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ELUCIDATING THE PHYTOCHEMICAL COMPOSITION AND BIOLOGICAL PROPERTIES OF *CYPERUS FLAVESCENS*: A COMPREHENSIVE IN VITRO ANALYSIS

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Abstract

Despite the huge importance of allopathic drugs, the origin of drugs was basically from medicinal plants that can't be denied. In current research, one medicinal plant *Cyperus flavescens* L. was collected from Quaid-i-Azam University, Islamabad and preserved for evaluation and biological profiling of the plant. Qualitative evaluation depicted the presence of 16 different biologically active compounds. Methanolic and chloroform extracts of whole parts of the plant was tested in vitro for its phenolic content, flavonoid content, cytotoxic, antioxidant, and antibacterial assays. The quantitative evaluation displayed the maximum phenolic content (53.90 ± 0.01 GAE mg/g), and maximum flavonoid content (42.23 ± 0.07 QE mg/g) in methanolic extract of *C. flavescens.* Furthermore, *C. flavescens* in methanolic extract shown the maximum DPPH scavenging capacity (52.72 ± 0.3 µg/mL), the maximum antioxidant capacity (164.32 ± 0.37 µg/mL), and maximum total reducing power was (238.93 ± 0.61 µg/mL). Moreover, the anti-bacterial study revealed that the maximum inhibition was observed in *C. flavescens* (4 ± 0.4 mm) again *S. aureus* (Gram-positive), while the maximum ZOI against *R. jostii* (Gram-positive) in *C. flavescens* (3 ± 0.5 mm), and maximum ZOI against *S. saprophyticus* (Gram-positive) in *C. flavescens* (3 ± 0.8 mm). Highest cytotoxicity potential depicted in *C. flavescens* in methanolic extract with LC_{50} (136.98 ± 0.34 µg/mL), and analysis of functional groups including C-H rock, C-N stretch, C-H wag, C-H bends, and C-Br stretch in the plant extracts was done using Fourier transform infrared spectroscopy. Further, pronounced activities are also recommended for the discovery of new drugs.

Keywords: Anti-bacterial activity, biological screening, *Cyperus flavescens,* cytotoxicity, Fourier transform infrared spectroscopy, in vitro analysis, phytochemical composition, secondary metabolites

INTRODUCTION

The significance of medicinal plants is becoming more widely recognized, and the kingdom of plants has a vast array of potential cures. Medication made from plants is often available, inexpensive, safe, effective, and seldom has side effects. When looking at the present hunt for novel, therapeutically effective medications, including anticancer treatments, the most apparent option is to look at the plants that have been chosen for medical usage over thousands of years (Dewick, 2002). The World Health Organization has reported that over 80% of the world's population primarily receives their basic medical care from herbal medicines (Ortholand and Ganesan, 2004).

Certain organic components found in medicinal plants, such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids, have defined physiological effects on the human body. Medicinal herbs are natural, non-addictive, and

have no negative side effects. Higher plant natural products could provide a fresh source of antibacterial agents with potentially a different mode of action (Mudasar *et al.,* 2016). One of the most diversified floras in the world is formed by Pakistan's distinct geographic, climatic, and vegetative traits, which vary along latitudinal and longitudinal gradients. Pakistan's economy is centered on agriculture. Furthermore, a vast range of floral diversity exists across the nation because of diverse climatic and biogeographical circumstances. A larger portion of the population depends on plants for their economic, medical, and nutritional needs. These medicinal plants are utilized in a range of formulations, including extracts, decoctions, syrups, poultices, lozenges, and elixirs, because basic medical facilities are unavailable in remote places.

