

## ANTIMICROBIAL POTENTIAL OF METHANOLIC EXTRACTS OF LEAVES OF *EPIPREMNUM AUREUM* (LINDEN & ANDRE) G. S. BUNTING

RITA MEHTA<sup>1\*</sup>, ASHOK BHAGWAT<sup>2</sup>, CHHAYA SAWANT<sup>2</sup>

<sup>1</sup>School of Science, Narsee Monjee Institute of Management Studies, Vile Parle, Mumbai, India, <sup>2</sup>Shri C. B. Patel Research Centre, Vile Parle, Mumbai, India. Email: ritahmehta@gmail.com

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### ABSTRACT

**Objective:** The aim of the present study was to screen and evaluate antimicrobial efficacy of the methanolic extracts of the leaves of *E. aureum* (Linden & Andre) G.S. Bunting against different gram +ve and gram –ve test bacteria and fungi. Further, aim was to evaluate the Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MBC) for the susceptible microorganisms. The preliminary phytochemical analysis of the hot and cold methanolic extracts of the leaves was undertaken to detect the presence of different phytoconstituents.

**Material and Methods:** The antimicrobial efficacy study of the methanolic extracts of *E. aureum* was evaluated by agar well diffusion and further assay was carried out to determine MIC using broth dilution method followed by determination of MBC.

**Results:** The results of antimicrobial activity screening revealed excellent antibacterial activity of hot methanolic extract against selected gram –ve pathogenic bacterial strains, *Viz. S. typhi, P. aeruginosa, S. paratyphi A* and *S. aureus* and *S. mutans* amongst gram +ve bacteria. The hot methanolic extract exhibited better antibacterial efficacy as compared to the cold methanolic extract. Both hot and cold methanolic extracts were ineffective against fungal strain *Candida albicans*. The results of MIC of the hot methanolic extract of *E. aureum* against *P. aeruginosa; S. typhi;* and *S. paratyphi A* was found to be in the range between 3-6 mg/ml. Further, MBCs for the above bacterial strains were found to be 4mg/ml; 5mg/ml and 6mg/ml respectively. The result of preliminary phytochemical analysis showed the presence of secondary metabolites such as alkaloids, tannins, flavonoids, triterpenoids, and saponins that may be responsible for antimicrobial activity.

**Conclusions:** This preliminary screening of antibacterial efficacy study of the methanolic extracts of leaves of *E. aureum* indicates the medicinal importance of the plant in control of highly pathogenic bacteria i.e. *Salmonella* species as well as resistant pathogenic bacteria like *P. aeruginosa*.

The identification and efficacy of the bioactive phytoconstituents from the methanolic extracts of the plant will further aid in development of new antibacterial agent from the plant.

**Keywords:** Antimicrobial activity; *E. aureum* (Linden & Andre) G.S. Bunting; *P. aeruginosa; S. typhi; S. paratyphi A*.

### INTRODUCTION

In view of the rising incidences of the side effects and the resistance that pathogenic microorganisms build against the antibiotics, there is a need to develop alternative antimicrobial drugs with diverse chemical structures and novel mechanisms of action for the treatment of infectious diseases [1-2].

One of the most recent approaches is to screen locally available medicinal plants and biologically active compounds isolated from plant species used in traditional and herbal medicine for possible antimicrobial properties. The plant kingdom is a big source of potential drugs. Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is increasing interest in plants as sources of agents to fight microbial diseases [3]. Medicinal plants contain different secondary metabolites which have been shown to have antimicrobial properties and that are easily available from natural products at affordable price, at the same time may provide better remedies and these can serve as valuable alternative to available antimicrobial agents [4-5].

In India, herbal medicines have been the basis of treatment and cure for various diseases in traditional methods practiced such as Ayurveda, Unani and Siddha. Since ancient times a number of Indian medicinal plants have been used globally. There are many references to Indian medicinal plants and trade in spices in a number of historical documents [6].

