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Characterization of phytoconstituents by GC-MS from the whole plant chloroform extract of *Cynodon dactylon* (L.) Pers. responsible for neuroprotective activity

Vir Vikram[1], Gurpreet Kaur[2]*, Vijender kumar[3]

Professor, Department of Pharmacology, CT University, Punjab.
Research scholar, CT University, Punjab.
Department of Pharmacognosy, DPSRU, New Delhi
Corresponding Author:Gurpreet Kaur

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Abstract

Cynodon dactylon is the most sacred plant since Vedas used in medicines as well as Holy yogas. The whole plant of *Cynodon dactylon* is used for medicinal purpose, externally as well as internally. It is an elegant tenacious perennial creeping grass growing throughout the country and ascending to 2440m. Plants are the traditional sources for many chemicals used as pharmaceutical biochemicals, fragrances, food colours and flavours in different countries especially in India. *Cynodon dactylon* (L) Pers commonly known as Bermuda grass belongs to the family Poaceae. In ethnomedicinal practices, the plant *Cynodon dactylon* used in the treatment of various diseases and has pharmacological actions. The objective of the study was to investigate the phyto- components present in the chloroform extract of *Cynodon dactylon* (L.) Pers by GC-MS analysis to ascertain its usage by the local community as a plant possessing medicinal properties. In total, 52 compounds were identified. The major constituents were turmerone (0.60%), Octadecanoic acid (1.85%), hexadecenoic acid (10.80%), Neophytadiene (0.99%),Tetracosanol (0.59%), Phytol (0.53%), Stigmasterol (1.69%), Gamma-Sitosterol (1.57%), 2-methoxy-4-vinylphenol (2.50%). These findings support the traditional use of *Cynodon dactylon* in various disorders. The detected Phytoconstituents encourage future isolation of these remarkable substances for potential usage in the pharmaceutical industry.

Introduction

Cognitive dysfunction is a major health problem in the 21st century, and many neuropsychiatric disorders and neurodegenerative disorders, such as schizophrenia, depression, Alzheimer's Disease dementia, cerebrovascular impairment, seizure disorders, traumatic brain injury, and Parkinsonism, can be severly functionally debilitating in nature. In course of time, a number of neurotransmitters and signaling molecules have been identified which have been considered as therapeutic targets. Conventional as well newer molecules have been tried against these targets. Phytochemicals from medicinal plants play a vital role in maintaining the brain's chemical balance by influencing the function of receptors for the major inhibitory neurotransmitters. In traditional practice of medicine, several plants have been reported to treat cognitive disorder. Substantial evidence shows that a number of dietary or phytobioactive compounds have considerable anti-oxidant and anti-inflammatory effects, displaying an inhibitory role in the oxidative and inflammatory mechanisms associated with neurodegenerative diseases [1-2]. These compounds include polyphenols, phytosterols, terpenoids and other nutritious components such as propolis, ω-3 polyunsaturated fatty acids (PUFAs), and vitamin E, Ascorbic acid and their anti-oxidative and anti-inflammatory roles have been widely confirmed in-vitro and in-vivo, including inhibited neurotoxic effects, by eliminating or limiting the activities of the reactive oxygen species (ROS) and reactive nitrogen species (RNS) [3] from the oxidative stress pathway and toll-like receptors, NF-κB and cytokines (TNF-α, IL-6, IL-1β, and IFN-γ), from proinflammatory immune pathways [2]. Dietary supplementation can improve the recovery and regeneration of dopamine terminals in the striatum in the PD brain.

Modern-day synthetic and chemical drugs are often explored with hesitate as they exhibit side effects, while traditional herbals are gaining huge interests as they are more natural, environment-friendly and devoid of side effects. Hence, with all the benefits of modern synthetic medicines, people have still preferred plant-based natural medicines over synthetic medicines. Medicinal plants are rich in secondary metabolites with many biological activities including antioxidant, anti-inflammatory, anticancer, antiviral, antifungal, and antibacterial agents.

