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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL OF *CYPERUS ROTUNDUS* L EXTRACTS

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ABSTRACT

In the present study rhizomes of *Cyperus rotundus* L. were successively extracted with petroleum ether, chloroform, ethyl acetate and methanol using continuous extraction method. Mark obtained was subjected to aqueous extraction and lyophilized using freeze drier apparatus. The extracts were investigated for their antibacterial and antifungal activity using cup –plate agar diffusion method. Antimicrobial activity carried out against six standard against organism of bacteria and two standard organisms of fungi. Methanolic and E. acetate extracts showed moderate activity against all organisms except *E, coli*, which was sensitive to the two concentrations used. Petroleum ether extract was inactive against all the tested organisms, while chloroform and water showed weak exhibition results. E. acetate extract phytochemical screening showed the presences of sterols, oils, flavonoids, saponin, coumarins and alkaloids.

INTRODUCTION

C. rotundus, (family Cyperaceae), also known as purple nutsedge or nutgrass, is a common perennial weed with sender, scaly creeping rhizomes, bulbous at the base and arising singly from the tubers which are about 1-3 cm long. The tubers are externally blackish in colour and reddish white inside, with a characteristic odor. The stems grow to about 25 cm tall and the leaves are linear, dark green and grooved on the upper surface. Inflorescences are small, with 2-4 bracts, consisting of tiny flowers with a red-brown husk. The nut is three angled, oblong-ovate, yellow in colour and black when ripe. (Auld and Medd: 1987).

C. rotundus is native to Africa, southern Asia, and southern and central Europe; further, it has been successfully naturalized in many tropical and subtropical regions of the world (Srivastava et al., 2013). A member of the Cyperaceae family, this is considered a noxious perennial plant that can grow up to 40-cm height (Khalida and Siddiqui, 2014). Its accepted name is *C. rotundus* L, and it is usually recognized with many vernacular names and synonyms. The plant has an extensive network of underground slandered and creepy rhizomes with a bulbous base, which arise from a single tuber of 1–3-cm length (Bryson et al, 2009). Tubers are usually oblong ovate in shape and blackish to brown in color, with an internally reddish white form and a specific fragrance (Oladipupo and Oyedeji, 2009). Its leaves are dark green in color appearing linearly with an upper surface having grooves and no ligules or auricles. Inflorescences usually have two to four bracts composed of small purple to red-brown husked flowers with three stamina and three stigma carpels attached to an unbranched, erect, triangular cross-sectioned, dark green, and glabrous culm of unequal length (Bryson, *et al*,, 2009). New subspecies of *C. rotundus* might even be discovered in tropical and subtropical areas worldwide.

Previous studies identified many chemical constituents such as alkaloids, cyperol, flavonoids, fatty oils, furochromones, glycerol, linolenic acid, myristic acid, nootkatone, starch, saponins, sesquiterpenes, sitosterol, stearic acid, terpenoids, polyphenol, and valencene in the tubers and rhizomes of *C. rotundus* (Sivapalan, 2013 and Sharma. *Et. al.* 2014). These chemicals are responsible for several therapeutic, pesticidal, fungicidal, and insecticidal properties of *C. rotundus*.

MATERIALS AND METHODS PLANT MATERIAL

Rhizomes of *C. rotundus* L. were collected from Kosti, White Nile Province, Central Sudan, identified by Dr. Hayder Adbalgader and herbarium sheet was deposit at the herbarium of Medicinal and Aromatic Plants Research Institute. (MAPRI).

METHODS

Preparation of Successive Extracts

100 g of the rhizomes powder was successively with petroleum ether, chloroform, ethyl acetate and methanol using Soxhlet Apparatus. Extraction was carried out for about 4 hours for petroleum ether, twelve hours for chloroform, and 8 hours for Ethyl acetate and twelve hours for methanol. Extracts were filtered through filter paper and evaporated using rotary evaporator apparatus. Yield percentage of each extract was calculated as follow.

Weight of extract X100/ weight of the crude powder plant.

Preparation of the Aqueous Extract

Mark reminded after successive extraction was dried under air and extracted by soaking in sufficient hot distilled water for about four hours with continuous steering. After cooled extracts were filtered using filter paper and lyophilized using freeze dryer till complete dryness.