*E. aureum*, also known as Money plant; devil's Ivy, is a monocot that grows vigorously and rapidly covering a wide area. The plant has a very peculiar growth pattern; when it is grown on the ground and if unrestricted in the wild, this liana can grow up on trunks of huge trees by attaching its aerial roots to their surfaces, reaching 10-20 meters.

A study by B.C. Wolverton [7] reported the use of golden pothos (*E. aureum*) grown on an activated carbon filter system reduced the air

levels of benzene and trichloroethylene. Further from our literature review, it was observed that Roy S. *et al.* 2013 [8] in their review paper have reported strong antibacterial activity of crude extracts of many of the plants of the family Araceae but detailed study of the antimicrobial activity of the plant *E. aureum* was not included in their report. Apart from these reports, Srivasatava *et al.* 2011 [9] has reported antibacterial, antioxidant and antitermite activity of the ethanolic extract of the explant of *E. aureum* and Sonawane *et al.* 2011 [10] has reported antibacterial efficacy of the aqueous extract and ineffectiveness of the methanolic extract of the plant *E. aureum* against *S. aureus* and *E. coli*. The lack of reports for other biological efficacy of the plant *E. aureum*, and in view of all these contraindicating reports about the antibacterial efficacy of the plant, a detailed investigation is needed to evaluate antimicrobial efficacy of this plant and at the same time ascertain the medicinal importance of this commonly available plant.

The present study attempts to evaluate and screen methanolic extracts of the leaves of *E. aureum* (Linden & Andre) G.S. Bunting against the selected gram +ve and gram –ve bacteria and *Candida albicans* as representative of fungi. Further evaluation of minimum inhibitory concentration (MIC) and minimum microbicidal concentration of the hot methanolic extract was carried out for susceptible organisms. A preliminary phytochemical analysis of the methanolic extracts was performed for detection of various plant secondary metabolites.

### MATERIAL AND METHODS

#### Plant material collection and authentication

Fresh plant material was procured from the Maharashtra Nature Park, Mahim in Mumbai and was identified and authenticated at the BLATTER HERBARIUM, St. Xavier's College, Mumbai (Voucher specimen accession no. 78393). Leaves were washed under running

tap water thrice and later air dried in shade. Dried leaves were powdered and packed in an air tight container. Dried leaf powder was used for hot and cold methanolic extract preparation. The methanolic extracts were further used for preliminary phytochemical analysis; antibacterial efficacy screening; MIC and microbicidal concentration evaluation

#### Preparation of plant extracts

Air dried leaves were processed for hot methanolic extraction using a Soxhlet apparatus. For the cold methanolic extract, the air dried leaves were subjected to agitation with methanol for 12 hours on a rotary shaker at the ambient temperature. Extracts were filtered and evaporated to dryness. The percentage yields for hot methanolic extraction and cold methanolic extract were 2.05 % and 2.15 % respectively.

#### Preliminary phytochemical screening

The detection and presence of bioactive secondary metabolites in the plant extracts is highly indicative of pharmacological importance of the plant in terms of its medicinal uses. The hot and cold methanolic extracts of *E. aureum* were subjected to various chemical tests as per the standard procedures for qualitative phytochemical analysis [11-13].

#### Screening for antibacterial activity

##### Preparation of test cultures

The list of selected representatives of gram +ve and gram -ve bacterial strains and representative of fungal strain used for the present study are described in table 1.

