Global Journal of Research in
Life Sciences
Journal homepage: https://gsjournals.com/gjrls/
ISSN: 2980-4183 (Online)

(RESEARCH ARTICLE)

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Phytochemical analysis, antioxidant, and antibacterial activities of *Cyperus rotundus L.* rhizomes

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Global Journal of Research in Life Sciences, 2022, 01(01), 001–009

Publication history: Received on 14 August 2022; revised on 17 September 2022; accepted on 20 September 2022

Article DOI: https://doi.org/10.58175/gjrls.2022.1.1.0022

Abstract

Cyperus rotundus L. is a ubiquitous weed, commonly found in tropical, subtropical, and temperate regions around the world, belonging to the Cyperaceae family. This slender, erect, perennial sedge propagates through a fibrous root system, enabling it to thrive in diverse habitats. The rhizomes initially appear black, transitioning to a darker shade as they mature. This plant is a traditional medicinal herb renowned for treating stomach ailments, nausea, vomiting, intestinal parasites, food poisoning, indigestion, and bowel irritation. Phytochemical screening of the ethanolic crude extract of *C. rotundus L.* rhizomes was conducted, revealing the presence of flavonoids, polyphenols, terpenoids, tannins, steroids, carbohydrates, and fixed oils. The total phenolic content was measured at 51.48 ± 1.77 mg GAE/g, 159.63 ± 2.28 mg GAE/g, and 17.90 ± 2.64 mg GAE/g in the ethanolic crude extract, ethyl acetate, and hexane fractions, respectively. Similarly, the total flavonoid content in the ethanolic crude extract, ethyl acetate, and hexane fractions was determined as (21.00 ± 1.51), (118.07 ± 5.06), and (10.67 ± 2.53) mg GAE/g, respectively. The ethyl acetate fraction of *C. rotundus L.* rhizomes exhibited significant antioxidant activity with an IC_{50} value of 27.11 ± 1.46 µg/mL. Furthermore, the ethanolic crude extract of *C. rotundus* L. rhizomes displayed effective antibacterial activity against gram-positive bacteria *Staphylococcus aureus*, with a zone of inhibition (ZOI) of 8 mm.

Keywords: Antioxidant; Antibacterial; *Cyperus rotundus* L.; IC_{50}, *Staphylococcus aureus*

1 Introduction

Nature has been a source of medicinal agents for thousands of years and an inspiring number of modern drugs have been isolated from natural sources[1]. For an extensive duration, plants have served as a medicinal resource for addressing a diverse range of illnesses and survival of people from the commencement of human culture[2- 4]. Plants have emerged as significant contributors of innovative pharmacologically active substances, playing a pivotal role in the development of numerous successful drugs, either directly or indirectly sourced from botanical origins[4]. Currently, the demand for the herbal drug treatment of various ailments is increasing and plants are being explored globally for the development of newer drugs[5]. Plants offer a vast potential for a wide array of valuable natural compounds, including terpenoids, flavonoids, and alkaloids, which serve crucial ecological roles, safeguarding against pests, antioxidant, antibacterial, antifungal, antidiabetic, anti-inflammatory, UV-B damage, and environmental stressors, thus making them significant reservoirs for novel drug discovery[2,6–8].

With attributes conducive to easy cultivation, rapid reproduction, and robust adaptability to various challenging climatic conditions, *Cyperus rotundus* is widely distributed and belongs to the Cyperaceae family, comprising 109 genera and approximately 5500 species[9,10]. *Cyperus rotundus L.*, a cosmopolitan weed, is extensively distributed across tropical, subtropical, and temperate regions worldwide. This slender, erect, perennial sedge proliferates through a fibrous root system, enabling its growth in diverse habitats and environments. The tubers display a blackish external

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hue and a reddish-white interior, accompanied by a distinctive scent. The stem reaches a height of approximately 25 cm, while the leaves, linear in shape, are dark green and possess grooves on their upper surface[11, 12]. *Cyperus rotundus L.* is employed for its wide-ranging medicinal attributes, encompassing astringent, diaphoretic, analgesic, antispasmodic, carminative, antitussive, lithologic, sedative, stimulant, stomachic, tonic, and antibacterial effects. These biological activities are attributed to the presence of bioactive compounds such as glycosides, tannins, reducing sugars, alkaloids, flavonoids, polyphenols, terpenoids and saponins, [9, 13–15].

