Research Article

Chemical Evaluation and Antimicrobial Activity of Leaf Powder of *Cynodon dactylon*

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ABSTRACT

The use of medicinal plants in the world and especially in India, contributes significantly to primary health care. *Cynodon dactylon* (L.) Pers. is a type of perennial grass that possesses great medicinal values. Whole plant of the *Cynodon dactylon* is traditionally used to treat painful and inflammatory conditions. The present study describes the phytochemical evaluation and antimicrobial activity of *Cynodon dactylon*. For the present studies, the chemical studies are done using ethanolic extracts and antimicrobial activities are done using ethanolic extract. Phytoconstituents present in *Cynodon dactylon* are Saponins, Tannins, steroids, alkaloids and Flavonoids. In this study the ethanolic extract of *Cynodon dactylon* was used to determine the antimicrobial activity against Escherichia coli, *Bacillus subtilis*. The antimicrobial activity was determined using the cup plate method. The diameter of the clear zone of inhibition surrounding the well was measured. It can be concluded that ethanolic extract of the leaf powder of *Cynodon dactylon* may be considered as an antimicrobial agent.

Keywords: Leaf powder, *Cynodon dactylon*, extraction, evaluation.

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INTRODUCTION

Since time immemorial the extracts of plants have been recognized to possess important medicinal activities. Ayurveda, the most ancient medicinal system contributed by our country, includes in its Materia Medica, drugs from plant and animal sources.[1]

The use of medicinal plants as a source for relief from illness can be traced back over many years in written documents of the early civilizations in China, India and the near east, but it is doubtless an art as old as mankind. Even today plants are the almost exclusive source of drugs for the majority of the world's population[2]. In India around 3000 plants are used in traditional systems of medicines. Even in these group 200 plants are used extensively. Systems of medicines.

Ayurveda, the widely followed Indian system of medicine is not merely a system of medicine but elicits the science of living and its approach is more on health than disease, more on prevention than cure which is similar to the modern concept of positive health by World Health Organization (WHO).[3]

Cynodon dactylon is aperennial herb found in various regions of India. It has different names in different Indian languages such as "durva", "durba", "dhro", "garichgaddi", "arukampillu", "sharapova" etc. It contains many metabolites, mainly proteins, carbohydrates, minerals, flavonoids, carotenoids, alkaloids and glycosides.[4] It is found in warm climates all over the world between 45 degrees south and north latitudes. It is available throughout the year.

Cynodon dactylon also possesses immense medicinal value and may be applied both externally as well as internally. The plant is astringent, sweet, cooling, constipating, haemostatic, diuretic and tonic and is useful in impaired conditions of pitta and kapha, burning wounds, leprosy, diarrhoea, vomiting etc. The plant is a remedy for snake bites, gout and rheumatic infections.[5] Its anthelmintic activity has been successfully investigated. Apart from this it also possesses anti-inflammatory activity also. Three varieties namely 'mildura' with bluish or greenish stem,'shveta durva' with whitish stem and branches and 'gandharva' with nodulose stem are mentioned in the "Bhavaprakash nighantu".[6]

Phytochemistry

Cynodon dactylon consists of 28.17% enzymes, 11.79% ash, 10.47% proteins. Ash contains 0.77% calcium, 0.58% phosphorous, 0.34% manganese, 0.23% sodium, 2.08% potassium.[7] Dry grass contains per 400 grams 36.16% carbohydrates, 6.04% proteins. It contains phenolic phytotoxins ferulic, syringic, para coumaric, vanillic, para hydroxy benzoic and ortho hydroxy phenyl acetic acid.[8] Flavonoids and glycosides were found to be present in aqueous extract while alkaloids, glycosides and flavonoids are present in ethanol extract of the plant. Other compounds like vitamin C, beta-carotene, fats, and palmitic acid have also been reported.[9]

(I) FLAVONOIDS

Flavonoids are the major components isolated and identified from Cynodon .The term flavonoid covers a large group of naturally occurring compounds which include chalcones, dihydrochalcones, aurones, flavonoids, is flavonoids, flavones, flavanols, isoflavone, 2,3-dihydro flavanols (flavonols), flavone 3,4-diols (leucoantho cyanidins), anthocyanidins and Catechins.[10]

Flavonoids are present in plants both in the Free State and as glycosides. Unlike anthocyanins in which the sugar residue is invariably attached to the 3-hydroxyl (and in addition) often to the 5-hydroxyl; the sugar residue in flavonoids may be attached to a hydroxyl group in any position, the most frequent attachment being to the 3 or 7- hydroxyl. Differences in the oxygenation patterns of the aglycone and its modification by alkylation, type and position of sugar components give rise to an almost unlimited number of combinations.[11]

125 different flavonoid aglycones have been enumerated and six monosaccharides (D-glucose, D-galactose, L-rhamnose, arabinose, D-xylose, D-glucuronic acid), about disaccharides and six trisaccharides have been reported to be associated with flavonoids.[12] Bis-glycosides are not uncommon and numerous flavonoid C-glycosides are known. The sugar may be different or identical and may be attached at two different positions.

