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PHYTOCHEMICAL EVALUATION AND ANTIMICROBIAL ACTIVITY OF *DIGERA MURICATA (L.)*

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ABSTRACT

This study gives an updated information of phytochemical screening and antimicrobial activity of the aqueous and methanolic extracts of *Digera muricata (L.)* belongs to the *Amaranthaceae* family. Medicinal plant care is known to be very effective, as there are no or limited side effects. These medicinal plants are rich source of ingredients that can be used in synthetic drug development. *Digera muricata (L.)* is found as weed throughout India. Almost all parts of this plant are high in its medicinal value but roots and leaves are widely used for various purpose. The leaves of *Digera muricata (L.)* was extracted using distilled water and methanol in ascending order of polarity using soxhlation. These extract was subjected to phytoochemical evalution reveals the presence of alkaloids, flavonoids, glycosides, aminoacids, carbohydrates, proteins, tannins, steroids. These extract is tested to antimicrobial activity by using cup plate method. Zone of inhibition is observed with *Bacillus subtilis* and

Staphylococcus aureus. The results from our research could help in the development of novel antimicrobial agent.

Keywords: ***Digera muricata (L.), Amaranthaceae,*** **soxhlation, cup plate method, antimicrobial activity,** ***Bacillus subtilis, Staphylococcus aureus***

INTRODUCTION

An antimicrobial is any naturally occurring, semi-synthetic or synthetic material that kills or inhibits microorganism growth but does little to no harm to the host. Microorganisms have evolved with the production of antimicrobials and have become immune to past antimicrobial agents. The old antimicrobial technology was either based on poisons or heavy metals that may not have fully killed the microbe, allowing the microbe to live, alter, and become poison and/or heavy metals immune **[1]**. Modern phytomedicine is a timely and original manual that paves the way for success in the production of plant based drugs, addressing the problems faced by a pharmaceutical scientist who wants to turn a plant compound into a safe and effective drug. Antimicrobial plant derived agent has been largely ignored. The *digera muricata* (L.) Mart is therefore used in both the folk and traditional medicine systems .In recent years the use and quest for plant-derived medicines and nutritional supplements has increased **[2]**. Chemists, ethno pharmacologists, microbiologists, and botanists are searching the earth for phytochemical and "leads" that could be produced for infectious disease care. Although 25 to 50 percent of current medicines are plant-derived neither are being used as antimicrobials. Traditional healers have always adopted plants to avoid or treat infectious diseases; western medicine is seeking to replicate their achievements. Plants are abundant in a large range of secondary metabolites, such as tannins, flavonoids, terpenoids and alkaloids that have been shown in vitro to have antimicrobial properties. The field of antimicrobial botanical extracts has two reasons for clinical microbiologists to be involved in. First, these phytochemical are also likely to make their path to the arsenal of physician-prescribed antimicrobial medications; some have already been studied in humans. It is estimated that two or three microorganism-derived antibiotics are launched on average per year. In the 21st century, severe infectious induced by pathogens resistant to widely antimicrobials available for human use throughout the world has been smaller in recent years than in the past years **[3]**.

Digera muricata (L.) Mart is also known as Chenchalikura commonly referred as "*latmahuria*". *Digera arvensisforsk* belongs to the family Amaranthaceae **[4]**. It is

widespread in India and eastern tropical Africa (from Sudan and Ethiopia south to Tanzania), Madagascar and tropical and subtropical Asia (from Yemen to Afghanistan, Pakistan, India, Malaysia and Indonesia. (Plant use) these are alternatively arranged leaves, 1-9cm long and 0.2-5cm wide, are narrowly linear to ovate widely **[5]**. Leaf stalks are long, up to 5cm in length, base narrowed and tip pointing. Flowers on sender spike-like racemes that can be as large. The fruit is subglobose, slightly compressed 2-2.5 mm, ribbed bluntly on each side and surrounded by thick rim. *Digera muricata* leaves and young shoots are used as vegetables locally and are provided to relieve constipation **[6]**.It is also used internally against digestive disorders and for the prevention of urinary discharges in Indian flowers and seeds. The leaf paste is applied locally to prevent the development. Boiled root infusion given to mother for the purpose of lactation after childbirth **[7]**.

