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Full Length Research Article

Evaluation of Phytochemicals and Assessment of Antibacterial Activity of *Cinnamomum zeylanicum*, *Syzygium aromaticum* and *Vetiveria zizanioides*

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Abstract

Cinnamomum zeylanicum (Dalchini), *Syzygium aromaticum* (Clove), and *Vetiveria zizanioides* (Khus Khus) are commonly used as spices in the preparation of a variety of foods. The variety of spices includes clove, black pepper, ginger, cardamom, vetiver, cinnamon, turmeric, cumin, chili, and so on. Each of them has characteristic properties; some are added to enhance flavour, some are colouring agents, while others play the role of preservatives and also provide a distinctive aroma. In the phytochemical analysis of crude extracts, the presence of carbohydrates, amino acids, proteins, glycosides, tannins, terpenoids, phenols, alkaloids, and saponins was determined. Clinical isolates of *E. coli, Staphylococcus aureus, Streptococcus faecalis, Salmonella paratyphi B,* and *Pseudomonas aeruginosa were used as test organisms for antibacterial testing, and all test organisms were found sensitive to crude extracts of the spices Cinnamomum zeylanicum, Syzygium aromaticum, and Vetiveria zizanioides. A methanolic* extract of *Cinnamomum zeylanicum was found to be* moderately antibacterial; *the* higher diameter of the zone of inhibition measured was 29 mm against *E. coli*. The methanolic extract of *Syzygium aromaticum* exhibited a 26-mm-diameter inhibition zone against *E. coli*, whereas an ethanolic extract of *Vetiveria zizanioides* exhibited a 23-mm-diameter inhibition zone against *Salmonella paratyphi B*.

***Key words*:** Phytochemicals, Antibacterial activity, *Cinnamomum zeylanicum, Syzygium aromaticum, Vetiveria zizanioides*

Spices in foods are used for their properties to augment aroma, flavour, and their varied roles [1-2]. Spices and phytochemicals of the varieties of plants have proven their health benefits as well as their wide prospect in medicine and are used against preventing and treating most infections and diseases [3-6]. Spices have long been used as traditional medicines [2], [7]. Spices are obtained from a variety of sources, including phytoplankton and their parts such as leaves, fruits, seeds, and the bark of trees, as well as metabolic products etc. [4]. Each spice's phytochemicals, or secondary compounds, have a particular flavour and aroma, and they aid plants by protecting them from pathogenic agents such as bacteria, moulds, viruses, pests, and parasites [8-9]. Piperine, Eugenol, Cinnamaldehyde, Lignans, Linalool, Thymoquinones, Curcumin, and Allicin are bioactive compounds obtained from spices and condiments such as Black Pepper, Cloves, and Cinnamon [10-13]. The principal components of these spices are tannins, terpenoids, alkaloids, and flavonoids, as well as lectins, saponins, phenolic compounds, and so on [14-15]. Most spices and their ingredients have important pharmacological properties such as anti-growth, antiparasitic, analgesic, sedative, antiseptic, anti-diabetic, anti-malarial, blood sugar lowering, and cholesterol-lowering agents [9], [16-21]. Some of them are regarded as potent antioxidants and nurturers to the cells and tissues. The most important characteristic of spices, they do not contain any of those toxic substances for humans [11]. The present research investigation was conducted to evaluate the phytometabolites found in spices such as clove, dalchini, and khus khus and their bactericidal capabilities against clinical isolates for their unseen potential as antimicrobials.

MATERIALS AND METHODS

Collection of plant material

The stem bark of *Cinnamomum zeylanicum* (Dalchini), buds of *Syzygium aromaticum* (Clove), and seeds of *Vetiveria zizanioides* (Khus Khus) were purchased from local markets of Nashik district, Maharashtra, India, and brought to the laboratory. These were cleaned by washing them with distilled

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water three times to remove impurities, dust, and other particulate matter. The above substances were shade dried in the laboratory at room temperature for 5 days. The dried parts were ground to make a fine powder. Powdered samples were kept in airtight containers until the investigation.

