INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 January, 2014; received in revised form, 07 March, 2014; accepted, 26 April, 2014; published 01 July, 2014

PHYTOCONSTITUENTS INVESTIGATION BY LC-MS AND EVALUATION OF ANTI-MICROBIAL AND ANTI-PYRETIC PROPERTIES OF *CYNODON DACTYLON*

Zabin K. Bagewadi*, Siddanagouda R.S and Praveen G. Baligar

Department of Biotechnology, B.V. Bhoomaraddi College of Engineering & Technology, Hubli-580031, Karnataka, India

Keywords:
Cynodon dactylon, HPLC, FTIR, H^1-NMR, LC-MS, Antimicrobial, Antipyretic

Correspondence to Author:

Zabin K. Bagewadi

Assistant Professor, Department of Biotechnology, B.V. Bhoomaraddi College of Engineering & Technology, Vidyanagar, Hubli-580031, Karnataka, India

E-mail: zabinb@gmail.com

ABSTRACT: Among numerous species of plants growing in the wild in India, Garike, or Druva or taxonomically the *Cynodon dactylon sp*. occupies its unique place in the traditions, religions and cultures of different societies. In the present study, Phytochemical screening of methanolic, petroleum ether, ethanolic and aqueous extracts of *Cynodon dactylon* revealed the presence of 17 different phytoconstituents. Quantitative analysis of methanolic extract revealed the concentration of various bioactive constituents. Antimicrobial activity of both Ethanolic and Methanolic extracts of *Cynodon dactylon* showed good inhibitory effect for all the tested Bacterial and Fungal strains including E. *coli, B. subtilis, S. typhimurium, M. luteus, K. pneumoniae, S. aureus, P. vulgaris, P. aeruginosa, C. albicans, A. flavus,* A. *fumigatus, P. notatum, Sporothrix schenckii, Stachybotrys chartarum and C. neoformans* by Agar well method. TLC for all the extracts showed bands in U.V for the presence of Flavonoids, Alkaloids, Phenols and Glycosoids. HPLC analysis of methanolic extract gave peaks at retention time 2.779, 4.115 and 9.840. LC-MS of methanolic extract of *Cynodon dactylon* showed the presence of eight compounds. H^1-NMR showed groups relating to acyclic aliphatic compounds and α-mono substituted. FTIR analysis of Methanolic, Aqueous and Petroleum Ether extract revealed the presence of 1^0Amines, Alkanes (CH_3, CH_2 & CH), saturated Aldehydes etc. functional groups. Antipyretic activity of both Ethanolic and Methanolic extracts showed a significant ($P<0.05$) dose dependent antipyretic effect in yeast induced elevation of body temperature in experimental rats. Thus, the present study concludes the medicinal significance of *Cynodon dactylon* extracts having potential Antimicrobial and Antipyretic properties which leads to pharmaceutical applications.

INTRODUCTION: Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs [1]. Plants produce diverse types of bioactive molecules, making them a rich source of different types of medicines.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(7).2874-89

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(7).2874-89

This revival of worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in healthcare [2]. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts. In India, almost 95% of the prescriptions were plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha [3]. Extracts of medicinal plants are useful in the treatment of several health problems such as bacterial [4], including diabetes, cancer and infectious diseases. *Cynodon dactylon* (Family: *Poaceae*) is an important ingredient in various Ayurvedic preparations.

Cynodon dactylon is a perennial grass that has a variety of medicinal properties [5]. It is cultivated throughout the tropics and subtropics. Whole herb or its root & stalk are used for medicinal purpose [6]. It is reported to have important properties like anabolic, antiseptic, astringent, cyanogenetic, demulcent, depurative, laxative, diuretic and emollient [7].

C. dactylon is used by traditional healers for purifying the blood, anuria, biliousness, conjuctivitis, diarrhoea, gonorrhoea, itches and stomachache [8]. A decoction of the root is used as a diuretic in the treatment of dropsy and secondary syphilis.

An infusion of the root is used to stop bleeding from piles. The expressed juice of plant act as astringent and is applied to bleeding cuts and wounds to stop. Leaf, root and rhizome of the plant have been used in folk medicine of different countries [9], in Ayurveda, *Cynodon dactylon* shows many pharmacological activities like antidiabetic, antioxidant, antidiarrheal, hepatoprotective, anti-ulcer, immunomodulator, CNS depressant, antimicrobial and germicidalas [10], antihysteria, antipsychotic [11] antigonorrheal infection [12], anti-viral, as well as hypolipidemic [13], hypoglycemic agent [14].

In India, the plant is reputed for the treatment of melena, thirst, anorexia, burning sensations of the body, pruritis, miscarriage and erysipelas, and its leaf juice with a pinch of common salt has been used orally in stomachache [15]. Decoction of whole plant has been given orally to cure menstrual problem. In addition, studies by some Iranian cardiologists have shown cardio protective effects in the patients who used the plant traditionally. Santosh kumar and coworkers evaluated the anti diabetic activity of aqueous extract of *Cynodon dactylon* [16].

Garjani A and co staff evaluated the rhizomes of *Cynodon dactylon* were used for the treatment of heart failure in folk medicine [17]. Najafi M, along with Gajrani A investigated probable antiarrhythmic effects of *C. dactylon (L.) Pers.* (Family Poaceae) against ischemia/ reperfusion (I/R)-induced arrhythmias in isolated rat heart [18].

The present study was undertaken, since no detailed study was available regarding Analytical chemistry methods such as LC-MS, IR, H^1-NMR and HPLC. The cytomorphological report of the leaves of *Cynodon dactylon* has been found inadequate, therefore the detailed pharmacognostical study like Phytochemical screening, Analytical Characterization, Antipyretic and Antimicrobial activity of the leaves of *Cynodon dactylon* was decided to bring out the salient diagnostic features which would help in crude drug identification.

MATERIALS AND METHODS:

Collection and Authentication of Plant material: The plant *Cynodon dactylon* was identified for its ethno-medicinal uses and collected from the village called Adur nearby Haveri District, Karnataka, India. The plant was grown in warm temperate climatic conditions of about 28 -30^0c. The *Cynodon dactylon* plant was taxonomically identified [19, 20] and authenticated by Dr. B. D. Huddar, Professor and Head Department of Botany, S.K Arts College & H.S.K. Science Institute Vidyanagar, Hubli, Karnataka, India. The plant sample is deposited at Department of Biotechnology, B. V. Bhoomaraddi College of Engineering & Technology Hubli, Karnataka, India.

Preparation of Plant Extract: The whole plant was cleaned and shade dried for one week. The dried plants were pulverized by an electrical blender and passed through the 20 µ mesh sieve. The aqueous extraction and extraction using organic solvents such as, Ethanol, Methanol and Petroleum ether was carried out using Soxhlet apparatus. The extraction was carried out for 24 hrs at 60^oC. The extracts were concentrated at 45°C using Rotary vacuum evaporator (Scietek, MODEL: RE 300). The concentrated extracts were kept in refrigerator at 4^oC until further use [21].

Preliminary Phytochemical screening: Phytochemical screening of methanolic, ethanolic, petroleum ether and aqueous extract of *Cynodon dactylon* were carried out. In general, test for the presence or absence of phytochemical compounds using standard methods involves the addition of an appropriate chemical agent to all the extracts in a

test tube and shaken. The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Phytochemical screening of various fractions from Ethanolic, Methanolic, Petroleum ether and Aqueous extracts of *C. dactylon* leaves were carried out for metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols & triterepenoids, tannins, quinines, cumarins, resins, anthroquinones, p*hlobatannin*, catechol, acidic compounds, reducing sugars, carbohydrates, proteins & amino acids, phenols and glycosides [22,23,24].

Quantitative analysis of Total Alkoloids, Flavonoids and Phenols:

1. **Alkoloids** [25]: Bromocresol green solution (BCG) (1×10^{-4}) was prepared by heating 69.8 mg Bromocresol green with 3 ml of 2N NaOH and 5 ml of distilled water until completely dissolved and the solution was diluted to 1L with distilled water. Phosphate buffer (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water). Atropine (100µg/ml) was used as standard. Accurately measured aliquots (40-100µg/ml) of Atropine standard were taken. Then, required amount of phosphate buffer pH 4.7 was added to make-up a volume of 5ml. 5ml of BCG solution was added and mixture was shaken with 4 ml of chloroform. The absorbance of the complex in chloroform was measured at 470 nm in spectrophotometer against blank prepared as above without atropine. Already extracted plant sample using different solvents stored at 4^0c were taken in a separating funnel and washed with chloroform 3 times. Aliquots of plant extracts were taken and assayed in the same way as mentioned above.
2. **Flavonoids** [26]**:** Total flavonoids contents were estimated with the aluminium chloride colorimetric assay. Aqueous and Ethanolic extracts that has been adjusted to come under the linearity range i.e. (400µg/ml). 1mg/ml of standard Quercetin was prepared. Accurately measure aliquots (0.2- 1 mg/ml) of a standard were taken. 0.3ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3ml of 10% $AlCl_3$ was added. After 6 min, 2ml of 1 M NaOH was added and the total volume was made up to 10ml with distilled water. Then the solution was mixed well and the absorbance was measured against a freshly prepared reagent blank at 510 nm.
3. **Estimation of total phenols** [27]**:** To estimate the total phenols in the test sample, Gallic acid (a phenol) standard was used. A stock solution (40 mg/ml) of Gallic acid was prepared in 80% ethanol, from which 0.1 to 0.9 ml i.e., 4 mg/ml to 9 mg/ml was transferred in to test tubes and volume in each case was raised to 1 ml with 80% ethanol. 1 ml of Folin – Ciocalteau reagent (prepared by diluting the reagent with distilled water in 1:2 ratio just before use) accompanied by 2 ml of 20% of Na_2CO_3 solution was added and the mixture was shaken vigorously. Each of these were boiled on a water bath (1 min), cooled and diluted to 25 ml with distilled water. The absorbance was taken at 750 nm using a spectrophotometer against a blank. The test sample was processed in this similar manner.

