



Comparative study of In vitro antibacterial activity of essential oil of two *Zingiberaceae* family species

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Abstract

The studied plants in this research belongs to the family *Zingiberaceae* which is well known source of many spices and medicinal drugs. Many plants belonging to this family have been remained unexplored. Ginger and curcumin are also from this family which is used in India on regular basis as main spices due to their marvelous properties. The comparative studies helps to examine and explain if particular characteristic traits are followed by all the members belonging to same family. And also to discover the one which is best of all. The present investigation was performed to detect in vitro antibacterial activity of essential oils from leaves of *Roscoeia purpurea* and *Zingiber chrysanthum*. The essential oil was obtained by hydro distillation of plants using Clevenger apparatus. The Antibacterial effect was examined by Well diffusion method against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This study has shown that both the essential oils are effective against gram positive and gram negative bacteria. The present study results that these plants can be explored as new bacteriostatic agents.

Keywords: *Roscoeia purpurea*, *Zingiber chrysanthum*, antibacterial activity and essential oil

Introduction

Roscoeia purpurea has broad leaves, very strong and thick rhizome. The plants grows upto 40-50cm of height. Generally this plants is used as ornamental plant. The tubers of this plant is being used traditionally in Nepal for the treatment of urinary infection, diabetes, fever, diarrhoea. And also the tubers are used for edible purpose. *Roscoeia purpurea* is one of the main ingredients of Ashtvarga. Tubers of *R. purpurea* exhibit Antioxidant activity, antidiabetic activity, hypolipidemic activity and Immuno-modulatory^[1,6].

Zingiber chrysanthum belongs to ginger family. The studies conducted on *Zingiber officinale* (ginger) has revealed that it shows many biological activities. It is used traditionally for curing cough, cold and respiratory related problems. In India it is used as spice in daily food and tea. It possess many biological properties such as antimicrobial, diabetes, anticancer, anti-inflammatory, respiratory disorder and antioxidant activity^[7, 12]. Ginger contains compounds like terpenoids, alkaloids β -carotene, ascorbic acid, flavonoids etc^[13]. Ginger is also used as veterinary medicine for treatment of horses and cattles for Abdominal and digestion related problems^[14, 15]. The leaves of *Hedychium* are boiled and used for indigestion^[16, 17]. Aromatic and herbal plants are used in making cosmetics. Many cultivators grow medicinal plants and make herbal tea, herbal beauty products. Problems arising about the obtaining and use of gingers for cosmetics and Beauty care products such as sustainability, economics, reproducibility and quality control should be discussed by the industries^[18].

Today we are facing problems arising due regular use of chemicals in our day today life such as food, cosmetics etc. Thus people are moving towards the use of natural and herbal products. The consumer have realised the value of products that have natural preservatives and with no artificial ingredients. The antimicrobials used traditionally

for preservation of food have proven to be very effective. Thus the research is encouraging, but much more is to be studied to evaluate the importance of essential oils of *Zingiberaceae* family plants in support of their practical applications^[19].

Materials and methods

The aerial part of *Roscoeia pupurea* plant was collected in the month of July and the aerial part of *Zingiber chrysanthum* plant was collected in the month of October from Uttarakhand, India (above 2400m). The fresh plant leaves collected were cut into small pieces and hydro distilled in Clevenger apparatus about 7-8 h for extraction of essential oil and dried over anhydrous Na_2SO_4 .

Bacterial strains

The microbial strains used were, Gram-positive *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 737) and Gram-negative *Pseudomonas aeruginosa* (MTCC 424), *Escherichia coli* (MTCC 443), *Salmonella typhimurium* (MTCC 531) and *Klebsiella pneumonia* (MTCC 3384) which were obtained from IMTECH Chandigarh, India which are maintained by sub-culturing on nutrient agar after a time period of every 3 months at Department of Biotechnology, Bhimtal campus, Kumaun University, Bhimtal.

Antibacterial screening

The Antibacterial activity was determined by well diffusion method against six bacterial strains. The test of antibacterial activity was performed by determining the MIC and MBC using Well diffusion method. The preliminary screening of antibacterial activity was conducted at 200 $\mu\text{l/ml}$ concentration. The media was prepared and poured into sterilized petri plates. Then the media was kept until it solidify. About 1×10^6 CFU/ml of microbial strain inoculum were cultured and 50 μl of bacterial strains was taken to

spread over the solidified media. The well of diameter 6mm were made on the solidified plates. Chloroamphenicol (30 μ g) per disc was taken as positive control, while n-Hexane was taken as negative control for all bacterial strains. The essential oil solution of desired concentrations were prepared i.e., 50 μ l/ml, 100 μ l/ml, 200 μ l/ml and 400 μ l/ml to determine MIC values. With the help of pipette essential oil with desired concentration was poured into the well on petri plates and allowed to incubate in inverted position at 37 \pm 1 $^{\circ}$ C for 1 day. The diameter of the zone of Inhibition (ZOI) was measured in mm. MICs were determined as the lowest concentration of essential oil inhibiting the visible growth of organism on agar plates. All experiments were carried out in triplicates ^[20].

