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Table: 3, Figs: 2

Quantification of medicinal phytochemicals in *Cynodon dactylon* (L.) and *Ocimum sanctum* (L.) plants

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ABSTRACT

The herbal plants *Cynodon dactylon* (L.) and *Ocimum sanctum* (Tulsi) medicinal plants are in great demand in Ayurveda to cure many common diseases of human beings and animals. These plants exist throughout the year, tolerates water scarcity in summer season and are self-propagating properties (self-seeding of *Ocimum* and runner propagation of *Cynodon* plants). We estimated the qualitative and quantitative phytochemicals present in these plants extracts. These plants extracts contained phytochemicals (alkaloids, cardiac glycosides, terpenoids and steroids, saponins, phenolic compounds and flavonoids, carbohydrates and protein) in ethanol, chloroform and diethyl ether extracts. Ocimum plant is richest in (12.20 %) alkaloids, whereas it was 4.24 % in Cyanodon plants. Other phytochemicals (steroids, flavonoids, carbohydrates and protein) were present in optimum range in both these plants.

Keywords: Herbal plants, *Cynodon dactylon*, *Ocimum sanctum*, Phytochemicals, Quantification

INTRODUCTION

There are 7,500 medicinal plant species in India. The common Dub grass or *Cynodon dactylon* (L.) of Poaceae family and holy basil or *Cynodon dactylon* (L.) and *Ocimum sanctum* (L.) of Lamiaceae contains many phytochemicals in its plant parts with multipurpose medicinal value. Among the herbal plants, *Cynodon* and *Ocimum* are very important herbal plants due its phytochemicals with active ingredients for Ayurveda medicines (21). C*ynodon dactylon* (L) is perennial herb, which will be propagating through rhizomes and rooting at every node. The morphology of the plant shows the conspicuous ring of white hairs in the ligule, fringe of hairs on the keel of the lemma and gray-green appearance of the foliage (22). The medicinal plants are growing in regions with different habitat (7). The *Cynodon* plants are mainly used as animal feed, garlands, and landscape grass and further used for medicines preparation. The *Cynodon* plants contain the nutrients like Calcium, Phosphorous, Potash, Potassium, Sodium, Manganese, Proteins, Enzymes, Carbohydrates and Fibers. *Cynodon* and *Ocimum* contain phytochemicals like flavonoids and alkaloids and also have antiseptic, astringent, cyanogenetic, demulcent, antiarthiritis (4), depurative and diuretic properties (6). Phyto-chemicals in *C. dactylon* were tannins (5), saponins (18), flavonoids (1), alkaloids (24), fatty acids (3) and sterols (8).

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Cyanodon dactylon

Ocimum sanctum

Figure 1. Single plants of *Cynodon dactylon* and *Ocimum sanctum*

Ocimum sanctum (L) or basil is a heavily branched with hairs all over the plants. It attains the height of about 75-90 cm. It has round oval shaped leaves up to 5 cm long. The plant flowers are purple or ceramic in colour. It grows up to an altitude of 200 m from sea level in moist soil (5). There are many *Ocimum* spp viz., *Ocimum sanctum*, *Ocimum canum*, *Ocimum basilicum*, *Ocimum kilmand* and *Ocimum scharicum*. The secondary metabolites of *Ocimum* are tannins, carbohydrates, glycosides, flavonoids, saponins, terpenoid, phenol and fatty acids in leaf extract, and its Tannins have anti-diarrheal and hemostasis properties (2). Flavonoids have antioxidant and immune stimulatory properties. The alkaloids, flavanoids, steroids, phenols, tannins and aromatic compounds have the antimicrobial and defensive against herbivores and insects (14). The alkaloids, flavonoids, glycosides and saponins have the antibiotic principles with defensive mechanisms for plant pathogens. The saponins have anti-hyperlipedemic, cardiodepessive and hypotensive properties (17). Glycosides act as cardio stimulants in cases of cardiac failure (23). *Ocimum sanctum* leaves contain phenolic compounds and methyl eugenol and caryophylllene. The antioxidant nature phytochemicals in the medicinal plants are mainly used to prevent and treat chronic diseases (26).