This plant has recently attracted a great deal of attention due to the variety of chemical compositions and a broad range of biological activities. The phytochemical studies of plants shows the presence of secondary metabolites such as Luteolin, Apigenin-6-C-hexoside-8-C-pentoside, 2,3-butanediol, 1,4-dioxane-2,6-dione, Lactic acid, Camphene, Limonene, 5-hydroxymethylfurfural[16,17,18]. These secondary metabolites play a crucial role in development of novel drugs. Based on the literature, our focus has been on evaluating the antioxidant, and antibacterial properties of *Cyperus rotundus* L. rhizomes.

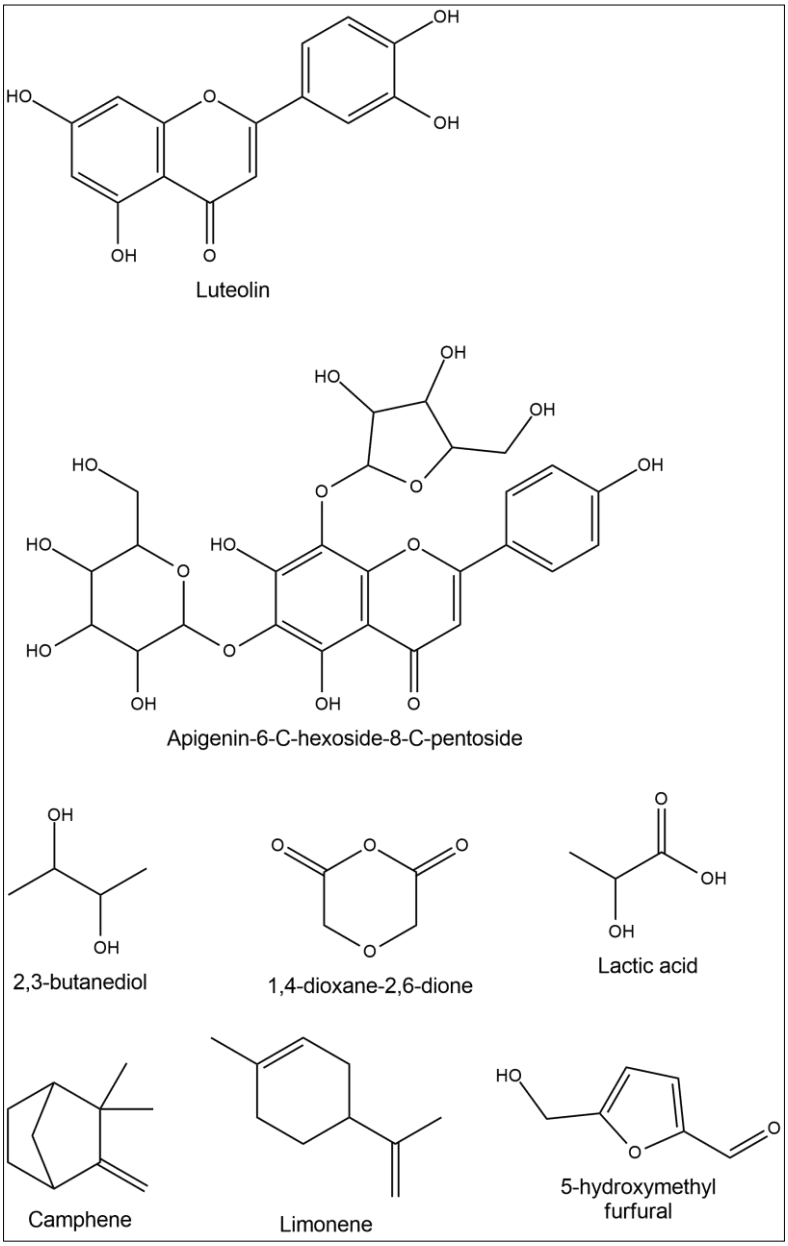

Figure 1 Chemical compound identified from *Cyperus rotundus* L.

2 Materials and Methods

2.1 Reagents and Chemicals

All chemicals and reagents used in research like, methanol (obtained from Fisher Scientific), acetone (from Fischer Scientific), DMSO (from Merck), hexane (from Merck), gallic acid, quercetin, ascorbic acid, and 1,1-diphenyl-2-picrylhydrazyl (purchased from Sigma Aldrich, St. Louis) were procured from a local supplier in Kathmandu, Nepal and were of analytical quality. Additionally, Folin–Ciocalteu phenol reagent, α-amylase enzyme were purchased from Sigma-Aldrich..

