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Antimicrobial activity of some Indian folklore medicinal plants against drug resistant bacteria and fungi isolated from clinical samples

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ABSTRACT

The present study was undertaken to screen the antimicrobial effect of folklore medicinal plants against clinical sample. The antimicrobial effect of medicinal plant extracts of gigantea, Musa paradisiaca and Crucuma amada were evaluated by agar well diffusion method against Staphylococcus aureus, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Proteus mirabilis, Candida albicans and Candida trophicalis. To study the susceptibility and minimum inhibitory concentration of aqueous and methanol herbal extract such as Calotropis gigantea, Musa paradisiaca and Crucuma amada against the drug resistant bacterial isolates. To study the anticandidal activity and minimum inhibitory concentration against Candida albicans and Candida trophicalis. The present results reveals that the methanolic extract of Musa paradisiaca showed maximum significant antimicrobial activity, in comparison to other two plants Curcuma amada and Calotropis gigantea. The present results revealed that medicinal plant could be used in treating diseases caused by the tested organisms.

Keywords: Medicinal plants, Antibacterial, Antifungal, *Musa paradisiacal*, Antimicrobial.

INTRODUCTION

Since ancient times, plants have been model source of medicines as they area a reservoir of chemical agents with therapeutic properties the general population is increasingly using herbal medicines as dietary supplements to relieve and treat different human ailments. Herbs and spices are a most important part of the human diet. They have been used for thousands of years to enhance the flavour, colour and aroma of food [1]. In addition to boosting flavour, herbs and spices are also known for their preservative [2], and medicinal value, which forms one of the oldest sciences [3]. Yet it is only in recent years that modern science has started paying attention to the properties of spices. Medicinal and spice plants are renewable raw materials. Their production is an alternative to the overproduction of traditional crops in agriculture. They also have an increasing economic importance spices can be defined as any dried. Fragrant, aromatic or pungent vegetables or plant substances in whole, broken or ground forms, that contribute flavour, whose primary function in food is seasoning rather than nutrition and that may contribute relish or piquancy of foods and beverages[4]. Although as natural substance spices and herbs are easily absorbed by our bodies and generally do not have any adverse effects, spices as medicine should be used judiciously. This is because substances being derived from a plant do not mean it is always harmless one drug used for one ailment could actually be deterimental to the treatment of another. The latest finding suggests that the chemicals present in spices can be allergens, carcinogens, mutagens and abortifacient.

Calotropis gigantea: Calotropis is used as traditional medicinal plant with unique properties. Calotropis is used alone or with other medicinals to treat common disease such as fevers, rheumatism, indigestion, cough, cold , eczema, asthuma, elephantiasis, nausea, vomiting and diarrhea, according to ayurveda, dried whole plant is a good

tonic, expectorant depurative and antihelminthic [5]. The root bark is febrifuge, antihelminthic, expectorant and laxative. The powdered root used in asthuma and bronchitis. The leaves are useful in the treatment of paralysis, arthralgia, swellings and intermittent fevers [5]. The flowers are bitter, digestive, astringent, antihelminthic and tonic Calotropis is also a reputed homoeopathic drug. Calotropis gigantean R.Br. Asclepiadaceae, commonly known as milkweed or swallowwort, is a common wasteland weed. Calotropis belongs to sclepiadaceae or milkweed or AK family which includes 280 genera and 2000 species of worldwide distribution. But most abundant in the sub tropics and tropics and rare in cold countries. Other familiar plants of Calotropis are milkweed or silkweed [asclepias syriaca. L] Butterfly weed [asclepias tuberose l.] and Calotropis procera (Ait). Ait.f.native to India Calotropis grows wild up to 900 meters. Throughout the country) on a variety of soils in different climates, sometimes where nothing else grows uses Calotropis yields a durable fiber (commercially known as bowstring of India) useful for ropes, carpets, fishing nets and sewing thread. Floss, obtained from seeds, is used for stuffing purposes. Fermented mixture of Calotropis and salt is used to remove the hair from goatskins for production of "Nari leather" and for sheepskins to make leather, which is much used for inexpensive book binding, fungicidal and insecticidal properties of Calotropis have been reported. Allelopathic effects of Calotropis on different agricultural crops have not been well studied. Extracts of different plant viz root; stem, leaf and stem+leaf of Calotropis affect germination and seedling vigor of many agricultural corps have been reported. However, extracts of Calotropis failed to produce any detrimental effects on weeds such as chenopodium album melilotus alba, melilotus indica, sphaeranthus indieus and phalaris minor.