**Table 1: Profile of organisms used in the present study**

Sr. No.	Test Organism	Associated Disease condition
<b>Gram +ve bacteria</b>		
1	<i>S. aureus</i> MTCC 737	Skin and tissue infection
2	<i>S. mutans</i> MTCC 890	Tooth cavity
<b>Gram -ve bacteria</b>		
3	<i>E. coli</i> MTCC 443	Pathogenic strain
4	<i>E. coli</i> ATCC 8739	Urinary Tract Infection and problematic in wound infection and surgery
5	<i>P. aeruginosa</i> MTCC 424	Mainly associated with Hospital infections
6	<i>P. vulgaris</i>	Urinary Tract Infection
7	<i>K. pneumoniae</i>	Pneumonia, septicemia, Urinary Tract Infection
8	<i>S. typhi</i> MTCC 733	Typhoid fever and food poisoning
9	<i>S. paratyphi</i> A	Paratyphoid
10	<i>S. paratyphi</i> B	Paratyphoid
<b>Fungal strain</b>		
11	<i>Candida albicans</i> MTCC 227	Opportunistic for oral and genital infections

Antimicrobial efficacy study was performed by agar cup diffusion method. The stock cultures were procured from the Microbial Type Culture Collection (MTCC), The Institute of Microbial Technology, Chandigarh, India. The stock cultures of *S. paratyphi* A; *S. paratyphi* B; *K. pneumoniae*; and *P. vulgaris* were clinical isolates.

The bacterial isolates were cultured on Nutrient agar media and were incubated at 37°C for 24hrs. The pure culture of test organisms was obtained by repeated sub-culturing. Morphological and biochemical reactions were carried out to ascertain proper identification. Stock cultures were maintained at 4°C on Nutrient agar slants. Active cultures for experiments were prepared by transferring a loopful of bacterial organisms from stock cultures to fresh Nutrient agar slants and incubated overnight at 37°C. The bacterial culture suspensions in sterile normal saline were prepared from the overnight culture of the respective bacterial strains by visual comparison with 0.5 McFarland standard so as to obtain the

approximate turbidity of the culture suspension to  $10^7 - 10^8$  CFU/ml [14].

*Candida albicans* inoculum was prepared from freshly grown culture suspended in sterile saline with approximate  $10^7 - 10^8$  cells/ml. The density estimation of the yeast culture suspension was done by counting cells in haemocytometer as per the AOAC method, 1995 [15]. For antifungal activity assay, 0.1 mL of this culture suspension was spreaded on Sabouraud Dextrose Agar (SAB) plates.

#### Preparation of media

All the media were obtained from Hi Media Laboratories Pvt. Ltd., Mumbai and were prepared as per the manufacturer's instructions. The Nutrient agar (NA) was used for the regular maintenance of test cultures. Mueller Hinton agar (MH) media was used for antibacterial activity assay and Sabouraud Dextrose Agar was used for antifungal activity assay.

#### Preparation of plant methanolic extracts as test samples

Both hot and cold methanolic extracts in the concentration range of 10mg/ml; 50mg/ml; and 100mg/ml were used for antimicrobial assay study. Methanol was used as control for methanolic extracts.

#### Agar well diffusion method

The agar cup diffusion method was employed to study the antimicrobial activity against the selected group of pathogenic bacteria. The experiment was performed essentially according to the method described in European Pharmacopeia with slight modification [16] in inoculum size where instead of 0.5 ml of  $10^4 - 10^5$  cells/ml, 0.1 ml of test inoculum with  $10^7 - 10^8$  cells/ml was swabbed on solidified Muller Hinton agar for bacteria and Sabouraud dextrose agar for yeasts [17]. Since methanol was used as a solvent for reconstituting the plant extracts, methanol control wells were maintained to test the antimicrobial activity in each plate. Results were noted after the appropriate incubation period by measuring the diameter of zone of Inhibition in millimeter (mm) scale.

All the antimicrobial assays were performed in triplicates.

#### Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

##### Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial agent that will inhibit the growth of a microorganism at the end of stipulated time of incubation period. Minimum microbicidal concentration (MBC) is that concentration of a microbial agent that will kill or eliminate a microorganism at the end of stipulated time of incubation period. For broth dilution method, test bacteria are inoculated into a liquid growth medium in the presence of different concentrations of the plant methanolic extract as an antimicrobial agent and the growth of test culture is assessed after overnight incubation and the MIC value is determined. The MIC value is generally regarded as the most basic measurement of the antimicrobial agent against a test organism.

