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The antibacterial activity of *Zingiber officinal* and *Ocimum sanctum Linn*. against *Bacillus subtilis* and *E. Coli*

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Abstract:
Objective: *To evaluate the antibacterial activity of Zingiber officinal and Ocimum sanctum Linn against Bacillus can reduce themselves to oval endospores and can remain in this dormant state for years. The endospore of one species from Morocco is reported to have survived being heated to 420 °C, E. coli and other facultative anaerobes constitute about 0.1% of gut microbiota,[11] and fecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination. Which are the most prevalent causes of infections in patients?*
Methods: *Antimicrobial activity of plant extract was evaluated and compared using disc diffusion method. The microorganism was initially cultured on sterilized nutritive agar medium both Escherichia coli and Bacillus subtilis were cultured for 24 h at 30 °C. After incubation, inhibition zone was measured in millimeters and antimicrobial activity of extracts was compared*
Results: *The best antibacterial activity, calculated as minimum inhibitory concentration values, against results showed that ethanolic extract of Zingiber officinale has best antioxidant activity among all the extracts. Ethanolic extract of ginger showed significantly higher efficacy against B. Subtilis while ethanolic extract of Ocimum sanctum Linn has higher antimicrobial efficacy against E. coli. Extracts were also good antioxidant activity. Phytochemical screening of Ocimum sanctum Linn and its extract showed the presence of flavonoids, alkaloids, saponins, quinones, tannins and sterols, while Zingiber officinale rhizomes only showed the presence of flavonoids and sterols/triterpenoids.*
Conclusions: *It can be concluded from the findings of the result that Ocimum tenuiflorum and Zingiber officinale have significant antioxidant and antimicrobial effect and can be used in pharmaceutical, food and cosmceuticals industry.*
Keywords: *Ocimum sanctum Linn leaf; Zingiber officinale rhizomes; herbal extract; antioxidants; antimicrobial effect.*

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I. Introduction

Antibiotic resistance has become a serious and widespread problem in developing countries, both in hospitals and the community, causing high mortality each year [1]. Inappropriate usage of antibiotics is the most influential factor of antibiotic resistance and the global emergence of multi-drug resistant bacteria is increasingly limiting the effectiveness of current drugs and significantly causing treatment failure [2]. Antibiotic resistance results in reduced efficacy of antibacterial drugs, making the treatment of patients difficult, costly, or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality [3]. New therapy classes of antibiotics have become a popular choice to reduce antibiotic resistance. However, antibiotic resistance is difficult to reduce. One strategy to avoid this is by using alternative therapeutic agents from plants that are effective against antibiotic resistant bacteria safe and have low cost. Consequently, one of the objectives of our research group is to investigate the potential antibacterial properties of traditional plants. In the present study, we used two plants that have the potential to be used as antibacterial agents against non-resistant bacteria [4–11], to conduct antibacterial activity assays against resistant bacteria isolated from in patients, such as Bacillus subtilis, and Escherichia coli. The bacterium can be grown and cultured easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. E. coli is a chemoheterotroph whose chemically defined medium must include a source of carbon and energy [15]. Bacillus includes both free-living (no parasitic) species, and two parasitic pathogenic species.

These two Bacillus species are medically significant: B. anthracis causes anthrax; and B. cereus causes food poisoning. Many species of Bacillus can produce copious amounts of enzymes, which are used in various industries, such as in the production of alpha amylase used in starch hydrolysis and the protease subtilisin used in detergents. B. subtilis is a valuable model for bacterial research. Some Bacillus species can synthesize and secrete lipopeptides, in particular surfactins and mycosubtilins [16, 17]

II. Materials and methods

Materials:

The ginger rhizome was purchased from the local market of Greater Noida U.P., India. All other chemicals and reagents used in the study were AR grade and were purchased from CDH, New Delhi. Tulsi plants were collected from a local area of Omicron 1A Greater Noida U.P.India, (28° 35' N, 77° 12' E).

