**In vitro** bioactivity and phytochemical screening of selected spices used in Mauritian foods

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**1. Introduction**

Spices, which include leaves (coriander, mint), buds (clove), bulbs (garlic, onion), fruits (red chili, black pepper), stem (cinnamon), rhizomes (ginger) and other plant parts, have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste, used to enhance the taste of foods[1]. Herbs and spices have been used during the Middle Ages for flavoring, food preservation, and/or medicinal purposes. Today many ethnic cuisines are recognized for their reliance on “signature” herbs and spices. Turmeric in Indian cuisine; basil, garlic, and oregano in Italian and Greek cuisines; and lemongrass, ginger, cilantro, and chili peppers in Thai food represent some of the cultural diversity in the use of herbs and spices. Satia–Abouta et al. reported that the cuisines of Asia, Southeast Asia, and the Mediterranean are perceived by many to be healthier than the typical Western diet[2]. Several studies have attributed the antimicrobial, antioxidant and pharmaceutical properties...
of spices and herbs to their phenolic compounds[3]. Mauritian cuisine is diverse and consists of a mixture of Indian, Chinese, European and African cuisines. Spices are basic ingredients in most of the dishes in Mauritius. Also, to treat minor ailments, several kinds of extracts from several endemic, exotic and indigenous plants are sold as decoctions or ‘tisanes’ in the Mauritian markets[4]. There is at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. and render spices and some herbs or their antioxidant components as preservative agents in food[5]. Antioxidants have a long history of use in the nutrition/health community and food industry. The traditional understanding has been that antioxidant chemicals promote health by removing reactive species that may otherwise exert harmful metabolic effects. In general, maximizing antioxidant concentrations is thought to minimize the risk for chronic disease[6]. Several studies have shown that spices are able to counteract oxidative stress in in vitro and in vivo systems[7,8]. According to a phytochemical database[9], the number of different antioxidants in some plants can reach up to 40. In the present study, an attempt was made to evaluate the antioxidant and antimicrobial activities besides the phytochemicals in six commonly used spices in Mauritian dishes with a view to evaluate their bioefficiency for their possible pharmaceutical applications.

2. Materials and methods

2.1. Spice materials

The following spices were used in this study and they were obtained from a local supermarket: ajwain seeds (Trachyspermum ammi), coriander seeds (Coriandrum sativum L), cinnamon bark (Cinnamomum verum), fennel seeds (Foeniculum vulgare), garlic cloves (Allium sativum), and dried turmeric powder (Curcuma longa).

2.2. Preparation of extracts

A decoction method was used to obtain aqueous extracts of the spices. A total of 500 g of each spice was added to 2.5 L of boiling distilled water and left to simmer for 1 h. The solutions were allowed to cool, filtered using muslin cloth and stored at −20 °C when not in use. Some of the solutions obtained were freeze-dried and the powdered samples obtained were stored at 4 °C. And 25 g of the fine powder was added to a conical flask together with different solvents (acetone, methanol, ethanol and water) for extraction of phytochemicals.

2.3. Phytochemical screening

A qualitative phytochemical test to detect the presence of alkaloids, flavonoids, saponins, steroids, tannins, coumarins and phenols was carried out using standard procedures[10–13].

2.4. Determination of total phenolic content

The total phenolic content of plant extract was determined using Folin–Ciocalteau reagent[14]. In brief, to 250 µL of Folin–Ciocalteu’s reagent, 10 µL of sample was added, followed by 3.5 mL of deionised water. After 3 min, 1 mL of 20% sodium carbonate was added. The mixture was vortexed and incubated at 40 °C for 40 min. It was allowed to cool in the dark. The absorbance was measured at 685 nm and all determinations were carried out in duplicates. A standard curve was obtained using various concentrations of gallic acid. The results were expressed as mg gallic acid equivalent/g dry weight of material.