For the present study, broth dilution method as described by CLSI 2006 [18] was used for determination of MIC values of the methanolic extracts of the plant. In brief, the plant methanolic extract was diluted in the range of 1 - 10 mg/ml in MH broth medium. Each tube was inoculated using 0.1 mL of the respective bacterial culture suspension. Both positive (Cefixime) and negative controls were also prepared simultaneously. All the assay tubes were incubated overnight at 37°C. The lowest concentration of the extract that produced no visible growth (turbidity) was recorded as the MIC values.

##### Minimum microbicidal concentration

For determination of minimum microbicidal concentration, a modified method described by British Society for Antimicrobial Chemotherapy Guide to Sensitivity Testing was undertaken. In brief, after MIC determination study, an aliquot of 0.1 mL of MH broth culture inoculum from all the tubes showing no visible growth (turbidity) was

spread on MH agar plates uniformly by using a sterile cotton swab and all the plates were incubated overnight at 37°C. After the incubation period, the MBC value of the plant extract was noted as the lowest concentration that kills > 99.9% of the initial bacterial population where no visible growth was observed on MH plates.

## RESULTS

### Preliminary phytochemical screening analysis

Preliminary phytochemical studies of the hot and cold methanolic extracts showed presence of flavonoids, alkaloids, tannins, triterpenoids, saponins and phenolic compounds (Table 2).

### Antibacterial activity assay

The results of *in vitro* antibacterial activity of the methanolic extracts of the leaves of *E. aureum* demonstrated remarkable inhibitory action against gram -ve bacteria (table 3; figure 1; and figure 2). The hot methanolic extract exhibited antibacterial activity against *E. coli* (Pathogenic strain); *P. aeruginosa*; *S. typhi*; *S. paratyphi* A; and *K. pneumonia* amongst gram -ve bacteria and *S.*

*aureus* and *S. mutans* from gram +ve bacterial strains. The hot methanolic extract was ineffective against *E. coli*; *P. vulgaris* and *S. paratyphi* B. It was observed that, the hot methanolic extract showed better antibacterial activity as compared to cold methanolic extract. Both hot and cold methanolic extracts were ineffective against *Candida albicans*.

**Table 2: Preliminary phytochemical analysis of methanolic extracts of *E. aureum***

Phytoconstituents	Hot methanolic extract	Cold methanolic extract
Tannins	+	+
Alkaloids	+	+
Flavonoids	+	+
Triterpenoids	+	+
Saponins	+	+
Sterols	-	-
Cardiac glycosides	-	-

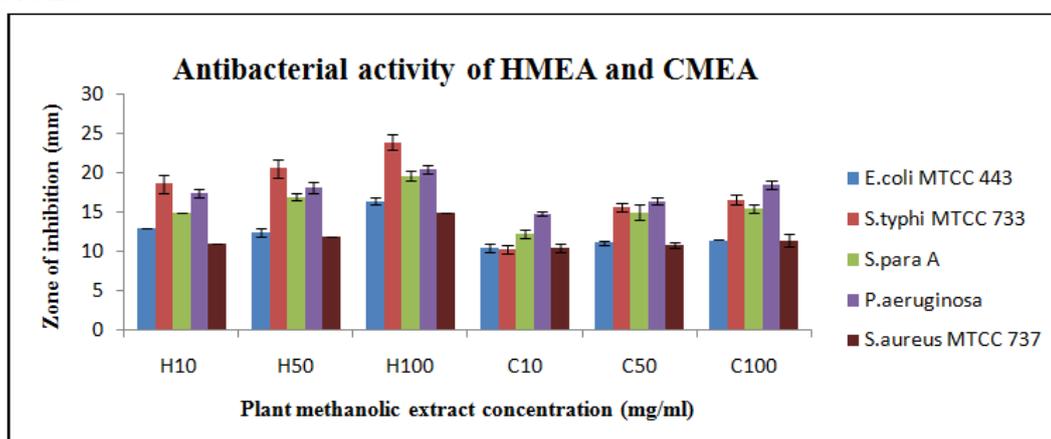
Key: +:- Phytoconstituent detected; -:- Phytoconstituent not detected