Medicinal plants are known to be the main source of drug therapy in traditional medicine. Medicinal plants are at great interest to drug industries, as herbal medicines and their derivative products are often prepared from crude plant extracts. Nature acts as an endless source of the medicinal entities, pharmacophores, novel chemophytes which contribute in the field of drug development for the betterment of the human illness since the ancient time. Medicinal plants have been used for thousands of years to cure various human diseases as the plants contain many constituents which have high therapeutic values *Cynodon dactylon* is commonly known as bermuda grass, belongs to family Poaceae. *Cynodon dactylon* is native to East Africa, Asia, Australia By Southern Europe. *Cynodon dactylon* has various medicinal properties. The plant is traditionally used as an agent to control diabetes. The extract of plant has been reported to be antidiabetic, antioxidant & hypolipidemic efficacy. The plant possesses antiviral and antimicrobial activity. The plant is astringent, sweet, cooling, haemostatic, depurative, vulnerary constipating, diuretic and tonic [4]. In the present study, we evaluated the phytochemicals, constituents of Chloroform extract of *Cynodon dactylon* by gas chromatography and Mass spectrometry (GC-MS) to provide the scientific information to develop potential phytomedicine.

Material and methods

Plants contain different phytochemicals, also known as secondary metabolites. Phytochemicals are useful in the treatment of certain disorders by their individual, additive, or synergic actions to improve health [4-5]. Phytochemicals are vital in pharmaceutical industry for development of new drugs and preparation of therapeutic agents [5]. The development of new drugs starts with identification of active principles from the natural sources. The screening of plant extracts is a new approach to find therapeutically active compounds in various plant species [4].

Plant material & preparation of extract

The whole plant of *Cynodon dactylon* was collected from the surrounding areas of Akal College of Pharmacy and technical education, Mastuana Sahib, Sangrur, in the month of November 2021. The plant was authenticated from CSIR-NIScPR New Delhi having authentification No- NISc PR/RHMD/ Consult 2021/ 3890-91. Whole plant of *Cynodon dactylon* was shade dried and coarsely powdered. The 500gm of powdered plant material is treated with various solvents by successive solvent extraction method. The extracts obtained were filtered & concentrated by using rota evaporator [6-7].

GC-MS analysis

The Chloroform extract of *Cynodon dactylon* was subjected to GC-MS detection. The detection was carried out with Gas chromatograph coupled with Mass spectrophotometer (GC-MS)(Shimadzu QP 2010 Mass spectrophotometer). Helium was employed as the carrier and its flow rate was adjusted to 1.2ml/min. The analytical column connected to the system was an RTx-5 capillary column. The column head pressure was adjusted to 100 Kpa. Column temperature programmed from 40.00 degree centigrade. The injector temperature was set at 230.0°C. The mass Spectra were screened range of M/Z 40-600amu. Compounds were identified by comparing mass spectra with library of the National Institute of Standard and Technology [8-9].

Result and Discussion:

GC-MS Analysis

The analysis and extraction of plant material play an important role in the development and quality control of herbal formulation. Hence present study was aimed to find out the bioactive compounds present in the chloroform extract of *Cynodon dactylon* by using gas chromatography and Mass spectroscopy. The active compounds with their peak number concentration (Peak area %) and retention time (R_t) were presented in figure 1 and table 1.

