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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *CYNODON DACTYLON* AGAINST HUMAN PATHOGENS.

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Abstract

Cynodon dactylon is a medicinally essential plant used for the treatment of various infectious diseases. The study of phytochemical and antibacterial activities of the extract of *Cynodon dactylon* showed potent activity against human pathogens. Four different solvents such as acetone, ethanol, methanol, chloroform and water were used to extract the compounds from the leaves of *C. dactylon* to evaluate the phytochemical components and antibacterial activity. The phytochemicals such as proteins, carbohydrate, flavonoids and alkaloids were detected in this study. The extracts showed 4 mm to 15 mm zone against the tested bacteria. Ethanol and methanol extract showed considerable activity than other solvents and hot water. The methanol extract of *C. dactylon* showed high activity against *S. aerus* and *P. aeruginosa*. However, ethanol extract was highly active against *E. coli* and *B. subtilis*. *C. dactylon* extracted with water, ethanol and methanol showed significant activity against *Enterobacter* sp. This study concluded that extracts of *Cynodon dactylon* have potent phytochemicals and significant activities against various human pathogens.

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Introduction:-

Medicinal plants are rich in various potential drugs and it holds harmless and healthier alternate to various synthetic drugs (Rai *et al.*, 2007). In recent years, there is a growing interest among the medical properties of plants in terms of antibacterial activity and antifungal activity. In addition, these medicinal plants are rich of bioactive compounds and play critical role in the development of novel drugs. Extracts of some of the medicinal plants are highly useful in the treatment of various health problems such as peptic ulcers, bacterial infections, arthritis and inflammation (Patwardhan *et al.*, 2010). Medicinal plants cure various diseases which are useful for investigation of potential sources of novel antimicrobial agents (Beegum and Devi, 2003). *Cynodon dactylon* is known as “Garike hullu” (Kanada), Dhoorva (Marathi), “Aruvaum pullu” (Tamil), “Garike and Thella gariki” (Telulgu) and “Doob” (Hindi). Different parts of medicinal plants such as root, leaf, stem, seed, fruit and park are mainly used to obtain several phytochemical constituents of plants. The extracts of medicinal plants are useful in the treatment of inflammatory diseases (Shah *et al.*, 2011).

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Cynodon is Bermuda grass and belongs to the family, Poaceae. It is native to East Asia, Africa, Southern Europe and Australia. *Cynodon* is generally considered as a weed and has been found to possess various potential medicinal properties (Singh *et al.*, 2009). The plant is widely used in India as potential agent to control diabetes. The extract of *C. dactylon* has been widely reported to be anti-diabetic, hypolipidemic and antioxidant efficacy, healing of minor injuries (Oudhia, 1999), hepatic antioxidant and immunomodulatory activities. The aqueous extract of *C. dactylon* rhizome was used for diuretic, purifying agent and dysentery (Sadki *et al.*, 2010). This plant extract has also potent application in secondary syphilis and dropsy (Kesari *et al.*, 2006), cardio protective (Garjani *et al.*, 2009) and wound healing properties (Oudhia and Pal, 2000). The extracts of *C. dactylon* had been reported to be effective for antimicrobial activity against fungal pathogens and bacterial pathogens (Kanimozhi and Rathbai, 2012). In this study, *Cynodon dactylon* was screened for its potential antibacterial activity.

Materials and methods:-

Plant material:



C. dactylon

Fresh shoots of *C. dactylon* was collected locally. The collected shoots were cleaned, washed with double distilled water and dried for 10 days (air drying) at room temperature (30 ± 2 °C). Then the plant material was powdered using a mixer grinder and stored in a clean plastic containers to protect from heat, light and moisture until further use.

Solvent extraction of antibacterial substances:-

Plant material was extracted with various solvents such as acetone, ethanol, methanol, chloroform and water. About 5 gm of dried powder was weighed and suspended in 50 ml of solvent separately. After 24 h, the suspension was filtered using Whatman No.1 filter paper. Then it was centrifuged for 5 min at 5000 rpm. This extraction procedure was repeated three times and pooled. The residual plant material was air dried completely to remove the respective solvents and the phytochemical components were analyzed.

Preparation of hot water extract from *C. dactylon*:-

About 5 gm of powdered plant material was weighed and suspended in 200 ml of double distilled water. Then, this mixture was heated continuously with stirring at 35 °C to 45 °C for 30 min. Further, the water extract was filtered using Whatman No.1 filter paper. Then it was centrifuged for 5 min at 5000 rpm and used for the analysis of antibacterial properties.

Qualitative analysis of phytochemicals from *C. dactylon*:-

Phytochemical test for the presence of bioactive chemical constituents in *C. dactylon* was carried out using various solvent using the standard procedures. All extracts were subject to phytochemical analysis.

Analysis of total protein:-

The presence of protein content in the shoot of *C. dactylon* was carried out by using standard method.

Analysis of carbohydrate:-

The presence of carbohydrates in the extract of *C. dactylon* was evaluated as suggested by Fehling's test. In this method, 1.0 ml of Fehling's reagent A was mixed with 1.0 ml of Fehling's reagent B and crude plant extract was added. Followed by gentle heating, the reagent mixture showed brick (Fehling, 1984).

Analysis of Flavonoids:-

To quantify flavonoids, 2 ml of 2% NaOH were added in 0.10 ml plant extract, which showed intense yellow colour and the colour disappeared for few min. Then addition of four drops of 1% aluminum solution added in each filtrate. Reappearance of yellow colour indicated the presence of flavonoids in the sample (Edeoga *et al.*, 2005).

