extracts, present limitless prospects for the discovery of novel antimicrobial agents[8].

Microbial infections in humans are a severe problem, and the most prevalent pathogens are microorganisms such as bacteria and fungi[9]. Antibiotic resistance in microorganisms has caused enormous clinical challenges in the treatment of infectious diseases[10]. So, during the previous three decades, the pharmaceutical industry has developed a variety of new antibiotics, while bacterial resistance to these drugs has been increasing[11]. Many infectious diseases caused by bacteria and fungi have evolved in recent decades, and the growth of many bacteria can be especially difficult to control due to their capacity to metabolize a wide range of substances[12,13,14]. Numerous drug-resistant pathogens have emerged, necessitating the discovery of antibacterial phyto molecules against a variety of pathogenic organisms[15].

Cyperus rotundus is also known as nutsedge, nutgrass, or java grass, and is a member of the *Cyperaceae* sedge family. *Cyperus rotundus* referred as Nagarmotha in Ayurveda, is the third largest monocotyledonous plant in the family[16]. According to the reports, the rhizomes of *C. rotundus* have been used in Asian countries to treat stomach and bowel diseases as well as conditions associated with several infections and inflammations[17,18]. New techniques are constantly being developed for natural leads to use as antimicrobial drug against infections[19]. Resistance to antimicrobial agents is serious and is observed frequently in organisms such as *Salmonella* and *Staphylococcus*[20]. Studies report the presence of flavonoids, alkaloids, cyperol, fatty oils, furochromones, glycerol, linolenic acid, myristic acid, nootkatone, starch, saponins, sesqui-terpenes, sitosterol, stearic acid, terpenoids, polyphenol, and new sesquiterpenoids in the tubers and rhizomes of *C.rotundus*[21].

The diverse pharmacological actions of coumarins, as well as the widespread usage of coumarin-containing medications in the clinic, contribute to the growing interest in the field. Several coumarin-based antibiotic hybrids have been produced in the previous decade indicating antibacterial potential [22]. Administration of antibiotics in an unscientific manner results in antibiotic resistance[23]. Therefore, a different approach to solve this phenomenon is required, which makes use of plants that have medical uses and active antimicrobial

components that can inhibit or even kill harmful bacteria. In our current study, we evaluated the antibacterial activity of the coumarin-rich fraction from the methanolic extract of the rhizome of *Cyperus rotundus* [24].

Materials And Methods

TEST ORGANISM, CULTURE MEDIA, CHEMICALS AND REAGENTS

Four bacterial species namely, *Staphylococcus aureus*-902, P*seudomonas aeruginosa-424, Bacillus cereus-430* and *Escherichia coli-443* were purchased from MTCC, Chandihar, India. Nutrient agar medium, nutrient broth, Gentamycin was purchased from Himedia, India. Test samples, Petriplates, test tubes, beakers and other glass wares used were from Borosil, India.

COLLECTION OF PLANT MATERIAL

Cyperus rotundus (Cyperacae) were collected from Kanchipuram district, Tamil Nadu during December. Rhizomes were collected in dry weather and dried under shade for 3 weeks. The plant was identified and authenticated by Siddha Central Reseach Institute, Arumbakkam. The shade dried rhizomes were coarsely powdered and used for further studies. A voucher specimen has been reserved in the Department of Pharmacognosy, SRM college of Pharmacy, Chengalpattu.

EXTRACTION AND ISOLATION OF COUMARIN RICH FRACTION

The dried, powdered rhizomes of *Cyperus rotundus* was extracted using Soxhlet in 80% methanol for 24 hours concentrated and evaporated to dryness and the percentage yield was calculated. Fractionation of methanolic extract was carried out by column chromatography. The fractions were collected and subjected to a phytochemical test for the presence of coumarins. The fractions that showed positive responses to the presence of coumarins were pooled together, evaporated to dryness, and used for further studies

FT-IR ANALYSIS

The FT-IR spectrometer (Alpha FT-IR Spectrometer from Bruker optics) is equipped with deuterated triglycine sulfate (DTGS) as a detector and a germanium beam splitter connected to a Windows-based computer and interfaced with OPUS operating system software (version 7. 0 Bruker Optic) was used for FT-IR analysis. The coumarin-enriched fraction of *C.rotundus* was mixed with 200 mg KBr (FT-IR Grade) and pressed into pellets and the spectra was recorded[25].