2.2 Collection and Identification of Plant Materials

The fresh rhizomes of *C. rotundus* were collected from Palpa District, Tansen. The plant was identified and authenticated as *Cyperus rotundus*L. by Professor Dr. Suresh Kumar Ghimire and a voucher specimen (**TUCH**-201023) was deposited in the Tribhuvan University Central Herbarium, Kirtipur, Kathmandu, Nepal.

2.3 Preparation of Plant Extract

The collected fresh rhizomes of *C. rotundus L.* were washed with tap water to remove the contaminants and dried under shade and ground to a fine powder and collected in airtight plastic bags until further use. The phytochemicals present in the powered rhizomes were extracted by the cold percolation method using ethanol as a solvent. Powered rhizomes (2 kg) of *C. rotundus* were kept in a clean and dry conical flask and 2 liters of dehydrated ethanol were added to the flask, kept for 7 days with frequent shaking, and obtained more than 3 times. The mixture was decanted and filtered with the help of a cotton plug and thus obtained filtrate was concentrated to crude form by Rotary Evaporator under reduced pressure at temperature 40 ℃. Then the crude extract was subjected to a hot plate to get the solid extract. The weight of solid extract was taken, collected in a glass bottle, labeled, and stored in a refrigerator until further use. The yield of different extracts was calculated by using the given formula:

$$\text{Percentage yield} = \frac{Wt.\ of\ the\ extract\ in\ g}{Wt.\ of\ the\ powered\ rhozomes\ in\ g} \times 100\%$$

2.4 Phytochemical Screening

The preliminary qualitative phytochemical screening of the rhizomes of *C. rotundus* L. was performed to identify the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, saponins, and steroids present in the crude extracts. The following chemical tests were carried out on the extracts by following standard procedures as previously described by Mbadianya *et aL.*, (2013)[19].

2.4.1 Determination of Total Phenol Content

The total phenol content of the extracts was measured using FC reagent by 96 well plate methods which were modified from the colorimetric method[20, 21]. At first 20 µL of different concentrations of standard 10, 20, 30, 40, 50, 60, 70, and 80 µg/mL gallic acid was loaded on 96 well. Then 20 µL of plant sample of 500 µg/mL was loaded on 96 well plates in triplicate. After that in each well-containing standard and sample 100 µL, FC reagent followed by 80 µL Na_2CO_3 was added separately. Then it was left in dark for 15 minutes absorbance was taken at 765 nm using Synergy LX Multi-Mode Reader with Gen5 3.08.01 software. Gallic acid was utilized to construct the standard curve, and the TPC was analyzed and it was subsequently expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of the extract..

2.4.2 Determination of Total Flavonoid Content

The total flavonoid content of the extracts was determined by the 96 well plate method which was modified from the colorimetric method[22]. Initially, 130 µL of various concentrations of quercetin standard (10, 20, 30, 40, 50, 60, 70, and 80 µg/mL) were loaded onto triplicate wells of a 96-well plate. Subsequently, 20 µL of a plant sample at a concentration of 500 µg/mL was loaded onto triplicate wells, followed by the addition of 110 µL of distilled water to each well containing the plant sample, resulting in a final volume of 130 µL. Next, 60 µL of ethanol, 5 µL of $AlCl_3$, and 5 µL of potassium acetate were individually added to each well containing the standard and plant sample. The plate was then kept in the dark for 30 minutes, and after this incubation period, absorbance readings were taken at 415 nm using a Synergy LX Multi-Mode Reader with Gen5 3.08.01 software.

2.4.3 Antioxidant Activity

The antioxidant activity of the extracts was determined by using the DPPH free radical scavenging assay[23, 24]. In the DPPH test, a positive control of quercetin at a concentration of 20 µg/mL and a negative control of 50% DMSO were employed. For each experimental set, 100 µL of the positive control quercetin, negative control DMSO, and the plant samples were loaded into separate wells of a 96-well plate in triplicate. Subsequently, 100 µL of DPPH reagent was added to each well, and the plate was incubated in darkness for 30 minutes. After this incubation period, absorbance readings were taken at 517 nm using a microplate reader. The capability to scavenge the DPPH radical was calculated by using the following equation:

$$\% \text{ Inhibition} = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Where A is the absorbance of the sample and control.