**Table 3: Antimicrobial effect of hot and cold methanolic extracts of *E. aureum***

Test Culture	Diameter of Zone of Inhibition (mm)						
	Methanol Control	Plant methanolic extract					
		HMEA (10mg/ml)	HMEA (50mg/ml)	HMEA (100mg/ml)	CMEA (10mg/ml)	CMEA (50mg/ml)	CMEA (10mg/ml)
<b>Gram +ve bacteria</b>							
<i>S. aureus</i> MTCC 737	NI	11.0 ± 0 #	12.0 ± 0 #	15.0 ± 0 #	10.5 ± 0.5 #	10.88 ± 0.29 #	11.5 ± 0.87 #
<i>S. mutans</i> MTCC 890	NI	27.5 ± 0.5 #	31.33 ± 0.58 #	37.67 ± 0.58 #	20.67 ± 0.58 #	23.0 ± 0 #	25.0 ± 0 #
<b>Gram -ve bacteria</b>							
<i>E. coli</i> ATCC 8739	NI	NI	NI	NI	NI	NI	NI
<i>E. coli</i> MTCC 443 (Pathogenic strain)	NI	13.0 ± 0 #	12.5 ± 0.5 #	16.5 ± 0.5 #	10.5 ± 0.5 #	11.17 ± 0.29 #	11.5 ± 0 #
<i>P. vulgaris</i> MTCC 424	NI	NI	NI	NI	NI	NI	NI
<i>P. aeruginosa</i> MTCC 424	NI	17.5 ± 0.5 #	18.17 ± 0.76 #	20.5 ± 0.5 #	14.83 ± 0.29 #	16.5 ± 0.5 #	18.5 ± 0.5 #
<i>K. pneumoniae</i> MTCC 733	NI	11.5 ± 0 #	10 ± 0.5 #	11 ± 0.5 #	NI	NI	NI
<i>S. typhi</i> MTCC 733	NI	18.67 ± 1.15 #	20.67 ± 1.15 #	24 ± 1 #	10.33 ± 0.58 #	15.67 ± 0.58 #	16.67 ± 0.58 #
<i>S. paratyphi</i> A	NI	15.0 ± 0 #	17.0 ± 0.5 #	19.67 ± 0.58 #	12.33 ± 0.58 #	15.0 ± 1 #	15.5 ± 0.5 #
<i>S. paratyphi</i> B	NI	NI	NI	NI	NI	NI	NI
<b>Fungal strain</b>							
<i>C. albicans</i> MTCC 227	15.0 ± 0 #	11.0 ± 0 #	11.0 ± 0 #	11.0 ± 0 #	11.0 ± 0 #	11.0 ± 0 #	11.0 ± 0 #

