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Production of Soap from *Muntingia calabura* against Wound infection causing microorganisms

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Abstract: *Muntingia calabura* (Elaecoparceae) is a medicinal plant traditionally used for relieve various pain such as headache, cold, gastric ulcer or to reduce the prostate gland swelling. It is widely cultivated in the world especially in India and Indonesia. The present study was carried out to elucidate the importance of *Muntingia calabura.* The leaves and flowers aqueous extract was evaluate for their *in vitro* antimicrobial activity by using well diffusion method against wound infection causing microorganism, such as *Staphylococcus sp., Pseudomonas sp., Klebsiella sp., Escherichia coli., Bacillus sp., Aspergillus sp., Candida sp.,*. Phytochemical screening was done, the bioactive constituents such as alkaloids, carbohydrates, steroids, saponins, flavonoids, tannins, Terpenoids, were found in the solvent. Antioxidant properties were assessed less than 1, 1- Diphenyl- 2-picryl hydroxyl (DPPH) radical assay and found to have high antioxidant activities. Antimicrobial soap was prepared which can be used as an external antiseptic prevention and treatment of microbial infections. From this finding, it was observed that M. calabura possess potential antimicrobial that could be attributed to its high content of Phenolic compounds, and thus, needs to be further explored

Key Words: *Muntingia calabura,* Phytochemical Analysis, Anti-Bacterial Activity, DPPH assay, Soap.

1. INTRODUCTION

The "Green Medicines" obtain greater accent from the public and medical profession due to greater advance in understanding the implement by which medicinal plants can positively influences healthy hygiene and uniqueness of life. The natural drugs are universal and affirmed that the herbs are healthier and safer than synthetic ones [1]. Medicinal plants were the main source of cosmeceutical product is highlighted with ecological friendly products prior to synthetic substances with equivalent properties. The best thing of the herbal cosmeceuticals and pharmaceuticals is free from side effects that it is purely made by the herbs and shrubs and offers a body in proper tune with nature [2]. Higher plants are able to produce photosynthesis and produce numerous chemical compounds with various biological activities.

Muntingia calabura is a plant belongs to the family Elaeocarpaceae and it is commonly known as Jamaica cherry or kerukup Siam. Every segment of *M. calabura* plant is used for house hold therapy against diverse illness from ancient times. *M. calabura* tree is largely harvested in yards and along roadsides for consumable and ornamental purposes and grows rapidly even poor soil [3]. *M. calabura* tree is being compassionated for reforestation for environmental protection. Since, the tree grows rapidly about 7-12min height extended with nearly horizontal branchlets. The flowers, borne singly, are approximately 1.25-2cm in width with green sepals and white petals and numerous yellow stamens and also used to treat headaches and as an ant dyspeptic, antispasmodic and diaphoretic. In Colombia, the infusions of flowers of these plants are druck as tranquillizer and tonic. Authentically, the flower of M. calabura possesses antiseptic properties and their extracts valued as antispasmodic activity [4]. The flower and bark are used to reduce swelling in lower extremities and as antiseptic properties. In Mexico, the plant is used in the treatment of measles, mouth pimples, stomach ache [5]. *M. calabura* has been revealed to possess antioxidant, antiproliferative, antipyretic and cardio protective effects. Active biotic and pharmaceutical potential compound from the plant for humans use is necessary to challenge the disease caused by micro organisms [6].

2. MATERIALS AND METHODS:

2.1 Collection of plant material

The leaves and flowers of Muntingia calabura were collected from in and around Coimbatore district.

2.2 Preparation of solvent extract

The leaves and flowers of *Muntingia calabura* were air-dried at room temperature for two weeks in shade shown in fig.1. The dried leaves and flowers were then ground into powered shown in fig.2 was soaked in aqueous at the ratio of 1:20 (w/v). The solvent was removed from the sample by evaporating at 65°C using a water bath. To remove solid plants material, the first supernatant was filtered using Whatsmann No.1 filter paper. The extract was stored in a air tight container and used for further studies shown in fig.3.[7].

Fig.1 The Leaves and Flowers of *Muntingia calabura* in Dried Form

Fig.2 The Leaves and Flowers of *Muntingia calabura* in Powered Form

Fig.3 The Aqueous Extraction of Leaves and Flowers of *Muntingia calabura*

2.3 Collection of Sample

Pus samples were collected from patients of wound infection from Sri Ramakrishna Hospital, Coimbatore and refrigerated at 4^0 C.

