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Preliminary phytochemical and antimicrobial screening of *Syzygium aromaticum, Elettaria cardamomum* and *Piper nigrum* extracts

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Abstract
Cardamom (*Elettaria cardamomum)*, pepper (*Piper nigrum*) and clove (*Syzygium aromaticum)* are some commonly used spices in households worldwide. Moreover, they have also been utilized as domestic medicine against various infections, a quality attributable to the existence of certain chemical moieties in them. In view of this, the current study was carried out to analyse the phytochemical and antimicrobial nature of these three spices in various extracts. Following a 6 stage extraction process, the extracts collected from these spices were exposed to a number of preliminary biochemical and phytochemical tests. The presence of tannins, saponins, terpenoids, flavonoids and alkaloids were revealed in some of the extracts used in the current investigation. The antimicrobial activity tested against two bacterial species, *Staphylococcus aureus* and a *Bacillus* species failed to inhibit the growth of these microbes in culture. However, from the aggregation of all our results, we arrived at a conclusion that the aforementioned spices definitely possess potential therapeutic properties of varying degrees owing to the presence of several phyto-active compounds in them.

Keywords: Phytochemical analysis, *Syzygium aromaticum, Elettaria cardamomum, Piper nigrum.*

1. Introduction
For aeons now, spices have been an irreplaceable part of cuisines all over the world and for the last couple of centuries, beginning from the founders of Ayurveda, these very spices and their constituents have been tested for their medicinal properties against several ailments. Cardamom, clove and pepper are some of the commonly used spices in the every Indian household for culinary purposes as well as in the treatment of various infections [1].

Elettaria cardamomum (cardamom) is a perennial shrub of the family Zingiberaceae. Its seeds are used as spice. It is called as the queen of spices [2]. It stands third in the list of expensive spices throughout the world preceded by saffron and vanilla. The medicinal use of cardamom is also mentioned in Unani and Ayurveda systems [3]. Apart from its use in cooking, cardamom also possesses a wide range of therapeutic features such as antifungal, antibacterial, antiviral, diuretic and carminative properties [4]. It is also employed to fight against cardiac diseases, renal problems, anorexia, asthma and bronchitis. Furthermore, it demonstrates antioxidant, antiplatelet aggregation, anti-hypersensitive and anti-cancerous attributes [5].

The spice clove is the withered flower bud of *Syzygium aromaticum* tree, which belongs to the family Myrtaceae. In Ayurveda and Chinese medicine, it is utilized for the treatment of various infections and diseases [6]. Eugenol is the major component of the clove's essential oils (70-90%). It is responsible for the characteristic, pungent odor of clove and is also accountable for the antimicrobial activity of the spice [7]. Likewise; it exhibits other characteristics such as antifungal, antibacterial, anti-carminative, antiseptic, anti-emetic, anti-oxidant, analgesic and insecticidal properties alongside as a flavouring agent in culinary [8].

Piper nigrum or Black pepper is a climbing woody stemmed plant which belongs to the family Piperaceae. It is native to India [9]. It is grown for its fruit, which is then dried to be used as a spice. This family consists of 12 genera and 1400 species; among these, the species belonging to the genus *Piper*, grown in South India are found to have high economic as well as medicinal importance [10]. *Piper nigrum* is also believed to be the king of spices because of its pungent eminence [11]. The active component in *Piper nigrum* is the alkaloid piperine. It promotes digestion by stimulating the secretion of the digestive enzymes from the pancreas. In addition to this, it also obstructs in vitro oxidation by extinguishing free radicals along with the inhibition of enzymatic biotransformation of drugs in the liver [12]. Apart from these, pepper is used to relieve pain, flu, cold, chills, rheumatism, fever and muscular aches [13]. It also expresses antimicrobial activity [14] as well as anti-mutagenic activity [15].

Taking into consideration all the above mentioned qualities of the three spices namely, *Syzygium aromaticum*, *Elettaria cardamomum* and *Piper nigrum*, the present study was carried out to assess their biochemical and phytochemical nature along with their the antimicrobial activities.

2. Materials and Methods

All the three spices were collected from a local market and dried in an oven for 24hrs. The dried spices were then powdered using an electric mixer. For the initial extraction, 5g of each of the powdered spice were added to petroleum ether in separate conical flasks in the ratio of 1:4 and placed in a mechanical shaker for 24hrs. The solution obtained was filtered and the filtrate was stored for the upcoming experimentations. The next solvent was added to the residue for further extraction. The order of addition of solvents was as follows: petroleum ether (pet.ether), benzene, chloroform, acetone, alcohol and finally water. The filtrates obtained from the above extractions were further subjected to phytochemical, biochemical and anti-microbial analyses.

2.1 Phytochemical analyses

Various extracts of the three spice samples were tested for Tannins, Saponins, Cardiac glycosides, Flavonoids and Alkaloids.

2.1.1. Test for Tannins [16]

2ml of each extract was added separately to 4ml of water and a few drops of 0.1% $FeCl_3$ were added to the extracts to form a blue coloured solution.

