ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 3, pp. 083-088, April, 2010

RESEARCH ARTICLE

SCREENING OF PHYTOCHEMICAL PROPERTIES AND ANTIBACTERIAL ACTIVITY OF *Cynodon dactylon L.*

Kaleeswaran B., Ilavenil S. and Ravikumar S*

Department of Biotechnology, PRIST University, Vallam, Thanjavur – 613 403

ARTICLE INFO

Article History:
Received 15th February, 2010
Received in revised form
5th March, 2010
Accepted 25th March, 2010
Published online 7th April

Key words:
Cynodon
Microdilution
GC-MS
Tricosane
Propanediol
Traditional healer

ABSTRACT

Traditionally, *Cynodon dactylon* L. juice is used for freshness and several common diseases. Preliminary phytochemical analysis proved that phenols, quinines and tannin were in ethanolic extract of *C. dactylon*. Then the ethanolic extract was analyzed by Gas Chromatography and Mass Spectrophometry (GC-MS) which showed 10 phytochemical components, some of them are not mentioned in the previous studies among these Tricosane (22.05 %), 1, 2-Propanediol (20.30%), 3-benzyloxy-1, 2-diacetyl (12.62%) were present at maximum level. Aqueous and ethanolic extract of *C. dactylon* (500 µg/ml) were investigated for their antibacterial activity against gram positive bacteria and gram negative bacteria using disc diffusion, well in agar and microdilution method. *E. coli, B. subtilis, S. aureus* and *A. hydrophila* were more susceptible in the ethanolic extract and no result was found in aqueous extract. Minimum inhibitory concentrations (MIC) value of the ethanolic extract was response between in the range of 125 - 62.5 µg/ml. Due to the presence of phyto components may control the bacterial growth which supports the usage of plant extract either in high concentration/long duration of traditional treatment by the traditional healers.

INTRODUCTION

Herbal products were being the effective source of both traditional and modern medicines which are used extensively to treat several medical problems. India is rich in biodiversity, which comprises the indigenous knowledge of traditional healers. In India, throughout its long history, has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of various diseases. Chemical studies of Indian medicinal plants afford an important material for the detection and development of new drugs of natural origin. In the recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju *et al*., 2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections (Balandrin *et al*., 1985). Hence, more studies pertaining to the use of this plant as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al*., 2005).

***Corresponding Author:** drravinikesh@yahoo.co.in
Tel: +91 94437 24611; Fax: +91 4362 265140

Cynodon dactylon (L) Pers. (Gramineae, Poaceae) is a herbal plant commonly known as 'Arugampul' in Tamilnadu in India which is treated as a blessed plant. This grass grows throughout India and in almost all parts of the world. It is a short C_4 grass, which is rhizomatous, stoloniferous and water-stress tolerant (Burton *et al*., 1988; Naidu and Harwood, 1997) and also recommended for soil revegetation (Osvaldo *et al*., 2005). Traditionally, juice of this plant is commonly consumed as health drink during the early morning in south India for healthy life. It forms an important part of Ayurvedic medicine, the juice of *C. dactylon* was used to treat hysteria, epilepsy and insanity (Rajvaidya, 1935). *C. dactylon* is used by traditional healers for purifying the blood, anuria, biliousness, conjuctivitis, diarrhoea, gonorrhoea, itches and Stomachache (Chellaiah Muthu *et al*., 2006).Present study has been designed to determine the role of plant extract of *C.dactylon*, both in aqueous and ethanol extract against pathogenic bacteria.

MATERIALS AND METHODS

Plant Material

The plant *C. dactylon* (L) were collected from Madurai and then transferred to PRIST University, Thanjavur, Tamilnadu, India. It was taxonomically identified and authenticated by Rev Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular systematic, St. Joseph College (Autonomous), Thiruchirapalli, Tamilnadu, India. The voucher specimens are deposited at the Rapinat herbarium and the voucher number is RHCD BP09.

Preparation of extract

The whole plant was cleaned and shade dried. The dried plants were pulverized by a electrical blender and passed through the 20 µ mesh sieve. A powdered plant was extracted successfully with ethanol by using soxhlet apparatus and water extracted by cooled maceration. The extraction was carried out for 24 hrs at room temperature with mild shaking (Chopra *et al*., 1992). The extract were filtered and concentrated at 45 °C using rotary vacuum evaporator. The extract obtained was vacuum dried and used for further investigation.

