RESEARCH ARTICLE

Antimicrobial Activities of Aqueous Extracts of Selected Indian Spices Against Food Borne and Enteropathogens

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Abstract

Spices have been used by the people for generations as flavorings, digestive, coloring, taste enhancer, preservatives, and as medications due to their various properties. Ayurveda says that they help to maintain body fluid balance. Post covid-19, the consumers are demanding foods with bio-preservatives; hence, this study aimed to evaluate antimicrobial abilities of various spices against common food borne pathogens to increase shelf life of the spices added food products without addition of harmful chemical preservatives. The confidence of people in the use of plant parts as natural medicines to prevent and cure diseases, has increased as they are considered safe and are as effective as allopathic medication without any side effects. Spices are plant parts like seeds, barks, stems, flowers, roots, leaves, etc. Each spice has characteristic aroma and is mainly used during cooking to impart flavor, color, to enhance taste of the dish, in spiritual rituals and also possess medicinal values. Various phytochemicals compounds like phenolics, tannins, flavonoids, saponins, and alkaloid etc are present in spices. The use of spices has been associated with several health benefits, including digestion and high nutritional value. Aqueous extract of various spices - turmeric *(Curcuma longa),* black pepper *(Piper nigrum),* fenugreek *(Trigonella foenum-graecum),* garlic *(Allium sativum), mustard (Brassica repa subsp. oleifera),* clove *(Syzygium aromaticum),* cumin *(Cuminum cyminum)* and cinnamon *(Cinnamomum verum)* were studied against common food borne and Enteropathogens *i.e Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633), *Shigella flexneri* (ATCC 12022), *Cronobacter sakazakii* (ATCC 29544), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6539), *Salmonella enterica* (ATCC 14028), and *Vibrio cholerae* (ATCC 3906) using agar well diffusion method. Black pepper was found highly active against *Staphylococcus aureus, Bacillus subtilis, Salmonella enterica,* and *Escherichia coli* whereas cinnamon was active against *Cronobacter sakazakii* and *Pseudomonas aeruginosa*. Aqueous extracts of clove exhibited antibacterial activity against *Shigella flexneri, Salmonella enterica* and *E. coli.* Cumin was moderately active against *E. coli*, *Cronobacter sakazakii*, *Salmonella enterica* and *Shigella flexneri*. Garlic exhibited high activity against *Salmonella flexneri, E. coli* and *Bacillus subtilis,* while turmeric was active against *Bacillus subtilis, Salmonella enterica* and *Staphylococcus aureus* only. *Vibrio cholerae* showed moderate sensitivity to all test spices turmeric, clove, garlic, cumin, cinnamon, black pepper, mustard and fenugreek. It is suggested that a combination of spices in Indian kitchen as additive in food products is advantageous over single use of an spice. We had planned and conducted all experiments with aqueous extracts with a view that only watersoluble phytochemicals are readily available to the microbes in the food products when they are added as flavoring, coloring or aromatic agents.

Keywords: phytochemicals; phenolics; tannins; flavonoids; alkaloids; phenolic; antimicrobial activities.

Introduction

Since the day break of time, spices have been significant to humans. The "Epic of Gilgamesh" and the "Bhagavad Gita," among other incredible literature, have mentioned their use for a variety of purposes. Several traditional kitchen formulations in India are frequently referred to as Ayurvedic nourishments having similarities with Ayurvedic dietetics and traditional cuisines [1]. Spices have been used by human for thousands of years, not only in India but were exported to various countries like Egypt, Iran, Africa and other ancient nations [2]. The ability of spices to conserve natural moisture of the foods made them excellent for preserving. They contribute to the preservation of the body's temperature, in understanding with Ayurveda [3]. Spices were used as flavoring agent, to impart color in food as well these were used as a treatment of various infections like cough, sneezing etc [4, 5]. The spices contained different types of bio-molecules commonly referred as phytocompounds such as flavonoids, tannins, alkaloids, etc. that perform certain organic capacities that fortify restorative exercises such as anti-cancer, anti-mutagenic properties, anti-inflammatory, and antioxidant and anti-microbial activities [6-10]. Spices are utilized within the pharmaceutical, naturopathic, nourishment additive, corrective, food and bio-pesticide industries, whereas they play a significant role in financial management and quality of products of the companies [11]. The garlic, a monocotyledonous herb *Allium sativum*, is the second most extensively distributed species of *Allium* in the globe [12], belongs to the family Alliaceae. Garlic is used to flavour the food and has several medicinal advantages [13]. It is crucial to maintain garlic as a staple export crop for a very long time [14]. Garlic is a good source of folic acid, vitamin C, calcium, iron, magnesium, potassium, zinc, and vitamins B2, B1, and B3, highly rich in nutrients, minerals and help in dehydration [15]. Flavonoids, phenolics, and other phytochemical components extracted from spices have the natural capacity to protect the foods by restraining microbial growth, oxidation, and specific chemical reactions in nourishment items [16]. Spices contain phytochemicals that are beneficial in the pharmaceutical, food, and meat industries, that are putting high emphasis on spice-based additives. Cloves, garlic, ginger, cinnamon, thyme, oregano, and basil are mostly used as seasonings [1, 7]. Food-borne infections may be caused by antimicrobial resistance in some bacteria. Many spices are used to extend the shelf life of food and to anticipate food-borne illnesses. While certain spices are essential in the food industry, many are also useful for repressing irreversible infections and inhibit pathogens, particularly in traditional healthcare [17]. Manufactured chemical additives are added to various food products that are now rejected by health conscious clients. Spices and herbs are safe alternatives to chemical additives used in food products [6].