2.1.2. Test for Terpenoids [17]

Salkowaski test was used. 5ml of various extracts was taken in different test tubes. To each of them 2ml of chloroform was added, along with it 3ml of concentrated sulphuric acid was added slowly to form a layer.

2.1.3. Test for Saponins [18]

1ml of the extract was added to 20ml of distilled water in a test tube and was shaken vigorously for 15 minutes. Formation of the foamy layer indicated the presence of saponins.

2.1.4. Test for Cardiac Glycosides [17]

Keller-Killani test was used. 2ml of glacial acetic acid was added to 5ml of the extracts containing a drop of $FeCl_3$ solution followed by the addition of 1ml of concentrated sulphuric acid.

2.1.5. Test for Flavonoids [17]

a. Aqueous filtrate along with concentrated sulphuric acid was taken in a test tube; 5ml of dilute ammonia solution was added.
b. To all the other filtrates few drops of 1% aluminium solution was added.

The presence of flavonoids was indicated by the development of yellow colour.

2.1.6. Test for Alkaloids [13]

1ml of the extracts and 10ml of acid alcohol were boiled and filtered. To 5ml of the filtrates, 2ml of dilute NH_3 and 5ml of $CHCl_3$ were added and shaken to extract the alkaloidal base. The chloroform layer was extracted with 10ml of acetic acid. This was divided into two portions. To one portion Mayer`s reagent was added and to the other Dragendorff`s reagent was added**.** Positive test with Mayer's reagent gives a cream coloured solution whereas Dragendroff's reagent gives orange colour.

2.2. Biochemical analyses

Biochemical analysis was done by conducting a panel of tests for different category of biomolecules. The tests carried out were Molisch's test for the detection of carbohydrates and Seliwanoff's test to distinguish between ketose and aldose. While Benedict's test was employed for testing the presence of reducing sugars, Barfoed's test was done for reducing monosaccharides. Osazone Formation test was used for the confirmation of monosaccharides. For the detection of proteins, Millon's test was carried out followed by Biuret test for detecting the peptide bonds. Ninhydrin test was applied for identifying the presence amino group whereas Xanthoproteic test was used for ascertaining the occurrence of aromatic amino acids. Aldehyde and Sakaguchi tests were conducted for detecting Arginine present in the protein and Pauly's test for the detection of Tryptophan and Histidine. Sulphur test was employed for checking the existence of sulphur containing amino acids.

2.3. Antimicrobial activity

The anti-bacterial activities of the aqueous extracts of cardamom, clove and pepper were checked against two different genera of bacteria S*taphylococcus aureus a*nd *Bacillus* sps. Discs were prepared using Whatmann filter paper no. 2. These discs were separately loaded with the aqueous extracts of the 3 spices and placed on separate cultures of the aforesaid bacteria and incubated for 24hrs.

3. Results and Discussion

3.1. Phytochemical analyses

The presence of active phytochemical constituents such as tannins, saponins, terpenoids, flavonoids and others are the principal reasons for a plant to exhibit medicinal activity. Flavonoids are well known antioxidants [9] and cardiac glycosides exhibit positive as well as negative effects on the heart. Saponins act as anti-feedants in plants [19] and likewise tannins shield the plants from phytophagous insects and herbivores [20]. Terpenoids provide plants their phytoalexin property. All these phyto-active compounds in conjunction elevate the therapeutic, commercial and economic values of such plants. In our analysis, tannins were tested to be positive in alcoholic and aqueous extract in clove as well as in pepper, but all the extracts of cardamom conveyed negative results. While saponins and cardiac glycosides were absent in all the extracts of cardamom and pepper, alcoholic and aqueous extracts of clove tested positive for the same. Similar to our findings another study revealed that glycosides and saponins were not found in the extracts of pepper [10].

Flavonoids were detected in most of the extracts except in case of benzene and acetone extracts of pepper; Pet.ether, chloroform and alcoholic extracts of clove; Pet.ether and alcoholic extracts of cardamom. In contrast to our results an

analysis reported that flavonoids were absent in various extracts of cardamom [21]. Likewise another report also states that clove oil and cardamom oil extracts display negative results for flavonoids [22].

A good number of extracts revealed positive results for terpenoids as shown in Tables 1, 2 and 3. In the test for alkaloids, only alcoholic and aqueous extracts of pepper and aqueous extract of clove presented with a positive outcome. A research paper stated that the presence of alkaloids was definite in hexane, dichloromethane, ethanolic and aqueous extracts of pepper [1]. Similarly, another investigation reported the incidence of alkaloids in dichloromethane extracts of clove and cardamom oil [22] which is in contrast to our findings, where alkaloids tested positive only in aqueous extract of clove and in alcoholic and aqueous extracts of pepper. The diversity in these results may be as a consequence of variations in climate, nutrition, plant chemotype, harvesting time and other factors [5].

The results for all the phytochemical screenings are displayed in Tables 1, 2, 3 for cardamom, clove and pepper respectively.