Colour reactions of the flavonoid compounds

Ferric chloride:

The production of ferric chloride is a general property of all classes of polyhydroxy flavonoid compounds. The production of ferric chloride is characteristic of 3-5 and 8-hydroxy flavones but not 6-7 or 4-hydroxy flavones 16 3-hydroxy flavones usually give brown color, the color given by 5-hydroxy flavones in green, purple, or brown.[13]

Neutral and Basic Lead Acetate:

Basic lead acetate will give colored precipitates with most of the flavonoid polyphenols. Which neutral lead acetate forms precipitate with compounds containing O-dihydroxy groupings or combination of this grouping with O-hydroxy carbonyl or 3-hydroxy chromone structural units.[14]

Sodium Hydroxide Solution:

The behavior or flavonoid pigment towards aqueous sodium hydroxide in the cold and on heating provides useful preliminary information on the basic structural type on the orientation of hydroxyl groups.[15] Flavonoids, for instance, form a yellow solution turning brown due to oxidative decomposition when air is passed through the solution. This relative instability to oxygen in alkaline solution distinguishes flavanols from flavones.[16] Color changes in alkaline buffer solutions give useful indications of the number and position of hydroxyl groups in flavones.[17]

Mineral acids:

Concentrated sulfuric acid dissolved many flavonoid compounds with production of colored solutions, the properties of which are often of diagnostic value in structure analysis. Murthy, Rajagopal anand Row have described the colors and fluorescence of a large number of chromones, flavones, etc., in sulfuric acid solution.[18]Flavone's dissolve in sulfuric acid with the production of lively orange to crimson colors.[19]

Reduction tests:

The reduction tests are of considerable use in distinguishing between various types of flavonoid compounds. One of the most used of the reduction tests utilizes magnesium-hydrochloric acid, the substance being taken in alcohol. Schinoda reduced synthetic and natural flavones and that the compounds with hydroxyl or methoxy substitution in the phenyl group gave red to red violet colors, among seven flavonoids tested all gave strong red violet to blue violet colors.[20] When magnesium is replaced by zinc, flavonols can be distinguished from flavonones and flavonol 3-glycosides from the Aglycones since only the former give deep colors.[21] Asahina and Incubus observed that flavones were reduced to anthocyanidins, as indicated by a red or pink color by treatment of an alcoholic solution with sodium amalgam and subsequent acidification; flavonols were reduced only by magnesium and hydrochloric acid and flavonoids under both conditions.[22] The two tests however, do not always distinguish between flavones and flavonols. Marini-Bettolo and Ballio have described a test with antimony pentachloride in carbon tetrachloride, which sharply distinguishes chalcones (intense red or violet precipitate) from flavonoids, flavones and flavanols (yellow precipitate).

(2) TRITERPENOID SAPOGENINS

Triterpenoidsare compounds with a carbon skeleton based on six isoprene units and they are derived biosynthetically from acyclic C_{30} hydrocarbon, squalene.[23] They have relatively complex cyclic structures, most being either alcohols, aldehydes or carboxylic acids. They are colourless, crystalline, often high melting point, optically active substances which are generally difficult to characterize because of their lack of chemical activity.[24]

The structures of their molecules follow the isoprene rule. However, certain substances with 30 carbon atoms such as lanosterol don't follow the classicalisoprene rule but follow a revised version of it called the biogenetic isoprene rule 41-43.[25] According to this rule terpenes are compounds formed by combination of isoprene units to aliphatic substances such as geraniol, fernosol, geranylgeraniol, squalene and others of similar kind and can be derived from these precursors by cyclization and in certain cases by rearrangement mechanisms.[26]