MATERIALS and METHODS

Plant materials:

Digeramuricata (lesua) plant is well known for antimicrobial activity and other medical properties. Fresh and disease-free leaves parts (leaves) of plant *Digera muricata* collected near farms of Chalapathi institute of pharmaceutical sciences. Plant is authenticated from Acharya Nagarjuna University.

Chemicals used:

Methanol, Peptone, Beef extract, Sodium chloride, Agar, Sulphuric acid, 10%Sodium hydroxide, Coppersulphate, Picric acid, Ninhydrin reagent, Aceticanhydride, Molish reagent, 10% Ferricchloride, Concentrated Hydrochloric acid, Distilled water.

Apparatus:

Autoclave, Laminar airflow, Incubator, Water bath, Petridish, Conical flask, 200ml Beaker, Test tubes.

Organisms used:

- *Bacillus subtilis*
- *Staphylococcus aureus*

Equipments used:

Soxhlet apparatus

METHOD:

Collection of plant materials:

Fresh leaves of *Digeramuricata* are collected from the farms near to the Chalapathi institute of pharmaceutical sciences, Guntur, Andhra Pradesh. Fresh leaves that are free from diseases are collected and washed them with the tap water and then with distilled water. Next the leaves are shade dried and pulverised in to powder. Then the powder is sealed in a airtight plastic bag and stored at room temperature till the extraction is carried out.

Preparation of extract:

The powder of leaves (10g) is subjected for extraction in 100ml of aqueous methanol. The content has been kept in continuous

shaker at room temperature for overnight. Then the extract is filtered twice with whatman filter paper no 1. Then the collected extracts are stored at 4 degrees centigrade and then subjected to the phytochemical analysis and antimicrobial studies **[8]**.

Seperation with column chromatography:

Methanol extract (20g) was subjected to silica gel column chromatography (100-200 mesh-Merck) packed and eluted with methanol mixture and water of increasing polarity to obtain fractions. Twenty grams of the process extract were chromatographed over a column of silica gel (Merck, India) and eluted began with 100-200 mesh). The admixture was packed on a column of silica gel (Merck, India) and eluted began with 100%hexane, and increased with 10:90 gave a colourless compound and the isolated compound and the isolated compound yield was 200mg after further purification with methanol **[9]**.

PHYTOCHEMICAL ANALYSIS

Alkaloids:

Approximately 2ml of extract was taken in a test tube to which the solution of picric acid was introduced. The presence of alkaloids suggested yellow colouration.

Flavonoids:

Approximately 4ml of extract solution was mixed with 1.5ml of 50 percent methanol solution. The solution was heated and added metal magnesium. Add 5-6 drops of concentrated hydrochloric acid is also added to this solution and red colour was observed for flavonoids **[10]**.

Glycosides:

Add 5ml of extract in a test tube and to it add 25ml of dilute sulphuric acid and boil it for 5 minutes and then cool it and neutralised it with 10% NaOH and then add 5ml Fehling's solution is added brick red precipitate indicates the presence of glycosides.

Proteins:

Take 2ml of extract in a test tube to it add equal volumes of 40% NaOH and two drops of 1%copper sulphate solution. Violet colour appearance indicates the presence of proteins.

Amino acids:

Take 2ml of extract in a test tube to it add 2 drops of freshly prepared 0.2% ninhydrin reagent and heat the solution. Appearance of pink or purple colour indicates the presence of amino acids **[11]**.

Steroids:

To 5ml of extract add 2ml of acetic anhydride and to it add 2ml of sulphuric acid. The colour changed from violet to blue indicates presence of steroids.

Carbohydrates:

Take 2ml of extract to it add 2 drops of molisch reagent. Then add 2ml of sulphuric acid to the solution through the walls of test

tube resulting violet ring in between two layers indicates presence of carbohydrates.

