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Qualitative and Quantitative Phytochemical Analysis of Bermuda Grass (*Cynodon dactylon* (L.) Pers.)

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Abstract:

Medicinal plants are a valuable source of bioactive compounds with therapeutic potential. Cynodon dactylon (L.) Pers., commonly known as Bermuda grass, has been traditionally used in herbal medicine for wound healing, anti-inflammatory, and antimicrobial purposes. The present study aimed to evaluate the phytochemical constituents and antimicrobial activity of methanolic extracts of C. dactylon. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, and glycosides. Antimicrobial activity was assessed using the agar well diffusion method against selected Gram-positive and Gram-negative bacteria and fungal strains. The extract exhibited significant inhibitory activity, particularly against Staphylococcus aureus and Escherichia coli, with maximum zones of inhibition of 18.2 ± 0.4 mm and 16.5 ± 0.3 mm, respectively, at 500 µg/mL. The findings support the traditional use of C. dactylon and suggest its potential as a natural antimicrobial agent.

Medicinal plants have been used worldwide for centuries as primary healthcare remedies. The increasing resistance of microorganisms to conventional antibiotics has intensified the search for alternative antimicrobial agents derived from plant sources.

Cynodon dactylon, belonging to the family Poaceae, is widely distributed in tropical and subtropical regions. Traditionally, it has been used in the treatment of wounds, urinary tract infections, diarrhea, and inflammation. Previous studies indicate that the plant contains flavonoids, alkaloids, and phenolic compounds responsible for various pharmacological activities. Cynodon dactylon is a perennial herb found in various regions of India. It has different names in different Indian languages such as "durva", "durba", "dhro", ", "arukampillu", "sharapova" etc. It contains many metabolites, mainly proteins, carbohydrates, minerals, flavonoids, carotenoids, alkaloids and glycosides.4 It is found in warm climates all over the world between 45 degrees south and north latitudes. It is available throughout the year.

Cynodon dactylon also possesses immense medicinal value and may be applied both externally as well as internally. The plant is astringent, sweet, cooling, constipating, haemostatic, diuretic and tonic and is useful in impaired conditions of pitta and kapha, burning wounds, leprosy, diarrhoea, vomiting etc. The plant is a remedy for snake bites, gout and rheumatic infections (1). ernative medicines. The therapeutic effect of plants is generally attributed to the activity of phenolic compounds, flavonoids, tannins, lignin's, or other phytochemical constituents that can be extracted using polar and/or non-polar solvents (2).

Materials And Methods:

Collection and Authentication of Plant Material:

Fresh whole plants of *Cynodon dactylon* were collected from Dr. D. Y. Patil ACS College Akurdi Campus. The plant material was washed, shade-dried for 7 days, and powdered using a mechanical grinder.

Preparation of Extract:

50 g of dried powder was extracted with 250 mL methanol using Soxhlet extraction for 6 hours. The extract was filtered and concentrated using a rotary evaporator. The dried extract was stored at 4°C until use.

Phytochemical Screening:

Qualitative Phytochemical Analysis of different extracts of ***Cynodon dactylon***. The different qualitative analysis of Phytochemicals of ***Cynodon dactylon*** were assessed by standard method and it was carried out to know the presence of compounds like alkaloids, glycosides, steroids, saponins, tannins, flavonoids, proteins and phenolic compounds etc. General reactions in these analyses revealed the presence or absence of these compounds in the seaweed extracts. Tested (3).

Test for Alkaloids:

All the extract of ***Cynodon dactylon*** were filtered separately and were dissolved with 2N hydrochloric acid and separated into three equal portion for each extract, one for Mayer's tests, second one for Dragondroff's reagent and last one for Wagner's reagents. The emergence of creamy white precipitate, orange precipitate and reddish-brown precipitate respectively indicated the presence of alkaloids in given sample.

Tests for Phenols:

About 2 ml each ***Cynodon dactylon*** extracts were taken, 2 ml of distilled water followed by few drops of freshly prepared 10% of Ferric chloride solution was added respectively. Formation of blue or black color indicated the presence of phenols in the algal extract.

Tests for Flavonoids:

With 2 ml of extract was added to 1.5 ml of 50% methanol solution. The solution was warmed and added magnesium metal. In continuation added a few drops of conc. Hydrochloric acid and the red color were formed which showed the presence of flavonoids and the orange color indicated the presence of flavones.