Methods:

Collection of (*Ocimum sanctum Linn*) leaves: Fresh plant leaves of *Ocimum sanctum L.* were collected from a local area of omicron 1A Greater Noida U.P. (28° 35' N, 77° 12' E).The leaves were washed thoroughly under tap water followed by sterile distilled water. Then leaves were dried under shaded condition at room temperature. Sampling of Ocimum sanctum L. was planted in the month of March. Middle aged fresh leaves of Ocimum sanctum L. were plucked during the month of September- October in the morning between 9-10 a.m. (IST) when dew was less and temperature was also not so high.

Collection of ginger stems (*Zingiber officinale*): Fresh ginger stem (Zingiber officinale) were procured from local market. The stem were washed with distilled water and dried in oven at 40 °C, about 5 to 10 min.

Extraction of plant material: Dry ginger was crushed to a coarse powder and extracted with 95 % ethanol by simple maceration process. Solvent was evaporated at room temperature. The residue obtained was dried. As well as the dried leaves material (in 20 gm) of *Ocimum sanctum L.* was extracted with 200 ml volumes of solvents, Ethanol, chloroform and n-butanol, separately at room temperature, in succession about 24 hours. The organic solvent was separated [21].

Bacterial preparation

The Bacillus subtilis and Escherichia coli were taken from isolated specimens who exhibited resistance to some antibiotics in hospitalized patients. They were taken based on ethical clearance approval from the ethical committee in the hospital. Test microorganism was obtained from the Department of Medical Lab Technology, School of Medical and Allied Sciences, Galgotias University, India, and comprised the gram-negative bacteria Escherichia coli and Bacillus subtilis. The microorganism was initially cultured on sterilized nutritive agar medium both Escherichia coli and Bacillus subtilis were cultured for 24 h at 30 °C. The bacteria cell suspensions were homogenized and adjusted to 0.5 McFarland standards (5 × 105 CFU/mL) using spectrophotometer.

Antimicrobial susceptibility assays:

The minimum inhibitory concentrations (MICs) of plant extracts were initially determined using Mueller–Hinton broth micro dilution [12]. MIC determination was performed by a serial dilution technique using 96-well microtiter plates. Plant extract (100 mL) was placed into the well/plate. Then, 100 mL bacterial cell suspensions were placed in each well/plate. Micro plates were incubated for 24 h at 37 C. The lowest concentrations without visible growth completely inhibited the bacteria (MICs). Ciprofloxacin was used as a control and Mueller–Hinton broth as negative control. Tetracycline and vancomycin were used as positive controls for *Zingiber officinale*, while cefotaxime and meropenem were used as positive controls for *Ocimum sanctum Linn*. The assay was repeated twice with three replicates per assay.

Phytochemical screening:

The selected plants which showed the MIC were screened for the presence of different classes of secondary metabolites including alkaloids, flavonoids, alkaloid, saponins, tannins, quinines and sterols/triterpenes using previously described methods [13].

III. Results and Discussion:

Results

Extracts yield

The ethanolic extracts of eight plants and the water extracts of two plants were calculated for the yield. T. indica showed the highest yield in water solvent, which showed that its constituents were relatively polar (Table 1).

The antibacterial activity

The antibacterial activity of the eight extracts was assayed in vitro by the agar micro dilution method against three resistant bacteria. The antibacterial activity against each bacterium was observed to be varied. Table 2 shows that among the eight plants, K. pandurata exhibited the smallest value of MIC against (256 mg/mL) (Figure 1), while had the same activity against. **Phytochemical analysis**

The screening of the phytochemical composition was conducted only for the two dried plants, S. alata and K. pandurata, as well as their extracts that showed the lowest MIC, because they have the potential to be developed as antibacterial agents. The secondary metabolites are shown in Table 3. All tested secondary metabolites were present in the S. alata extract. In K. pandurata, only flavonoids and steroid/triterpenoids were present.

