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**ASSESSMENT OF PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND
ANTIBACTERIAL ACTIVITIES OF SOME FLOWERS**

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ABSTRACT

This study reports the antioxidant properties of three medicinal plant's flowers namely *Lantana camara* (Lcf), *Euphorbia milii* (Emf) and *Ixora coccinea* (Icf) from India. Preliminary phytochemical investigations were on carbohydrates, proteins, alkaloids, flavonoids, saponins, total phenols, steroids and terpenoids. Antioxidant and antibacterial activities from dried materials of these flowers were studied using DPPH and FRAP assays. *L. camara* flower was ranked the highest inhibition of free radicals (81.25%) followed by *I. coccinea* flower extract (73.78%) and lowest inhibition of free radicals was observed in *E. milii* flower extract showed least inhibition of 68% activity and IC₅₀ of 0.696 mg/ml for DPPH for *L. camara* flowers. The highest FRAP value was reported in Lcf showed 70% inhibition. Similarly, flower extracts of Icf exhibited 67% inhibition of FRAP values, when compared with Emf respectively. The antibacterial activity of all flowers showed an appreciable broad spectrum activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* with MIC value ranges between 6 to 9mm of inhibition respectively. The studied flowers possess considerable antioxidant and antibacterial activities and may contribute to the well-being of individuals who consume them.

KEYWORDS

Medicinal plants, Flowers, Antioxidant and Antibacterial activities.

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INTRODUCTION

Now a days medicinal plants are gaining more importance against almost all human diseases in the form of raw materials or in the product form. Most of the medicinal plants contains major constituents like alkaloids, flavonoids, saponins, terpenes, phenols carbohydrates and proteins which plays a major role in clinical trials. Moreover, usage of high quantities of synthetic chemicals and allopathic

drug are causing side effects. To overcome all these problems medicinal plants and plant products signifies safety and ecofriendly to human and environment (Merlin, 2003)¹.

Increase in the population have increased with unaffordable cost of treatments. Furthermore, increased side effects from several allopathic drugs and confirmed its development of resistance to many infectious diseases. This made researchers to work on eco friendly and natural plant materials as a source to avoid all the drugs for the source of medicines for the variety of human diseases. The World Health Organization (WHO) has also recommended the usage of medicinal plants against many diseases where we can utilize the effectiveness of plants as a modern drug. It also suggests that this plant sources will have lesser side effects than allopathic drugs and can be used as ecofriendly in nature. (Anonymous, 1998)².

Phytochemicals are the major constituents of plant origin and they are considered as secondary metabolites which can be used has drugs. These secondary metabolites will be produced from any part of the plants body such as leaves, bark, stem root, flowers, fruits, seeds etc., This plant part may contain large quantities and different qualities of active compounds based on nature of the plants (Mojab, 2003)³. Moreover, there is lack of information on the distribution of the active compounds present in different plant parts which are related to the distribution of active compounds present in other plants. (Parekh and Chanda, 2008)⁴. *Lantana camara* an ornamental and commonly called as “Fencing weed” belongs to family *Verbenaceae*, seen in many parts of India. The plant grows to a height of 2-3 m and spreads its branches to 1-2m width. Leaves and stem part contains several triterpenoids, flavonoids, alkaloids, and glycosides because of this chemical constituents plant showed an anti-tumor, antibacterial and antihypertensive activities, roots were used for the treatment of malaria, rheumatism and skin rashes. Extract from the leaves of *L. camara* possessed larvicidal activity while extract from flowers also showed repellent activity against mosquitoes.

Euphorbia milii which belongs to Euphorbiaceae family, a flowering plant commonly known as “Christ thrown”. *Euphorbia milii* plant is widely spread in Asian European, South American and South Brazil. All parts of plant used in ayurvedic medicine for the treatment of warts in South Brazil. Cancer. *Ixora coccinea* L. belongs to Rubiaceae family commonly grows to 4-6 ft height with a dense, multi-branched evergreen shrub. Leaves are widely used in Ayurveda and Sidda medicine for the treatment of many diseases (Yuan, et al, 2012)⁵. Hence the present study was aimed to know the phytochemical analysis, antioxidant and antibacterial activities of three medicinal plants flowers of *L. camara*, *E. milii* and *I. coccinea*. No reports were reported on the flowers till now.

