

## Extraction, Identification and Antibacterial activity of Cyperus oil from Iraqi *C. rotundus*

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### Abstract

*Cyperus rotundus* has many different uses and these were based on the different parts of plant. The medical uses of cyperus have been used in medicine for thousands of years. The parts of the cyperus used are its leaves, seeds and oil. The Extraction process was carried out by steam distillation. Optimum organic extractant determined. The collected oil was identified via Thin Layer Chromatography (TLC) using a mixture of Ethylacetate: toluene (1:9) as chromatographic eluent. This study was designed to extract and identify of essential cyperus oil from *C. rotundus*. The Antibacterial activity of Cyperus oil was studied for various microorganisms (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Streptococcus pyogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*) using inhibition zone method (Aromatogram). The MIC and MBC for each microbe were estimated. The oil of *C. rotundus* was shown a remarkable activity against gram-positive bacteria, less antibacterial activity was found against gram-negative bacteria and no activity was observed with the oil against *Pseudomonas aeruginosa* and *Proteus vulgaris*. Novel method for extraction and identification of chemical composition for Iraqi cyperus oil was conducted. The study of the biological activities of this oil is very important because of needing to be determined whether there is any correlation between the biological activities and one or more of the chemical compounds purified from *C. rotundus* oil.

**Keywords:** cyperus oil, antibacterial activity, thin layer chromatography.

MIC

9:1

MBC

## Introduction

*C. rotundus* has many different uses were based on the different parts of the plant. The medical use of cyperus has been used in medicine for thousands of years. The parts of the cyperus used are its leaves, seeds and oil. The fresh leaves and ripe seeds have quite different aromas and uses. Both the leaves and seeds are rich in volatile oils that act mainly on the digestive system, stimulating the appetite and relieving irritation and as an expectorant. The oil is fungicidal and bactericidal. The, leaves were widely used to flavor food, especially in the Middle East, and Southeast Asia. The seeds are also an ingredient of curries and pickling spices, dishes a la grecque, and bakery products. Medicinally, cyperus was used internally for minor digestive problems, and externally for hemorrhoids and painful joints (seeds). Seeds reduce griping in laxative preparations based on *Rheum officinal* and *Cassia angustifolia*. The oil adds to the flavor of gin, vermouth and Chartreuse, and was also prized in perfumery (1). General references to cyperus's medical uses were also found in classical Greek and Latin literature (2). The medical uses of cyperus in the modern era were described by (3). In India, the fruits were considered carminative, diuretic, tonic, stomachic, antibilious, and refrigerant.

*C. rotundus* L., was widely distributed in the Mediterranean basin areas. This plant, which grows naturally in tropical, subtropical and temperate regions, is widespread in northeast (4).

The tubular part of *C. rotundus* is one of the oldest known medicinal

plants used for the treatment of dysmenorrhoea and menstrual irregularities. It was also used as analgesic, sedative, antispasmodic and to relieve diarrhea. *C. rotundus* has been widely investigated by several authors. It is a medicinal plant appearing among Indian, Chinese and Japanese traditional drugs that were used against spasms, stomach disorders and anti-inflammatory diseases (5).

Other pharmacological investigations indicated that *C. rotundus* had remarkable hypotensive, anti-inflammatory and antipyretic effects. Previous phytochemical studies showed that the major chemical components of this herb were essential oil, flavonoids, sesquiterpenes and cardiac glycosides (6).

The aim of this research is to establish a new procedure for extraction and characterization of cyperus oil and to study its antimicrobial activity using various microorganisms and to determine whether there is any correlation between the biological activities and one or more of the chemical fractions purified from *C. rotundus* oil.

## Materials and Method

**Plant:** *C. rotundus* collected from different Iraqi regions, Identify friendly by taxonomy professor (Dr. Ali Al-Musawy, Baghdad university ,College of sciences).

**Chemicals :** Ethanol absolute 99.9% (BDH), Ethanol 98% (BDH) , Ethylacetate (Fluka), Toluene (Fluka), Methanol 98% (BDH) , chloroform (Merck,Drmsstadt) ,Hexane (AnalaR), sodium sulphate (BDH). Sodium Flurescein (BDH). Cyperol ,Cyperene, Rotundine, Cyperone all from sigma. TLC plate

" Whatman " Silica gel GF60 .

**Sterilization:** Cultures media were sterilized by autocleaving at 121°C, 15 pound/In<sup>2</sup> for 15 minutes.

**Test organisms and inoculation preparation:** Organisms were obtained from cultures collected from mouth, wound swabs and urine of patients referred to central health Laboratories. The microorganisms were identified by using laboratories methods which including gram stain and biochemical tests. Isolates were as follows; *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, and *Escherichia coli* isolated from mouth swabs, *Pseudomonas aeruginosa* was isolated from wounds and *proteus vulgaris* was isolated from urine samples. Isolates were maintained on nutrient agar overnight, cultures were prepared by inoculating 2-3 colonies into 2-3 mL nutrient broth and incubating overnight at 37° C with shaking for the agar dilution assay, overnight culture were diluted in nutrient broth to approximately 10<sup>8</sup> cell/mL according to MacFerland tubes.

