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Comparative Study of Pharmacological and PhytochemicalAnalysis on Cynodon Dactylon And Rubia Cordifolia

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Abstract

Plants consist of various phytoconstituents which are responsible for antibiotic propertiestowards human pathogens such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosaand Bacillus subtilis. The present study was aimed to pharmacological and phytochemical analysis ofCondon dactylic and Rubia cord folia. Crude methanol and aqueous extracts of the leaves of Cynodondactylon and Rubia cord folia were used for the study. Different dilutions of each extracts were studiedfor the phytochemical screening and antibacterial activity. Antibacterial activities of each extract werecarried out by using agar cup technique by using Tetracycline as a standard. All the extracts exhibitedsignificant antibacterial activity but S aureus organism were showing the resistivity towards aqueousand metabolic extracts of Rubia cordifolia.

Keywords: Agar cup technique, Cynodon dactylon, Phytoconstituents, Rubia cordifolia, Tetracycline

Introduction: The term phytoconstituents defines the diverse range of chemical compound derivedand isolated from the plants. The interest on the phytoconstituents have been started from Vedic erawhich was seems to be useful to human being at that era and till present it is still very useful. Compoundsand extracts derived from plants have been found useful in medicine such as allopathic, homeopathicand Ayurveda beauty products and health products in developed and developing society. Due to the development of bacterial resistance to presently available antibiotics has necessitated the search ofnew antibacterial agents able to fight against resistant pathogenic bacteria. So the antibacterial activityof the plant extracts of Cynodon dactylonand Rubia cordifolia were studied against various pathogenic bacteria such as Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis.Cynodon dactylon commonly known as "Doob grass "belonging to the family Poaceae. It is aperennial grass distributed all over the world and native to the warm temperature and tropical region.The plant has been rich in metabolites notably proteins Carbohydrates, minerals, carotenoids, alkaloids, glycosides andTriterpenoids. Rubia cordifolia commonly known as Indian madder and Manjishtha in Sanskrit is perennial, Herbaceous, prickly climber with long and cylindrical root with a thin red bark. Belonging to the family Rubiaceae, The plant roots contain an organic compound calledalizarin that gives red color. The roots and stems are well known sourceof Anthraquinones. The rootshave also been reported as antioxidant, antiinflammatory anticancer immunomodulator and hepatoprotective and are extensively used against blood urinary and skin diseases. The present studywas aimed to screening of phytoconstituents and Investigation of the antibacterial potential of crudemethanol extract and aqueous extract of the Cynodon dactylon and Rubia cordifolia on Human pathogens suchas Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis by using agar cup technique taking tetracycline as a standard drug.

MATERIALS AND METHOD:

Plant material: Fresh plant materials of Cynodon dactylon and Rubia cordifolia were collected fromthe locality of kattigenhalli. The leaves of Cynodon dactylon and Rubia cordifolia were initially separatedfrom the main plants body and rinsed with distilled water. After wards the samples were dried under shade paper towel in laboratory and then homogenized into fine powder.

Extraction

Aqueous extraction: 10gm of dried powder of each plant material was weighed and soaked separatelyin a 50ml cold water in a stoppered conical flask and then kept in rotary shaker at

200rpm for 24hrs after completion of 24hrs extract were filtered by using What man's filter paper No.1. The extracts wasconcentrated in a thermostat water bath at 80o C temperature and obtained extract were kept in thedesiccator for the experiment.

Solvent extraction: 10gm of dried powder of each plant material was weighed and soaked separatelyin a 50ml extrapure methanol in a stoppered conical flask and then kept in rotary shaker at 200rpm for24hrs after completion of 24hrs extractwere filtered by using whatmans filter paper No.1. The extractswas concentrated In a thermostat water bath at 80oC temperature and obtained

Extract were kept in the desiccator for the experiment.

Drugs and Chemicals: Tetracycline (pFizer pharmaceuticals Ltd. Thane) Methanol (Merck). All the chemicals used are laboratory and analytical grade. Qualitative Phytochemical Screening. Detectionof alkaloids

Mayer's Test: The extract were dissolved individually in dilute HCL and filtered. Filtrate were treatedwith Mayer's reagent. Formation of yellow color precipitate specifies the presence of alkaloids.

Detection of carbohydrates

Molish test: 10mg of extract dissolved in 2ml of molish reagent and shake properly. Add 2ml of concH2so4 from the Sides of the test-tube. Appearance of violet ring at the Interface indicates the presenceof carbohydrates.

Test for flavonoids:

Alkaline reagent test: 10mg of extract dissolved in 2ml of 2% NAOH solution. And intense yellow colour formed which turn colourless on addition of few drops of dil HCL which indicated the presence offlavonoids.

Detection of tannins

Ferric chloride test: 5mg of extract was taken and 0.5ml of 5% ferric chloride was added. The development of dark bluish black color indicates the presence of tannins.

