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Screening for antibacterial activity of Cynodon dactylon (Durva) on selected microorganisms

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ABSTRACT:
Cynodon dactylon of the family Poaceae is extensively used in clinical practice and has reported various pharmacological activities with persistent findings till date. It is a very familiar plant of our surroundings and almost available in all corners of the world. In ethno-medicinal practices, the juice of the plant is used as an astringent and is applied to fresh cuts and wounds. It is used internally in the treatment of chronic diarrhoea and dysentery. It is also useful in the treatment of catarrhal ophthalmia. The leaves of Cynodon dactylon are also used in the treatment of hysteria, epilepsy and insanity. In this experimentation, first the whole dried plant of Cynodon dactylon was subjected to methanolic extraction followed by incubation in a shaker incubator at 50 r.p.m for 24 hours. After incubation the extract was filtered using whatmann filter paper no.1 and collected in a beaker which was further diluted for testing its antibacterial activity. The in-vitro evaluation of antibacterial activity was done against infectious disease causing bacterial pathogens such as Staphylococcus aureus,Escherichia coli by Agar cup method. The plant exhibits antibacterial activity and can be used to treat many infections as they are available and can be grown easily to treat the infections efficiently.

Keywords: Cynodon dactylon, Methanolic extraction, In-vitro evaluation, Antibacterial activity, Agar cup method, Escherichia coli, Staphylococcus aureus.

I. INTRODUCTION:

Medicinal plants are rich in various potential drugs and it holds harmless and healthier alternate to various synthetic drugs. 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. Medicinal plants cure various diseases which are useful for investigation of potential sources of novel antimicrobial agents .Cynodon dactylon is known as “Garike hullu” (Kanada), "Dhurva" (Marathi), “Aruvaum pullu” (Tamil), “Garike and "Gerichagaddi” (Telugu) and “Doob” (Hindi). Different parts of medicinal plants such as root, leaf, stem, seed, and fruit are mainly used to obtain several phytochemical constituents of plants. The extracts of medicinal plants are useful in the treatment of inflammatory diseases. (2)

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 weed and has been found to possess various potential medicinal properties. The plant is widely used in India as a potential agent to control diabetes and also to treat diabetic retionopathy. (3)

The extract of Cynodon dactylon has been widely reported to be anti-diabetic, hypolipidemic and antioxidant efficacy, healing of minor injuries, hepatic antioxidant and immunomodulatory activities. (2) In recent years, a large advance in chemical and pharmacological studies has contributed to the knowledge about new therapeutically active compounds obtained from the natural products. Cynodon dactylon occurs on almost all soil types especially in fertile soil. e.g. loamy soil. It is common in disturbed areas such as gardens, roadsides, overgrazed, trampled areas, uncultivated lands, localities with high levels of nitrogen, and is often found in moist sites along rivers. It is suitable for cultivation under dry land conditions. Cynodon dactylon plays an important role in conservation, because it prevents soil erosion. It provides good grazing, is very useful as

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a lawn grass and is recommended for the protection of waterways. (4)

Scientific Classification :(5)

- Kingdom: Plantae –Plants.
- Subkingdom: Tracheobionta- Vascular plants.
- Super division: Spermatophyta- Seed plants.
- Division: Magnoliophyta- Flowering plants.
- Class: Liliopsida- Monocotyledons.
- Subclass: Commelinidae.
- Order: Cyperales.
- Family: Poaceae- Grass family.
- Genus: Cynodon Rich- Bermuda grass.
- Species: Cynodon dactylon.

Vernacular names:
Hindi- Doob, Dub, Dubra, Khabbal, Kaligas, Neelee Doob.
English- Creeping panic grass, Couch grass, Bahama grass, Bermuda grass, Dun. Grass, Devil's grass, Doab grass, Doorwa.
Sanskrit- Sataparva, Satavalli, Niladurva.
Bengali- Durva, Dub, Dubla, Durba, Doorva, Neel Doorva.
Gujrati- Khadadhro, Lilidhro, Dhro, Dhrokad, Gharo.
Marathi - Doorva, Harali, Dhurva, Karala.
Kannad- Garike hullu, Kudigarike, Kudigarikai.
Punjabi- Dubada, Daurva, Dun, Dubra, Khabbal, Tilla, Talla, Dhub.
Tamil- Aruvam pillu, Hariali, Muyalphul, Arugam Pullu.
Telgu- Garika, Pacchgaddi, Ghericha, Garicagaddi, Gerike, Harvali.
Urdu- Doob ghas, Doob. (6)

Side effects: Durva (Doob) grass does not really have any side effects, but overdose of it may sometimes lead to problems like; paraesthesia oral, rash, skin burning sensation. (6)

General appearance: Leaves of Cynodon dactylon are lanceolate, about 2 to 10cm long and 1.25 to 3mm wide. Flowers are characterized by the presence of spikelets with 1 perfect floret. Glumes are lanceolate and extend up to 2mm in length.