Consequently, these plants constitute a vital part of the nation's healthcare system. In current research, one plant is collected based on its medicinal importance of family and genus along with the consideration of local use of *Cyperus flavescens* plant by native people. This plant is collected from Quaid-I-Azam University, Islamabad. The selected plant sample is tested for evaluating phytochemical screening and its biological activities. It is commonly referred to as yellow flatsedge, found in various regions worldwide. *Cyperus flavescens* L. (= *Pycreus flavescens* L.) is a species with a broad cosmopolitan range (Marciniuk *et al.,* 2020). It found in suitable wetland habitat in various region of Pakistan. According to Govaerts *et al,* (2021), sedges, or Cyperaceae, are the third-largest monocot family with over 5600 species. Cyperus is the biggest genus in the family Cyperaceae in the tropics and subtropics. About 700 species are included in Cyperus. This genus has numerous species with grass-like appearance and adaptability to wet environments. The genus grows in a wide range of habitats, especially in wetlands, marshes, riverbanks and paddy fields in both tropical and temperate climates.

Cyperus flavescens was traditionally use in Oaxaca and Santa María Tecomavaca region for treatment of depression, extracted from roots (Guzmán Gutiérrez *et al.,* 2014). The medicinal properties include in treatment of fever and gastrointestinal disorders. The essential oils, flavonoids, and tannins of plants have great contribution in inflammation, gastrointestinal discomfort as well as therapeutic potential. It is distributed across several countries, especially in Southern and central Europe, North America, Central and South America, Asia in various regions, including Pakistan, China, Japan, and Southeast Asia, Africa, and Australia. In Pakistan, it widely distributed in wetland marches, paddy fields, and along riverbanks. The appearance of *C. flavescens* is smooth and slender texture with perennial herbaceous plants.

It typically grows up to 30 cm tall with triangular shape of stem in cross-section. The green color leaves consist of basal and alternate shape which are narrow, linear and grass-like in shape. The type of inflorescence compound umbel. The flowers are typically ovate and yellowish-brown, 3 stamens and single pistil with 2-3 branched style. The flowers are small and inconspicuous, with no petals. They are wind pollinated. The fruit shape is elliptical to obovoid, small, 1-1.5 mm long with smooth and brown surface. The root system is fibrous and extensive with short rhizomes, helping the plant to spread and stabilize the soil. The seeds are achenes,

which are small, 1-1.5 mm long, dark brown, dry, one-seeded fruit that do not open at maturity (Figure 1). The current research was conducted to analysis the medicinal potential of *Cyperus flavescens.* The aims and objectives of this study include the qualitative and quantitative analysis of phytochemical compounds, biological screening including antioxidant and antibacterial activities, evaluation of cytotoxicity potential, and functional groups identification of selected medicinal plant using FTIR (Fourier Transform Infrared Spectroscopy) from selected plants.

MATERIAL AND METHODS

Collection of Plants and Preparation of Crude Extract: The present research includes selection of one plant, namely *Cyperus flavescens* from different areas of Quaid-i-Azam (QAU), Islamabad. The plant was identified from Prof. Dr Zafar Iqbal, through QAU Herbarium and Flora of Pakistan. The plant samples were rinsed with tap water and then with de-ionized water. It was dried, chopped, crushed and powdered with electrical grinder. To prepare crude extract, two solvents were used: polar methanol and non-polar chloroform (Guha *et al.,* 2011 & Harborne, 1998). A weighing mechanic was used to measure 30g of plant powder, which was then dissolved in 300 mL of solvents. The resulting blend was then shaken for 10 minutes at 200 rpm on an incubator shaker. At room temperature, the extract was then left for a further seven days. The plant extract was sieved utilizing Whatman filter paper #1, after seven days. The extract was poured into petri plates and allowed to evaporate in the fume hood chamber for 2 days. The dried filtrate was scratched with the aid of a spatula. The extract was transferred to Eppendorf tubes and kept at 4 °C for further procedures.

Phytochemical Compound Screening: Various tests were used to investigate phytochemical compound found in plant extract as both quantitative and qualitative test were carried out.