Phytochemical analysis tests

Phytochemical analysis of the isolated fractions from ethanolic extract of *C. dactylon* leaves for secondary metabolites such as glycosides, flavonoids, alkaloids, triterpenoids, tannins, phenols, saponins, quinines, anthraquiones, coumarins and steroids was done using standard methods (Horbone, 1984).

GC Mass Analysis

Ethanolic extract of *C.dactylon* was analyzed by GC equipped with mass spectrometry (GC-MS-QP2010-Shimadzu). The chromatographic conditions were as follows: Column: DB-% ms (length 30.0 m, Diameter 0.25 mm, Film thickness 0.25 µm). The 1µl DG ethanolic extract was injected into the GC-MS in split less mode at 200 °C. The column oven temperature was held at 45 °C for 1 minute, then programmed at 10 different rates upto 280 °C and held for 15 minutes. Helium carrier gas was maintained at a flow rate of 1.4 ml/min.

Microorganisms used

For this study, both Gram positive bacteria (*Staphylococcus aureus* ATCC 11632 and *Bacillus subtilis* ATCC 23859) and Gram Negative bacteria *(Escherichia coli* ATCC 25922, *Klebsiella pneumonia* ATCC 10031, *Pseudomonus aeroginosa* ATCC 10145, *Aeromonas hydrophila* ATCC 35654) were used to determine the antibacterial activity of leaves of *C. dactylon*.

Determination of antibacterial assay

Disc diffusion method

Disc diffusion method was used to determine the zone of inhibition against chosen bacteria by the *C. dactylon* plant extract (Rabe and Van Staden, 1997). The diluted bacterial cultures were spread over nutrient agar plates using sterile glass L rod and 500 µg/ml of concentration of the ethanol and aqueous extract were applied in filter paper, and then allowed to dry before being placed on the top layer of the agar plate. The plates were incubated at 37 °C for 24 hrs and growth of inhibition zones were measured.

Well in agar method

Antibacterial activity of plant extract was tested by a modified well in agar method (Sinclair and Dhingra, 1995). Inoculum suspension was spread over the agar plates using sterile glass L rod. Subsequently, using sterile borer, well of 0.5 cm diameter was made in the inoculated media and then 500 µg/ml concentration of each extract (aqueous and ethanol) was 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 at the end of the 24 – 48 hrs.

Broth dilution method

Quantitative evaluation of antibacterial effect of ethanolic and water extract of *C. dactylon* was determined by the broth dilution method. The concentration of 50 µg/ml of plant extracts were added to 10 ml nutrient broth in 20 ml test tubes. The tubes were then inoculated with appropriate bacteria at 10^5 CFU/ml and incubated at 27°C in temperature controlled orbital shaker (Scigenics-Orbitek) at 100 rpm. The inhibition of bacterial growth was determined by measuring the absorbance at 625 nm after 12, 24 and 36 hrs.

Micro dilution assay

The Minimal Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were also studied for the microorganisms which were determined as sensitive to the extracts in the disc diffusion assay. The inocula of the microorganisms were prepared from 12 hr broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Determination of MIC and MBC values of the extracts was determined using two fold serial micro dilution method. Final concentration of the plant extracts ranging from 500 - 3.9 µg/ml. The tested extracts were added to sterile nutrient broth into micro titer plates before the diluted bacterial suspension (Final inoculums of 10^5 bacteria) were added. Each extract was assayed in triplicate. The antibiotic (Penicillin and Streptomycin) were used as positive control ranging from 50 – 0.039 µg/ml. The plate was covered with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for 10 seconds and then incubated at 37 °C for 24 h. Microbial growth was determined by absorbance at 620 nm using the RT-2100C micro plate reader (Rayto Life and analytical Sciences Co.Ltd, Shenzhen, China). The MIC values were taken as the lowest concentration of the extracts in the wells of the microtitre plate that showed no turbidity of the wells in the microtitre plate was interpreted as visible growth in a subculture of the well showing no apparent growth in a sterile agar plate. The least concentration showing no visible growth on agar subculture was taken as MBC value (Ahmet Adiguzel *et al*., 2005).