Extraction of plants material

The plants material extracts were prepared as follows:

1. *Preparation of aqueous extracts for volatile components*

Dried clove buds were ground into a fine powder and dissolved in sterile distilled water (10% w/v) separately in an Erlenmeyer flask to make an aqueous extract. The flasks were incubated at room temperature on an orbital shaker for 48 hours for the extraction process. A widely accepted method, hydro distillation with some modifications [21], was used for the extraction of the bioactive profile from clove powder. The extract was then evaporated using a distillation unit with a round bottom flask at 40°C. The lower temperature is purposefully used for the probable retention of volatile bioactive compounds. The cooled vapours were condensed and collected in a closed container for the imprisonment of volatile components. After cooling, the final extract was stored in an airtight container and kept at 4°C. Similarly, the aqueous extracts for volatile components of *Cinnamomum zeylanicum* stem bark powder and *Vetiveria zizanioides* were prepared.

2. *Preparation of aqueous extracts*

Fine powder, each plant material extracted with sterile distilled water. 20 gm of powder was soaked in 200 ml of sterile distilled water [22]. The extraction process was carried out for 72 hours at room temperature on an orbital shaker. The extracts were dried under a reduced pressure at 40°C using a rotary vacuum evaporator. The dried extracts were stored in sterile bottles at 4°C until further use.

3. *Extraction in organic solvents*

Each sample was ground into a fine powder and extracted with organic solvents (ethanol and methanol). Solvent extraction was performed as available in reported studies with some modifications of 20 gm of powder, each selected plant material was soaked in 200 mL of each solvent [3], [23-25]. The extraction process was carried out for 72 hours at room temperature on an orbital shaker. The extracts were dried under a reduced pressure at 40°C using a rotary vacuum evaporator. The dried extracts were stored in sterile bottles at 4°C until further use.

Preliminary phytochemical analysis of crude extracts

The phytochemical analysis of all crude extracts was conducted for the detection of carbohydrates, amino acids, proteins, glycosides, tannins, terpenoids, phenols, alkaloids, and saponins, as adopted from previous reports [14], [22], [26-28].

Preparation of plant extracts for antibacterial activity

Stock solutions of *Cinnamomum zeylanicum, Syzygium aromaticum, and Vetiveria zizanioides extracts* were prepared by mixing them with sterile distilled water. 1 mg of each extract was mixed with 10 mL of sterile distilled water and designated as stock solution. Two concentrations, i.e., 50 and 100 µg/mL, were prepared with the sterile distilled water [29]. These concentrations were used for the evaluation of an antibacterial activity.

Antibacterial activity by diffusion assay

The agar diffusion method has been used for the evaluation of the antibacterial potency of crude extracts. The two dilutions of the extracts were prepared, i.e., 50 and 100 µg/mL using sterile distilled water. In this test, extracts were impregnated on paper discs of size 6 mm and used for assaying [25], [30]. The disc diffusion assay has performed the testing of the antibacterial potential of each extract. Clinical isolates previously isolated, such as *E. coli, Staphylococcus aureus, Streptococcus faecalis, Salmonella paratyphi B,* and *Pseudomonas aeruginosa* were used as test organisms for the diffusion assay. Mueller Hinton agar containing Beef extract: 2.0 g/L, Acid hydrolysate of casein: 17.5 g/L, Starch: 1.5 g/L, Agar-agar: 17.0 g/L, and pH: 7.3±0.2 was used as a basal and seed medium for the antibacterial assay. Sterile basal agar plates and seed agar butts were prepared. After the pouring and solidification of seed agar, sterile filter paper discs were impregnated with the respective crude extract dilution and placed on the surface of the medium in each plate. The discs impregnated with sterile distilled water were also placed on an agar surface as a negative control. Standard antibiotic streptomycin was used in the antimicrobial assay study as a positive control. Then all plates were incubated at 37°C for 24 hours. After incubation, the diameters of the zones of inhibition obtained were measured in mm and recorded.

RESULTS AND DISCUSSION

Qualitative detection of phytochemicals such as carbohydrates, amino acids, proteins, glycosides, tannins, terpenoids, phenols, alkaloids, flavonoids, and saponins in *Cinnamomum zeylanicum, Syzygium aromaticum, and Vetiveria zizanioides* crude extracts was conducted. Phytochemicals or the presence of metabolites in plants suggest their numerous activities, including protection against various pathogens, may be regarded as safeguards, as well as their pharmacological properties [7], [16], [31-32]. The phytochemicals analysed in all three crude extracts for volatile components are summarised in (Table 1).