Qualitative Screening: 2mg of methanol and chloroform plant extract was screened as part of the current investigation to look for the occurrence of several phytochemicals like Phenols, Terpenoids, Saponins, Carbohydrates, Protein (Yadav and Agarwala, 2011), Quinone, Tannins, Cardiac Glycosides, Anthocyanin and Beta Cyanin (Roghini and Vijayalakshmi, 2018), Flavonoids (Wadood *et al.,* 2013), Alkaloids (Devika and Koilpillai, 2012), Steroids (Sonam *et al.,* 2017), Phlobatannins (Rauf *et al.,* 2013), Coumarins (Vishwakarma *et al.,* 2016), Fats and Oil (Roopalatha and Nair, 2013) and Phytosterol (Shaik *et al.,* 2020).

Quantitative Screening: Following protocols were used to estimate the total flavonoids and phenolic content.

Determination of Total Phenolic Content: 1 mL of dimethyl sulfoxide was poured in 1 mg of plant extract to make the stock solution. The concentration of gallic acid used is 400 µg/mL. The following mixes were let sit at room temperature for 60 minutes. The optical density was observed at 630 nm (Chandra *et al.,* 2014).

Determination of Total flavonoid Content: The stock solution for the plant samples was made by combining one milligram of each extract with one milliliter of Dimethyl sulfoxide (DMSO). In the following process, quercetin was used as a standard at a concentration of 100 µg/mL. The resulting mixture was then incubated at 37 °C for a minimum of 30 minutes. Using a microplate reader, optical

density was measured at 405 nm following incubation (Guglani *et al.,* 2020).

Antioxidant assays: It includes 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, total antioxidant capacity, total reducing power.

DPPH Radical Scavenging Assay: Methanol was used as a solvent to prepare DPPH solution (0.004%). The standard was ascorbic acid, while the negative control was DMSO. For 30 minutes, the mixes were incubated at 25 °C without any light. The testing models' absorbance was set at 517 nm using a micro-plate reader (Din *et al., 2*013).

Total Antioxidant Capacity (TAC): 4 mg plant sample was liquefied in 1 mL of DMSO to create the sample's stock solution. The test was run using dilutions ranging from 31.25 µg/mL to 500 µg/mL. The TAC reagent was prepared. After that, the mixture was heated to 95 °C for 90 minutes. The absorbance of each plant sample was determined at 630 nm using a microplate reader (Sun and Sun, 2022).

Total Reducing Power Assay: 1mL of plant extract and one milliliter of DMSO were mixed. From 31.25 µg/mL to 500 µg/mL, dilutions were used to perform the test. Gallic acid was taken as positive control and DMSO as a negative control. The mixture was centrifuged for five minutes at 3,000 rpm, (Ghoora *et al.,* 2020).

Antibacterial assay: Each plant extract (20 mg) was dissolved in 1 mL of DMSO for the preparation of the stock solution in different dilutions. Oxytetracycline was utilized as positive control, and Methanol was negative control. Three cultures were used (*Staphylococcus aureus, Rhodococcus jostii, and Styphylococcus saprophyticus)*. Using absorbance detection mode, colonies were constantly maintained at an optical density of 0.5 at 600 nm. Subsequently, the petri plates were filled with autoclaved medium and allowed to settle (Baskaran *et al.,* 2018).

Brine shrimps' cytotoxicity assay: To create the stock solution, 50 mg/mL of the substance was diluted in mL of methanol. Serial dilution of the extracts was performed (31.25 µg/mL to 1000 µg/mL). To determine the cytotoxic capability of plant extracts, protocol proposed by Krishnaraju *et al,* (2005) is used. Methanol was taken as a negative control and for positive control, 4 mg/mL vincristine sulphate was taken.

Fourier Transform Infrared Spectroscopy (FTIR): It was performed following the method narrated by Jain *et al,* (2016a). The mixture was placed in a sample cup with a diffuse reflectance accessory to contain the disc. The infrared spectra were obtained using the Vertex 70 infrared spectrometer. The scan range for the material was 4000–400 cm^{-1}. The peak values of the FTIR were assessed. At that time, functional groupings were investigated.