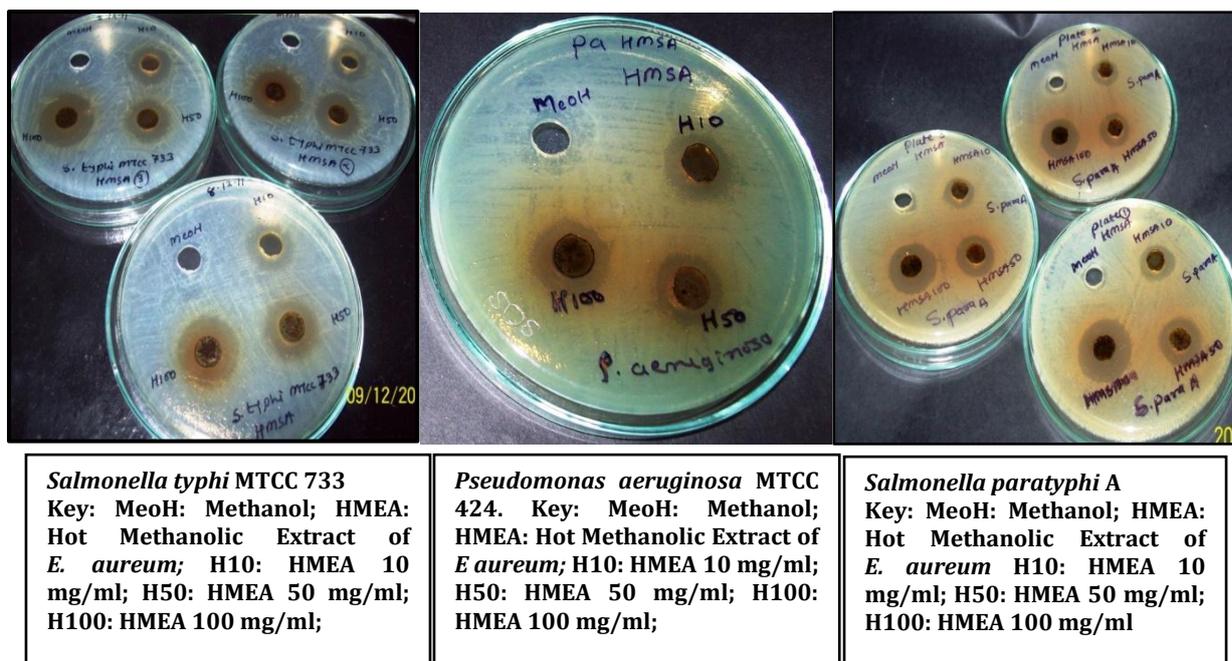
It can be hence concluded that the antibacterial efficacy of the plant *E. aureum* is attributed to the individual or synergistic effect of phytoconstituents present in the methanolic extract.

Key: HMEA: - Hot methanolic extract of *E. aureum*; CMEA:-Cold methanolic extract of *E. aureum*; NI = No zone of inhibition; # = Values are mean ±SD for triplicate results



**Fig. 1: Antimicrobial effect of hot and cold methanolic extracts of *E. aureum***

Key: HMEA:-Hot methanolic extract of *E. aureum*; CMEA:-Cold methanolic extract of *E. aureum*. Values are mean ±SD for triplicate results; H10: - Hot ethanolic extract 10 mg/ml; C10: - Cold methanolic extract 10 mg/ml



**Salmonella typhi MTCC 733**  
Key: MeoH: Methanol; HMEA: Hot Methanolic Extract of *E. aureum*; H10: HMEA 10 mg/ml; H50: HMEA 50 mg/ml; H100: HMEA 100 mg/ml;

**Pseudomonas aeruginosa MTCC 424.** Key: MeoH: Methanol; HMEA: Hot Methanolic Extract of *E. aureum*; H10: HMEA 10 mg/ml; H50: HMEA 50 mg/ml; H100: HMEA 100 mg/ml;

**Salmonella paratyphi A**  
Key: MeoH: Methanol; HMEA: Hot Methanolic Extract of *E. aureum* H10: HMEA 10 mg/ml; H50: HMEA 50 mg/ml; H100: HMEA 100 mg/ml

Fig. 2: Antibacterial effects of methanolic extracts of the plant *E. aureum*

#### MIC and MBC evaluation analysis

The MIC test is a rapid, easy and reliable method to evaluate biostatic efficacy of any antimicrobial agent. MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents.

MIC and MBC values of the plant extract were determined for *P. aeruginosa*; *S. typhi*; and *S. paratyphi* A. The MIC values range between 3-6 mg/ml for the respective organism. Whereas MBC values for the above bacterial strains were found to be 4mg/ml; 5mg/ml and 6mg/ml respectively. The MIC values range between 1-10 µg/ml for the standard drug cefixime for *S. typhi* and *S. paratyphi* A. The results are described in table 4.