Antimicrobial activity:

1. **Agar well method:** Antibacterial activity of all plant extracts were tested by modified Agar well method. Inoculum suspension was spread over the agar plates using sterile L-shaped glass rod. Well of 0.5 cm in diameter was made in inoculated media and 150µl plant extracts were aseptically filled into the well. Later the plates were placed at room temperature for an hour to allow diffusion of extract into the agar. Then the plates were incubated for 24 hrs at 37˚C. The results were recorded by measuring the diameter of inhibitory zone using a transparent meter rule at the end of 24-48 hrs [28].
2. **Broth Dilution method:** Quantitative analysis of Antibacterial effect of methanolic extracts of *Cynodon dactylon* were determined by Broth dilution method. 1ml of plant extract was added to 10 ml nutrient broth in 50 ml of Erlenmeyer flask. The tubes were then inoculated with appropriate test bacteria and incubated at room temperature in a controlled orbital shaker at 120 rpm.

The inhibition of Bacterial growth was determined by measuring the absorbance at 625 nm at different time interval.

Thin Layer Chromatography: The methanol and ethanol extracts of the *Cynodon dactylon* were subjected to Thin Layer Chromatographic analysis, to find the presence of number of chemical constituents to support the chemical test. For the detection of alkaloids in all plant extracts, the standard used was atropine. The alkaloid spots were separated using the solvent mixture Toluene: Ethyl Acetate: Acetic Acid (8.7:1.0:0.3).The colour and R_f values of the separated alkaloids spots were recorded both under ultraviolet (254nm) and visible light. Some spots were detected by spraying Dragendorff's reagent. The phenols were separated using Toluene: Propanol (70:30) solvent mixture. The colour and R_f values of these phenols were recorded under visible light after spraying the plates with Folin-Ciocalteu's reagent and heating at 80^{o}C for 10min [29].

Liquid Chromatography-Mass Spectroscopy (LC-MS): LC-MS analysis of the methanolic root extract of *Cynodon dactylon* was carried out using Thermo/Finnigan Surveyor System consisting of a degasser, binary pump, auto sampler, and column heater. The column outlet was coupled to a Thermo fleet (LCQ-Fleet) Ion Trap mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out in a personal computer with Data Analysis software (Qual Browser; Thermo Electron, San Jose, CA). For the chromatographic separation, a phenomenex luna 5-µm C8 column (250 × 4.6 mm) was used. The column was held at 95% Solvent A (0.1% acetic acid in water) and 5% Solvent B (0.1% acetic acid in acetonitrile) for 1 min, followed by an 11 min step gradient from 5% B to 100% B, then 4 min with 100% B. Finally, elution was achieved with a linear gradient from 100% B to 5% B for 2 min. The flow rate was 200 µl / min and injection volume was 5 µl. The following parameters were used throughout the MS experiment: for electro spray ionization with positive ion polarity the capillary voltage was set to 20 V, the capillary temperature to 300°C, the nebulizer pressure to 40 psi, and the drying gas flow rate to 15 L/ min.

Fourier Transform Infrared Spectrophotometer (FTIR): ATR model FTIR Spectrophotometer (Bruker Co., Germany) was used for analysis of the methanolic, petroleum ether, aqueous crude extract of *Cynodon dactylon.* The spectrum was focus in the mid IR region of 400-40000 cm^{-1} by the KBr pellet technique. The spectrum was recorded using Attenuated Total Reflectance (ATR) technique beach measurement.

HPLC Analysis: A modular Shimadzu LC-10 system comprised of a LC-10AD pump, a CTO-10A column oven, a SPD-10A UV-DAD detector, a CBM-10A interface and a LC-10 Workstation was utilized. A LC-18 column (250 mm x 4 mm i.d. x 5 mm) from Supelco (Bellefonte, USA) was employed, at 30^{o}C. Separations were done in the isocratic mode, using acetonitrile: water (40:60) v/v at a flow rate of 1mL min^{-1}; with an injection volume of 20 µl; UV detection was at 274 nm.

H^1-NMR Analysis: H^1-NMR spectra of methanol extract of *Cynodon dactylon* was recorded on a NMR-400 MHz (with multi-nuclei analysis from H^1) and chemical shifts were recorded as δ values. The result graph was compared with the reference chart and possible functional group present in the plant were determined [30].

ANTIPYRETIC ACTIVITY:

Brewer's yeast induced Hyperpyrexia method: Wistar albino rats of either sex weighing 150-200 g were procured from animal house of K. L. E. University's College of Pharmacy, Hubli, were used for the study after the clearance from Institutional Animal Ethics Committee (KLECOPH/IAEC/ Pharmacology/08-09/08).The study protocols were duly approved by the Institutional Animal Ethics Committee (IAEC) of KLE Pharmacy College, Hubli. Studies were performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. Wistar rats (150-200 g) of either sex were selected and kept for one week to acclimatize to laboratory conditions before starting the experiment. They were subjected to standard diet and water *ad libitum*, but 12 hr prior to an experiment, the rats were deprived of food but not water.

A dose of 200 mg/kg was taken as an effective dose for both methanolic and aqueous extracts of *Cynodon dactylon* to evaluate antipyretic activity. Wistar rats were divided into four groups of six rats each. Animals were fevered by injecting 20 mg/kg of 20% suspension of Brewer's yeast subcutaneously [31]. Initial rectal temperature was recorded. After 18h, animals that showed an increase of 0.3-0.5^{o}C in rectal temperature were selected. The test extracts, reference standard Paracetamol (150 mg/kg) and control saline vehicle were administered orally and rectal temperature was recorded by digital thermometer 30 min before and 0.5, 1, 2, 3, 4, 5 and 6 h after extracts/ drug administration.

RESULTS:

Preliminary Phytochemical Screening: The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The extracts of *Cynodon dactylon* have revealed the presences of alkaloids, flavonoids, glycosides, phenols, saponins, sterols & triterpenoids, tannins, quinines, coumarins, resins, anthraquinones, *phlobatannin*, catechol, acidic compounds, reducing sugars, carbohydrates, proteins & amino acids, phenols and glycosides. Results of preliminary screening of *Cynodon dactylon* plant extracts are shown in **Table 1.**

TABLE 1: PHYTOCHEMICAL ANALYSIS OF EXTRACTS OF *CYNODON DACTYLON*

Sl. No.	Tests	Aqueous Extract	Methanol Extract	Petroleum Ether Extract	Ethanol Extract
1.	**Alkaloids**				
	a. Mayer's test	-	+	-	-
	b. Dragendorff's test	-	+	-	-
	c. Wagner's test	-	+	-	-
	d. Hager's test	-	+	-	-
	e. Tannic acid test	-	+	-	-
2.	**Flavonoids**				
	a. Shinoda test	-	+	-	-
	b. Alkaline reagent test	-	+	-	-
	c. Ammonium test	-	+	-	-
	d. Aluminium chloride test	-	+	-	-
3.	**Saponins**				
	a. Emulsion test	+	-	-	-
	b. Frothing test	+	-	-	-
4.	**Steroids & Triterpenoids**				
	a. Liebermann- Burchard test	-	+	-	+
	b. Salkowski test	-	+	-	+
5.	**Tannins**				
	a. Lead sub-acetate test	+	+	-	+
	b. Ferric chloride test	+	+	-	+
6.	**Phenols**				
	a. Bromine water test	+	+	-	+
	b. Ferric chloride test	+	+	-	+
7.	**Quinones**	+	+	+	+
8.	**Glycosides**				
	a. Raymond's test	+	+	-	+
	b. Legal's test	+	+	-	+
9.	**Carbohydrates**				
	a. Molisch's test	+	+	+	+
	b. Camnelisation	+	+	+	+
	c. Selwinoff's test	+	+	+	+
10.	**Anthraquinones**	-	-	-	-
11.	**Resins**				
	a. Precipitate test	+	+	-	+
	b. Colour test	+	+	-	+

