Antimicrobial Activity of *Cyperus rotundus* Linn. Extracts and Phytochemical Screening

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Abstract: This study aimed to evaluate the antimicrobial activity of *C. rotundus* rhizomes and tuber together, leaves and oil against several bacteria and three species of fungi. The plant was collected from Degala, Erbil, Kurdistan, Iraq. Antimicrobial activity of Crude aqueous and ethanolic extracts was examined by inoculating bacteria and fungi on media containing plant extract, and agar well diffusion method. The study revealed the effect of plant by evaluating microbial growth and inhibition zone measurement. Aqueous extract of leave had ability to suppress growth of *E. coli*, and both aqueous and ethanolic extracts of rhizome-tuber figured out antibacterial activity against *S. aureus* and *E. coli*, positively. Moreover, all selected bacteria and fungi were susceptible to ethanolic extract of rhizome-tuber, with different inhibition zones. Cyperus oil had no potential inhibitory effect against bacteria and fungi, excluding *C. albicans*. By performing phytochemical screening for rhizome-tuber extracts eleven secondary metabolites were detected.

Keywords: *Cyperus Rotundus*, Antimicrobial Activity, Aqueous Extract, Ethanolic Extract, Phytochemicals

1. Introduction

Antimicrobial activity of crude whole plant of *C. rotundus* have efficiency against some clinical isolates of bacteria (Prasad, 2014). According to World Health Organization, plants belonging to clinical aspect are considered as the worthiest choice in drug production (Santos et al., 1995). Modern mechanisms continuously used to detect chemical structures of herbs and utilizing them as antimicrobial agents against infectious diseases (Rojas et al., 2003).

Cyperus rotundus L. is common perennial weed belongs to the family Cyperaceae. It is recognized with many dialect names and synonyms, in Iraq known as Soad or Al Saad, and in other areas known as Nut grass, Nagarmotha, Nutsedge, Purple nutsedge, and others. Rhizome of this plant is cylindrical shape, scaly creeping, bulbous at the base and arising singly from the tubers which is appeared blackish in color from outside and reddish white inside, with a characteristic odour. It is widely distributed in tropical, subtropical and temperate regions throughout the world (Uddin et al.,

2006; Sivapalan, 2013; Himaja et al., 2014; Peerzada et al., 2015).

Locally *C. rotundus* used in traditional medicine as decoction for flatulence, nausea, vomiting, regulating hormones (prolactin), tonic hypoglycemic and diuretic (Naqishbandi, 2014).

The purple nutsedge contains allelochemicals, vis, polyphenols (Komai et al., 1997), sesquiterpenes (Komai & Ueki,1975), flavonol glycoside (Singh & Singh,1986), saponin (Singh & Singh,1980). The plant decrease product and quality of crops via competing for growth factors and allelopathic effects (Narwal et al., 1992). Moreover, other researchers concluded that *C.rotundus* affects the crops by allelopathy, competition, and also as an alternative host for insects and pathogens (Singhet al., 2009).

Leaves were used to flavour food, especially in the Middle East, and Southeast Asia. The seeds are also a carrier and pickling of spices, and bakery products (Nima et al., 2008). Additionally, some types of perfume have been produced from tubers and rhizomes of *C. rotundus*, it was also used to spices, and Ayurveda remedies in Arab countries, Africa, China, and India for centuries (Sharma & Gupta, 2007). They possess antidiarrheal, anti-oxidant, anti-inflammatory, anti-mutagenic, antiperiodic, anticonvulsant, anti-saturative, anti pyretic, antifungal, antidiabetic, antimalarial, antilipidemic, anti-bacterial, antiviral, anti-tumoral, cardio protective and wound- healing properties (Kilani et al., 2008; Sundaram et al., 2008; Dang et al., 2011). Its tubers have long been used as a natural remedy to cure spasms, diarrhea, dysmenorrhea, and menstrual irregularities (Bhattarai, 1993; Al-Massarani et al., 2016). From the ancient time rhizomes and tubers of *C. rotundus* have long been used as herbal remedy to treat bowel and stomach disorders in several countries including China, India, Iran, and Japan (Al-Massarani et al., 2016). Furthermore, in antimicrobial investigation on the rhizomes *of C. rotundus*, they found that the ethanolic extract exhibited highest activity against tested bacteria, whereas all extracts were in effective against fungal strains (Sharma & Singh, 2011).