PLANT MATERIAL

Flowers of *Lantana camara* was collected from local area of Nadanahalli, Mysore (District), Karnataka as shown in (Figure No.1). Flowers of *Euphorbia milii* and *Ixora coccinea* was collected from Mysore. All the flowers were thoroughly washed in running water, sterilized by 70 % alcohol and air dried for 7-8 days and grounded to fine powder.

Preparation of extracts

The powdered plant samples (50g/250ml,) were extracted successively with aqueous extract using Soxhlet apparatus at 60°C for 8 to 10 hours to extract the compounds according to (Borris, 1996)⁶.

Preliminary phytochemical investigations

Analysis on the presence of both primary metabolites and secondary metabolites such as proteins, carbohydrates, alkaloids, saponins, phenols, tannins, flavonoids, steroids and terpenoids were assessed for flower extracts of *L. camara* (Lcf), *E. milii* (Emf) and *I. coccinea* (Icf) according to the standard procedure, as described below, were used (Harborne et al,1998)⁷.

Test for Proteins

Biuret test

Test solution containing flower aqueous extracts was treated with equal volume of 10% sodium hydroxide (NaOH) solution and added two drops of

1% copper sulphate solution, later it was mixed thoroughly and observed for the formation of violet/pink color. If pink/violet color appears it represents the presence of proteins in the test solution.

Test for Carbohydrates

Benedict's test

Test solution containing flower aqueous extracts, to this few drop of Benedict's reagent (alkaline solution containing cupric citrate complex) later it was kept in water bath for 10 minutes till the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Molisch's Test

This test is also used to check the presence of carbohydrates. Test solution containing flower aqueous extracts, filtrates were treated with 2 drops of alcoholic α -naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Test for Alkaloids

Wagner's Test

Test solution containing flower aqueous extracts was treated with 3-5drops of Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of water) kept for 10 minutes in water bath and observed for the formation of reddish brown precipitate which indicates the presence of alkaloids.

Mayer's Test

Test solution containing flower aqueous extracts filtrates were treated with Mayer's reagent (1% Potassium Mercuric Iodide) taken in test tubes kept for 20 minutes in water bath. Formation of a yellow colored precipitate indicates the presence of alkaloids.

Test for Saponins

Foam Test

Test solution containing flower aqueous extracts filtrate was mixed with water and shaken and observed for the formation of froth, which should be stable for 10-15 minutes. This result indicates the presence of Saponins present in the test solution.

Froth Test

Test solution containing flower aqueous extracts were diluted with 20mL distilled water and this was shaken in a graduated cylinder for 15 minutes. Later formation of 1cm layer of foam indicates the presence of saponins.

Test for Flavonoids

Alkaline reagent test

Test solution containing flower aqueous extracts filtrate was mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless when added with of few drops of diluted acid which indicates the presence of flavonoids.

Test for Phenolic compounds

Ferric Chloride test

The extract (50mg) is dissolved in 5ml of distilled water. To this few drop of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compound.

Lead acetate test

The extract (50mg) is dissolved in of distilled water and to this 3ml of 10% lead acetate solution is added. A bulky white precipitate indicates the presence of phenolic compounds.

Tests for Steroids and Terpenoids

Salkowski's Test

Test solution containing flower aqueous extracts filtrate a few drops of concentrated sulphuric acid (H_2SO_4), later it was shaken well and allow to stand for some time, red color appears in the lower layer indicate the presence of sterols and formation of yellow colored lower layer indicate the presence of steroids (Borris, 1996)⁶.

Determination of antioxidant activities

To know antioxidant activity, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric ion reducing antioxidant power (FRAP) and were used for different plant extracts to know antioxidant potential.

Determination of free-radical scavenging activity (DPPH assay)

The effect of (Lcf), (Emf) and (Icf) aqueous extracts of different concentrations (20, 40, 60 and 80mg/mL) was estimated by the method (Liyana-

Pathirana and Shahidi, 2005)⁸. 190µl of reagent and 2660µl of distilled water was mixed with 150µl of sample solution 10µl of sample and 140µl of distilled water in dark condition and the reaction was usually measured at 517nm. The ascorbic acid (aa) linear standard was plotted ranged from 25 to 800µM. The DPPH additional dilution values were measured by comparing with standard curve. Three replicates were maintained to each sample. The per cent inhibition of DPPH activity of each sample was calculated using standard formula.