2.5. Determination of total flavonoid content

Total flavonoids were measured by AlCl₃ colorimetric assay[15]. Briefly, to 500 µL of extract and 2000 µL of distilled water, 5% of sodium nitrate was added. After 5 min, 150 µL of 10% AlCl₃ was added. A total of 2000 µL of sodium hydroxide (1 mol) was added after 1 min followed by 1200 µL of distilled water. The mixture was vortexed and incubated for 30 min. The absorbance was measured at 510 nm against a prepared blank. A yellow color indicated the presence of flavonoids. Quercetin was used as a standard to generate a standard curve. The total flavonoids content of samples were determined in duplicates and the results were expressed as mg quercetin acid equivalent/g dry weight of material.

2.6. Antioxidant activity

A modified Benzie & Strain ferric reducing antioxidant potential (FRAP) method was used to obtain the total antioxidant capacity[16]. FRAP assay depends on the reduction of Fe³–TPTZ to Fe²–TPTZ. The FRAP reagent consisting of 10 mL of 10 mmol TPTZ solution in 40 mmol/ L HCl and 10 mL of 20 mmol/L ferric chloride in 200 mL of 0.25 mol sodium acetate buffer (pH 3.6) was freshly prepared and warmed at 37 °C. The spice extract (20 µL) was allowed to react with 4500 µL of the FRAP solution and 480 µL of water for 30 min in the dark at 37 °C. All determinations
were carried out in duplicate. Absorbance was read at 593 nm. The standard curve was linear between 0 and 1 mmol/L FeSO$_4$·H$_2$O.

2.7. Antimicrobial activity

An agar–well diffusion method was employed for the determination of antibacterial activities of the aqueous extracts of the spices. And 3.5 g of the each freeze–dried extracts were weighed and dissolved in distilled water. And 100 µL of either S. aureus or E. coli suspension was spread on EMB medium. Five wells of 0.8 mm diameter were punched over the agar plates using sterile gel puncher (cork borer). Then, 100 µL of the spice extracts were added into the wells using a micropipette. There were five replicates for each spice and for each bacterium tested. The plates were incubated at 37 °C for 24 h. The zone of clearance around each well after the incubation period confirms the antimicrobial activity of each spice extract.

2.8. Statistical analysis

The spectrophotometric tests were performed in duplicates and the results were expressed as mean±range. The antibacterial results were statistically analysed using Minitab statistical software version 16 for Windows at a 5% significance level. One–way analysis of variance (ANOVA) and Tukey’s test were carried out to test any significant differences among different aqueous spice extracts. Values of $P<0.05$ were considered as significantly different ($\alpha=0.05$),

3. Results

3.1. Phytochemical screening

Phytochemical screening of the extracts showed that the solvent extracts contain most of the phytochemicals like alkaloids, coumarins, flavonoids, saponins, steroids, tannins and phenols (Table 1).

### Table 1

Phytochemical constituents of the six spices.

| Phytochemicals | Spices | Cinnamon | Ajwain | Fennel | Garlic | Turmeric | Coriander |
|----------------|--------|----------|--------|--------|--------|----------|----------|
| Alkaloids      | +      | +        | -      | -      | +      | +        | +        |
| Coumarins     | +      | +        | +      | +      | +      | +        | +        |
| Flavonoids    | +      | +        | +      | +      | -      | -        | -        |
| Saponins      | +      | +        | +      | +      | +      | -        | -        |
| Steroids      | +      | +        | +      | +      | +      | +        | +        |
| Tannins       | +      | +        | -      | -      | +      | +        | +        |
| Phenols       | +      | +        | +      | -      | -      | -        | +        |

*: presence of phytochemical; -: absence of phytochemical.

3.2. Total phenolic content

The amount of phenolics varied widely, ranging between 177 and 1 890 mg GAE/g DW (Table 2). The aqueous extract of cinnamon showed the highest level of phenolics while turmeric showed the lowest ($P<0.05$). The total phenolic content of fennel and coriander was not significantly different from each other. Also, there were no significant differences between phenolic content of garlic and coriander.

### Table 2

Total phenol and total flavonoid contents of the six spices ($n=2$).

| Spices       | Total phenol$^a$ | Total flavonoid$^b$ |
|--------------|------------------|---------------------|
| Cinnamon     | 1890.360±27.353  | 20.910±1.491        |
| Ajwain       | 1227.240±0.00    | 37.570±3.946        |
| Fennel       | 779.980±112.100  | 18.040±0.00         |
| Garlic       | 271.030±10.522   | 3.990±0.126         |
| Turmeric     | 176.870±10.305   | 2.810±0.062         |
| Coriander    | 594.780±42.685   | 10.560±0.545        |

Data are expressed as mean±standard deviation.