Table 1 Phytochemical detected in crude extracts for volatile components

Phytochemicals	Aqueous extracts for volatile components		
	Cinnamomum zeylanicum	*Syzygium aromaticum*	*Vetiveria zizanioides*
Carbohydrates	-	-	-
Amino acids and peptides	-	-	-
Glycosides	-	-	-
Tannins	+	-	+
Terpenoids	+	+	+
Phenols	+	+	+
Saponins	-	-	+
Alkaloids	+	+	-

Present: +; Absent: -

Phytochemicals detected in crude aqueous, ethanol, and methanol extracts of *Cinnamomum zeylanicum, Syzygium aromaticum,* and *Vetiveria zizanioides* are depicted in **(**Tables 2-4**)**. It was reported that phytochemicals like alkaloids, flavonoids, tannins, saponins, steroids, glycosides, and terpenoids in the extract of clove, dalchini, and khus khus, etc. were majorly responsible for their vital role as pharmacological constituents for the preparation of various drugs [2]. In all three extracts of *Cinnamomum zeylanicum, Syzygium aromaticum,* and *Vetiveria zizanioides,* the presence of carbohydrates, amino acids, proteins, glycosides, tannins, terpenoids, phenols,

alkaloids, flavonoids, and saponins was detected. In a previous report, it was mentioned that *Vetiveria zizanioides* extracts evaluated the presence of glycosides, tannins, terpenoids, alkaloids, flavonoids, and saponins [33].

Table 2 Phytochemicals detected in crude extracts of *Cinnamomum zeylanicum*

Phytochemicals	*Cinnamomum zeylanicum* extracts		
	Aqueous	Ethanol	Methanol
Carbohydrates	+	+	+
Amino acids and peptides	-	-	-
Glycosides	-	+	+
Tannins	-	+	+
Terpenoids	+	+	+
Phenols	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Flavonoids	-	+	-

Present: +; Absent: -

Table 3 Phytochemicals detected in crude extracts *Syzygium aromaticum*

Phytochemicals	*Syzygium aromaticum* extracts		
	Aqueous	Ethanol	Methanol
Carbohydrates	+	+	+
Amino acids and peptides	-	-	+
Glycosides	+	+	+
Tannins	-	+	+
Terpenoids	-	+	+
Phenols	+	+	+
Saponins	-	+	+
Alkaloids	-	+	+
Flavonoids	-	+	+

Present: +; Absent: -

Antibacterial assay

The antibacterial disc diffusion assay was performed using clinical isolates of *E. coli, Staphylococcus aureus, Streptococcus faecalis, Salmonella paratyphi B,* and *Pseudomonas aeruginosa* as test organisms. *Cinnamomum zeylanicum* oil is reported to have antibacterial as well as antifungal properties and used for a variety of purposes in medicine [34]. Aqueous extract showed less antimicrobial activity as compared to ethanol and methanol extracts. The 100 µg/mL concentration of all extracts found a higher zone of inhibition against all tested organisms (Tables 5-7). Test organisms *E. coli* and *Streptococcus faecalis* were found susceptible to both concentrations of extracts of all three spices. All extracts exhibited antibacterial activity against the test organisms in comparison with the control. In the present study, the antibacterial activity of the three extracts was evaluated against both Gram-positive and Gram-negative organisms. *Cinnamomum zeylanicum* extract exhibited a higher diameter of the inhibition zone, i.e., 29 and 26 mm against *Escherichia coli*, followed by an extract of *Syzygium aromaticum* with 26 and 25 mm.

Table 4 Phytochemicals detected in crude extracts *Vetiveria zizanioides*

Phytochemicals	*Vetiveria zizanioides* extracts		
	Aqueous	Ethanol	Methanol
Carbohydrates	+	+	+
Amino acids and peptides	+	+	+
Glycosides	+	+	+
Tannins	-	-	+
Terpenoids	+	+	+
Phenols	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Flavonoids	-	+	+

Present: +; Absent: -

Plants that are rich in tannins have antibacterial potential due to their basic character, which may allow them to react with enzymes and proteins and weaken or pervasion of the plasma membrane, thereby being regarded as bactericidal by altering metabolism [35]. The zones of inhibition were obtained against clinical isolates are depicted in (Table 5-7), which suggests that plant extracts may contain antibacterial components, some of which were analysed as above.