Table 1 The herbal plants extract yield

Plants	Part of plants	Solvent	Yield (%)
Z. officinale	Rhizome	Ethanol (96%)	13.53
O. sanctum	Leaf	Ethanol (96%)	6.19

Table 2 The antibacterial activity of plants extracts towards Bacillus subtilis and Escherichia coli

Plant extracts	MIC (mg/mL)	
	Bacillus subtilis	Escherichia coli
Z. officinale	> 8192	> 8192
O. sanctum	> 8192	> 8192

Fig 1. MIC value of *Ocimum sanctum Linn* leaf; *Zingiber officinale* rhizomes against Bacillus subtilis and Escherichia coli at 256 mg/mL at after 36 Hours

Fig 2. MIC value of *Ocimum sanctum Linn* leaf; *Zingiber officinale* rhizomes against Bacillus subtilis and Escherichia coli at 256 mg/mL after 72 Hours

Table 3 Description Title

Bacteria type	Antimicrobial activity of extract											
	Zone of inhibition (mm)											
	Ginger chloroform (µg/ml)		Ginger ethanol (µg/ml)		Tulsi chloroform (µg/ml)		Tulsi ethanol (µg/ml)		Tulsi n-butanol (µg/ml)		Ciprofloxacin (µg/ml)	
	100	200	100	200	100	200	100	200	100	200	100	200
B. Subtilis	2	3	5	**9**	3	5	4	**6**	2	2	36	38
E. Coli	2	3	4	4	3	5	4	**6**	2	2	32	38

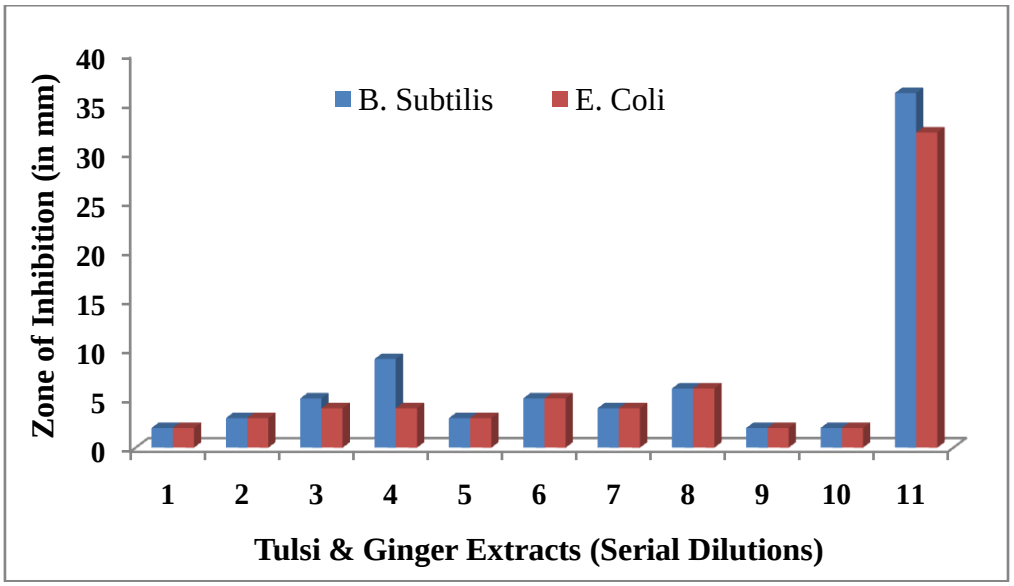

Fig: 3 Tulsi and Ginger extract serial dilution showing the Zone of inhibition (in mm)

Table 4 Results of phytochemical analysis of dried S. alata and its extracts

Secondary metabolites	Z. officinale		O. sanctum	
	Dried Extract	Extract	Dried Extract	Extract
Flavonoid	+	+	+	+
Quinnon	+	+	–	–
Saponin	+	+	–	–
Tannin	+	+	–	–
Alcaloid	+	+	–	–
Steroid/triterpenoid	+	+	+	+

+: Present; −: Absent.