2.4. Identification of Bacterial isolates

Pus samples were inoculated on to Nutrient Agar, Blood agar.

According to Cappuccino Laboratory Manual and Bergey's manual of Determinative Bacteriology microscopic examination and different biochemical parameters were performed to identify bacterial isolates. The Bacterial isolates were designated as B1, B2, and B3…etc

2.5 Lacto phenol cotton blue staining

The Colonies grown on Rose Bengal agar were subjected to Lacto phenol cotton blue staining**.** This method is used for making semi- permanent microscope preparation of fungi. The stain contains phenol which serves as fungicide,

lactic acid which acts as cleaning agent, cotton blue which stains the outer wall of fungus and glycerine which gives a semi- permanent preparation.

A small amount of fungal culture was transferred to a drop of stain on a glass slide using an inoculation needle and covered with a cover slip. The slide was examined under low power and high power microscopic view according to Cappuccino Laboratory Manual. The Fungal isolates were designated as F1, F2….etc

2.6 Phytochemical screening [8]

The phytochemical screening tests were performed on leaf and flower extracts to detect various phytoconstitutents present in them such as: alkaloids, flavonoids, saponins, tannins, carbohydrates, trepenoids etc., following the standard procedures.

2.6.1 Test for alkaloids (Mayer's test)

To 1ml of the sample treated with few drops of Mayer's reagent. The presence of alkaloid shows turbidity or creamy precipitation.

2.6.2 Test for amino acid and proteins (Ninhydrin test)

To 1ml of sample was treated with few drops of ninhydrin reagent was added. Then the mixture was heated in a water bath for 2-3 minutes. The presence of amino acid indicates yellow color while the protein indicates bluish-blackish color.

2.6.3 Test for carbohydrates (Benedict's test)

To 1ml of the sample was treated with 2ml of Benedict's reagent. The presence of carbohydrate shows color change of blue or green or orange or red.

2.6.4 Test for flavonoids

To 5ml of ammonia solution, 1ml of sample was treated with few drops of concentrated sulphuric acid. The presence of flavonoids indicates yellow color.

2.6.5 Test for phenol

The aqueous extract was mixed with 5ml of Folins-ciocalteu reagent and 4ml of sodium carbonate were vortexed for 15 seconds and allowed at 40°C for 30 minutes for color development. The presence of phenols indicates blue color.

2.6.6 Test for reducing agents

To the aqueous extract, a little amount of Fehling's reagent were added and the mixture was boiled for 2 min. The presence of reducing agents indicates a brick red color.

2.6.7 Test for saponins (Foam test)

The aqueous extract was diluted with distilled water and shook well for 15 min. About length of 1cm foam indicates the presence of saponins.

2.6.8 Test for Tannins (Lead acetate test)

To 2-3ml of extract, 0.5ml of 1% of lead acetate was added and the formation of a white precipitate indicates the presence of tannins and Phenolic compounds.

2.6.9 Test for Trepenoids

About 2ml of chloroform and 3ml of concentrated sulphuric acid was added with 5ml of extract. The presence of trepenoids appears reddish-brown color.

2.6.10 Test for Steroids

To 1ml of extract was treated with chloroform and acetic anhydride solution and few drops of concentrated sulphuric acid was added. The presence of steroids indicates dark pink or red colour precipitate.

2.6.11 Test for Phlobatannins

To 1ml of sample was boiled with 1% of aqueous hydrochloric acid. Formation of red precipitate indicates the presence of Phlobatannins.

2.7 Antimicrobial activity

The agar well diffusion method was employed to determine the antibacterial and antifungal activities of *Muntingia calabura* leaf and flower extracts. Standardized inoculums suspension (0.1ml) of each bacterial strain was spread on nutrient agar plates while fungal on Rose Bengal agar plates with sterile swabs. The agar plates were punched with a sterile cork borer size of 6mm and different concentrations of 20, 40, 80, 100µl of the sample was added with a micropipette. Plates were allowed to stand at room temperature for 30min and bacterial and fungal plates were incubated at 37°C for 24 hours and 20ºC for 4-5 days. After incubation the zone of inhibition was measured (mm) [9].