Table 1: Phytochemical Analyses of Cardamom

	Pet.ether extract	Benzene Extract	Chloroform extract	Acetone extract	Alcohol Extract	Water extract
Tannins	-	-	-	-	-	-
Saponins	-	-	-	-	-	-
Terpenoids	-	-	-	-	-	-
Cardiac Glycosides	-	-	-	-	-	-
Flavonoids	-	+	+	+	-	+
Alkaloids						
1.Mayers reagent	-	-	-	-	-	-
2.Dragondrof's reagent	-	-	-	-	-	-

Table 2: Phytochemical Analyses of Clove

	Pet.Ether extract	Benzene Extract	Chloroform extract	Acetone extract	Alcohol extract	Water extract
Tannins	-	-	-	-	+	+
Saponins	-	-	-	-	+	+
Terpenoids	+	-	+	-	+	+
Cardiac Glycosides	-	-	-	-	-	-
Flavonoids	-	+	-	+	-	+
Alkaloids						
1.Mayers reagent	-	-	-	-	-	-
2.Dragendroff's reagent	-	-	-	-	-	+

Table 3: Phytochemical Analyses of Pepper

	Pet.Ether extract	Benzene Extract	Chloroform extract	Acetone extract	Alcohol extract	Water extract
Tannins	-	-	-	-	+	+
Saponins	-	-	-	-	-	-
Terpenoids	+	-	+	-	-	+
Cardiac Glycosides	-	-	-	-	-	-
Flavonoids	+	-	+	-	+	+
Alkaloids						
1.Mayers reagent	-	-	-	-	+	+
2.Dragendroff's reagent	-	-	-	-	-	-

3.2. Biochemical analyses

The test for the presence of carbohydrates i.e. Molisch's test exhibited positive results for all three spices (Table 4), on the contrary the outcome for all other tests for carbohydrates were negative. Supporting our findings a study reported that extracts of clove and cardamom oils tested positive for Molisch's and negative for Fehling's test [22]. The presence of proteins in the extracts were proved by Xanthoproteic test for clove; Millon's, Xanthoproteic, Aldehyde and Sulphur test for pepper extracts; Xanthoproteic and Sulphur test for cardamom extracts, but the outcome for the other tests except Biuret test were negative (Table 5).

Table 4: Test for Carbohydrates

Test	**Results**		
	Cardamom	**Clove**	**Pepper**
Molisch's Test	+	+	+
Fehling's Test	-	-	-
Benedicts Test	-	-	-
Barfoed's Test	-	-	-
Seliwanoff's Test	-	-	-

Table 5: Test for Proteins

Test	Results		
	Cardamom	**Clove**	**Pepper**
Biuret Test	+	+	+
Ninhydrin Test	-	-	-
Millon's Test	-	-	+
Xanthoproteic Test	+	+	+
Aldehyde Test	-	-	+
Sakaguchi Test	-	-	-
Sulphur Test	+	-	+
Paulys Test	-	-	-

3.3. Antimicrobial activity

The aqueous extracts of all the three spices did not show any antimicrobial activities or zones of inhibition against *Staphylococcus aureus* and *Bacillus* in the disc diffusion method. Supporting our results a report revealed that *Piper nigrum* shows no zone of inhibition against *Bacillus subtallis*[13]. Likewise, another study also revealed that aqueous extract of pepper shows less activity or a very small zone of inhibition for *Bacillus* species when compared to other organisms [10]. A recent article declared that methanolic extract of clove is effective in the inhibition of both gram positive and gram negative bacteria [23]. It was found that hot and cold aqueous extracts of clove inhibits the growth of *S. mutans* to a great degree when compared to ethanolic and methanolic extracts of clove. Contrastingly, another species *S. aureus* was completely resistant to aqueous extracts, here only methanolic and ethanolic extracts of clove showed inhibition [24]. This finding supports our study where aqueous extract of clove showed no zone of inhibition for *S. aureus*. Table 6 depicts the results of the assessment of antimicrobial activities of the aqueous extracts of the 3 different spices carried out in the current study. The microbes showed resistance to all the three spices used. This may be either due of the usage of aqueous extract or because of the usage of crude extracts of the spices instead of pure extracts.

Table 6: Antimicrobial Analysis

Microbes	Solvent	Results		
		Cardamom	Clove	Pepper
Bacillus sps.	water	Resistant	Resistant	Resistant
Staphylococcus aureus	water	Resistant	Resistant	Resistant

4. Conclusion

In our study we detected the presence of only flavonoids in the extracts of cardamom whereas, in the extracts of clove only cardiac glycosides were not identified while the rest of phytochemicals were present. Pepper extracts responded positively for most of the phytochemical tests except for saponins and cardiac glycosides. All the afore-mentioned phytochemicals have a key role in the medicinal activities exhibited by the plant. Thus the presence or absence of any one such phytochemical has a huge impact on its therapeutic properties (antibacterial, anti-oxidant, analgesic, insecticidal etc.). Although our investigation revealed microbial resistance to the aqueous extracts of the three spices, antimicrobial activities of these spices are well documented. Further studies can be conducted using different extracts of the spices or by using a pure extract of a particular phyto-constituent for detailed results on its impact as a therapeutic agent.

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