RESULTS

The preliminary phytochemical screening of the *C. dactylon* ethanolic extract is having tannin, quinones and phenols (Table 1). Based on the GC-MS analysis, 10 phyto components were identified in the ethanolic extract of *C.dactylon* (Table 2). Among this, Tricosane (22.05%), 1, 2-Propanediol 3-benzyloxy-1,2-diacetyl (20.30%) and then Dibutyl phthalate (12.62 %), Phthalic acid, Butyl undecyl ester (10.22 %) occupied the major portion (Fig 1) and in addition with five more components were identified in the ethanolic extract of *C.dactylon* .

Based on the above results, the ethanolic and aqueous extracts of *C.dactylon* were evaluated for the effect of antibacterial activity against several pathogenic bacterial strains included with gram positive and gram negative strains. Penicillin-G and Streptomycin were used as a standard. The ethanolic extract and aqueous extract of *C. dactylon* were tested against the bacterial strains by using disc diffusion (Table 3) and well diffusion method (Table 4). Unfortunately, no result was observed in the aqueous extract. But in ethanolic extract, the bacterial species *E. coli* (13 ± 0.32), *B. subtilis* (11 ± 0.27),

Fig. 1. GC-MS Chromatogram obtained from ethanolic extract of *C. dactylon*

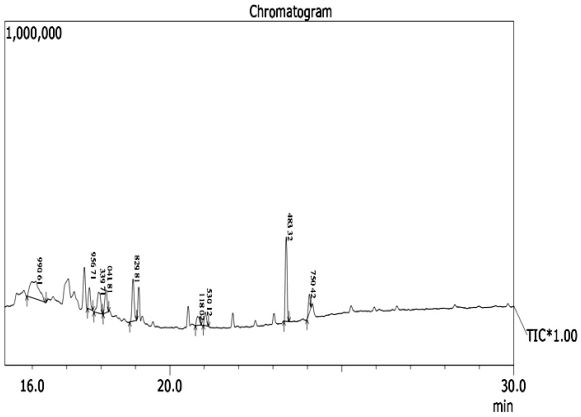


Fig. 2. Effect of ethanolic extract on pathogenic bacteria in broth dilution method

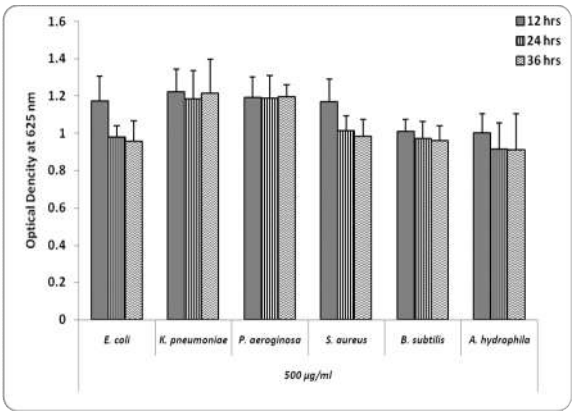
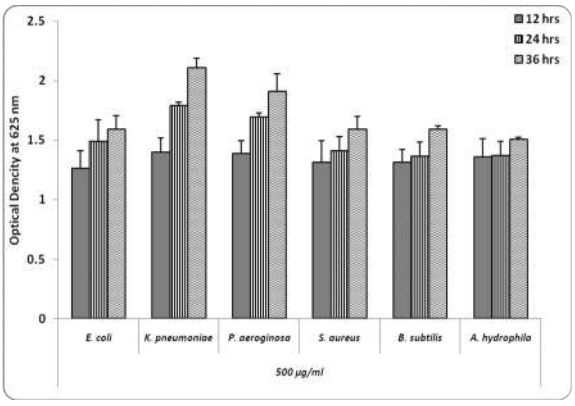


Fig. 3. Effect of Aqueous extract on pathogenic bacteria in broth dilution method



A. hydrophila (10 ± 0.28) and *S. aureus* (10 ± 0.15) showed maximum susceptibility whereas *K. pneumonia* (6 ± 0.18) and *P. aeroginosa* (9 ± 0.12) showed high resistance in disc diffusion method. In well diffusion method, *B. subtilis* , *S. aureus, A. hydrophila* and *E. coli* found to be more susceptibility with 14 to 15 mm zone of inhibition, whereas *K. pneumonia , P. aeroginosa* showed high resistance with 8 ± 0.25 mm and 11 ± 0.15 mm zone of inhibition respectively. From this result, the antibacterial activity effect of plant extract was observed in both disc and well diffusion method.