Spices are largely used in the food market, but they are also used in the pharmaceutical, beauty care products, perfume, and pharmaceutical industries [18]. Mustard (*Brassica spp*.) is a key group among the world's oil seed crops. It is the world's third most important source of edible oil. Mustard's nutritional and functional qualities are attributed to their fatty acid composition and fatty acid distribution pattern within the triacylglycerol molecule [10, 19]. Clove oil is extracted from the flower buds of *Syzygium aromaticum*, a plant belonging to the family Myrtaceae, is a precious spice and has been used as for food preservation and medicine for millennia due to its antibacterial and antioxidant characteristics [6,11].

Since ancient times, people have used turmeric (*Curcuma longa*), which is now a major ingredient in many Asian dishes with numerous medicinal value. Turmeric, India's sacred spice is frequently used as a condiment, flavoring ingredient, culinary additive, and powerful additive [20]. It has been designated as a medicinal agent in the treatment of several illnesses in traditional Ayurvedic, Unani, and Siddha pharmaceutical systems [21]. Turmeric has traditionally been used as a home remedy in the treatment of various infections such as anorexia, attack, sickness, and digestive system clutter. Non-curcuminoids found in turmeric include calebin A, turmerone, elemen, bisacurone, cardion, bisabolone, caseren, furanodiene, ferulic corrosive, and coumaric corrosive, Demethoxycurcumin (about 18%), bisdemethoxycurcumin (about 5%), and curcumin constitute the majority of commercially available curcuminoids. Curcumin has been shown to be the most physiologically dynamic phytochemical of them all [22]. Turmeric contains polyphenolic component that is used to treat diabetes mellitus all over the world [23]. Turmeric has also been associated with pharmacological effects such as anti-inflammatory, antibacterial, anticancer, antioxidant with hypolipidemic effects. It is crucial to note that the majority of these effects are related to the proximity of bioactive phenolic acids [15]. An antioxidant potential of 45 genotypes in turmeric of was investigated, and it was discovered that both non-curcuminoids and curcuminoids contribute to turmeric's antioxidant effect [24]. Spices containing phenolic chemicals are well-known for their significant antioxidant and antibacterial properties [25]. Turmeric's physiologically active components, ar-turmerone, and -turmerone, have been shown to have antibacterial, antioxidant, anti-inflammatory, and anticancer properties [26]. Turmeric oil is also commonly utilized in pharmacological and therapeutic products. As a result, the foundation of turmeric's beneficial effects is made up of both curcuminoids and unstable fixes [27].

Black pepper (*Piper nigrum*) is an Indian spice, and its essential oil has been shown to have antimicrobial activity [28]. In Black pepper, phenolic components are considered to be responsible for its antibacterial activity, deteriorating bacterial films, and inhibiting bacterial growth [29]. Piperine, pyrrolein B, and piperamide have been discovered to be the most important critical constituents in black pepper. Based on acetone extraction, this essential oil has been shown to be effective in inhibiting mycelial growth of *Fusarium graminearum* and *Penicillium viridcatum* [11]. Black pepper has been shown to exhibit antibacterial activity with inhibitory doses ranging from 50 to 500 ppm. It appears to be a significant inhibitor of the growth of Gram-positive microscopic organisms such as *Staphylococcus aureus, Bacillus cereus*, and *Streptococcus faecalis,* as well as a few Gram-negative microscopic species such as *Pseudomonas aeruginosa* [29]. Fenugreek (*Trigonella faenum-graecum*) used as a spice and herb in various cuisines, and the only edible portions of the plant are green leaves and seeds. Leaves are used to flavor meals or eaten as greens in Indian cuisine, while seeds are used as seasonings or crushed to make curry powders and pastes. Fenugreek is used as a bread addition in African cuisine. The presence of galactomannan, a source of soluble dietary fiber in seed endosperm, increases the bread's nutritional and physicochemical qualities [30]. It is used as mouth freshner and possesses antimicrobial activity [31a].

Microbial contamination is a key source of food decomposition and deterioration. Spices are used to protect foods against bacteria, fungi such as mould and yeast [9]. The aim of this study was to determine the antimicrobial activities of selected Indian spices- turmeric *(Curcuma longa),* black pepper *(Piper nigrum),* fenugreek *(Trigonella foenum-graecum),* garlic *(Allium sativum), mustard (Brassica repa),* clove *(Syzygium aromaticum),* cumin *(Cuminum cyminum)* and cinnamon *(Cinnamomum verum)* on different common food borne and Enteropathogens bacteria that included *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633), *Shigella flexneri* (ATCC 12022), *Cronobacter sakazakii* (ATCC 29544), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6539), *Salmonella enterica* (ATCC 14028), and *Vibrio cholerae* (ATCC 3906) using agar well diffusion method. Phytochemical components of their extracts were also analyzed in order to their antibacterial properties.

Materials And Methods

Collection Of Sample And Their Preparation

Fresh turmeric *(Curcuma longa),* black pepper *(Piper nigrum),* fenugreek *(Trigonella foenum-graecum),* garlic *(Allium sativum), mustard (Brassica repa),* clove *(Syzygium aromaticum),* cumin *(Cuminum cyminum)* and cinnamon *(Cinnamomum verum)* were purchased from the local market of Meerut, Uttar Pradesh. These were sorted out to remove visible impurities if any, gently washed using running tap water, wiped with a clean dry cloth, then sun-dried [32 a,b]. The spices were then crushed into powder using a mortar and pestle [33].