Detection of sterols and steroids

Salkowski's test: 5mg of extract was dissolved in 2ml of chloroform and equal volume of conc sulphuricacid was added Along the sides of the test tube. The upper layer turns red and lower layer turns yellowwith green fluorescence, Indicating the presence of steroids and sterols compound in the extract.

f) Test for proteins

Xanthoproteic Test: The extracts were treated with few drops of concentrated nitric acid. Occurrenceof yellow colour indicates the presence of protein.

g) Test for saponins

Froth test: Crude extract was mixed with 5ml of distilled water in test tube and it was shaken vigorously.The formation of stable froth indicates the presence of saponins.

Antibacterial Activity

Agar Cup Technique: Bacteriostatic activity of crude plant extract were tested by using agar cup technique.

Media preparation: Muller Hinton agar were used as a growth medium to study the antibacterial activity Bacterial suspension 24hrs old culture of Ecoli, S. aureus, P. aeruginosa and B. subtilis were used forthe inoculation.

Protocol: Assay of minimum inhibition concentration: Crude plant extract were diluted as 30%,50%, 70% and 90% dilution with sterile distilled water. MH agar plate were inoculated with 24hrs oldbacterial culture by using sterile cotton swabs. Later wells were punched 4 in one plate by using sterile well borer. Wells were filled with diluted plant extractand incubated for 24hrs at 37oC.after incubation period zone of inhibition were observed and measured. The least concentration showing visible growth was taken as MIC value.

Results and Discussion: Phytochemical characteristics of Cynodon dactylon and Rubia

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cordifolia were tested and summarized in table 1 Table 2. The results revealed that flavonoid, sterols, were Present in both the plants. Different dilutions of crude plant extracts were used and agar cup technique were used to optimize minimal inhibitoryconcentration to obtain zone of inhibitions summarized below.

Table 1: Phytochemical screening of aqueous extract of Cynodon dactylon and Rubia cordifolia

Extracts		
Phytoconstituents	C.dactylon	R.cordifolia
Alkaloid	++	--
Flavanoid	++	++
Tannin	++	--
Sterols	++	++
Coarbohydrates	++	++
Proteins	--	++
Saponin	++	++

Table 2: Antibacterial activity of Methanolic extract. Antibacterial activity against E.coli organism

Conc of plant ext.(in %)	Zone of inhibition *Cynodon dactylon*	MIC of *Cynodon dactylon*(mm)	Zone of inhibition *Rubia cordifolia*	MIC of *Rubia cordifolia*(mm)
30	++	12	++	13
50	++	16	++	14
70	++	17	++	18
90	++	20	++	19

Table 3: Antibacterial activity against S.aureus organism

Conc of plant ext. (in %)	Zone of inhibition *Cynodon dactylon*	MIC of *Cynodon dactylon* (mm)	Zone of inhibition *Rubia cordifolia*	MIC of *Rubia cordifolia* (mm)
30	++	13	++	13
50	++	15	++	15
70	++	16	++	18
90	++	19	++	21

Table 4: Antibacterial activity against B.subtilis organism

Conc of plant ext. (in %)	Zone of inhibition *Cynodon dactylon*	MIC of *Cynodon dactylon* (mm)	Zone of inhibition *Rubia cordifolia*	MIC of *Rubia cordifolia* (mm)
30	++	12	++	14
50	++	15	++	16
70	++	15	++	19
90	++	18	++	21

Table 5: Antibacterial activity against P.aeruginosa organism

Conc of plant ext.(in %)	Zone of inhibition *Cynodon dactylon*	MIC of *Cynodon dactylon* (mm)	Zone of inhibition *Rubia cordifolia*	MIC of *Rubia cordifolia* (mm)
30	++	14	++	16
50	++	14	++	17
70	++	15	++	18
90	++	16	++	21

Discussion: Phytochemical screening of plant extract revealed the Presence of phytoconstituents which exhibit medicinal property. Analysis of plantextract reveals the presence of phytoconstituents such as alkaloid, flavonoid, saponin, sterols, tannins, proteins and carbohydrates. Phytoconstituents possess biological property such as Antiapoptos is, antiageing, cardiovas cularprotection, anti-diabetic, anti-arthritic, anti-inflammation etc. The plant extract reveals to contain saponin which possess anti- inflammatory activity, sterols have antibacterial as well as anti-arthritic activity. Alkaloidpossess antibacterial and analgesic activity. The antibacterial activity experiment was conducted using Crude plant extract. Standard antibiotic (Tetracycline) were Included to monitor the experimental conditions and to facilitate better comparative analysis. The susceptibility testing of plant extract showed that Cynodon dactylon have potent broad spectrum antibacterial activity.MIC was performed by using differentdilutions of crude plant extract.

Conclusion: The result obtained from present and earlier study confirms the phytochemicals to be bioactive. The plant extracts could be an effective source of medicine. Through our study of the plant extracts showed better antibacterial activity against 4 human pathogenic microorganisms further ithas been found that Cynodon dactylon and Rubia cordifolia both shows broad spectrum antibiotic activity against human pathogenic organisms. Through my study foundthat 30% dilutions shows moderate effect against four bacteria used for the study. For future aspects the purification and colourization of the phytoconstituents can be done which may lead to development of important pharmaceutical compounds.

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