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ORIGINAL ARTICLE

Phytochemical Screening and Antimicrobial Activity of *Lantana camara*

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ABSTRACT
Lantana camara Linn, is a medicinal plant known in traditional medicinal system. From the study it was found that the result of phytochemical test of methanol extract of both leaves and flower contains tannin, flavanoids, phenol, alkaloids. In ethanol extract tannin was found to be present in leaves whereas, the flower contains flavanoids and alkaloids. In DPPH scavenging the result was found to be prominent in both ethanol and methanol extracts of leaves and flowers. In the antimicrobial activity, the leaves and the flowers of methanol extract posses the clear zone of inhibition in E coli and S typhi at 2.5% concentration. The study revealed that the plant Lantana camara possess good antioxidant property and rich in medicinal uses and other bioactive components. Hence the need to explore the potential of this plant, especially in the area of traditional medicine ad pharmaceuticals industries, arises.
Keywords: *Lantana camara, Phytochemical screening, Antimicrobial activity, Antioxidant activity*

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INTRODUCTION

Medicinal plants have an important source of the medically important compounds Medicinal plants are used to cure several types of health problems from the ancient time Plants provides a variety of bioactive molecules when systematically analyzed for the development of newer pharmaceutical products [11]. Medicinal plants provide livelihood and health security to a large segment of Indian population and found as a major resource base for the traditional medicine and herbal industry It has been reported that more than 80% of drug substances were natural product or their development was inspired by natural compounds. The traditional folk medicines of India use medicinal plant based drugs for preventing or suppressing various diseases [12]. In pharmacological evaluation various plants are used in different traditional system of medicine. By advanced scientific techniques and studies it was concluded that many of traditionally known plants have various medicinal properties via. Anticancer, anti inflammatory, anti diabetic, antifungal, antibacterial, antioxidant activity etc [8].

From the viewpoint, this study was undertaken to make a proper documentation of medicinal plants. From the outcome a plant species namely *L. camara* was selected for study. The present study aimed the analysis of phyto chemistry and medicinal properties of *L. camara.*

MATERIAL AND METHODS

Identification of plant: The plant identification was based on morphological floral characters. Herbarium of the selected plant was prepared and deposited at Regional Research Laboratory (RRL). CSIR-NEIST, Jorhat,2019. It was identified at RRL, ACCESSION NO. 0128. (Fig-1)

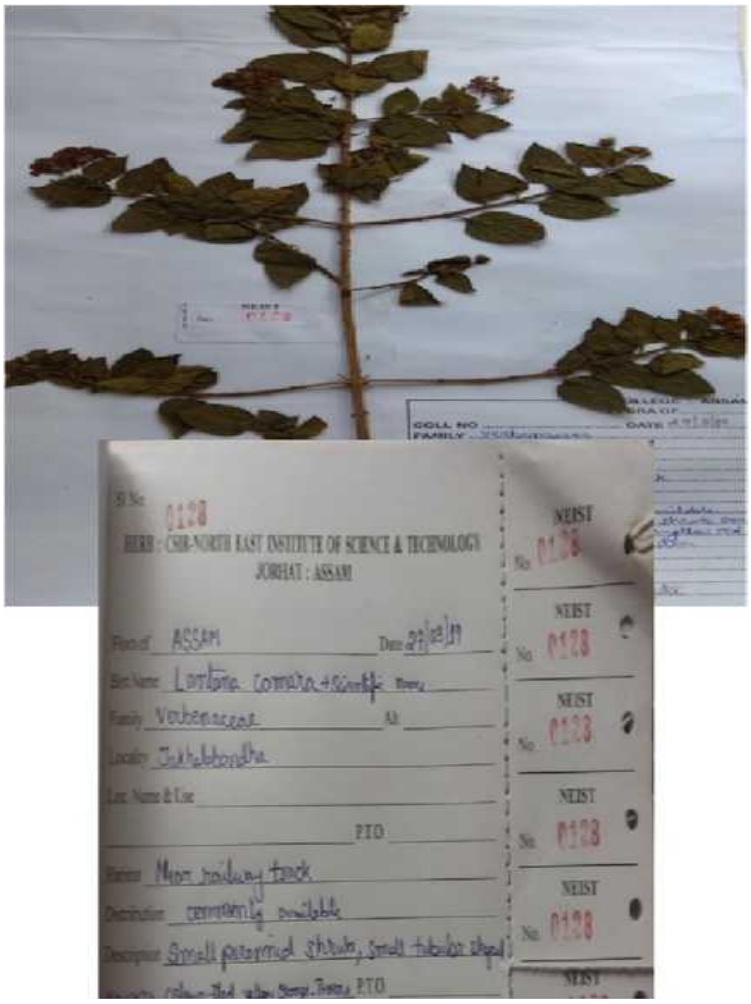

Figure 2: HERBARIUM SHEET OF *LANTANA CAMARA*

Collection of plant: Fresh leaves and flowers of the plant were collected from Kuwaritol, Nagaon, Assam on February, 2019. Washed separately thoroughly with water and were shade dried and with the help of grinder crushed into powder.

Preparation of plant extracts

Powdered leaves and flowers (50gm) of the plant sample were taken in a conical flask of appropriate size. The plant materials were immerged in methanol and ethanol separately for 72 hours by maintaining dark room. After 72 hours, the extracts were filtered using Whatmann Filter paper No.l. The filters were dried by using hot water bath. This dried extracts formed methanol and ethanol extracts of 100% concentration. Both the leaves and flowers extracts were kept in air tight glass vials separately at 4°C. The crude solvent extracts of the plant material so obtained were considered as stock extracts of the plant material and were used for further experimental purposes.

Photochemical Analysis: Phytochemical tests were carried out as per methods described by with some modification [3-5, 10].