Extract Preparation

2 g (w/w) of powder of above spices was suspended in 20 mL of sterile distilled water overnight, and then centrifuged at 5000 rpm for 15 min at 4°C. The supernatant was collected and filtered through Whatman's filter paper No. 1 [34].

$$\text{Yield\%} = \frac{\text{Yield (gram)} \times 100}{\text{Initial weight (gram)}}$$

Proximate Analysis Of Spices.

For proximate analysis of spices, moisture content, solubility in water and ash content were determined using AOAC method (2005). Moisture content was calculated by subtracting the initial weight placed in oven from final weight of spices after drying in oven at 55°C [35]. The solubility was determined by total amount of water added in spices subtracted from total weight of spices after water evaporated while total ash was estimated by heating known amount of spice at temperature 550°C for 5 to 6 hours in muffle furnace [32(a, b), 36] using following formulae.

$$\text{Ash content\%} = \frac{\text{weight of ash} \times 100}{\text{Weight of sample}}$$

Preliminary Phytochemicals Analysis Of Spices

AOAS methods described by Trease and Evans (1990) [see: 32 a, b] were used for phytochemicals screening of an aqueous extract of spices prepared.

I. Detection Of Alkaloids

(a) Mayer's Test: 2 mL of the aqueous extract sample was mixed with 4-5 drops of Mayer's reagent composed of 1.36 g mercuric chloride and 5 g potassium iodide in 100mL of distilled water. The presence of alkaloids was demonstrated by the formation of a white precipitate [32 a b].

(b) Wagner's Test: Wagner's reagent (1.3 g of iodine and 3g of potassium iodide added into 100mL of distilled water) mixed with 2mL of water extract in the sterile test tube. The presence of alkaloid showed by reddish brown precipitation [33].

Detection Of Flavonoids

(a) **Shinoda's test:** Aqueous extract of the sample was mixed with 2 ml of 95% ethanol. Two to three drops of concentrated hydrochloric acid (HCl) were mixed together. After adding two to three pieces of magnesium ribbon, heat was applied. The colours were changed to yellow, orange, red, and pink which indicated the presence of flavonoids [32 a, b].

(b) Sulphuric acid test: 2 mL aqueous extract of sample was mixed with few drops of concentrated sulphuric acid (H_2SO_4). The formation of red or orange precipitation indicated the presence of flavonoids [37].

Detection Of Tannins

(a) Feeric Chloride test: 1 ml of aqueous extract of sample was transferred in a test tube, and 5% ferric chloride was added. A colour change to brownish green indicated the presence of tannin [32 a, b].

(b) Lead acetate test: 1mL of aqueous extract of the sample was taken in a sterile test tube to which few drop of 10% lead acetate were added. The formation of white precipitate indicated the presence of tannins [38].

Detection Of Terpinoids

(a) Salkowski Test: 1mL of aqueous extract of the sample was placed in a sterile test tube, mixed with 0.5mL of chloroform, and a few drops of concentrated sulphuric acid were added. The presence of terpenoids was detected by the creation of a reddish-brown precipitate [32 a, b].

(b) Phenolic Test: 1mL of aqueous extract of the sample was mixed with 2 mL distilled water and 10 mL ferric chloride in a sterile test tube. Formation of reddish brown or blue precipitation indicated the presence of terpenoids [32 a, b].

Detection Of Carbohydrate

(a)Molisch's test: 1 mL of aqueous extract was combined with alpha-naphthol, ethanol, and a few drops of concentrated sulphuric acid. The formation of a violet, blue, and purple colour indicated the presence of carbohydrates [32 a, b].

(b) Bendict's test: 1 mL aqueous extract was mixed with Bendict's reagent and heated for 2 -3 minutes before cooling. Colour changed into green, yellow, brown and red indicated the presence of carbohydrates [32 a, b].

Detection Of Protein

(a) Xanthoprotein test: 1 mL of aqueous extract was added into a sterile test tube and mixed with two to three drops of nitric acid. The presence of protein was shown by the colour changing to yellow [39].

(b) Ninhydrin test: 1mL aqueous extract of the sample was mixed with 2-3 drops of ninhydrin's solution. The formation of purple colour showed the presence of proteins [39].

Antimicrobial Activity Using Agar Well Diffusion Method

Agar well diffusion methods was used for evaluating the antimicrobial effect of aqueous extracts of spices [6]. Fresh cultures of selected food-borne bacteria were made in nutrient broth for 24 h at 37°C using pure culture from our stocks of Microbial Culture Collection maintained at -20 and -80°C. 100μL of test bacterial inoculum was aseptically added onto a sterilized Müller-Hinton Agar plate and was uniformly spread using a sterilized L-shaped spreader (Hi Media, Mumbai, India). The plates were now allowed to dry and the wells were cut with a sterile cork borer (6mm in diameter). 50 lμL of aqueous extract of each sample was added aseptically into the wells cut on the agar plates using micropipette. Sterile distilled water was used as control. The plates were incubated in the BOD incubator for 48 h in an upright posture at 37±1°C to allow for radial distribution of the test sample. The diameter of the zone of inhibition (in mm) was measured against *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633), *Shigella flexneri* (ATCC 12022), *Cronobacter sakazakii* (ATCC 29544), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Salmonella enteric* (ATCC 14028), and *Vibrio cholera* (ATCC 3906) and the zone of antimicrobial activity was determined using following formulae

$$\textbf{Zone of antimicrobial activity} = \frac{\textbf{(X+Y) - 2× Diameter of cork borer}}{\textbf{4}}$$

Whereas, X = Diameter on X axis, Y = Diameter on Y axis.