$^a$ mg gallic acid equivalent/g DW; $^b$ mg quercetin equivalent/g DW; DW: Dry weight.

3.3. Total flavonoid content

With regards to the flavonoid contents, they varied from (2.800±0.062) to (37.570±3.946) (Table 2). Ajwain had the highest flavonoid content (37.6±3.95 mg QE/g DW) whilst turmeric had the lowest (2.8±0.06 mg QE/g DW) ($P<0.05$). Flavonoid contents of cinnamon and fennel were not significantly different. The total flavonoids of garlic was also not significantly different from those of turmeric and coriander. The decreasing order of flavonoid contents was ajwain>cinnamon>fennel>coriander>garlic>turmeric.

3.4. Antioxidant activity

The antioxidant effectiveness of the six spice extracts was assessed by the FRAP assay. The results are summarized in Table 3. The highest FRAP value ($P<0.05$) was observed in cinnamon with 24.93±0.198 µmol Fe$^{3+}$/g DW while turmeric showed the lowest antioxidant activity ($P<0.05$) with (5.980±0.313) µmol Fe$^{3+}$/g DW. Significant differences were observed between the total antioxidant activities of the spices ($P<0.05$) except between garlic and fennel. The decreasing order of antioxidant activity was: cinnamon>coriander>ajwain>fennel>garlic>turmeric.

### Table 3

Total antioxidant capacity (µmol Fe$^{3+}$ equivalent per g DW) of the six spice extracts ($n=2$).

| Spice Extract | FRAP (µmol Fe$^{3+}$/g DW) |
|---------------|---------------------------|
| Cinnamon      | 24.93±0.198               |
| Ajwain        | 13.570±0.126              |
| Fennel        | 12.140±0.115              |
| Garlic        | 10.800±0.489              |
| Turmeric      | 5.980±0.313               |
| Coriander     | 18.530±0.517              |

Data are expressed as mean±range.

Data was analysed by One–way ANOVA followed by Tukey’s multiple comparison test; Different letters between rows represent significant difference between samples ($P<0.05$).
3.5. Antimicrobial activity

The antibacterial activities of the spices’ extracts are summarised in Table 4. All the aqueous extracts of the spices showed antibacterial activities against both *S. aureus* and *E. coli*. In the case of *E. coli*, the zones of inhibition varied from (11.20±0.74) mm to (24.00±1.10) mm while in the case of *S. aureus* it varied from (11.20±0.74) to (24.00±1.10). The aqueous extract of turmeric showed the highest antibacterial activity (P<0.05) against *S. aureus* with a (26.10±2.09) mm inhibition zone while the extract of garlic showed the lowest antibacterial activity (P<0.05) against both *E. coli* and *S. aureus* with an inhibition zone of (11.90±1.29) and (11.90±1.29) mm respectively. When *S. aureus* was used as the test organism, the differences between the antibacterial activities of the aqueous extract of the spices were statistically significant (P<0.05) except for ajwain and fennel with no significant differences between them. In the case of *E. coli*, there were no significant differences between the antibacterial activities of cinnamon and ajwain as well as between the activities of ajwain and fennel, and the activities of turmeric and coriander (P>0.05).

### Table 4

| Spice Extract | *E. coli* (mm) | *S. aureus* (mm) |
|---------------|----------------|------------------|
| Cinnamon      | 24.0±1.10      | 17.6±0.78        |
| Ajwain        | 22.5±1.58ab    | 19.6±1.87        |
| Fennel        | 21.8±0.96c     | 21.0±2.18        |
| Garlic        | 11.2±0.74d     | 11.9±1.29        |
| Turmeric      | 18.0±2.04ab    | 26.1±2.09        |
| Coriander     | 17.9±0.78      | 23.0±1.51        |

Data are expressed as mean±standard deviation, (n=15)

Data was analysed by One-way ANOVA followed by Tukey’s multiple comparison test.

Different letters between rows represent significant difference between samples (P<0.05).