2.4.4 Antimicrobial Assay

The antibacterial activity of plant extract against American Type Culture Collection (ATCC) strains such as Gram-positive (*Staphylococcus aureus* ATCC 43300) and Gram-negative (*Escherichia coli* ATCC 2591, *Klebsiella pneumonia* ATCC 700603, and *Salmonella typhi* ATCC 14028) bacteria was determined by Agar well diffusion method as described previously with slight modifications[25].

The inoculum of the target microorganism was evenly spread across the entire agar surface using a sterile cotton swab saturated with its suspension. Following this, aseptic wells with a diameter of 6 mm were created in the agar medium using a sterile cork borer. These wells were then filled with 50 µL (50 mg/mL) of the plant extract. The plates were allowed to stand at room temperature for 2 hours to allow diffusion. For comparison, 50 µL of neomycin (1 mg/mL) and DMSO were respectively used as the positive and negative controls. Subsequently, the zone of inhibition was determined by measuring the diameter of growth on the plates after incubating them at 37 °C for 24 hours. The zones of inhibition were measured in millimeters using a ruler.

2.5 Statistical Analysis

All the experiments were performed in triplicates and data were presented in mean ± standard error of the mean. The TPC, TFC, and antioxidant assay were processed by using Gen5 Microplate Data Collection and Analysis Software and then by MS Excel 2016. The IC_{50} values value was calculated using GraphPad Prism software version 8. The structure of compounds were drawn by using Chem Draw Professional version 16.0.1.1.

3 Results

In order to assess the abundance of secondary metabolites within the plant, the percentage yield of the plant extract was computed. The yield percentage of the ethanolic extracts has been presented in **Table 1**.

The outcomes of the phytochemical analysis of the ethanolic extract of *C. rotundus L.* rhizome is detailed in **Table 2**. The analysis revealed the presence of flavonoids, terpenoids, tannins, carbohydrates, and phenols in the plant extract. The presence of secondary metabolites were confirmed by colour changes. The presence of metabolites was indicated by the use of a '+' sign, while '-' sign was used to indicate their absence.

The quantities of phenolic and flavonoid contents were assessed using a gallic acid calibration curve for TPC and a quercetin calibration curve for TFC. For TPC, the ethyl acetate fraction exhibited the highest content at 159.63 ± 2.28 mg GAE/g, followed by the crude ethanolic extract at 51.48 ± 1.77 mg GAE/g, and the hexane fraction at 17.90 ± 2.64 mg GAE/g.

In terms of TFC, the ethyl acetate fraction displayed the highest value of 118.07 ± 2.921 mg QE/g, followed by the crude ethanolic extract at 21.00 ± 0.872 mg QE/g, and the hexane fraction at 10.67 ± 1.461 mg QE/g. The TPC and TFC data is outlined in **Table 3**, while the gallic acid curve and quercetin curve is visualized in **Figure 2**.

The total antioxidant activity of the examined plant fractions was quantified using IC_{50} and compared to quercetin. Among the studied fractions, the ethyl acetate fraction of *C. rotundus L.* demonstrated potent antioxidant activity with an IC_{50} value of 27.11 ± 1.46 µg/mL. In comparison, with the IC_{50} value of quercetin, which stood at 13.41 ± 1.94 µg/mL. The results of radical scavenging assays are shown in **Table 3**.

The crude ethanolic extract of *C. rotundus L.* exhibited a zone of inhibition against *Staphylococcus aureus* ATCC 43300 with a diameter of 8 mm. The results of antibacterial activities is shown in **Table 4**.

Table 1 Presents the percentage yield of the ethanolic crude extract of *C. rotundus L.*

Scientific name of the plant	% Yield of ethanolic extract
Cyperus rotundus L.	9.28

Table 2 Phytochemical Screening Results of Ethanolic Crude Extract of *C. rotundus* L.