Table 5 The antibacterial effect exhibited by extracts of *Cinnamomum zeylanicum*

Test organism	Concentration of extract, µg/ml	Diameter of Zone of Inhibition, mm			Streptomycin, 100 µg/ml
		Aqueous	Ethanol	Methanol	
Staphylococcus aureus	50	9	12	12	18
	100	8	14	17	
Streptococcus faecalis	50	6	13	12	16
	100	8	16	13	
E. coli	50	18	21	26	32
	100	23	24	29	
Salmonella paratyphi B	50	8	16	14	29
	100	11	14	19	
Pseudomonas aeruginosa	50	7	13	11	21
	100	9	13	15	

Cinnamomum zeylanicum proved its antimicrobial, anticancer, anti-inflammatory, and antidiabetic properties [36]. *Cinnamomum zeylanicum* extracts exhibited higher antibacterial activity against *Escherichia coli* at both concentrations of 50 and 100 µg/mL. All test organisms were found to be sensitive to the *Cinnamomum zeylanicum* extracts. Bark oil extracts of *Cinnamomum zeylanicum* have been reported as antimicrobials against pathogenic organisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Peptococcus*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *S. mutans* [1], [24-25], [37-38]. Clove extract has been evaluated as highly inhibitory against *Botrytis cinerea* in laboratory investigation [39]. Ethanol and methanol extracts of

Syzygium aromaticum exerted an antibacterial effect on all test organisms, while *Pseudomonas aeruginosa was* found to be the moderately sensitive. Previously, it was reported that *Syzygium aromaticum* organic extracts were found to be antibacterial against Gram-positive as well as Gram-negative test organisms [1-2], [16]. Oils from the bark extract of *Cinnamomum zeylanicum have been* assessed as antimicrobial and antifungal [41]. *Vetiveria zizanoides* extracts were found antibacterial against bacteria such as *Pseudomonas aeruginosa*, *Salmonella paratyphi*, and *Stapylococcus aureus* [42]. It was reported that the *Vetiveria zizanioides* used in various preparations such as juice or sharbat, cosmetics, and soaps, shampoos and perfumes, as well as in many skin disorders, and other causes, which demonstrates its usefulness for society [43]. An ethanolic extract of *Vetiveria zizanioides previously exerted an* antibacterial effect against *Mycobacterium tuberculosis* [44]. *Vetiveria zizanioides* was exhibited an effectiveness against dermatophytes and *C. albicans* [45].

Table 6 The antibacterial effect exhibited by extracts of *Syzygium aromaticum*

Test organism	Concentration of extract, µg/ml	Diameter of Zone of Inhibition, mm			Streptomycin, 100 µg/ml
		Aqueous	Ethanol	Methanol	
Staphylococcus aureus	50	7	11	14	18
	100	8	13	17	
Streptococcus faecalis	50	5	14	13	23
	100	10	17	19	
E. coli	50	13	21	22	28
	100	19	25	26	
Salmonella paratyphi B	50	9	12	10	22
	100	10	13	12	
Pseudomonas aeruginosa	50	6	18	21	26
	100	7	21	23	

Table 7 The antibacterial effect exhibited by extracts of *Vetiveria zizanioides*

Test organism	Concentration of extract, µg/ml	Diameter of Zone of Inhibition, mm			Streptomycin, 100 µg/ml
		Aqueous	Ethanol	Methanol	
Staphylococcus aureus	50	-	8	8	18
	100	7	9	10	
Streptococcus faecalis	50	12	10	14	22
	100	10	12	16	
E. coli	50	18	18	19	28
	100	19	19	23	
Salmonella paratyphi B	50	12	19	14	24
	100	14	23	15	
Pseudomonas aeruginosa	50	10	11	16	23
	100	10	14	12	

CONCLUSION

The efficient antibacterial potential and phytochemical content of each spice were assessed as part of the present study. All three of these spices, evidently having potential as antibacterial agents, will may help in the preparation of novel plant-originated preparations and drugs in combinations.

Conflict of interest: *The authors have no conflicts of interest regarding this investigation*.

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