2.8 Antioxidant assay

2.8.1 DPPH free radical scavenging activity

The free radical scavenging activity of plant extract was measured by using 2, 2- Diphenyl- 1- PicrylHydrazl (DPPH). The aliquot of 3 ml of 0.004% DPPH solution in methanol and 10 to 50 µl of plant extract at various concentrations were mixed and the mixture was shaken vigorously and allowed at room temperature for 30 minutes. Decolourization of DPPH was determined by measuring the absorbance at 517 nm [10]. Scavenging activity was calculated as the percentage inhibition (%) using the following formula:

$$\%\text{DPPH anti-radical activity} = \frac{(\text{Control absorbance}) - (\text{sample absorbance})}{\text{Control absorbance}} \times 100$$

2.9 Preparation of soap

One gram of NaOH was dissolved in 5ml of distilled water, to which one gram of sodium silicate was added and the mixture cooled to room temperature. Seven grams (7 g) of coconut oil was then added to 8% of flower extract. Stirring was started until a thick paste of homogenous soap resulted. The soap sample was left to solidify [11].

2.9.1 Antimicrobial activity of soap by using *Muntingia calabura* flower extract

Antimicrobial activities of herbal soap were evaluated by 24 hours broth cultures of the test organisms were spread on nutrient agar plates with sterile swabs. The medicinal soap (2cm) size of *M. calabura* flower extract was gently pressed to the inoculated plates transversely across the inoculums to ensure intimate contact with agar surface. The inoculated plates were incubated at 37º C for 24 hours. Incubated plates were examined for the interruption of growth along the inoculums beneath the specimen and for a clear zone of inhibition beyond its edge.

3. RESULTS AND DISCUSSION

3.1 Identification of Bacterial isolates

Randomly five bacterial colonies were selected from the growth in Nutrient agar and Blood agar and designated as B1,B2,B3,B4 and B5.

3.1.1 Microscopic examination

3.1.1.1 Gram staining

The purple color rods and coccus shape bacteria was observed which indicates the presence of gram positive organism, pink colored rods indicates the presence of gram negative organism. The Gram staining and biochemical results are tabulated in Table (1). From the result the bacterial isolates B1,B2,B3,B4 and B5 was found to be *Escherichia coli., Klebsiella sp., Pseudomonas sp., Staphylococcus sp., and Bacillus sp.*, respectively.

TABLE 1 Result for Gram Staining and Biochemical Test

BIOCHEMICAL TEST	BACTERIAL ISOLATES				
	B 1	**B2**	**B3**	**B4**	**B5**
Indole	Positive	Negative	Negative	Positive	Negative
Methyl red test	Positive	Negative	Negative	Positive	Negative
Voges - proskauer test	Negative	Positive	Negative	Positive	Positive
Citrate utilization test	Negative	Positive	Positive	Positive	Positive
Carbohydrate fermentation test	Positive	Positive	Positive	Negative	Negative
Triple sugar iron agar test	Positive	A/A	Negative	Positive	Negative
Hydrogen sulphide production	Negative	Negative	Negative	Negative	Negative
Starch hydrolysis	Negative	Negative	Negative	Positive	Negative
Oxidase test	Negative	Negative	Positive	Negative	Negative
Catalase test	Positive	Positive	Positive	Positive	Positive
Urease test	Negative	Positive	Negative	Positive	Positive
Nitrate reduction test	Positive	Positive	Positive	Negative	Negative
Gelatin hydrolysis	Negative	Negative	Positive	negative	Positive
Casein hydrolysis	Negative	Negative	Negative	Negative	Negative

3.2 Identification of Fungus

Randomly two fungal colonies were selected from the growth in Rose Bengal agar and designated as F1 and F2.

3.2.1Lacto phenol cotton blue staining

Delicate blue hyphae are observed and fruiting structures with a pale blue background.
Hyphae are septate; conidiophores are short and inflated. Conidia are black with thick walls.
From the result the fungal isolates F1 and F2 was found to be *Aspergillus sp., Candida sp.,*

3.3 Phytochemical Analysis of Leaves and Flowers *Muntingia calabura*

The leaf and flower extract of *M. calabura* were analysed for different phytochemical shown in table 2. Fig.4 shows the results of phytochemical analysis of leaves and Fig.5 shows the results of phytochemical analysis of flowers.

