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Investigation on phytochemical screening, thin layer chromatographic profiling and antibacterial potential of ten widely used spices in Bangladesh

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Abstract

From ancient civilization, spices and herbs have played an important role in the lifestyle of people around the world. As Spices are being used from generation to generation as food additives and traditional medicines, these have been used as study materials to investigate its different bioactive properties and phytochemicals for their potential usage in the pharmaceutical industries. Phytochemicals are the compounds produced by plants, but not directly used for the growth and development rather engaged in protection against environmental and pathogenic stress. Plant materials were collected and water extraction method was applied for the preparation of extracts. After extraction all of the experiments were done according to standard protocols. Our study focused on the screening of the presence of valuable phytochemicals in ten widely used spices in Bangladesh. Among phytochemical screened tannin, glycosides and phenolic compounds are most abundant in all spices. The highest phenolic content was found in *Syzygium aromaticum* (67.77 µg GAE/ml of extract) and the lowest phenolic content was found in *Coriandrum sativum* (4.22 µg GAE/ml of extract). Among the spices, *Myristica fragrans* contained the highest tannin content (78.75 µg TAE/ml Extract) and *Elettaria cardamomum* contained the lowest tannin content (61 µg TAE/ml Extract). The study also employed the antibacterial activity of the spices extracts. The extracts showed the strongest antibacterial activity against the gram-positive bacteria when compared to the gram-negative bacteria. The highest zone of inhibition for *Escherichia coli*, *Bacillus subtilis,* and *Salmonella typhi* were found 8.31 mm, 10.4 mm and 8.61 mm in *Nigella sativa, Elettaria cardamomum and Amomum subulatum* respectively, and the lowest zone of inhibition for *Escherichia coli*, *Bacillus subtilis,* and *Salmonella typhi* were found 5.18 mm, 5.36 mm and 5.86 mm in *Coriandrum sativum, Coriandrum sativum and Syzygium aromaticum* respectively. Thin layer chromatographic (TLC) analysis was also done to check the presence of phyto-constituents. Our study revealed several spots (From 1 to 3) for different samples and their retention factors (R_f) were calculated. It is concluded that, results of these investigations had provided valuable information on further discovery of noble drugs.

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Introduction

Spices are the dried parts of plants, whole plants or the plant products which are aromatic or pungent, and are used to season food. These spices are used in the food to increase taste and color. Most spices are derived from different parts of the plants including fruits, seed, bark, flower, and roots. These are not considered as food as they lack nutritious value (Nahar *et al*., 2017). Though Spices lack of nutritional values, these contain a huge amount of the bioactive compounds. Such as tannins, coumarin, phenolic compound, essential oil, saponin etc. Essential oils are being found from the spices used in various industries like the perfume industry, aftershave lotion and insect repellants (Sachan *et al.,* 2018).The science behind the usage of spices as ethnomedicine is the presence of phytochemicals.

Secondary metabolites or phytochemicals are the different bioactive compounds that are being produced by the plants itself and are not used directly in growth and development of the plants. These bioactive compounds are being used as the protector of plants against both external stress and pathogenic attack (Chew *et al.,* 2011). These phytochemicals have various health benefits including the antimicrobial, anti-inflammatory, anti-diabetic, anti-hyperglycemic and also anti-cancer properties. There are a huge number of phytochemicals present in various parts of different plants. Among them, alkaloids, tannin, saponin, glycoside, flavonoids, phenol, coumarin and reducing sugar are most important (Kumar *et al.,* 2015).

Coriandrum sativum(Coriander)is a spice and herb. This is mostly cultivated in the winter and summer seasons considering as an annual crop. Its seeds and leaves show antimicrobial and phytochemical properties and have a very strong effect against gram-negative bacteria, such as *E. coli* (Patel and Vakilwala, 2016). *Amomum subulatum* (Black cardamom) is commonly known as the large cardamom and a perennial herb. Its fruits are prescribed to treat vomiting, abdominal pain, indigestion, gastric troubles, and liver complication, etc. Phytochemical analysis of *A. subulatum* confirms the presence of many valuable phytoconstituents (Puttanna *et al.,* 2016). *Elettaria cardamomum* (True cardamom)is one of the important family members of Zingiberaceae. It has been widely used spices in Bangladesh, India, and Sri Lanka. Seeds of the cardamom have a stringent aroma and are used in aromatherapy to stimulate energy and its oil is highly effective as an antioxidant (Kumari and Rao, 2015). *Cuminum cyminum* (Cumin) is the flowering plant belonging to the Apiaceae family. Phytochemical screening revealed that cumin contained alkaloid, flavonoid, glycosides, tannin, protein, and steroid and also exerted antimicrobial, anticancer, anti-inflammatory and antioxidant activities (Al-snafi, 2016). *Nigella sativa* (Black cumin) is a spice plant commonly known as black cumin or black seed. Traditionally its seed and oil are being used to treat various diseases such as cough, jaundice, fever, paralysis, etc. Black cumin contained different phytochemicals such as flavonoids, alkaloids, fatty acids, anthocyanin and essential oil (Desai *et al.,* 2015).