Inhibition (%) = $A_{\text{control}} - A_{\text{sample}} / A_{\text{control}} \times 100$

Where A_{control} is the absorbance of the DPPH radical with ethanol, A_{sample} is the absorbance of DPPH radical with sample extract/ standard.

The DPPH activity of flower extracts was expressed as IC_{50} , the concentration of extract (µg/mL) required to scavenge 50% of DPPH radicals. IC_{50} values were estimated by a linear regression analysis. This IC_{50} values were used for remaining antioxidant assays for access the percentage of inhibition.

FRAP assay

For FRAP assay 25ml of acetate buffer (300mM, pH 3.6) was added to 2.5ml of 2, 4, 6-tris-(2-pyridyl)-S-triazine (TPTZ) solution (10mM TPTZ in 40mM HCl) taken in a test tube and later 2.5ml $FeCl_3$ (20mM) aqueous solution was added and kept in room temperature for 15 minutes. Later 150µl of (Lcf), (Emf) and (Icf) aqueous extract samples of different concentrations (20, 40, 60 and 80mg/mL) was estimated using 0.5mg ml/l methanol and added to freshly prepared FRAP reagent (4.5ml) and the solution was mixed. The reaction mixers absorbance was measured at 593nm and the FRAP solution was referred as blank (Szollosi R, Szollosi, 2002⁹; Tomic, *et al.*, 2008)¹⁰. The activity was compared with standard L-ascorbic acid.

Antibacterial activity

Inoculum of microorganisms

Less than 24 h incubated bacterial cultures concentrations (1.5×10^8 CFU/mL) were used for antibacterial activity. 24 hrs pure culture of *Staphylococcus aureus*, *Bacillus subtilis*,

Escherichia coli, *Pseudomonas aeruginosa* were used for the experiments.

Disc diffusion method

24 h growth bacterial suspension (100µl) was spread uniformly on Nutrient Agar medium using glass rod for each bacterial species. The impregnated with different extracts discs were placed on bacterial suspension containing media separately and plates were incubated at 35 ± 2 °C for 2 days. To determine cell viability, different concentrations of 10 nm were used. Aliquots were taken every 1 h for 12 hrs to determine cell viability by the method of serial dilutions and counting of colonies on plates (Yildirim, *et al.*, 2001)¹¹.

RESULTS

Phytochemical Screening

Results obtained for qualitative screening of phytochemicals in (Lcf), (Emf) and (Icf) were presented in Table No.1. Of the eight phytochemicals screened for, six were found to be present in various solvent extracts. They are alkaloids, saponins, phenols, flavonoids, steroids and triterpenoids. These compounds have significant application against human pathogens, including those that cause enteric infections (El Mahmood, *et al.*, 2008)¹². The result indicates that (Lcf) extracts, hold promises as source of pharmaceutically important phytochemicals when compared to (Emf) and (Icf). Alkaloids play some metabolic role and control development in living system. They are also involved in protective function in animals and are used as medicine, especially the steroidal alkaloids.

In all, more phytochemicals were found present in extract prepared with distilled water. Remarkably, carbohydrates were found in Lcf extract and proteins were not present in any of the extracts. Among the extracts tested maximum result was observed in Lcf extract as shown in Table No.1. According to (Parekh and Chanda, 2007)¹³ the factors affecting the choice of polar and non polar concentration of different compounds extracted. The logic in using different solvents when screening

for phytochemicals in plant materials was clearly validated in present study.

