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Determination of Bioactive Components of Cynodon dactylon by GC-MS Analysis & it's *In Vitro* Antimicrobial Activity

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Abstract

Cynodon dactylon (L.) Pers. (family –Poaceae), is traditionally used for curing different aliments. Hence the present investigation was carried out to determine the possible chemical components from *C.dactylon* leaves by GC-MS Technique. This analysis revealed that *C.dactylon* leaves contain 2-Propanol, 1-hydrazino- (24.37%), Glycerin (3.45%), n-Hexadecanoic acid (14.90%), Hexadecanoic acid, ethyl ester (1.83%), 1-Triacontanol (12.88%), 9,12-Octadecatrienoic acid (Z,Z), Phytol (5.52%) and Stigmasterol (6.68%) justifying the use of this plant to treat many aliments in folk and herbal medicine. The in-vitro antibacterial activity of *Cynodon dactylon (L.) Pers.* extract in ethanol was carried out by using the Well Diffusion method. The Streptomycin (100µg/ml) was used as Standard Control antibacterial agent. The antibacterial activity was investigated by using different test organisms. The Zone Diameter of Inhibition and the diameter of the well were recorded. Each assay was carried out for each test organisms used in this project work. *Staphylococcus aureus, Escherichia coli, Salmonella typhi Proteus mirabillis & Streptococcus pyogens* show nearly equal Zone of Inhibition with respect to Streptomycin.

Key-Words: *Cynodon dactylon*, GC-MS Analysis, Antimicrobial Activity, Glycerin, Phytol, Stigmasterol, Streptomycin.

Introduction

Scientific Classification:
Kingdom: Plantae (Plants), Subkingdom: Tracheobionta (Vascular plants), Super division: Spermatophyta (seed plants), Division: Magnoliophyta (Flowering plants), Class: Liliopsida (Monocotyledons), Subclass: Commelinidae, Order: Cyperales, Family: Poaceae (Grass family), Genus: Cynodon, Species: Cynodon dactylon (L.) Pers. (Durva Grass).

Cynodon dactylon is perennial and found abundant as weed along the roadsides, in lawns, localities with high level of nitrogen [1] and can readily take possession of any uncultivated area.[6] *C. dactylon* is widely cultivated in warm climates all over the world between about 30° S and 30° N latitude, and that get between 625 and 1,750 mm (24.6 and 68.9 in) of rainfall a year (or less, if irrigation is available). It is fast-growing and tough, making it popular and useful for sports fields, as when damaged it will recover quickly.[6]

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It plays an important role in soil conservation, because it prevents soil erosion.[1] The mother earth is endowed with a rich wealth of medicinal plants. They are important sources of new chemical substances that have beneficial therapeutic effects. Medicinal plants are the source of many potent and powerful drugs. They present a healthier and safer alternative to the synthetic drugs. Different parts of medicinal plants like root, stem, flower, fruit, seed etc. are used to obtain pharmacologically active constituents.[10] Medicinal activities of plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins and terpenoids present in these plants. Medicinal plants are found useful in the treatment of variety of health problems such as bacterial infections, inflammation, arthritis, peptic ulcers etc.[6]

According to an estimation of the World Health Organization (WHO), about 80 percent of the world's population relies on herbs for its primary healthcare needs. More than 35,000 plant species are being used around the world for the medicinal purposes in traditional and ethno-medicinal practices.[2] Currently, there is a renewed global interest in the study and use

of medicinal plants because such investigations provide novel and active molecules for therapeutic importance. For this reason traditional plant based remedies are back and find increasing application as source of direct therapeutic agents.[6] Among numerous species of plants growing in the wild in India, Doob Ghas, or Durva or taxonomically *Cynodon dactylon (L.) Pers.*, family Poaceae occupies its unique place and key position in ethno-medicinal practices and traditional medical (Ayurvedic, Unani, Nepalese, and Chinese) knowledge systems. Whole herb and its root stalk are used for medicinal use. It is the most sacred plant of India next to Tulsi. Hindus worship the God Ganesha with the leaves Durva religiously.[3] The plant contains crude proteins, carbohydrates, mineral constituents, oxides of magnesium, phosphorous, calcium, sodium and potassium.[2] The plant affords β-sitosterol, flavonoids, alkaloids, glycosides and triterpenoides.[9] Other compounds like vitamin C, carotene, fats, palmitic acid etc. are also reported. Green grass contains 10.47% crude protein, 28.17% fiber and 11.75% of total ash.[6]