Curcuma amada: Description: A Ginger with stout underground rhizomes. Foliage dies down in late in autumn and the rhizomes remain dormant in winter. The inflorescence appears in spring from the base of the rhizome. The peduncle grows to about 8 to 10 inches tall. Leaves appear avert the flowers. When in full growth the plants can reach a height of about 3 feet tall. Leaves are broad and very decorative. Good for cut-flower use with a vase life of about 10 days for a fresh stem.

Habitat: This species is grown in villages. Rhizomes have an aroma of green mango. The fresh and dried rhizomes are used for flavouring curries.

Ethno botany: Rhizomes used to some extent in villages. For flavouring curries. There are 25 – 80 species in the genus musa, depending on the taxonomist, but it is likely that the higher number stems from subspecies being given species status. Musa is important not only for fruit production, but the genus has provided man with food, clothing, tools and shelter prior to recorded history. Manila hemp (M.textilis) is grown, for fiber, not fruit, which is derived from its pseudostem. This fiber can be made into strong rope or abaca cloth. Edible musa spp.originated in southeastern. Asia, from India east and south to northern. Australia early Filipinos probably spread the banana eastward to the pacific islands, including Hawaii, prior to recorded history. Westward, banana likely followed the major trade routes that transported other fruits, and it is known to have arrived in east Africa around 500AD. Bananas were not carried to Europe until the 10^{th} century, and Portuguese traders, obtained it from West Africa, not Southeast Asia, during the age of discovery. Plants were taken from West Africa to the Canary Islands and South America in the 16^{th} century, and spread throughout the Caribbean with settlement of the area in the 16^{th}-17^{th} centuries. Bananas are bow grown pan tropically in130 countries, more than any other fruit crop in the world.

Medicinal Properties and Non - Food Usage of Musa paradisiaca

Bananas are wide spread in poor, tropical countries, and due to the abundance of folk remedies in those countries and/or lack of western medicines, bananas have found a number of medicinal uses. Many of the uses are poorly documented, but include treatments for ailments of the skin, back and blood, headaches, fever and flu, both diarrhea and constipation, and several others including gray hair and syphilis. Bananas contain moderate amounts of Potassium, although the utility of bananas for restoring electrolyte or regulating blood pressure may be overstated. Some extracts of banana have shown hypoglycemic activity in lab studies. Banana fruit contain the active principals such as serotonin, nor-epinephrine and dopamine. Banana leaves and stems contain large amount of fertilizer elements, and are often cut from old plants to serve as mulch and nutrition for the growing crop. Fertilizers are made from dried, chopped stems and leaves. Ash from burned leaves and stems is used as salt. Live stock is often fed banana culls and other plant parts. The fibers from the ground banana rachis can be mixed with wood fibers to make paper, novelty items such as notepads, envelopes and wrapping paper are sold in casta rice. Banana leaves are among the largest of all plants. They can be used effectively as umbrellas, wrapping material for food or other loose items, plates for vessels for liquids, as well as thatch. Tent bats chew along the midrib of leaf undersides, causing the leaf to fold, creating a dry sleeping place (hence their name). Plant: Both banana and plantain are large herbaceous