Anti-oxidant Assay

DPPH radical scavenging activity

DDPH radical scavenging is specifically used to determine chain breaking activity in the proliferation phase of protein and lipid oxidation as shown in Figure No.2. Antioxidant effects on DPPH scavenging may be due to their hydrogen donation capacity (Mao, *et al*, 2006)¹⁴. All the extracts showed dose-dependent increase in DPPH scavenging activities. Lcf inhibit the free radicals significantly at 80µg/ml concentration when compared to other extracts Emf and Icf. Lcf water extracts (80mg/ml) showed highest inhibition of free radicals (81.25%) followed by Icf (80mg/ml) of extract (73.78%) and lowest inhibition of free radicals was observed in Emf extract (80mg/ml) showed 68% as shown in Figure No.3. In particular Lcf showed 73% activity which is almost equal to standard ascorbic acid (84%).

Table No.2 shows antioxidant activity with IC₅₀ values of Lcf, Emf and Icf measured by DPPH radical-scavenging assays. Overall, Lcf extract is showing the best antioxidant properties when compare to all extracts (significantly lower IC₅₀ values = 0.696 ± 0.31mg/mL) and the Emf extracts possess poor radical scavenging activity (0.820 ± 0.75mg/mL). Icf extracts revealed a moderate antioxidant activity (0.735±1.10mg/mL). This data proves that the Lcf extract exhibit a strong scavenging activity than all the flower extracts used. Similar reports showed the presence of solvent extracts and the compounds present will increase the scavenging activity may be due to the presence of phenols, vitamin and flavonoids and phytochemicals present in the medicinal plants possessed the strong effects on reducing DPPH radical scavenging 65% comparing with standards (Kumar *et al*, 2014)¹⁵.

FRAP assay measures the reducing power of a potential antioxidant reduces the ferric ion (Fe³⁺) to the ferrous ion (Fe²⁺) leading to the formation of a deep blue complex ferric tripyridyl triazine. Flower samples of Lcf exhibited higher antioxidant activity

than the two other flowers, Emf and Icf. The highest FRAP value was reported in Lcf showed 70% inhibition. Similarly, flower extracts of Icf exhibited 67% inhibition of FRAP values, when compared with Emf. All the extracts showed lower FRAP values than the positive control, ascorbic acid 78.45% as shown in Figure No.4. FRAP assay is based on electron transfer reaction, whereas, DPPH assays are based on electron and H atom transfer (Prior, *et al*, 2005)¹⁶. Furthermore, antioxidant activity provides a meaningful method for direct comparison with known potent antioxidant widely distributed in plant samples (Kim, *et al*, 2002)¹⁷. (Floegel, *et al*, 2011)¹⁸ recently reported a strong correlation between DPPH and FRAP methods and between these methods and phenolics and flavonoids like our results.

Anti-bacterial activities

Lcf extracts showed highest zone of inhibition against all the microorganisms (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) used. Based on all the results, the Lcf showed the highest zone of inhibition compared to Emf and Icf against all the tested microorganisms. Lcf extract showed 8mm against *S. aureus*, 9mm against *B. subtilis*, 9mm against *E. coli* and 8mm against *P. aeruginosa* at 200µL. Emf and Icf also showed zone of inhibition against all the pathogenic bacteria but lesser activity than Lcf but showed more activity than Emf. In the other hand D. water (control) did not exhibit any effect on the tested microorganisms. In all the test samples antibiotic (chloramphenicol) showed highest zone of inhibition of 24mm as shown in Table No.3.

From these results Lcf extracts were found to be the most effective, with a broad antimicrobial spectrum against both Gram-positive bacteria *Staphylococcus* and Gram-negative bacteria *Pseudomonas*. These finding are found to be more effective, because these bacteria are resistant to a number of antibiotics. Phenols, flavonoids, tannins and antioxidants have previously been reported to have a wide spectrum of biological activities including antioxidants and antimicrobial activities (Cushnie and Lamb, 2005)¹⁹. Our findings are closely related

to all the published data *P. aeruginosa* inhibited by the extract of *Brachylaena elliptica* and *Brachylaena ilicifolia* supported the report of (Sagbo et al, 2015)²⁰ reported the antibacterial properties of an aqueous extract of *B. ilicifolia*, contains flavonoids, tannins, alkaloids and polyphenols compounds are responsible for antibacterial properties (Oyedemi and Bradley, 2010)²¹.