Piper nigrum (Black pepper) is a flowering plant cultivated for its fruits. These are generally dried and used as spices. It is widely used for the treatment of cough, bronchitis, asthma, etc. Various studies show that it contains many important phytochemicals, such as tannin, alkaloid, phenol, flavonoids etc. which are highly capable of producing definite action on disease-causing microorganisms (Ganesh *et al.,* 2014). *Myristica fragrans* (Nutmeg) is an important spice used all over the world and native in India. Phytochemical studies revealed that there were 12 important types of phytochemicals found to have tremendous physiological effects (Francis *et al.,* 2019).

Laurus nobilis (Bay leaf)is an industrial plant used in food, cosmetics, and drug. Due to the presence of various important types of phytochemical, various biological and pharmacological properties have been reported such as: antibacterial, antifungal and antioxidant (Chahal *et al.,* 2017). *Syzygium*

aromaticum (clove) is one of the most valuable traditional herbs having a broad spectrum of biological activity. Major phytochemical were found in the clove oil which exerts some pharmacological properties such as insecticides, antibacterial, antifungal and anti-carcinogenic (Mittall *et al.,* 2014). *Cinnamomum verum* (Cinnamon), an evergreen tropical tree belonging to the Lauraceae family has a tremendous effect on the treatment of type 2 diabetes and insulin resistance (Vakilwala *et al.,* 2017).

Many plants have antimicrobial properties due to the presence of secondary metabolites or phytochemical. The Essential oil or tannin is mostly responsible for the antimicrobial activity of the plants (Selvamohan *et al.,* 2012).The incidence of the multiple antibiotic resistances in human pathogenic microorganisms is increasing day by day at an alarming rate due to the indiscriminate use of antimicrobial drugs especially for the treatment of infectious diseases. This alarming phenomenon has forced scientists to find new antimicrobial substances from various sources, like different plant varieties (Soma *et al.,* 2018).

The thin layer chromatographic profiling is a basic chromatographic technique, which aims at separating the non-volatile compounds. Thin layer chromatographic analysis is done on a sheet of aluminum plate or glass which is generally coated with various absorbent materials such as silica gel, cellulosic materials, and aluminum oxide. The samples are put as a drop by the use of capillary tube and an appropriate solvent is used to separate the phytoconstituents. There are two phases, one is the mobile phase (the solvent) and another is the stationary phase (the absorbent materials) (Factor, 1991).Migration of the active compound and the spot obtained is highly solvent dependent. By using thin layer chromatographic analysis, the active compound of extracts can be separated (Ahamed *et al.,* 2017).

The aim of this study was to investigate the presence of phytochemical and also the determination of total phenolic and tannin content in the extracts. This study also aimed at investigation f antibacterial properties of spices extracts against both gram-positive and gram-negative bacteria as well as thin-layer chromatographic profiling of the extracts.

Materials and methods

Plant materials

This experiment was conducted at the Cell Genetics and Plant Biotechnology Laboratory, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh (23°53'14" N, 90°15'56" E). Ten spices such as Cinnamon, Coriander, Cumin, Clove, Black pepper, Black cardamom, Bay leaf, True cardamom, Black caraway, and Nutmeg were used in this experiment. All of the spices were collected from the local market (Savar Nama Bazar). List of plant materials along with their scientific and family name and used part is represented in Table 1.The study materials are presented in the Fig. 1.

Preparation of extract

The collected plant materials were washed thoroughly by running tap water and then sun-dried and powdered by using a mechanical grinder. The powdered materials were taken in air-tight plastic bags with labeling and kept in cool chamber (15-25°C) for further usage. Five grams of powder were taken in a conical flask and 25ml distilled water was added and vortexed properly. Mixtures were heated at 60°C for 45 minutes in a water bath. Then the mixtures were filtered through Whatman filter paper (0.40 µm) overnight. After this filtration, these filtrates were centrifuged at 2500 rpm for 25 minutes. The supernatants were collected and kept at 4°C for further screening (Ahamed *et al.,* 2017).

Qualitative phytochemical screening

Crude extracts that were screened for the presence and the absence of secondary metabolites specially saponin, alkaloid, tannin, coumarin, flavonoids, reducing sugar, glycoside, and phenol by using the following methods.

