The Journal of Phytopharmacology 2021; 10(3):162-165
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2021; 10(3): 162-165
May- June
Received: 03-02-2021
Accepted: 20-05-2021

doi: 10.31254/phyto.2021.10303

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Comparative evaluation of antibacterial activity of solvent fractions and essential oil of *Cyperus rotundus* L. rhizomes

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ABSTRACT

Cyperus rotundus L. belongs to the family Cyperaceae. It is used to treat constipation, stomach disorders, dysmenorrhea, skin diseases, wounds, leprosy, fever, etc., in indigenous medicine. Also, they are used to treat itchy pus-filled pimples in Sri Lanka. This study aimed to evaluate *in vitro* antibacterial activity of different solvent fractions and essential oil of *Cyperus rotundus* L. rhizomes. Soxhlet apparatus was used to obtain the methanolic and petroleum ether extracts from rhizomes, and essential oil was extracted using steam distillation. The antibacterial activity of extracts and essential oils was determined against *Staphylococcus aureus* and *Pseudomonas aeruginosa* using the agar disc diffusion method. The activity was compared with Gentamycin as standard. The activity of extracts and essential oil were compared with one-way ANOVA followed by Dunnett's test. The results showed that both extracts and essential oil of *Cyperus rotundus* L. rhizomes showed a statistically significant difference in antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* compare to the standard drug, Gentamycin. The essential oil of *Cyperus rotundus* L. rhizomes showed comparatively higher antibacterial activity than extracts. *Staphylococcus aureus* was more susceptible to methanolic extract and essential oil than petroleum ether extract. *Pseudomonas aeruginosa* was more susceptible to essential oil and petroleum ether extract than methanol extract. *Cyperus rotundus* L. rhizomes possess good antibacterial activity, and further studies are required to isolate active phytochemicals and screen for antimicrobial activity.

Keywords: *Cyperus rotundus* L. rhizomes, antibacterial activity, essential oil, extract

INTRODUCTION

Antibiotic resistance is emerging as a serious problem worldwide. Due to bacterial resistance to many antibiotics, plants have gained attention in searching for new drug molecules. Medicinal plants are source for producing new drug candidates because they have been used from ancient times in indigenous medicine. Plants consist of different phytochemicals such as flavonoids, tannins, glycosides, alkaloids, saponins, terpenoids, phenolic compounds, etc. These phytochemicals showed antimicrobial properties in various *in vitro* and *in vivo* studies [1].

Cyperus rotundus L. belongs to the family Cyperaceae and is commonly known as nutgrass. It is considered one of the world's worst weeds [2]. It is an erect, glabrous, grass-like herb with fibrous roots that typically grows from 7-40 cm tall. It reproduces by tubers and rhizomes. Rhizomes of this plant possess initially white and fleshy with scaly leaves and then become fibrous, wiry, and very dark brown with age [3].

Cyperus rotundus L. rhizomes are used in different diseases like chronic diarrhea, inflammation, skin rashes, and excess bleeding. Also, it has anti-estrogenic, antimicrobial, anti-helminthic, antiemetic, and anti-diabetic activities [4]. Furthermore, they have analgesic, anticonvulsant, gastro-protective, anti-obesity, hemodynamic, wound healing and anti-cancer, antimalarial, antioxidant activities [3]. *Cyperus rotundus* L. rhizomes also were used to treat skin diseases, wounds, and the treatment of itchy pus-filled pimples and red-bumps.

This plant is used in many infectious diseases due to its' antimicrobial property. Even though some studies evaluated this plant's antibacterial activity, we have conducted a comparative study on the antibacterial activity of the rhizomes of *Cyperus rotundus* L. using polar, nonpolar solvents and essential oil.

MATERIALS AND METHODS

Collection and extraction of rhizomes

The rhizomes of *Cyperus rotundus* L were collected, cleaned, and dried under shade for 15 days.

Then it was coarsely powdered. Powdered rhizomes were used for the preparation of methanol and petroleum ether extractions and essential oil.

Methanol and petroleum ether fractions of powdered rhizomes were prepared using Soxhlet apparatus based on previous studies with slight modifications [2]. Soxhlet apparatus was used for the extraction process. Resultant extracts were evaporated under reduced pressure using a rotary evaporator. The essential oil was extracted by steam distillation according to the previous studies with minor modifications [5].

Preliminary phytochemical screening

According to the previous studies, phytochemical screening was conducted for the methanol and petroleum ether crude extracts of rhizomes of *Cyperus rotundus* L.[6] These extracts were qualitatively analyzed for Phyto compounds such as alkaloids, flavonoids, saponins, tannins, glycosides, polyphenols, etc.

