esearch article Available online www.ijsrr.org ISSN: 2279-0543

International Journal of Scientific Research and Reviews

Evaluation of Phytochemical Antioxidant Antimicrobial Activity Determination of Bioactive Components of Ethanolic Extract of Aerial And Underground Parts of *Cynodon dactylon L.*

Kanimozhi.D, V.Ratha bai*

Department of Zoology, Presidency College, Chennai-600 005, Tamilnadu, India

ABSTRACT

Cynodon dactylon(L.) Pers. belongs to the Family Poaceae. The present study was carried out to investigate the Phytochemical, antioxidant, antimicrobial activity, determination of bioactive components of ethanolic extract of aerial and underground parts of *Cynodon dactylon*. Tannins, Saponins, Flavonoids, Alkaloids, Proteins, Steroids and Anthraquinones were assessed for different phytochemical test in ethanolic extract of *Cynodon dactylon*. The ethanolic extract shows positive result for tannin, saponins, proteins and steroids. *In-vitro* antioxidant activity of ethanolic extract of *Cynodon dactylon* were determined by DPPH free radical scavenging assay. The reducing power of the extract was also determined. Ascorbic acid was used as standard and positive control for both the analysis. The ethanolic extract of *Cynodon dactylon* had shown very significant DPPH radical scavenging activity compared to standard antioxidant. The DPPH radical scavenging activity of the extract was increased with increasing concentration. In DPPH free radical scavenging assay was found to be 65%. Analysis of gc-ms revealed that ethanolic extract of *C.dactylon* contains 2-Hexadecen-1-Ol, 3,7,11,15 Tetramethyl(12.05%), Hexadecanoic Acid, Ethyl Ester(6.49%), gamma.-Sitosterol(6.45%), 9 Octadecenoic acid, methyl ester(6.17%), Tetracontane(5.99%), N-Nonacosane(5.01%).

KEY WORDS: Antioxidant, Reducing power Assay, Antimicrobial, Stigamasterol.

*Corresponding Author

Dr. (Mrs).V.Ratha bai
Associate Professor
PG & Research Department of Zoology,
Presidency College,
Chennai-5, Tamilnadu, India
E mail: kanphd5@gmail.com

INTRODUCTION

Plants with antioxidant activities have been reported to possess free radical scavenging activity[1] molecules like vitamins, terpenoids, phenols,lignins, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity[2,3]. Several studies have reported that these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities[4,5]. Free radicals are known as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defence mechanism[6]. A systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in pharmaceutical and drug research.

Cynodon dactylon.(L). Pers. belongs to the family of Poaceae[7] and is said to have many medicinal properties including Antihelmentic[8], Antidiuretic, Anti inflammatory, Hepatoprotective activity[9] as well as treatment of Urinary tract infections[10], Prostatitis, and Dysentery. Traditionally it is used in diabetes[11,12] jaundice, kidney problems[13], urinary disease, Gastrointestinal disorder[14], Constipation and abdominal pain. The whole plant is used for diuretic, dropsy, syphilis, wound infection and piles. *Cynodon dactylon* is used as antihaemorrhagic in dysentery and nasal bleeding[15].The juice of the plant is astringent and is applied externally to fresh cuts and wounds. It is used in the treatment of catarrhal opthalmia, hysteria, epilepsy, insanity, and chronic diarrhea. The plant is folk remedy for anasarca, calculus, carbuncles, cough, hypertension, snake bites, gout and rheumatic affections. *Cynodon dactylon* is a valuable herbal medicine and used for first aid for minor injuries[16,17]. *Cynodon dactylon* is bitter, sharp hot taste, good odor, laxative, brain and heart tonic, aphrodisiac, expectorant, carminative and useful against grippe in children and for pains, inflammations, and toothache[18]. Virus-affected discolored leaves of *Cynodon* are used for the treatment of liver complaints. In Homoeopathic systems of medicine, it is used to treat all types of bleeding and skin troubles[19]. The Ethanolic extract of aerial parts of *C. dactylon* showed marked protection against convulsions induced by chemo convulsive agents in mice[20]. Ethanolic extract of defatted *C. dactylon* has high antidiabetic potential along with good hypolipidemic profile[21]. This suggests the potential for *Cynodon dactylon* to become an alternative to current diabetes medications. The antimicrobial activity of Ethanol, Methanol, Acetone, Chloroform, Hexane and Petroleum ether extract of *Cynodon dactylon* was tested against infectious disease causing bacterial pathogens such as such as *E.Coli, Pseudomonas aeruginosa,*

Staphylococcus aureus and *Klebsiella pneumonia* fungus like *Aspergillus niger, Candida albicans, Candida kefyr* and *Candida tropicalis* using the Agar Well diffusion method[22].