Test for Saponin: 1ml of the extract was mixed with 3 ml of distilled water and then the mixture was shaken for 5-6 minutes in a falcon tube. The presence of 1cm

of foam for 10 minutes indicated the presence of saponin in the extract (Kumar *et al.*, 2009).

Test for Alkaloid: About 4-6 drops of Wagner's reagents [2gm of potassium iodide and 1.27gm iodine in 100 ml of water] was added in 1ml of extract. The radish brown precipitate confirmed the presence of alkaloids (Ahmed *et al.,* 2018).

Test for Tannin: 1ml of distilled water was mixed with the 0.5ml of extract and then 5-6 drops of 1% ferric chloride solution were added. The presence of the gallic tannin and cathecholic tannin were confirmed by the blue color and greenish-black color of the extract respectively (Patil and Nasreen, 2016).

Test for Coumarin: 1.5ml of 10% NaOH was added with 1ml of prepared extract. The presence of coumarin was confirmed by the production of yellow color through a chemical reaction (Rizk, 1982).

Test for Flavonoids: 2 ml of extract was taken in a falcon tube and then 3-4 drops of 20% NaOH was added. The chemical reaction produced an intense yellow color and became colorless when 4-5 drops of dilute HCl was added. This indicated the presence of Flavonoids (Ugochukwu *et al.*, 2013).

Test for Reducing Sugar: 2ml of distilled water was added in 1ml of extract. Then the solution was heated at 55-60°Cfor 15 minutes and then 5-6 drops of Fehling's solution A and B were added respectively. The formation of a brick-red precipitate indicated the presence of reducing sugar (Patil and Nasreen, 2016).
Test for Glycosides: 1ml of glacial acetic acid was mixed with 1ml of extract and then 5-6 drops of 1% Ferric chloride solution was added. Brown colored ring was produced at the top and this indicated the presence of glycosides (Patil and Nasreen, 2016).

Test for Phenol: 1ml of the extract was mixed with 1ml of ethanol. Then the solution was mixed with 6-7 drops of 1% ferric solution. The formation of blue, green and purple color of the solution indicated the presence of phenol (Ahamed *et al.,* 2017).

Determination of total tannin content

Total tannin content was determined by the Folin-Ciocalteu method. About 0.1 ml of each sample extract was added to a falcon tube (15 ml) containing 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent, 1 ml of 35 % sodium carbonate (Na_2CO_3) solution and diluted to 0.9 ml of distilled water. The full mixture volume was finally 10 ml. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Tannic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in the same manner as described earlier. Absorbance for tests and standard solutions were measured against the blank at 725 nm with a UV/Visible spectrophotometer (T60 UV-Visible Spectrophotometer, PG Instruments Ltd., United Kingdom). The tannin content was expressed in terms of µg of TAE/ml of extract (Ukoha *et al.,* 2011).The estimation of total tannin contents was carried out in triplicate.

Determination of total phenolic content

Total phenolic content of extracts was determined with the Folin-Ciocalteu assay. 0.4 ml of each sample was mixed with1.6 ml of 7.5% of the sodium carbonate solution. After 5 minutes, 2 ml of 10 fold diluted Folin-Ciocalteu's phenol reagent was added to the mixture and mixed thoroughly. The mixture was kept in the dark for 1 hour at 27°C, absorbance was measured at 760 nm. The total phenolic content was determined from the extrapolation of calibration curve which was made by preparing a Gallic acid solution. The estimation of phenolic compounds was carried out in triplicate. The TPC was expressed as micrograms of Gallic acid equivalents (GAE)/ml of extract (Kauro *et al.,* 2005).

Evaluation of antibacterial activity

Test for microorganisms

A total of three bacterial species were tested including *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi*. These were identified by the standard biochemical test of the stock culture of gram-negative organisms (*Salmonella typhi* and *Escherichia coli*) and gram-positive organisms (*Bacillus subtilis*).

These cultures were maintained on LB agar slope media at 4°C and sub-cultured in Luria Broth by using Picking-up Technique (Aneja, 2017). Sixteen hours old pure cultures were prepared for each time use.

Preparation of sample discs with test samples

Sterile antimicrobial discs (Oxoid, UK) were taken to blank Petri plates. These discs were soaked with different concentration (2.5 µg/µl, 5 µg/µl, 7.5 µg/µl and 10 µg/µl) of the sample extracts and then dried properly.

Determination of antibacterial activity by disc diffusion method

Antibacterial activity of ten extracts was investigated by disc diffusion method (adopted from Polash *et al.,* 2017). LB medium was used to grow bacteria. Dried and sterile antibacterial disc (5 mm) containing different spices extracts of different known concentrations (2.5µg/µL, 5µg/µL, 7.5 µg/µL and 10 µg/µL) were placed on the nutrient agar medium uniformly seeded with test microorganisms. Then the plates were incubated for 24 hours at 37°C for optimum growth of the microorganisms. The spices extracts having antibacterial property prevent bacterial growth in the media surrounding discs and produced a clear, transparent area which is defined as 'zone of inhibition'. Antibacterial activity of the extract samples was determined by their ability to prevent the growth of microorganisms surrounding the discs. The antibacterial activities of extract samples were determined by measuring the diameter of the zone of inhibition in millimeters with slide calipers.