Preparation of test sample

Dried extract (5mg) were dissolved in 5ml of dilute hydrochloric acid and then filtered. The test solution i.e. the filtrate used to test the presence of alkaloids. For the remaining test, test solutions were prepared by dissolving the extract in DMSO in the concentration of l mg/ml.

Tests for Alkaloids:

Hager's Test: Hager's reagent (saturated aqueous solution of Picric acid) few drops in 3 ml of test solution were added to observe the yellow precipitate formation.

Mayer's Test: In 3ml of test solution, Mayer's reagent (Potassium mercuric iodide solution) few drops were added and observed the formation of white or cream colored precipitate.

Wagner's Test: Wagner's reagent (iodine in potassium iodide) in the amount of 3ml of test solution, were added in drops and observed for the formation of red brown precipitate.

Tests for Amino Acids and Proteins:

Biuret Test: To 2ml of the test solution, 2 ml of 10% NaOH and 2 drops of 0.1% $CuSO_4$ solutions were added, and observed for the violet or pink color formation.

Tests for Carbohydrate:

Benedict's Test: Benedict's reagent (sodium citrate and sodium carbonate) at equal volume and test solutions were mixed. The mixture was heated in hot water bath for 5 minutes and observed for the formation of characteristic colored precipitate (green, yellow, red, depends on amount of reducing sugar present in the filtrate).

Fehling's Test: One ml of Fehling's solution A and 1 ml of Fehling solution B mixed together and boiled for 1 minute. Equal volume of sample was added and heated in a hot water bath for 5-10 minutes and to observe the formation of brick red colored precipitate.

Tests for Flavonoids: dilute ammonia solution (5ml) was added to a small amount of test solution followed bt addition of concentrated sulphuric acid and observed the formation of yellow coloration.

Tests for Phenolic Compound and Tannins: For the appearance of the corresponding endpoints small quantities of extracts were treated with the following reagent.

With 5% solution of ferric chloride was added to form a deep blue-black color, which indicates that phenolic compound was presence.

With 10% solution of lead acetate was added for the white precipitate formation, which signifies the presence of tannins.

Antioxidant Activity Analysis:

Determination of DPPH radical scavenging activity

The antioxidant activity of any compound can be determined by the scavenging activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH, stable free radical) [1].

Chemicals used: DPPH, Ascorbic acid, Methanol

Preparation of Standard Solution: Here standard L-Ascorbic acid were used. By dissolving 10 mg of Ascorbic acid in 10ml of methanol stock solution of standard was prepared.

For test solution 20, 40, 60, 80 and 160 µl of the stock solution was taken in test tubes separately and by adding methanol final volume of 100 µl was adjusted.

Preparation of Test Sample: Stock solution of plant sample in concentration of lmg/ml was prepared by dissolving extract in methanol. As like the standard, the sample solution of 20, 40, 60, 80 and 100 µl were taken separately in test tubes. Then the volume was made 100 µl by adding methanol.

Preparation of 0.3 mM DPPH Solution: DPPH (11.82mg) was dissolved in 100ml of methanol and was kept covering the tubes with aluminium foil to protect it from light.

Estimation of DPPH Scavenging Activity: DPPH scavenging activity were estimated by the protocol described earlier (Blois 1958) with some modification. The tubes containing standard and sample were incubated at dark at room temperature for 30 minutes. For control, 1 ml of 0.3 mM DPPH solution was added to 2 ml of methanol and allowed to incubate at dark for 30 minutes at room temperature Absorbance were measured after 30 minutes at 517 nm taking methanol as blank using a UV- visible spectrophotometer (SPECTRA max PLUS 384). The percentage inhibition was calculated by comparing the absorbance values of the control and the test samples. In triplicate all the tests extracts were performed. As the reference compound ascorbic acid was used. The capacity to scavenge the DPPH activity was calculated as the inhibition percentage of free radical by the sample or standard using the below given formula.

$$\% \text{ Inhibition of DPPH scavenging activity} = \{(Ao - A_t)/ Ao\} X 100$$

Where Ao is absorbance of the control reaction, and A_t is the absorbance of test/ standard.

The antioxidant activity of the extract was expressed as $IC_{50.}$ The IC_{50} values of the antioxidant activity were calculated by linear regression, the concentration of the tested plant extract was represented by abscissa and the average percent was represented by ordinate. The IC_{50} value can be defined as the concentration (µg/ml) of extract inhibits the formation of DPPH radicals by 50%.

Antimicrobial Activity:

Preparation of test extract: Initially 20% concentration of stock solution was prepared by dissolving the required amount of stock extract in Dimethyl Sulphoxide (DMSO) and volume adjusted by adding distilled water. From the stock solution, the test extract of desired concentrations (2.5%, I 25% and 0.625%) were prepared by adding distilled water. Test extracts so prepared were used for conducting in vitro

experiments for antimicrobial efficacy against the pathogens cited above employing agar well diffusion method [7].

Culture media: Nutrient agar (NA) and Nutrient broth (NA) were used for culture of the test microorganisms.

Preparation of Nutrient Agar (NA) Media:

28gm of commercially available Nutrient Agar (NA) media in 1000ml of distilled water were dissolved and stirred it well in a shaker. The media was then healed in an electronic heater till it reaches 100°C and becomes sticky then the media is autoclaved at 151b pressure (I21°C) for 15 minutes to avoid contamination.

Preparation of Nutrient Broth (NB) Media:

1.3gm of nutrient broth (NA) in 100 ml distilled water was dissolved (standard I3gm/1000ml), stirred it well in a shaker and the media is then autoclaved.