3.6 Correlation between antibacterial activities of spice extracts and total antioxidant capacity

The correlation between total antioxidant capacity and antibacterial activities of the aqueous extracts is shown in Figure 1. The linear correlation coefficient data revealed that the antibacterial activities of the extracts against *S. aureus* were negatively correlated (r=-0.231) with the total antioxidant capacities of the spices whereas for *E. coli*, the antibacterial activities were positively correlated (0.493) to the antioxidant activity.

![Figure 1](image1.png)

**Figure 1.** Relationship between total antioxidant capacity of the spice extracts and antibacterial activity.

![Figure 2](image2.png)

**Figure 2.** Relationship between total phenolic contents of the spice extracts and antibacterial activity.
3.7. Correlation between antibacterial activities of spice extracts and total phenolic content

The correlation between antibacterial activity and total phenolic contents (Figure 2) showed that in the case of *E. coli*, the antibacterial activity was strongly correlated ($r = 0.790$) with the total phenolic contents of the spice extracts while in the case of *S. aureus*, the antibacterial activity showed a poor negative correlation ($r = -0.186$) with the total phenolics.

3.8. Correlation between antibacterial activities of spice extracts and total flavonoid content

It was observed that the antibacterial activities of the extracts against *S. aureus* was very poor ($r = -0.058$). However, a strong correlation ($r = 0.728$) was observed between the antibacterial activities of the spices against *E. coli* and total flavonoids (Figure 3).

3.9. Correlation between total phenolic content, total flavonoid content and antioxidant potential

The correlation between total antioxidant capacities of the aqueous spice extracts and phenolic content was also assessed. The data revealed that both total phenolic and flavonoid contents were positively correlated with the antioxidant capacities of the extracts (Figure 4). The linear correlation coefficient values showed that the total antioxidant capacity was more strongly correlated to total phenols ($r = 0.822$) than it was to total flavonoid ($r = 0.376$).

4. Discussion

The results of the present study suggested that several phytochemicals as well as antioxidant properties are present in all the six spices extracts. The presence of the phytochemicals can be correlated with the fact that solvent extracts showed antibacterial activity against the bacterial strains. Phytochemicals give plants their colour, flavour, smell and are part of a plant’s natural defense system and protect them against herbivorous insects and vertebrates, fungi, pathogens, and parasites[17,18]. The phytochemicals alkaloids, coumarins, flavonoids, saponins, steroids, tannins and phenols were present in the aqueous extracts of cinnamon, fennel and ajwain. The results are in accordance with the findings of other authors who have studied these spices[19-23]. The cinnamic acid pathway has phenylalanine as starting material and produces many phenolic acids, coumarins, flavonoids, isoflavonoids and lignans[24]. Alkaloids were absent in extracts of garlic, turmeric and coriander while tannins and phenols were absent in extracts of both garlic and turmeric. Some of the important
components detected in fennel recently were trans-anethole, fenchone, estragole (methyl chavicol), and α-phellandrene[25]. On the other hand, the active ingredients of turmeric include the curcuminoids diferuloylmethane (curcumin I), demethoxycurcumin (curcumin II), bisdemethoxycurcumin (curcumin III) and cyclocurcumin[26]. Perusal of literature revealed that the phytochemical content of spices may be influenced by a number of factors including: physiological and morphological characteristics intrinsic to the cultivar, the level of expression of the genes, and pedo-climatic growth conditions[27].