Thin layer chromatographic (TLC) profiling

The extracts of different spices samples were subjected to thin-layer chromatographic studies. The dimensional ascending method was used for this TLC analysis (Ahamed *et al.,* 2017). The dimension of the TLC plate (Merck, India) was 20×20 cm and coated with silica gel 60G F254. These TLC plates were cut with a scissor in 14×3 cm shape. Then the TLC plate was marked with pencil softly 1.5 cm far from both top and bottom. For spotting the samples on the bottom line of the TLC plate glass capillaries were used. After spotting, these plates were taken in the fume hood to dry the plate and loaded the sample again in the previous spot until a dark spot is obtained. Then about 20 ml of solvent Ethyl acetate: Hexane: Acetic acid (6:6:3) was taken into the TLC chamber. The TLC plate was placed into the chamber lining on the top. The solvent quantity should be the beneath of the spotted line. After a run, the plates were taken in fume hood and then used to detect the spots.

Detection of the spot

After completion of the run, the plates were dried and the spots were detected with the help of UV light at 254 nm and 366 nm (Ahamed *et al.,* 2017). Movement of the activecompound was expressed by the retention factor (R_f).

$$\text{Retention factor (Rf)} = \frac{\text{Distance traveled by the solvent}}{\text{Distance traveled by the solute}}$$

Results and discussion

Yield extract (%)

The highest yield (37.4%) was obtained from the *Coriandrum sativum* extract and the lowest yield (15.9%) was obtained from the *Amomum subulatum* extract (Table 2).

Qualitative phytochemical screening

Our results showed that the spices extract contain significant amount of tannin, phenol, glycosides, and alkaloids while saponins, flavonoid, reducing sugar and coumarins present at a considerable amount (Table 3). Among all the spices,*C. cyminum* and *A. subulatum* contain a considerable amount of mostof the phytochemical screened in this study. The qualitative phytochemical screening of various plant extracts is very much essential to obtain the required information regarding the chemical constituents for pharmacological discoveries of novel drugs. The extraction solvent might affect the result of the qualitative phytochemical screening (Savithramma *et al.,* 2011).

Table 1. Description of the study materials.

Sample I.D	Family name	Scientific name	Common name	Local name	Used parts
1	Apiaceae	*Coriandrum sativum*	Coriander	Dhonia	Leaves
2	Zingiberaceae	*Amomum subulatum*	Black cardamom	Kaloelach	Seeds
3	Zingiberaceae	*Elettaria cardamomum*	True cardamom	Sadaelach	Seeds
4	Apiaceae	*Cuminum cyminum*	Cumin	Zira	Seeds
5	Ranunculaceae	*Nigella sativa*	Black cumin	Kalozira	Seeds
6	Piperaceae	*Piper nigrum*	Black Pepper	Golmorich	Fruits
7	Myristiacaceae	*Myristica fragrans*	Nutmeg	Jaifol	Fruits
8	Lauraceae	*Laurus nobilis*	Bay leaf	Tejpata	Leaves
9	Myrtaceae	*Syzygium aromaticum*	clove	Lobongo	Flower bud
10	Lauraceae	*Cinnamomum verum*	Cinnamon	Darchini	Bark

Table 2. Percentage yield (%) of different samples after extraction.

Spices			Yield in gram	Percentage Yield (%)
Sample I.D	Local Name	Scientific Name		
1	Dhonia	*Coriandrum sativum*	0.374	37.4
2	Kaloelach	*Amomum subulatum*	0.159	15.9
3	Sadaelach	*Elettaria cardamomum*	0.223	22.3
4	Zira	*Cuminum cyminum*	0.314	31.4
5	Kalozira	*Nigella sativa*	0.285	28.5
6	Golmorich	*Piper nigrum*	0.290	29.0
7	Jaifol	*Myristica fragrans*	0.331	33.1
8	Tejpata	*Laurus nobilis*	0.285	28.5
9	Lobongo	*Syzygium aromaticum*	0.346	34.6
10	Darchini	*Cinnamomum verum*	0.363	36.3

Determination of total phenolic content

The highest phenolic content was found in *S. aromaticum* (67.77 µg GAE/ml of extract) and the lowest phenolic content was found in *C. sativum* (4.22 µg GAE/ml of extract) (Fig. 2). The order of amount for the total phenolic content of different spices extracts is as follows *S. aromaticum* > *L. nobilis* > *C. cyminum* > *C. verum* > *M. fragrans* > *A. subulatum* > *P. nigrum* > *E. cardamomum* > *N. sativa* > *C. sativum*. There are different types of phenolic contents in the plant. Phenolic compounds are the natural antioxidants and which may occur in all parts of the plant and function as the antibiotics and natural antioxidants (Silva-Beltran *et al.*, 2015).