The triterpenoids are widely distributed in nature for the most part in the vegetable kingdom. Amongst the

exceptions are the hydrocarbon squalene, first obtained from shark liver oil, ambrein from ambergrin and a number of tetracyclic substances isolated from wool fat. A few triterpenes eg: Zeroin, lanosterol etc, are also known to occur as metabolic products of both higher and lower fungi.[27]The triterpenoids occur either in the free state or as esters or as glycosides (saponins). Amongst the naturally occurring esters are the cinnamate, palmitate, and stearate.[28]

There are probably more than 500 natural triterpenoids of established structures. Although the isolation of many well-known triterpenoid's dates back to the century, the first correct structures were not assigned until the time of the 2nd world war. Thus, the parent substances β-amyrin, α–amyrin and lupeol were correctly formulated respectively in 1937, 1949 and 1951 respectively.[29]

Reactions of saponins, steroids, tannins and phlebotomine

1) Without chemical treatment: -

Saponins are detectable by exposure to u.v at 254, 365 nm[30]

2) Colour reactions: -

1) Foam test: - Small amount of extract is shaken with little quantity of water, the foam produced persists for 10 minutes. It confirms the presence of saponins.[31]

2) Haemolysis test: - To 2 ml of 1.8% sodium chloride solution in the two test tubes, 2 ml distilled water is added to one and 2 ml of 1% extract to the other. 5 drops of blood are added to each tube and gently mixed with the contents. Haemolysis observed under the microscope in the tube containing the extract indicates the presence of saponins.[32]

3) The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer is tested for steroids.[33]

A) Salkowskitest: - Few drops of concentrated sulphuric acid are added to the chloroform layer, shaken and on standing; lower layer turns yellow colour changes to deep red.[34]

B) Liebermann Burchard reaction: - Generally pentacyclic triterpenoids give prominent pink colour. Karioyone applied this test for micro detection of triterpenes in plants.

water and heated in a water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins

Steroids: 2 ml of acetic anhydride was added to 0.5 g of the extract of each with 2 ml of H2S04.The color changed from violet to blue or green in some samples indicating the presence of steroids.[35]

Table 1: phytochemical constituents of *Cynodon dactylon*

Phytochemical	Positive or Negative
Alkaloids	+
Tannins	+
Steroids	+
Flavonoids	+
Saponins	+

MATERIALS AND METHODS

Leaf powder extraction

Approximately, 100 g of plant powder was soaked into 200 mL of ethanol solvents and shaken on a platform shaker (LabCompanionTM) at 150 rpm with temperature of 25 C to obtain plant extract. The soaking process was repeated three times for to obtain a complete extraction. The extracts obtained were then evaporated and concentrated under reduced pressure (768–7 mmhg) using Rota-VaporTM (BUCHI) to obtain 1 ml of extract per 10 g of plant sample. Aliquots were then kept in -20 C temperature StrataTM-X 33um Polymeric Sorbent reverse-phase (200 mg/6 mL) (Phenomenex)cartridges with 12-cartridge manifold system were used.[36].

Ethanol absolute (1 ml) was used to activate the sorbent and further equilibrated with sterile deionised distilled water (1 ml). Samples were then loaded into the cartridges and washed with 1 % ethanol (1 ml) to remove any impurities from the samples.

Anti-microbial activity

Micro organisms are isolated from various substances or sources like soil, air, water, food etc. In the laboratory they can be separated into pure cultures. These cultures are suitable for study of their morphological, cultural, biological, serological and genetic properties. In pure cultures techniques colonies are isolated and visible masses representing multiplication of single organisms.[38] A culture which contains more than one kind of micro-organisms is called a mixed culture.

The assay of is based on comparison of inhibition of growth of microorganisms under examination with that produced by known concentration of standard preparation having a known activity. Cup plate method is used in this research.[39]

Cup plate method:

Preparation of agar medium:

Agar - 20gms [1000ml]

Peptone - 10gms [1000ml]

Beef extract - 10gms [1000ml]

Nacl - 5mg [1000ml]

1.Peptone, Nacl and beef extract are heated and agar is added to this broth. Gel formation takes place. Add this to boiling tube and seal it with cotton plug. Now they are kept in autoclave for 15 minutes at 121 degrees at 15 Lb pressure.[40]Inoculate a previously liquefied medium appropriate to assay with requisite quantity of suspension of microorganisms

2.Add the suspension to the medium at a temperature between 40-50 degrees and immediately pour inoculated medium into petri dishes to give a depth of 3-4 mm. Put the plate on a level surface.[41]

3.Store the prepared dishes in a manner so as to ensure that no significant growth on the surface of agar.

4.Prepare solutions of known concentrations of 25 ml, 50ml, 75ml and 100ml.