12	**Resins**				
	a. Precipitate test	+	+	-	+
	b. Colour test	+	+	-	+
13	**Coumarins**	+	+	-	+
14	**Acidic compounds**	-	+	-	-
15	**Phlabotannins**	-	+	-	-
16	**Catechol**	-	+	-	+
17	**Proteins & Amino Acids**				
	a. Millons test	+	-	-	+
	b. Ninhydrin test	+	-	-	+

Quantitative analysis of Total Alkaloids, Flavonoids and Phenols: For alkaloids, a yellow-colored complex with a maximum absorption was developed and standard used was atropine. This complex was completely extractable by chloroform at pH 4.7.Concentration of alkaloids in methanolic root extract was 14.6 µg/ml. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenols. These compounds possess abroad spectrum of chemical and biological activities including radical scavenging properties. Using the standard plot of quercetin (y = 0.001x + 0.005) concentration of Flavonoids in Methanolic root extract was found to be 0.05 mg/ml quercetin equivalent/g of dry sample. Quantification of Phenols of *Cynodon dactylon* was carried out by FCR method with Gallic acid standard, concentration of phenols in Methanol root extract was found to be 0.283 mg/ml and comparatively high concentration was found in the roots**.**

Antimicrobial Activity: The Methanolic and Ethanolic extract of *C. dactylon* (150 µl) were investigated for their antimicrobial properties against gram positive bacteria and gram negative bacteria using agar well method. *E. coli*, *B. subtilis*, *S. aureus* and were more susceptible in the Ethanolic extract and no result was found in aqueous extract. Ethanolic extract showed highest zone of inhibition for *Proteus vulgaris* (12mm). Methanolic extract showed good zone of inhibition for *Streptococcus aureus* (8mm). The extracts were also tested for anti-fungal activity, both Methanolic and Ethanolic extracts showed significant results. Ethanolic extract showed highest zone of inhibition for *Candida albicans* (12mm). Methanolic extract showed good zone of inhibition for *Aspergillus fumigates* (8mm). Results of Antimicrobial activity of both extracts are shown in **Table 2** and **Table 3** **Figures 1-7** shows the zone of inhibition for different bacteria.

TABLE 2: ANTIBACTERIAL ACTIVITY OF DIFFERENT EXRACT

Micro organisms	Ethanolic extract (150µL) (Zone of inhibition in mm)	Methanolic extract (150µL) (Zone of inhibition in mm)
E. coli	3	6
Bacillus subtilis	8	7
Proteus vulgaris	12	3
Micrococcus leutus	5	4
Salmonella typhimurium	6	3
Streptococcus aureus	9	8
Pseudomonas aeruginosa	2	4
Klebsiella pneumonia	9	5

TABLE 3: ANTI FUNGAL ACTIVITY OF DIFFERENT EXTRACTS (ZONE OF INHIBITION IN MM)

Microorganisms	Ethanolic extract (150µl)	Methanolic extract (150µl)
Candida albicans	9	6
Aspergillus fumigates	6	7
Sporothrix schenckii	1	1.1
Pencillium notatum	2	2.6
Aspergillus flavus	4	5
C. Neoformans	1.3	1
Stachybotrys chartarum	1.2	3

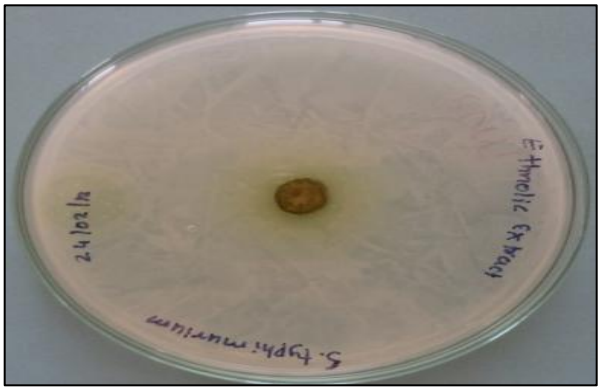

FIGURE 1: ZONE OF INHIBITION FOR *S.TYPHIMURIUM*

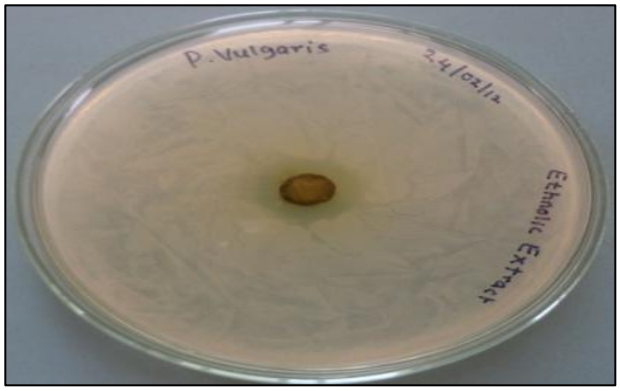

FIGURE 2: ZONE OF INHIBITION FOR *P.VULGARIS*

FIGURE 3: ZONE OF INHIBITION FOR *S.AUREUS*

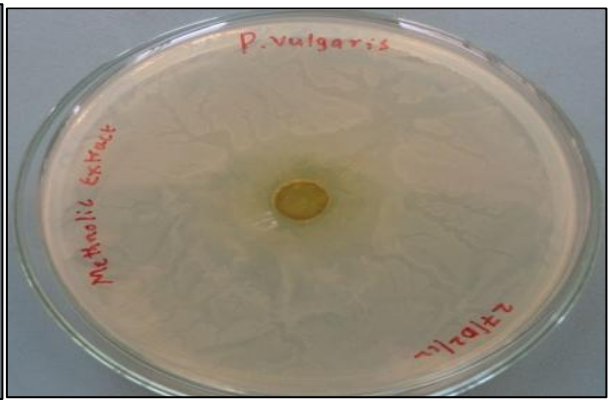

FIGURE 4: ZONE OF INHIBITION FOR *P.VULGARIS*

FIGURE 5: ZONE OF INHIBITION FOR *B.SUBTILLIS*

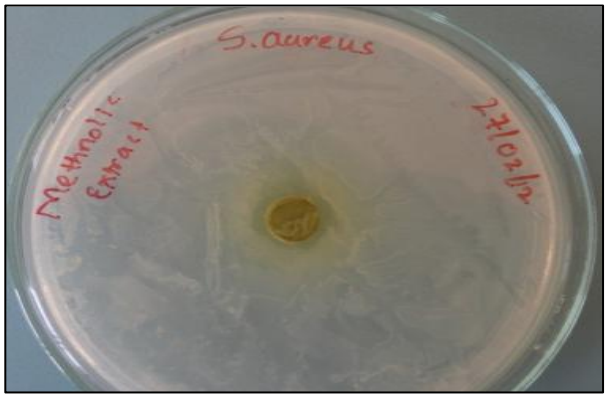

FIGURE 6: ZONE OF INHIBITION FOR *S.AUREUS*

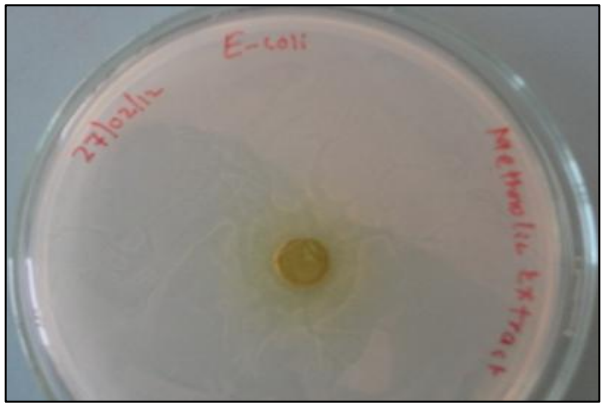

FIGURE 7: ZONE OF INHIBITION FOR *E.COLI*

FIGURE 8: *CYNODON DACTYLON* PLANT

Broth dilution method: Results of broth dilution technique showed, the Ethanolic extract successfully controlled the growth of *E. coli, B. subtilis, streptococcus aureus, Proteus vulgaris* and *Salmonella typhimurium* at different time intervals. Methanolic extract successfully controlled the growth of *E. coli, Klebsiella pneumonia, B. subtilis, streptococcus aureus, Proteus vulgaris* and *Salmonella typhimurium* at different time intervals. **Figures 9-16** shows the rate of inhibition for different bacteria using methanolic root extracts.