Table 3. Qualitative phytochemical screening of spices extracts.

Spices		Saponin	Alkaloid	Tanin	Coumarin	Flavonoid	Reducing sugar	Glycoside	Phenol
Sample I.D	Scientific name								
1	*Coriandrum sativum*	-	-	++	-	++	-	++	+
2	*Amomum subulatum*	++	++	+	+	-	+	+	++
3	*Elettaria cardamomum*	-	+	+	++	-	-	+	+
4	*Cuminum cyminum*	-	++	+ ++	+	+	-	++	+
5	*Nigella sativa*	-	-	+	-	-	-	+	+
6	*Piper nigrum*	-	+	+	-	+	-	+++	+
7	*Myristica fragrans*	-	+++	++	-	-	+	+	+
8	*Laurus nobilis*	+	-	+	+	-	++	+	+++
9	*Syzygium aromaticum*	++	++	+	-	-	+	-	+
10	*Cinnamomum verum*	-	-	+++	-	-	+++	++	+

Determination of total tannin content

Among the spices, *M. fragrans* contain the highest tannin content (78.75 µg TAE/ml Extract) and *E. cardamomum* contains the lowest tannin content (61 µg TAE/ml Extract) (Fig. 3). The order of amount for total tannin content of different spices extracts- *M. fragrans* > *C. verum* > *A. subulatum* > *L. nobilis* > *P. nigrum* > *C. sativum* > *C. cyminum*> *S. aromaticum*> *N. sativa*> *E. cardamomum*.

Table 4. Thin layer chromatographic (TLC) profiling of spices extracts.

Spices extracts		Number of spot(s) detected	R_f Value
Sample I.D	Scientific name		
1	*Coriandrum sativum*	2	0.47
			0.53
2	*Amomum subulatum*	1	0.78
3	*Elettaria cardamomum*	3	0.34
			0.56
			0.64
4	*Cuminum cyminum*	2	0.20
			0.32
5	*Nigella sativa*	1	0.76
6	*Piper nigrum*	2	0.45
			0.64
7	*Myristica fragrans*	3	0.62
			0.68
			0.76
8	*Laurus nobilis*	2	0.30
			0.36
9	*Syzygium aromaticum*	1	0.22
10	*Cinnamomum verum*	3	0.30
			0.56
			0.65

Tannins act as protection against various pathogens and are natural polyphenol ubiquitously distributed in vegetables, fruits, and seed. Tannins provide a different type of astringent flavor which causes lessening of the appetite of herbivorous animals and insects and in this way, tannins represent a defense of natural line (Swain, 1977).

Table 5. Antibacterial activity of the spices extract against *E. coli*. [Four different concentrations (2.5 µg/µl, 5.0 µg/µl, 7.5 µg/µl and 10 µg/µl) of different spices extracts used for the antibacterial activity. Values represent Mean ± SD of three different replications.

Spices		Diameter of the zone of inhibition (mm)			
Sample I.D	Scientific name	2.5 µg/µl	5.0 µg/µl	7.5 µg/µl	10 µg/µl
1	*Coriandrum sativum*	5.18 ± 0.06	6.7 ± 0.24	6.84 ± 0.19	6.66 ± 0.55
2	*Amomum subulatum*	5.99 ± 0.07	6.22 ± 0.09	6.44 ± 0.09	8.01 ± 0.04
3	*Elettaria cardamomum*	6.4 ± 0.41	6.95 ± 0.17	6.45 ± 0.67	6.98 ± 0.06
4	*Cuminum cyminum*	6.23 ± 0.82	7.65 ± 0.44	8.16 ± 0.54	7.94 ± 0.43
5	*Nigella sativa*	5.45 ± 0.44	5.67 ± 0.35	7.46 ± 0.29	8.31 ± 0.12
6	*Piper nigrum*	5.87 ± 0.1	6.18 ± 0.07	6.52 ± 0.09	6.49 ± 0.03
7	*Myristica fragrans*	5.2 ± 0.18	5.53 ± 0.12	5.64 ± 0.16	6.55 ± 0.11
8	*Laurus nobilis*	6.49 ± 0.08	7.22 ± 0.13	7.27 ± 0.09	7.71 ± 0.2
9	*Syzygium aromaticum*	6.27 ± 0.05	6.92 ± 0.03	7.42 ± 0.04	7.48 ± 0.04
10	*Cinnamomum verum*	7.07 ± 0.05	7.49 ± 0.13	7.53 ± 0.1	7.72 ± 0.26

Thin layer chromatographic (TLC) analysis

The highest number of spots was found (3) for *E. cardamomum*, *M. fragrans* and *C. verum* extract and the lowest number of spots (1) were found for *A. subulatum*, *N.sativa* and *S. aromaticum*. The highest R_f value (0.78) was found for the *A. subulatum* extract and the lowest R_f value (0.20) was found for the *C. cyminum* extract (Table 4).