Inoculum:

Stock culture was sub cultured at regular interval and used for experimental purposes. Broth inoculums was prepared by adding one loop full of pure culture of the test microbes containing 50ml of NB in a conical flask and then incubated at 37±2 ˚C for 24 hr**s.**

Agar well diffusion method: (Das *et al*., 2010) Agar media of l5-20 ml was poured on glass Petri dishes (diameter: 9mm) and allowed to solidify. With broth culture the NA plates were swabbed for the test pathogens using a sterile spreader. Well of 6mm diameter was made in the inoculated plates (2 wells/plate) with a sterile cork borer. Each well was filled with100µl of the test extracts by using micropipette. The plates were kept for 30 minutes and to diffuse for 2 hours at room temperature. Then the incubation of the plates was for 24 hrs at 37±2˚C. The antimicrobial activities of the extracts were determined by the diameter measuring the zone of inhibition around the well [2, 9].

RESULTS AND DISCUSSION

Phytochemical Analysis:

Phytochemistry is the study of phytochemicals that is derived from plants. Phytochemicals may be classified into two classes namely Primary and secondary Primary phytochemicals are required by the plants for growth and other metabolism. Secondary phytochemicals are used by the plants for defense mechanism. Results of the phytochemicals analysis are summarized below (Table 1-4) (fig 2-3)

Fig 2-Phytochemical activity of *Lantana camara* leaves (ethanol, methanol) extract

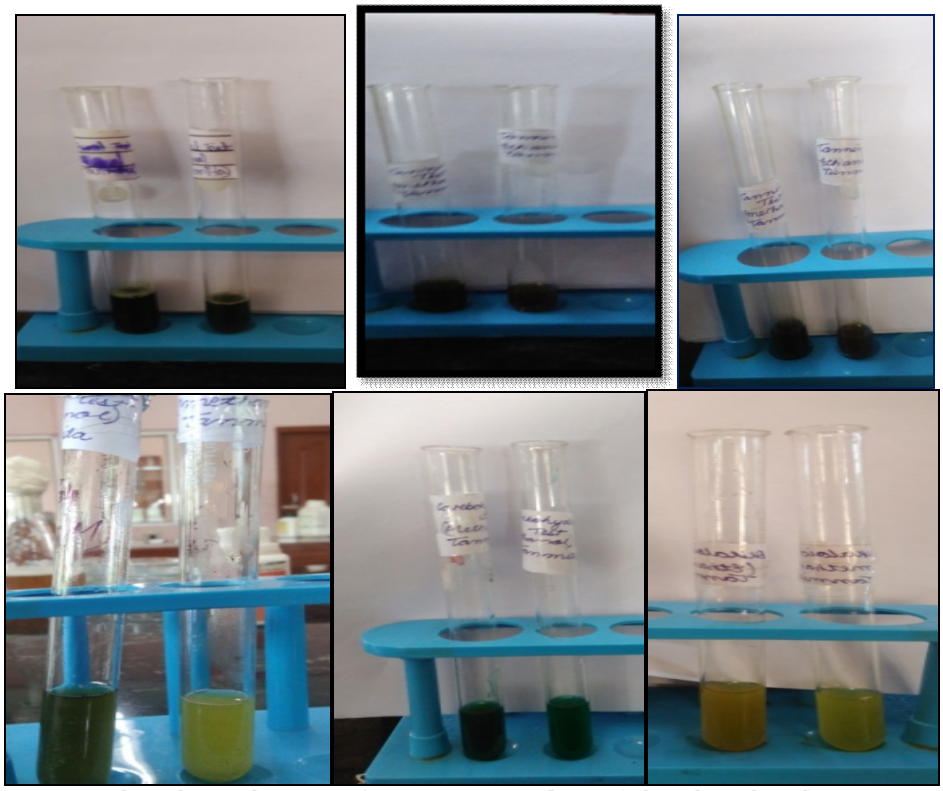

Fig3- Phytochemical activity of *Lantana camara* flower (ethanol, methanol) extract

Table 1: Phytochemical analysis of methanol extract of *Lantana camara* (leaves):

Phytochemical	Observation	Result
Tannin test	Formation of brownish green color	Present
Flavanoid test	Yellow color formation	Present
Phenol test	Dark blue/black color formation	Present
Alkaloid test	Yellow precipitate formation	Present
Carbohydrate test	No brick red coloration	Absent
Amino acid test	No purple / bluish coloration	Absent

Table 2: Phytochcmical analysis of ethanol extract of *Lantana camara (*leaves):

Phytochemical	Observation	Result
Tannin test	Formation of brownish green color	Present
Flavanoid test	No yellow color formation	Absent
Phenol test	No dark blue/black color formation	Absent
Alkaloid test	No yellow precipitate formation	Absent
Carbohydrate test	No brick red coloration	Absent
Amino acid test	No purple / bluish coloration	Absent

Table 3: Phytochemical analysis of methanol extract of Lantana camara (flower):

Phytochemical	Observation	Result
Tannin test	Formation of brownish green color	Present
Flavanoid test	Yellow color formation	Present
Phenol test	Dark blue/black color formation	Present
Alkaloid test	Yellow precipitate formation	Present
Carbohydrate test	Formation of brick red coloration	Present
Amino acid test	No purple / bluish coloration	Absent

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Table 4: Phytochemical analysis of ethanol extract of *Lantana camara* (flower):

Phytochemical	Observation	Result
Tannin test	No formation of brownish green color	Absent
Flavanoid test	Yellow color formation	Present
Phenol test	No dark blue/black color formation	Absent
Alkaloid test	Yellow precipitate formation	Present
Carbohydrate test	No formation of brick red coloration	Absent
Amino acid test	No purple / bluish coloration	Absent