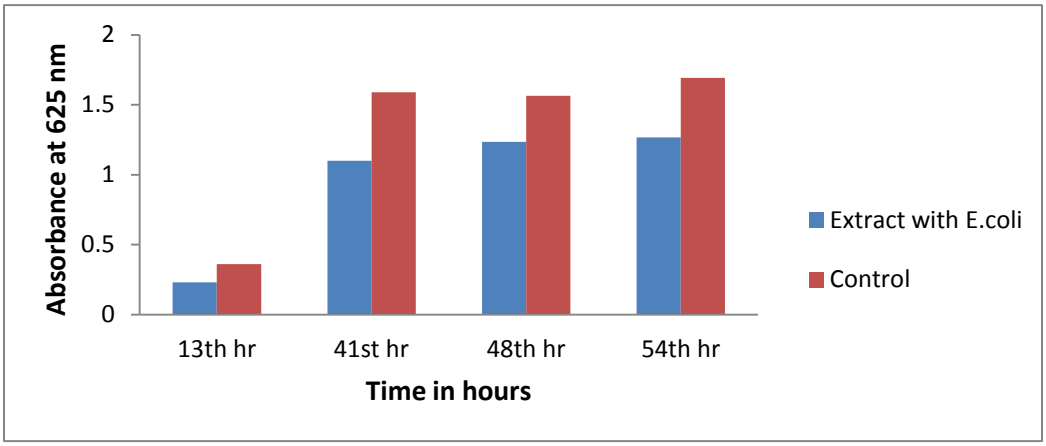

FIGURE 9: THE RATE OF INHIBITION FOR *ESCHERICHIA COLI* BY METHANOLIC ROOT EXTRACT

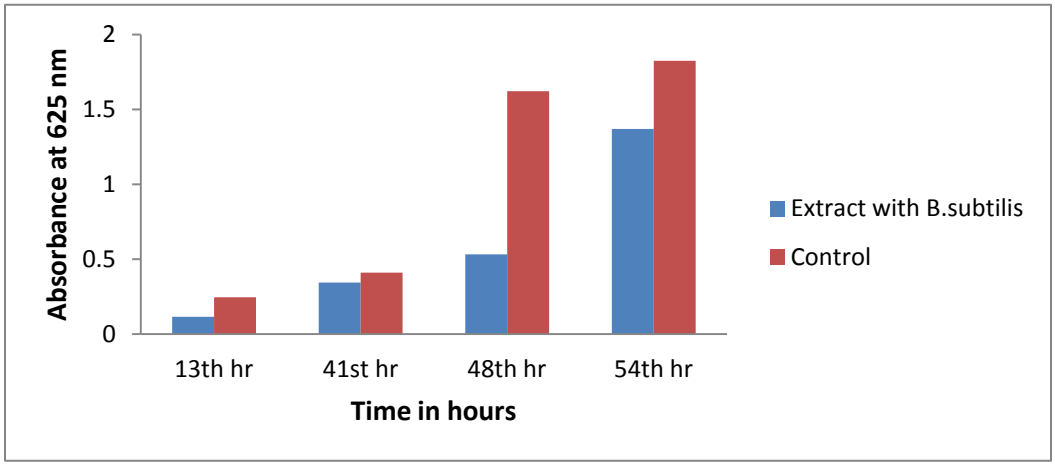

FIGURE 10: THE RATE OF INHIBITION FOR *BACILLUS SUBTILIS* BY METHANOLIC ROOT EXTRACT

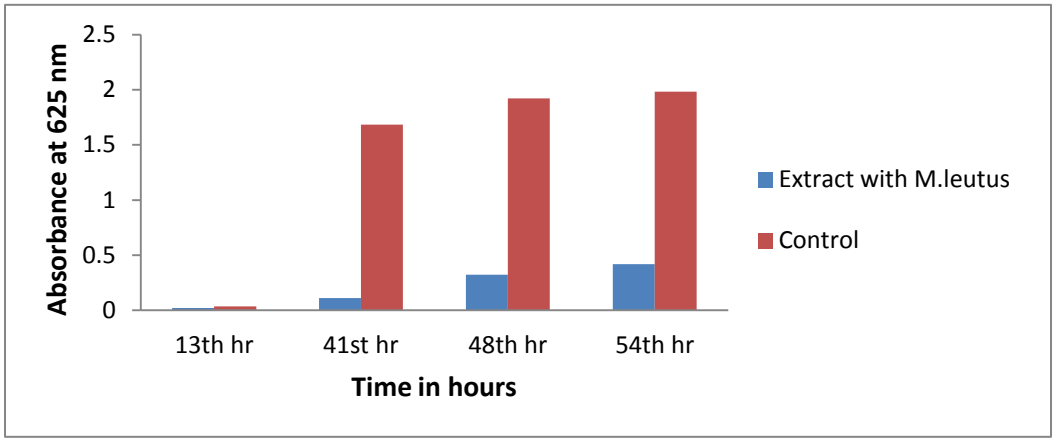

FIGURE 11: THE RATE OF INHIBITION FOR *MICROCOCCUS LEUTUS* BY METHANOLIC ROOT EXTRACT

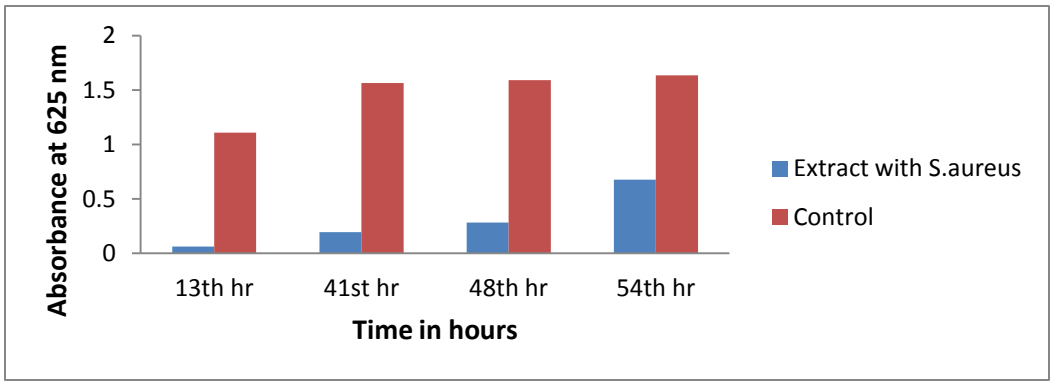

FIGURE 12: THE RATE OF INHIBITION FOR *STREPTOCOCCUS AUREUS* BY METHANOLIC ROOT EXTRACT

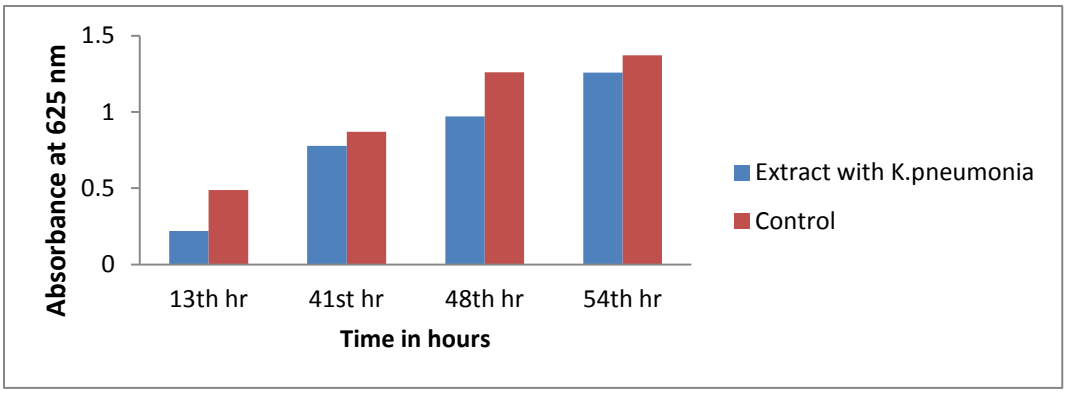

FIGURE 13: THE RATE OF INHIBITION FOR *KLEBSIELLA PNEUMONIA* BY METHANOLIC ROOT EXTRACT

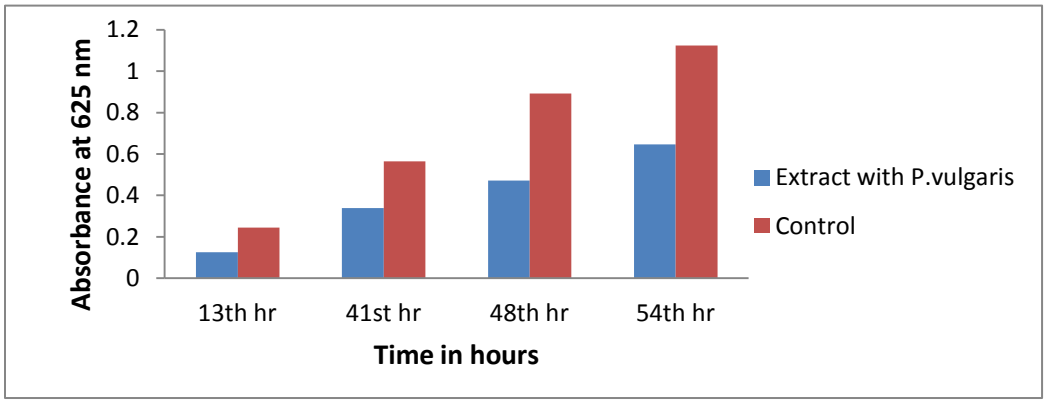

FIGURE 14: THE RATE OF INHIBITION FOR *PROTEUS VULGARIS* BY METHANOLIC ROOT EXTRACT

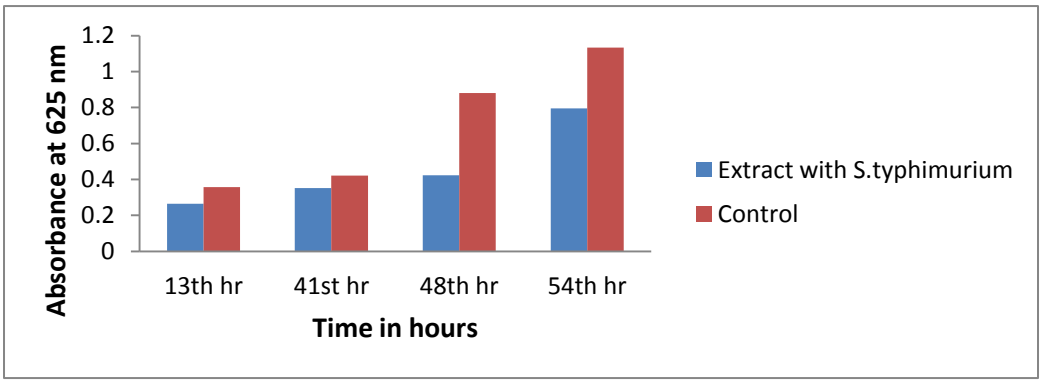

FIGURE 15: THE RATE OF INHIBITION FOR *SALMONELLA TYPHIMURIUM* BY METHANOLIC ROOT EXTRACT

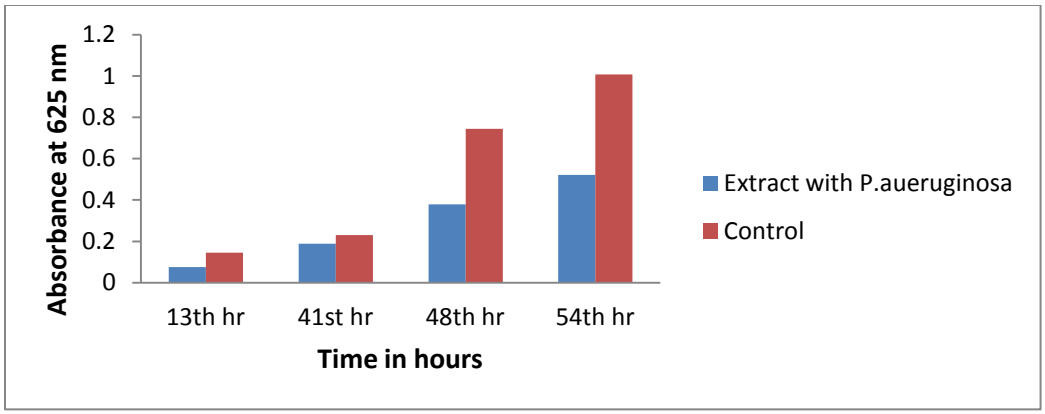

FIGURE 16: THE RATE OF INHIBITION FOR *PSEUDOMONAS AUERUGINOSA* BY METHANOLIC ROOT EXTRACT

Thin Layer Chromatography: Thin Layer Chromatography was used to detect spots from different solvent extracts. The standard used for the Thin Layer Chromatography of alkaloids was Atropine. An orange pink colored Spot was observed for the standard on using the system Toluene: Ethyl Acetate: Acetic Acid (8.7:1.0:0.3) and spraying with Dragendorff's reagent. The Rf value for the Alkaloids when compared with Atropine was found to be 0.92. The sample spots of methanolic whole plant extract, Ethanolic root extract, and methanolic root extract also showed similar colored spot on development. For Phenols standard used for the Thin Layer Chromatography of Phenols is Gallic acid. A green color spots were observed on using the solvent system Toluene: Propanol (70:30). The spraying reagent used was FCR. The Rf value was found to be 0.70.The sample spots of methanolic whole plant extract, Ethanolic root extract, and methanolic root extract also showed similar colored spot on development.

Liquid Chromatography-Mass Spectroscopy (LC-MS): The Liquid Chromatography Mass Spectroscopy (LC-MS) of Methanolic root extract of *Cynodon dactylon* shown in **Figure 17** showed molecular peak (base peak) at m/z 383.3 is good agreement with the compound (-) – cornigerine (1). The compound showed molecular peak (base peak) at m/z 120 is good agreement with empirical formula $C_8H_{18}O$ i.e. (2-Ethylhexanol) (2).The fragmentation has showed the peak at m/z 