MATERIALS AND METHODS

Study Area

Medicinal garden of Tamil Nadu Agricultural University, Agricultural College and Research Institute, Vazhavachanur, Thiruvannamalai District, Tamil Nadu, India. The habitat, biological characters, and qualitative and quantitative analysis of phytochemical were studied.

Collection of Samples

The fresh leaves of selected medicinal herbal plants *Cynodon dactylon* (L.) and *Ocimum sanctum* (L.) were collected from our Farm during 2022-23. The collected leaves were washed with tap water and further rinsed with distilled water to remove soil

and dust particles and then shade dried for 96 h. The dried plants parts were grinded to get the powdered samples.

The extract of leaves was prepared by mixing 10 g powdered samples separately in 200 ml ethanol or chloroform or diethyl ether for 12 h. It was in the shaker at 150 rpm at 25 ^{O}C to get the extracts. The plant extracts obtained were evaporated and concentrated under reduced pressure to obtain 1 ml of extract for analysis. The phytochemical tests were done for Alkaloids, Glycosides, Phenolic compounds, Flavonoids, Tannin, Terpenoid, Steroid, Saponin, Carbohydrate (Glucose) and Protein as per standard analytical procedure (10).

Phytochemical Analysis

Phytochemical tests were done in aqueous extracts of *Cynodon dactylon* (*L*.) and *Ocimum sanctum* (L.) Plant (2 ml solution) for various secondary metabolites based standard methods as under.

(i). Alkaloids: The plant extract of diethyl ether based *C. dactylon* and *O. sanctum* were taken in a test tube and then added 2-3 drops of Dragendroff's reagent (potassium bismuth iodide solution) appearance of pale-yellow colour indicates that absence of alkaloids in this extract (22). Again, perform the same experiment with chloroform and ethanol extract in another test tube appearance of pink colour indicates that absence of alkaloids in these extracts. Appearance of brown colour indicates that presence of alkaloids.

(ii). Glycosides: All the three extract of these plants separately taken and then add dilute sulphuric acid. The solution was boiled and filtered. The filtrate was cooled, and then adds 2-3 drops of benzene. The solution was shaken well, organic layer got separated. After this added the equal volume of ammonia solution to the organic layer, ammoniacal layer did not turn pink, which indicates absence of glycosides in these extracts.

(iii). Flavonoids : The plant extract of diethyl ether based *C. dactylon* and *O. sanctum* were taken in a test tube and then added few fragments of magnesium ribbon and after this added the concentrated hydrochloric acid drop wise, absence of colour means absence of flavonoids in this extract. The same test was repeated with chloroform extract of *C. dactylon* and *O. sanctum* and also absence of colour. In methanol extract, there is appearance of reddish colour shows the presence of flavonoids in these extracts.

(iv). Tannins: The plant extract of diethyl ether based *C. dactylon* and *O. sanctum* were taken in a test tube and added few drops of ferric chloride solution and the result show that the pale-yellow colour appears, in chloroform extract shows yellow colour and in methanol extract appearance of brownish black colour indicates the absence of tannins in all these extracts.

(v). Steroids and Terpenoids: The plant extract of diethyl ether based *C. dactylon* and *O. sanctum* were taken in a test tube and added few drops of conc. sulphuric acid then shake well, no colour appears in these two indicates disappearance of sterols and triterpenoids. In the chloroform and methanol and water extract was done and

appearance of reddish colour in the lower layer means presence of steroids and appearance of yellow colour in the lower layer indicates presence of triterpenoids.

(vi). Saponins: The plant extract of three solvents based *C. dactylon* and *O. sanctum* were taken in a test tube and added some water and shaken well, no persistent foam indicates absence of saponins**.**

(vii). Glycosides/ Carbohydrates: The plant extract of three solvents based *C. dactylon* and *O. sanctum* were taken in a test tube and added with Benedict's reagent (alkaline solution of cupric citrate complex) and boiled on water bath, absence of red precipitates indicates the absence of carbohydrates.