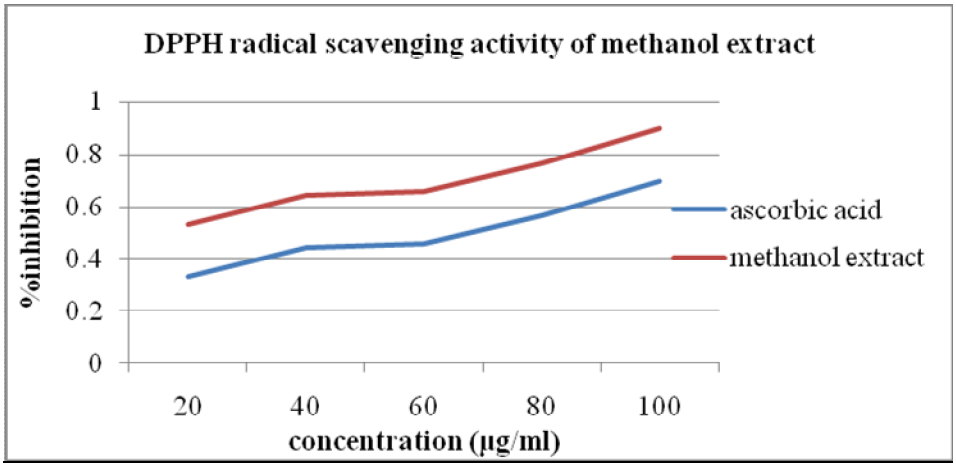
It has been observed that in methanol extract leaf and flower, tannin, flavanoids, phenol, alkaloids were present in both. Tannin was only present in ethanol extract of leaves whereas flavanoid and alkaloid were present in ethanol extract of flower. *L.camara* possesses essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids and tannin from the different parts such as roots, leaves and flowers as major phytochemical [6].

Antioxidant Activity Analysis

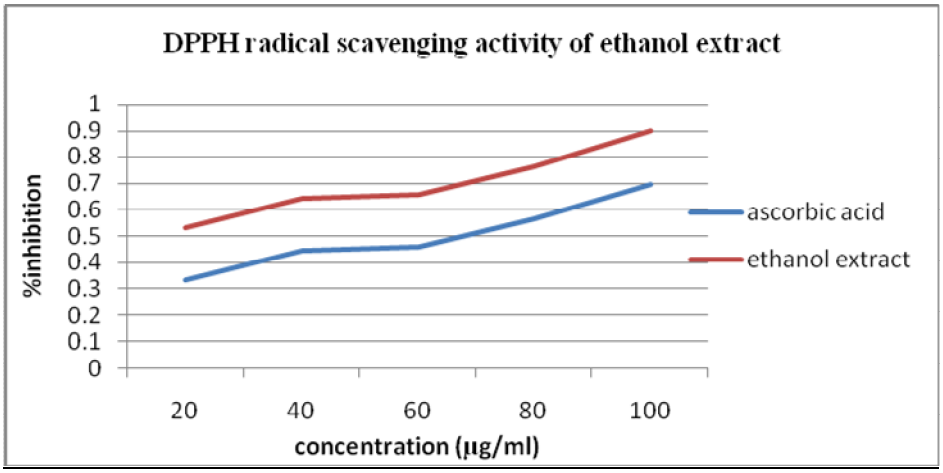
Some phytonutrients act as antioxidants that are scavengers of free radicals. An antioxidant can provide reactive free radicals that prevent in oxidation of other molecules and can have health promoting effects in prevention of diseases. For estimation of efficiency of the antioxidant, many techniques are employed, and out of which DPPH radical scavenging activities were experimented to assess the antioxidant property of different solvents.

DPPH Radical Scavenging activity

The result of the DPPH activity of *L.camara* in different solvent extracts were displayed **in graph 1-4**



Graph 1: DPPH scavenging activity of methanol extract of *L.camara* leaves



Graph 2: DPPH scavenging activity of ethanol extract of *L camara* leaves

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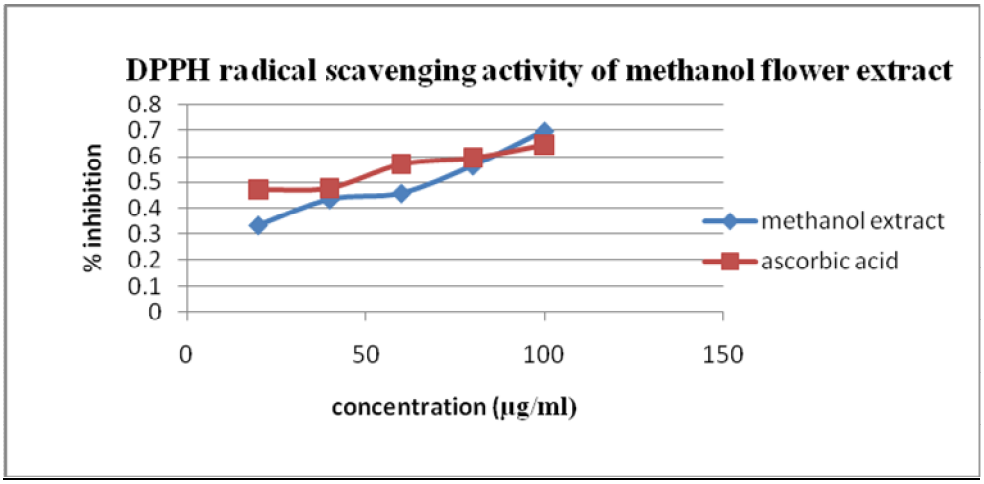
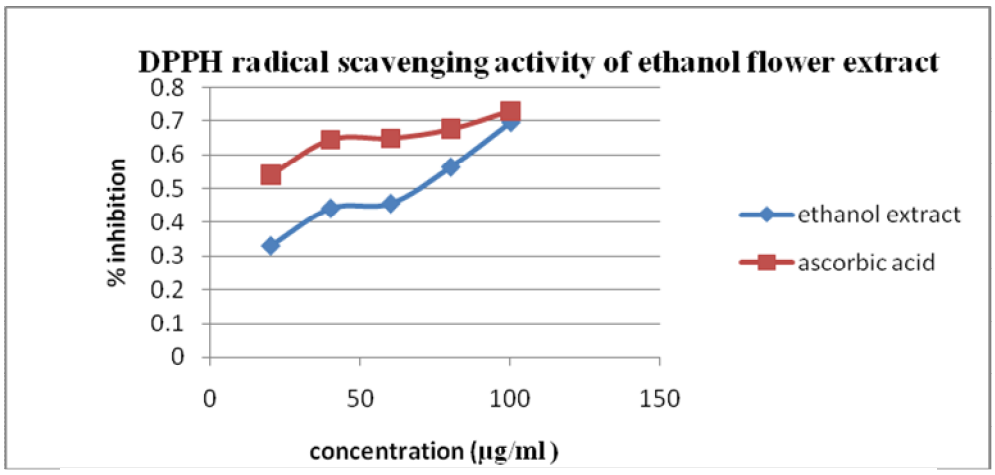
In DPPH radical scavenging assay, good and prominent antioxidant activity was displayed by methanol and ethanol extract.