Evaluation of antibacterial activity

The *in-vitro* antibacterial activity of different extracts of *Cyperus rotundus* L. rhizomes was conducted by the agar disc diffusion method [7]. All the procedures were done under aseptic conditions using a class II biosafety cabinet. Gentamicin (10µg/ml) was used as a positive control. DMSO and Tween 80 were used as a negative control. All experiments were conducted in triplicate.

Bacterial strains

Staphylococcus aureus and *Pseudomonas aeruginosa* were used for *in vitro* antimicrobial study. Both strains were maintained at 4 °C over nutrient agar slants throughout the experiment.

Procedure for antibacterial study

Methanol and petroleum ether extracts were dissolved in dimethyl sulfoxide [8], and essential oil was dissolved in Tween 80 [9]. A series of different concentrations were prepared (50mg/ml, 100mg/ml, and 200mg/ml).

Agar culture plates were prepared according to guidelines. Agar was allowed to cool at 45-50°C after autoclaving it. Around 30ml of culture, the medium was poured into each 100mm diameter sterile Petri dishes to get a uniform depth of approximately 4mm. After adding agar into plates, they were placed in a biosafety cabinet at room temperature to solidify until excess surface moisture was removed by evaporation. Antimicrobial discs were prepared using blank sterile ABST discs. They were impregnated in 50 microliters of each test sample and kept under laminar flow for about 30 minutes to absorb and dry.

The microbial inoculum was prepared by dissolving a loop full of colonies of test microbes in 10ml of physiological saline until the turbidity is at 0.5McFarland standard. (equivalent to 1.5×10^6 CFU/ml). Within 15 minutes after adjusting the inoculum suspension's turbidity, A sterile swab was dipped into the adjusted bacterial suspension. The excess inoculum in the swab was removed by rotating several times and pressing firmly on the inside of the tube's wall above the fluid level. Then it was inoculated in the dried surface of a Mueller-Hinton agar plate by streaking the swab over the entire sterile agar surface. The procedure was repeated two more times, and the plate was rotated for 60^0C each time to ensure an even distribution of inoculum. The plate's top was replaced, and it was allowed 3-5 minutes, but no longer than 15 minutes, for any excess surface moisture to be absorbed before applying the antibiotic discs. Then appropriate discs were placed on the surface (no closure than 24mm from the center to center) on the agar plate's surface using sterile forceps. The plates were kept in inverted position in the incubator at 35^0C within 15 minutes after discs were applied. The plates were incubated aerobically. It was incubated for 16-18 hours.

After 16-18 hours of incubation, each plate was examined. The diameter of the zone of complete inhibition, including the diameter of the disc, was measured. Zones were measured in millimeter using a sliding caliper.

Data analysis

The diameter of the zone of inhibition (in millimeters) was used as an indicator for antimicrobial activity. The test results were presented as mean with standard deviation. One-way ANOVA followed by Dunnett's test was used to compare the activity. Differences in activities were considered significant for *P*-values < 0.05.

RESULTS

The yield percentage of methanol, petroleum ether, and essential oil were 1.975, 0.916, and 0.6 % W/W.

According to the preliminary phytochemical screening test depicted in Table 1, methanol crude extract contained alkaloids, phenolic compounds, glycosides, flavonoids and saponins, and tannins were absent. In petroleum ether crude extract, alkaloids, phenolic compounds, and flavonoids were present, while saponins, tannins, and glycosides were absent. Tannins were absent in both extracts.

Table 1: Phytochemical screening

Phyto constituents	chemical tests	Methanol extract	Petroleum ether extract
Alkaloids	Wagner's test	+	+
Saponins	Froth test	+	_
Flavonoids	Lead acetate test	+	+
Tannins	Gelatin test	_	_
Glycosides	Keller –killiani's test	+	_
Polyphenols	Test for polyphenols	+	+

(+) indicate present, (-) indicate absent

The result of zones of inhibition of extracts and essential oil of Cyperus *rotundus* L. rhizomes were depicted in Table 2. The essential oil showed a maximum inhibition zone at 200mg/mL for both gram-negative and positive strains compare to extracts. Methanolic extracts showed a high inhibition zone for *Staphylococcus aureus* compares to petroleum ether extract. Petroleum ether extract showed high inhibition zone against *Pseudomonas aeruginosa* compared to methanol extract.