GAS CHROMATOGRAPHY –MASS SPECTROMETRY (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) was performed with an Agilent 7890B-5977A instrument equipped with an HP-5MS 5% phenylmethyl siloxane capillary column (30 m × 0.25mm × 0.25 µm) and an Agilent 5977A MS detector. One microliter sample was injected into the GC-MS in partial mode. Helium carrier gas was used at a flow rate of 1 ml/min. The temperature of the injector was 280°C. The operation was performed at the column temperature maintained at 50 °C for 2 min, then increased to 270 °C at a rate of 5 °C/min, and finally kept at the same temperature for 10 minutes. The mass spectra thus obtained in the scan range of 40-600 amu were interpreted using the NIST 2011 library[26,27].

ANTIBACTERIAL ASSAY

The successive extracts at different concentrations (500, 250, 100 and 50µg/mL) in their respective solvents were evaluated for antibacterial effect in comparison with the standard antibiotic, gentamycin (10µg/mL) by agar-well diffusion method. The medium was prepared by dissolving 2.8gm of the commercially available nutrient agar medium (HiMedia) in 100mL of distilled water and the dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes.

AGAR WELL DIFFUSION METHOD

The antibacterial activity was studied by agar well diffusion method.[28,29,30]. The agar plates were inoculated with a standardized inoculum of the microorganism to be tested. Filter paper discs of about 6 mm in diameter containing the various concentrations of coumarin fraction are placed on the surface of the agar and incubated. The antimicrobial agent diffuses into the agar and inhibits the germination and growth of the test microorganism, after which the diameter of the inhibition zones were measured[31].

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION:

Based on the results of antibacterial assays, the most active extract was further selected for the minimum inhibitory concentration (MIC) determination. The MIC was determined by the method followed by Vianney et al., 2018. Accordingly, 50µl of the coumarin enriched fraction at a concentration (0.9-500µg/ml) Bacterial suspensions were added to a microplate well at a 100-fold dilution centrifuged at 700pm for 1 minute and

incubated at 37°C for 24 h in a microplate reader (ROBONIK, India). Additionally, to determine the cell viability, 10 µL of 2,3,5-triphenyltetrazolium chloride (TTC) at 20 mg/mL was added to each well and incubated at 37°C for 30 minutes in dark. The formation of a red formazan precipitate indicates viability. The precipitate formed was then resuspended in 20 µL of absolute ethanol and the difference between the absorbance of the blank and the sample was observed at 485 nm. The blank was prepared using sterile nutrient broth without bacterial suspension[32,33].

Results and discussion:

Currently, microbial infections have become an important clinical threat, associated with morbidity and mortality which may be primarily due to the development of microbial resistance to the existing antimicrobial agents. Hence there has been a continuous surge in antimicrobial susceptibility testing for the discovery of novel antimicrobial agents[34]. For the development of potentially beneficial new chemotherapeutic drugs, plants remain an essential source[35].

In our present study, the coumarin enriched fraction from rhizomes of *C.rotundus* obtained by column chromatography was subjected to FT-IR and GC-MS analysis followed by antibacterial study. The FT-IR spectrum showed identification of different functional groups as observed in The FT-IR analysis of *C.rotundus* extracts indicated the existence of functional groups such as alcohols, carboxylic acids, alkanes, aldehydes, amines, primary alcohols, aromatic compounds, aliphatic ketones, alkenes (Figure 1 and Table 1). The 3360cm^{-1} has shown broad and is specific to phenolic OH. The sharp bands at 1043 cm^{-1} and 879cm^{-1} are characteristics of aromatic C=C ring. The band at 2974cm^{-1} is due to amine salt N-H bend and the weak band at 608cm^{-1} is specific to the halo group.