5.Prepare whatman filter paper discs and add them to the solutions.

6.Inoculate the discs in their respective marked places on the petri dish under sterilised conditions.

7.Incubate the petri dishes in a hot air oven for 24 hours for the growth of microorganisms.

8.Measure the zone of inhibition.

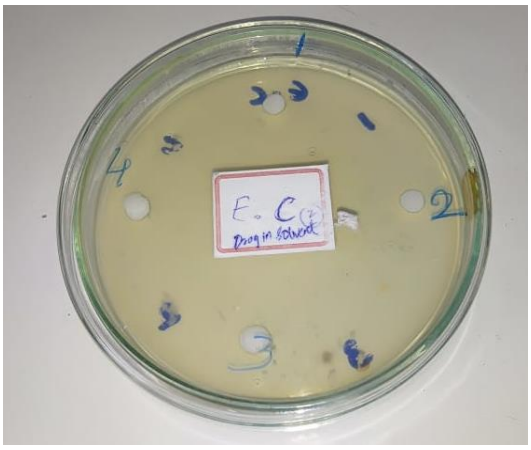

Figure 1: Antimicrobial activity against *Escherichia coli*

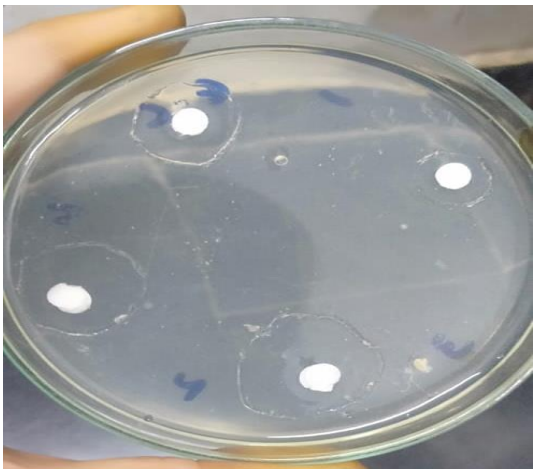

Figure 2: Anti-microbial activity against Bacillus subtilis

RESULTS AND DISCUSSION

The presence of antimicrobial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contributions towards human health. 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. Phytochemical analysis of ethanolic extract showed the presence of tannins, steroids, flavonoids, saponins, and absence of alkaloids and phlobatannins. Phytochemical constituents are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores. The primary phytochemical analysis revealed that the extracts contained some phytoconstituents such as saponins, steroids, tannins flavonoids, which could be responsible for the observed antimicrobial property. These bioactive compounds are known to act by different mechanisms and exert antimicrobial action.

Tannins bind to proline rich proteins and interfere with the protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls.

Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell Steroids have been reported to have antimicrobial properties, the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes. The test organisms used in this study are associated with various forms of human infections. From a clinical point of view, E. coli causes septicemias and can infect the gallbladder, meninges, surgical wounds, skin lesions and the lungs, especially in debilitate and immunodeficient patients.

Bacillus subtilis causes wound infections and urinary tract infections in the elderly and young males often following catheterization or cystoscopy, and it is a secondary invader of ulcers and pressure sores. The demonstration of activity against both gram negative and gram-positive bacteria is an indication that the plant can be a source of bioactive substances that could be of broad spectrum of activity. The fact that the plant was active against both clinical and laboratory isolates is also an indication that it can be a source of very potent antimicrobial substances that can be used against drug resistant microorganisms prevalent in hospital environments. In the present study, the ethanolic extract proved to be a potent antimicrobial.

CONCLUSION

Ethanolic extract demonstrated a broad spectrum of activity against antimicrobial activity. The broad-spectrum antimicrobial activities of the plant extract, possibly due to the identified phytochemical constituents. Bioactive substances from this plant can therefore be employed in the formulation of antimicrobial agents for the treatment of various diseases including gonorrhoea, pneumonia, eye infections. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation. From the present study we can draw a conclusion that the traditional use of plant *Cynodon dactylon* for the infectious disease is promising, mainly against wide range of microorganisms.

The result obtained showed antimicrobial activity through cup plate method for ethanolic extract of leaf powder of *Cynodon dactylon*, which is evident by the formation of clear zone. For ethanol extraction, in terms of zone diameter measurements showed maximum clear zone indicating their greater sensitivity to this specific extract.

The zone of inhibitions obtained for different concentrations are:

25ml - 11mm

50ml - 17mm

75ml - 22mm

100ml - 29mm

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