118.8 and it showed the presence of compound Benzofuran 2-3-dihydro- (3) .The mass spectrum showed the molecular peak (base peak) at m/z 150 is good agreement with empirical formula $C_9H_{10}O_2$ i.e. (Ethyl benzoate) (4). The fragmentation has showed the peak at m/z 149.9 and it showed the presence of 2-methyle-4-vinylphenyle (5). The compound showed molecular peak (base peak) at m/z 228 is exactly in agreement with the empirical formula $C_{10}H_9ClO_4$ i.e. Methyl 2-(2-chloroacetoxy) benzoate. The fragmentation has also showed the exact value at m/z 228.2 so it showed the presence of Benzaldehyde, 3-(chloroacetoxy) -4-methoxy (7). The compound showed molecular peak (base peak) at m/z 431 is good agreement with the compound Ergosta-7, 22-dien-3β-5α, 6β-triol (8).

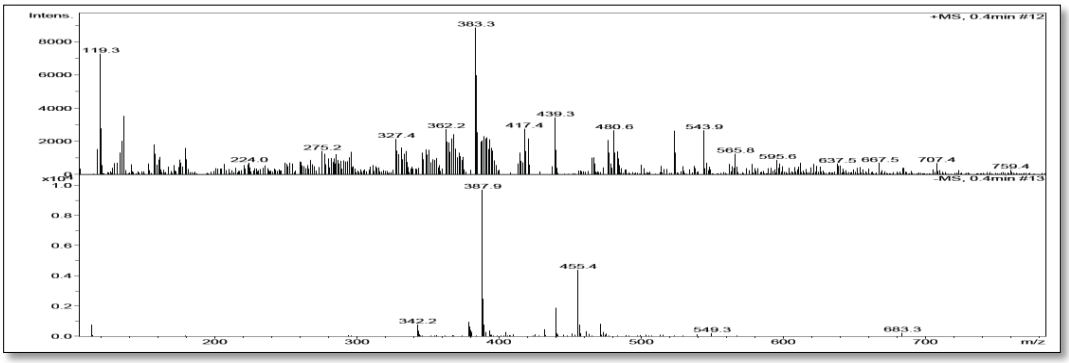

FIGURE 17: LC-MS ANALYSIS OF METHANOLIC EXTRACT OF *CYNODON DACTYLON*

Fourier Transform Infrared Spectroscopy (FTIR): The infrared spectrum of the Methanolic extract of *Cynodon dactylon* shown in **Figure 18** showed characteristic absorption band of NH (1^0Amines), 2 bands at 3402.2 cm^{-1}, Alkanes CH, CH_2 and CH_3 stretching at 2990.0 cm^{-1} and at 2850 cm^{-1}, C=O (saturated Aldehyde) stretching at 1739cm^{-1}, NH_2 scissoring (10 – amines) med stretching at 1627.8 cm^{-1}, N-H (2^0 - amide) med at 1512.1 cm^{-1}, oxalic acid (C-O-H bending) at 1419.5 cm^{-1}, C-O-H bending at 1114.8 cm^{-1}, O-C stretching at 1056 cm^{-1}, =C-H &=CH_2 stretching at 925.8 cm^{-1}, C- H deformation stretching at 617.2 cm^{-1}. The infrared spectrum of the aqueous extract of *Cynodon dactylon* shown in **Figure 19** showed characteristic absorption band of OH (H-bonded) at 3355.9 cm^{-1}, Alkanes (CH_3, CH_2 and CH) stretching at 2935.5cm^{-1}, Alkenes C=C (symmetry reduces intensity) at 1635.5cm^{-1}, N-H (2^0amide)|| band at 1512.1 cm^{-1} , CH_2 & CH_3 deformation at 1407.9 cm^{-1}, C-O stretching at 1076.2 cm^{-1}, =C–H & =CH_2 stretching at 926.6 cm^{-1}. The infrared spectrum of Petroleum ether extract of *Cynodon dactylon* shown in **Figure 20** showed characteristic absorption band of week N-H (1^0-Amines) at 3398.3 cm^{-1}, Alkanes (CH_3,CH_2 & CH) stretching at 2916.2 cm^{-1} and 2850.6 cm^{-1}, C=O (saturated Aldehyde) stretching at 1735 cm^{-1}, C=O (amide | bond) stretching at 1635.5 cm^{-1}, N-H (2^0 – amide) || bond medium at 1515.9 cm^{-1}, CH_2& CH_3 deformation at 1461 cm^{-1} & 1377.1 cm $^{-1}$, OH bond (out of plane) at 721.3 cm $^{-1}$.