Graph 3: DPPH scavenging activity of methanol extract of *L.camara* flower



Graph 4: DPPH scavenging activity of ethanol extract of *L.camara* flower

In DPPH scavenging activity analysis 1, 1-diphenyl-2-picryl-hydrazy 1, the molecule is characterized as a stable free radical by the delocalisation over the molecule as a spare electron, so that the molecules do not dimerise, with most other free radicals. The delocalization gives deep violet color which has been characterized by an absorption band at 517nm. DPPH solution was mixed with a substance which can donate a hydrogen atom, which gives rise to the loss of the violet color in the reducing form. Sometimes due to the picryl group there may be a residual pale yellow color.

Antimicrobial Analysis

The results indicated that the test extracts were shown to possess antimicrobial activity towards *E.coli, S.epidermis, C. albican* and *S.typh*i in a concentration of 30µl. The details of the results showed in the table 5 and 6. The sensitivity of the test were found to be highest in *E.coli,* exhibiting the zone of inhibition maximum of 30mm in both leaf and flower extract at 2.5% . The zone of inhibition at 2 5% by methanol leaf extract were 30mm in *E.coli* and *S.typhi*, 28mm in *C. albican* and 27mm in *S. epidermidis.* At 1.25%, 28mm in *E.coli* and *S.typhi*, 23mm in *C.albican* and 27mm in *S epidermidis.*At 0.625%. 24mm in *E.coli,* 15mm in *S.typhi*, 20mm in *C.albican* and 25mm in *S.epidermis.*

The zone of inhibition at 2.5% by methanol flower extract were 30mm in *E.coli* , 20mm *S.typhi*, 15mm in *C.albican* and 12mm in *S.epidermidis.* At 1.25%, 18mm in *E.coli*, 13 in *S.typhi* and *Sepidermidis,* 9mm in *C.albican.* At 0.625%, 13mm in *E.coli* and 12mm in *S.typhi.* No zones were found in *S.epidermidis* and *C. albican.*

Different varieties of *L. camara* leaves and flowers were reported for antibacterial activity [6]. Solvent of leaves and flowers of three different extract of four different varities of *L. camara* exhibited antibacterial activity in *E. coli, Bacillus subtilis* and *P.aeruginosa* and against *Staphylococcus aureus* exhibited poor

antibacterial activity. Leaves and roots ethanolic extracts of *L. camara* were observed for antibacterial activity. The extracts were found to exhibit antimicrobial activity against *Staphylococcus aureus, vulgaris, Pseudomonas aeruginosa, Vibrio cholareae, Escherichia coli.* The highest activity was showed in the leaves extract of *L. camara* against Gram positive *Bacillus cereus* a Gram positive and *Salmonella typhi* a Gram negative.

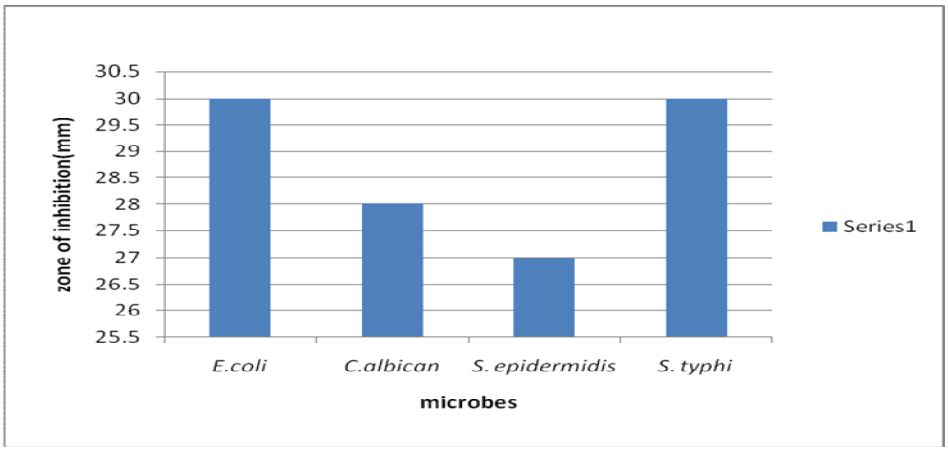
Antimicrobial activity: methanol extract derived from *L. camara* leaves and flowers were tested for antimicrobial activity.