In broth dilution, the ethanolic extract of *C.dactylon* showed more inhibitory effect than the aqueous extract in different time interval (Fig 2 & 3). From this, the ethanolic extract of *C. dactylon* exhibits the efficiency to control the *E. coli, B. subtilis*, *S. aureus* and *A. hydrophila*. The aqueous extract of *C. dactylon* exhibited no activity against all bacterial strains. It is evident that the ethanolic extract is better than the aqueous extract for controlling the bacterial growth. In microdilution, Minimal inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of ethanolic extract of *C. dactylon* against six pathogenic bacteria were shown in Table 5. MIC has the values of the bacterial strains sensitive to 3.9 - 500 µg/ml. The MIC value of ethanolic extract were observed at 62.5 µg/ml against *E. coli, A. hydrophila* and 125 µg/ml against *K. pneumonia, P. aeroginosa, B. subtilis* , *S. aureus*. However, there was no MBC observed in all pathogenic bacteria which point out that the plant extract only obsessed bacteriostatic effect. Suppose MBC values may be observed when the concentration of this plant extract will be increased against the tested bacteria.

DISCUSSION

Tannin, quinones and phenols are acting as antimicrobial agents (Marjorie Murphy Cowan 1999; Mohammad Ali Ebrahimzadeh *et al.,* 2008, Brano 2007; Jigna and Sumitra 2007; Takuo Okudu, 2005) and our result also confirmed by the positive result of these components through the phytochemical screening of ethanolic extract of *C. dactylon*. Alkaloids of *C. dactylon* exhibit antimicrobial activity against pathogenic bacteria and dermatophytes. This noted antimicrobial activity of *C. dactylon* is attributed to the presence of the alkaloids, tyrptamine, tyramine and gramine (Raman *et al*., 2002) and the aerial parts of *C.dactylon* were reported to contain cynodin, hydrocyanic acid, triticin, beta carotene (Kirtikar and Basu, 1980). Previous study was showed the compounds including arundoin, furfural, furfuralcohol, β-ionone,2-(4′-hydroxyphenyl)-propionic,4hydroxybenzoic, 2-(3′-methoxy-4-hydroxyphenyl) propionic, 3-methoxy-4-hydroxybenzoic acids, phytol, β-sitosterol-d-glucoside, stigmasterol acetate and phytone had been isolated from *C. dactylon* (Kirtikar and Basu, 1980; Miller 1967; Ottmoto *et al*., 1970; Rizk *et al*., 1986).

In addition with, Tricosane was identified in the GC-MS analysis of ethanolic extract of our study plant. This component was also present in several plants like *Azadiracta indica, Staphylea* sp. which was known for its antimicrobial activity (Wafaa *et al.*, 2007; Lacikova *et al.,* 2007). Transphytol is another compound present in *C. dactylon* extract was represented in other extracts like *Phlomis* species, *Gynandropsis gynandra* and in diatom *Navicula delognei*, all were exhibiting antifungal, antibacterial, antitumour and repellants properties (Findlay and Ashok, 1984; Yuan Zhang and Zhe-Zhi Wang, 2008; Lwande *et al.,* 1999). *C. dactylon* has propanediol, the same component was found in *Scapania verrucosa* and it was studied for antifungal and antitumour activities (Philip Domenico *et al*., 1997).

Finally, Adamantane and Benzyloxy components were also present smaller quantity in the extract of *C. dactylon* and also they have antimicrobial activity (Adnan *et al.,* 2007).

method maximum susceptibility with 14 – 15 mm zone of inhibition was observed at the level of 500 µg/ml concentrations against several pathogenic bacteria. It was clearly evident that our experimental plant has the antimicrobial/antibacterial property against several bacterial species. Likewise, in vitro antimicrobial activity of methanolic extract of *C. dactylon* showed the inhibitory

Table 1. Preliminary phytochemical analysis for ethanolic extract of C. dactylon

Sl.No	Phytochemicals	Ethanolic extract
1	Alkaloids	-
2	Anthraquinones	-
3	Coumarins	-
4	Flavonoids	-
5	Glycosides	-
6	Phenols	+
7	Quinones	+
8	Saponins	-
9	Steroids	-
10	Tannin	+
11	Triterpenoids	-

(+) present; (-) absent

Table 2. Identified components of ethanolic extract of *C.dactylon* by GC-MS.