IV. Discussion

The research of antibacterial activity of plant extracts (TE and GE) against *Escherichia coli* and *Bacillus subtilis* with different concentration. The least Zones of inhabitation were displayed by negative control and ciprofloxacin exhibited the wide zone of inhabitation. Ocimum sanctum leaves extracts showed increasing zone of inhibition for new antibacterial agents has become a very important endeavor, especially in recent times, considering the escalating levels of antibiotic resistance among pathogenic bacteria. One of the efforts in this research is focused on the use of medicinal plants, which are widely available resources, less if no side effects, less expensive and have shown antimicrobial properties [14]. Also, the therapeutic properties of medicinal plants are well recognized at a global level, especially for antibiotic development. Thus, the research of alternative and effective medicines from plants against such resistant bacteria has become an important concern all over the world [15]. Antibiotic misuse has been considered as a major cause of antibiotic resistant bacteria. As a result, bacteria become resistant to antibiotics, which are in turn less effective after extended periods of use. Pharmaceutical companies whose efforts are focused on the production and manufacture of antibiotics strive to manufacture new generations of antibiotics which are capable of treating such antibiotic-resistant bacterial strains [16]. The evolution of antibiotic-resistant bacteria has left researchers scrambling to develop new and stronger antibiotics. It is a major pathogen causing nosocomial and community acquired infection throughout the world. It is also one of the leading causes of skin; soft tissue, bone, joint; abscess and normal heart valve infections and its infections in humans have been associated with excess morbidity, mortality and increased length of hospitalization [14]. Tulsi is an enzyme that mediates resistance to third generation cephalosporins (e.g., ceftazidime, cefotaxime and ceftriaxone) and monobactams (aztreonam), but do not affect cephamycins (cefoxitin and cefotetan) or carbapenems (meropenem and imipenem). Escherichia coli, Klebsiella pneumoniae and Klebsiella oxymora are the most common. In this method, susceptibility panels in 96- well microtiter plates contained various concentrations of antimicrobial agents. Then, standardized numbers of bacteria were inoculated into the wells of the microtiter plates and incubated overnight at 35 C. The MIC value was observed as the lowest concentration where no viability was observed in the wells after incubation. Compared with agar-based methods, broth micro dilution can decrease much labor and time [18]. Each of the tested extracts displayed antibacterial activity against Bacillus subtilis and Escherichia coli producing bacteria

and CRE. Furthermore, all extracts showed highly varying MIC values against those resistant bacteria, but the lowest MIC value belonged to S. alata leaf extract (512 mg/mL) and K. pandurata (256 mg/mL) against. A previous study has shown that *Zingiber officinal* is susceptible to Streptococcus pyogenes and Ocimum *sanctum Linn*, and the ethanolic extract of antibacterial activity against methicillin-susceptible aureus and Salmonella typhi at varying values of MIC [7]. Escherichia coli have the ability to grow in the presence of beta-lactams and its derivatives, including cephalosporin and penicillin. This resistance is intrinsic and can be transferred to susceptible strains through horizontal transfer of the mecA gene [16]. The results obtained from the present study provide evidence that ethanolic extracts of tulsi. Pandurata exhibit antibacterial activities against isolated Bacillus subtilis strain, which suggests that they may be clinically useful. Further researches in respect of these findings are needed and are promising. Preliminary phytochemical analyses revealed that only consisted of flavonoids and sterols/triterpenoids while contained flavonoids, alkaloids, saponins, tannins, quinones, and sterols/triterpenoids. These bioactive compounds have been reported to be used by plants for protection against bacterial and are responsible for antimicrobial activity [7, 19, 20]. *Zingiber officinal* and *Ocimum sanctum Linn* were shown to be potentially developed as antibacterial agents, especially for Bacillus subtilis strain. Further in vivo research and discovery of mode action are needed to shed light on their antibacterial effects.

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