Table 5: Antimicrobial activity of *Lantana camara* leaf extract

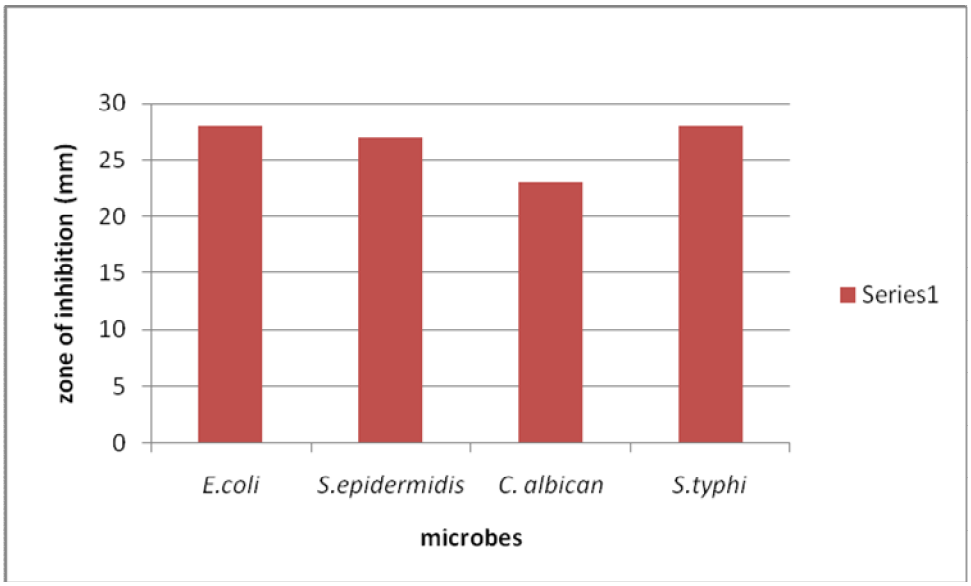
Concentration / Strain	2.5%	1.25%	0.625%
E. coli	30 mm	28 mm	24 mm
C. albican	28 mm	23 mm	20 mm
S,epidermidis	27 mm	27 mm	25 mm
S.typhi	30 mm	28 mm	15 mm

Table 6: Antimicrobial activity of *Lantana camara* flower extract

Concentration / Strain	2.5%	1.25%	0.625%
E. coli	30 mm	18 mm	13 mm
C. albican	15 mm	13 mm	0 mm
S,epidermidis	12 mm	09 mm	0 mm
S.typhi	20 mm	13 mm	12 mm



Graph 5: Antimicrobial activities of *L.camara* methanol leaf extract (2.5% concentration)



Graph 6: Antimicrobial activities of *L.camara* methanol leaf extract (1.25% concentration)

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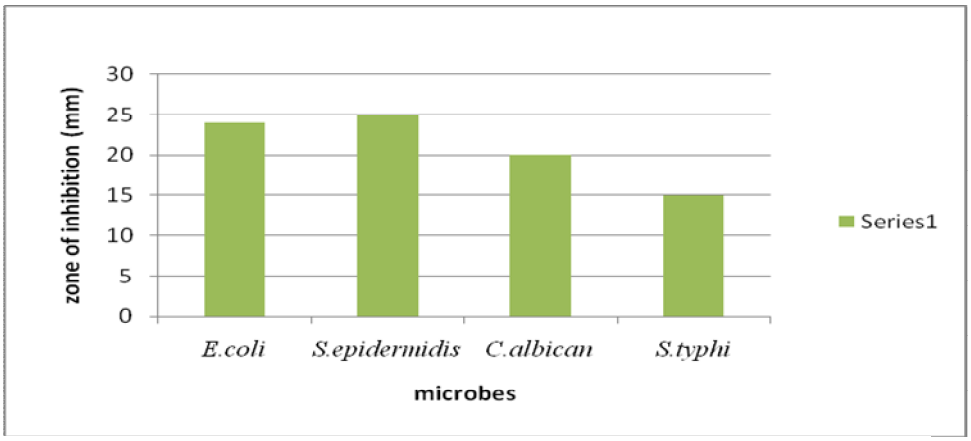
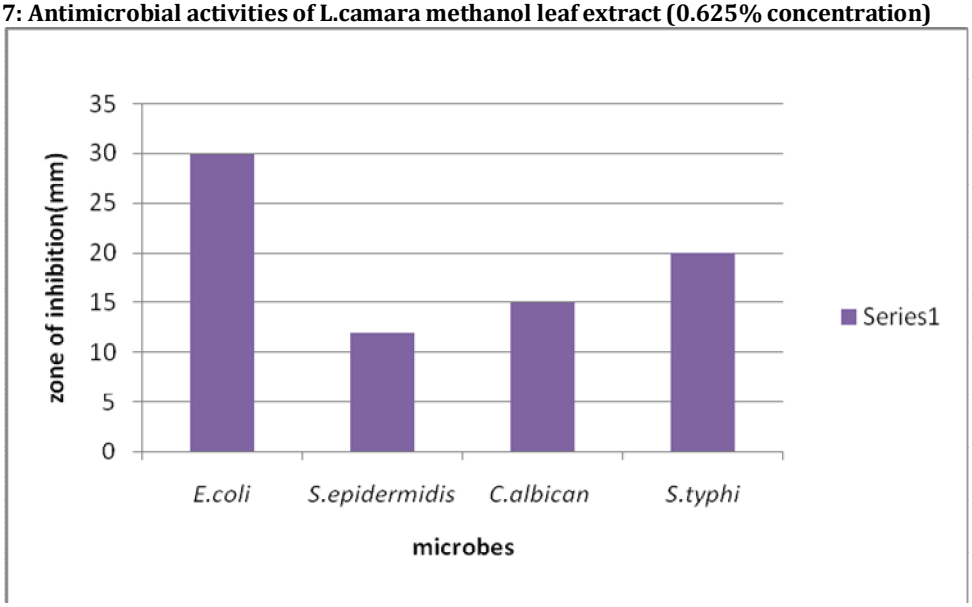
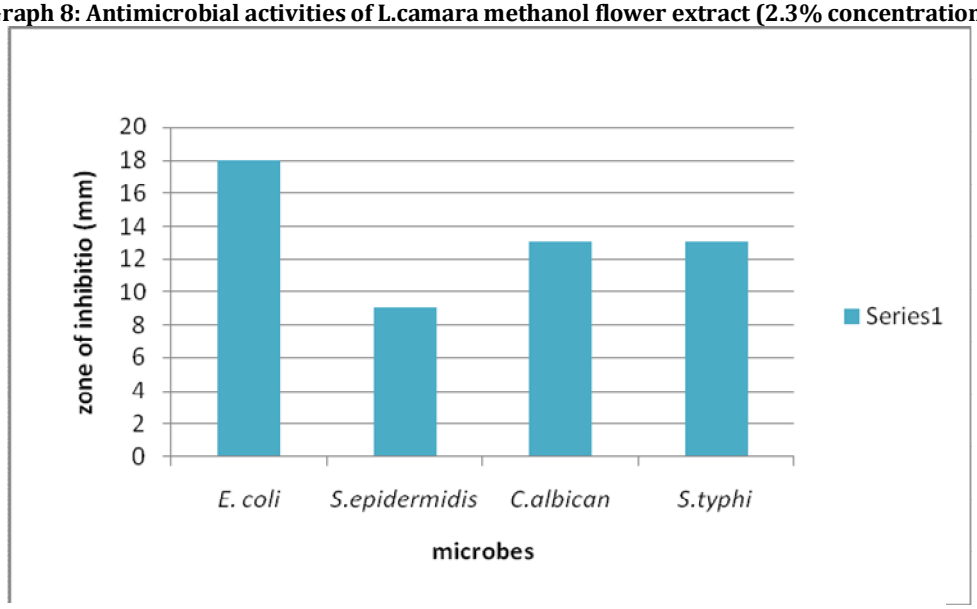


