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RESEARCH ARTICLE

EVALUATION OF ANTIMICROBIAL ACTIVITY, MINIMUM INHIBITORY CONCENTRATION (MIC) & ANTI-TUMOR ACTIVITY OF *ELETTARIACARDAMOMUM AND FERULA ASSA-FOETIDA* LEAVES

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ABSTRACT

Aim: In the present study we compared the antimicrobial and Anti Tumoractivity of two varieties of Indian medicinal plants. Tetracycline was used as the positive control in anti-microbial assay.
Methods: Agar well diffusion assay, Carrot disc assay.
Results: Methanoic extracts of *Elettariacardamomum* and *Ferula assa-foetida* leaves were tested against *Escherichia coli* (gram negative bacteria), *Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans* (gram positive bacteria) *and Candida vulgaris* (fungi) using agar well diffusion assay. The Anti-tumor activity was evaluated by Carrot disc assay. The range varied from 4 mm to 7 mm against *E.coli*, 7 to 11 mm against *Staphylococcus aureus*, 12 to 15 mm against *B. subtilis*, 4 to 10 mm *Streptococcus mutans* and lastly mild antifungal activity of 2 mm was observed for *Candida vulgaris.* All the extracts presented comparatively higher MIC values than that of tetracycline (a standard antibiotic used as positive control). Both plants exhibit significant anti-tumor activity.
Significance and impact of study: Based on these findings presents a good scope for developing a potential antimicrobial and Anti-tumor agent in suitable compounds formulation with least amount of side effects.

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INTRODUCTION

Antibiotic chemotherapy has been one of the most significant medical successes of the twentieth century. Various microbiological infections have been treated with the help of medicinal plants. In recent years, the occurrence of antimicrobial resistance among main microbial pathogens such as *Staphylococcus aureus*, *Moraxella, Klebsiella*, *Haemophilus*, *Neisseria*, *Streptococcus pneumonia* and *Enterococcusfaecalis* is growing at an shockingrate globally (Cohen, 1992, Gold, 1996, Kaushik *et al.,* 2008). Sometimes many antibiotics can no longer be used for the treatment ofinfections caused by such organisms and the threat to the practise of other drugs is steadily increasing (Courvalin *et al*., 1996). Natural products particularly plant-based derivatives have played a significant role throughout the world in treating and inhibiting human diseases (Newman 2000, Chin, 2006; Duncan *et al*., 1999). Thus, this idea has become the base for the development of a remedy medicine for the development of new drugs (Goyal *et al*., 2008). The broad-spectrum effectiveness of these plant derivatives may provide a suitable basis for new antimicrobial therapies (Kaushik *et al*., 2003) *Elettariacardamomum* is an important member of family Zingiberaceae. It is commonly known as Chhotielaichiisknown as the 'Queen of Spices' (Abraham *et al*., 1965). The main compound which was observed is 1,8-cineole (representing 50% or more), with minor amounts of camphor, α-terpineol, limonene, borneol, α-terpenyl acetate, and α-pinene (anti-timour) (Agaoglu *et al*., 2005, Miyazawa *et al*., 1975). Additionally Cardamom seeds are used in aromatherapy to stimulate energy (Lawrence BM *et al*., 1979, Kaushik *et al*., 1988). It also acts as Ayurvedic remedy in case of asthma, bronchitis, digestive problems, and urinary complaints and several other human ailments (Wyk *et al*., 2004, Unnikrishn *et al*., 1988). Assafoetidais dried latex (oleo-gum-resin), contains about 40- 64% resin, 25% endogenous gum, 10-17% volatile oil and 1.5-10% ash. The resin portion is known to contain asareninotannols 'A' and 'B', ferulic acid, umbelliferone and four unidentified compounds (Lee *et al.,* 2009). Use ofassafoetida is beneficial in treatment of asthma, excessive and painful menstruation, tooth ache, sexual impotency fever, and whooping cough (Ballabh and Chaurasia, 2007). There is reporting that a *ssafoetida* also possesses anti-influenza A (H(1)N(1)), antiviral and cytotoxic effects (Lee *et al.,* 2009). It has been postulated that certain compounds like luteolin, Ferulic acid, α-pineneand Indole 3carbinoletc are

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present in plants which posses anti tumor activity. (Unnikrishn *et al*., 1988) Present study was aimed and focussed to evaluate the antimicrobial and anti tumor potential of metanoic extracts of *E. Cardamomum* and *Ferula assa-foetida*leaves.