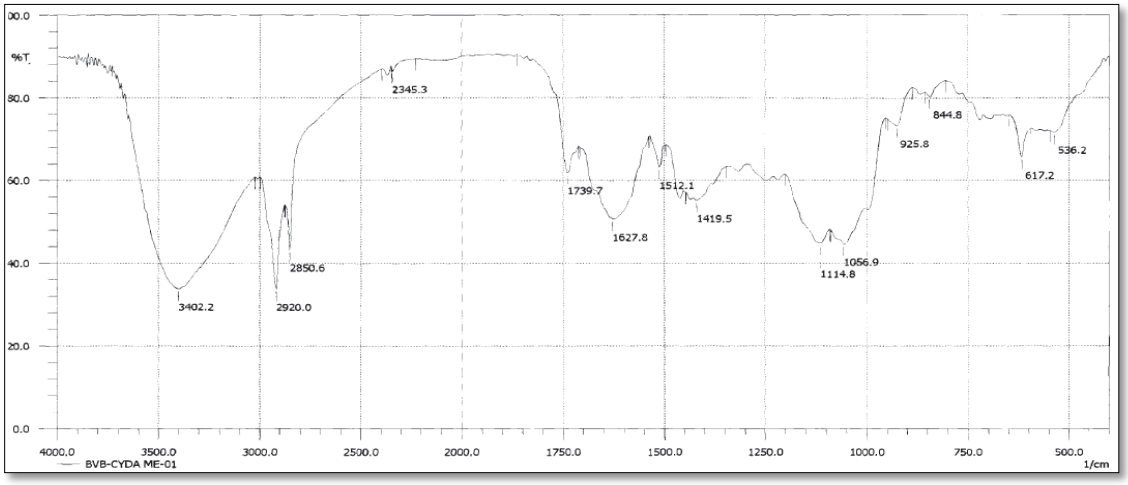

FIGURE 18: FTIR ANALYSIS OF METHANOLIC CRUDE EXTRACT OF *CYNODON DACTYLON*

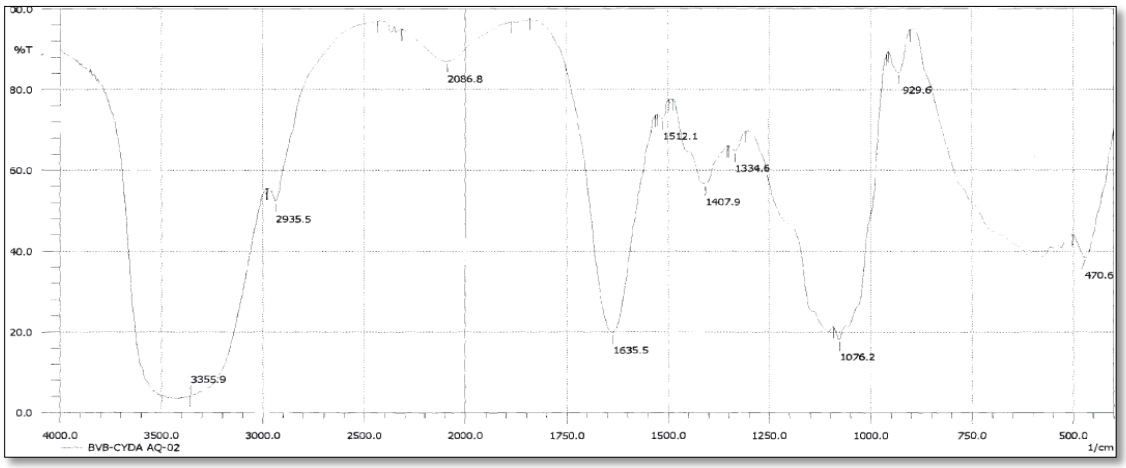

FIGURE 19: FTIR ANALYSIS OF CRUDE AQUEOUS EXTRACT OF *CYNODON DACTYLON*

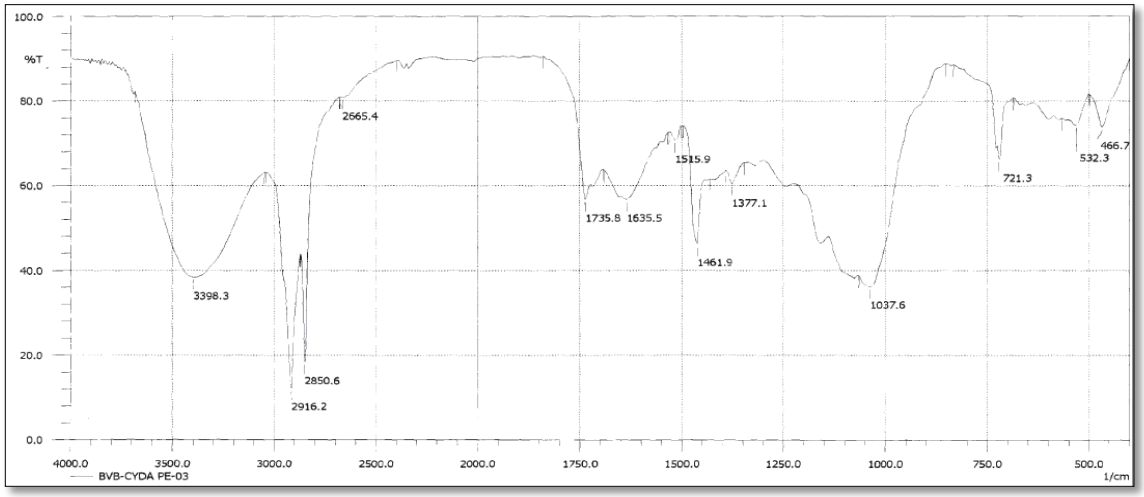

FIGURE 20: FTIR ANALYSIS OF PETROLEUM ETHER EXTRACT OF *CYNODON DACTYLON*

HPLC and H^1-NMR: Further analytical HPLC and H^1-NMR spectroscopy was undertaken to assess the various components present in the extract and to find out the functional group present in the crude extract. HPLC analysis of methanol extract of leaf of *Cynodon dactylon* was carried out with the mobile phase methanol: acetonitrile: water in the ratio 25:35:40 and gave a total of 3 peaks at retention time 2.779 min, 4.115 min and 9.840 min (shown in **Figure 21**). The highest peak was seen at the retention time 2.779 min. After comparing with HPLC Chromatogram of standard, shows the presence of Phytochemicals like Alkaloids, Phenols and Flavonoids. H^1-NMR analysis of the same methanol extract of *Cynodon dactylon* showed a number of peaks in between δ 0 to 2 and δ 3 to 5 as shown in **Figure 22**. Both the group of peaks may be acyclic aliphatic compounds and α - mono substituted mentioned in **Table 4.**