Analysis of Steroids:-

The plant extracts mixed with 2 ml of chloroform and Conc. H₂SO₄. Development of red color in the chloroform layer indicated the presence of steroids. Then few drops of acetic acid was added. Development of greenish colour indicated steroids positive.

Antibacterial test:-

Microorganisms used in the present study mainly represent pathogenic species commonly associated with human infections.

Test microorganisms:-

The following pathogenic strains were used for the antibacterial studies. The bacteria such as, *Staphylococcus aureus*, *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Enterobacter* sp. were used.

Preparation of inoculum:-

Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to the nutrient broth (g/l) (peptic digest of animal tissue, 5.0; beef extract, 1.5; yeast extract and 1.5; sodium chloride, 5.0). The Erlenmeyer flasks were incubated at 37 °C for 18 h and were used as the inoculum.

Antimicrobial susceptibility tests:-

Antimicrobial susceptibility testing of the crude plant extracts using well diffusion method. In this method, the antibacterial substances diffuse from the well through a solidified agar layer in a Petri plate to an extent so that the growth of added micro organisms is inhibited entirely in a circular area or zone. The antibacterial activity is expressed as the zone of inhibition in millimeters, which is measured with a zone reader. The presence of definite zone of inhibition of any size around the well indicated antibacterial activity and the zone of inhibition was measured.

Results and discussion:-

In the present study, proteins, carbohydrate, flavonoids and alkaloids were detected from *Cynodon dactylon* (Table 1). This result was in accordance with observations made previously by various research groups. In *C. dactylon* various compounds have been extracted and quantified from different parts. It contains carbohydrates, minerals, proteins, and other compounds like palmitic acid, vitamin C, terpenoids and alkaloids. It was reported that the green grass contains 11.75% of total ash, 28.17% fiber and 10.47% crude protein (Paranjpe, 2001). The important phytochemical constituents such as, flavonoids, luteolin carotenoids, glycosides, phytosterols, saponins and volatile oils were reported from *C. dactylon* (Annapurna *et al.*, 2013). The phytochemical analysis in *C. dactylon* showed that the plant contained flavanoids, alkaloids, glycosides, terpenoides, triterpenoids, steroids, saponins, tannins, resins, phytosterols, reducing sugars, carbohydrates, proteins, volatile oils and fixed oils (Dande and Khan, 2012).

Table 1:- Qualitative analysis of crude ethanolic extract of *Cynodon dactylon*

Sl No.	Experiment	Acetone extract	Ethanol extract	Methanol extract	Chloroform extract	Water extract
1	Total protein	---	++	++	---	++
2	Carbohydrate test	---	++	++	---	++
3	Flavonoids test	++	++	---	---	++
4	Steroids	---	---	---	---	++

++Present; -- Absent

The phytochemicals such as quinines, tannin and phenols are mainly responsible for antimicrobial properties. The alkaloids of *C. dactylon* exhibit antimicrobial activity against human pathogens. It was previously reported that the solvent extract of *C. dactylon* exhibited antimicrobial activity because of the presence of gramine, tyramine, tyrtamine and alkaloids (Raman *et al.*, 2002). The aerial parts of *C. dactylon* contain cynodin, triticin, hydrocyanic acid and beta carotene (Kirtikar and Basu, 1980). In the present study, well diffusion method was followed to elucidate antibacterial activity. The extracts showed 4 mm to 15 mm zone against the tested bacteria. Ethanol and methanol extract showed considerable activity than other solvents and hot water. The methanol extract of *C. dactylon* showed high activity against *S. aureus* and *P. aeruginosa*. However, ethanol extract was highly active against *E. coli* and *B. subtilis*. *C. dactylon* extracted with water, ethanol and methanol showed significant activity against *Enterobacter* sp. (Table 2). This result was in accordance the observations made previously with other medicinal plants. Likewise, the methanolic extract of *Solanum palinacanthum* was observed the zone of inhibition on *B. subtilis*, *A. hydrophila* and *S. aureus* (Aline *et al.*, 2008). The petroleum ether extract of the medicinal plant, *Capparis zeylanica* showed more activity against *K. pneumonia*, *P. vulgaris*, *S. aureus* and *B. subtilis*. The inhibitory zone ranged from 10 to 16 mm at a concentration of 10 µg/ml (Chopade *et al.*, 2008). Chloroform extracts of the medicinal plant exhibited least antibacterial activity against the selected bacterial pathogens. In the present study also, chloroform extract showed very less activity than other solvents and hot water extract. It was also observed that selected gram negative bacteria were more sensitive to most of the extracts tested compared to gram-positive bacteria. The gram-negative bacteria were highly resistant to antibiotics than that of gram positive bacteria. And, this resistance is mainly due to the variations in their cell wall composition (Paz *et al.*, 1995).

Table 2:- Antibacterial activity of *Cynodon dactylon* against various human pathogens

Extract	Zone of inhibition (mm)				
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterobacter</i> sp.
Acetone	12	9	11	13	9
Ethanol	13	16	7	12	8
Methanol	12	11	10	8	7
Chloroform	6	-----	7	4	7
Water	5	-----	9	----	11

In an another study six various organic solvents were employed to extract the antibacterial compounds from the leaves of *C. dactylon* against the pathogenic stain such as *Streptococcus pyogens*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabillis* and *Pseudomonas aeruginosa*. Among the solvents tested butanolic extract of *Cynodon dactylon* was found to be good than other tested solvents (Chaudhari *et al.*, 2011). The present work revealed that the solvent and water extract of *C. dactylon* was found to be effective against various human pathogens. From this study we draw a conclusion that the traditional use of plant *C. dactylon* for the infectious disease is promising against bacteria.

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