(viii). Proteins: The plant extract of three solvents based *C. dactylon* and *O. sanctum* were taken in a test tube and added with 1ml of 40 per cent sodium hydroxide and few drops of 1 per cent copper sulphate. Formation of violet colour indicates the presence of proteins.

Statistical analysis

All the data were analyzed on Statistics 8.1 software by using ANOVA followed by LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

I. Qualitative analysis of phytochemical constituents: The results of phytochemicals like Alkaloids, Glycosides, Phenolic compounds, Flavonoids, Tannins, Terpenoids, Steroid, Saponins, Carbohydrates (Glucose) and Proteins content of *Cynodon dactylon* (L.) and *Ocimum sanctum* (L.) plants in different solvents (diethyl ether, ethanol, chloroform and water) were given in Tables 1 and 2. Antioxidant activity and phenolic contents in *Ocimum sanctum and Ocimum bascilicum* has been reported (1).

Table 1. Study of phytochemical compounds of C. *dactylon* plant extract using different solvents

S. No.	Phytochemical constituents	Types solvents			
		Diethyl ether	Ethanol	Chloroform	Water
1	Test for alkaloids: (Dragendroff's test)	Absent	Present	Absent	Absent
2	Test for glycosides: (Borntrager's test)	Absent	Present	Absent	Absent
3	Test for flavonoids:(Shinoda test)	Absent	Present	Absent	Present
4	Test for tannins: (Ferric chloride test)	Absent	Absent	Absent	Absent
5	Test for steroids and terpenoids (Salkowaski test)	Present	Present	Absent	Present
6	Saponin Frothing test	Absent	Present	Absent	Present
7	Test for carbohydrates Borntrager's test)	Absent	Present	Absent	Absent
8	Protein Biuret test	Absent	Present	Present	Absent

(i). Phytochemical constituents of *Cynodon dactylon*

The plant extracts of *Cynodon dactylon* showed the positive response for the presence of alkaloids and glycosides. The flavonoids, steroids and terpenoids, saponins and proteins were present in ethanol extract, whereas the negative response for the absence of tannins in all extracts. The steroids and terpenoids were shown the positive response in the diethyl ether and water solvents and saponins shown the negative response in diethyl ether and proteins was found in ethanol and chloroform solvents and were present in the Table-1. Phytochemical analysis of *Cynodon dactylon* leaves extract was carried out and found the phytochemicals (9). The Phytomedicinal properties of *Cynodon dactylon* (25) were found for medicinal purpose.

(ii). phytochemical constituents of *Ocimum sanctum:* The plant extract of Tulsi showed the positive response for the presence of alkaloids and glycosides were positive in all the four extracts. The phytochemicals like flavonoids, steroids and terpenoids, saponins, proteins and carbohydrates were positive in ethanol extracts and absence of tannins in all four extracts. The alkaloids were positive responsive to diethyl ether and water solvents and saponins showed positive in water solvent and were present in the Table-2.

Table 2. Study of phytochemical compounds of *O. sanctum* plant extract using different solvents

S. No.	Phytochemical constituents	Types solvents			
		Diethyl ether	Ethanol	Chloroform	Water
1	Test for alkaloids: (Dragendroff's test)	Present	Present	Absent	Present
2	Test for glycosides: (Borntrager's test)	Absent	Present	Absent	Absent
3	Test for flavonoids: (Shinoda test)	Absent	Present	Absent	Present
4	Test for tannins: (Ferric chloride test)	Absent	Absent	Absent	Absent
5	Test for steroids and terpenoids (Salkowaski test)	Absent	Present	Absent	Absent
6	Saponin Frothing test	Absent	Present	Absent	Present
7	Test for carbohydrates (Borntrager's test)	Absent	Present	Absent	Absent
8	Protein Biuret test	Absent	Present	Absent	Absent

II. Quantitative analysis for phytochemical constituents of *Cynodon* and *Ocimum* plants

The Quantitative analysis of phytochemical constituents like alkaloids, steroids and terpenoids, flavonoids, carbohydrates and proteins were done in *Cynodon dactylon* and *Ocimum sanctum* and presented in Table-3 and Figure-2. The same quantification was carried in medicinal plants (11).