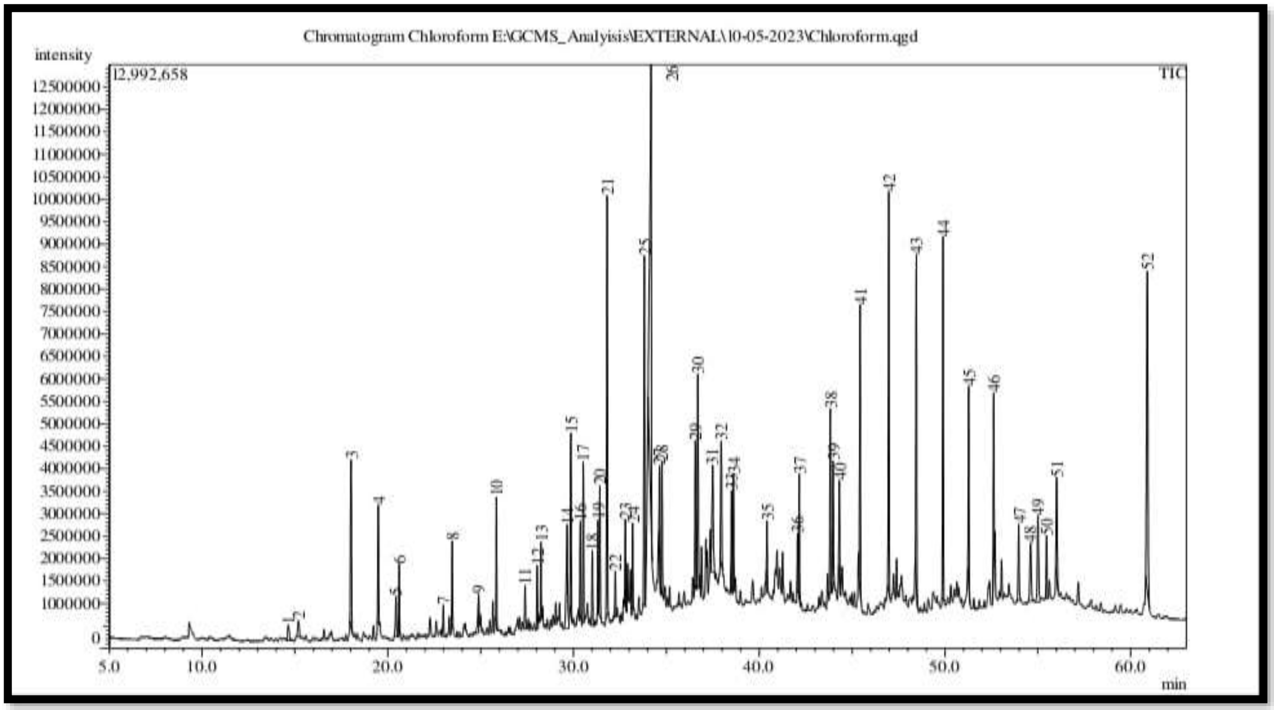


Figure no.1 GC-MS chromatogram of chloroform extract of *Cynodon dactylon* (L.) Pers.

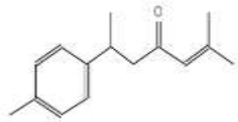
Table no.1 GC-MS spectral analysis of Chloroform extract of *Cynodon dactylon* (L.) Pers.

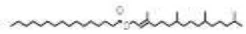
Peak#	Retention Time	Area%	Compound
1.	14.643	0.37	Dodecane
2.	15.187	0.40	Benzene, (ethenyloxy)-
3.	18.021	2.50	2-Methoxy-4-vinylphenol
4.	19.490	1.51	2-Hydroxy-3-acetyl-6-methyl-4-pyrone
5.	20.441	0.63	Vanillin
6.	20.612	0.66	Tetradecane
7.	22.992	0.45	Eicosane
8.	23.466	0.90	2,4-Di-tert-butylphenol
9.	24.891	0.59	Dodecanoic acid
10.	25.848	1.29	Nonadecane
11.	27.393	0.60	aR-Turmerone
12.	28.040	0.77	hexadecyl acrylate
13.	28.243	0.79	Eicosane
14.	29.660	2.20	Tetradecanoic acid
15.	29.856	2.26	Benzyl Benzoate
16.	30.367	1.03	1-Nonadecene
17.	30.527	1.74	Heneicosane
18.	31.014	0.93	Isopropyl myristate