In terms of phytochemicals, Previous researches detected many chemical constituents such as flavonoids, alkaloids, cyperol, fatty oils, furochromones, glycerol, linolenic acid, myristicacid, nootkatone, starch, saponins, sesqui-terpenes, sitosterol, stearic acid, terpenoids, polyphenol, and novel sesquiterpenoids in the tubers and rhizomes of *C. rotundus* (Sivapalan, 2013; Himaja et al., 2014; Peerzada et al., 2015). These chemicals are responsible for therapeutic, pesticidal, fungicidal, and insecticidal properties of *C. rotundus.*

This study was designed to investigate antibacterial and antifungal activity of rhizomes-tubers, leaves and oil *of C. rotundus*, collected from Erbil–Kurdistan region against pathogenic isolates of bacteria and fungi that could be used as new antimicrobial agents and enhance to control infectious diseases.

2- Material and Methods

2.1- Plant Preparation

C.rotundus plants were collected from Bardbar village, Degala, Erbil, Kurdistan, Iraq. The botanical identification was carried out by. Mr. Shorish Gorony (biology department, Science College, Soran University). The plant was dried in shade and grounded into fine powder by electrical grinder.

Two 50gm of each rhizomes-tubers and leaves powders were extracted separately, one of them with

250 ml of 70% ethanol and the other with 250ml distilled water, each mixture was agitated for 24 hours. Then, the extracts were filtered, dried and weighted to determine the concentration for preparing stock solution which was 500 mg/ml (Harbone, 1984). Also the *C. rotundus* oil was purchased from the local market.

2.2- Microorganisms

Several clinical isolates of bacteria and fungi were used to demonstrate antimicrobial potential of plant extracts. Bacteria were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and fungi were *Candida albicans*, *Candida tropicalis* and *Aspergillus niger*. Firstly, they were inoculated into broth media and incubated overnight to activate them. Concentration of microorganisms in broth media were optimized, and cultured on media to indicate antimicrobial potential of extracts and microbial susceptibility to performed extracts.

2.3- Media Preparation

In this study, different media were prepared such as; peptone water to activate microorganisms, Mueller Hinton agar used as a selective medium for antimicrobial susceptibility test, Sabouraud Dextrose Agar (SDA) also established for fungal growth and estimating suppressed fungi by plant extract.

2.4- Antimicrobial Susceptibility Test

This test was performed in two ways, culturing bacteria and fungi on media (Nutrient agar and SDA), enriched by 1ml of plant extracts. The next method was agar well diffusion, which is the most common method concerning susceptibility of microorganisms to antimicrobial agents, by inoculating 0.4 ml of extracts into wells with 12mm diameter.

2.5- Phytochemical Screening

Phytochemical screening was performed for eleven secondary metabolites within aqueous and ethanolic extracts of rhizomes-tubers of the plant. Various reagents were used for specific secondary metabolites such as; Wagner's reagent, Kelnar-kiliani, sodium hydroxide, Ferric chloride, hydrochloric acid and Ethanolic acitic acid.

3- Results and Discussion

Extracts of rhizomes-tubers, leave and oil of *Cyperus rotundus* plant were applied as antibacterial and antifungal agent against six pathogenic isolated bacteria and three species of fungi. *C. rotundus* is estimated as a promised plant, due to the presence of antimicrobial activity in many diseases caused by micoorganisms, such as; stomach, diarrhoea, dysentery and skin (Him-Che, 1985; Bown, 1995). *C. rotundus* in aqueous and ethanol extracts was used in randomly growing microbes on media enriched by 1% of extract, and agar well diffusion method by inoculating 0.4ml/well.

According to growth of targeted microorganisms on enriched medium by plant extract, both aqueous and ethanolic extracts of rhizomes-tubers had antibacterial activity on *E. coli* and *S. aureus*, while

only ethanol extract exhibited anti fungal activity against *A. niger*. In case of leave extract, only *E. coli* was susceptible to aqueous extract, but ethanolic extract had no affect on microorganisms. Furthermore, *C. rotundus* oil had antifungal potential against *C. albicans*, and no antimicrobial activity to others, as shown in (Table 1 and 2).