MATERIALS AND METHODS

Plant materials

Cynodon dactylon(L). aerial and underground parts were collected during January, February from in and around Maduravoyal region, were authenticated by Department of Plant biology and Biotechnology, Aringnar Anna College, Walajapet, Tamilnadu. The voucher specimen were kept in the PG & Research Department of Zoology, Chennai, Tamilnadu, India and used for this study. GC-MS technique was carried out in Sargam laboratory, Chennai, Tamilnadu.

Preparation of Extracts

The aerial and underground parts of *Cynodon dactylon* were washed with fresh water and dried under shade at room temperature, cut into small pieces and powdered in a mixer grinder. *Cynodon dactylon* grass were powered and stored in sterile containers for further use. Then this powdered sample (100gm/100ml) were kept in ethanol extract for overnight at room temperature[23,24]. Soxhlet apparatus are used for this extraction. The extract were evaporated at 50^0c until solvent layer evaporated completely. The ethanolic crude sample of *Cynodon dactylon* were subjected to different tests three consecutive soaking are pooled and evaporated under pressure. The crude sample were subjected to phytochemical screening for the presence of proteins, saponins, flavonoids, alkaloids, protein, tannins, steroids and anthraquinones.

Phytochemical screening

To test for alkaloids, about 1ml of the extract was taken to that a few drops of Dragendorff's reagent were added observed for orange red color and turbidity or precipitation with this reagent was taken as evidence for the presence of alkaloids. 1ml of extract was added to 2ml of H_2O(shaken vigorously), Frothing which persisted on warming was taken as preliminary evidence for saponins. To 1ml of the extract a few drops of 0.1% ferric chloride and observed for blue color/brownish green, indicates the presence of tannins. Borntrager's test was used for detecting the presence of anthraquinones. In this case 1ml of the plant extract shaken with benzene layer separated and half of its own volume of 10%

ammonia solution added. A pink, red or violet coloration in the ammoniacal phase indicated the presence of anthraquinone. To 1ml of the extract conc.Hcl and magnesium chloride, pink tomato red color indicates the presence of flavonoids. To 1ml of the extract Bradford reagent was added and observed for blue color indicates the presence of proteins. To 1ml of the extract 10% H_2SO_4 was added, green color indicates the presence of steroids[25,26].

Antioxidant assay

The antioxidant activity of Plant extracts were determined by different *in vitro* methods such as, the DPPH(1,1-diphenyl-2-picryl-hydrozyl), and assay of reducing power by FRAP method free radical scavenging assay and reducing power methods. All the assays were carried out in triplicate and average values were considered.

Dpph Radical Scavenging Activity

The antioxidant activity of ethanolic extracts of *Cynodon dactylon*(Leaves, stem and root). and the standard compound BHT was measured in terms of hydrogen donating radical scavenging ability using the stable DPPH method[27]. 1ml of extract was added to 3.7mL of methanol solution. After centrifugation, the supernatant is collected 200 ml of DPPH solution is added. Kept in the dark for 45 min and the resulting decrease in absorbance at 517 nm were recorded against blank using a UV-Vis Spectrophotometer. The radical scavenging activity on DPPH was expressed as,

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample) / (Absorbance Of control)] x 100

Frap Assay of Reducing Power

A simple automated test measuring the ferric reducing ability of plasma, the FRAP assay, is presented as novel method for assessing ‘‘antioxidant power.’’ Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. FRAP values are obtained by comparing the absorbance change at 593nm in test reaction mixtures with those containing ferrous ions in known concentration. Absorbance changes are linear over a wide concentration range with antioxidant mixtures, including plasma and with solutions containing one antioxidant in purified form.

Reagent preparation.

Reagents included 300mmol/acetate buffer, pH 3.6(3.1g sodium acetate trihydrate($C_2H_3NaO_2.3H_2O$) and 16ml $C_2H_4O_2$ per liter of buffer solution); 10mmol/liter TPTZ (2,4,6 tripyridyl-*s*-triazine) in 40mmol/liter HCl ; 20mmol/liter $FeCl_3.6H_2O$. Working FRAP reagent was prepared as required by mixing 25ml acetate buffer, 2.5ml TPTZ solution, 2.5ml $FeCl_3.6H_2O$ solution[28]. A 300ml freshly prepared FRAP reagent was warmed to 37^0C and a reagent blank reading was taken at 593nm,10ml of sample was then added, along with 30ml H_2O, final dilution of sample in reaction mixture was, therefore, 1/34. Absorbance readings were taken after 4minutes. The change in absorbance is measured at 593nm. The increase in absorbance of the reaction mixture indicated reducing power.

FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 minute / change in absorbance of standard from 0 to 4 minutes) x FRAP value of standard (1000µM)

Note: FRAP value of Ascorbic acid is 2.

Determination of antimicrobial assay

Test organisms

The bacterial species used for the test were *Proteus mirabilis*, *Salmonella typhii*, *Enterococcus faecalis*. The fungal species used for the test were *Trochoderma viride, Candida albicans* used to determine the antimicrobial activity of ethanolic extract of aerial and underground parts of *Cynodon dactylon*. *Streptomycin* is used as the standard antibiotic in both Antibacterial and antifungal activity. All the stock cultures were obtained from Chromopark Research Center, Tamilnadu, India.

Culture Media and Inoculumn Preparation

Nutrient agar broth(Himedia, India) were used as the media for the culturing of bacterial strains. Loops full of all the bacterial cultures were inoculated in the nutrient broth and incubated at 37˚c for 72hrs and potato dextrose agar and potato dextrose broth(Himedia, India) were used as the media for the culturing strains. Loops full of all the fungus were inoculated in the Potato dextrose broth(PDA) and incubated at room temperature for 72hrs.

Disc diffusion method

Antimicrobial activity of aerial and underground parts of ethanolic extract of *Cynodon dactylon* was determined by disc diffusion method on Muller Hinton agar (MHA) medium The plates were

incubated at 37 °C for 24 hrs and growth of inhibition zones were measured. Disc diffusion method was used to determine the zone of inhibition against chosen bacteria by the *C. dactylon*. The diluted bacterial and fungal cultures were spread over nutrient agar plates using sterile glass L rod. The disc were placed in MHA plate at the concentration of 1000µg/ml, 500µg/ml, 250µg/ml, 100µg/ml, 10µg/ml of ethanolic extract of *Cynodon dactylon* and then allowed to dry before being placed on the top layer of the agar plate. The plates microbial growth was determined by measuring the diameter of zone of inhibition. The invitro antibacterial activity was performed by agar disc diffusion[29] and agar well diffusion method.

Thin layer chromatography

TLC was used to monitor the identity of each extracts and fractions, additionally to screen the qualitative purity of the isolated compound. It was also developed to optimize the solvent system that would be applied for column chromatography. Analytical TLC was performed qualitatively on precoated TLC plates with Silica gel plate (Himedia, India) using diverse solvent systems for mostly semi-polar compounds. However, solvent system containing Chloroform:Methanol(19:1) was used. The compound was then detected by their UV transilluminator at wavelength 254 and 366nm.

Gas chromatography- mass spectrum analysis(gc-ms)

GC-MS technique was used in this study to identify the bioactive components present in the extract. GC-MS technique was carried out at Sargam laboratory, Chennai, Tamil Nadu. GC-MS analysis of this extract was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column (Length : 30.0m, Diameter : 0.25mm, Film thickness : 0.25µm Composed of 100% Dimethyl poly 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 1.51ml/min and an injection volume of 1ml was employed (split ratio: 10). Injector temperature 240°C; Ion source temperature 200°C. The oven temperature was programmed from 70°C (isothermal for 2 min.), with an increase of 300°C for 10 min. Mass spectra were taken at 70eV; a scan interval of 5 minutes with scan range of 40 – 1000 m/z. Total GC running time was 35 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 TurboMass.

Identification of components

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique (NIST08s), WILEY8 and FAME having more patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The Name, Molecular weight, Molecular formula and Structure of the component of the test material was ascertained.

RESULTS AND DISCUSSION

In this present study the ethanolic extract of phytochemical screening and antioxidant activity of the Ethanolic extract of *Cynodon dactylon* were investigated by using DPPH scavenging assay and reducing power of the extract. The phytochemical screening of ethanolic extract of *Cynodon dactylon* shows the presence of tannin, saponin, proteins and steroids are shown in table1.

Table1: Phytochemical analysis of ethanolic extract of *Cynodon dactylon*

S.NO	Phytochemicals	Test performed	*Cynodon dactylon*
1.	Alkaloids	Dragendorff's test	-
2.	Saponins	Frothe test	+
3.	Tannins	Ferric chloride test	+
4.	Anthraquinones	Born trager's test	-
5.	Flavonoids	Shinoda test	-
6.	Proteins	Bradford test	+
7.	Steroids	Liber mann-Buchard test	+

DPPH radical scavenging activity of ethanolic extract of *Cynodon dacton* and ascorbic acid are presented in figure1. Reducing power of ethanolic extract of *Cynodon dactylon* were presented in figure2.