monocots, reaching 25 ft in some cultivars, but generally 6 – 15 fit tall cultivation. Plantains are often larger than bananas. The "trunk" or pseudostem is not a true stem, but only the clustered, cylindrical aggregation of leaf stalk bases. Leaves are among the largest of all plants, becoming up to 9 ft long and 2 ft wide. Margins are entire and venation is pinnate; leaves tear along the veins in windy conditions, giving a feathered or tattered look. there are 5 - 15 leaves on each plant, with 10 considered the minimum for properly maturing a bunch of fruit. A shoot may produce a total of up to 50 leaves during its life cycle, but leaves are functional only for about 2 months. In the humid tropic, about 1 new leaf per week is produced. The perennial portion of the plant is the rhizome, which may weigh several pounds. It is often called a corm. It produces suckers, or vegetative shoots, which are thinned to 2 per plant- one "parent" suckers, for fruiting and one "follower" to take the place of the parent after it fruits and dies back. It also produces roots and serves as a storage organ for the plant. The vegetative apex of the rhizome spontaneously initiates a reproductive meristem after 40 leaves have been produced, usually 9 months after initiation of a sucker. A banana plant bears fruit 10 - 12 months after planting, plantains can taken longer, 14 – 19 months, particularly in cooler areas.

Flowers: The inflorescence is a spike originating from the rhizome. Initially, it appears above the last leaves in an upright position. As the bud opens, the narrow, white, tubular, toothed flowers are revealed, clustered in whorled double rows along the stalk, each cluster covered by a thick, purple, hood- like bract. The bract lifts from the first hands in 3 – 10 days. Female flowers, with inferior ovaries, occupy the lower 5 to 15 rows on the stalk, with neuter or hermaphrodite flowers in the center, and males at the top. Male flowers and bracts are shed immediately after opening, leaving the terminal potion of the Salk naked expect for the large, purple, fleshy bud at the tip containing unopened male flowers (expect dwarf Cavendish bananas and 'frenchiplantoin' males persistent). The flower stalk begins to drop down under its own weight shortly after opening; the flowers are negatively geotropic and turn upright during the first 10 weeks of growth.

In this study, we have selected three medicinal plants, in an attempt to screen for antibacterial and antifungal activities. This includes erukkum (*Caltropis gigantea*), banana (*Musa paradisiaca*) and mango ginger (*Curcuma amada*).

MATERIALS AND METHODS

Plant Materials

Calotropis giganteaand Musa paradisiaca is used the part of flower extract. *Curcuma amada* is used the part of root extract. For extraction the following are used aqueous extract and methanol. Bacterial cultures employed are *Staphylococcus aureus*, *Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae* and *Pseudomonas aeruginosa*. Fungal Cultures employed are *Candida albicans* and *Candida trophicalis*. Media used are Muller Hinton agar (MHA), Sabouraud`s Dextrose agar (SDA), Nutrient broth and Sabouraud`s Dextrose broth. Other materials used are test tubes, conical flasks, Petri plates, Beaker, Soxhelet apparatus and thin layer chromatography apparatus.

Preparation of Extract

The flower and root were washed with water, then surface sterilized with 10% sodium hydrochloride solution, rinsed with sterile distilled water and were air dried at room temperature. The samples were ground into a fine powder. Preparation of aqueous extract the powdered plant samples were homogenized in sterile distilled water and filtered. The filtrate was evaporated in vacuo at 40 - 60˚c using a strong evaporated. Methanol extracts are 50g of powdered plant product was loaded in Soxhlet apparatus and fractionated in 250 ml methanol (40 -60˚c).The fraction obtained were rotary evaporated to dryness for yield the solvent residues and were stored at -20˚c until they were used for antibacterial and antifungal assay. Preparation of test solution was prepared with known weight of crude extract dissolved in 50% dimethyl sulfoxide [DMSO] to give a concentration of 125 µg/ml.

Micro organism tested

The clinical isolates were isolated from clinical samples and conducted antimicrobial susceptibility test by disc diffusion method (kirbuy bauer method). The drug resistant bacteria isolated from clinical samples were *Staphylococcus aureus*, *Salmonella typhi*, shigella dysenteriae, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The drug resistant fungi were *Candida albicans* and *Candida trophicalis*. Preparation of inoculums 24 hours old cultured of selected bacteria and fungi were mixed with physiological saline and turbidity was corrected by adding standard of 0.5 (10-6 cfu/ml).