Table 4: MIC and MBC values of hot methanolic extract of *E. aureum*

S. No.	Test Culture	MIC value	MBC value
1	<i>P. aeruginosa</i> MTCC 424	4 mg /ml	4 mg /ml
2	<i>S. typhi</i> MTCC 733	5 mg/ml	5 mg /ml
3	<i>S. paratyphi</i> A	6 mg/ml	6 mg /ml

#### DISCUSSION

The result of the preliminary phytochemical analysis of the hot and cold methanolic extracts showed that the extracts of the plant *E. aureum* contains phytoconstituents such as tannins, flavonoids, phenolic compounds, alkaloids, proteins, triterpenoids and saponins. These secondary metabolites may have potentially significant application against some of the human pathogenic bacteria including that cause enteric infections. The antimicrobial activity of phenolics (tannins, flavonoids) and saponins have been established in some plants [19-20]. Flavonoids are becoming the subject of anti-infective research, and some researchers have isolated and identified the structures of flavonoids possessing antifungal, antiviral and antibacterial activity [21].

Srivatasava. et al. 2011 [9] have evaluated antibacterial efficacy against very few of the test strains, viz. *B. subtilis* and *M. luteus* and *E. coli*. They have reported that ethanolic and methanolic extracts of the leaves of *E. aureum* are effective against *B. subtilis* and *M. luteus* while ethanolic root extracts is active against *E. coli*. Whereas Sonawane et al. 2011 [10] has reported that only aqueous extract of *E. aureum* Linn. has significant antimicrobial activity against *E. coli*

and *S. aureus* whereas no activity was reported with methanolic extract against *E. coli* and *S. aureus*.

The results of the present study on *in vitro* antibacterial activity screening indicates that the methanolic extracts of the leaves of *E. aureum* exhibited remarkable inhibitory action against gram -ve bacteria viz. *E. coli* (Pathogenic strain); *P. aeruginosa*; *S. typhi*; *S. paratyphi* A; and *K. pneumonia*. The difference in the antibacterial activity of the present study for the plant *E. aureum* (Linden & Andre) G.S. Bunting and other reports can be due to the difference and variation in plant species variety, location where the plant is grown as well as method of extraction at the same time strains of the test bacteria used.

Moreover it was interesting to note that the plant methanolic extract is showing significant antibacterial activity against *S. typhi* and *S. paratyphi* A but it is ineffective against *S. paratyphi* B. This is significantly important keeping in view of the antibacterial therapy for paratyphoid fevers that are caused by different species of *Salmonella*. Enteric fever is a major global problem. Emergence of antibacterial resistance threatens to render current treatments ineffective. There is little research or public health effort directed toward *Salmonella paratyphi* A, because it is assumed to cause less severe enteric fever than does *S. typhi* [22]. In view of these reports, the result of this investigation becomes highly significant to explore potential of the bioactive constituents of the methanolic extract of leaves of *E. aureum* to develop and prepare formulations of antimicrobial therapy against *S. typhi* and *S. paratyphi* A.

In addition to these results, it was observed that the plant extracts exhibited remarkable antibacterial activity against *S. mutans* which is commonly found in the human oral cavity and is a significant contributor to tooth decay [23]. This aspect of the plant extract opens up new avenues for development of anticariogenic agent for oral health.

#### CONCLUSION

The findings of the present study suggest the strong evidences of the antibacterial efficacy of the plant *E. aureum*. The present investigation will prove useful in exploring the antibacterial efficacy of the active secondary metabolites present in the methanolic extract of *E. aureum*. These results suggested that the preparations incorporating flavonoids and/or tannins from *E. aureum* could be of interest for further development as antimicrobial agent as an alternative treatment. However further detailed research on the

isolation of bioactive compounds would enable to check efficacy and potential of the natural antibacterials against pathogenic bacterial strains.

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