Graph 7: Antimicrobial activities of L.camara methanol leaf extract (0.625% concentration)



Graph 8: Antimicrobial activities of L.camara methanol flower extract (2.3% concentration)



Graph 9: Antimicrobial activities of L.camara methanol flower extract (1.25% concentration)

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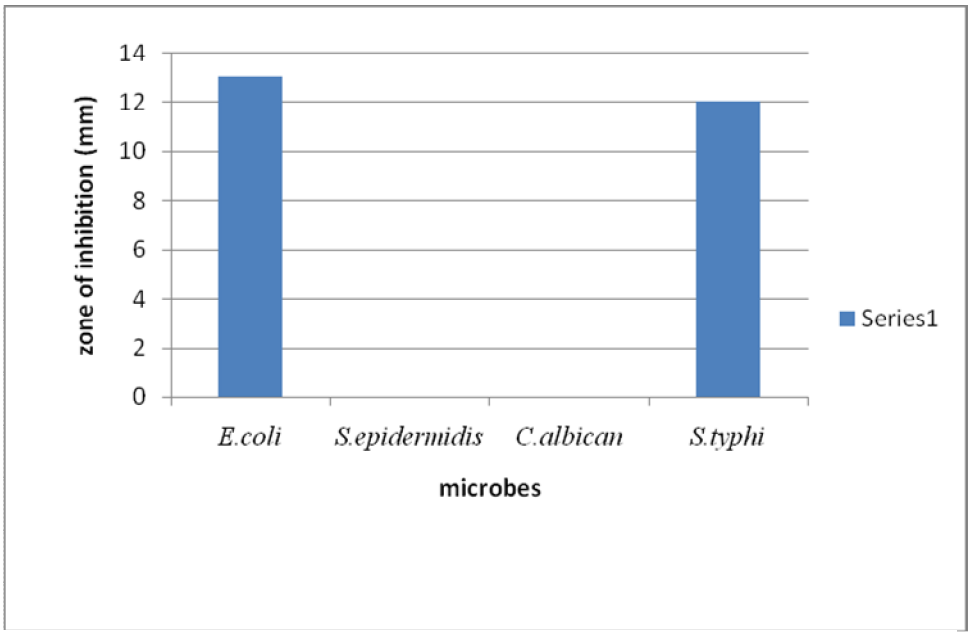

Graph 10: Antimicrobial activities of *L.camara* methanol flower extract (0.625% concentration)