Table 3. Quantitative analysis of phytochemical constituents (%) of C. *dactylon* and O. *sanctum* plant extract using different solvents

S. No.	Plants	Alkaloids	Steroids and Terpenoids	Flavonoids	Carbohydrates	Proteins
1.	*Cynodon dactylon*	04.2± 0.07	0.23±0.03	0.045±0.21	01.3±0.03	02.1±0.10
2.	*Ocimum sanctum*	12.2±0.19	0.67±0.15	0.812±0.33	02.2±0.05	04.2±0.11

Flavonoids: Ten g plant samples of *Cynodon dactylon* and *Ocimum sanctum* extracted with 100 ml of 80 per cent aqueous methanol at room temperature. The whole solution is then filtered through filter paper and the filtrate is later on transferred into a water bath and solution is evaporated into dryness. The sample is then weighed until a constant weight. The phytochemical compound chemical like phenolic, alkaloids, saponin compounds found in *Cynodon dactylon (*22) and protein compounds (23) and antiarthritic controlling compounds (24).

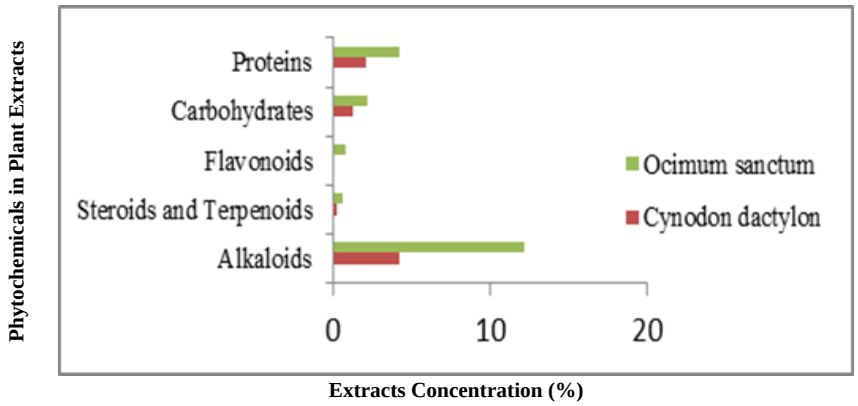

Figure 2. Quantity of phytochemical constituents (%) in *C. dactylon* and *O. sanctum* plant extract in different solvents

Saponins: The plant samples were ground and 20 g of each *Cynodon dactylon* and *Ocimum sanctum* put a conical flask and 100 ml of 20 per cent ethanol s added to the plant samples. The sample was heated over a hot water bath for 4 h with continuous stirring at about 55ºC. The mixture is then filtered and the residue of the samples were dried in the oven to a constant weight and then quantified.

CONCLUSIONS

The plants have allelopathic importance due to their phytochemicals of medicinal values. The qualitative analysis revealed the presence/absence of alkaloids, glycosides, flavonoids, steroids and terpenoids, saponin and protein in *Cynodon dactylon* (L.) and *Ocimum sanctum* (L.) plant extracts. These are responsible to enhance the stamina and improve immune system to cure many diseases and also provide antioxidants and other nutrients. These plant extracts possess compounds with antimicrobial properties (alkaloids, cardiac glycosides, terpenoids and steroids, saponins, phenolic compounds and flavonoids, carbohydrates and proteins), to cure skin diseases, fever, syphilitic, indigestion, inflammations, cut wounds ulcer, antispasmodic, anticancer, eye disease, cardio protective, arthritis, antifertility, hepatoprotective, chronic fever, antiemetic, etc., as natural medicines without any harmful health side effects.

FUTURE RESEARCH

There is possible research that the plant extract of *Cynondon dactylon* (L) and *Ocimum sanctum* (L.) can be used for synthesis of new formulations and antimicrobial drugs due to its abundance and perennial nature.

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CONFLICTS OF INTEREST

The authors of this article declare no conflict of interest in this research.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

ETHICAL STATEMENT

This is to inform you that in this study, we have not been involved in any animal and human studies.

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