In vitro testing of extracts for antimicrobial activity

Tested microorganisms

All microbial strains, 7 reference bacterial strains representing the gram negatives (*Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 53651, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* NCTC 8196, *Salmonella typhi* NCTC 0650) and gram positives (*Bacillus cereus* NCTC 8236, *Staphylococcus aureus* ATCC 25923), and two references and(*Aspergillus Niger* ATcc 9763) fungal strain (*Candida albicans* ATCC 7596) were used. Bacterial and fungal strains used in the study were obtained from the Department of Microbiology, of the Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI) and National Health Laboratory of Khartoum in Sudan. The bacterial cultures were maintained on nutrient agar and incubated at 37℃ for 18 h and then used for the antimicrobial test.

Testing for antibacterial Activity

The cup-plate agar diffusion method (Kavanagh, 1972) was adopted with some minor modifications to assess the antibacterial of the prepared extracts. One ml of the standardized bacterial stock suspension 108–109 C.F.U/ ml were thoroughly mixed with 100 ml of molten sterile nutrient agar which was maintained at 45 ºC. 20 ml aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agars was left to set and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. Alternate cups were filled with 0.1 ml sample of the oil using automatic microlitre pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours. Two replicates were carried out for the oil against each of the tested organisms. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were shown in table (4).

Testing for antifungal activity

The same method as for bacteria were adopted, instead of Nutirent agar, Sabouraud dextrose agar was used the inoculated medium was incubated at 25ºC for two days for *C. albicans* and three days for *A. niger*. After incubation the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were shown in table (4).

Antimicrobial activity of standard reference drugs

Four standard reference drugs (ciprofloxacin and gentamicin for antibacterial and nystatin and clotimazole for antifungal were used. Different concentrations were prepared by dissolving specific weight of each drug in specific volume of distilled water and tested against standard organism using the same method above. Results are shown in table (5).

Phytochemical Screening

General phytochemical screening for the active constituents was carried out for ethyl acetate fraction which showed the highest antimicrobial activity using the methods described by (Martinez & Valencia (1999), Sofowora (1993), and Harborne (1984) with many few modifications.

Test of Tannins

0.2 g of the fraction was dissolved in 10 ml of hot saline solution and divided in two tests tubes. To one tube 2-3 drops of ferric chloride added and to the other one 2 – 3 drops of gelatin salts reagent added. The occurrence of a blackish blue color in the first test tube and turbidity in the second one denotes the presence of tannins.

Test of Sterols and Triterpenes

0.2 g of the fraction was dissolved in 10 of chloroform. To 5 ml of the solution 0.5 ml acetic anhydride was added and then 3 drops of conc. Sulphuric acid at the bottom of the test tube. At the contact zone of the two liquids a The gradual appearance of green, blue pink to purple color was taken as an evidence of the presence of sterols (green to blue) and or triterpenses (pink to purple) in the sample.

Test for Alkaloids

0.5 g of the fraction was dissolved in 10 ml of 2N Hcl in water bath and stirred while heating for 10 minutes, cooled filtered and divided into two test tubes. To one test tube few drops of Mayer’s reagent was added while to the other tube few drops of Valser’s reagent was added.

A slight turbidity or heavy precipitate in either of the tow test tubes was tanked as presumptive evidence for the presence of alkaloids.

Tests for Flavonoids

0.5 g of the fraction was dissolved in 30 ml of 80% ethanol and filtered. The filtrate was used for following tests: -

A/ to 3 ml of the filtrate in a test tube 1ml of 1% aluminum chloride solution was in methanol was added. Formation of a yellow color indicated the presence of Flavonoids. Flavones or and chalcone.

B/ to 3ml of the filtrate in a test tube 1ml of 1% potassium hydroxide solution was added. A dark yellow color indicated the presence of Flavonoids compounds (flavones or flavonenes) chalcone and or flavonols.

C/ to 2ml of the filtrate 0.5ml of magnesium turnings were added. Producing of defiant color color to pink or red was taken as presumptive evidence that flavonenes were present in the plant sample.

Test for Saponins

0.3 g of the fraction was placed in a clean test tube. 10 ml of distilled water was added, the tube stoppered and vigorously shaken for about 30 seconds. The tube was then allowed to stand and observed for the formation of foam, which persisted for least an hour, was taken as evidence for presence of saponins.