Screening of Antibacterial and Antifungal Activity in Plants

The sterile Muller Hinton agar (MHA) and sabouraud`s dextrose agar (SDA) medium was poured into a petridish in a uniform thickness and kept aside for solidification. Using sterilized swabs, even distribution of lawn culture was prepared using desired bacteria such as *Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis, Shigella dysenteriae* and fungi such as *Candida albicans* and *Candida tropicalis* in Muller Hinton agar (MHA) plates. Fungi such as *Candida albicans* and *Candida tropicalis* in sabouraud`s dextrose agar (SDA) in medium. The inoculated plates were kept aside for few minutes. Using well cutter 2 wells are made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol. Using sterilized micro pipette, 20µl/ml of aqueous and methanol herbal extract 500µg/ml was added in to one well and in another well the same volume of corresponding solvent without herbal extract were added and maintained as control. The plates were incubated at 37ºc for 24 hours for bacteria and fungi after incubation, the inhibition of growth was analyzed and results were recorded.

Muller Hinton Agar Medium (MHA)

Beef extract-0.2gm, Peptone-1.75gm, Starch-0.15gm, Agar-1.70gm, distilled water- 100ml and PH-7.5.

Sabouraud`s Dextrose Agar (SDA)

Dextrose-4.0gm, Peptone-1.0gm, Agar-1.5gm, distilled water-100ml and PH-7.5.

Determination of Minimum Inhibition Concentration (MIC)

1 ml of plant extract (1mg/ml) was incorporated into 1 ml of nutrient broth and sabouraud`s dextrose broth and was serially diluted to obtain concentration of 1000mg/ml, 500mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml respectively. 20µg/ml of the inoculum was added to each of the test tubes. The tube without the extract served as control. The tubes were incubated 37ºc for 24 hours the readings were recorded. Minimum Inhibition Concentration was recorded as the lowest concentration of the extract at which no visible growth of the bacterial and fungal occurred after a period of 24 hours incubation.

Extraction and Purification of Antimicrobial Compound Soxhlet Extraction

The sample was placed into a Soxhlet extractor covered with a filter paper. A 200ml of solvent methanol was transferred in a flask the bottom of the apparatus. The flask, Soxhlet extractor glassware and water-cooled condenser were assembled. The heat mantle boiled the solvent and vapour was directed to the extractor to the top-mounted condenser. The condensed vapour dripped down into the sample diffused from the sample in to the solvent, which siphons bags into the lower flask when the cup was filled. The clean solvent methanol vapour continues to condense, refilled the Soxhlet cup and flushed back into the flask. The cycling action extracted the methanol soluble chemical from the sample and collected then in the flask. After the flasks cooled, the solvent and extract were typically condensed down to approximately 10ml and taken for Thin Layer Analysis (TLC).

Thin Layer Chromatography (TLC)

Preparation of TLC plates:

The slurry was placed in the applicator and applied evenly to a required thickness using a metal scale.

Activation of absorbent:

After making thin layers on plates. The liquid associated with the thin layer was removed, the thin layer plate was dried for 30 minutes in air and then in an oven at 110ºc for another 30 minutes. The drying made the absorbent layer active.

Sample application:

Capillary tubes were used for transferring the sample solution to the thin layer for quantitative work solvents used for sample solutions were volatile and as non-polar as possible.

Development tank

The TLC plate was placed in a development chamber at an angle of 45º. The bottom of the chamber is covered up to nearly 1mm by the solvent. Three sides of the tank are lined with solvent impregnated paper while top was covered tightly with the lid. The TLC that development chamber was perfectly saturated with solvent vapour by closing it by a lid.

Solvent System
The plates were developed in a solvent mixture of acetone 50%, methanol 40% and distilled water 10% upper layer.

Development methods
Ascending technique was used in which the solvents were allowed to rise to the height of about 15-18 cm on the normal 20 cm tall plate. This is quite a quick process, which required 20-40 minutes. At the end of this time, the plate was removed from the developing tank. Then, solvent front was marked and plate was finally allowed to dry.

Detection of components:
By spraying the plates with 9:1 ethanolic ferric chloride, presence of flavonoids can be detected. Flavonoids compounds – bluish grey colour and Non-flavonoids compounds – no such colour.