The plant contains crude proteins, carbohydrates, and mineral constituents, oxides of magnesium, phosphorus, calcium, sodium and potassium. The plant affords β-sitosterol, flavonoids, alkaloids, glycosides and triterpenoids. Other compounds like vitamin C, carotene, fats, palmitic acid etc. are also reported. Green grass contains 10.47% crude protein, 28.17% fiber and 11.75% of total ash.

Fig 1- Cynodon dactylon

Uses: Cynodon has a renowned position in Indian systems of medicine and many parts of the plant are assumed to have medicinal properties. Cynodon is a valuable herbal medicine and used as first aid for minor injuries because the juice of the plant is used as an astringent and is applied to fresh cuts and wounds. Farmers traditionally apply crushed leaves to minor wounds as a styptic to stop bleeding. The whole plant is extremely beneficial externally in wounds and the paste of the plant is applied on the forehead during headache. The roots in the form of paste with water are taken internally against fevers. The aqueous fluid extract of the rhizome is used as anti-inflammatory, diuretic, purifying agent and also in dysentery. Cynodon plant is useful for pains, inflammations and

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toothache it also possesses anti diabetic, anti-ulcer, diuretic, antimicrobial, and immune-modulatory activities. As well as treatment of urinary tract infections, prostatitis and syphilis. It has been observed that in most of the studies primarily the research being conducted on C. dactylon involves its glycemic potential (food's effect on a person's blood glucose also called blood sugar level.), which is involved in the treatment of diabetes. The paste made of the plant mixed with honey is used in epistaxis (nose bleeding). Oral administration of the juice of the plant with honey 2-3times a day for few days effectively treats menorrhagia (abnormal heavy and prolonged menstrual period). (7)

Different part of the medicinal plant could be the better source to extract a variety of phytocompounds and used as drugs. Phytochemicals have different structural diversity with biological activities which offer unique platform for drug discovery. Many weeds, herbs, shrubs and plants of our surroundings are often containing very powerful medicinal activity to overcome many of our today's major health problems such as UTI. (8)

II. MATERIAL AND METHODS:

Bacterial strains

Bacterial species used were Escherichia coli, and Staphylococcus aureus.

Preparation for reviving the bacteria

Nutrient agar was prepared for reviving above cultures. The media was prepared and autoclaved at 121°C for 15 minutes. After incubation, each organism was streaked on nutrient agar slant from their respective stock cultures. The tubes were incubated at 37 °C for 24 hours.

Plant material.

Fresh leaves of Cynodon dactylonwere collected. The collected leaves were cleaned and washed properly with sterile double distilled water and dried for 10 days (air drying) at room temperature. The plant material was powdered using a mortar and pestle and stored in a clean plastic container to protect from heat, light and moisture until further use. The commercially available Cynodon dactylon was already in powdered form.

Filtration wasdone using Whatmann filter paper no.1 so that any solid precipitate does not interfere with the results. The agar diffusion test is qualitative, simple and easy to perform. The methodology includes the inoculation of bacterial cells on nutrient agar petri dishes and test samples which are laid over these dishes.

Afterwards, the dishes were incubated for 18–24hr at 37°C and thereafter. The presence of antibacterial activity is indicated by the absence of bacterial growth directly below the test sample.

This zone is influenced by a number of variables, including the susceptibility test medium, the concentration of the test organism, the rate of growth of the test organism, the concentration of test sample, the diffusion of the test sample in the agar and the susceptibility of the organism to the test sample. The first five variables are standardized by CLSI; therefore, if the test is properly performed, the size of the zone of inhibited growth is directly related to the susceptibility of the organism—the larger the zone, the more susceptible the organism is to the test sample. As it would be expected, the results of the dilution tests and diffusion tests are related.

Fig 2: Natural dried Durva leaves

Fig 3: Durva crushed using methanol

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Solvent extraction of antibacterial substances.