Table 1: Anti-bacterial activity of rhizomes-tubers, leaf and oil of C. *rotundus*

Extract / Bacteria	Rhizome D.W	Rhizome Ethanol	Leaf D.W	Leaf Ethanol	Cyperus Oil
S. aureus	++	++	-	-	**None**
E. coli	++	+	+	-	**None**
B. cereus	-	-	-	-	**None**
P. aeruginosa	-	-	-	-	**None**
p. mirabilis	-	-	-	-	**None**

'+': effective '-': non effective

Table 2: Antifungal activity of rhizome-tuber, leave and oil extracts *C. rotundus*

Extract / Fungi	Rhizome D.W	Rhizome Ethanol	Leaf D.W	Leaf Ethanol	Cyperus Oil
C. albicans	-	-	-	-	++
A. niger	-	++	-	-	-

In agar well diffusion method, both types extract of rhizome were applied on Mueller Hinton agar. Rhizome and tuber extract is evaluated as the promised part of *C. rotundus*, due to the presence of most crucial secondary metabolites (Himaja et al., 2014). Ethanolic extract of rhizome revealed a wide spectrum antimicrobial activity, while no activity seen from aqueous extract. According to (Parekh & Chanda, 2006), microbial activity of plant extracts in organic solvents is much higher than that in water. Inhibition zones were 35mm for *Bacillus cereus*, 34mm, 33mm and 27mm for *P. aeruginosa*, *S. aureus* and *K. pneumoniae*, respectively. Furthermore, it was 26mm for *C. albicans* and 25mm for *C. tropicalis*. Our result indicated that capsulated bacteria (*K. pneumoniae*) is less susceptible than other non-capsulated bacteria, and fungi are more resistant than bacteria to antimicrobial agents. The inhibition zone was reported at 24 and 48 hours of incubation, the susceptibility of bacteria and fungi after 48 hours was the same susceptibility at 24 hours earlier, excluding *P. aeruginosa* which was changed from 34 to 37mm after 48 hours as indicated in (Table 3).

Table 3: Antimicrobial activity of rhizome-tuber extracts

Extract / Bacteria	Rhizome D.W/mm at 24hrs.	Rhizome Ethanol/mm at 24hrs.
S. aureus	**-**	**33**
E. coli	**-**	**-**
Bacillus sp.	**-**	**35**
P. aeruginosa	**-**	**34**
p. mirabilis	**-**	**-**
K. pneumonia	**-**	**27**
C. albicans	**-**	**25**
C. tropicals	**-**	**26**

- : No effect

This study agree with the result of (Kabbashi**,** et al., 2015), whom concluded that high activity of whole extract of *C. rotundus* showed (31 & 30 mm) against (*S. aureus* & *B. subtilis***)** and (20 & 26 mm) against (*A. niger* & *C. albicans*). It also showed (19 & 20 mm) against (*E. coli* & *P. aeruginosa*).

Phytochemical screening was performed for eleven types of chemical components in aqueous and ethanolic rhizome of *C. rotundus*, by using specific tests. As a result, six phytochemicals were existed in both aqueous and ethanol extracts (Alkaloids, Phenols, Phlobatannins, Saponins, Terpenoids and Tannins. While, Only Cardiac glycosides was seen in ethanolic extract and Flavanoids in aqueous extract, but no sterols, Quinones and Oxalates were observed in plant extracts, as shown (Table 4).

Table 4: Phytochemical screening of secondary metabolites from rhizome of *C. rotundus*

Phytochemical compound	Test	Observation	Aqueous extract	Ethanolic extract
Alkaloids	Wagner, (Kage etal., 2009)	Red/brown precipitate	+	+
Cardiac glycosides	Kelnar-kiliani, (Dugger 2002)	Brown & violate ring	-	+
Flavonoids	Alkaline reagent (NaOH) (Dugger, 2002)	Yellow to colorless	+	-
Phenols	Ferric chloride (Mutalib, 2015)	Deep blue to black	+	+
Phlobatannins	Precipitate (Sydor etal., 2006)	Red	+	+
Saponins	Foam(Aharoni etal 2005)	Foam formation	+	+
Sterols	Liermannn-Bercard (Dugger, 2002)	Dark pink	-	-
Tannins	Braymer (Mutalib, 2015)	Blue to greenish	+	+
Terpenoids	Salkowaski (Aharoni etal 2005)	Reddish-brown precipitate	+	+
Quinones	HCl(Sydor etal., 2006)	Yellow precipitate	-	-
Oxalate	Eth. Acitic acid (Sydor etal., 2006)	Greenish black	-	-

'+': Present '-': Absent

4- Conclusion

In this investigation, rhizomes - tubers together had the highest antibacterial and antifungal activity, due to the presence of secondary metabolites. Ethanolic extracts were more effective than water extracts. Also bacteria were showed more susceptibility than fungi against the plant extracts. All bacterial and fungal inhibitory effect was the same at 24 and 48 hours of incubation time, excluding *P. aeruginosa* which was more affected even after 24 hours. More studies are needed to detect the main responsible components of antimicrobial activity of *C.rotundus* extracts.

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