TABLE 2 Results for Phytochemical Analysis

CONSTITUENTS	LEAF EXTRACT	FLOWER EXTRACT
Alkaloids (Mayer's test)	+	-
Amino acid and protein (Ninhydrin test)	Protein (+)	Protein (+)
Carbohydrate (Benedict's test)	+	-
Flavonoids	+	-
Phenols	-	-
Reducing agent	-	+
Saponins	+	+
Tannins	-	-
Terpenoids	-	+
Steroids	-	+
Phlobatannins	-	+

Fig.4 Phytochemical Analysis of Leaves

Fig.4 Phytochemical Analysis of Flowers

3.4 Antimicrobial activity of leaves and flower extract

The well diffusion assay showed that the leaves and flowers have different degrees of microbial growth inhibition, depending on the strains. The antimicrobial activity of the aqueous extract of *Muntingia calabura* leaf and flower was studied against wound infection causing micro organisms were observed as shown in table 3, 4.

TABLE 3 Antimicrobial activity of aqueous extracts of *Muntingia calabura* leaves

S. no	organisms	Diameter of inhibition zone in mm			
		Aqueous extract (*M. calabura* leaves)			
		20µl	40µl	60µl	80µl
1	*Escherichia coli*	15µl	21µl	22µl	25µl
2	*Klebsiella sp*	-	14µl	17µl	-
3	*Pseudomonas sp*	11µl	17µl	14µl	-
4	*Staphylococcus sp*	25µl	26µl	22µl	20µl
5	*Bacillus sp*	13µl	18µl	21µl	-
6	*Aspergillus sp*	16µl	20µl	17µl	16µl
7	*Candida sp*	-	19µl	21µl	-

TABLE 4 Antimicrobial activity of aqueous extracts of *Muntingia calabura* flowers

S. no	organisms	Diameter of inhibition zone in mm			
		Aqueous extract (*M. calabura*flowers)			
		20µl	40µl	60µl	80µl
1	*Escherichia coli*	12µl	20µl	15µl	-
2	*Klebsiella sp*	-	14µl	16µl	19µl
3	*Pseudomonas sp*	16µl	17µl	-	-
4	*Staphylococcus sp*	13µl	15µl	18µl	22µl
5	*Bacillus sp*	17µl	18µl	19µl	15µl
6	*Aspergillus sp*	19µl	17µl	18µl	-
7	*Candida sp*	-	15µl	18µl	16µl

The result obtained that gram positive organism *Staphylococcus sp* was found to be most susceptible Gram-positive bacteria while *Escherichia coli* was found to be most susceptible Gram-negative bacteria and *Candida sp* was found to be the most susceptible fungi in aqueous extract of *M. calabura l*eaf. In *M. calabura* flower that gram positive organism Staphylococcus sp was found to be most susceptible Gram-positive bacteria while *Escherichia coli* was found to be most Gram-negative bacteria and *Aspergillus sp* was found to be most susceptible fungi.

3.5 Antioxidant activity of leaf and flower

The DPPH antioxidant assay is best on the ability of 1-1-diphenyl-2-picrylhydrazyl, is a stable free radical to decolorize in the presence of antioxidants. The DPPH free radical contains an odd electron, which is responsible for decolorized (visible deep purple color) and quantitatively measured from the changes in absorbance. The DPPH (1,1-diphenyl -2-picrylhydrazyl) radical scavenging activity of Muntingia calabura leaf and flower shown in (Table 5 & Figure 6, 7).

TABLE 5 Antioxidant activity of aqueous extracts of Leaves and Flowers of *Muntingia calabura*

Solvent	Absorbance at 517nm				
	Concentration of solvent				
	10	20	30	40	50
Muntingia calabura leaves	0.153	0.169	0.187	0.230	0.256
	60%	56%	51%	40%	34%
Muntingia calabura flowers	0.177	0.216	0.229	0.248	0.272
	54%	44%	41%	35%	29%
Standard (without DPPH) leaves	47%	38%	32%	27%	14%
Standard (without DPPH) flowers	55%	42%	35%	22%	18%