Table 6. Antibacterial activity of the spices extracts against*B. subtilis*. [Four different concentrations (2.5 µg/µl, 5.0 µg/µl, 7.5 µg/µl and 10 µg/µl) of different spices extracts used for the antibacterial activity. Values represent Mean ± SD of three different replications.

Spices		Diameter of the zone of inhibition (mm)			
Sample I.D	Scientific name	2.5 µg/µl	5.0 µg/µl	7.5 µg/µl	10 µg/µl
1	*Coriandrum sativum*	5.36 ± 0.5	5.46 ± 0.07	5.58 ± 0.06	6.56 ± 0.37
2	*Amomum subulatum*	6.42 ± 0.04	8.23 ± 0.05	7.59 ± 0.08	8.16 ± 0.05
3	*Elettaria cardamomum*	5.87 ± 0.25	9.12 ± 0.48	10.4 ± 0.73	10.4 ± 0.73
4	*Cuminum cyminum*	5.43 ± 0.21	8.99 ± 0.98	8.83 ± 0.59	9.37 ± 0.11
5	*Nigella sativa*	5.36 ± 0.06	5.81 ± 0.34	8.14 ± 0.17	9.66 ± 0.53
6	*Piper nigrum*	6.18 ± 0.03	6.36 ± 0.19	6.96 ± 0.07	7.87 ± 0.09
7	*Myristica fragrans*	6.18 ± 0.03	6.77 ± 0.27	6.8 ± 0.04	7.16 ± 0.95
8	*Laurus nobilis*	5.59 ± 0.07	7.26 ± 0.06	7.38 ± 0.04	7.68 ± 0.04
9	*Syzygium aromaticum*	6.19 ± 0.05	6.53 ± 0.08	7.48 ± 0.06	7.58 ± 0.04
10	*Cinnamomum verum*	6.18 ± 0.11	6.33 ± 0.13	6.61 ± 0.04	7.27 ± 0.16

Table 7. Antibacterial activity of the spices extracts against *S. typhi*. [Four different concentrations (2.5 µg/µl, 5.0 µg/µl, 7.5 µg/µl and 10 µg/µl) of different spices extracts used for the antibacterial activity. Values represent Mean ± SD of three different replications.

Spices		Diameter of the zone of inhibition (mm)			
Sample I.D	Scientific name	2.5 µg/µl	5.0 µg/µl	7.5 µg/µl	10 µg/µl
1	*Coriandrum sativum*	6.99 ± 0.09	7.31 ± 0.02	7.85 ± 0.02	7.77 ± 0.12
2	*Amomum subulatum*	6.84 ± 0.14	7.3 ± 0.36	7.86 ± 0.23	8.61 ± 0.53
3	*Elettaria cardamomum*	7.32 ± 0.06	7.35 ± 0.06	8.21 ± 0.62	7.84 ± 0.06
4	*Cuminum cyminum*	6.31 ± 0.1	6.66 ± 0.14	7.08 ± 0.11	7.68 ± 0.33
5	*Nigella sativa*	7.34 ± 0.2	7.56 ± 0.02	7.89 ± 0.2	8.23 ± 0.11
6	*Piper nigrum*	6.50 ± 0.14	6.71 ± 0.18	6.68 ± 0.12	7.48 ± 0.04
7	*Myristica fragrans*	7.15 ± 0.06	7.64 ± 0.37	8.07 ± 0.36	8.17 ± 0.31
8	*Laurus nobilis*	6.31 ± 0.07	7.03 ± 0.14	7.05 ± 0.07	7.4 ± 0.05
9	*Syzygium aromaticum*	5.86 ± 0.07	6.67 ± 0.21	7.25 ± 0.06	7.54 ± 0.1
10	*Cinnamomum verum*	6.62 ± 0.06	6.72 ± 0.03	7.62 ± 0.37	7.78 ± 0.26

Different R_f values of the extracts indicate the presence of different phytochemicals. The changes in R_f value of the phytochemical provide an important understanding of the polarity and suggest an appropriate selection of solvent system for the separation of pure compound. By analyzing the R_f values of compounds in different solvent systems, an appropriate solvent system may be selected for a particular plant extract (Sharma *et al.,* 2013). The Results of TLC represented here, are not the actual indicator of particular compound present in the extracts. More research should be done to identify the presence of particular compounds by more advanced technique such as HPLC, GC-MS.