MATERIALS AND METHODS

Materials

Muller-Hinton Agar, Potato Dextrose Agar, Tetracycline. Particularly, methanol was used as solvent purchased from Sigma-Aldrich Delhi (INDIA).

Methods

Plant material collection

The leaves of *Elettariacardamomum* and *Ferula assa-foetida* leaves were collected from Hapurchungi Road nursery, Ghaziabad.

Preparation of methanolic extract of leaves

The collected leaves *Elettariacardamomum and Ferula assa-foetida* leaveswere cut into small pieces when got properly dried with the help of mixer. The powdered leaves (5 g) was weighed and soaked in 100 ml of methanol in a conical flask. The flask containing the leaves was shaken, corked and left to stand on shaker for 48 h at room temperature. After 48 h, the mixture was filtered by What man filter no1 and the extract was collected and concentrated by evaporation to dryness in evaporating dish (Trease *et al*., 1997). Keep it foe 50-72 hours in desiccator. The dried methanol extract was stored in refrigerator for, antimicrobial activity, anti-tumor activity study.

Microbial strains and culture

All microbial strains were procured from Institute of Microbial Technology, Sector 39A, Chandigarh, India. (Table 1)

Preparation of inoculums

All gram positive and gram negative were precultured in nutrient broth overnight in a rotary shaker at 37°C for E. coli and 30°C for B. subtilis. Potato Dextrose Agar has been used for fungi. *Agrobacterium tumefaciens* was cultivated in LB media. The cultures were used when A600 of 0.6 was obtained.

In vitro Antimicrobial activity testing using Agar well diffusion assay (Wayne *et al*., 2002; NCCLS)

Agar-Well diffusion test was used for testing the antimicrobial activity of crude extracts. For the antimicrobial test, sterile Muller-Hinton Agar for bacteria and Potato Dextrose Agar for fungi were used. With sterile technique, microorganisms were inoculated by swabbing thoroughly over the entire sterile agar surface of a plate to obtain a confluent lawn of microbial grow. Each test sample solution of different concentration (2.5 to 10 mg/ml) 100µl was introduced with pipette into each well as labelled. And then, the plates were placed in an incubator at 37°C for 22-24 hour. After respective incubation time for microorganism, the plates were examined and the diameters of the zones of complete inhibition were measured to the nearest whole millimeter with a ruler. The strains were designated arbitrarily as sensitive or resistant. Sterilized distilled water was used as negative control. Tetracycline (5 µg/ml) was used as a standard antibiotic (i.e. positive control).

Assessment of minimum inhibitory concentration (MIC)

Active extracts obtained by agar well diffusion assay were further used to determine the MIC required for the bacteriostatic effects by standard broth microdilution methodology (18). Stock solution of each active extracts were serially diluted with Mueller-Hinton broth to gain different concentration .Plates were then kept at 37°C for an overnight incubation. Then MIC was defined and calculated as the lowest concentration of the extract which inhibit the visible growth of microbial strains.

Determination of Anti-tumor Activity by using Carrot Disc Assay

Test for anti-tumor activity was done using carrot discbioassay with minor modification (Chen *et al*., 1999). Selected plant extracts were prepared with 50 ppm and 100 ppm concentration. Carrot (*Daucascarota* L.) samples were sterilized with commercial bleach (cocorax) followed by washing with sterilized deionized water for three times. Each disc was overlaid with 100ul of *Agrobacterium tumefaciens* inoculums (108cfumL-1). A 50ul aliquot of each extract with different concentration was then added using syringe into disc. Petri dishes were sealed by para-film and incubated at 30°C. After 2 weeks, the discs were checked for development of young galls (tumors) from the meristematic tissue around the central vascular system.

Statistical Analysis

Mean value and standard deviation were calculated for each test bacteria, and fungi. Data were analysed by one-way ANOVA and p values were considered significant at $p > 0.05$ (Singariya et al., 2011c). MS Office 2010 has been used in graphical presentation.

RESULTS

Plants are a potential source of therapeutic and beneficial activities due to the presence of active bioactive components. Many reports are available on the antiviral, antibacterial, antifungal, antihelmintic, antimolluscal and anti-inflammatory properties of plants (20, 21, 22). These studies have aided in identifying the active principles which can be explored for developing drugs for the therapeutic use in human beings. Some are in clinical trails. However, not many reports are available on the therapeutic uses of leaves of both plants. In this phase we have determined Antimicrobial Activity by Agar Well Diffusion and Anti-tumor activities of *Elettariacardamomum* and *Ferula assa-foetida* leaves.