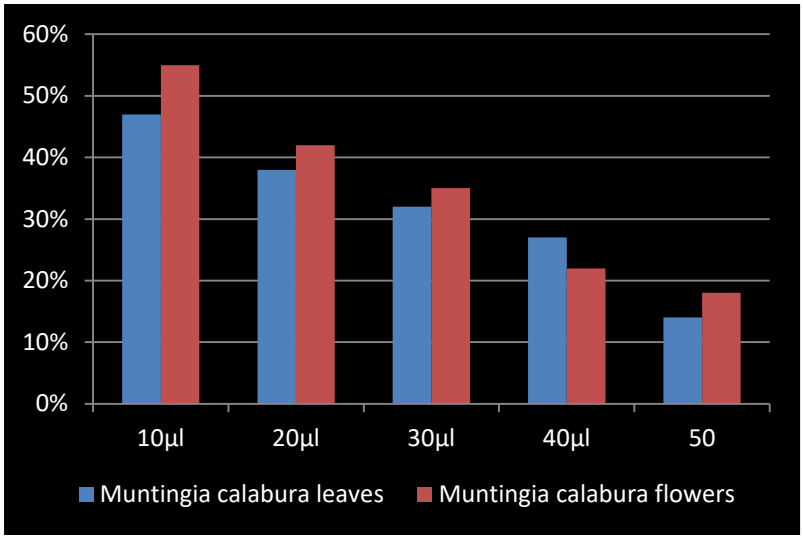

Fig.6 The graph shows the decreased in absorbance spectrum without DPPH

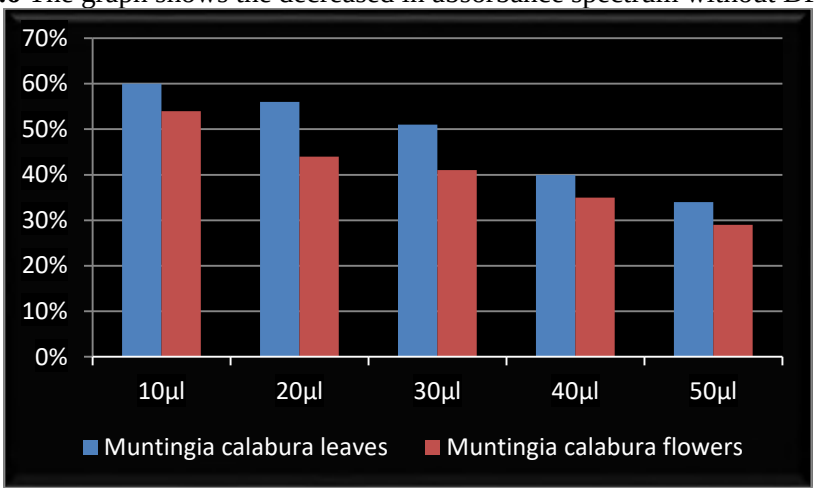

Fig.7 The graph shows the increased in absorbance spectrum with DPPH

3.6 Production of Soap

Production of Soap is highly used for the Wound infection causing micro- organisms. The aqueous extract of *Muntingia calabura* was used to prepare the medicinal soap and shown in figure 8.

Fig.8 *The figure showing the Soap extracted from Muntingia calabura flower*

3.7 Antibacterial Activity of soap from *Muntingia calabura* flower

The aqueous extracts of *Muntingia calabura* were used to prepare the medicinal soap and were subjected to antibacterial analysis. The *M. calabura* flower extract in soap showed sensitivity towards *Escherichia coli., Klebsiella sp., Pseudomonas sp., Staphylococcus sp., Bacillus sp.*

The alkalinity (NaOH) is useful in removing acidic, fatty and oily soils. The sodium silicate acts as corrosion inhibitors. About half of coconut oil consists of lauric acid components. The lauric acid is used as an antioxidant

(protects against free- radical formation and damage). So this combination of coconut oil, NaOH, sodium silicate and plant extract will enhance antimicrobial activity of prepared soap were shown in figure.9. Tables 6 are shown the result of measured zone of inhibition.