Sl.No	Retention Time	Area	Formula	Area%	Name
1	16.099	1030779	$C_{23}H_{48}$	22.05	Tricosane
2	17.659	282965	$C_{20}H_{40}O$	6.05	Trans-Phytol
3	17.933	478069	$C_{23}H_{36}O_4$	10.22	Phthalic acid, Butyl undecyl ester
4	18.140	349258	$C_{22}H_{31}OP$	7.47	Phosphine Oxide, Bis(Pentamethylphenyl)-
5	18.928	589936	$C_{16}H_{22}O_4$	12.62	Dibutyl phthalate
6	20.811	167096	$C_{15}H_{30}O$	3.57	(Z)6-Pentadecen-1-ol
7	21.035	179293	$C_{20}H_{26}O_3$	3.83	1-Adamantanecarboxylic acid, 2-Isopropoxyphenyl ester
8	23.384	949056	$C_{14}H_{18}O_5$	20.30	1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-
9	24.057	222114	$C_{16}H_{22}O_4$	4.75	Butylaldehyde, 4-benzyloxy-4-[2,2,-dimethyl-4-dioxolanyl]-
10	30.411	427022	$C_{47}H_{82}O_2$	9.13	Stigmast-5-En-3-Ol, Oleat
	Total	4675588		100.00	

Table 3. Antimicrobial activity of ethanolic extract of *C. dactylon* (Disc diffusion method) (Zone of inhibition in mm)

Concentration (µg/disc)	*E. coli*	*K. pneumoniae*	*P. aeroginosa*	*S. aureus*	*B. subtilis*	*A. hydrophila*
Ethanolic extract	13 ± 0.32	6 ± 0.18	9 ± 0.12	10 ± 0.15	11 ± 0.27	10 ± 0.28
Penicillin-G	20 ± 0.12	16 ± 0.21	18 ± 0.32	16 ± 0.33	20 ± 0.15	22 ± 0.15
Streptomycin	18 ± 0.18	14 ± 0.22	19 ± 0.28	16 ± 0.18	28 ± 0.18	21 ± 0.15

Table 4. Antimicrobial activity of ethanolic extract of *C. dactylon* (Well in agar method) (Zone of inhibition in mm)

Concentration (mg/ml)	*E. coli*	*K. pneumoniae*	*P. aeroginosa*	*S. aureus*	*B. subtilis*	*A. hydrophila*
Ethanolic extract	14 ± 0.27	8 ± 0.25	11 ± 0.15	15 ± 0.16	15 ± 0.21	14 ± 0.31
Penicillin-G	22 ± 0.21	16 ± 0.33	13 ± 0.18	17 ± 0.14	22 ± 0.4	28 ± 0.31
Streptomycin	19 ± 0.32	15 ± 0.21	22 ± 0.21	18 ± 0.12	25 ± 0.41	21 ± 0.28

Table 5. Minimal Inhibitory Concentration (MIC) and Minimal Bacterial Concentration (MBC) of the ethanolic extract of *Cynodon dactylon* (values in µg/ml)

Microorganism	EC		KP		PA		SA		BS		AH	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Cynodon dactylon	62.5	-	125	-	125	-	125	-	125	-	62.5	-
Penicillin-G	3.125	>6.25	6.25	12.5	6.25	>12.5	3.125	>6.25	3.125	>6.25	1.56	3.125
Streptomycin	0.39	>1.56	12.5	>25	6.25	>12.5	1.56	3.125	1.56	3.125	0.39	>1.56

EC - *Escherichia coli*; **KP** - *Klebsiella pneumonia*; **PA** - *Pseudomonas aeroginosa*;
SA - *Staphylococcus aureus*; **BS** - *Bascillus subtilis*; **AH** – *Aeromonas hydrophila*