Table No.1: Results showing screening of phytochemicals in (Lcf), (Emf) and (Icf)

S.No	Phytochemicals	Test	(Lcf)	(Emf)	(Icf)
1	Alkaloids	Wagner's	+	-	-
		Mayer's	+	-	-
2	Flavonoids	Alkaline reagent	+	+	+
3	Phenols	Ferric Chloride	+	-	+
		Lead acetate	+	+	+
4	Saponins	Foam	+	-	-
		Froth	+	+	+
5	Proteins	Biuret	-	-	-
6	Carbohydrates	Benedict's	+	-	-
7		Molisch's	+	+	+
8	Steroids and Terpenoids	Salkowski's	+	-	+

Table No.2: The antioxidant activity with IC₅₀ values of Lcf, Emf and Icf measured by DPPH radical-scavenging assays

S.No	Concentrations (mg/ml)	Lcf	Emf	R Icf
1	20	0.204±0.31	0.421±0.40	0.311±0.33
2	40	0.456±0.31	0.538±0.30	0.444±0.45
3	60	0.558±0.45	0.619±0.45	0.545±0.45
4	80	0.696±0.31	0.820±0.75	0.735±1.10

Table No.3: Antibacterial activity of different concentration of ethyl extract of *E. milii* flower

S.No	Microorganisms	Concentration of	Lcf	Emf	R Icf
1	<i>S Staphylococcus aureus</i>	50µL	7±0.7	7±0.7	5±0.5
		100µL	8±0.6	6±0.6	6±0.5
		200µL	8±0.6	5±0.6	6±0.5
		Std	24±0.6	24±0.6	24±0.6
		control	0±0.0	0±0.0	0±0.0
2	<i>Bacillus subtilis</i>	50µL	5±0.4	2±0.4	2±0.4
		100µL	7±0.3	1±0.3	1±0.3
		200µL	9±0.3	1±0.3	1±0.3
		Std	24±0.6	24±0.6	24±0.6
		control	0.0±0.0	0.0±0.0	0.0±0.0
3	<i>Escherichia coli</i>	50µL	5±0.4	5±0.5	5±0.5
		100µL	9±0.4	6±0.5	6±0.5
		200µL	9±0.5	7±0.5	7±0.5
		Std	24±0.6	24±0.6	24±0.6
		control	0±0.0	0.0±0.0	0.0±0.0
4	<i>Pseudomonas aeruginosa</i>	50µL	2±0.5	6±0.5	6±0.5
		100µL	6±0.5	6±0.5	6±0.5
		200µL	8±0.5	6±0.5	5±0.7
		Std	24±0.6	24±0.6	24±0.6
		control	0.0±0.0	0.0±0.0	0.0±0.0