S. N.	Phytochemicals	Colours	Ethanolic crude extract
1	Alkaloid	Reddish-brown	-
2	Flavonoid	Orange	+
3	Saponin	Light maroon	-
4	Terpenoid	Reddish grey	+
5	Tannin	Blue-black	+
6	Glycosides	Red	-
7	Carbohydrate	Deep violet	+
8	Phenol	Greenish blue	+
9	Steroid	Green	+
10	Fixed oil	-	+

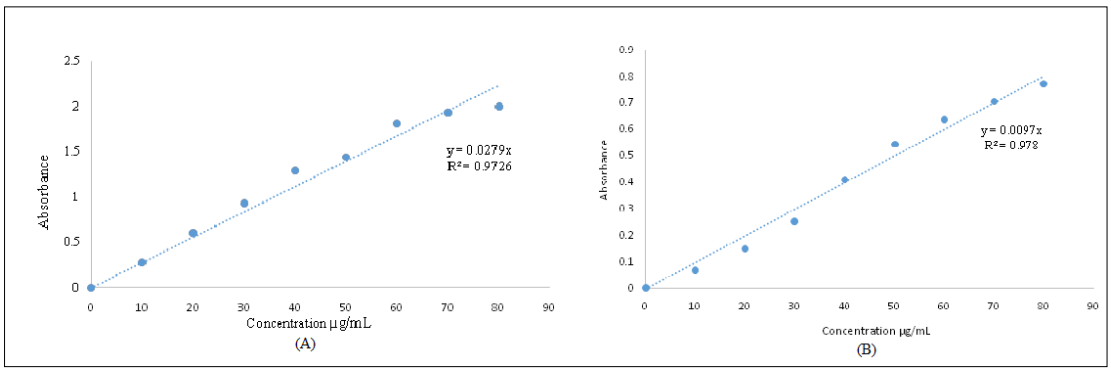

Figure 2(A) Calibration curve of standard gallic acid and (B) Calibration curve of standard quercetin

Table 3 Total Phenolic Content and flavonoid Content of *C. rotundus* L. and : IC_{50} (µg/mL) Values of Different Fractions of Plant Extracts against DPPH

Extract's name	TPC (mg GAE/g)	TFC (mg QE/g)	IC_{50}(µg/mL)
*C. rotundus*L. ethanol extract	51.48 ± 1.022	21.00 ± 0.872	366.85 ± 8.9409
*C. rotundus*L. ethyl acetate fraction	159.63 ± 1.316	118.07 ± 2.921	27.11 ± 1.46
*C. rotundus*L. hexane fraction	17.90 ± 1.524	10.67 ± 1.461	262.63± 9.23
Quercetin (standard)	-	-	13.41 ± 1.94