Test for Cumarins

0.2 g of the fraction dissolved in 10 ml distilled water in test tube and filter paper attached to the test tube to be saturated with the vapor after a spot of 0.5N KoH put on it. Then the filter paper was inspected under UV light, the presence of coumrins was indicated if the spot has found to be adsorbed the UV light.

Test for Anthraquinone Glycoside

0.2 g of the fraction was boiled with 10 ml of 0.5N KoH containing 1ml of 3% hydrogen peroxide solution. The mixture was extracted by shaking with 10 ml of benzene. 5ml of the benzene solution was shacked with 3ml of 10% ammonium hydroxide solution and the two layers were allowed to separate. The presence of anthraquinones was indicated if the alkaline layer was found to have assumed pink or red color.

Test for Cyanogenic Glycoside

0.2 g of the fraction was placed in Erlenmeyer flask and sufficient amount of water was added to moisten the sample, followed by 1ml of chloroform (to enhance every activity). A piece of freshly prepared sodium picrate paper was carefully inserted between a split crock which was used to stopper the flask, a change in color of the sodium picrate paper from yellow to various shades of red was taken as an indication of the presence of cyanogenic glycoside.

RESULTS AND DISCUSSION

Table 1: Yield Percentages of *C. rotundus* extracts.

Solvent	Weight of extract (g)	Yield %	Colour	Condition
Petroleum Ether	5.23	5.23	Dark oily	Semi-solid
Chloroform	3.25	3.25	Dark green	Semi-solid
Ethyl acetate	2.56	2.56	Brown	Gummy
Methanol	23.11	23.11	Dark brown	Gummy
Water	17.87	17.87	Brown	Powder

Table 2: Inhibition zones of different extracts against standard organisium.

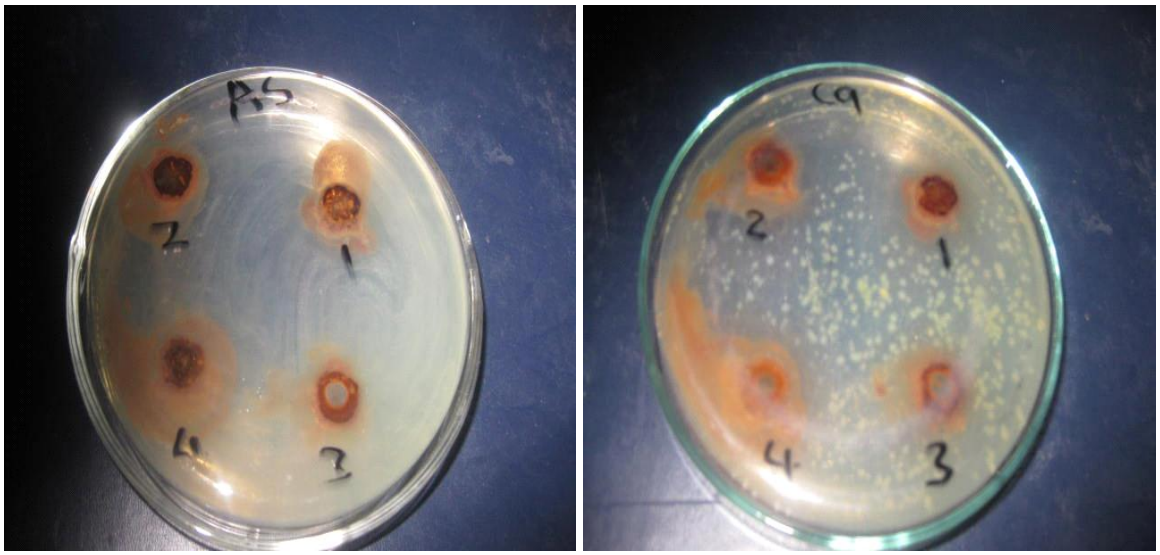
Microorganism	P.E		CHCL3		MeoH		E. acetate	
Conc	10%	20%	10%	20%	10%	20%	10%	20%
S. typhii	-	14	17	17	14	15	17	20
K.. pneumoniae	-	-	-	14	-	16	14	20
P. vulgaris	-	14	-	17	15	17	20	30
E. coli	-	-	14	15	-	15	14	20
S. aureus	-	-	14		16	16	20	25
P. aeruginosa	-	-	14	17	17	19	19	30
C. albicans	14	15	-	-	15	16	17	25
A. niger	14	16	14	15	14	14	14	15

Preliminary Phytochemical Screening

Table 3: Result OF Phytochemical Screening of different extracts.