Figure No.1: Flowers of *L. camara*, *E. milii* and *I. coccinea*



Figure No.2: Test tubes showing the inhibition of free radicals

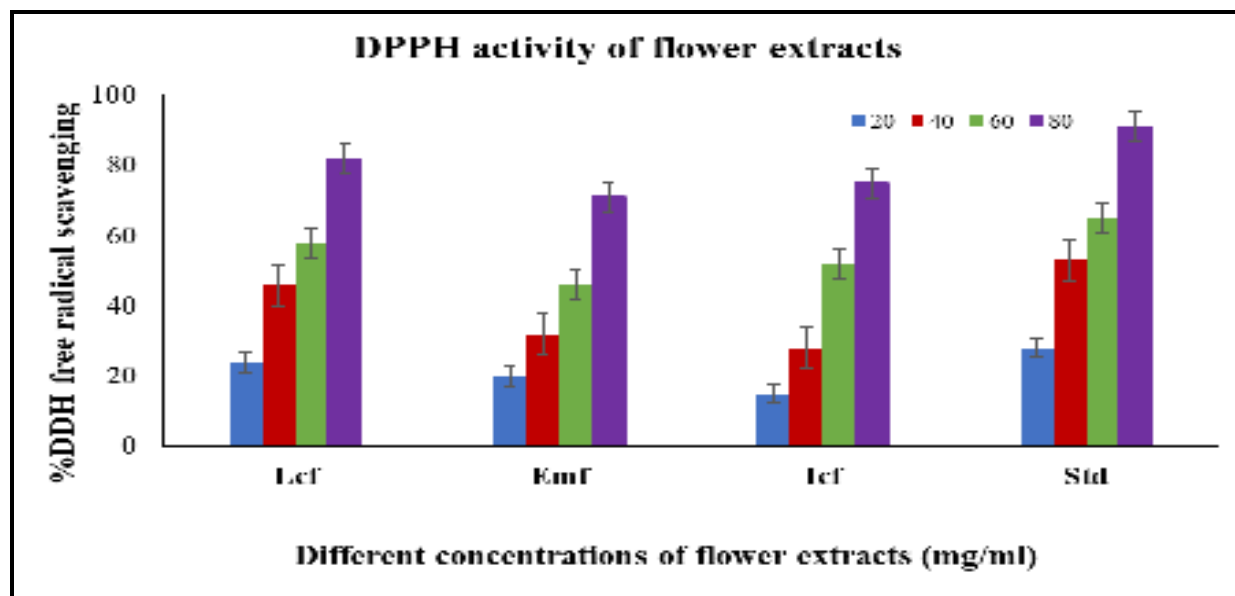


Figure No.3: Antioxidant activity of different extracts of flowers measured by DPPH

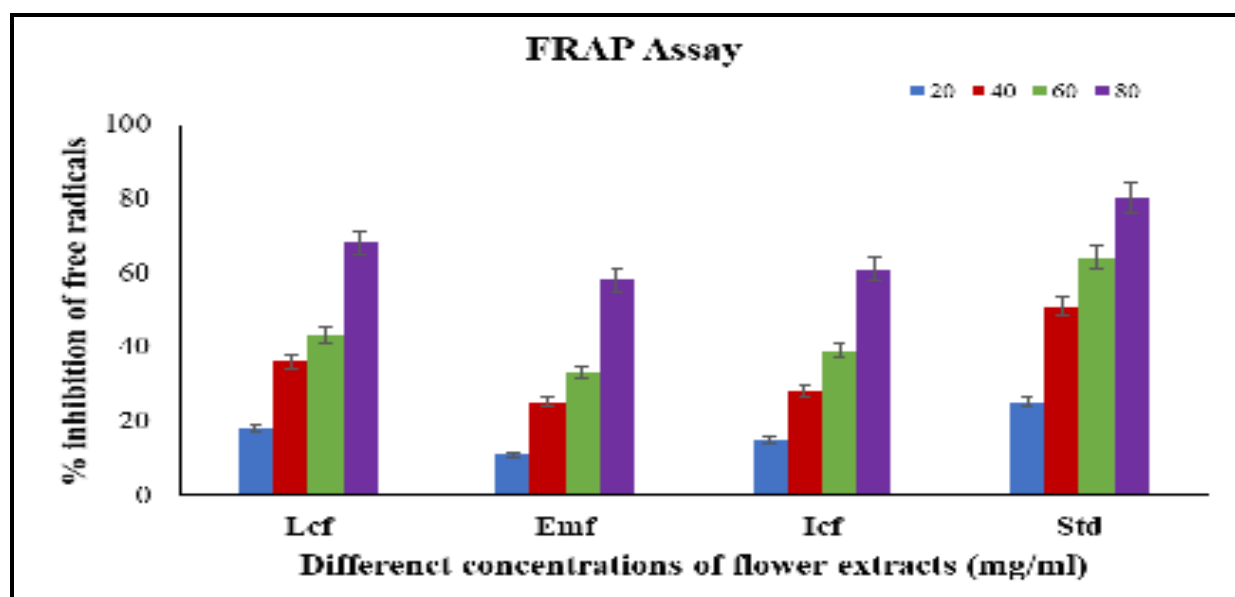


Figure No.4: Antioxidant activity of different extracts of flowers measured by FRAP

CONCLUSION

The three flowers of medicinal plants collected from various places of Mysore, India exhibited variable but considerable antioxidant and antibacterial activities. Among three flowers studied, *Lantana camara* flowers, possessed the highest activities in the antioxidant methods and exhibited free radical scavenging properties which may be due to the presence of flavonoids, tannin, alkaloid and polyphenol compounds. The antibacterial activity of the extract against tested bacteria shows that it has the potential to be used for the treatment of wound infections caused by these bacterial infections.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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