RESULTS

The yield of extracts evaluated from plant was different in both methanol and chloroform solvents. Methanolic extract *C. flavescens* exhibited more yield than chloroform extract Table. 1.

Qualitative screening: The screening was done for the confirmation and detection of different secondary metabolites including phenol, flavonoids, alkaloids, terpenoids, saponins, tannins, cardiac glycosides, steroids, phytosterol, coumarins, anthocyanin, ß-cyanin, fats, oils, quinone, and phlobatannins. In

general, selected plant in methanolic extract displayed more positive results than chloroform extract. Tannins, Phlobatannins, quinone, Anthocyanin and Beta cyanin showed negative results in chloroform extract of selected plant mention in Table. 2.

Quantitative screening: For both extracts, TPC and TFC were investigated.

Total phenolic content: Folin-Ciocalteu colorimeter technique was used to evaluate Total phenolic content (TPC). TPC was assessed using the gallic acid (GAE mg/g) calibration curve. The results of this study show that the methanolic extract of *C. flavescens* had the highest phenolic content (53.90 ± 0.01 GAE mg/g), while the chloroform extract of *C. flavescens* had the lowest phenolic content (28.37 ± 0.01 GAE mg/g), seen in Figure 2a**.**

Total Flavonoid Content: Utilizing quercetin as the standard and the quercetin (QE mg/g) calibration curve, the total flavonoid content (TFC) was ascertained. The TFC of plant samples were assessed by regression method. The methanolic extract of C. flavescens has the highest flavonoid concentration (42.23 ± 0.07 QE mg/g), according to the analysis of the chloroform and methanolic extracts. Conversely, the minimal flavonoid content (20.02 ± 0.02 QE mg/g) in the C. flavescens chloroform extract was investigated as shown in Figure 2b.

Antioxidant assays: The ability of a plant to neutralize free radical oxygen species (ROS), which can injure the body. The activities include DPPH, TAC, and TRP.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay: The test for DPPH, Methanol and chloroform extract of *C. flavescens* was used with five dilutions (31.25 µg/mL to 500 µg/mL). The standard for DPPH is ascorbic acid. The maximum potential scavenging activity was displayed by methanolic extract of *Cyperus flavescens* (CFM) (52.72 ± 0.3 µg/mL) and the least potential was (19.43 ± 0.23 µg/mL). while minimum potential scavenging activity was displayed by chloroform extract of *Cyperus flavescens* (CFC) (14.10 ± 0.2 µg/mL) with the highest potential was shown as (42.76 ± 0.3 µg/mL). So, it was perceived that CFM displayed the highest potential scavenging activity than the CFC as shown in Figure 2c**.**

Total antioxidant capacity (TAC): Different dilutions of plant extracts (31.25 µg/mL to 500 µg/mL) were taken in the estimation of total antioxidant capacity. As Ascorbic acid was taken as standard the results were expressed in the ascorbic acid equivalent calibration curve. Among both extracts, maximum TAC was observed in the methanolic extract of *C. flavescens* from (164.32 ± 0.37 µg/mL to 54.07 ± 0.93 µg/mL) while the minimum TAC was observed in the chloroform extract which was from (42.59 ± 1.40 µg/mL to 105.80 ± 0.64 µg/mL) **(**Figure 2d**).** The data revealed that chloroform extracts showed minimum TAC potential as compared to methanolic extracts.