Antibacterial activity of the Spices Extracts

All the spices extracts were investigated for their antibacterial activity against both Gram-negative (*E.*

coli and *S. typhi*) and Gram-positive (*B. subtilis*) bacteria by the simple agar diffusion method. Four different concentrations of the extract were used in this study. These different concentrations were 2.5 µg/µl, 5 µg/µl, 7.5 µg/µl and 10 µg/µl.

The antibacterial activity of the extracts against *E. coli*is expressed in millimeter and represented in Table 5. For *E. coli*, the highest (8.31 mm) zone of inhibition was observed for *N. sativa* and the lowest (5.18 mm) zone of inhibition was observed for *C. sativum*. The antibacterial activity (Diameter zone of inhibition) of the spices extract against *B. subtilis* was expressed in millimeter and represented in Table 6. For *B. subtilis*, the highest (10.4 mm) zone of inhibition was observed for *E. cardamomum* and the lowest (5.36 mm) zone of inhibition was observed for *C. sativum* and *N. sativa*.

Fig. 1. Study materials are shown with their scientific and local names.

The antibacterial activity (Diameter of zone of inhibition) of the spices extract against *S. typhi*is expressed in millimeter and represented in Table 7. For S. *typhi*, the highest (8.61 mm) zone of inhibition was observed for *A. subulatum* and the lowest (5.86 mm) zone of inhibition was observed for *S.aromaticum*. In our study, all of the spices show the zone of inhibition thus the antibacterial activity. The reason behind the antibacterial activity of the plant extract is due to the presence of the different types of secondary metabolites, such as saponins, glycosides, and tannin (Panathula *et al.,* 2014). Our results of the study validate this information as the spices are rich in both glycosides and tannin which are being confirmed by the qualitative phytochemical screening.

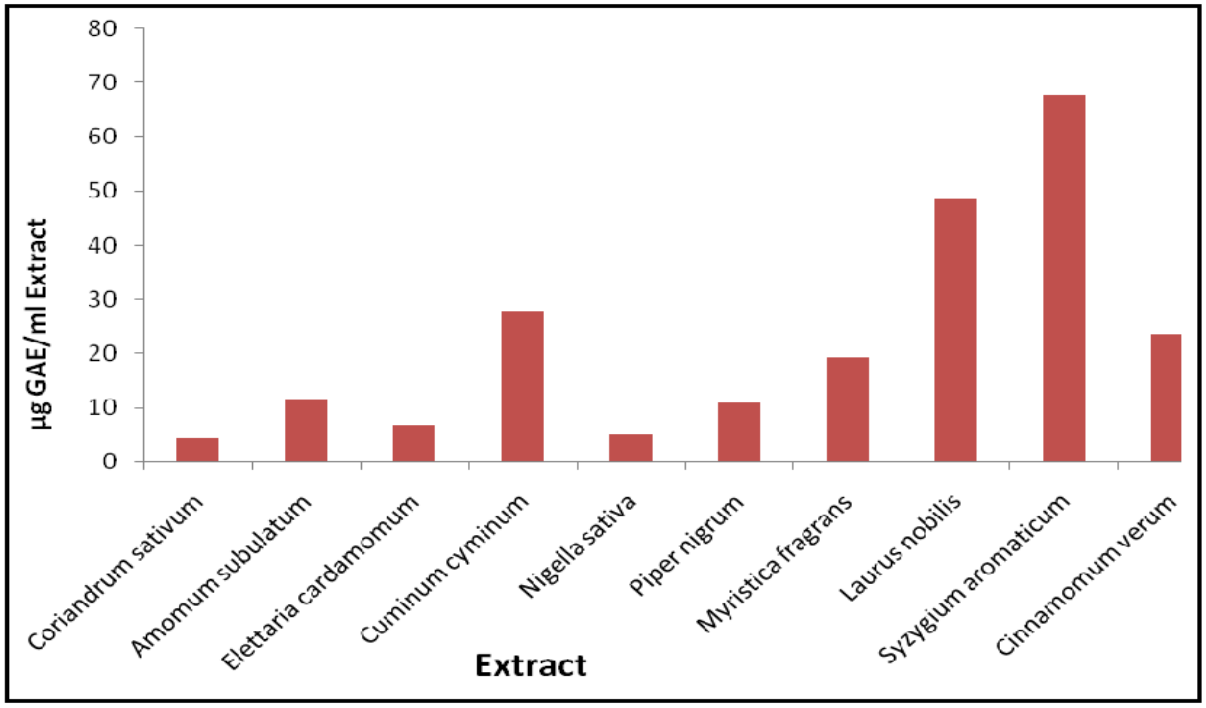

Fig. 2. Estimation of the total phenolic content of spices extracts expressed as µg of Gallic acid Equivalent/ml of extract.