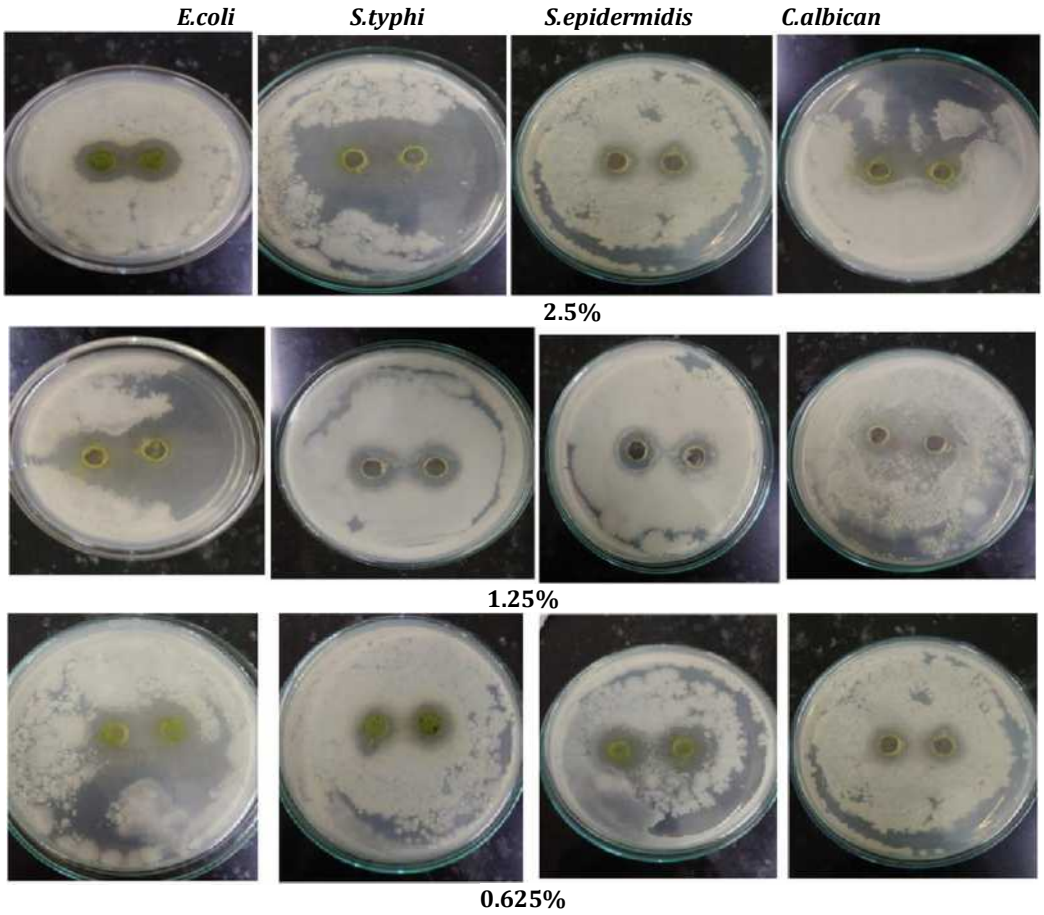

Figure 5: The Zone of inhibition caused by *L. camara* leaf methanol extract

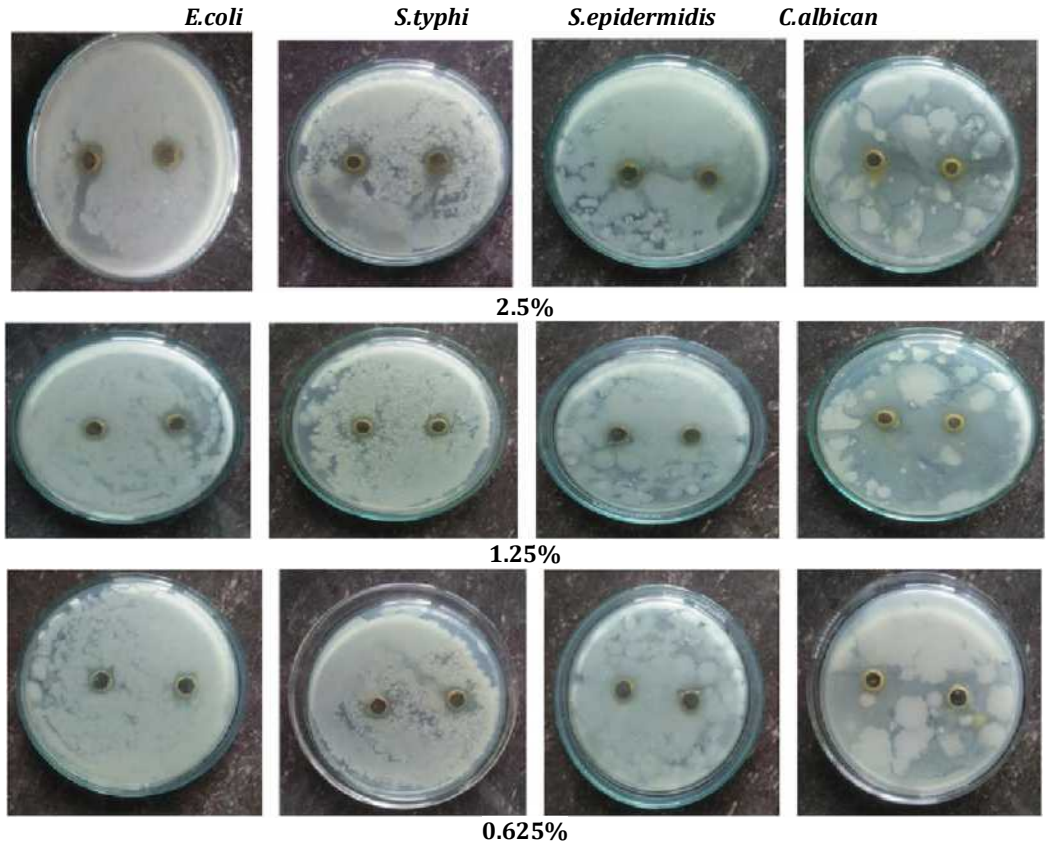

Figure 6: The Zone of inhibition caused by *L. camara* **flower methanol extract**

CONCLUSION

The results from the above study of the plant *Lantana camara* revealed that the plant possess good and prominent antioxidant property which is rich in medicinal uses and other bioactive components. Thus *Lantana* plant should be promoted and cultivated in area where other plants are difficult to grow or in harsh environmental conditions. Efforts should be made to explore this plant for its potential medicinal utility and development into healthcare products for betterment of animals and humans which would be effective, easily available, and low cost and alternatively be used as household commodities in area.

From the above study it was found that, in phytochemical analysis of the extract that is methanol extract of both leaves as well as flower contains tannin, flavanoids. phenol, alkaloids. In ethanol extract tannin was found to be present leaves whereas the flower contains flavanoids and alkaloids. In DPPH scavenging the result was found to be prominent in both ethanol and methanol extracts of leaves and flowers of *L.camara*. In antimicrobial activity of the leaves and the flowers of *L.camara* methanol extract posses the clear zone of inhibition in *E.coli* and *S.typhi* at 2.5% concentration.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

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