Cynodon has a renowned position in Indian systems of medicine and many parts of the plant are assumed to have medicinal properties. Doob ghas is a valuable herbal medicine and used as first aid for minor injuries because the juice of the plant is used as an astringent and is applied to fresh cuts and wounds.[5] Farmers traditionally apply crushed leaves to minor wounds as a styptic to stop bleeding. The whole plant is extremely beneficial externally in wounds and the paste of the plant is applied on forehead during headache. The roots in the form of paste with water are taken internally against fevers. The aqueous fluid extract of the rhizome is used as anti-inflammatory, diuretic, purifying agent and also in dysentery.[11] *Cynodon* plant is useful for pains, inflammations and toothache it also possess anti-diabetic, anti-ulcer, diuretic, antimicrobial, hepato-protective, cardio-protective and immunomodulatory activities.[11] As well as treatment of urinary tract infections, prostatitis and syphilis. It has been observed that in most of the studies primarily the research being conducted on *C. dactylon* involves its glycemic potential (food's effect on a person's blood glucose also called blood sugar level.), which is involved in the treatment of diabetes. The paste made of the plant mixed with honey is used in epitaxis (nose bleeding). Oral administration of the juice of the plant with honey 2-3times a day for few days effectively treats menorrhagia (abnormal heavy and prolonged menstrual period). Local application in the form of paste of the plant extract upon the lower abdomen reduces severe bleeding in vagina. A decoction of *Cynodon dactylon* mixed with sugar is useful in the problem of urine retention.[5]

According to Ayurvedic system of medicine it acts as an appetizer, anthelmintic, antipyretic, alexiteric (Resisting poison) agent. Durvadi kvatha, Durvadya ghrta, Durvadya taila and Durvadi yoga are some classical Ayurvedic preparations of the plant. According to Unani system of medicine, *Cynodon dactylon* is used as a laxative, coolant and expectorant, carminative and as a brain and heart tonic. In Homoeopathic systems of medicine, it is used to treat all types of bleeding and skin troubles.[3] Besides, about 100 g juice of the plant is taken daily to control the blood pressure as a time tested remedy for blood pressure in various parts of the country.[2]

Material and Methods

Plant material and extraction procedure:

Cynodon dactylon (L.) Pers. (Durva grass) used for this project work was collected from Botanical Garden of CBC college Nashik Road. *Cynodon dactylon (L.) Pers.* (Durva grass) was first identified in the college Botanical garden under the guidance of head of botany department. Then the whole plant of *Cynodon dactylon (L.) Pers.* was collected, rinsed & washed with tap water followed by distilled water. These washed plant material was then air dried for 3-4 hours. After air drying plant material was dried in hot air oven at 50 °C till plant material is dried completely. These dried plant material was fine powdered using mixer grinder. It was then stored in dry container for further use.

Solvent absolute ethanol was selected for the extraction of bioactive components using Soxhlet Extraction apparatus. 20 gm Powder was filled in the empty tea bag and kept in the thimble-holder. Soxhlet Extraction apparatus was assembled in the water bath at 85 °C. These Soxhlet Extraction apparatus was then filled with 500 ml ethanol. After completion of repeated 20-25 cycle when the colour in the Soxhlet apparatus containing *Cynodon dactylon* powder becomes faint or clears the solvent, then the process of extraction was stopped. Total extract present in the round bottom flask was collected in a beaker. This collected extract was then concentrated by evaporating excess amount of ethanol using magnetic stirrer. After concentrating the extract it is stored in the autoclaved bottle at 4 °C till its further use.

Gas Chromatography- Mass Spectrum Analysis (GC-MS):

GC-MS technique was used in this study to identify the phytocomponents present in the extract. GC-MS technique was carried out at Central Instrument Center, K.T.H.M College, Nashik. GC-MS analysis of this extract was performed using Shimadzu GC-MS QP-

2010 Ultra system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Rxi® 5ms fused silica capillary column (30 m x 0.25µ Mdf. Composed of 5%Diphenyl / 95% Dimethyl siloxane). For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min. and an injection volume of 2µl was employed (split ratio of 10:1). Injector temperature 225°C; Ion-source temperature 280°C. The oven temperature was programmed from 70°C (isothermal for 1min.), with an increase of 10°C /min, to 300°C , then 5°C/ min. to 310°C, ending with a 9min. isothermal at 310°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 41 to 450 Da. Total GC running time was 34 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Lab Solution Version 2.53.

Identification of components:

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The Compound Name, Molecular weight of the test material was ascertained. Thirteen compounds were identified in *C.dactylon* leaves extract by GC-MS analysis. The active principle Molecular Weight (MW), Concentration (%), Molecular Formula (MF), Retention Time (RT) and their bioactivity are presented in Figure 1 & Table1&2. The prevailing compounds were 2-Propanol, 1-hydrazino- (24.37%), Glycerin (3.45%),n-Hexadecanoic acid (14.90%), Hexadecanoic acid, ethyl ester (1.83%), 1-Triacontanol (12.88%), 9,12-Octadecatrienoic acid (Z,Z), Phytol (5.52%) and Stigmasterol (6.68%).