For naturally available Cynodon dactylon: 10 gm. of dried powder of plant material extract was re-suspended in 50 ml of solvent separately, and for commercially available Cynodon dactylon the powder material was suspended in 50 ml of solvent and both the solutions were placed in a shaker incubator for 50 r.p.m for 24 hour. After 24hour, the suspension was filtered using Whatmann filter paper no.1 .The residual plant was dried completely to remove the respective solvents. The extract was washed once with distilled water to remove remaining solvents.

Fig 4: Commercially available powdered form of Cynodon dactylon

Fig 5a&5b: Filtration of the suspension using Whatmann filter paper no. 1.

Antibacterial susceptibility test

For this test, the crude extract of both naturally available Cynodon dactylon and its commercially available powder was diluted using distilled water which were as following – 1:2, 1:4,1:8, 1:10 and it was undiluted with controls- Antibiotic (ciprofloxacin), Methanol, Distilled water respectively.

After dilution , media was poured in sterile petri-plate under aseptic conditions followed by preparation of well using sterile metal cork borer with internal diameter of 10mm. Respective dilutions were loaded into the well along with controls and were incubated at 37 °C for 24 hours. After incubation zone of inhibition were measured in mm.

III. RESULTS & DISCUSSION

The antibacterial results reveals that the activity of the crude extract of Cynodon dactylon

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plant is encouraging. Antibacterial activity was done by using Agar well diffusion method; Ciprofloxacin was used as standard for comparing results for antibacterial activity. The methanolic extract was washed once with distilled water and gently the water was discarded so that any remaining methanol would wash away, this step is done because methanol would suppress the antibacterial activity of the Cynodon dactylon and might have given a larger zone of inhibition. Dilutions were done using distilled water, because methanol tends to kill the microorganism leading to a larger zone of inhibition.

The natural and commercially available Cynodon dactylon were collected and extracted separately in a respective solvent i.e. sterile distilled water. The extract of the both were extracted for antibacterial activity and showed positive result i.e. both the extract showed antibacterial activity against Gram positive (S.aureus) and Gram negative (E.coli) bacteria. Similar study was conducted by several other scientist and similar results were found.

The extract prepared in sterile distilled water was tested for antibacterial activity against E.coli and S.aureus using agar well diffusion method. The results were observed after 24 hrs. of incubation at 37 ^{0}C.

The zone of inhibition for naturally available Durva was 32mm and 38 mm for E.coliand S.aureus respectively, whereas the zone of inhibition for commercially available Durva was 31 mm and 30 mm for E.coliand S.aureus respectively. This results are for 1:2 dilution after extraction.

Similar results were found by Amita S. Rao, Nayantara A.K., RashmiKaup S., Arjun sharma where the aqueous extraction of Cynodon dactylon of concentration 400mg/ml gave zone of inhibition of 14mm and 10mm for E.coli and S.aureus respectively. (Rao.et al., IJPSR) (9)

In another study of antibacterial activity of 1000ug/ml concentration of Cynodon dactylon methanol extract exhibit the most effective result i.e. zone of inhibition against S.aureus 14mm and E.coli12mm (10).

The methanolic extracts inhibited the growth of pathogenic bacteria by 70%. The broad spectrum of antibacterial activity of the extract was due to presence of active principle in the extracts which may be polar compounds such as Saponins. (11)

In other study all the multi-drug bacterial isolates were sensitive to different concentrations of C.dactylon root hydroalcoholic extract, the most sensitive bacterial isolates to Bermuda grass root extracts were P. aeruginosa isolates, also A.calcuaceticus, E.coliand S.aureus(12)

Table No. – 1
For commercially available product

Dilutions	Zone of Inhibition (in mm)	
	E.coli	*S.aureus*
1:2	31	30
1:4	28	26
1:8	21	22
1:10	19	21
1:15	21	19
1:19	17	19
1:24	15	18
Undiluted	29	31
Distilled water	No inhibition	No inhibition
Antibiotic	42	40
Methanol	30	36

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Table No. - 2
For naturally available Cynodon dactylon

Dilutions	Zone of Inhibition(in mm)	
	E.coli	*S.aureus*
1:2	32	38
1:4	36	36
1:8	31	29
1:10	28	28
Undiluted	40	38
Distilled water	No inhibition	No inhibition
Antibiotic	41	42
Methanol	42	42