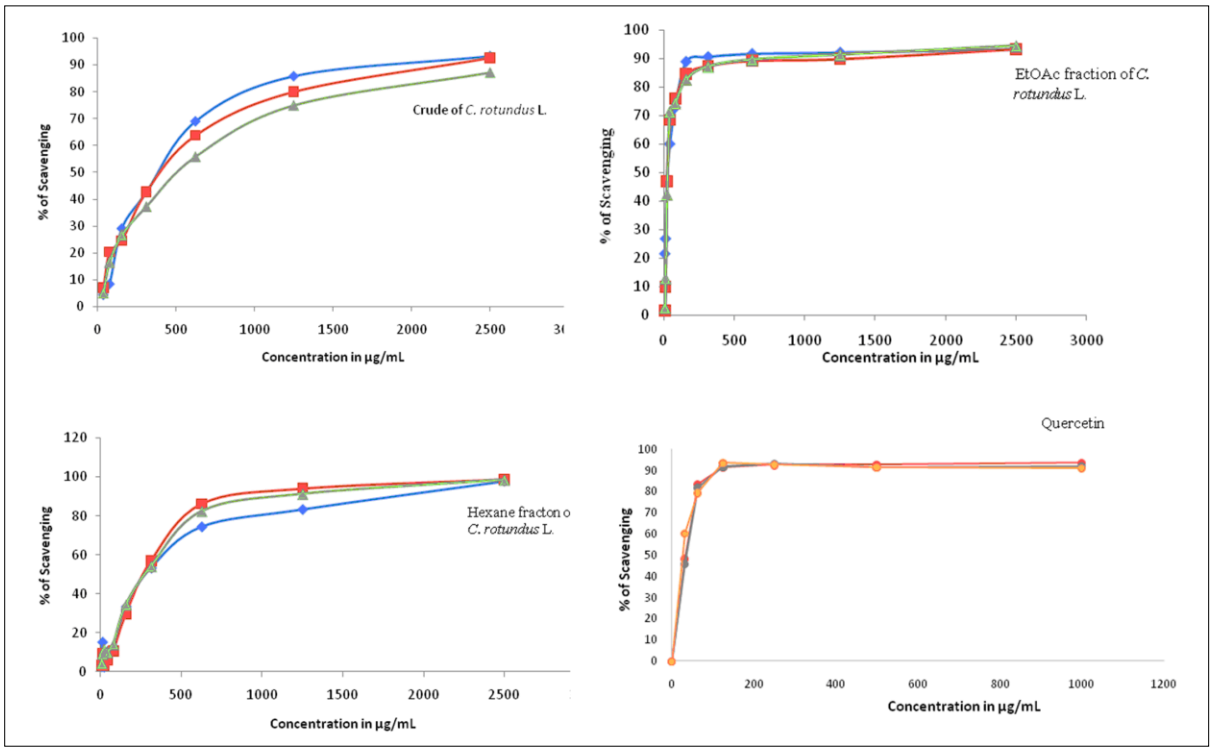

Figure 3 DPPH scavenging activity of different sample

Table 4 Zone of inhibition of crude extract of *C. rotundus L.* against tested microorganism

S. N.	Name of micro-organism	ZoI Value(mm)		
		Crude ethanolic extract	Positive control	Negative control
1	*Staphylococcus aureus* ATCC 43300	8 mm	10 mm	-
2	*Escherichia coli* ATCC 2591	-	18 mm	-
3	*Klebsiella pneumonia* ATCC 700603	-	19 mm	-
4	*Salmonella typhi* ATCC 14028	-	17 mm	-

4 Discussion

The utilization of medicinal plants for their pharmacological attributes is growing worldwide, with over 25% of prescription drugs derived from plant sources according to the World Health Organization[26]. This study attempted to investigate the potential source of antioxidant and antibacterial agents from the *C. rotundus* L..

The phytochemical analysis of crude ethanolic extracts from *C. rotundus* L. rhizomes revealed the presence of flavonoids, polyphenols, terpenoids, tannins, steroids, carbohydrates, and fixed oils, underscoring the richness of phytoconstituents in the selected plant. Previous research on the same plants revealed the presence of various groups of secondary metabolites, including alkaloids, saponins in methanol extract[27,16].

Phenolics and flavonoids, prominent secondary metabolites found predominantly in fruits and plants are often attributed to diverse pharmacological effects[28]. Hence, in this study total phenolics and flavonoids content were evaluated in the ethanolic extract and its derived fractions. The total phenolics content in the ethyl acetate fraction of *C. rotundus* L. is relatively high as compared to that in ethanolic crude extract and hexane fraction. The amount of phenolics

in the ethyl acetate fraction of *C. rotundus* L. is 159.63 ± 1.316 mg GAE/g sample whereas, ethanolic crude extract and hexane fraction contain 51.48 ± 1.022 and 17.90 ± 1.524 mg GAE/g respectively. This might be one of the major factors for ethyl acetate fraction of rhizomes of *C. rotundus* L. exhibiting strong antioxidant. In the previous study, the ethyl acetate fraction exhibited a TPC value of 567.35 ± 7.89 mg GAE/g, while the 90% methanol extract demonstrated a TPC value of 174.66 ± 2.35 mg GAE/g[16]. This study also demonstrates that the plant contains a substantial amount of phenolic content.

Similarly, total flavonoid present in the ethyl acetate fraction of *C. rotundus* L. is 118.07± 2.921 mg QE/g sample whereas, ethanolic crude extract and hexane fraction have21.00 ± 0.872 and 10.67 ± 1.461 mg QE/g sample respectively. However, from the recent research the 90% methanol extract exhibited a TFC value of 121.71 ± 1.67 mg RE/g, while the ethyl acetate fraction demonstrated a TFC value of 316.32 ± 2.59 mg RE/g[16]. These results shed light on the flavonoid composition of *C. rotundus* L. offer insights into their potential pharmacological significance.

The antioxidant activities were measured by measuring the DPPH free radical scavenging assays. The IC_{50} value for DPPH inhibition of ethanolic crude extract of rhizomes of *C. rotundus* L. was 366.85 ± 8.94 µg/mL. The IC_{50}values of different fractions of *C. rotundus* L. exhibited that the ethyl acetate fraction was the most potent natural antioxidant among all extracts with IC_{50}value 27.11 ± 1.46 µg/mL. The IC_{50} values obtained for the hexane fraction was 262.63 ± 9.23µg/mL, are termed as the poor antioxidant. Higher amounts of phenolic and flavonoid could be responsible for the highest antioxidant potential of *C. rotundus* L[29]. The antioxidant compounds contained in plant extracts have several functions and their activity and mechanism of action strongly depend on their composition and environmental conditions[30].