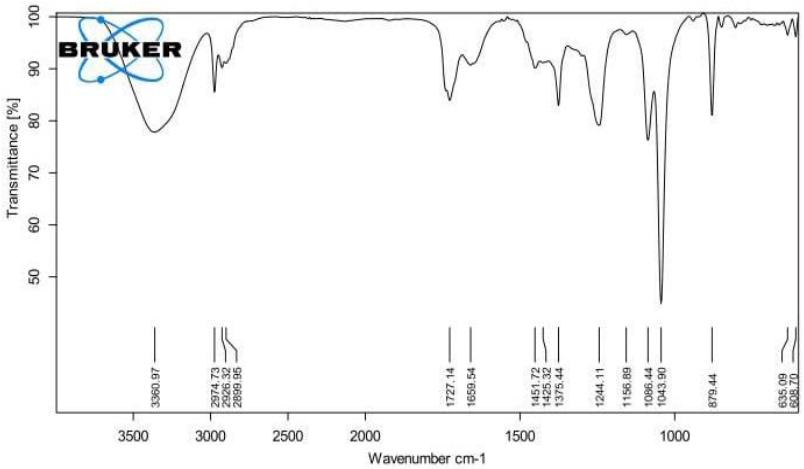

Figure 1. FT-IR interpretation of coumarin enriched extract of *Cyperus rotundus* rhizomes.

Table 1. FT-IR interpretation of isolated fraction from coumarin extract of *Cyperus rotundus.*

Wavenumber (cm -1)	Intensity	Group	Compound class
3360	Broad band	O-H stretching	Alcohol
2974	Sharp band	N-H stretching	Amine
2926	Weak band	C-H stretching	alkane
2899	Weak band	C-H stretching	Aldehyde
1727	Moderate band	C=0 stretching	Carboxylic acid
1659	Moderate band	C=C Stretching	Alkene
1451	Moderate band	C-H stretching	Alkane
1425	Weak band	O-H stretching	alcohol
1375	Sharp band	S=O stretching	Sulfonamide
1244	Moderate band	C-N stretching	Amine
1156	Sharp band	C-O stretching	Aliphatic amine

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1086	Sharp band	C-O stretching	Primary alcohol
1043	Sharp band	CO-O-CO stretching	Anhydride
879	Sharp band	C=C stretching	Alkene
635	Weak band	C-I stretching	Halo compound
608	Weak band	C-Br Stretching	Halo compound

Further, the volatile chemical components present in the extracts of *Cyperus rotundus* were studied using GC–MS analysis. The volatile components along with their respective retention times and abundance were recorded (Figure 2 & Table 2)). The GC-MS of coumarin rich extract showed various bio-actives, including 2,4-bis(1,1-dimethylethyl) Phenol, cetene, E-15-Heptadecenal, 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, 5-Eicosene, (E), 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro Indazol-4-one, 3-cyclopentylpropionic acid, 2-pentadecyl ester, n-Tetracosanol-1, 10-Heneicosene (c, t), 17-Pentatriacontene; 1,8,15,22-Tetraaza-2,7,16,21-cyclooctacosanetetrone, 4H-Cyclopropa[5',6']Benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a -dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl(1aα,1bβ,1cβ,2aβ,3aβ,6aα,6bα,7α,8β,8aα), Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester. A total of 13 compounds were detected. The component, 2,4-bis(1,1-dimethylethyl) phenol, detected at 22.01 showed the maximum abundance of 16.6%. Additionally, the presence of E-15-Heptadecenal; 5-Eicosene, (E); 3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro, Indazol-4-one; n-Tetracosanol-1; Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester having the peak area of 12.8%, 12.7%, 11.7%, 7.6%, 8.1% were also detected in the coumarin fraction of *C rotundus* extract.