The disc diffusion method showed maximum susceptibility with 10 - 13 mm and in well diffusion zone of diameter 17 mm, 12 mm and 17 mm against *S. aureus, E. coli* and *K. pneumonia* respectively and there

was no results observed in aqueous extract (Jigna and Sumitra 2007). Similarly, the methanolic extract of *Solanum palinacanthum* was showed the zone of inhibition on *A. hydrophila*, *B. subtilis* and *S. aureus* (Aline *et al*., 2008). In terms of specific inhibition by petroleum ether extract of *Capparis zeylanica* against *S. aureus, B.subtilis, K. pneumoniae* and *P. vulgaris* antibacterial activity and produced inhibition zone ranging from 10 to 16 mm at a concentration of 16.5 µg/ml, whereas chloroform, ethanol and water extracts showed inhibitory activity against all six bacterial strains at concentrations of 13.5, 14.0 and 14.0 µg/ml respectively (Chopade *et al*., 2008).

From our results in broth dilution technique, the ethanolic extract of *C. dactylon* successfully control the *E. coli, B. subtilis*, *S. aureus* and *A. hydrophila*. MIC value also revealed that almost all tested bacterial strains were sensitive to the ethanolic extract of our study plant. From the earlier studies, it is also revealed that the organic solvent extract is better than aqueous extracts (Okigbo and Emoghene, 2004; Nair *et al*., 2005). Our result also showed the aqueous extract possess no antibacterial didn't possess any antibacterial activity. Similarly, the ethanolic extract of *Punica granatum* was most active against *E. coli* (Veeramuthu *et al*., 2006) and the methanolic rootstock extract of *Euphorbia fusiformis* exhibited significant antibacterial activity against *Staphylococcus aureus, E.coli, Pseudomonas aeroginosa, K.pneumonia, Proteus vulgaris, S. typhii* A *and S.typhii* B (Natarajan *et al*., 2005); *Solanum. torvum* showed antimicrobial activity against *B. subtilis*, *B. cereus*, *P. aeruginosa* and *S. aureus* (Wiart *et al*., 2004), while *S. nigrum* was active against *Salmonella typhi* (Rani *et al*., 2004). On the other hand, reported methanolic extracts of almost all the plants exhibited antibacterial activity towards one or another bacterium (Venkatesan *et al*., 2005). Now, the present study also supported that the ethanolic extract is also effective control the bacterial growth than the aqueous extract. This probably indicates that there are bioactive ingredients that are able to inhibit the growth of these common pathogens (Etani *et al*., 1998; Okigbo *et al*., 2005).

According to this study, plant based antimicrobial drug have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobial agents. The results revealed that the extract of *C. dactylon* was effective against both gram positive and gram negative bacteria. Presence of chemical components of *C. dactylon* may inhibit the bacterial growth. Traditionally, *C. dactylon* was employed using/mixing with aqueous for treating the antibacterial and other infections. Naturally, the biological active compounds whose activity can be enhanced in the presence of ethanol could have been produced number of active compound responsible for antibacterial activity. The present study provides the scientific information about the plant extract of *C. dactylon* and supports the usage of this plant for curing many bacterial diseases by traditional healers. Further, phytochemical separation and immunological studies of this plant is in under progress.

ACKNOWLEDGEMENTS

Financial and excellent technical supports from PRIST University, Thanjavur, Tamilnadu, India is gratefully acknowledged.

REFERENCES

Adnan A. Kadi, Nasser R. El-Brollosy , Omar A. Al-Deeb, Elsayed E. Habib ,Tarek M. Ibrahim , Ahmet Adiguzel, Medine Gulluce, Meryem Segul, Hatice Ogutcu, Fikrettin Sahin and Isa Karaman. 2005. Antimicrobial Effects of *Ocimum basillicum* (Labiatae) Extract. Turk.j. Biol., 29. 155 – 160. Marjorie Murphy Cowan. 1999. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews,* 12 (4): 564–582.

Ali A. El-Emam. 2007. Synthesis, antimicrobial, and anti-inflammatory activities of novel 2-(1-adamantyl) -5-substituted-1,3,4-oxadiazoles and 2-(1adamantylamino)-5-substituted-1,3,4-thiadiazoles. *Euro. J. of Medicinal Chem.,* 42: 235-242

Aline C. Pereira, Denilson F. Oliveira, Geraldo H. Silva, Henrique C.P. Figueiredo, Alberto J. Cavalheiro, Douglas A. Carvalho,Luciana P. Souza And Sara M. Chalfoun. 2008. Identification of the antimicrobial substances produced by *Solanum palinacanthum* (Solanaceae). Ann. of the Brazil. Academy of Sci., 80(3): 427 – 432.