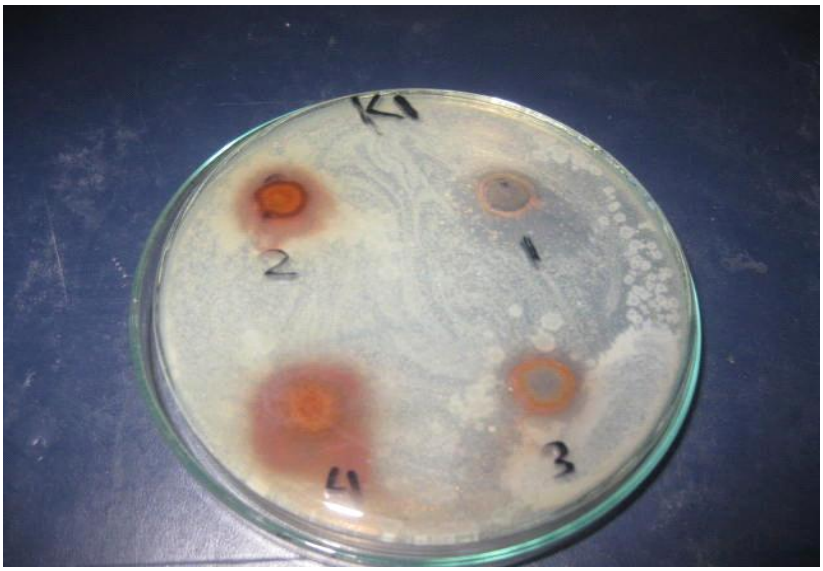
Extract Test	Petroleum ether	Chloroform	Methanol	Ethyl acetate	Water
Alkaloids	+	+	+	+	+
Sterols	-	+	-	+	+
Tritepenes	-	+	+++	+++	+
Flavonoids	-	+	++	+++	+
Saponins	+	+	+++	++	+
Cumarins	-	+	++	+++	+
Tannins	-	-	+	+	+
Anthraquenones	-	-	-	-	-
Cyanogenic	-	-	-	-	-

Key: +Trace, ++ Moderate, +++ High, - Negat Results showed that significant differences between yield percentages of all solvents used. The highest yield was of methanol (23.11%) followed by water (17.87%). Petroleum ether (5.23%), chloroform (3.25%) and the lowest yield was of ethyl acetate (2.56%.). Yield percentages of extract depends mainly on the amount of extractable matters contained by the plant sample and it clear that the content of the polar compounds are higher than non-polar.