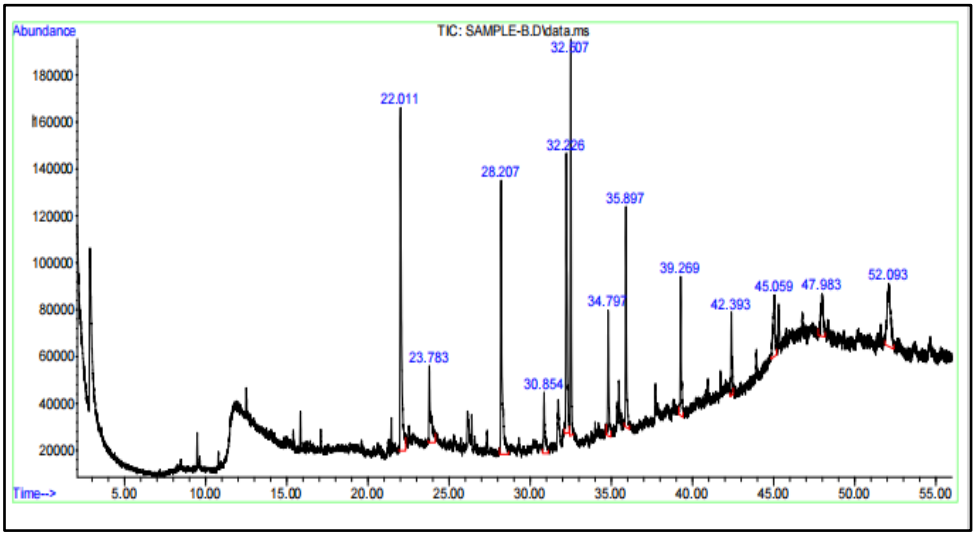

Figure 2. GC-MS chromatogram of the bioactive compounds present in coumarin rich fraction extract of *Cyperus rotundus*.

Table 2. Compounds identified in GC–MS fingerprint of coumarin enriched extract of whole plant of *Cyperus rotundus*

S.NO	Retention time (mins)	Name of the compound	Molecular formula	Molecular weight (MW)	Peak area %
1	22.011	2,4-bis(1,1-dimethylethyl), Phenol	$C_{14}H_{22}O$	206	16.6%
2	23.783	Cetene	$C_{16}H_{32}$	224	5.0%
3	28.207	E-15-Heptadecenal	$C_{17}H_{32}O$	252	12.8%
4	30.854	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	3.2%
5	32.226	5-Eicosene, (E)	$C_{20}H_{40}$	280	12.7%
6	32.507	3,6,6-trimethyl-1-phthalazin-1-yl-1,5,6,7-tetrahydro, Indazol-4-one	$C_{18}H_{18}N_4O$	306	11.7%
7	34.797	3-Cyclopentylpropionic acid, 2-pentadecyl ester	$C_{23}H_{44}O_2$	352	4.9%

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8	35.897	n-Tetracosanol-1	$C_{24}H_{50}O$	354	7.6%
9	39.262	10-Heneicosene (c,t)	$C_{21}H_{42}$	294	5.0%
10	42.392	17-Pentatriacontene	$C_{35}H_{70}$	490	3.4%
11	45.059	1,8,15,22-Tetraaza-2,7,16,21-cyclooctacosanetetrone	$C_{24}H_{44}N_4O_4$	452	5.1%
12	47.983	4H-Cyclopropa[5',6']Benz[1',2':7,8]azuleno[5,6-b]oxiren-4-one, 8-(acetyloxy)-1,1a,1b,1c,2a,3,3a,6a,6b,7,8,8a -dodecahydro-3a,6b,8a-trihydroxy-2a-(hydroxymethyl)-1,1,5,7-tetramethyl(1aα,1bβ,1cβ,2aβ,3aβ,6aα,6bα,7α,8β,8aα)	$C_{22}H_{30}O_8$	422	3.9%
13	52.093	Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	$C_{27}H_{52}O_5$	456	8.1%