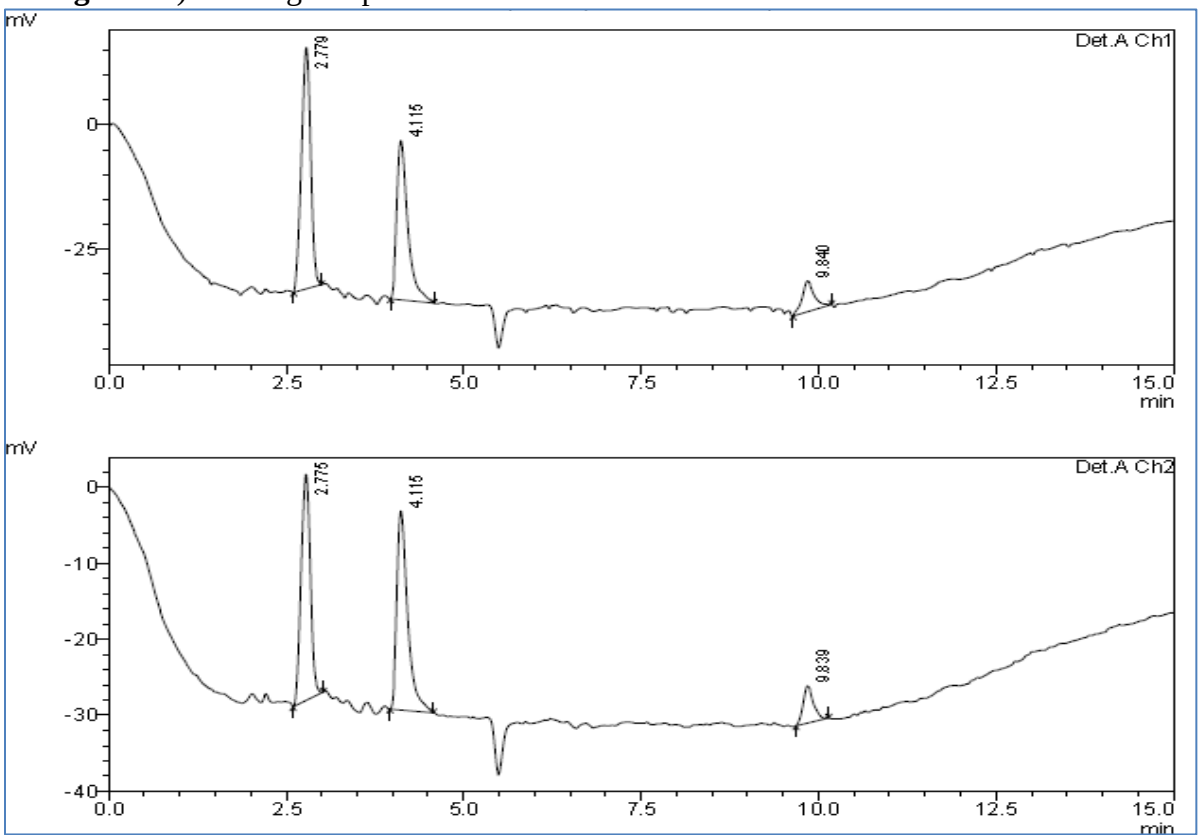

FIGURE 21: HPLC ANALYSIS OF METHANOLIC EXTRACTS OF *CYNODON DACTYLON*

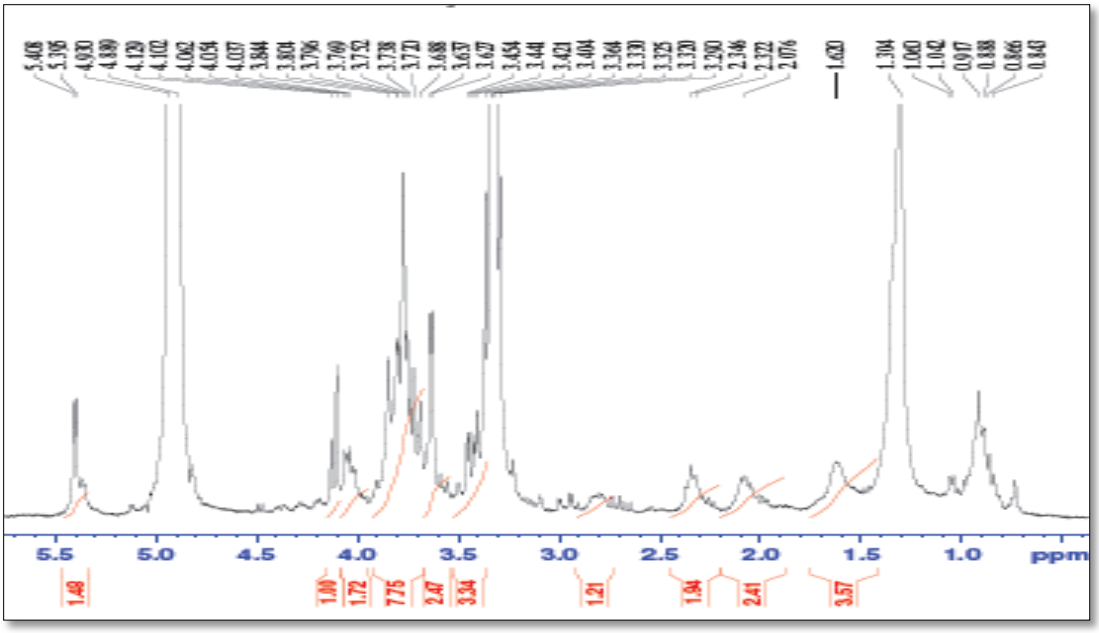

FIGURE 22: H^1 NMR ANALYSIS OF METHANOLIC EXTRACT *CYNODON DACTYLON*

TABLE 4: POSSIBLE FUNTIONAL GROUPS PRESENT IN METHANOLIC EXTRACT OF *CYNODON DACTYLON* ANALYZED BY H^1-NMR

Peak in ppm	Possible type of group of compound
0-2	Aliphatic alicyclic compounds
1-2	β-substituted Aliphatic compounds
2-5	α- mono substituted aliphatic compounds

Antipyretic Activity: The Antipyretic activity results were expressed as the mean±SEM. The results obtained from the present study were analyzed using One way ANOVA followed by Dunnett's multiple comparison tests. $p<0.05$ was used to indicate statistical significance. Data was computed for statistical analysis by using Figure Pad Prism Software. The results of the antipyretic effect of the test compounds, standard (paracetamol 150 mg/kg) and control are presented in **Table 5**. The paracetamol as well as methanolic and aqueous extracts at dose of 200 mg/kg started showing effective antipyretic activity after 1h of post-dosing, when compared with control. Antipyretic activity was observed up to 4hrs after paracetamol and test extracts administration. Values expressed as mean ±SEM, n=6 animals in each group. The results were analyzed using One way ANOVA followed by Dunnett's multiple comparison tests. *$p<0.05$ was used to indicate statistical significance when compared to control.

TABLE 5: VANTI-PYRETIC ACTIVITY OF METHANOLIC AND AQUEOUS LEAF EXTRACTS OF *CYNODON DACTYLON*

Treatment	Dosage mg/kg	Rectal temperature (^{0}C)								
		Before yeast	18h after	Temperature after treatment (^{0}C)						
				30 min	1h	2h	3h	4h	5h	6h
Control	Normal saline	37.3±0.1	37.8±0.1	38.0±0.1	38.1±0.1	38.2±0.1	38.4±0.1	38.5±0.1	38.6±0.1	38.7±0.1
Paracetamol	150	37.7±0.1	38.5±0.1	38.3±0.1	37.9±0.1*	37.7±0.1	37.5±0.1*	37.7±0.1*	37.9±0.1	38.1±0.1
Methanolic extract	200	37.5±0.1	38.0±0.1	37.8±0.1	37.7±0.1	37.6±0.1*	37.5±0.1*	37.6±0.1*	37.8±0.1	38.1±0.1
Aqueous extract	200	37.6±0.1	38.3±0.1	38.3±0.1	37.9±0.1*	37.8±0.1*	37.7±0.1*	37.8±0.1*	38.2±0.1	38.4±0.1

DISCUSSIONS: Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health [32]. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug [33]. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [34]. Many reports are available on the antiviral, antibacterial, antifungal and anti-inflammatory properties of plants. . Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. In Present study, not many reports are available on the Antipyretic Properties of

Cynodon dactylon plant is used for developing commercial formulations. Hence, the present study establishes the Antipyretic and Antimicrobial activity of different extracts of *Cynodon dactylon* plant in models used. From the above results, phytochemical screening has shown presence of many secondary metabolites in different extracts of *Cynodon dactylon.* The potent activity of Antimicrobial and Antipyretic activity may be attributed to the presence of many secondary metabolites. Many of these phytochemicals screened are known to show medicinal as well as physiological activity [35]. The increase of antibiotic resistance of microorganism to conventional drugs has necessitated the search for new efficient and cost effective ways for the control of infectious diseases. Many reports show the effectiveness of traditional herbs against microorganisms as a result plants have become one of the bases of modern medicine [36].

Antipyretic activity of *Cynodon dactylon* plant extracts, both extracts markedly decreased the rectal temperature of pyretic rats. This postulation is supported by the antipyretic effect of the extract, evidenced by its impact on the pathogenic fever induced by the administration of a yeast injection.

Its etiology includes production of prostaglandins in central nervous system, which is the final common pathway responsible for fever induction [37]. Inhibition of prostaglandin synthesis could then be the possible mechanism of antipyretic actions of these extracts as that of paracetamol [38]. Thus the present study concludes that the methanolic and aqueous extracts have antipyretic activity in animals at the dose of 200 mg/kg.

According to authors [39] HPLC fingerprinting is the best way for chemical characterization. HPLC profile differentiation is one such important and powerful procedure which has often employed for this purpose. Each and every metabolite has a specific role and functions in harmony with other metabolites within the organizational framework of cells in the defence mechanism of the plants [40].

The H^1-NMR analysis of the methanol extract of *Cynodon dactylon* showed presence of aliphatic group of compounds which might be responsible for the bioactive properties of the plant extract. Indeed, NMR spectroscopy is by far the most powerful spectroscopic techniques for obtaining detailed structural information about organic compounds in solution. The data generated from the experiment have provided the chemical basis for bioactivity and the therapeutic use of *Cynodon dactylon* for various ailments.

CONCLUSION: The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. Antibacterial and antipyretic efficacy shown by this plant provides a scientific basis and thus, validates their traditional uses as homemade remedies. Isolation and purification of different phytochemicals may further yield significant antibacterial agents. The present findings suggest that, the plant widely available all over the world, could be a prominent source of medicinally important natural compounds.

ACKNOWLEDGMENT: We Acknowledge Vision Group on Science and Technology Department of Information Technology, Biotechnology and Science & Technology, Science Projects in College Education (SPiCE / (SSP-UG)), Government of Karnataka for funding the project. Authors are thankful to B. V Bhoomaraddi College of Engineering & Technology, Hubli, Karnataka, India for providing necessary facilities to carry out this study.