Antimicrobial Activity by Agar Well Diffusion Test

According to the testing results, methanol fraction of crude extract shows best activity against *S. aureus* (11mm), Bacillus subtilis (15mm) in inhibition zone diameter respectively. Although positive control Tetracycline showed sensitivity against selected bacterial strains except fungi. This indicated that both extracts showed distinct growth inhibition and wider spectrum of its potential antimicrobial activity. (Table 2 and 3)

Table 1. List if microbial strains used in assay

Anti- Microbial Assay					Anti- Tumor Assay
Gram negative *Escherichia coli* (MTCC 443)	Gram positive *Staphylococcus aureus* (MTCC 3160)	*Bacillus subtilis* (MTCC 121)	*Streptococcus mutans* (MTCC 890)	Fungi *Candida vulgaris*	Bacteria *Agrobacterium tumefaciens*

Table 2. In-vitro antimicrobial activity of Elettariacardamomum leaves

Zone of inhibition in mm					
Concentration (mg/ml)	Gram negative *Escherichia coli*	*Staphylococcus aureus*	Gram positive *Bacillus subtilis*	*Streptococcus mutans*	Fungi *Candida vulgaris*
Control	0	0	0	0	0
Tetracycline	33.60 ± 2.31*	33.85 ± 1.80*	40.00± 0.25*	5 ± 0.38*	0
2.5 mg/mL	4± 0.33*	7± 0.61*	12± 0.95 *	4± 0.34*	0
5 mg/mL	5± 0.38*	9± 0.84*	14± 1.12*	6± 0 .60*	0
10 mg/mL	7± 0.62*	11± 0.93*	15± 1.20*	10± 0.91*	2± 0.23*

*Values are represented as mean ± SD of triplicates.

Table 3. In-vitro antimicrobial activity of Ferulaassa-foetidaleaves

Zone of inhibition in mm						
Concentration (mg/ml)	Gram negative Escherichia coli	Staphylococcus aureus	Gram positive Bacillus subtilis	Streptococcus mutans	Fungi *Candida vulgaris*	
Control	0	0	0	0	0	0
Tetracycline	33.60 ±2.31*	33.85 ± 1.80*	40.00 ± 0.25*	5 ± 0.38*	0	
2.5 mg/mL	2 ± 0.32*	5 ± 0.80*	11 ± 1.20*	4 ± 0.70 *	0	0
5 mg/mL	2.3 ± 0.67*	7 ± 0.88*	13 ± 1.48*	6 ± 0.78 *	1 ± 0.20 *	0
10 mg/mL	5.1 ± 0.82*	10 ± 0.92*	17 ± 1.60*	10 ± 0.92 *	2.5 ± 0.34*	2

*Values are represented as mean ± SD of triplicates.

Table 4. In-vitro antimicrobial activity of Elettariacardamomum and Ferula assa-foetidaleaves

Minimum inhibitory concentration (MIC) - mg/ml					
Concentration (mg/ml)	Gram negative *Escherichia coli*	Gram positive *Staphylococcus aureus*	*Bacillus subtilis*	*Streptococcus mutans*	Fungi *Candida vulgaris*
Tetracycline	1.42	1.56	1.60	1.48	0.72
E. cardamomum	1.65	1.58	1.79	1.49	0
F. foetida.	1.57	1.64	1.65	1.55	0