In the evaluation of the antibacterial activity of the fractions of ethanolic extract of rhizomes, *C.rotundus* L. showed significant inhibitory activity against the growth of *Staphylococcus aureus* among the number of tested bacteria. However, prior reports have indicated that diverse extracts yielded promising outcomes against *Escherichia coli* and *Klebsiella pneumonia*. This variation in biological activities could potentially be attributed to factors such as the timing of harvest, origin, soil conditions, and altitude[11, 31, 32].

5 Conclusion

The findings from this investigation unveiled the antioxidative and antibacterial capabilities inherent in the examined medicinal plants, *Cyperus rotundus* L. Moreover, *Cyperus rotundus* L. holds promise as a natural reservoir of antioxidants. Expanding upon the purification and extraction of bioactive compounds from these plant extracts, particularly the ethyl acetate fraction, may offer potential avenues for the formulation of novel pharmaceuticals.

Compliance with ethical standards

Acknowledgments

We acknowledge the Central Department of Chemistry, Tribhuvan University, Nepal, for graciously providing laboratory facilities, and extend our gratitude to Professor Dr. Suresh Kumar Ghimire for his expertise and contribution in plant identification.

Disclosure of conflict of interest

The authors declare to have no conflict of interest.

Availability of data and materials

All data collected in the study has been presented and visualized in the manuscript.

Statement of informed consent

No personal data are published.

References

[1] Srivastava PK. Achyranthesaspera: A potent immunostimulating plant for traditional medicine. Int J Pharm Sci Res 2014; 5: 1601–1611.

[2] Twilley D, Lall N. The Role of Natural Products From Plants in the Development of Anticancer Agents. In: Natural Products and Drug Discovery. Elsevier, pp. 139–178.

[3] Devanesan AA, Zipora T, G. Smilin BA, et al. Phytochemical and pharmacological status of indigenous medicinal plant Pedalium murex L.—A review. Biomed Pharmacother 2018; 103: 1456–1463.

[4] Veeresham C. Natural products derived from plants as a source of drugs. J Adv Pharm Technol Res 2012; 3: 200–201.

[5] Ogbole OO, Segun PA, Fasinu PS. Antimicrobial and antiprotozoal activities of twenty-four Nigerian medicinal plant extracts. South Afr J Bot 2018; 117: 240–246.

[6] Sheel DrR, Nisha K, Kumar P. Preliminary Phytochemical Screening of Methanolic Extract Of Clerodendron infortunatum. IOSR J Appl Chem 2014; 7: 10–13.

[7] Stanković N, Mihajilov-Krstev T, Zlatković B, et al. Antibacterial and Antioxidant Activity of Traditional Medicinal Plants from the Balkan Peninsula. NJAS - Wagening J Life Sci 2016; 78: 21–28.

[8] Plant Natural Products. OpenPlant, https://www.openplant.org/plant-natural-products (accessed 13 August 2023).

[9] Ngoc QN, Minh TN. Cyperus rotundus cyperaceae: a study of phytochemistry, total polyphenol content, flavonoid content, and antioxidant activity. E3S Web Conf 2021; 332: 06003.

[10] Mohamed AI, Beseni BK, Msomi NZ, et al. The antioxidant and antidiabetic potentials of polyphenolic-rich extracts of Cyperus rotundus (Linn.). J Biomol Struct Dyn 2022; 40: 12075–12087.

[11] R A, A R, S I, et al. Anti-oxidant, Anti-microbial Evaluation and GC-MS Analysis of Cyperus rotundus L. Rhizomes Chloroform Fraction. Am J Ethnomedicine; 2, (2015, accessed 4 March 2021).

[12] Al-Snafi AE. Medicinal plants with cardiovascular effects (part 2) : plant based review Prof Dr. 2016. Epub ahead of print 2016. DOI: 10.9790/3013-067034362.

[13] Singh N, Pandey BR, Verma P, et al. Phyto-pharmacotherapeutics of Cyperus rotundus Linn. (Motha): Phyto-pharmacotherapeutics-of-Cyperus-rotundus (2012, accessed 13 August 2023).