Results And Discussion

Moisture content is essential for proper flavor and aroma formation of the spices. Fig 1 shows that garlic (32.4%) possessed highest percentage of moisture content, followed by turmeric (28.6%), mustard (26.62%), cinnamon (20.45%), clove (18.5%) and cumin (10.96) whereas black pepper (9.2%) had the lowest moisture content when compared to turmeric, clove, cumin, cinnamon, mustard, and fenugreek (Fig. 1).

The percentage of solubility is significant because the constituent phytochemicals of the spices must be transmitted in water soluble form for a proper metabolic functions [40]. We found that garlic (42.4%) had the highest solubility in water followed by cumin, turmeric, cinnamon, clove, black pepper, fenugreek and mustard (6.3%) had the lowest aqueous solubility percentage (Fig. 1), possibly because of the presence of insoluble fatty acids as its constituent [41-45].

The ash content of spices may influence their physiochemical as well as nutritional properties [15]. The ash content of the spice showed that mustard, cumin, garlic and black pepper had high ash content, whereas cinnamon (2.4%) had a low ash content as compared to turmeric, clove, cumin, garlic, black pepper, whereas turmeric, fenugreek and clove yielded lesser ash content and cinnamon possessed the lowest (Fig. 1). High percent of ash content suggest high mineral concentration, and lower ash content shows a low mineral concentration, however, other properties of spices also contribute to their medicinal and nutritional value [8].

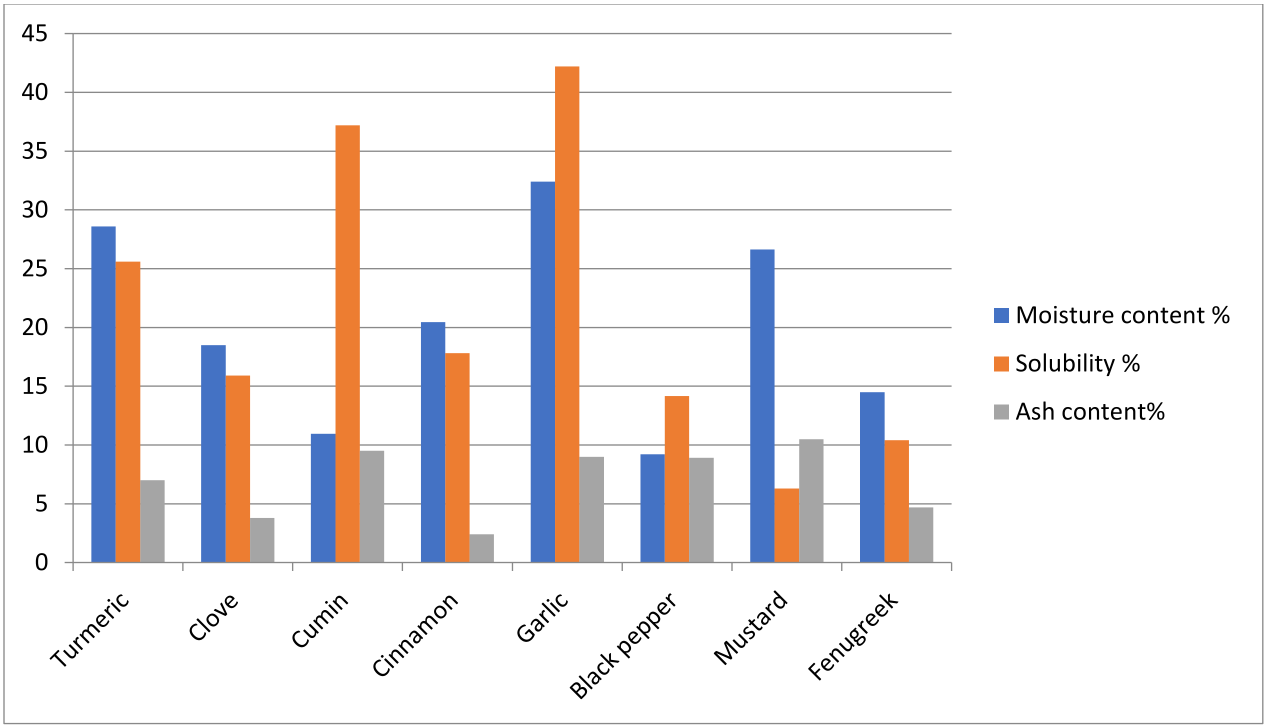


Fig 1: Percent moisture content, solubility and ash content of turmeric, clove, cumin, cinnamon, garlic, black pepper, mustard and fenugreek.