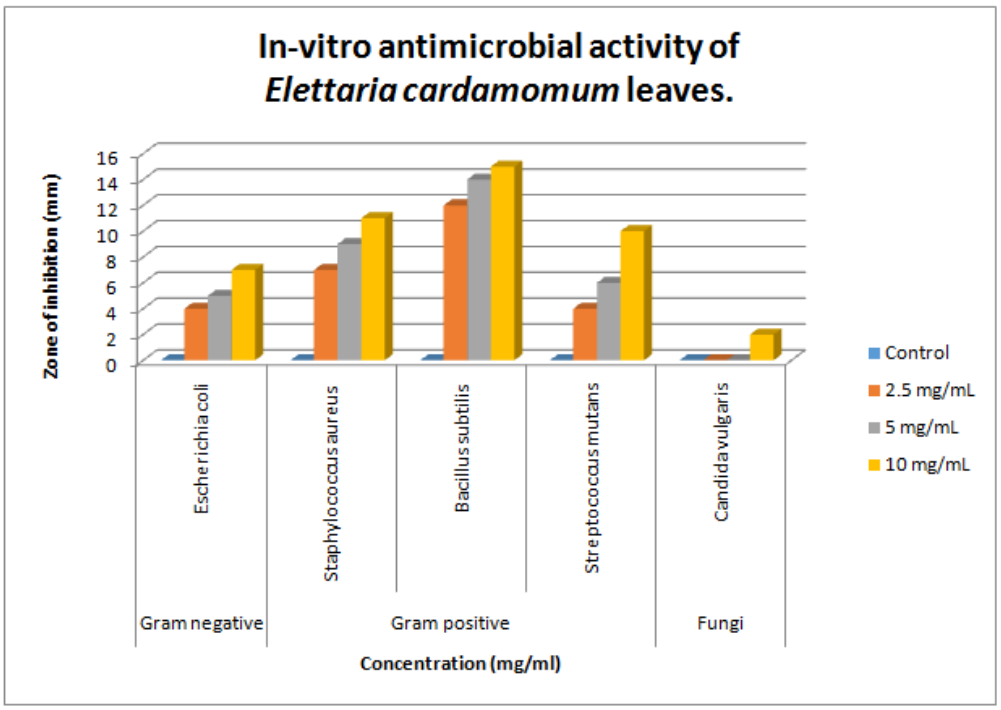

Fig.1. In-vitro antimicrobial activity of *Elettariacardamomum* leaves

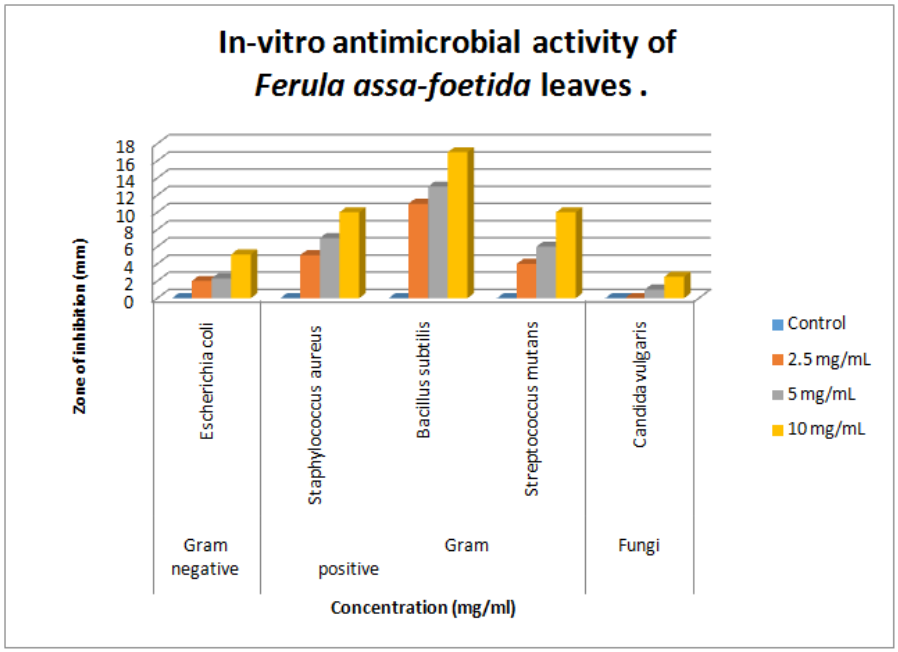

Fig.2. In-vitro antimicrobial activity of *Ferulaassa-foetida* leaves

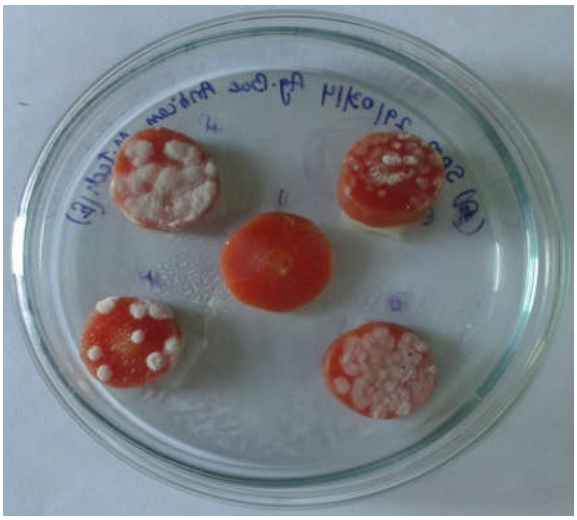

Fig.3. Anti-tumor Activity of *Elettariacardamomum*on Carrot-disc Assay with *A.tumefaciens*