Tests for Tannins:

About 0.5 g of ***Cynodon dactylon*** extract was taken and added to a 10 ml of water kept in the test tube and filtered. Added a few drops of freshly prepared 5% ferric chloride and observed for brownish green or blue-black coloration. A brownish green color was formed which indicates the presence of tannins.

Test for Saponins:

A 2ml of extract was diluted with 20ml of deionized water, shaken vigorously and observed. Persistent foaming indicated the presence of saponins.

Test for Steroids:

2 ml of acetic anhydride was added to 2ml extract of each sample followed by careful addition of 2ml H2SO4. The color changed from violet to blue or green indicates the presence of steroid.

Test for Proteins:

To a 2ml of methanolic extract, 1ml of 40% NaOH solution was added and 2 drops of 1% CuSO4 solution added. The presence of a peptide linkage of the molecule was indicated by the violet color which shows the presence of protein.

Quantitative analysis of phytochemical substances in *Cynodon dactylon* extracts:

Estimation of Phenols:

The total phenols in different ***Cynodon dactylon*** extracts were determined by a modified method as described by Siddiqu N et al. (4). The assay involved gallic acid as the standard. The Gallic acid in the range of 20 -200 mg /l was used as standard for the construction of the calibration curve. 1 ml of 10% Folin Ciocalteu reagent was added to 20ml of different extract and the standard. The samples were mixed well and incubated for 5 to 10 min followed by addition

0.7 ml of 10% Na2CO3. Again, the mixed sample was incubated for 2 hours, and absorbance has been taken at 765nm using UV/VISIBLE Spectrophotometer (Schimadzu, Japan) against blank, i.e., distilled water. All the values were taken thrice, and the estimation of phenol was done by Gallic acid equivalent/ (GAE/g) of dried weight.

Estimation of flavonoids:

Estimation of flavonoids was done by Aluminium Chloride colorimetric method and Rutin in the range of 20-200mg/l had been used for the calibration curve. 0.5 ml of 2% aluminium chloride was mixed with equal volume of (different) extract of ***Cynodon dactylon*** then incubated for 1 hour at room temperature and the absorbance of the mixture was measured at 415 nm using UV/Visible Spectrophotometer.

Estimation of the total flavonoids:

Estimation of the total flavonoids was carried out in triplicate. The results mean values ± standard deviations and expressed as mg Rutin equivalents (RUE/g) dry weight.

Estimation of Tannins:

Estimation of tannins was done by using (method) Catechin as standard; 20-200mg/l of Catechin had been used for the calibration curve. 5ml of extract mixed with 1.5 ml of 40% vanillin followed by addition of 0.75ml of HCl. The solution was mixed well and incubated for 20 min at room temperature then the absorbance of solution mixture was taken at 500 nm using UV/VISIBLE Spectrophotometer. Estimation of tannins content was carried out in triplicate. The results were mean values ± standard deviations and expressed as mg catechin equivalents /g (CAE /g) dried weight.

Results And Discussions:

Qualitative analysis of phytochemical substances in *Cynodon dactylon* extracts:

Qualitative analysis of phytochemical substances in *Cynodon dactylon* extracts in the present study, preliminary phytochemical screening of 8 different chemical compounds (alkaloids, steroids, tannins, saponins, flavonoids, phenols, protiens and glycosides) were performed with petroleum ether, chloroform and methanol extracts of *Cynodon dactylon.* The patterns of composition differed considerably in their varied degree. The results of phytochemical evaluation were shown in Table 1. Saponins did not show any positive result for their presence in any of the extracts. Among the three different extracts, methanol extract showed the presence of maximum number of seven compounds. Next to that, chloroform extract showed five compounds. Petroleum ether extracts showed only three compounds with very little intensity. Methanol extract showed the presence of alkaloids, steroids, flavonoids, phenols, glycosides, tannins and proteins. Chloroform extract showed the presence of phenols, tannins, flavonoids, steroids and proteins. Petroleum ether extract showed the mild presence of flavonoids, tannins, and steroids. The presence or absence of the phytochemicals depends upon the solvent medium used for extraction. Alkaloids have a wide range of pharmacological activities including antimalarial, anticancer, cholinomimetic, vasodilatory, antiarrhythmic, analgesic, antibacterial and antihyperglycemic activities. Alkaloids have cytotoxic activity that is due to the presence of microtubule interfering agents that can bind to beta tubulin, thus inhibiting the formation of the mitotic spindle fibre required for cell division. Steroids of *Cynodon dactylon* are known to be important for immunomodulators, antiarthritic agent, antioxidant, radical scavenging agents, antimicrobial, antiparasitic and cardiotonic