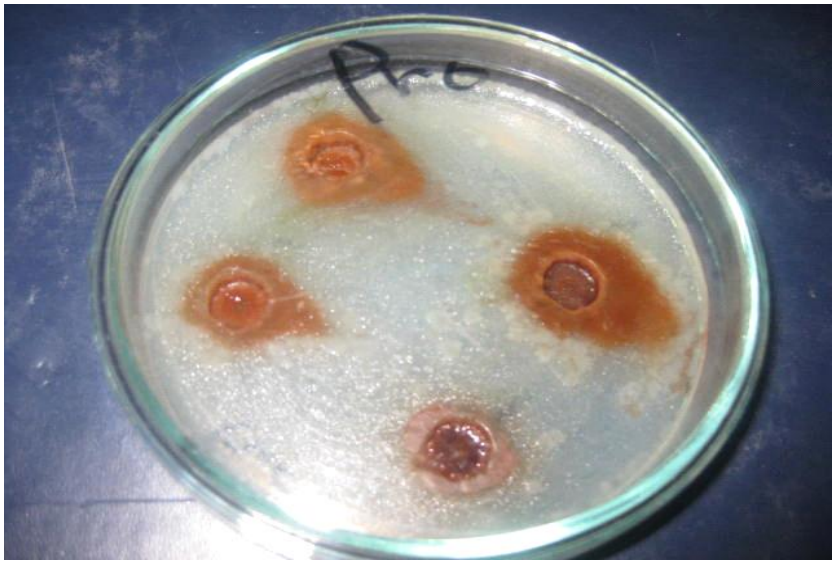


Inhibition zones (mm) of *C. rotundus*

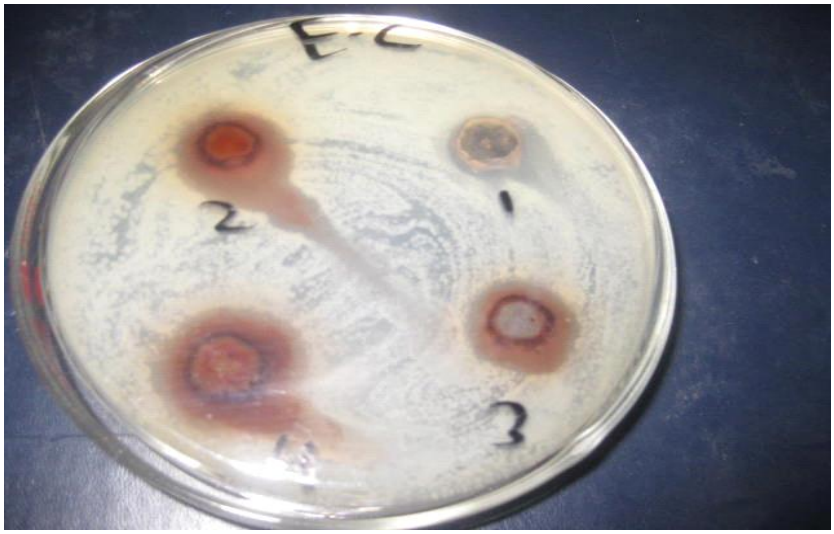
Extracts against *C.albican*



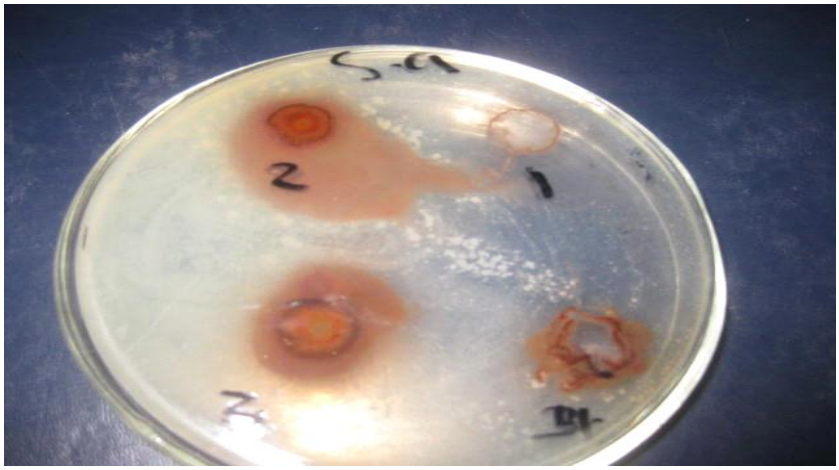
Inhibition zones (mm) of *C. Rotundus* four extraction against *Klebsiella pneumonia*



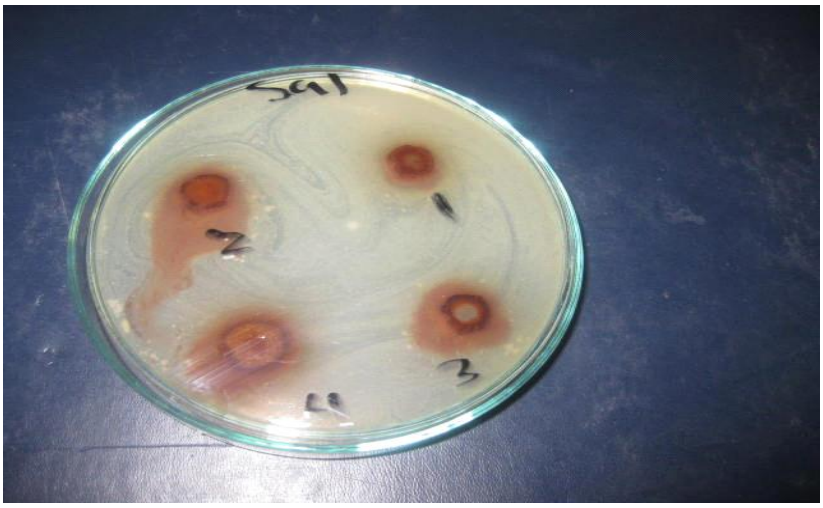
Inhibition zones (mm) of *C. Rotundus* four extraction against *Proteus vulgaris*