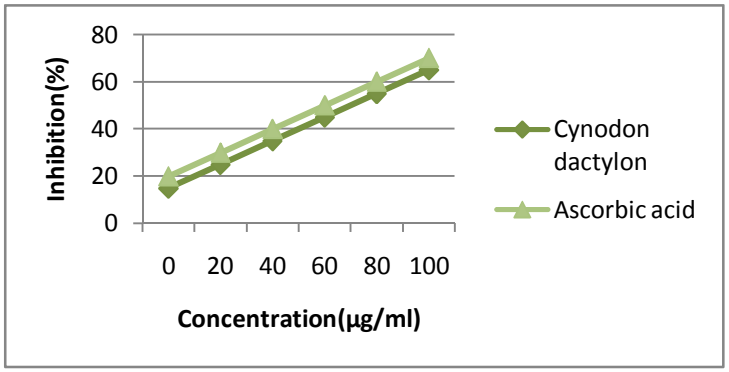

Fig. 1: DPPH radical scavenging activity of ethanolic extract of *Cynodon dactylon* . Shows inhibition of *Cynodon dactylon* and standard Ascorbic acid

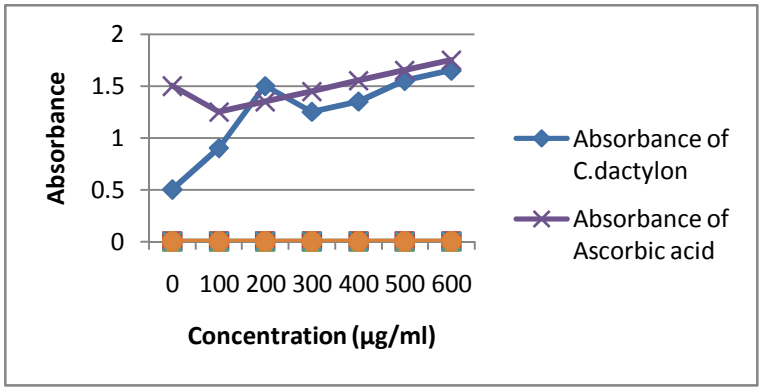

Fig2: Reducing power of ethanolic extract of *Cynodon dactylon* as compared to Ascorbic acid.

Ethanolic extract has got profound antioxidant activity. Both methods have proven the effectiveness of the ethanol extract compared to the reference standard antioxidant ascorbic acid. The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. Comparison of the antioxidant activity of the extract and ascorbic acid is shown in Figure1. The ethanol extract of *Cynodon dactylon* exhibited a significant dose dependent inhibition of DPPH activity. The ethanol extract of *Cynodon dactylon* and Ascorbic acid were found to be at 65% and 70% respectively

The reducing ability of a compound generally depends on the presence of reductants which have been exhibited antioxidative potential by breaking the free radical chain, donating a hydrogen atom. The

presence of reductants (i.e.antioxidants) in *Cynodon dactylon* extract causes the reduction of the Fe 3+/ferricyanide complex to the ferrous form. Therefore, the Fe 2+ can be monitored by measuring the formation of Perl's Prussian blue at 593 nm. Figure 2 shows the re-ductive capabilities of *Cynodon dactylon* extracts compared to ascorbic acid. The reducing power of ethanolic extract of *Cynodon dactylon* was very potent and the power of the extract was increased with quantity of sample.The reducing power of bioactive compounds has been reported to be associated with their antioxidant activity. The reducing power of *Cynodon dactylon* was found to be 1.65µg/ml.

Antimicrobial activity

The efficacy of the ethanolic extract of *Cynodon dactylon* is shown in Table 2. The ethanolic extract was more effective against *Salmonella typhii* than *Proteus mirabilis* and *Enterococcus faecalis*. The fungal species *Candida albicans* shows more effective against *Trichoderma viridie*. The results of Antimicrobial activity are given in fig3. which clearly show that the ethanolic extract of *Cynodon dactylon* has both Antibacterial and antifungal activity against the tested organisms.