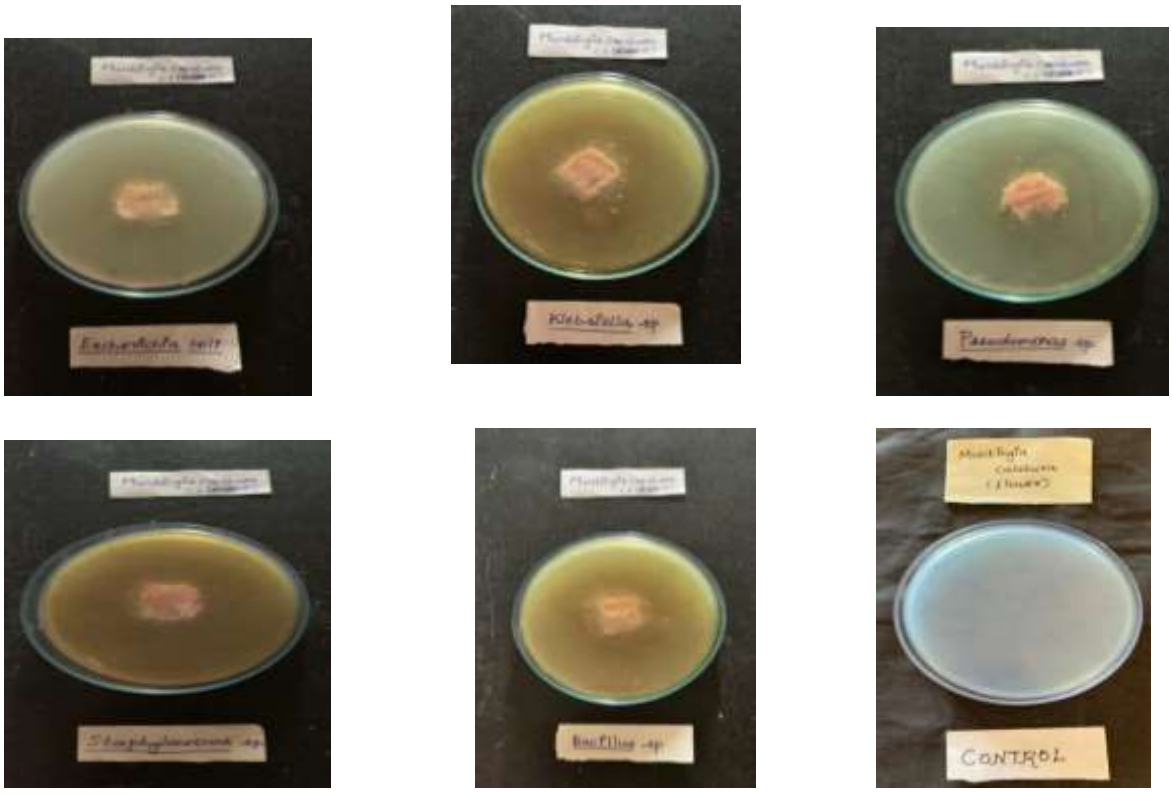

Fig.9 Zone formation that highly effective against to wound infection causing microorganisms

TABLE 5 Antibacterial activity of Soap of Flower

S. no	organisms	Diameter of inhibition zone in mm
		Aqueous extract (*M. calabura* flowers)
1	*Escherichia coli*	19mm
2	*Klebsiella sp*	21mm
3	*Pseudomonas sp*	18mm
4	*Staphylococcus sp*	23mm
5	*Bacillus sp*	22mm

4. CONCLUSION:

Plants have been known to be a source of bioactive secondary metabolites for various pharmacological purposes. One of such plants with wide ethanomedicinal use is *Muntingia calabura.*

The leaf and flower of *Muntingia calabura* collected in Coimbatore District and aqueous extracts were prepared.

The phytochemical screening of the leaf and flower of *Muntingia calabura* were performed which showed the presence of alkaloids, protein, carbohydrate, flavonoids, saponins for leaves and protein, reducing agent, saponins, Terpenoids, steroids, Phlobatannins for flowers.

The aqueous extracts of *Muntingia calabura* leaf and flower showed antibacterial and antifungal activity against wound infection causing microorganisms such as *Escherichia coli, Klebsiella sp., Pseudomonas sp., Bacillus sp., Staphylococcus sp., Aspergillus sp., Candida sp.*, by well- diffusion method.

The anti- oxidant activity was evaluated using DPPH assay and was found to be high which showed the aqueous extracts of leaf and flower of *Muntingia calabura* have effective radical scavenging activity.

In conclusion, the flower of *M. calabura* naturally contains antiseptic properties, due to its medicinal values, the soap were produced by using aqueous extracts of flower and subjected to antimicrobial activity against wound infection causing microorganisms which showed sensitivity to *Escherichia coli., Klebsiella sp., Bacillus sp., Staphylococcus sp.*, ie., most of the wound infection causing microorganisms and measured by zone of inhibition was done to determine the effectiveness of soap and found that highly effective.

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