Table 2: Inhibition zone values obtained from different extracts of *Cyperus rotundus* L. rhizomes and standard

Test organism	Essential oil(mg/ml)			Methanol extract(mg/ml)			Petroleum ether(mg/ml)			Gentamycin (µg/ml)
	200	100	50	200	100	50	200	100	50	10
Pseudomonas aeruginosa	13.83 ± 0.29	12.33 ± 0.29	10±0	6±0	4.17 ± 0.29	2.83 ± 0.29	9.33 ± 0.58	7.17 ± 0.29	6.17 ± 0.29	17.67±0.58
Staphylococcus aureus	17.67 ±0.58	15.83 ± 0.29	14±0	12.17 ± 0.29	10.33 ± 0.29	8.17 ± 0.29	8.67 ± 0.58	6.17 ± 0.29	4.17 ± 0.29	19.67±0.58

DISCUSSION

The presence of different phytochemicals in the extracts depends on their solubility. Even though some phytochemicals present in both extracts, their concentration may be varied. Methanol extracts the most polar phytochemical compounds, and petroleum ether, which is nonpolar solvents, extracts most nonpolar compounds. Thus, methanol showed a high yield percentage due to a comparatively higher number of polar compounds present in the plant.

In the previous study on phytochemical analysis, glycosides were present the petroleum ether extract [10]. In this study, glycosides were absent in the petroleum ether extract. This could be due to less quantity of glycosides present in the sample and the extraction process's efficiency. According to the results, most phytochemicals were present in the methanol crude extract of *Cyperus rotundus* L. rhizomes.

All the extracts showed concentration-dependent antibacterial activity against both gram-negative and positive bacteria. However, the activity's efficiency was differed based on the type of extract and the bacterial strain used. A wide range of phytochemicals showed antimicrobial activity. These phytochemicals, also known as secondary metabolites, showed potent activity against a wide range of bacteria [11]. Alkaloids and flavonoids [12] and phenolic compounds [13, 14], and other phytochemicals showed good antimicrobial activity.

Interestingly, in our study, these phytocompounds such as alkaloids, flavonoids, and phenolic compounds were present in both extracts, and they could contribute antimicrobial activity of these extracts. Terpenes and their derivatives are secondary metabolites that are commonly present in essential oils [15]. Terpenes possess good antimicrobial activity [16]. The lipophilicity of terpenes is one of the determining elements of their antibacterial activity [17].

Staphylococcus aureus was more susceptible to essential oil and methanol extract. *Pseudomonas aeruginosa* was more susceptible to essential oil and petroleum ether extract. Differences in extracts and essential oil against these bacterial strains could be due to the presence of different phytocompounds in different levels. Also, the penetration ability of these compounds through the bacterial wall may contribute to their antimicrobial activity. Gram-positive bacterial cell walls contain a single layer, whereas gram-negative cell walls contain a multilayered structure bound by the outer cell membrane [18]. Methanolic extract showed higher activity against gram-positive bacteria is due to that compounds present in the methanolic extracts could penetrate the gram-positive cell wall compared to the gram-negative cell wall. The essential oil contains terpenoids, which are lipophilic, and petroleum ether extract, a nonpolar solvent, has lipophilic compounds. Due to these lipophilic compounds in both essential oil and petroleum ether extract, they were more active against gram negative bacteria. These lipophilic compounds can easily penetrate the gram-negative cell wall. This could be the reason for the less effectiveness of methanolic extract against gram-negative bacteria.

Both extracts and essential oil have considerable good antibacterial activity. According to one-way ANOVA followed by Dunnett's test, these extracts and essential oil's antibacterial activity showed a statistically significant difference compared to Gentamycin ($P<0.05$). Extracts contain a mixture of several phytocompounds. The concentration of phytocompounds that possess antimicrobial activity may not be insufficient to exert maximum antibacterial activity. Further studies needed to isolate and screen the individual phytocompounds for antimicrobial activity to explore effective plant-derived antimicrobial agents.

CONCLUSION

Both extracts and essential oil inhibited the growth of both *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* (Gram-positive bacteria) was more susceptible to essential oil and methanol extract. *Pseudomonas aeruginosa* (Gram-negative bacteria) was more susceptible to petroleum ether extract. Even though extracts showed good antibacterial activity, compared to the reference drug, they showed less activity. Further studies needed to isolate the plant's phytoconstituents and screen antimicrobial activity to explore active antimicrobials.

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HOW TO CITE THIS ARTICLE

Thuvaragan S, Nishadini NVC, Gnanakarunyan T, Srikaran R. Comparative evaluation of antibacterial activity of solvent fractions and essential oil of *Cyperus rotundus* L. rhizomes. J Phytopharmacol 2021; 10(3):162-165.