(1) without any treatment (2) *A. tumefaciens*+ *Elettariacardamomum*extract (50 ppm*) (3) *A. tumefaciens*+ *Elettariacardamomum*extract (100 ppm*) (4) (+)ve control (*A. tumefaciens*+ 70%EtOH) (5) (-)ve control (*A. tumefaciens*)

Fig.4. Anti-tumor Activity of *Ferula assa-foetida*on Carrot-disc Assay with *A.tumefaciens*

(1) without any treatment (2) *A. tumefaciens*+ *Ferula assa-foetida* extract (50 ppm*) (3) *A. tumefaciens*+ *Ferula assa-foetida* extract (100 ppm*) (4) (+)ve control (*A. tumefaciens*+ 70% EtOH) (5) (-)ve control (*A. tumefaciens*) *parts per million

In-vitro antimicrobial activity of *Elettaria cardamom* and *Ferula assa-foetida* revealed that maximum zone of inhibition was seen in case of *bacillus subtillus* (gram positive bacteria) as shown in figure Graph 1 and 2. Antimicrobial sensitivity was observed in the following order:

Bacillus subtillis> *Staphylococcus aureus* > *Streptococcus mutans*>Escherichia coli > *Candida vulgaris.*

Minimum Inhibitory Concentration (MIC)

Table 4 represents the MIC values of extracts of *E. cardamom* and *F. foetida* leaves against susceptible microbes. All the tested extracts showed significant variations in MIC values depending upon the test strain. *S. mutans*, the most sensitive bacteria showed lowest MIC range. MIC was not observed against *Candida vulgaris*in case of methanoic extract. Both extracts could inhibit *E. coli* at moderate concentrations.

Anti-tumor Activity by Carrot-Disc Assay

Test for anti-tumor activity was done using carrot disc overlaid with 100ul of *Agrobacterium tumefaciens* (108cfumL-1). *A. tumefaciens* (*Rhizobium radiobacter*) is an indigenous soil bacterium known for its phytopathogenic effects. It causes crown gall tumor disease in a extensive range of plants including most dicotyledons, some monocots and some of gymnosperms species. Upon infection, the bacterium transmit part of its plasmid DNA to the plant. The Ti-plasmid causes the plant's cells to grow rapidly without going through apoptosis, resulting in tumor development similar in nucleic acid and histology to human and animal cancers (21). It plays a major role in aspect of anti-tumor studies. The T-DNA has also been transferred to human cells, representing the diversity of insertion application. The mechanisms by which *Agrobacterium* inserts materials into human cells also by type IV system, is very similar to mechanisms used by animal pathogens to insert materials (mostly proteins) into human cells also type IV secretion. Besides, it plays a major role in aspect of antitumor studies. After 2 weeks incubation of *A. tumefaciens*on each carrot disc in this research, negative control which use only for pathogenicity test showed young galls (tumors) developing from the meristematic tissue around the central vascular system. All extracts of selected plants showed anti-tumor activity. No gall was detected in carrot discs treated with *Elettariacardamomum and Ferula assa-foetida*extracts in the dose of 50 ppm and100 ppm. The test results are shown in Fig. 3, and Fig.4. 70% EtOH treated on the test disc was used in this case as positive control.

DISCUSSION AND CONCLUSION

The phytochemical screening of plants extract revealed the presence of bio-active constituents which are known to exhibit medicinal as well as physiological activities. The results obtained in the present study indicate that methanolic extract leaves of *Ferula assa-foetida* and *Elettariacardamomum* leavespossess significant antimicrobial activity at different concentration. In-vitro antimicrobial activity of Elettariacardamomum and Ferula assa-foetidaleavesreveals that maximum zone of inhibition was seen in case of bacillus subtillus (gram positive bacteria). Antimicrobial sensitivity in both plants was observed in the following order: Bacillus subtillis> Staphylococcus aureus > Streptococcus mutans> Escherichia coli>Candida vulgaris.

Significant antitumor activity was observed in both the plants. Based on these findings presents a good scope for developing a potential antimicrobial and Anti-tumor agent in suitable compounds formulation with least amount of side effects.

Conflicts of interest

The authors report no conflicts of interest.

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