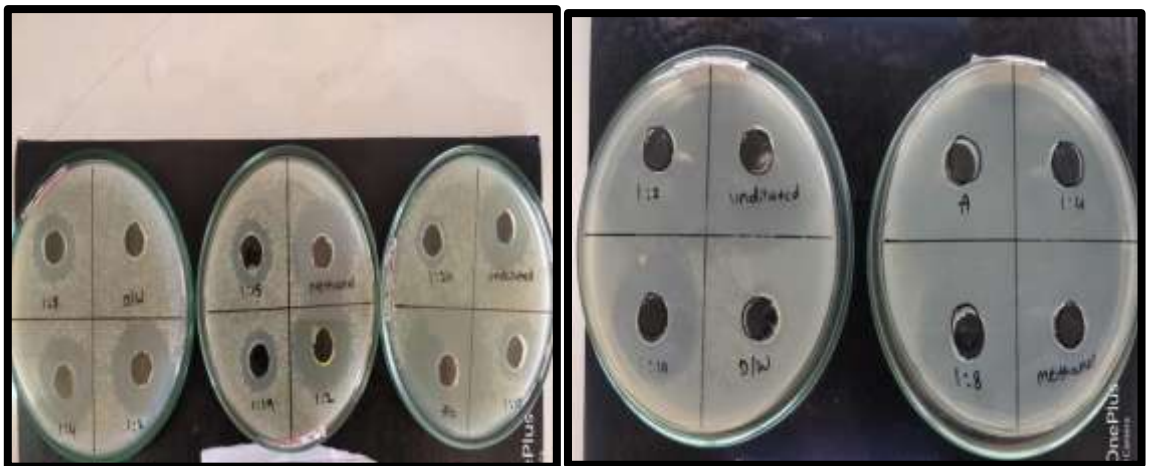

Fig 6a& 6b: Agar Cup Method of naturally available Cynodon dactylon against E.coli and S.aureu srespectively.

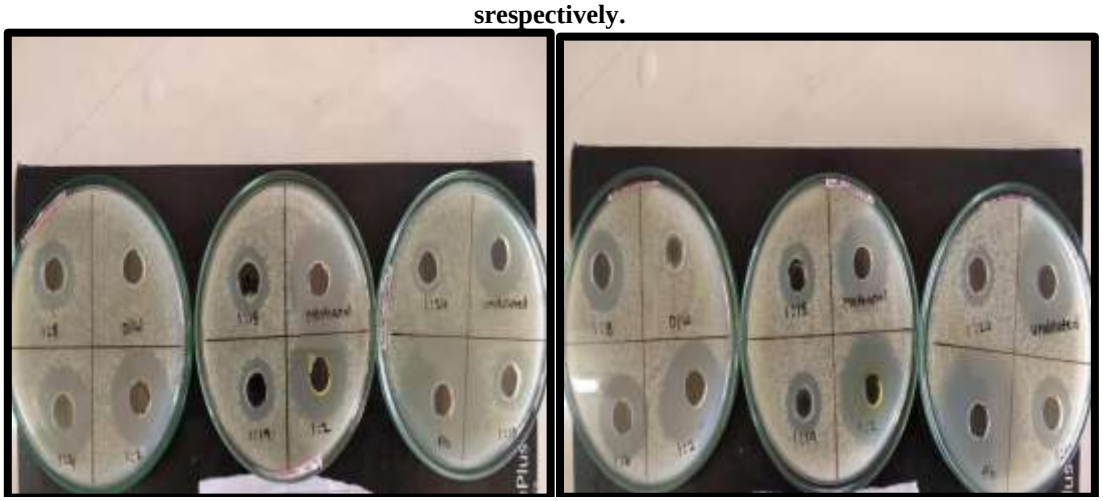

Fig 7a: Agar Cup Method of commercially available Cynodon dactylon against E.coli & S.aureus

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IV. CONCLUSION:

From the above results, it can be concluded that the plant Cynodon dactylon showed significant antibacterial activity. The experimental evidence obtained in the laboratory could provide a rationale for the traditional use of this plant as antibacterial for infections. The activity was performed according to the standard laboratory procedures. The plant may be further explored for its phytochemical profile to recognize the active constituent accountable for antibacterial activity. Thus the present experiment from methanolic extract of Cynodon dactylon has been scientifically proved to be beneficial as an antibacterial agent for specific bacterial infections.

Naturally available Cynodon dactylon gave more zones of inhibition than commercially available Cynodon dactylon .It suggests that preferably natural Durva should be used for effective results. More active ingredients in Cynodon dactylon worked together to give the antibacterial activity, and in case of commercial one the action is suppressed by other added ingredients.