Our results of antibacterial activity of the spices extracts support the previously published report that Gram-positive bacteria are more sensitive to plant extracts when these are compared to the Gram-negative bacteria (Polash *et al.,* 2017). Therefore, these spices extracts were tested against both Gram-positive (*B. subtilis*) and Gram-negative (*E. coli* and *S. typhi*) bacteria. Antibacterial activity of most of the spices extracts at the same concentration against *B. subtilis* was stronger when compared to *S. typhi* and *E.coli*.

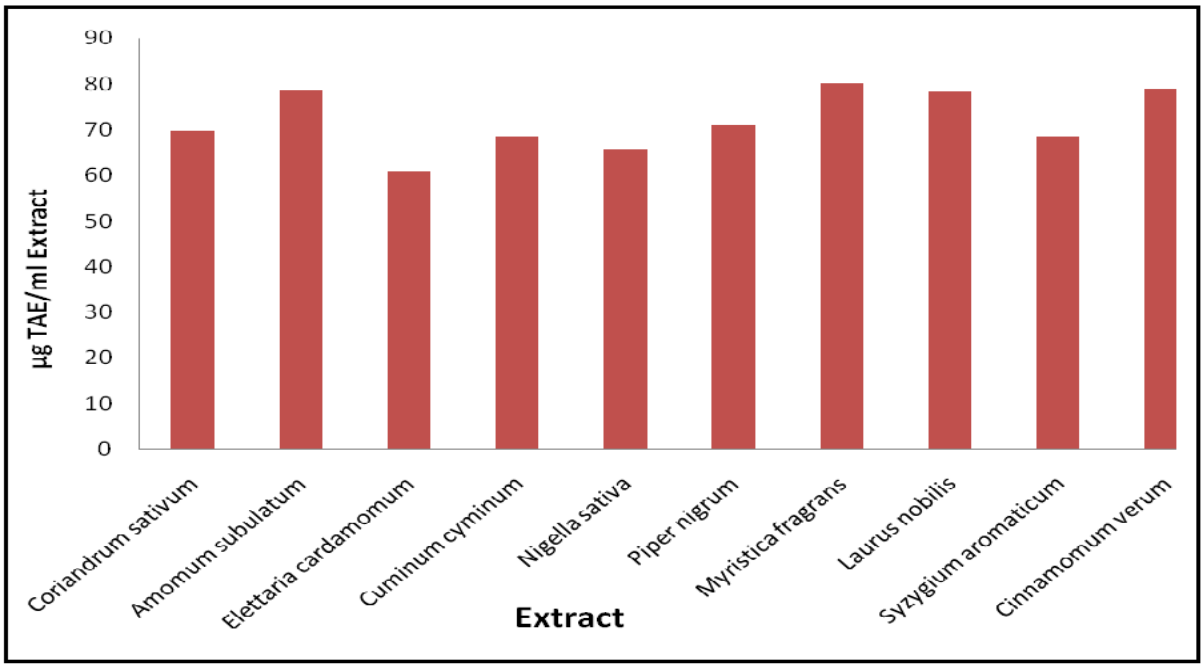

Fig. 3. Estimation of total tannin content of spices extracts expressed as µg of Tannic acid Equivalent/ml of extract.

This variation in antibacterial activity of the Gram-positive and the Gram-negative bacteria may be due to the significant differences in the outer layer. Gram-negative bacteria possess a unique outer membrane and periplasmic space which are not found in gram-positive bacteria (Duffy and Power, 2001). This is why; they showed different antibacterial activity tested against different extracts.

Conclusion

In this study, we have evaluated the qualitative phytochemical contents, total tannin, and phenol content, thin layer chromatographic analysis and the antibacterial activity against both gram-positive and gram-negative bacteria. All the spices extracts were made by using water as a solvent.

The qualitative phytochemical screening evaluates the presence of the many important phytochemicals. R_f values of the TLC analysis also confirm the presence of phytochemical though much research needs to be carried out in order to identify the particular phytochemical.

These spices are rich in tannin and phenolic compounds. Spices extracts also have antibacterial activity and show much antibacterial activity against Gram-positive bacteria compared to Gram-negative ones. Though these spices are added to the food and curry to increase the taste, these have some medicinal properties too. Due to these properties, these spices can be screened against various disease-causing pathogens and can also be used as novel drug candidates.

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Conflict of Interest

The authors declare no conflict of interest.

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