Table2: Inhibition zone of diameter of ethanolic extract of *Cynodon dactylon*

S.No	Organisms	Concentration Zone of inhibition in mm					
		1000µg	500µg	250µg	100µg	10µg	Streptomycin
1.	*Proteus mirabilis*	20	18	16	14	-	21
2.	*Salmonella typhii*	26	22	16	10	-	24
3.	*Enterococcus faecalis*	9	8	6	5	-	22
4.	*Candida albicans*	14	12	11	9	-	18
5.	*Trichoderma viride*	9	8	5	4	-	15

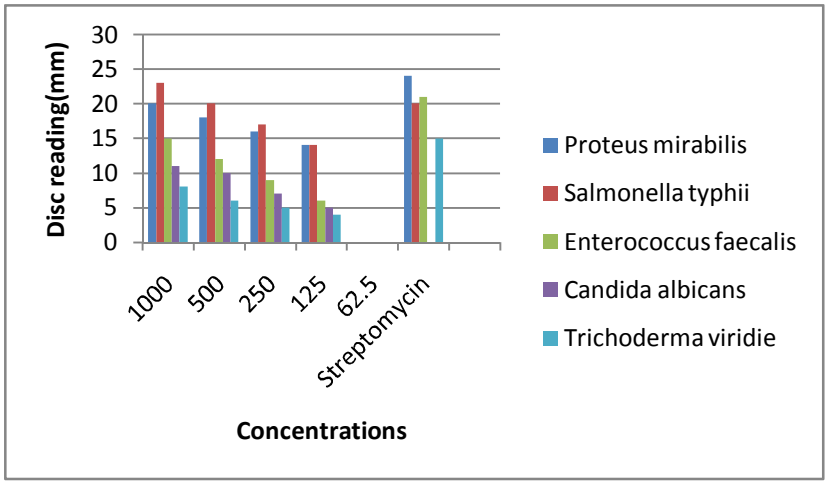

Figure 3: Antimicrobial activity of ethanolic extract of *Cynodon dactylon*

Thin layer chromatography

In order to identify the compounds, the extract was subjected to fractionation and purification of its components. Hence TLC was performed on aluminum sheets precoated with silica gel using capillary tube (2-5 l) and allowed the plates to dry. The plate was developed in Chloroform: Methanol(19:1) and visualized under UV illuminator. The color change indicates the presence of bioactive components in ethanolic extract of *Cynodon dactylon*.

Fig.4 : TLC of Ethanolic extract of *Cynodon dactylon* visualized under uv transilluminator

GC-MS technique

Twenty six compounds were identified in ethanolic extract of aerial and underground parts of *C. dactylon* by GC-MS analysis. The active principle Molecular Weight(MW),Concentration(%),

Molecular Formula(MF),Retention Time(RT) and their bioactivity are presented in Figure5 & Table3. The prevailing compounds were 2-Hexadecen-1-Ol, 3,7,11,15 Tetramethyl(12.05%), Hexadecanoic Acid, Ethyl Ester(6.49%), gamma.-Sitosterol(6.45%), 9-Octadecenoic acid, methyl ester(6.17%), Tetracontane(5.99%), N-Nonacosane(5.01%).