**Extraction of Cyperus oil:**

*C. rotundus* ripe dried fruits were broken into small pieces under sterile conditions; 20 g of powdered fruits were dissolved with 150 mL of ethanol, ethyl acetate, methanol and chloroform solvents then extracted by using steam distillation apparatus for 30 minutes. The determination of Cyperus oil was carried out by the steam distillation apparatus. The distillate was collected in a graduated separatory funnel. The water-free mixture of volatile oil was recovered as follows: 0.1 ml of 0.1% w/v solution of *sodium fluorescein*( to colored the aqueous layer) and 0.5 ml of water was introduced to the distillate and leave to stand for 5 minutes then water separated from the lower tab of sparatory funnel.

The volume of Cyperus oil measured directly by adding xylene to take up the Cyperus oil. The content of oil was expressed as a percentage v/w (oil volume / weight of *C. rotundus* powder). Optimum solvent extractant was determined. The extract thus obtained was injected into dark sterilized container.

**Identification of cyperus oil composition (8):**

Examine by thin-layer chromatography using a silica gel as the coating substance.

**Test solution.** 0.50 mL of cyperus oil was shaken with 5.0 ml of hexane for 2 min to 3 min and filter over 2 g of anhydrous sodium sulphate

**Reference solution.** 15 µl of each of the following standard solutions cyperol, cyperene, cyperone, rotundine, caryophyllene and 25 µl of olive oil were dissolved in 5.0 ml of hexane immediately before use.

Apply to the plate as bands 20 µl of the test solution and 10 µl of the reference solution. The chromatogram was developed over a path of 10 cm using a mixture of 10 volumes of ethyl acetate and 90 volumes of toluene. The plates were dried in air and developed again in the same conditions. The plate was sprayed with anisaldehyde solution and examine in daylight while heating at 100°C to 105°C for 5 min to 10 min. The chromatogram obtained with the test solution shows zones similar in position, colour and R<sub>f</sub> value to the zones in the chromatogram obtained with the reference solution. Several violet-grey to brownish zones, including the zone corresponding to geraniol, are between the starting point and the zone due to cyperol in the chromatogram obtained with the reference solution. It may also show several faint violet-grey zones between the zone due to triglycerides and that due to cyperol in the

chromatogram obtained with the reference solution.

**Determination of MIC and MBC:**

The following concentration of cyperus oil were prepared using nutrient broth, 2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40 g/dL. Tween-80 was added all assay at final concentration of 0.001% (v/v) to enhance oil solubility. The MIC was defined as the lowest concentration of cyperus oil preventing visible growth. The MBC was defined as the lowest concentration of cyperus oil resulting in death of 99.99% of the inoculation. All broth dilution tests were performed at least twice, if results varied tests were repeated and model selected.

**Disk diffusion method (9):** 0.1mL of  $10^8$  cell/mL of each microbe used in this experiments onto Nutrient agar except *Streptococcus pyogenes* onto Brain-Heart Infusion broth (BHI). The inocula were spreaded using glass spreader or sterile cotton swab. To study the effect of cyperus oil in microbes growth, we prepared filter paper disks (whattman no.1) saturated with different concentrations of cyperus oil by adding 0.1mL for each concentration to a container contains 10 sterilized disks, then the cultures inoculated at 37°C for (14-16) h. The following antibiotic disks Tobramycin, Cefalexin, Gentamycin and Ampicillin were utilized as a positive control to the microbes.

**Results and Discussion**

In this study, extraction procedure was conducted by steam distillation, the best method for extraction of the essential oil containing hydroxyl groups or other polar groups via formation oxonium extraction system, Optimum extractant was estimated depending on the quantity of cyperol that extracted

by the following solvents; ethanol, ethyl acetate, methanol and chloroform in the study we conclude that Methanol was the best extractant, Table (1).

**Oil composition:** The oil extracted by steam distillation from the *C. rotundus* was obtained in a yield of 72 % (w/w). Representing 98.6% of the total composition of the oil. The TLC analysis shows the following compounds as a major component in the oil based on standards shapes and  $R_f$  values as shown in Table (2).