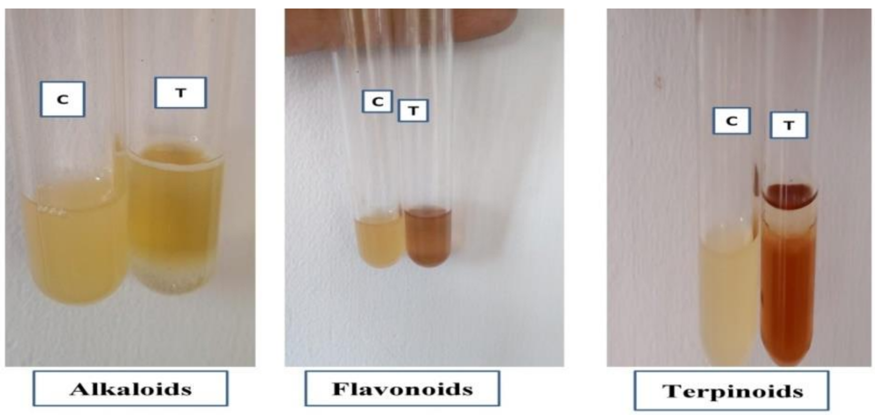
The preliminary phytochemical screening of turmeric *(Curcuma longa)*, black pepper *(Piper nigrum)*, fenugreek *(Trigonella foenum-graecum)*, garlic *(Allium sativum)*, *mustard (Brassica repa)*, clove *(Syzygium aromaticum)*, cumin *(Cuminum cyminum)* and cinnamon *(Cinnamomum verum)* revealed the presence of different kinds of bioactive secondary metabolites that included alkaloids, tannins, terpenoids, flavonoids, carbohydrates and proteins test spices which confirm the earlier findings of Oladeji *et al*. [5]. The qualitative analysis of spices for the presence of different phytochemical of spices in aqueous extract is shown in Table 2 and the tests in Fig. 2. We used water extracts for phytochemical analysis in view of the solubility aspects of available biomolecules during metabolism of food products when the spices are incorporated with foods for various purposes. The previous authors have used various organic solvents for extraction of biomolecules from spices and the antimicrobial activities were determined by them using DMSO as solvent after extraction and evaporation in different organic solvents. Such method and approach of the previous investigators is not close to natural conditions, hence, we conducted all experiments with water extracts of the spices as these will be available to the human during their consumption with various food products.

Table 2: Phytochemical screening of aqueous extract of selected Indian spices. ('+'indicates the presence of phytochemicals and '–' as the absence.

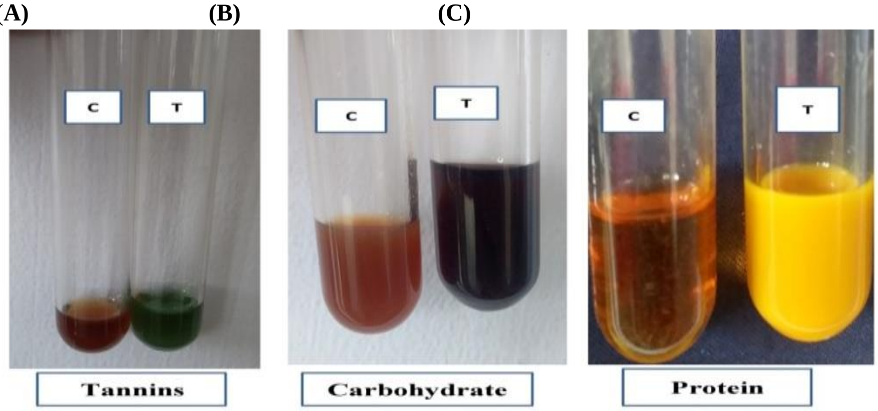
Phytochemicals	**Alkaloids**	**Flavonoids**	**Tannin**	**Terpinoid**	**Carbohydrate**	**Protein**
Spices						
Turmeric	+	+	+	+	+	+
Garlic	+	+	-	+	+	+
Fenugreek	+	+	-	+	+	+
Black pepper	+	+	-	+	+	+

Cumin	+	+	+	+	+	+
Cinnamon	+	+	+	+	+	+
Clove	+	+	+	+	+	+
Mustard	+	+	-	+	+	+

Alkaloids are significant bioactive molecules that have antibacterial, antiviral, and insecticidal properties as well as they show antimicrobial activity against a variety of food-borne illnesses causing bacteria [46]. Flavonoids are necessary class of natural products and their abundance in these spices have many medicinal properties. Flavonoids are important for plants as they remove harmful light, as photoreceptors, optical attractants, bug deterrents, antibiotics, antioxidants, disinfectants, and antimicrobial. Flavonoids possess antiviral, anti-inflammatory, and vasodilators properties. Flavonoids demonstrate antioxidant activity because they can both eliminate the production of free radicals and scavenge existing ones. However, flavonoids' antioxidant activity is a result of their capacity to both prevent the production of free radicals and scavenge existing ones [47]. Terpenoids have numerous biological properties, including tumor chemopreventive, antibacterial, antifungal, antiviral, anti-hyperglycemic, anti-inflammatory, anti-parasitic action, and memory boosters [32 a, b]. Terpene can also be used to increase skin penetration and protect against autoimmune disorders. Terpenes can also be converted into adhesives, glues, pesticides, curing agents, and other compounds utilized in agriculture, chemistry, and other fields [48]. Tannins are widely used in alcoholic beverages as a clarifying agents and as a flavoring component in both alcoholic as well as nonalcoholic beverages and juices. Tannins originated from a variety of plant-based sources and are also frequently utilized in the wine industry. Tannins can also be used as a mordant, which is especially useful in the natural dyeing of cellulose fibers like cotton [27].



(**A)** **(B)** **(C)**



(D) **(E)** **(F)**

Figure 2: Qualitative analysis of phytochemicals of spices (A) Alkaloid, (B). Flavonoids, (C) Terpenoids, (D) Tannins, (E) Carbohydrates (F) Proteins.

Carbohydrates metabolism is an important process that provides a constant supply of energy for living cells. The body produces glucose from carbohydrates, which is then turned into energy to power biological functions and physical activities. Carbohydrates are abundant and have several biological roles [10]. Carbohydrate-based or carbohydrate-modified medications are frequently employed in cardiovascular and haematological treatments spanning from inflammatory disorders and antithrombotic therapy to wound healing [30]. They give energy, help in the regulation of blood sugar and insulin metabolism, aid in the metabolism of cholesterol and triglycerides, and promote fermentation [32 a, b]. When sugar is ingested, the digestive tract begins the process of breaking it down into glucose, which is required for energy. Proteins help to repair and development of bodily tissues and boosts immunity while stimulating metabolic activities, balancing pH and fluid balance. Protein also helps in boost our immune system [49].