Total reducing power: The assay used to evaluate total reducing power was the ferric reducing power assay. It is also referring as the phosphor-molybdenum assay. Gallic acid was used as standard and it was observed that the methanolic extract of *C. flavescens* from (238.93 ± 0.61 µg/mL to 132.66 ± 0.4 µg/mL) showed maximum as compared to the chloroform extract of *C. flavescens* from 148.26 ± 0.69 µg/mL to 64.53 ± 0.6 µg/mL indicated minimum TRP shown in Figure 2e**.**

Cytotoxicity assay: The results were compiled and LC_{50} (50 % lethal concentration of mortality) was also determined. The highest cytotoxic potential was displayed by CFM (93.33 ± 0.94 µg/mL) and the least cytotoxic potential (10 ± 0.81) with the LC50 value (136.98 ± 0.34 µg/mL). The maximum cytotoxic potential was observed in CFC (96.66 ± 0.5 µg/mL) and the minimum potential was displayed (10 ± 0.00 µg/mL) with LC_{50} value (172.56 ± 0.5 µg/mL). It was observed that the highest LC_{50} came from chloroform extracts then methanolic extracts showed less LC_{50} value. The trend of LC_{50} value for CFC (172.56 ± 0.5 µg/mL) > CFM (136.98 ± 0.34 µg/mL). Thus, it was perceived that the methanolic extract of C. *flavescens* has the highest cytotoxicity potential as compared to chloroform extract due to lower value of LC_{50}. The lower the value of LC_{50}; the higher the cytotoxicity potential. The percentage mortality of both extract is given in Figure 2f.

Antibacterial activity: Different concentrations of methanolic and chloroform extracts were taken to perform the activity. Agar disk diffusion method was used with three different strains of bacteria (Gram-positive: *Staphylococcus aureus, Rhodococcus jostii, and Styphylococcus saprophyticus*). The methanol extracts of *C. flavescens* showed that the maximum zone of inhibition (ZOI) was displayed (4 ± 0.4 mm) while the minimum ZOI (1 ± 0.5 mm) against *S. aureus*. Using *S. aureus* strains, chloroform extracts showed maximum ZOI (3 ± 0.5 mm) on the other hand the least ZOI (1 ± 0.3 mm). In the case of *R. jostii* strain in chloroform extracts maximum ZOI displayed (2 ± 0.5 mm) while the lowest ZOI (1 ± 0.4 mm) while in methanolic extract the maximum ZOI was displayed (3 ± 0.5 mm) and minimum ZOI (1 ± 0.5 mm). In the case of methanolic extracts of *C. flavescens* showed maximum ZOI (3 ± 0.8 mm) with minimum ZOI displayed (1 ± 0.32 mm) against *S. saprophyticus* strain. Furthermore, CFC (2 ± 0.9 mm) exhibited maximum ZOI against *S. saprophyticus* and minimum ZOI was exhibited by CFC (1 ± 0.5 mm). The results demonstrated that altogether maximum ZOI was observed in methanolic extracts as compared to chloroform extracts. Oxytetracycline was used as a control and a sample of this drug shows the maximum ZOI as compared to all other extracts. Figure 2g indicated the MIC of the methanolic and chloroform extracts *C. flavescens*.

Fourier Transform Infrared Spectroscopy: Both chloroform and methanolic samples were used for the analysis of functional groups and various functional groups were detected. The functional groups identified in methanolic extract of *C. flavescens* include N-H stretch, C-H stretch, N-O stretch, C-Br stretch, N-H bend. Similarly, chloroform extract of *C. flavescens* showed the presence of functional groups including C-H rock, N-H wag, C-H bend, N-H bend, C=O stretch, C-N stretch, N-O stretch, C-Br stretch.

Table. 1: Plant and its extract selected for the present study

Sr. No	Plant name	Part use	Collection area	Solvents 300 g/ extract	abbreviations
1	*Cyperus flavescens*	Whole plant/ 30 g	Quiad-i-Azam University, Islamabad	Methanol/ 4.9 g Chloroform/ 3.2 g	CFM CFC

Figure. 1: ***Cyperus flavescens***

Table. 2: Qualitative phytochemical analysis of *C. flavescens*

Sr No.	Secondary metabolites	CFM	CFC
1,2	**Phenol, Terpenoids**	+++	+++
3,4,5,6	**Flavonoids, Cardiac glycosides, Phytosterol, Carbohydrates**	+++	+
7	**Saponins**	+	+++
8,9.10	**Tannins, Quinone, Anthocyanin and Beta cyanin**	+++	-
11	**Alkaloids**	++	+
12	**Steroids**	++	+++
13,14,15	**Fats and oils, Proteins, Coumarins**	**+++**	**++**
16	**Phlobatannins**	+	-