REFERENCES:

1. N. Savithramma, M. Ling Rao and S. Ankanna: Preliminary Phytochemical Screening of Some Important Medicinal Plants. Middle-East Journal of Scientific Research 2012; 2-1:139-145.
2. N. Md. Azmathullah, M. Asrar Sheriff and A.K. Sultan Maiden: Phytochemical Screening of *Calotropis procera* Flower Extracts and their Bio-Control Potential on *Culex sp*. Mosquito Larvae and Pupae. International Journal of Pharmaceutical & Biological Archives 2011; 26:1718-1721.
3. N. Savithramma, M. Linga Rao and D. Suhrulatha: Screening of Medicinal Plants for Secondary Metabolites. Middle-East Journal of Scientific Research 2011; 8(3):579-584.
4. Solanki R.: Some medicinal plants with antibacterial activity. International Journal of Comprehensive Pharmacy 2010 1(4):1-4.
5. Dahiru, D, William, E.T. and Nadroi M.S: Protective effects of Ziziphus mauritiana leaf extract on carbon tetrachloride-induced liver injury. African Journal Biotechnology 2005; 4(10):1177-1179.
6. S.K Singh, P.K Rai, S Mehta, R.K Gupta and G Watal: Curative effect of *Cynodon dactylon* against STZ induced hepatic injury in diabetic rats. Indian Journal Clinical Biochemistry 2009; 24:410-413.
7. K.K. Kritikar and B.D. Basu: Indian Medicinal Plants (Cynodon dactylon). International Book Distributors, Second Edition 1980.
8. Duke. J: Handbook of Energy Crops. Purdue University 1995.
9. Chellaiah Muthu, Marriappan Ayyanar, Nagappan Raja and Savarimuthu Ignacimuthu: Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu India. Journal of Ethnobiology and Ethnomedicine 2006; 2(43):1–10.
10. Abhishek B, Anita Thakur: Anthelmintic activity of Cynodon dactylon. Journal of Pharmacognosy and Phytochemistry 2012 1(3):1-3
11. Vipin Kumar Garg and Sarvesh Kumar Paliwal: Anti-Inflammatory activity of aqueous extract of *Cynodon dactylon*. International Journal Pharmacology 2011; 7(3):370-375.
12. Deepak Bharti, Pradnya Jagtap, vaishali Undale and Ashok Bhosale: Aerial parts of Aqueous extract of *Cynodon dactylon* shows hypotensive effect in high fructose treated Wistar rats. International Journal of Research in Pharmaceutical and Biomedical Sciences 2012; 3(2):591.
13. Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, Tripathi PC, Seal T and Mukherjee B.: Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. Journal of Ethnopharmacol 2003; 84:131-138.
14. Yesilada E, Sezik E, Honda G, Takaishi Y, Takeda, Y and Tanaka T: Traditional medicine in Turkey IX: Folk medicine in north-west Anatolia. Journal of Ethnopharmacol 1999; 64:195–210.
15. Biswas TK and Mukherjee B: Plant medicines of Indian origin for wound healing activity. International Journal of Lower Extremity Wounds 2003; 2:25-39.
16. Santosh Kumar Singh, Prashant Kumar Rai, Shikha Mehta, Rakesh Kumar Singh and Geeta Watal: Assessment of Anti diabetic Potential of Cynodon dactylon extract in Streptozocin diabetic rats. 2009; 24-4.
17. Alireza Garjani, Arash Afrooziyan, Hossein Nazemiyeh, Moslem Najafi, Ali Kharazmkia and Nasrin Maleki-Dizaji: Protective effects of hydroalcoholic extract from rhizomes of Cynodon dactylon (L.) Pers. on compensated right heart failure in rats. BMC Complementary Alternative Medecine 2009; 9:28.
18. M Najafi, H Ghavimi, A Gharakhani and A Garjani: Effects of hydroalcoholic extract of Cynodon dactylon (L.) pers. on ischemia/reperfusion-induced arrhythmias. DARU Journal of Pharmaceutical Sciences 2008: 16(4):233-238.
19. Magadi R Gurudeva: Botanical and vernacular name of South Indian Plants. Divyachandra prakashan, *Bangalore*, 144, 2001.
20. Theodore Cooke, Bishen Singh and Mahendra pal Singh: The Flora of Presidency of Bombay, Dehardun, 2: 1032-1083, 1908.
21. Kaleeswaran B, Ilavenil S. and Ravikumar S: Screening of Phytochemical properties and Antibacterial Activity of *Cynodon Dactylon L.* International Journal of Current Research 2010; 3:083-088.
22. S. De, Y. N. Dey and A. K. Ghosh: Phytochemical Investigation and Chromatography Evaluation of the different Extracts of Tuber of *Amorphaphallus Paeoniifolius* (Araceae). International Journal of Pharmaceutical and Biomedical Research 2010*;* 1(5):150-157.
23. Pius O. Ukoha, Egbuonu A. C. Cemaluk, Obasi L. Nnamdi and Ejikeme P. Madus: Tannins and other phytochemical of the *Samanaea saman* pods and their antimicrobial activities. African Journal of Pure and Applied Chemistry 2011; 5(8):237-244.
24. Majumdar, S.H, Chakraborthy G.S, Kulkarni K.S: Medicinal Potentials of Semecarpus anacardium Nut- A review. Journal of Herbal Medicine & Toxicology 2008; 2(2):9-13.
25. Fazel Shamsa, Hamidreza Monsef, Rouhollah Ghamooshi and Mohammadreza Verdian-rizi: Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai Journal of Pharmaceutical Sciences 2008; 32:17-20.
26. Mohamad Asif, S.H.Ansari, Md.Rafiul Haque and Nazia Kalam: Estimation of Phenolic, Flavonoid contents and Antioxidant activity in nut galls of *Quercus infectoria* Olivier. Journal of Pharmacy Research 2012; 5(7):3855-3857.
27. Rabe T and Van Staden J: Isolation of an Antibacterial Sesquiterpenoid from *Warburgia salutary*. Journal of Ethanopharmocology 1997; 73:171-174.
28. P.B.Mallikharjuna, L.N.Rajanna, Y.N. Seetharam and G.K.Sharanabasappa: Phytochemical Studies of *Strychnos potatorum* L.f. A Medicinal Plant. Journal of Chemistry 2007; 24:510-518.
29. Silverstein RM, Webster FX, Kiemle DJ: Proton NMR spectrometry and Spectrometric identification of organic compounds. John Wiley and Sons, Seventh edition 2005, 127-204.
30. R. A. Turner: Screening method in Pharmacology. Academic Press, New York & London 1965, 298.
31. O. O. Igbinosa, E. O. Igbinosa and O. A. Aiyegoro: Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). African Journal of Pharmacy and Pharmacology 2009; 3(2):058-062.
32. Suresh K, Deepa P, Harisaranraj R and Vaira Achudhan V: Antimicrobial and Phytochemical Investigation of the Leaves of *Carica papaya* L., *Cynodon dactylon* (L.) Pers.,

Euphorbia hirta L., *Melia azedarach* L. and *Psidium guajava* L. Ethnobotanical Leaflets 2008; 12:1184-1191.
33. B. Mahesh and S. Satish: Antimicrobial Activity of Some Important Medicinal Plant against Plant and Human Pathogens. World Journal of Agricultural Sciences 2008; 4 (S): 839-843.
34. Sofowara A: Medicinal plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria, 289.
35. Syed Hidayathulla, Keshava Chandra K and K.R. Chandrashekar: Phytochemical Evaluation and Antibacterial activity of *Pterospermum diversifolium Blume*. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(2): 165-167.
36. Sutapa Das, Rana Datta and Subhangkar Nandy: Antipyretic and Analgesic Effect of Methanolic Extract of different Parts of *Abroma Augusta* Linn. Asian Journal of Pharmaceutical and Clinical Research 2012; 5(4):129-133.
37. Tjolsen A, Gerge OG and Hunskaar S: The formalin test: an evaluation of method. Pain 1992, 5:13-17.
38. Springfield EP, Eagles PKF and Scott G: Quality assessment of South African herbal medicines by means of HPLC finger printing. Journal of Ethnopharmacology 2005; 101: 75-83.
39. Patil AG, Koli SP, Patil DA and Chnadra N. Phamacognostical standardization and HPTLC finger print of *Crataeva tapia* Linn. SSP. Odora (Jacob.) Almeida leaves. International Journal of Pharma and Bio Sciences 2010; 1(2):1-14.
40. Saraf A: Phytochemical and antimicrobial studies of medicinal plant Costus speciosus (Koen.). Journal of Chemistry 2010; 7(1):405-413.

How to cite this article:
Bagewadi ZK, Siddanagouda RS and Baligar PG: Phytoconstituents investigation by LC-MS and evaluation of antimicrobial and antipyretic properties of *Cynodon dactylon*. *Int J Pharm Sci Res* 2014; 5(7): 2874-89.doi: 10.13040/IJPSR.0975-8232.5 (7).2874-89.