RESULTS AND DISCUSSION

In the present study the screening of antibacterial and anti candidal activity of plants such as Calotropis gigantea, Musa paradisiacal and *Curcuma amada* was evaluated by well diffusion method and were over their minimum inhibitory concentration were determined by the tube test. The antimicrobial activity of the aqueous extract in well diffusion method revealed that *Calotropis gigantea*showed maximum activity followed by *Curcuma amada* and *Musa paradisiaca* out of the seven clinical bacterial and fungal isolates tested, the *Staphylococcus aureus* was maximum sensitive against all the plants *Calotropis gigantea*(14mm), *Curcuma amada* (13mm) and *Musa paradisiaca* (11mm). Against *Shigella dysenteriae* was minimum sensitive against *Calotropis gigantea*, *Curcuma amada* (13mm), *Musa paradisiaca* (9mm), followed by *Salmonella typhi* sensitive against Calotropis gigantea, *Curcuma amada* (11mm), *Musa paradisiaca* (10mm), *Pseudomonas aeruginosa* sensitive against Calotropis gigantea, *Curcuma amada* (8mm) and *Musa paradisiaca* (9mm), *Proteus mirabilis* sensitive against Calotropis gigantea, *Curcuma amada* (9mm) and *Musa paradisiaca* (8mm), *Candida albicans* sensitive against *Calotropis gigantea*(10mm), *Curcuma amada* (9mm), *Musa paradisiaca* (8mm) and *Candida tropicalis* sensitive against *Calotropis gigantea*, *Curcuma amada* (8mm) and *Musa paradisiaca* (11mm)- Table-1.

Table – 1: Antimicrobial activity of aqueous extract of plant product using well diffusion method

S.No	Name of the Organisms	Zone of Inhibition (mm)		
		Calotropis gigantean **(500µg/ml)**	***Musa paradisiaca*** **(500µg/ml)**	***Curcuma amada*** **(500µg/ml)**
1	*Staphylococcus aureus*	14	11	13
2	*Salmonella typhi*	11	10	11
3	*Pseudomonas aeruginosa*	8	9	8
4	*Shigella dysenteriae*	13	9	13
5	*Proteus mirabilis*	9	8	9
6	*Candida albicans*	10	8	8
7	*Candida tropicalis*	8	11	8

Table – 2: Antimicrobial activity of methanol extract of plant product using well diffusion method

S.No	Name of the Organisms	Zone of Inhibition (mm)		
		Calotropis gigantean **(500µg/ml)**	***Musa paradisiaca*** **(500µg/ml)**	***Curcuma amada*** **(500µg/ml)**
1	*Staphylococcus aureus*	15	19	15
2	*Salmonella typhi*	14	19	10
3	*Pseudomonas aeruginosa*	13	21	21
4	*Shigella dysenteriae*	15	25	15
5	*Proteus mirabilis*	11	21	16
6	*Candida albicans*	12	20	18
7	*Candida tropicalis*	10	18	9

The methanol extract of plants was evaluated by well diffusion method, that among the three plants tested, *Musa paradisiaca* was significantly sensitive to all test clinical isolates the maximum sensitive to *Shigella dysenteriae* (25mm) followed by *Pseudomonas aeruginosa*, *Proteus mirabilis* (21mm), *Candida albicans* (20mm), *Staphylococcus aureus* and *Salmonella typhi* (19mm) and Calotropis tropicalis (18mm). *Curcuma amada* showed maximum sensitive to *Shigella dysenteriae* (25mm) followed by *Pseudomonas aeruginosa* (21mm), *Proteus*