[14] Al-Awar M, Alqabbani T. Anti-diabetic, Antioxidants, Anti-hepatorenaltoxicity Activities of Cyperus rotundus Rhizome Extract in Alloxan-induced Diabetic Rats. Epub ahead of print 10 December 2021. DOI: 10.20944/preprints202112.0179.v1.

[15] Sivapalan SRS. Medicinal uses and Pharmacological activities of Cyperus rotundus Linn – A Review. Int J Sci Res Publ Vol 3 Issue 5 May 2013 ISSN 2250-3153 2013; 3: 2250–3153.

[16] El-Wakil ES, Shaker S, Aboushousha T, et al. In vitro and in vivo anthelmintic and chemical studies of Cyperus rotundus L. extracts. BMC Complement Med Ther 2023; 23: 1–17.

[17] R A, A R, S I, et al. Anti-oxidant, Anti-microbial Evaluation and GC-MS Analysis of Cyperus rotundus L. Rhizomes Chloroform Fraction. Am J Ethnomedicine; 2015, accessed 14 August 2023).

[18] Abo-Altemen RA, Al-Shammari AM, Shawkat MS. GC-MS Analysis and Chemical Composition identification of Cyperus rotundus L. from Iraq. Energy Procedia 2019; 157: 1462–1474.

[19] Mbadianya JI, Echezona BC, Ugwuoke KI. Phytochemical Constituents of some Medicinal Plants. 2013; 2: 5.

[20] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc 2007; 2: 875–877.

[21] Lu X, Wang J, Al-Qadiri HM, et al. Determination of total phenolic content and antioxidant capacity of onion (Allium cepa) and shallot (Allium oschaninii) using infrared spectroscopy. Food Chem 2011; 129: 637–644.

[22] Chang C-C, Yang M-H, Wen H-M, et al. Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal; 10. Epub ahead of print 14 July 2020. DOI: 10.38212/2224-6614.2748.

[23] Sabudak T, Demirkiran O, Ozturk M, et al. Phenolic compounds from Trifolium echinatum Bieb. and investigation of their tyrosinase inhibitory and antioxidant activities. Phytochemistry 2013; 96: 305–311.

[24] Subedi A, Amatya MP, Shrestha TM, et al. Antioxidant And Antibacterial Activity Of Methanolic Extract Of Machilus Odoratissima. Kathmandu Univ J Sci Eng Technol 2012; 8: 73–80.

[25] Tagg JR, McGiven AR. Assay System for Bacteriocins. Appl Microbiol 1971; 21: 943.

[26] Ndezo Bisso B, Njikang Epie Nkwelle R, Tchuenguem Tchuenteu R, et al. Phytochemical Screening, Antioxidant, and Antimicrobial Activities of Seven Underinvestigated Medicinal Plants against Microbial Pathogens. Adv Pharmacol Pharm Sci 2022; 2022: e1998808.

[27] El-Wakil E, Morsi E, Abdel-Hady H. PHYTOCHEMICAL SCREENING, ANTIMICROBIAL EVALUATION AND GC-MS ANALYSIS OF CYPERUS ROTUNDUS. WORLD J Pharm Pharm Sci 2019; 8: 129–139.

[28] Guo H, Saravanakumar K, Wang M-H. Total phenolic, flavonoid contents and free radical scavenging capacity of extracts from tubers of Stachys affinis. Biocatal Agric Biotechnol 2018; 15: 235–239.

[29] Kamala A, Middha SK, Gopinath C, et al. In vitro Antioxidant Potentials of Cyperus rotundus L. Rhizome Extracts and Their Phytochemical Analysis. Pharmacogn Mag 2018; 14: 261–267.

[30] Zargoosh Z, Ghavam M, Bacchetta G, et al. Effects of ecological factors on the antioxidant potential and total phenol content of Scrophularia striata Boiss. Sci Rep 2019; 9: 16021.

[31] Karzan K, Shnawa B, Gorony S. Antimicrobial Activity of Cyperus rotundus Linn. Extracts and Phytochemical Screening. Eurasian J Sci Eng; 3. Epub ahead of print 2017. DOI: 10.23918/eajse.v3i2p82.

[32] El-Wakil E, Morsi E, Abdel Hady H. Phytochemical Screening, Antimicrobial Evaluation And GC-MS Analysis Of *Cyperus Rotundus*. *WORLD J Pharm Pharm Sci* 2019; 8: 129–139.