Evaluation of Antimicrobial Activity:

Antimicrobial activity of *Cynodon dactylon* (Durva grass) was carried by Well diffusion method In this method clear zone i.e. zone of inhibition diameter around Well was measured against the organisms sensitive to *Cynodon dactylon* which was inoculated in the nutrient agar plates. The in-vitro antibacterial activity of *Cynodon dactylon (L.) Pers.* extract was carried out by using the Well diffusion method. The extract in ethanol was used as standard antibacterial agent. The antibacterial activity was investigated by using different test organisms. The test organisms are sub-cultured on nutrient agar slopes at 37 °C for 24 h. Colonies of fresh cultures of the different microorganisms from overnight growth were picked with sterile inoculating loop and suspended in 0.85% sterile saline contained in sterile test tubes. Thereafter, solidified nutrient agar plates were separately flooded with the liquid inoculums of the different test organisms using sterile pipette. The plates were drained and allowed to dry at 37°C for 30 mins after which four equidistant wells of 5 mm in diameter were punched using a sterile cork borer at different sites on the plates. 100 µL of the Cynodon dactylon extract was separately placed in the different punched wells with 1 mL sterile syringe. The plates were allowed to stay for 20 mins in refrigerator for diffusion of extract take place and followed by an overnight incubation that lasted for 24 hrs at 37°C. The Zone Diameter of Inhibition and the diameter of the well were recorded. Each assay was carried out for each test organisms used in this project work.

Collection of Microbial culture:

Microbial cultures used in this project work are *Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli, Proteus mirabilis* & *Salmonella typhi* which were collected from MLT department of Jadhav Hospital, Nashik Road.

Results and Discussion

Figure 1: Chromatogram obtained from the GC- MS with the extract of Cynodon dactylon

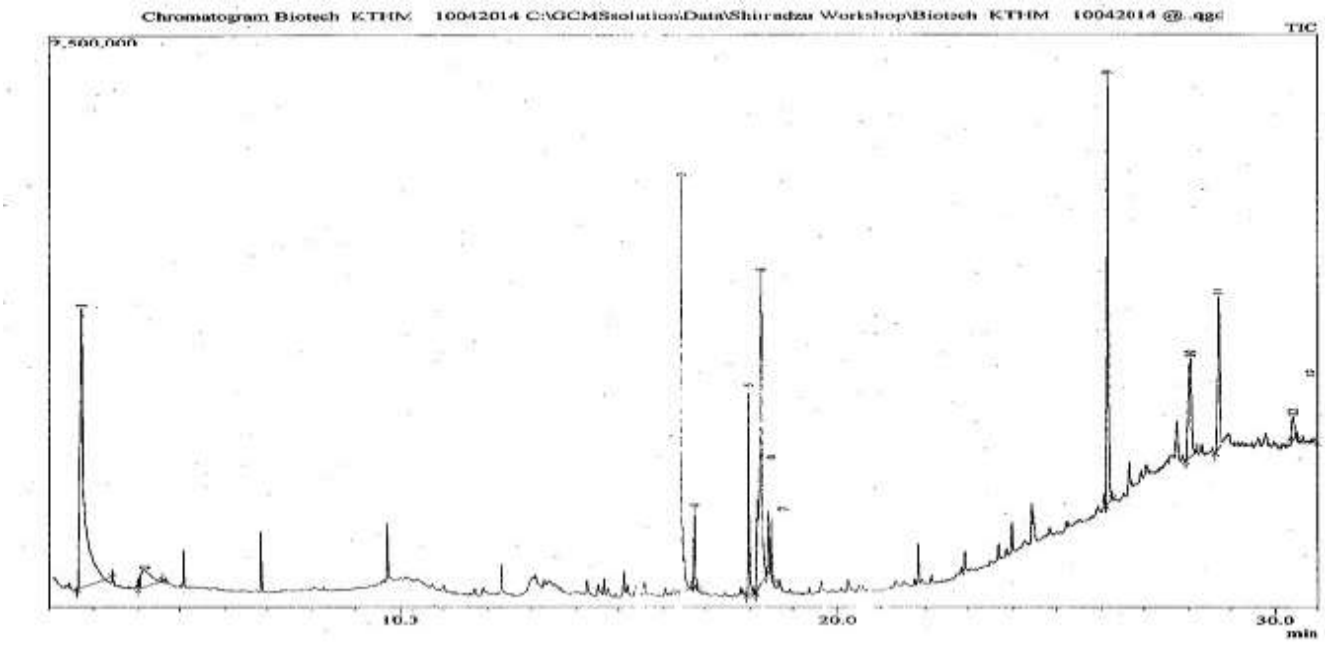

Table 1: Total ionic chromatogram (GC-MS) of Cynodon dactylon obtained with 70eV using an Rxi® 5ms fused silica capillary column with He gas as the carrier