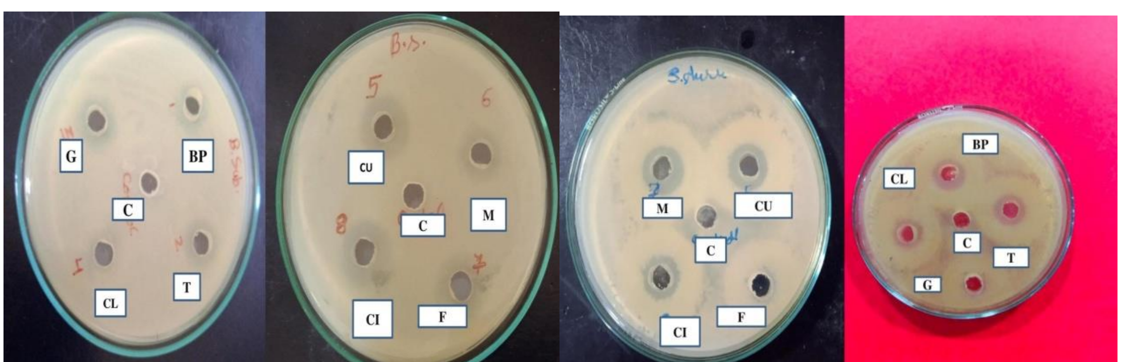
All experiments to measure the zone of antibacterial activity (inhibition) were conducted in triplets, and the zone of activity was determined in mm in diameter by considering both the X and Y axes, from which the disc diameter (X2) was subtracted and then divided by 4 to calculate the average zone of inhibition. On comparison of data presented in Table 3 with simple standard error, reveals that aqueous extract of black pepper was highly active against *Staphylococcus aureus* (ATCC6539), *Bacillus subtilis* (ATCC6633), *Salmonella enterica* (ATCC14028) and *Escherichia coli* (ATCC8739) while water extract of clove showed high activity only against *Shigella flexneri* (ATCC12022) and *S. enterica*. It may be because of presence of large amounts water insoluble clove oil not extracted in aqueous extract. Gupta *et al.* [6a, b, & 50] had earlier showed high activity of cinnamon, herbal and clove oils with organic solvents and tested their anti-microbial activities with Dimethyl sulfoxide (DMSO) as solvent. The biomolecules soluble in organic solvents are not normally available to the organisms in active form under natural conditions, and the present study with aqueous extract of spices against food borne and Enteropathogens is more relevant as far as their availability in natural conditions is concerned. *Salmonella enterica* (ATCC14028) and *Escherichia coli* (ATCC8739) were inhibited by aqueous extract of all test spices - turmeric, black pepper, fenugreek, garlic, mustard, clove, cumin and cinnamon (Table 3). *Bacillus subtillis* (ATCC6633) was inhibited by turmeric, black pepper and garlic while *Staphylococcus aureus* (ATCC6539 was highly sensitive to black pepper and turmeric. Prakash *et al*. (2023) [51] in our laboratories have isolated a large number of mupirocin resistant *Staphylococcus aureus* strains and we found that some of these resistant strains were sensitive to aqueous extracts of black pepper and we are conducting further experiments to find out the active biomolecules that can inhibit the growth of antibiotic resistant Staphylococci. *Cronobacter sakazakii* (ATCC28544) was selectively sensitive to fenugreek, cumin and cinnamon while *Shigella flexneri* (ATCC12022) was sensitive to clove, mustard and cinnamon and *Pseudomonas aeruginosa* (ATCC9027) showed sensitive to aqueous extracts of cinnamon alone. *Vibrio cholerae* (ATCC 3906) showed resistant against all aqueous extracts of the test spices. This selective anti-bacterial activities of the spices may be attributed to varying amounts of phytochemicals in aqueous extracts of the test spices (Table 3, Fig 3).

For thousands of years, spices have been used in food preservation, medicines, supplements, additives in foods for flavour taste and colour, including as herbal remedies [52]. Scientific research on traditionally used medicinal herbs in their natural available state as aqueous extracts is critical for improving medical standards [40]. Spices and their byproducts are perfect substitutes in food preservation methods, maintaining the consistency of biological and nutritional qualities, as well as the quality and safety of the food products [53]. The plant's therapeutic qualities may be due to the presence of various phytochemicals that exhibit antioxidant and antibacterial effects [40]. Phytochemical screening suggested the presence of phytochemicals such as alkaloids, saponins, flavonoids, phenols, tannins, glycosides, and carbohydrates in aqueous extracts of the spices tested in the present study which confirms the earlier reports of Abdisa and Kenea [54].