Result

Table 1 summarizes the antibacterial activity revealed by the essential oil samples of *Roscoea purpurea* and *Zingiber chrysanthum*. Both the samples have shown a broad spectrum of antibacterial test. The inhibitory effect of samples are different as per bacterial strain. The essential oil of *Roscoea purpurea* is effective against four bacterial strains i.e *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The zone of inhibition observed for essential oil from *Roscoea purpurea* leaves was maximum against *Klebsiella pneumonia* and *Bacillus subtilis*, while no activity was

shown against *Salmonella typhimurium* and *Staphylococcus aureus*. The MIC values observed for different bacteria are 50(μ l/ml) for *Staphylococcus aureus* and *Bacillus subtilis*, 100(μ l/ml) for *Escherichia coli* and *Pseudomonas aeruginosa*. *Zingiber chrysanthum* leaves essential oil has shown antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The maximum efficacy was shown against *Pseudomonas aeruginosa* followed by *Escherichia coli*, and minimum ZOI for *Salmonella typhimurium*. The MIC value for *Pseudomonas aeruginosa* and *Escherichia coli* is 50(μ l/ml), *Bacillus subtilis* and *Staphylococcus aureus* is 100(μ l/ml), while for *Salmonella typhimurium* is 200 μ l/ml.

Conclusion

The present study indicated that essential oil of *Roscoea purpurea* is much more effective against bacterial strains than compared to essential oil of *Zingiber chrysanthum* leaves. Both the plants belongs to similar family, but shows different biological properties to different extent. This may be due to presence of different chemical constituents in essential oil of plants or presence of different area percentage of similar chemical compounds. The present study suggests that *Roscoea pupurea* and *Zingiber chrysanthum* can be explored as new antibacterial agents to fight pathogenic microorganisms.

Table 1: Antibacterial activity of *Roscoea purpurea* and *Zingiber chrysanthum* leaves by well diffusion method.

Microbial strain	Plant	Zone of inhibition (mm) CA	Zone of inhibition at concentration (μ l)			
			400	200	100	50
<i>Escherichia coli</i> (MTCC 443)	R.P	21.15 \pm 0.1	14.13 \pm 0.30	11.96 \pm 0.15	9.96 \pm 0.15	8.76 \pm 0.20 [▲]
	Z.C	19.18 \pm 0.33	13 \pm 0.20	10.43 \pm 0.20	9.26 \pm 0.15	9.03 \pm 0.25 [▲]
<i>Bacillus subtilis</i> (MTCC 441)	R.P	25.12 \pm 0.15	19.86 \pm 0.20	17.76 \pm 0.15	14.53 \pm 0.15	12.10 \pm 0.20 [▲]
	Z.C	20.08 \pm 0.20	N/O	N/O	N/O	N/O
<i>Klebsiella pneumonia</i> (MTCC 3384)	R.P	24.80 \pm 0.15	18.06 \pm 0.15	16.46 \pm 0.15	13.06 \pm 0.15	9 \pm 0.10 [▲]
	Z.C	23.20 \pm 0.20	N/O	N/O	N/O	N/O
<i>Staphylococcus aureus</i> (MTCC 737)	R.P	26.08 \pm 0.25	N/O	N/O	N/O	N/O
	Z.C	20.19 \pm 0.10	11.10 \pm 0.10	9.66 \pm 0.15	7.76 \pm 0.15 [▲]	N/O
<i>Pseudomonas aeruginosa</i> (MTCC 424)	R.P	18.90 \pm 0.25	10 \pm 0.10	8.96 \pm 0.15 [▲]	N/O	N/O
	Z.C	25.12 \pm 0.20	19.63 \pm 0.15	15.60 \pm 0.10	14.10 \pm 0.20	12.26 \pm 0.15 [▲]
<i>Salmonella typhimurium</i> (MTCC 531)	R.P	23.40 \pm 0.21	N/O	N/O	N/O	N/O
	Z.C	20.30 \pm 0.15	10.8 \pm 0.10	9.16 \pm 0.15 [▲]	N/O	N/O

CA: Chloroamphenicol

R.P: *Roscoea pupurea*,

Z.C: *Zingiber chrysanthum*

▲: Minimum inhibitory concentration (MIC)

N/O: Not Observed

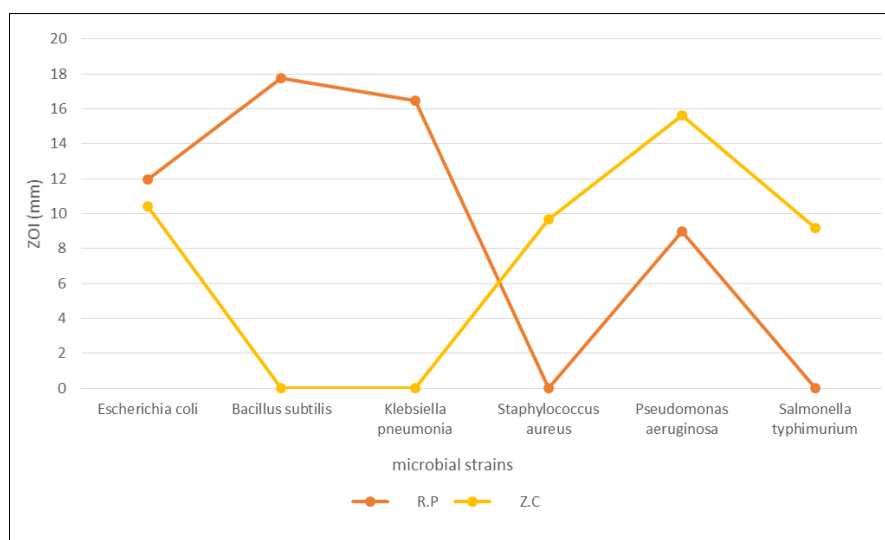


Fig 1: Antibacterial activity of R.P and Z.C leaves Essential oil samples against bacteria at 200 μ L/mL

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Conflict of interest

We declare that there is no conflict of interest.

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