Controls such as Distilled water was loaded to ensure the antibacterial effect was due to the natural product selected and not due to solvent. Methanol was also loaded into the well to ensure any interference of methanol.

After incubation at 37 °C for 24 hours different dilutions gave different ranges of zones of inhibition. The least diluted that is 1:2, 1:4 gave a larger zone of inhibition than other dilution.

Considering the above results it is evident that Cynodon dactylon gives positive results against Gram positive and Gram negative microorganisms.

Future perspective:

Cynodon dactylon occupies a key position in ethno medicinal practices and traditional medicinal systems. It is extremely useful in a wide variety of diseases and disorders. Various pharmacognostic and pharmacological actions of Cynodon dactylon have been investigated by researchers all around the world, supporting its medicinal uses mentioned in the traditional medical knowledge systems. Therefore, further investigations on therapeutic actions of individual phytochemicals present in Cynodon dactylon at cellular and molecular level can be encouraged. (13)

Cynodon dactylon were previously studied to possess antiarrhythmic activity against ischemia or reperfusion-induced arrhythmias and cardio protective properties in tested rat. Carbohydrate and fatty acid derivatives from natural sources have been proven to possess broad-spectrum antimicrobial activity. The antibacterial activity of methanol extract was believed to be due to the presence of active principle in the extracts such as saponins, phenolics, and terpenoids which might be responsible for the broad spectrum of antibacterial activity compared to the other extracts. Higher resolving strength of ethanol in regards to its yield percentage consequently enables it to resolve comparatively more bioactive compounds which might explain the considerable antimicrobial activity compared to the other solvents. Meanwhile, other solvents such as ethyl acetate can be able toresolve other trace bioactive constituents which are not being able to be resolved by methanol in greater amounts, explaining the significant antimicrobial activity. Generally, gram-negative bacteria weremore resistant to antibiotics than gram-positive bacteria. The resistance is due to the differences in their cell wall composition. (14)It is known that since the cell wall of gram-negative organisms possesses a thick murine coat, they show more antibiotic resistance than gram-positive organisms. (10)

Medicinal herbs have a long history of use as drugs in Asia either as crude or in extract form added in food or administered orally. These herbs possess bio active components, and this has brought about an improved comprehension of their numerous capable clinical, physiological, and therapeutic applications in the current medical fraternity. (15) Antimicrobial agents are the drugs helps reducing the worldwide burden of infectious illness. But overuse of these agents is leading to the resistance of bacteria to these drugs. However, given the evidence of the rapid global spread of resistant clinical isolates, the need for new antimicrobial agents is critical; however, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents suggests that even new families of antimicrobial agents will have a short life expectancy. A large variety of medicinal plants have been identified as important sources of natural antibacterial substances that may be effective in the treatment of these troublesome bacterial illnesses. The World Health Organization (WHO) believes that medicinal plants are the best source ofa wide range of medications. (3)According to Unani system of medicine, Cynodon dactylon is used as a laxative, expectorant, carminative, coolant and as a brain and heart tonic. In Homoeopathic systems of

medicine, it is used to treat all types of bleeding and skin troubles. (16). Also studies show that herbal face wash of Durva have anti-microbial and anti-inflammatory actions. (17)

In other studies it is seen that the area of the zone of inhibition of the Bermuda grass oil is greater than that of the negative control. Thus, it is encouraged to the manufacturers of antibacterial products to use Bermuda grass oil as an antibacterial agent. (18) C. dactylon dietary interventions have shown most promising results on Bovine Coronavirus (BCV or BCov) and other respiratory diseases in calves. BCovInfection causes calf enteritis and contributes to the enzootic pneumonia complex in calves. A previous study of Bovine Coronavirus, in which calves fed high-concentrate C. dactylon grass diet were evaluated for Bovine Coronavirus for 2-week conditioning period, obtained all calves tested negative. (19)

The effect of Cynodon dactylon in restoration of the male reproductive dysfunction induced by immobilization stress, was studied by evaluation sexual behavioural, sexual performance, fructose content of the seminal vesicles, epididymal sperm concentration and histopathological examinations. Treatment of rats under stress with methanolic extract of Cynodon dactylon has shown a promising effect in overcoming stress-induced sexual dysfunction, sexual performance, fructose content, sperm concentration and its effect on accessory sexual organs and body weight. The authors concluded that Cynodon dactylon methanolic extract had a potent aphrodisiac and male fertility activity. (20)

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