Key: (+): Slightly present, (++): Moderately present, (+++): Highly present, (-): Absent

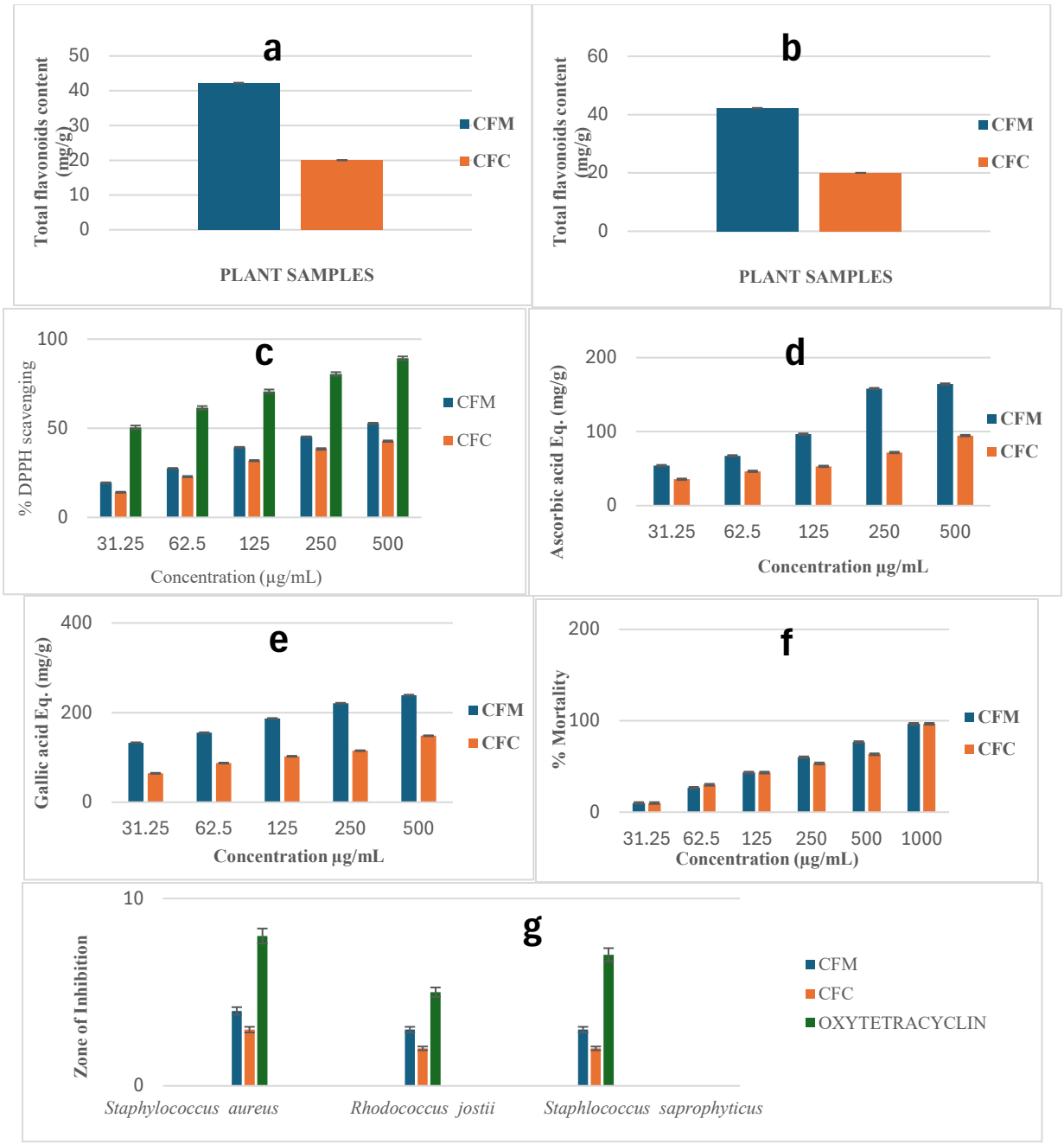

Figure 2: (a) Total phenolic content (b) Total flavonoid content (c) DPPH activity (d) Total antioxidant capacity (e) Total reducing power assay (f) Cytotoxicity assay and (g) Antibacterial activity of *Cyperus flavescens*

DISCUSSION

Several medical schools have suggested treating a variety of illnesses with medicinal products either on their own or in conjunction with prescription drugs (Yadav and Agarwala, 2011). The presence of alkaloids, tannins and steroids in the entire selected species screened coupled with flavonoids and saponins in the *Cyperus esculentus* studied inferred that they may be pharmaceutically useful (Zhang *et al.,* 2022). Alkaloids are valuable in

pharmacological applications of stimulants and anesthetics in the central nervous system (Abulude *et al.,* 2022). Flavonoids comprise one of the most important groups of polyphenols; making up over 60% and serve to bring down the danger of coronary heart disease. Additionally, they are used as cancer prevention agents and employed as a natural antioxidant due to their capacity to scavenge free radicals (Azeez *et al.,* 2022). The current study was carried out for phytochemical evaluation and biological screening.

Due to their antibacterial action, tannins can help cure gastrointestinal diseases. Anti-inflammatory, antioxidant, antiviral, antibacterial, antiparasitic, anticancer, antiseptic, and antidiuretic effects of tannins have been documented in studies (Bouzada *et al.,* 2009). According to Francis *et al,* (2002), saponins assist in lowering cholesterol levels, which in turn lowers the risk of cardiovascular disorders including hypertension. Furthermore, the food and beverage sector as well as the cosmetics business employ certain saponins (Price *et al.,* 1987). Cardiac glycosides offer potential anti-cancer action and aid in the treatment of heart-related conditions (Prassas and Diamandis, 2008).