Artizzu, N., Bonsignore, L., Cottiglia, F., Loy, G. 1996. Studies on the diuretic and antimicrobial activity of *Cynodon dactylon* essential oil. Fitoterapia, 67:174–176.

Balandrin M.F.J., Kjocke A, Wurtele E. 1985. Natural plant chemicals: Sources of Industrial and Medicinal materials. Science, 228: 1154-1160.

Brano N.Lenta. 2007. Anthroquinones from the stem bark of *Stereospermum Zenkeri* with antimicrobial activity. *Phytochemistry*, 68: 1595 – 1599.

Burton, G.W., Hook, J.E., Butler, J.L and Hellwig, R.E. 1988. Effect of temperature, daylength and solar radiation on production of coastal bermudagrass. *Agron., J.* 80: 557 – 560.

Chellaiah Muthu, Marriappan Ayyanar, Nagappan Raja and Savarimuthu Ignacimuthu. 2006. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu. *India. J. of Ethanobio and Ethnomed.,* 2(43): 1 – 10.

Chopade, V.V.,Tankar, A.N., Ganjiwale, R.O. and Yeole, P.G. 2008. Antimicrobial activity of *Capparis zeylanica* Linn. roots. *Int. J of green Pharmacy,* 28 – 30.

Chopra RN, Nair SL, Chopra JC. 1992. Glossary of Indian medicinal plants. CSIR publication, New Delhi, 50.

Etani E., Agani M., Tsujihata S., Tsukamoto T., and Ohta M. 1998. Antibacterial action of vinegar against food-borne pathogenic bacteria including *Escherichia coli* 01 57:H7. J. *Food protection,* 6: 953-959.

Horbone SB. A Guide to Modern Techniques of Plant Analysis. 1984. Chapman and Hall, London, 3: 4 – 80.

Iwu M.W., Duncan A.R., Okunji C.O. 1999. New Antimicrobials of Plant Origin. In: Janick J. (ed.): Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA., 457–462.

Jigna Parekh, Sumitra V. Chand A. 2007. In vitro Antimicrobial Activity and Phytochemical Analysis of Some Indian Medicinal Plants. Turk J Biol., 31: 53-58.

John A.Findlay and Ashok D Patil. 1984. Antibacterial constituents of the diatom *Navicula delognei*. J. of Natural product, 47 (5): 815 – 818.

Kirtikar, K.K., Basu, B.D. 1980. Indian Medicinal Plants, second ed. Lalit Mohan Publication, India, 2650.

Krishnaraju A.V., Rao T.V.N, Sundararaju D. 2005. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (*Artemia salina*) lethality assay. Int J Appl Sci Eng., 2: 125-134.

Lacikova L., E. Svajdlenka, I. Masterova and D. Grancai. 2007. Isolation and Identification of Flower Oil Components from Four Staphylea L. Species. , Chem. Pap., 61 (6): 512—514.

Lwande W., A.J. Ndakala, A. Hassanali, L. Moreka, E. Nyandat, M. Ndungu,H. Amiani, P.M. Gitu, M.M. Malonza, D.K. Punyua. 1999. Gynandropsis gynandra essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. Phytochemistry, 50: 401-405.

Miller, R.P. 1967. Oestrogenic activity in Central African Highland grasses. Rhodesian Zambia Malawi. *Journal of Agricultural Research,* 5: 179.

Mohammad Ali Ebrahimzadeh, Fereshteh Pourmorad and Ahmed Reza Bekhradnia. 2008. Iron Chelating activity, Phenol and Flavonoid content of some meditional plants from Iran. *Afric. J. Biotech.,* 7(18): 3188 – 3192.

Naidu, B.P. & Harwood,m.R. 1997. Opportunities for landscape stabilization revegetating disturbed landsin streeful environments with exotic or native forages. *Trop. Grasslands.,* 31: 364 – 369.