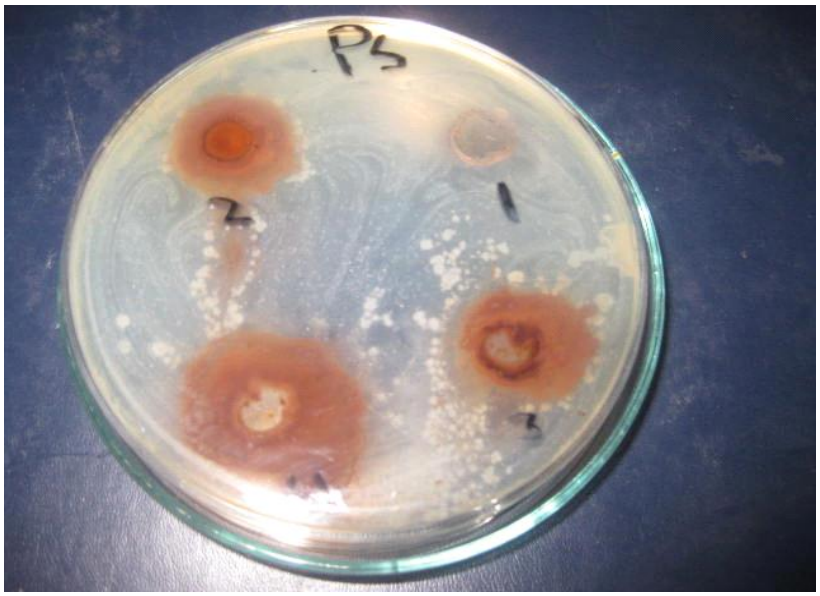
Inhibition zones (mm) of C. *Rotundus* four extraction against *E.coli*



Inhibition zones (mm) of C.*Rotundus* four extraction against *staphylococcus aureus.*



Inhibition zones (mm) of C. *Rotundus* four extraction against *Salomounila.*



Inhibition zones (mm) of C. *rotundus* four extraction against *P.seudomonas.*

Antimicrobial activity results showed that ethyl acetate extract was the most active one compared to the other extracts. It showed high activity against all standard organisms of bacteria and fungi used except against *A. niger.* Inhibition zones ranged between 20 to 30 mm for all organisms used when 20% concentration used and against *A. niger* it resulted 14 to 15 mm. At 10% concentration, inhibition zones ranged between 14 to 20 mm among all of the organisms used. Other extracts showed different activities against all organisms ranged between moderate, weak and inactive. Petroleum ether extract was the weakest one compared to the other extracts. It was inactive against most of the organisms used and showed moderate activity against *C. albicans* and *A. niger*, Inhibition zones ranged between 14 to 16 mm.

These results found to be on line with previous studies through around the word. Which indicated that different extracts of *C. routduns* have antimicrobial activity.

Results of phytohemial screening showed the presence of different type of secondary metabolites compounds among all extracts obtained. Alkaloids, triterpens, sterols, tannins, flavonoids, saponins and cumarins showed positives results while cyanogenic glycosides and anthraquenone glycosides were absent through all extracts. These results were in agreement with which, Previous studies identified many chemical constituents such as alkaloids, cyperol, flavonoids, fatty oils, furochromones, glycerol, linolenic acid, myristic acid, nootkatone, starch, saponins, sesquiterpenes, sitosterol, stearic acid, terpenoids, polyphenol, and valencene in the tubers and rhizomes of *C. rotundus* (Sivapalan, 2013 and Sharma. *et. al.* 2014). These chemicals are responsible for several therapeutic, pesticidal, fungicidal, and insecticidal properties of *C. rotundus*.

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