Terpenoids have antifungal and antibacterial properties (Amaral *et al.,* 1998). Our results showed that the methanolic extract of *C. flavescens* had the most phenolic content, which is consistent with the findings of researchers who looked at the amounts of phenols in different plant parts and discovered that the highest number of phenols (Hussain *et al.,* 2009). The total flavonoid content was found to range from 42.23 ± 0.07 QE mg/g, whereas the total phenolic content ranged from 53.90 ± 0.01 GAE mg/g. According to Kilani-Jaziri *et al*, (2009), the main antioxidants found in *Cyperus rotundus* include flavonoid components like quercetin, luteolin, afzelechin, and catechin and phenolic compounds such 3-hydroxy-4-methoxy-benzoic acid, galloyl quinic acid, and ferulic acid. The endogenous antioxidant molecules, antioxidant supplements, vitamins C and E, and several antioxidant enzymes make up our body's natural antioxidant system (Yoshihara *et al.,* 2010). An entire family of extremely reactive chemicals known as ROS is produced during the metabolism of oxygen.

The methanolic extract's DPPH free radical scavenging experiment revealed its maximum potential when compared to chloroform extracts, with a CFM shown 52.72 ± 0.3 µg/mL. However, it was discovered that our findings and the study of Harput *et al,* (2011), whose *Verbena officinalis* was 40.93 + 0.25 g/mL, were connected. A biological sample's total antioxidant capacity measurement techniques are categorized as inhibitory approaches using reactive species. Ginsburg *et al,* (2011) stated that phenolic compounds have been reported to be responsible for the antioxidant activity of plants which was applied to our results as CFM showed maximum phenolic content and also showed maximum antioxidant potential and vice versa. The current research was also aligned with the results of Toma *et al,* (2015) presented that *V. officinalis* showed total antioxidant capacity of 157.99 + 6.58 µg/mL and TAC of *V. orchidea* (155.41 + 1.58 µg/mL) in comparison to those our results of *C. flavescens* extract showed the TAC observed was 164.32 ± 0.37 µg/mL.