The oil of *C. rotundus* was mainly composed of Cyperol (40%), sesquiterpene hydrocarbons (Caryophyllene) (30.5%), cyperene (30.9%), rotundine (7.6%) and cyperone (4.5%). The cyperus oil possesses an inhibitory effect against the growth of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *Streptococcus pyogenes* at the concentration of 40%, 35%, while at the concentration of 25%, 30% the inhibitory effect was observed against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. At the concentration of 20% and 15% only *Staphylococcus aureus* growth was inhibited. No inhibition effect was observed against all microorganisms at the concentration of 2.5%, 5%, 7.5%, 10%. The cyperus oil has no inhibitory effect on the growth of *Pseudomonas aeruginosa* and *Proteus vulgaris* at all concentrations (2.5, 5, 7.5, 10, 15, 20, 25, 30, 35, 40)%. The results were summarized in table (3).

The oil of *C. rotundus* shows a remarkable activity against the Gram-positive bacteria: *Staphylococcus aureus* and less important antibacterial activity were found against *Streptococcus pyogenes*. The results for

antibacterial activity tests were given in Table (3).

Less important antibacterial activity was found against the Gram-negative bacteria. Whereas, no activity was observed with the oil against *Pseudomonas aeruginosa* and *Proteus vulgaris*. Therefore, it seems that oil-containing hydrocarbon terpenoids are more active against Gram-positive than to Gram-negative (10).

In general, depending on the site of action, pharmaceutical studies of antimicrobial classified into, drug that inhibit cell wall synthesis, drug that inhibit nucleic acids synthesis, drugs that inhibit proteins synthesis and drugs that affect cytoplasmic membrane (11).

In general, Gram-negative bacteria

were found to be more resistant to the oil than Gram-positive bacteria, possibly because of their cell wall lipopolysaccharides. Although the oil tested displayed some antibacterial activities, the efficiency differed depending on the concentration of the oil as well as on the bacterial strain used. It is interesting to note that the oil showed antibacterial activity towards organisms of interest to the medical field such as *Staphylococcus* and *Enterococcus* (12).

The study of the biological activities of this oil is very important since it needs to determine whether there is any correlation between the biological activities and one or more of the chemical fractions purified from *C. rotundus* oil.

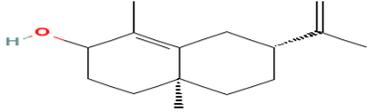
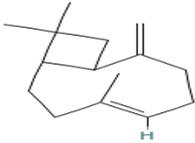
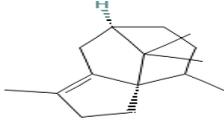
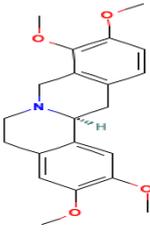
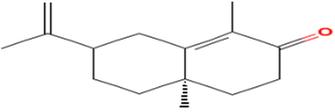
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**Table (1): Percent Extraction for Cyperus oil with various extractant (20 g of powdered fruits *C. rotundus*).**

Solvent (Extractant)	Extracted Cyperus oil (mL)	Extraction Percent%(v/w)
<i>Ethanol</i>	13.0	65
<i>Ethyl acetate</i>	07.4	37
<i>Methanol</i>	14.4	72
<i>Chloroform</i>	12.0	60

Table (2): TLC analysis results,  $R_f$  values and identified compounds.

<i>Compound</i>	<i>R<sub>f</sub>reference</i>	<i>R<sub>f</sub>test</i>	<i>MW(g/mol)</i>	<i>Structure</i>
<b>Cyperol</b>	0.842	0.846	220.35	
<b>Caryophyllene</b>	0.401	0.392	204.35	
<b>Cyperene</b>	0.329	0.312	204.35	
<b>Rotundine</b>	0.930	0.932	369.45	
<b>Cyperone</b>	0.812	0.804	218.33	

**Table (3): Antibacterial activity of cyperus oil depending on inhibition zones, MIC is 15% for *Staphylococcus aureus*, 25% for *E.coli*, 30% for *Klebsiella pneumoniae* and 35% for *Streptococcus pyogenes***

Microorganisms	40% <sup>(1)</sup>	35%	30%	25%	20%	15%	10%	7.5%	5%	2.5%
<i>S.aureus</i>	++++ <sup>(2)</sup>	+++	+++	++	+	+	R <sup>(3)</sup>	R	R	R
<i>S. pyogenes</i>	++	+	R	R	R	R	R	R	R	R
<i>K. pneumoniae</i>	+++	++	+	+	R	R	R	R	R	R
<i>E.coli</i>	+++	++	+	+	R	R	R	R	R	R
<i>P. aeruginosa</i>	R	R	R	R	R	R	R	R	R	R
<i>P. vulgaris</i>	R	R	R	R	R	R	R	R	R	R

(1): Concentration in g/dL(%), (2):Each (+) represents 1 millimeter, (3):(R) represents Resistant to oil activity