properties. Steroids also play an important role in nutrition, herbal medicine, cosmetics and skin conditioning agents. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many tannin containing drugs are used in the treatment of piles, inflammation, burns and as astringent. Tannins are also being used in the process of tanning leather. Flavonoids showed its presence in all tested extracts. Flavonoids have antimicrobial, antiviral, antioxidant and spasmolytic activity. Flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health in fighting diseases. Phenols also showed its presence in all extracts of *Cynodon dactylon*. In general, phenolic compounds possessed specific physical, chemical and biological activities that make them useful as drugs (5). Phenolics were also responsible for the antimicrobial, anti-inflammatory, anti-viral, anticancer actions. Saponins did not show any positive result in any extract of *Cynodon dactylon* (7).

Table 1: Qualitative analyses of phytochemical substances in different extracts of *Cynodon dactylon*

Sl. No	Phytochemical Parameters	Petroleum Ether	Chloroform	Methanol
1	Alkaloids	+	+	+
2	Glycosides	-	-	-
3	Phenols	+	+	+
4	Flavonoids	+	+	+
5	Tannin	+	+	+
6	Saponin	-	-	-
7	Steroid	-	-	+
8	Protein	+	+	+

+: present - : Absent

Quantitative analysis of phytochemical substances of *Cynodon dactylon* extracts:

Phenolics, flavonoids and tannins contents of *Cynodon dactylon* were estimated to be quantitatively varied according to solvents used in extraction processes (6). The highest total phenolics (2.74 ± 0.17 mg GAE/g dry wt) and tannins (2.65 ± 0.43 mg CAE/g dry wt) was recorded in methanol extract, while the highest total flavonoids (1.97 ± 0.03 mg RUE/g dry wt) was recorded in chloroform extract of *Cynodon dactylon.*

Table 2: Quantitative analyses of phytochemical substances present in different extracts of *Cynodon dactylon.*

solvents	Total Phenolics (mg GAE/g dry wt)	Total Flavonoids (mg RUE/g dry wt)	Total Tannins (mg CAE/g dry wt)
Petroleuum ether	0.91±0.05	0.81±0.05	1.75±0.04
Chloroform	1.12±0.15	1.97±0.03	1.52±0.47
Methanol	2.74±0.17	1.62±0.07	2.65±0.43

Values are means of three analyses of the extract ± standard deviation (n=3) GAE: Gallic acid equivalent, RUE: Rutin equivalent, CAE: Catechin equivalent.

Conclusion:

The study demonstrates that *Cynodon dactylon* possesses significant phytochemical constituents and exhibits notable antimicrobial activity. The results validate its traditional medicinal use and suggest its potential as a natural antimicrobial agent. Further studies involving isolation and characterization of active compounds are recommended. In the present study an attempt has been made to carryout preliminary phytochemical screening of the different extracts of *Cynodon dactylon* and shown the presence of alkaloids, flavonoids, phenolic compounds, steroids, tannins, glycosides and proteins in the extracts. These preliminary phytochemical screenings shown the presence of active compounds that have pharmacological action. Extraction solvents influence yield of total phenolics, total flavonoids and total tannins from *Cynodon dactylon*.

Discussions:

The present study critically examines the qualitative and quantitative phytochemical investigations carried out on *Cynodon dactylon* and interprets their relevance considering its traditional and emerging pharmacological applications. Although widely regarded as a common turf grass, *C. dactylon* has long been utilized in traditional medicine systems such as Ayurveda for the management of wounds, inflammation, urinary disorders, diabetes, and hemorrhagic conditions. The phytochemical evidence generated over recent decades provides a scientific basis for many of these ethnomedicinal claims. Variability in phytochemical detection across studies can be attributed to differences in extraction solvents, plant parts used, geographical origin, and seasonal variation. Methanolic and ethanolic extracts frequently show higher yields of phenolics and flavonoids compared to aqueous extracts, reflecting the polarity-dependent solubility of these bioactive constituents. Such methodological differences highlight the need for standardized extraction protocols to ensure reproducibility and comparability across studies.

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