The "ferric reducing antioxidant power" (FRAP) assay, an approach is based on antioxidants' capacity to convert Fe^{3+} to Fe^{2+}. The reducing

capacity of the substance, a crucial factor in determining whether a chemical would be a good antioxidant, is directly measured by the FRAP assay (Firuzi *et al.,* 2005). Methanolic extract of *C. flavescens* showed TRP from 238.93 ± 0.61 µg/mL to 132.66 ± 0.4 µg/mL represents the maximum TRP of all the extracts, our findings are comparable to the report of Sharifi-Rad *et al,* (2016) in which they mentioned the TRP of *V. persica* extracts measured as 65.22 µg/mL and 43.82 µg/mL. Multiple illnesses and diseases have been linked to the oxidation of biological components by free radicals, including lipids, proteins, and DNA (Wiseman and Halliwell, 1996). The brine shrimp lethality assay is thought to be a valuable technique for determining toxicity in the early stages. Additionally, it has been recommended for testing the pharmacological effects of plant extracts (Carballo *et al.,* 2002). The present study concluded that the LC_{50} of the methanolic extract of *C. flavescens* depicted was 93.33 ± 0.94 µg/mL and, our results are in accordance with the results of Safaepour *et al,* (2009) stated the LC_{50} value of brine shrimps' cytotoxicity assay from the family Boraginaceae. In present research, the disk diffusion method was used for the examination of the antibacterial potential of different plant extracts against three bacterial strains (Gram-positive: *S. aureus, R. jostii, and S. saprophyticus*).).

Our outcomes depicted that methanolic extracts of most plant samples shows good resistance against the bacterial strains represented a strong correlation with the findings of Matu and Van Staden, (2003), where they analyzed *Conyza schimperiana* extracts against disease-causing bacteria shows that among all the extracts methanolic extracts displayed maximum zone of inhibition. Previously Hassan and Ullah, (2019) reported that Veronica biloba showed ZOI of 10.5±1mm against *S. aureus* which was relatable to our data where CFM showed ZOI of 4 ± 0.4 mm against *S. aureus.* Fourier transform infrared spectroscopy brought the concept that plant samples may contain a wide range of biologically active compounds. The report of Jain *et al,* (2016b) stated that existence of functional groups in *M. spicata* as exhibited by our extracts *C. flavescens* including C-H rock, N-H wag, C-H bend, N-H bend, C=O stretch, C-N stretch, N-O stretch, C-Br stretch, N-H stretch, C-H stretch, N-O stretch.

CONCLUSION

Cyperus flavescens is commonly used to treat many disorders like roots are found to be effective in antidepressant issues, whole plant traditionally uses in folk medicine to treat fever, rheumatism, and digestive issues, such as diarrhea and dysentery. The results confirm the hypothesis that a large number of plants in the Cyperaceae family are a viable source of antioxidants. The current information would surely be helpful in evaluating the chosen medicinal plants as potential sources of natural antioxidants for use in functional food applications and nutraceuticals. Taking the antibacterial activity of our plant extracts into account, it was depicted that they could be used as bare or in conjugation after characterization as a good source of antibacterial agents and could also be used in the discovery of new drugs as this research field continue to attract much interest these days. The phytochemical analysis and in-vitro biological screening of *Cyperus flavescens* was done before, so all results were stated and analyzed first time here.

CONFLICT OF INTEREST:

There is no conflict of interest regarding research work in this publication.

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AUTHOR'S CONTRIBUTION

All authors contributed significantly to the conception, design, data collection, analysis, and interpretation of the study. Each author participated in drafting and revising the manuscript and approved the final version for publication.

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