Antimicrobial activity of black pepper is very popular [4] and has great pharmaceutical value in the prevention and cure of cough and cold including the oral congestion [31a, b] have found *Staphylococcus aureus* as the most common causal organism of bacterial infection in the mouth and the high antibacterial activity of black pepper in the present study against *Staphylococcus aureus* confirm its medicinal property. Black pepper has considerable antibacterial activity and is used in traditional medicine to treat a variety of diseases [55]. Turmeric has been used to treat coughs, bronchitis, chest asthma, and colds for centuries, and its therapeutic effectiveness has been related to the existence of natural metabolites present in its root (rhizome), notably curcumin and cinole, which have anticancer and anti-inflammatory activities [56]. Scientific literature shows that garlic possess considerable levels of different important elements like vitamin C and vitamin B6, which are required for human health [23] besides several antimicrobial components. Fenugreek (*Trigonella foenum-graecum)* contains trigonelline, choline, gentianine, and carpaine, that show high biological activity in the neurological, cardiovascular, and anti-diabetic systems [57], was active *Salmonella enterica*, *E. coli* and *Cronobacter sakazakii*. Use of spice consumption has been linked to a variety of health benefits [30] including significant antimicrobial actions [58]. These properties are mostly due to the presence of biologically active chemicals that inhibit the growth of pathogenic and food-spoilage organisms [59]. Yeasts like *Candida albicans, Aspergillus spp.* and *Penicillium spp.* [60] and bacteria like *E. coli* and *Staphylococcus aureus*, well-known opportunistic microbes (normal components of human microbiota) can cause diseases in gastrointestinal tract, skin, soft tissue, circulatory system, respiratory system, and urogenital system [61]. Screening and identification of natural herbal parts as antimicrobial agents can reduce the need for antibiotics to treat specific conditions and also by preventing the formation of antibiotic resistance [62] Around 80 % of the world population continue to rely on plants for their healthcare, and 95% of modern medications have been derived from traditional medicinal plants [63]. This study is unique from the point of view that throughout the investigation we have used aqueous extract of spices for phytochemical screening as well as for antibacterial testing to find out their actual role as additives in food products.

Table 3. Zone (mm) of antibacterial (inhibition) of aqueous extract of spices against test bacteria on Müller-Hinton agar medium. Each figure is an average of three independent replicates

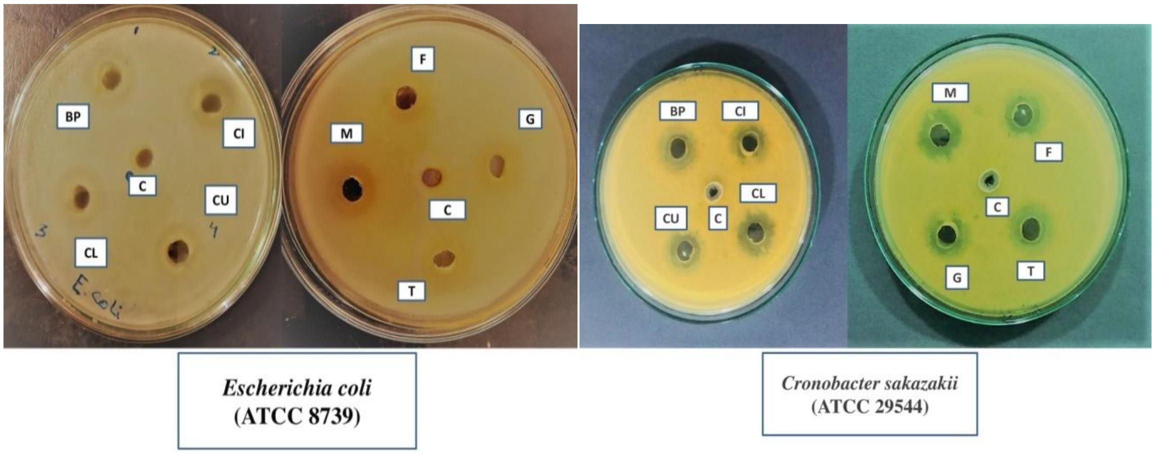
Spices / Pathogens	Turmeric	Black pepper	Fenugreek	Garlic	Mustard	Clove	Cumin	Cinnamon
Bacillus subtilis (ATCC 6633)	15±1.1	**20.5±1.1**	11±1.0	14.25±1.2	10.6±1.4	6.4±1.1	6.4±1.0	6.7±1.5
Salmonella enterica (ATCC 14028)	14±1.0	**16±1.0**	15±1.0	15±1.3	13±1	15±1.2	13.3±1.2	9.2±1.0
Staphylococcus aureus (ATCC 6539)	15±1.2	**21.5±1.3**	8.5±1.1	11.5±1.0	7±1.0	3.1±1.1	5.4±1.1	5±1.0
Escherichia coli (ATCC 8739)	11±1.0	**15.25±1.2**	12.75±1.2	14.75±1.1	12.4±1.5	13.4±1.0	14.5±1.0	6±1.1
Cronobacter sakazakii (ATCC 29544)	6±1.0	11.2±1.2	12.1±1.1	9±1.3	9±1.0	6±1.2	14±1.2	**16±1.0**
Shigella flexneri (ATCC 12022)	6±1.3	7±1.2	11±1.0	9.5±1.2	12.1±1.1	**16±1.0**	13±1.0	12±1.2
Pseudomonas aeruginosa (ATCC 9027)	9±1.2	8±1.0	10.5±1.2	11±1.0	9.3±1.2	11±1.2	9.1±1.1	**12.5±1.2**
Vibrio cholerae (ATCC 3906)	10±1.0	7.1±1.1	5.5±1.2	7.4±1.1	8.3±1.2	7±1.0	8±1.0	7.5±1.2