Nair R, Kalariya T, Chanda S. 2005. Antibacterial activity of some selected Indian medicinal flora. *Turk. J. Biol*, 29: 41-47.

Natarajan B., John Brito S., Srinivasan K., Nagamurugan N., Mohanasundari C., Perumal G. 2005. Anti-bacterial activity of *Euphorbia fusiformis*- A rare maedicinal herb. J. Ethnopharm., 102:123-126.

Okigbo R.N., Emoghene A.O. 2004. Antifungal activity of leaf extracts of some plants species on Mycosphaerella fijensis Morelet, the casual organisms of black sigatoka disease in banana (*Musa acuminate*). KMITL Sci.J., 4:20-31.

Okigbo R.N.,Ajalie A.N. 2005.Inhibition of Some Human with Tropical Plants Extracts *Chromolaena odorata* and *Citrus aurantifolia* and some antibiotics. Int. J. of *Molecular med and Advance Sci.,* 1(1): 34-40.

Osvaldo R.Vignolio, Carmen Biel, Felicidad de Herralde, Jose P.L.Araujo-Alves and Robert Save. 2005. Use of water stress tolerant *Lotus creticus* and *Cynodon dactylon* in soil re-vegetation on different slopes in a Mediterranean climate. *Ann.Bot.Fennici.,* 42: 195-205.

Ottmoto, T., Ikuse, M.,Natori, S. 1970. Triterpenoids of the Graminae. *Phytochemistry*, 9: 2137.

Philip Domenico, Richard J. Salo, Sabrina G. Novick, Paul E. Schoch, Ken Van Horn, and Burke A. Cunha. 1997. Enhancement of Bismuth Antibacterial Activity with Lipophilic Thiol Chelators. Antimicrob. *Agents and Chemother.,* 41: 1697–1703.

Rabe T, Van Staden J. 1997. Isolation of an Antibacterial Sesquiterpenoid from *Warburgia salutaris*. J. of Ethanopharm., 73:171-174.

Rajvaidya, S. G. 1935. Shankar Nighantu, Banousadhi Bhander,Jabalpur, MP, India. Chellaiah Muthu, Marriappan Ayyanar, Nagappan Raja and Savarimuthu Ignacimuthu. 2006. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J.of Ethanobio and Ethnomed., 2(43): 1 – 10.

Raman, Nanjian,Radha, A.,Balasubramanian, K., Raghunathan, R., Priyadarshini, R. 2002. Biodiversity: Biomolecular aspects of Biodiversity and Innovative Utilization, Proceedings of the IUPAC International Conference on Biodiversity, Kluwer academic/Plenum Publishers, New York, 253-256.

Rani P And Khullar N. 2004. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. *Phytother Res.,* 18: 670–673.

Rizk, A.M., Heiba, H.I., Ma'Ayergi, H.A., Batanocing, K.H. 1986. Constituents of plants growing in Qatar. *Fitoterapia*, 57: 3–9.

Sinclair JB, Dhingra OD. 1995. Basic Plant Pathology Methods. 287-305.

Takuo Okudu. 2005. Systematics and health effects. *Phytochem*., 66: 2012 – 2031.

Veeramuthu Duraipandiyan, Muniappan Ayyanar andVenkatesan M, Vishwanathan MB, Ramesh N. 2005, Antibacterial potential from Indian *Suregada angustifolia*. *J. of Ethnopharm*., 99: 349-352.

Wafaa A. Helmy, Howaida I. Abd-Alla, Hassan Amer and El-Safty, M.M. 2007. Chemical Composition and 'In vitro' Antiviral Activity of *Azadirachta indica* A. Juss (Neem) Leaves and Fruits Against Newcastle Disease Virus and Infectious Bursal Disease Virus . *Aus. J. of Basic and Applied Sci.,* 1(4): 801-812.

Wiart C, Mogana S, Khalifah S, Mahan M, Ismail S, Buckle M, Narayana Ak And Sulaiman M. 2004. Antimicrobial screening of plants used for traditional medicine in the state of Perak, Peninsular Malaysia. *Fitoterapia*, 75: 68–73.

Yuan Zhang, Zhe-Zhi Wang. 2008. Comparative analysis of essential oil components of three Phlomis species in Qinling Mountains of China. *J. of Pharmaceut. and Biomed. Anal.*, 47: 213–217.

****