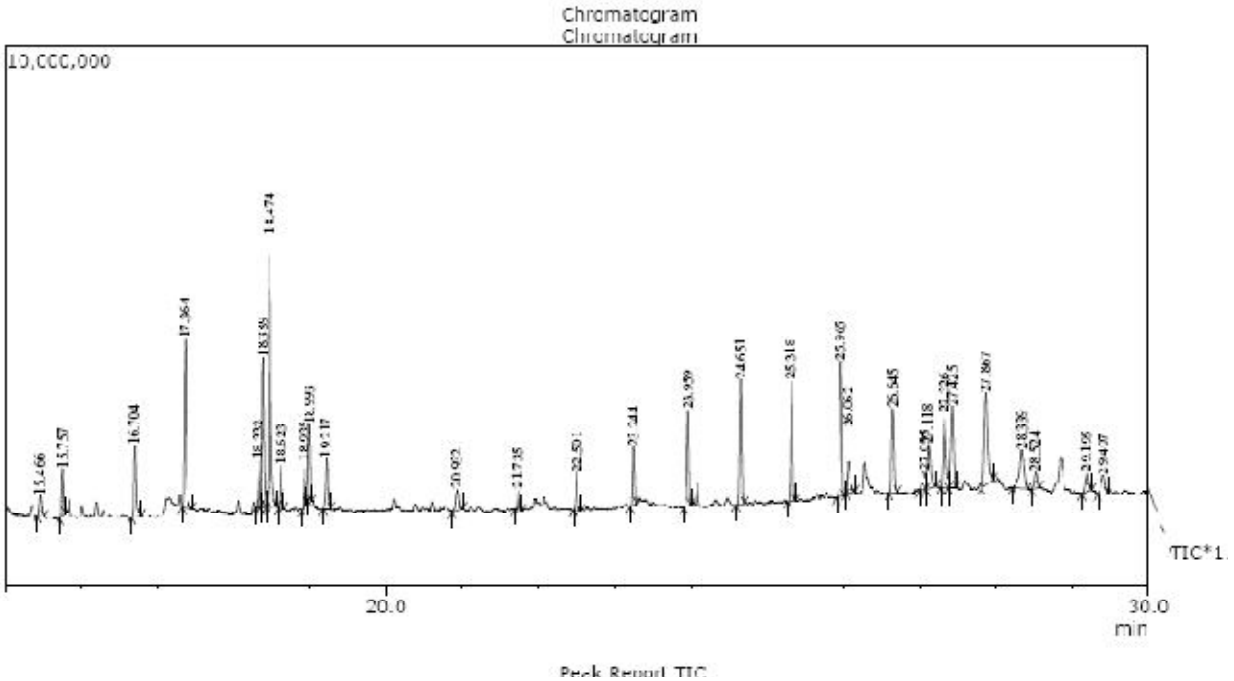

Figure 5: Chromatogram obtained from the GC- MS with the ethanolic extract of *Cynodon dactylon*

Table 3: Total ionic chromatogram (GC-MS) of *Cynodon dactylon* obtained with 70eV using an Elite -1 fused silica capillary column with He gas as the carrier.

No	RT	Name of the compound	Molecular Formula	Molecular weight	Peak area %	Common name
1.	15.466	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	$C_{13}H_{18}O_3$	222	0.97	-
2.	15.757	3,7,11,15-tetramethylhexadec-2-en-1-ol	$C_{20}H_{40}O$	296	1.74	Phytanic acid
3.	16.704	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O2$	270	3.07	Palmitic acid
4.	17.364	Hexadecanoic Acid, Ethyl Ester	$C_{18}H_{36}O2$	284	1.68	Palmitic acid ethyl este
5.	18.332	9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester	$C_{19}H_{34}O2$	294	6.17	Linoleic acid ethyl ester
6.	18.389	9-Octadecenoic Acid, Methyl Ester, (E)-	$C_{19}H_{36}O2$	296	12.05	Elaidic acid methyl ester
7.	18.474	2-Hexadecen-1-Ol, 3,7,11,15-Tetramethyl	$C_{20}H_{40}O$	296	1.61	Phytol
8.	18.623	Octadecanoic Acid, Methyl Ester	$C_{19}H_{38}O2$	298	1.61	Methyl stearate

9.	18.935	Linoleic acid ethyl ester	$C_{20}H_{36}O2$	308	1.61	Ethyl linoate
10	18.993	Octadeca-9,12,15-Trien-1-Ol	$C_{18}H_{32}O$	264	3.00	Linolenic acid
11.	19.217	Stearic acid, ethyl ester	$C_{20}H_{40}O2$	312	2.25	Ethyl stearate
12.	20.932	Octadecane, 1,1'-[(1-Methyl-1,2-Ethanediyl)Bis(Oxy)]Bis-	$C_{39}H_{80}O2$	580	1.25	-
13.	21.735	Eicosane	$C_{20}H_{42}$	282	0.84	Icosane
14.	22.501	Celidoniol, Deoxy-	$C_{29}H_{60}$	408	1.56	Nonacosane
15.	23.959	Tetratetracontane	$C_{44}H_{90}$	618	3.64	-
16.	25.965	Tetracontane	$C_{40}H_{82}$	562	5.99	-
17.	26.062	1-Eicosanol	$C_{20}H_{42}O$	298	1.80	Arachidyl alcohol
18.	26.645	Dotriacontane	$C_{32}H_{66}$	450	4.30	Dicetyl;
19.	27.055	Hexadecanal	$C_{16}H_{20}$	240	1.55	Palmitoyl
20.	27.118	Ergost-5-En-3-Ol, (3.Beta.,24r)-	$C_{28}H_{48}O$	400	2.86	Campsterol
21	27.326	Stigmasterol	$C_{29}H_{48}O$	412	4.81	-
22.	27.425	Pentatriacontane	$C_{35}N_{72}$	492	4.60	-
23.	27.867	Gamma.-Sitosterol	$C_{29}H_{50}O$	414	6.45	-
24.	28.524	Stigmasta-4,22-Dien-3-One	$C_{29}H_{46}O$	410	1.01	-
25.	29.195	Delta.4-Sitosterol-3-One	$C_{29}H_{48}O$	412	1.45	-
26.	29.407	Hexatriacontane	$C_{36}H_{74}$	506	1.58	-