Sr. No	RT	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area %
1.	2.731	2-Propanol, 1-hydrazino	$C_3H_{10}N_2O$	90.1243	24.37
2.	4.199	Glycerin	$C_3H_8O_3$	92	3.45
3.	16.445	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.90
4.	16.750	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	1.83
5.	17.964	Phytol	$C_{20}H_{40}O$	296	5.52
6.	18.249	9,12,15-Octadecatrienoic acid, (Z,Z,Z) -	$C_{18}H_{30}O_2$	278.42	14.79
7.	18.421	9,12-Octadecadienoic (Z,Z) -	$C_{18}H_{32}O_2$	280.45	1.83
8.	18.498	9,12,15-Octadecatrienoic acid, (Z,Z,Z) -	$C_{18}H_{30}O_2$	278.43	2.66
9.	26.144	1-Triacontanol	$C_{30}H_{62}O$	438.81	12.88
10.	28.048	Stigmasterol	$C_{29}H_{48}O$	412.69	6.68
11.	28.688	.gamma.-Sitosterol	$C_{29}H_{50}O$	414.71	6.52
12.	30.405	2-Dodecen-1-yl (-) succinic anhydride	$C_{16}H_{26}O_3$	266.37	1.17
13.	31.077	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.40

Table 2: Biological activity of Phytocomponents identified in Cynodon dactylon

Sr. No	Name of the Compound	Molecular Formula	Activity
1.	2-Propanol, 1-hydrazino	$C_3H_{10}N_2O$	Antidepressants, Drugs for disorders of the nervous system
2.	Glycerin	$C_3H_8O_3$	Antimicrobial, Anti-inflammatory
3.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Antiandrogenic, Flavor Hemolytic,5-Alpha reductase

			inhibitor
4.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Unknown
5.	Phytol	$C_{20}H_{40}O$	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic
6.	9,12,15-Octadecatrienoic acid, (Z,Z,Z) -	$C_{18}H_{30}O_2$	Drugs for genital or sexual disorders; Contraceptives
7.	9,12-Octadecadienoic (Z,Z) -	$C_{18}H_{32}O_2$	Drugs for disorders of the urinary system, Expectorants
8.	9,12,15-Octadecatrienoic acid, (Z,Z,Z) -	$C_{18}H_{30}O_2$	Drugs for genital or sexual disorders; Contraceptives
9.	1-Triacontanol	$C_{30}H_{62}O$	Anti-acne agents, Drugs for Dermatological disorders for treating wounds, ulcers, burns, scars, keloids,
10.	Stigmasterol	$C_{29}H_{48}O$	Antiasthmatics, Antimicrobial, Anti-inflammatory
11.	.gamma.-Sitosterol	$C_{29}H_{50}O$	Use for joint disorders, e.g. arthritis, arthrosis, Non-central analgesic, antipyretic or anti-inflammatory agents
12.	2-Dodecen-1-yl (-) succinic anhydride	$C_{16}H_{26}O_3$	Antineoplastic agents, Antioxidants, Antimicrobial
13.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	Antimicrobial, Anti-inflammatory

Table 3: Antimicrobial activity of *Cynodon Dactylon (L.) Pers*. by Well diffusion method

Test Organism	Zone of Inhibition Test Organism in diameter (mm) Well Diffusion Technique	
	Durva grass	**Streptomycin (100µg/ml) Control**
Streptococcus pyogens	18	23
Staphylococcus aureus	30	35
Escherichia coli	25	38
Proteus mirabillis	23	30
Salmonella typhi	11	18

Conclusion

From this study it can be concluded that Ethanolic Extracts of Durva (*Cynodon dactylon*) showed wide range of antibacterial activity. It is a valuable alternative for the classical wound dressings, especially considering the increasing antibiotic resistance. It is cheap in use the purchase price is low and the patient heals faster than with a conventional treatment. Fewer surgical excisions and grafts are needed and care can be continued at home sooner. In addition, it is biodegradable. The ethanolic extract of *Cynodon* was investigated against the selected clinical pathogens such as *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Proteus mirabillis & Streptococcus pyogens* by agar Well diffusion method. All the wells have shown varying degrees of antimicrobial activities against the clinical pathogens tested. As *Cynodon dactylon* show its antimicrobial activity against microbes causing wound infection this can heal wound in vivo too. GC MS studies of the extract showed the presence of selected active constituents in significant amount. Almost all of them possess multifaceted properties. The wide range of antimicrobial activity of *Cynodon* dactylon can be used and administered in the ethnomedical practice. It can be a source of novel useful drugs and of greater pharmacological importance. Thus, there is a need to carry out further research work for effective utilization of such plants.

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