Tannins:

Add 3-4 drops of 10percent ferric chloride solution to a portion of the extract diluted with water. A blue colour for Gallic tannins is observed and green for catecholic tannins is observed **[12]**.

ANTIMICROBIAL SCREENING

CUP PLATE METHOD:

Cultivation of microorganism:

The following microorganisms were used to study the antibacterial activity.

Bacillus subtilis – Gram positive bacteria

Staphylococcus aureus – Gram positive bacteria

Preparation of the medium:

Composition of nutrient agar medium

Beef extract……..10g

Peptone……..10g

Sodium chloride…….5g

Agar……20g

Purified water…….1000ml

pH 7.2± 0.2

The medium was prepared by heating on a water bath by dissolving the required quantity of the dehydrated medium in distilled water, and was dispenced in conical flasks of volume 100ml. the conical flasks were sealed with cotton plugs and sterilized at 121˚C (15 lb psig) for 15 minutes by autoclaving. The conical flask contents have been aseptically poured into sterile. Petridishes and are allowed to solidify. Such sterilized media were used to subculture the culture of bacteria **[13-16]**.

Procedure:

Every petridish was filed with a nutrient agar medium agar medium to a depth of 4-5mm that had previously been innoulated with suitable test organism inoculums, and then allowed to solidify. Petridish was placed on a level surface to ensure the medium layer is in uniform thickness. The petridishes were sterilized in a hot air oven at 160-170˚C for 30 minutes prior to use. Small sterile borer of uniform size, with an inner diameter of approximately 6-8mm and made of aluminum or stainless steel, was mounted at 10cm height. Each plate was divided up along the diameter into three equal parts. With the aid of a sterile borer, one cylindrical cavity was rendered in medium to each section. They incubated the petridishes for 18 hours at 37˚C. The inhibition zone diameter was determined **[15]**.

RESULTS AND DISCUSSION

The methanol extract was prepared by soxhleation. The fraction obtained in 4^{th} fraction showed most chemical constitutents **(Table 1)**.

In antimicrobial screening zone of inhibition is clearly observed in plates containing *Bacillus subtilis*, *Staphylococcus aureus*. Zone diameter was tabulated in the following **Tables 2, 3**.

Table 1: Phytochemical analysis

Plant Constituents	Present(+)/ Absent(-)
Alkaloids	+
Flavonoids	+
Glycosides	+
Amino acids	+
Fatty acids	-
Carbohydrates	+
Saponins	-
Proteins	+
Tannins	+
Steroids	+

Table 2: Zone diameter for *Bacillus subtilis*

I.	26.8
II.	27.6
III.	21.5

Table 3: Zone diameter of *Staphylococcus aureus*

I.	25.8
II	24.1
III	27.1

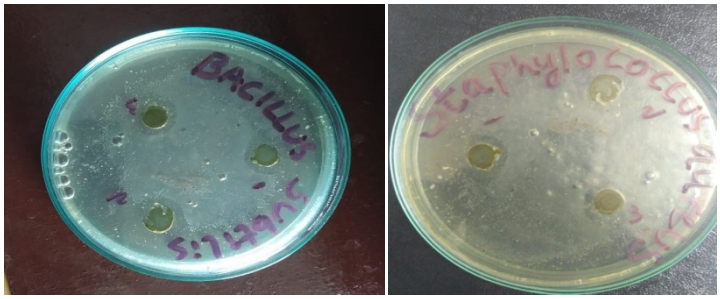

Figure 1: Antimicrobial activity on *Bacillus subtilis and Staphylococcus aureus*

CONCLUSION

Digera muricata is a weed plant that generally found in our surroundings and also in agricultural fields. This plant has a wide uses and it is used in natural medicine for treatment various disorders. Further there is an increasing resistant shown by the pathogens to the frequently used antimicrobials.

Plant leaves were collected and extraction is carried out through methanol and water and to this extract phytochemical analysis is performed, then it is screened for antimicrobial activity and this extract was found to be effective against *Bacillus subtilis* and *Staphylococcus aureus.*

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