DISSCUSSION

Nonacosane is a straight-chain hydrocarbon it plays a role in the chemical communication of several insects, including the female *Anopheles stephensi* mosquito[30]. Nonacosane occurs naturally and has been identified within several essential oils. It can also be prepared synthetically[31]. Stigmasterol also known as Wulzen anti-stiffness factor is one of a group of plant sterols, or phytosterols, that include β-sitosterol, campesterol, ergosterol (provitamin D_2), brassicasterol, delta-7-stigmasterol and delta-7-avenasterol, that are chemically similar to animal cholesterol. Phytosterols are Stigmasterol is used as a precursor in the manufacture of semisynthetic progesterone[32] a valuable human hormone that plays an

important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D_3[33]. The Upjohn company used stigmasterol as the starting raw material for the synthesis of cortisone[34] insoluble in water but soluble in most organic solvents and contain one alcohol functional group. Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast, and colon cancers. Studies have also indicated that a diet high in phytoesterols may inhibit the absorption of cholesterol and lower serum cholesterol levels by competing for intestinal absorption. Studies with laboratory animals fed stigmasterol found that both cholesterol and sitosterol absorption decreased 23% and 30%, respectively, over a 6-week period. It was demonstrated that it inhibits several pro-inflammatory and matrix degradation mediators typically involved in osteoarthritis-induced cartilage degradation[35]. It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties[36].

CONCLUSIONS

The results of this work supports the importance of *Cynodon dactylon* in various aspects. The present work confirms that ethanolic extract of aerial and underground parts of ethanolic extract of *Cynodon dactylon* can act as a good source of medicine in natural origin. The presence of various components reveals that the plant as nature can act as a drug for various disease like epilepsy, diabetes, cancer etc. and can serve as a secondary metabolite in the target of many receptors.

REFERENCES

1. Das NP, Pereira TA. Effect of flavonoids on thermal auto-oxidation of palm oil: structure Activity relationship**.** J Am oil Chem Soc. 1990; 67: 255-258.
2. Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs**.** J Agric Food Chem. 2001; 49(11): 5165-5170.
3. Cai YZ, Sun M, Corke H. Antioxidant activity of betalains from plants of the Amaranthaceae**.** J Agric Food Chem. 2003; 51(8): 2288-2294.
4. Sala A, Recio MD, Giner RM, Manez S, Tournier H, Schinella G, Rios JL. Anti inflammatory and antioxidant properties of *Helichrysum italicum***.** J Pharm Pharmacol 2002; 54(3): 365-371.

5. Rice-Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridham JB. The relative activities of Plant-derived polyphenolic flavonoid**.** Free radical Res. 1995; 22:375-383.
6. Parr A, Bolwell GP. Phenols in the plant and in man: The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile**.** J Sci Food Agric. 2000; 80: 985-1012.
7. Harlan J. *Cynodon* species and their value for grazing and Hay. Herbage Abstract. 1970; 40(3): 233-238.
8. Sujon MA, Mostofa M, Jahan MS, Das AR and Rob S. Studies on Medicinal plants against Gastrointestinal Nematodes of Goats**.** Bangl. J. Vet. Med. 2008; 6(2): 179–183.
9. Singh SK, Rai PK, Mehta S, Singh RK, Watal G. Curative Effect Of *Cynodon dactylon* Against Stz Induced Hepatic Injury In Diabetic Rats. Indian Journal Of Clinical Biochemistry. 2009; 24(4): 410-413.
10. Cheryl A L. Ethnomedicines used in Trinidad and Tobago for urinary problems and Diabetes mellitus. Journal of Ethnobiology and Ethnomedicine. 2006; 45(2): 1746-4269.
11. Singh SK, Kesari AN, Gupta RK, Jaiswal D, Watal G. Assessment of antidiabetic potential of *Cynodon dactylon* extract in streptozotocin diabetic rats. J Ethnopharmaco. 2007; 114(2): 174-179.
12. Jarald E E, Joshi S B, Jain D C. Antidiabetic activity of aqueous extract and non polysaccharide fraction on *Cynodon dactylon* Pers. Indian journal of Experimental Biology. 2008; 46(9): 660 -667.
13. Khajavi Rad A, Hadzadeh M A, Rajaei Z, Mohammadian N, Valiollahi S, Sonei M. The beneficial effect of *Cynodon dactylon* on ethylene glycol-induced kidney calculi in rats. Vrol J.2011; 8(3): 179 -84.
14. Das S, Dutta Choudhury M. Plants Used Against Gastro-Intestinal Disorders and As AntiHemorrhagic by Three Tribes of North Tripura District, Tripura, India. Ethnobotanical Leaflets.2010; 10(4): 467-78.
15. Kunja B. Satapathy, Binod B.Sahu, Gouri Shankar jena. Crop weeds diversity and their ethnomedicinal uses in the treatment of common ailments in Jaipur district of odisha(India). Int.J.Med.Arom.Plants. 2012; 2(1): 80 -89.
16. Oudhia P. Medicinal weeds in rice fields of Chhattisgarh (India). Int. Rice Res.1999; 24(1): 40.