19.	31.317	0.99	Neophytadiene
20.	31.418	1.51	2-Pentadecanone, 6,10,14-trimethyl-
21.	31.810	5.57	2-Pentadecanone, 6,10,14-trimethyl-
22.	32.249	0.53	Phytol
23.	32.791	0.84	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-
24.	33.185	0.97	Hexadecanoic acid, methyl ester
25.	33.812	4.45	Dibutyl phthalate
26.	34.174	10.80	n-Hexadecanoic acid
27.	34.628	0.84	1-Nonadecene
28.	34.762	1.46	Heneicosane
29.	36.561	1.70	9,12-Octadecadienoic acid (Z,Z)-, met
30.	36.694	3.15	6-Octadecenoic acid, methyl ester, (Z)-
31.	37.491	2.24	2H-Pyran, 2-(2-heptadecynyloxy) tetra
32.	37.955	1.85	Octadecanoic acid
33.	38.506	0.95	n-Tetracosanol-1
34.	38.615	1.26	Heneicosane
35.	40.416	0.91	2-Methylhexacosane
36.	42.062	0.59	n-Tetracosanol-1
37.	42.154	1.37	Heneicosane
38.	43.825	2.18	Eicosane
39.	43.976	1.69	Hexadecanoic acid, 2-hydroxy-1-(hydr
40.	44.313	1.14	Bis(2-ethylhexyl) phthalate
41.	45.426	3.06	Hexatriacontane
42.	46.973	4.74	Hexatriacontane
43.	48.457	3.67	Hexatriacontane
44.	49.896	4.33	Hexatriacontane
45.	51.278	2.21	Hexatriacontane
46.	52.627	1.92	Hexatriacontane
47.	53.971	1.17	Hexatriacontane
48.	54.604	1.30	Ergost-5-en-3-ol, (3.beta.)
49.	55.009	1.69	Stigmasterol
50.	55.473	0.88	Hexatriacontane
51.	56.014	1.57	gamma.-Sitosterol
52.	60.896	6.90	Tris(2,4-di-tert-butylphenyl) phosphate

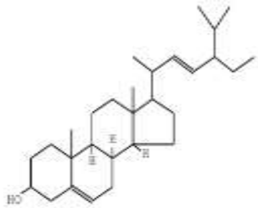
The Percentage content of compounds is Dodecanoic acid (R_{t}-24.891), Turmerone (R_{t}-27.393), Hexadecanoic acid (R_{t}-34.174), Neophytadiene (R_{t}-31.317), Octadecadienoic acid (R_{t}-37.955), phytol (R_{t}-32.249), Stigmasterol (R_{t}-55.009), Gamma-sitosterol (R_{t}-56.014), 2-Methoxy-4-vinylphenol (R_{t}-18.021). The detected Phytoconstituents encourage future isolation of these remarkable substances for potential usage in the pharmaceutical industry. Structures of active compounds were presented in figure 2.