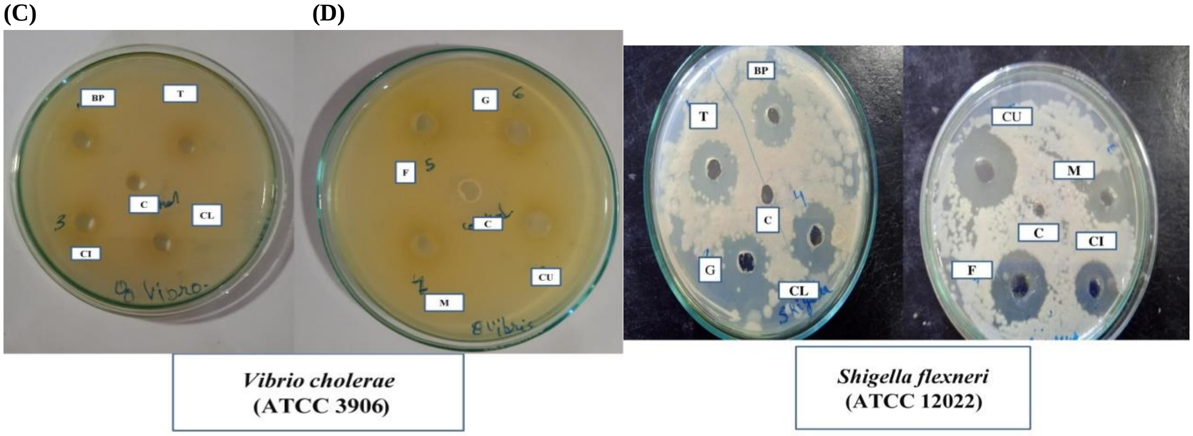
Bacillus subtilis **(ATCC 6633)**

Staphylococcus aureus **(ATCC 6538)**

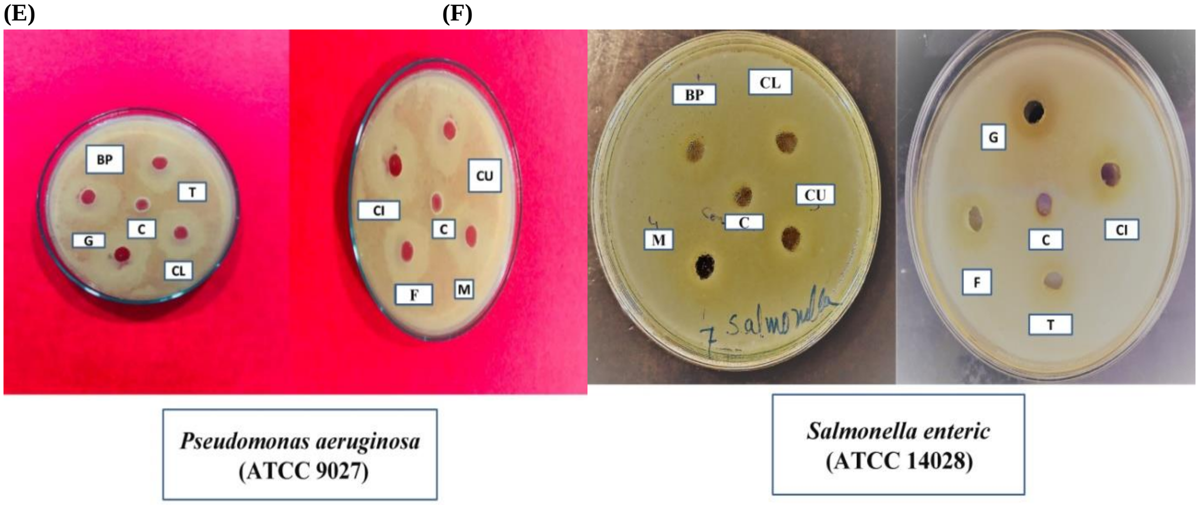
(A) (B)



(C) **(D)**



(E) **(F)**



(G) **(H)**

Fig 3: Antimicrobial Activity of spices against common food borne pathogens zone of Inhibition (A) *Bacillus subtilis* (ATCC 6633), (B) *Staphylococcus aureus* (ATCC 6538), (C) *Escherichia coli* (ATCC 8739),(D) *Cronobacter sakazakii* (ATCC 29544) (E) *Vibrio cholerae* (ATCC 3906) (F) *Shigella flexneri* (ATCC 12022) (G) *Pseudomonas aeruginosa* (ATCC 9027) and (H) *Salmonella enterica* (ATCC 14028).

Conclusions

Black pepper exhibited highest activity against *Staphylococcus aureus, Bacillus subtilis, Salmonella enterica,* and *Escherichia coli* as compared to turmeric, garlic, cumin, clove, cinnamon, mustard and fenugreek whereas cinnamon showed highest zone of antibacterial (inhibition) against *Cronobacter sakazakii* and *Pseudomonas aeruginosa*. Aqueous extracts of clove exhibited antibacterial activity against *Shigella flexneri, Salmonella enterica* and *E. coli*. Cumin was moderately active against *E. coli, Cronobacter sakazakii, Salmonella enterica* and *Shigella flexneri*. Similarly, garlic was active against *Salmonella flexneri, E. coli* and *Bacillus subtilis*. Turmeric showed highest zone of antibacterial activity against *Bacillus subtilis, Salmonella enterica* and *Staphylococcus aureus* while *Vibrio cholerae* showed moderate sensitivity to test spices turmeric, clove, garlic, cumin, cinnamon, black pepper, mustard and fenugreek. It may safely be concluded that all spices have antibacterial activities against one or the other food borne and Enteropathogens and none of the spice is active against all test pathogens. It, is therefore, suggested that a combination of spices in Indian kitchen as additive in food products is advantageous over single use of an spice. The constituent phytochemicals of Indian spices have pharmaceutical and medicinal properties and their active water soluble components need to be further purified, identified and documented as part of Ayurveda.

Conflict Of Interest: The authors declare that they have no conflict of interest.

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