17. Oudhia P. Medicinal weeds in groundnut fields of Chhattisgarh (India). Int. Arachis Newslett.1999; 19: 62-64.
18. Agharkar S P. Medicinal plants of Bombay presidency. Scientific Publ. Jodhpur, India. 1991; p. 80-87.
19. Oudhia P, Joshi BS and Kosta VK. The possibilities of preparing homeopathic drugs from the obnoxious weeds of Chhattisgarh. Bhartiya Krishi Anusandhan Patrika.1998; 13(1/2):53-57.
20. Dilip Kumar Pal. Determination of Brain Biogenic Amines in *Cynodon dactylon* pers & *Cyperus rotundus* treated mice. International Journal of Pharmacy and Pharmaceutical Science. 2009; 1(1): 190-197.
21. Santosh Kumar Singh, Prashant Kumar Rai, Dolly Jaiswal, and Geeta Watal. Evidence based Critical Evaluation of Glycemic Potential of *Cynodon dactylon*. Evidence-based Complementary and Alternative Medicine, 2007; 5(4): 415-420.
22. Evaluation of Anti Microbial Activity of *Cynodon dactylon.* Kanimozhi,D, Ratha bai V. IJRPS 2012; 2(2): 34-43.
23. Grouch IJ, Smith MT, Vanstadan J , Lewis MJ and Hoad GV. Identification of auximin a commercial seaweeds concentrate. J. Plant Physiol. 1992; 139:590- 594.
24. Matanjun P, Matanjun S Mohamadm , Mustapha NM, Muhammed K and Ming GH. Antioxidant activity of Phenolic content of eight sps of seaweeds from the north Borneo.J Appl Phycol. 2008; 20 (4): 367-373.
25. Harborne, J.B.(1973) Phytochemical methods: Aguide to modern techniques of plant analysis. Chapmanand Hall Ltd. London.
26. Trease GE, Evans WC (1983). Textbook of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher, London. pp. 343-383.(edeoga 29)
27. Eberhardt MV, Lee CY, Liu RH. Antioxidant activity of fresh apple. Nature. 2000; 405: 903-904.
28. Iris FF, Benzie I and Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: The FRAP Assay. Analytical Biochemistry. 1996; 239: 70–76.
29. Jigna Parekh, Sumitra V. Chanda. In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk J Biol. 2007; 31: 53-58.

30. Brei B, Edman JD, Gerade B, Clark JM . Relative abundance of two cuticular hydrocarbons indicates whether a mosquito is old enough to transmit malaria parasites. J. Med. Entomol. 2004; 41 (4): 807–9.
31. Bentley HR, Henry JA, Irvine DS, Mukerji D and Spring FS. Triterpenoids cyclolaudenol, a triterpenoid alcohol from opium. J. Chem. Soc. 1995; 32: 596–602.
32. Sundararaman P, Djerassi C. A convenient synthesis of progesterone from stigmasterol. J Org Chem.1997; 42 (22): 3633–3634.
33. Kametani T, Furuyama H. Synthesis of vitamin D3 and related compounds. Med Res Rev. 1987; 7 (2): 147–171.
34. Hogg John A. Steroids, the steroid community, and Upjohn in perspective: A profile of innovation. Steroids. 1992; 57 (12): 593–616.
35. Gabay O, Sanchez C, Salvat C, Chevy F, Breton M, Nourissat G, Wolf C, Jacques C, Berenbaum F. Stigmasterol: a phytosterol with potential anti-osteoarthritic properties. Am J Clin Nutr.2010; 18 (1): 106–116.
36. Panda S, Jafri M, Kar A, Meheta BK. Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from Butea monosperma. Fitoterapia. 2009; 80 (2): 123–126.