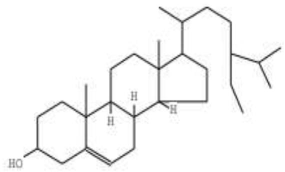

Dodecanoic acid

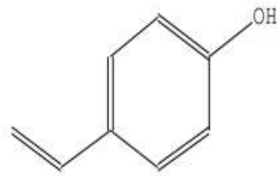

Turmerone


Hexadecanoic acid


Neophytadiene


Stigmasterol


Gamma-sitosterol



4-Vinylphenol

Figure 2: Major phytoconstituents present in chloroform extract of *C. dectylon*

Decanoic acid, is a saturated, medium-chain fatty acid and is popularly known as capric acid or decylic acid. Decanoic acid is naturally found in coconut oil and palm kernel oil. Medium-chain fatty acids are easily absorbed when compared with other fatty acids and are metabolized within the liver mitochondria to produce ketone bodies. As it is helpful in producing acetyl-CoA, a vital part of the citric acid cycle to deliver ATP, cerebral energy metabolism can be improved by ketone bodies during periods with inadequate glucose availability or uptake. Daily consumption of medium-chain fatty acids has been reported to reduce minor to moderate cognitive impairment in patients with Alzheimer's disease [10]. Turmerones are major bioactive compounds of Curcuma species with several beneficial pharmacological activities. In addition, various *in-vivo* and *in-vitro* studies noted that turmerones could affect different cytokines, metabolic pathways, and targets. Turmerones will have the potential to be a candidate agent to lessen many pathological and immunological conditions as a result of these pharmacological activities [11]. aR-Tumerones are aromatic turmerones and they display a variety of activities, such as antimutagenicity, anti-hyperglycemic, cell proliferative and anti-inflammatory actions [12]. It has been also suggested that aR-turmerone inhibits microglia activation, a property that may be useful in treating neurodegenerative disease [13].

Aparna V. et al., (2012) reported that n-hexadecanoic acid, is an inhibitor of phospholipase A(2), hence, an anti-inflammatory compound [14]. Neophytadiene (NPT) is a diterpene found in various plants reported with anxiolytic-like activity, sedative properties, and antidepressant-like actions [15]. Stigmasterol ($C_{29}H_{48}O$), a naturally occurring steroid derivative, is found in many plants. Stigmasterol has various pharmacological effects such as anticancer, anti-osteoarthritis, anti-inflammatory, anti-diabetic, immunomodulatory, antiparasitic, antifungal, antibacterial, antioxidant, and neuroprotective properties [16]. It is reported that Stigmasterol can effectively reduce neurological deficits and infarct damage induced by the ischemic/reperfusion injury, improve histopathology changes, and restore the levels of the endogenous antioxidant defense system in a dose–response mode [17]. Sundarraj S. et al., (2012) suggest that γ-Sitosterol exerts potential anticancer activity through the growth inhibition, cell cycle arrest and the apoptosis on cancer cells [18]. Polyphenols exert neuroprotective effects, inhibiting cell death and attenuating the depolarization of the mitochondrial membrane induced by the activation of glutamate receptors, to reduce the entry of Ca^{2+} [19]. A diterpene alcohol phytol might produce sedative and anxiolytic effects. Phytol could be an alternative for treatment of anxiety [20]. Vanillin promotes early neurofunctional development on neonatal rats following hypoxic-ischemic brain damage. Vanillin ameliorates brain infarct volume, brain edema and histomorphological damage after HIBD in neonatal rats. Vanillin alleviates neonatal HIBD possibly by attenuating oxidative damage and preserving BBB integrity. Vanillin might be a promising neuroprotective candidate for neonatal hypoxic-ischemic encephalopathy [21].

Conclusion

Medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development., the data generated from these experiment provide the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments. This study offers a plant for using *Cynodon dactylon* as herbal alternative for various diseases. GCMS analysis revealed 52 phytoconstituents in *Cynodon dactylon*. The presence of various bio-active compounds detected after GC-MS analysis using the chloroform extract of *Cynodon dactylon* justifies the use of whole plant for various elements by traditional practitioner.

However, isolation of individual phytochemical constituents and subjecting it to the neuroprotective activity will be definitely giving fruitful results and will open a new area of investigation of individual components and their pharmacological potency. From these results, it could be concluded that "*Cynodon dactylon* " contains various bio-active compounds. Evaluation of pharmacological activity is under progress. Therefore, it is recommended as a plant of phytopharmaceutical importance. From the above analysis it was concluded that the main constituents i.e., Turmerone, 2-methoxy-4-vinylphenol, Hexadecanoic acid, Phytol, Stigmasterol, Neophytidine, Gamma-stigmasterol can be used in the management of neurogenerative disorders in neuronal ischemia as anti-inflammatory, antiulcer, antioxidant, anticancer. This study reports a neuroprotective function for ar-turmerone, providing new insights into the potential therapeutic applications of ar-turmerone for neurological disorders.

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