mirabilis (15mm), *Staphylococcus aureus* (15mm), *Salmonella typhi* and *Candida albicans* (18mm) and *Candida tropicalis* (9mm). The *Calotropis gigantea*showed maximum sensitive to Shigella dysenteriae, *Staphylococcus aureus* (15mm), *Salmonella typhi* (14mm), *Pseudomonas aeruginosa* (13mm), *Candida albicans* (12mm), *Proteus mirabilis* (11mm) and *Candida tropicalis* (10mm), (Table-2). The present results reveals that the metallic extract of *Musa paradisiaca* showed maximum significant antimicrobial activity, in comparison to other two plants *Curcuma amada* and Calotropis gigantea. The lowest concentration of plant extract required to inhibit the clinical drug resistant bacterial and fungal isolates were evaluated by the tube dilution assay. The minimum inhibitory concentration of aqueous extract of *Calotropis gigantea*showed that the lowest concentration for *Shigella dysenteriae* and *Proteus mirabilis* was 125µg/ml, followed by *Staphylococcus aureus*, *Salmonella typhi* and *Candida tropicalis* 250µg/ml, *Candida albicans* and *Pseudomonas aeruginosa* 500µg/ml. (Table-3)

Table – 3: Determination of Minimum inhibitory concentration (MIC) for aqueous extract of Calotropis gigantea

S.No	Name of the Organisms	Minimum Inhibitory Concentration (µg/ml)					
		1000	500	250	125	62.5	31.25
1	*Staphylococcus aureus*	+	+	+	-	-	-
2	*Salmonella typhi*	+	+	+	-	-	-
3	*Pseudomonas aeruginosa*	+	+	-	-	-	-
4	*Shigella dysenteriae*	+	+	+	+	-	-
5	*Proteus mirabilis*	+	+	+	+	-	-
6	*Candida albicans*	+	+	-	-	-	-
7	*Candida tropicalis*	+	+	+	-	-	-

+ Presence of growth
- Absence of growth

Table – 4: Determination of Minimum inhibitory concentration (MIC) forMethanol extract of *Calotropis gigantea*

S.No	Name of the Organisms	Minimum Inhibitory Concentration (µg/ml)					
		1000	500	250	125	62.5	31.25
1	*Staphylococcus aureus*	+	+	+	+	+	-
2	*Salmonella typhi*	+	+	+	-	-	-
3	*Pseudomonas aeruginosa*	+	+	-	-	-	-
4	*Shigella dysenteriae*	+	+	+	+	+	-
5	*Proteus mirabilis*	+	+	+	+	-	-
6	*Candida albicans*	+	+	+	-	-	-
7	*Candida tropicalis*	+	+	+	+	-	-

+ Presence of growth
- Absence of growth

Table – 5: Determination of Minimum inhibitory concentration (MIC) for Aqueous extract of *Musa paradisiaca*

S.No	Name of the Organisms	Minimum Inhibitory Concentration (µg/ml)					
		1000	500	250	125	62.5	31.25
1	*Staphylococcus aureus*	+	+	+	-	-	-
2	*Salmonella typhi*	+	+	-	-	-	-
3	*Pseudomonas aeruginosa*	+	+	+	-	-	-
4	*Shigella dysenteriae*	+	+	+	-	-	-
5	*Proteus mirabilis*	+	+	-	-	-	-
6	*Candida albicans*	+	+	+	-	-	-
7	*Candida tropicalis*	+	+	-	-	-	-

+ Presence of growth
- Absence of growth

The Minimum Inhibitory Concentration (MIC) of methanol extract of *Calotropis gigantea*showed lowest concentration to *Shigella dysenteriae* 62.5µg/ml, *Staphylococcus aureus* 62.5µg/ml, *Proteus mirabilis* and *Candida albicans* 125µg/ml, *Salmonella typhi* and *Candida albicans* 250µg/ml, *Pseudomonas aeruginosa* 500µg/ml. (Table-4). The Minimum Inhibitory Concentration of aqueous extract of *Musa paradisiaca* to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Candida albicans* is 250µg/ml, were as *Proteus mirabilis*, *Salmonella typhi* and *Candida tropicalis* is 500µg/ml. (Table-5). The Minimum Inhibitory Concentration of methanol extract of *Musa paradisiaca* to *Pseudomonas aeruginosa*, Shigella dysenteriae, *Candida albicans* is 62.5µg/ml, were as *Salmonella typhi*, *Proteus mirabilis* is 250µg/ml and *Candida tropicalis* 500µg/ml. (Table-6).