The results of agar well diffusion carried out in coumarin enriched extract of *C.Rotundus* rhizomes against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and E. coli* were displayed in Figure 3 and Table 3

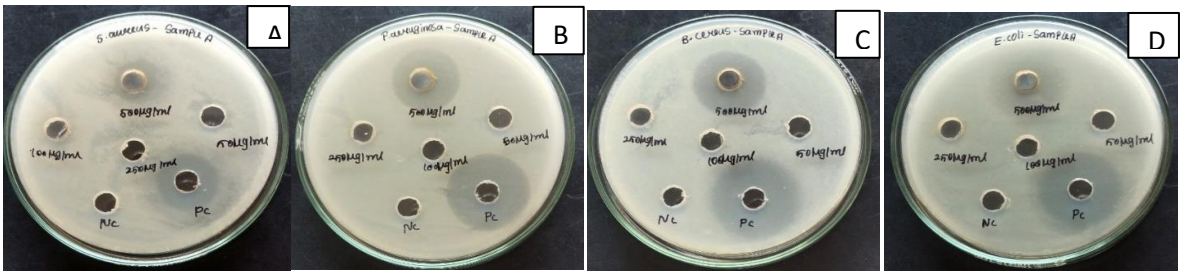

Fig 3: Antibacterial effect of coumarin enriched fraction at 500µg/ml, 250µg/ml, 100µg/ml and 50 µg/ml. A) *Staphylococcus aureus* B) *Pseudomonas aeruginosa C) Bacillus cereus D)* E. coli.

Table 3. Zone of inhibition (mm) upon treatment of *C. rotundus* coumarin enriched fraction

S. No	Name of the test organism	Zone of inhibition (mm)				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	Gentamycin
1.	*Staphylococcus aureus*	13±1.41	11.5±0.7071	9.25±0.35	6.25±0.30	17.5±0.70
2.	*Pseudomonas aeruginosa*	15.5±0.70	5.25±0.3	-	-	18.5±0.7
3.	*Bacillus cereus*	13±1.06	10.25±0.35	7.2±0.28	4.15±0.21	19.25±0.3
4.	*Escherichia coli*	17.5±0.70	0	0	0	20.25±0.35

Values are expressed as Mean ± SD, n=3

The coumarin-enriched fraction showed a concentration-dependent antibacterial effect against *Staphylococcus aureus and Bacillus cereus.* At the maximum concentration of 500µg/ml the coumarin enriched extract showed the highest anti-bacterial activity against *E. coli* with a zone of inhibition of (17.5 ± 0.70 nm) compared to standard Gentamycin (20.25 ± 0.35nm). However, bacterial growth inhibition was not observed at concentrations against *E. coli and Pseudomonas aeruginosa.*

The MIC of the most effective plant extracts (*Cyperus rotundus*) was employed by the disc diffusion method to evaluate their bacteriostatic and bactericidal properties. The inhibitory effect of *Cyperus rotundus* extract was found to be at 0.9µg/ml with percentage inhibition zones of 26.3 against *S. aureus and at 0*.45µg/ml with a percentage inhibition zone of 7 against *B.cereus.*

Conclusion:

Our data depicts the significant antimicrobial activity of coumarin enriched extracts of rhizomes of *Cyperus rotundus. Our* study shows ethanol and ethyl acetate as effective solvents in extracting important bioactive compounds. The FT-IR spectrum and GC-MS profile of the coumarin enriched extract showed the presence of various functional groups and volatile bioactives, responsible for the antimicrobial activity. Thus the result of the studies revealed that *Cyperus rotundus* extract can be used as an antibacterial agent in inhibiting the growth of *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus* and *Escherichia coli*.

Conflict of interest:

The authors have no conflicts of interest regarding this investigation

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