Table – 6: Determination of Minimum inhibitory concentration (MIC) for methanol extract of *Musa paradisiaca*

S.No	Name of the Organisms	Minimum Inhibitory Concentration (µg/ml)					
		1000	500	250	125	62.5	31.25
1	*Staphylococcus aureus*	+	+	+	+	-	-
2	*Salmonella typhi*	+	+	+	-	-	-
3	*Pseudomonas aeruginosa*	+	+	+	+	+	-
4	*Shigella dysenteriae*	+	+	+	+	+	-
5	*Proteus mirabilis*	+	+	+	-	-	-
6	*Candida albicans*	+	+	+	+	+	-
7	*Candida tropicalis*	+	+	-	-	-	-

+ Presence of growth
- Absence of growth

Table – 7: Determination of Minimum inhibitory concentration (MIC) for aqueous extract of *Curcuma amada*

S.No	Name of the Organisms	Minimum Inhibitory Concentration (µg/ml)					
		1000	500	250	125	62.5	31.25
1	*Staphylococcus aureus*	+	+	-	-	-	-
2	*Salmonella typhi*	+	+	-	-	-	-
3	*Pseudomonas aeruginosa*	+	+	-	-	-	-
4	*Shigella dysenteriae*	+	+	+	+	-	-
5	*Proteus mirabilis*	+	+	+	-	-	-
6	*Candida albicans*	+	+	+	+	-	-
7	*Candida tropicalis*	+	+	-	-	-	-

+ Presence of growth
- Absence of growth

Table – 8: Determination of Minimum inhibitory concentration (MIC) for methanol extract of *Curcuma amada*

S.No	Name of the Organisms	Minimum Inhibitory Concentration (µg/ml)					
		1000	500	250	125	62.5	31.25
1	*Staphylococcus aureus*	+	+	+	+	-	-
2	*Salmonella typhi*	+	+	+	-	-	-
3	*Pseudomonas aeruginosa*	+	+	+	+	-	-
4	*Shigella dysenteriae*	+	+	+	+	+	-
5	*Proteus mirabilis*	+	+	+	+	-	-
6	*Candida albicans*	+	+	+	+	+	-
7	*Candida tropicalis*	+	+	+	-	-	-

+ Presence of growth
- Absence of growth

Table – 9: Separation of active compounds from three medicinal plants by using TLC method

S. No	Name of the plant	Colour developed	Component detected
1	*Calotropis gigantea*	Bluish grey	Flavonoid
2	*Musa paradisiaca*	Bluish grey	Flavonoid
3	*Curcuma amada*	Bluish grey	Flavonoid

The Minimum Inhibitory Concentration of aqueous extract of *Curcuma amada* against *Shigella dysenteriae* and *Candida albicans* was 125µg/ml, *Proteus mirabilis* 250µg/ml, were as other organism such as *Staphylococcus aureus*, *Candida tropicalis* 500µg/ml and *Salmonella typhi* and *Pseudomonas aeruginosa* 500µg/ml. (Table-7). The Minimum Inhibitory Concentration of methanol extract of *Curcuma amada* against *Shigella dysenteriae*, *Candida albicans* was 62.5µg/ml, followed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* is 125µg/ml. were as *Salmonella typhi* and *Candida albicans* showed 250µg/ml. (Table-8).The active compound present in all three plants was extracted using Soxhlet apparatus with methanol. The methanol extract of plant product was separated using TLC and the nature of active compound was quality activated detected as flavonoids compounds. (Table-9). Flavonoids are a major group of phenolic compounds reported for their spasmolytic [6], antimicrobial [7], and antiviral [8], effects.

CONCLUSION

It is concluded that the three folklore medicinal plant extract possess antimicrobial activity against human pathogens. The screening antibacterial and anti candidal of activity of all three plants *Calotropis gigantea*, *Musa*

paradisiaca and *Curcuma amada* showed significant results with test clinical isolates thus plants product will be used to treated the bacterial candidal diseases after extending the their drug standardization in vivo antimicrobial assay and clinical trials. Presence of flavoniods in three plants might be antimicrobial activity against tested organisms. Further probe are needed to isolate and characterize the biomolecule/ bioactive compounds to develop new antimicrobial drugs against human pathogens.

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