Phenolic compounds can be classified into phenolic acids, flavonoids, tannins, phenolic volatile oils and others. Phenolic acids include two subclasses, namely hydroxycinnamic acids (e.g. coumaric acid, ferulic acid, caffeic acid, and chlorogenic acid) and hydroxybenzoic acids (e.g. protocatechuic acid, gallic acid, and vanillic acid). Flavonoids constitute the largest class of phenolic compounds, and they are ubiquitous in plants. Flavonoids have the basic skeleton of diphenylpropanes (C6–C3–C6) with different oxidation of the central pyran ring, and further subclassified as flavones (e.g. apigenin and luteolin), flavonols (e.g. galangin, kaempferol, quercetin, rutin, and isorhamnetin), flavanones (e.g. naringenin, naringin, and hesperetin), isoflavones (e.g. genistin and daizin), and anthocyanidins (e.g. apigeninid and cyanidin). The number of natural polyphenols has been estimated to be over one million, because they often occur as glycosides with one or more sugar moieties[28–30]. Phenolic compounds are commonly present in spices and have been reported to have several biological activities including antioxidant activity. In our studied spices we found high amount of phenolic compounds, an indication of their strong antioxidant capacities. Phenolic compounds are considered as one of the most effective antioxidants[31]. They have been reported to possess many biological activities such as cytotoxic antitumour activity, vascular activity, antioxidant activity, anti-allergic activity, antimicrobial activity, anti-inflammatory activity, enzyme inhibition and antimicrobial activity[32]. Quantitative results of phenolic acids and flavonoids in the spices studied were displayed. The highest total phenolic content was obtained in cinnamon bark extract (1890.36±27.353 mg GAE/g DW) and the lowest in turmeric extract (176.87±10.305 mg GAE/g DW). The phenolic compounds present in cinnamon include 2-hydroxycinnamaldehyde, cinnamyl aldehyde derivatives and flavan–3–ols[33]. The figures obtained for turmeric in our study however, is higher than the ethanolic extract (120.34 mg GAE/g) and ethanol/water extract (120.05 mg GAE/g) obtained by another author[34]. In terms of total flavonoids content, the highest value was obtained for ajwain (37.57±3.946 mg QE/g DW) and the lowest for turmeric (2.81±0.062 mg QE/g DW) extracts. The differences in the total phenolic and total flavonoid of the spices may be accounted for by the different phenolic compounds present in these spices as well as in different amounts. For example, protocatechuic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, apigenin, luteolin, quercetin, rutin, galangal, naringin and catechinaldehyde are known to be absent in cinnamon while chlorogenic acid rutin are known to be present in fennel extract[33]. Fennel is also known to contain 1,5-odicaffeoylquinicacid, 3–caffeoylquinic acid, 4–caffeoylquinic acid, quercetin–3–O–galactoside, rosmarinic acid, eriodictyol–7–O–rutinoside, kaempferol–3–O–glucoside and kaempferol–3–O–rutinoside as phenolics[36].

In order to defend against damage from oxidation and reactive oxygen species (ROS), organisms have developed complex antioxidant systems to scavenge ROS thereby protecting key biological sites from oxidative damage. These antioxidants are either produced by the body or derived from diets. Consumption of food rich in antioxidants has been shown to play an essential role in the prevention of cardiovascular diseases, cancers, neurodegenerative diseases, inflammation and problems caused by cutaneous aging[37–42]. Phenolic compounds account for a major portion of the antioxidant capacity in many plants[43]. The antioxidant activity of phenolics may be due to their ability to terminate free radicals and chelate metal ions by rapid donation of a hydrogen atom or electrons to the oxidized molecule or radicals[44]. In our study the highest antioxidant properties were detected in cinnamon extracts (24.93±0.198 µmol Fe^2+/g DW) and the lowest in turmeric (5.98±0.313 µmol Fe^2+/g DW). The strong correlations we observed between antioxidant activities and phenolic content indicate phenolic compounds were a major contributor to the antioxidant activities in these spices. This is in agreement with several other studies that found strong correlations between high phenol content and high antioxidant activities in grapes, tomato, vegetables and herbs and medicinal plants[45–48]. Different phenolic compounds are responsible for the antioxidant properties of the six spices studied. The antioxidant property of garlic preparation in hot water is due to the production of dimethyl, diallyl, methyl allyl from thiosulfonates; the antioxidant capacity of the aqueous extract of coriander is due to the presence of limonanol, flavonoids, coumarins, catechins, terpenes and polyphenolic while curcumin is responsible for the antioxidant properties of turmeric[49,50]. Environmental variables such as processing and cooking may affect to a significant extent the concentrations and biological activities of the antioxidants in the spices, and consequently the amount of antioxidants available to the consumer. Reports of the effects of cooking on the antioxidant capacity of plant–based foods indicate this effect may be negative, positive or none at all[51–55].

Plants produce a high diversity of secondary metabolites in order to protect themselves from biotic stresses as well as abiotic stresses. There are many published reports on spice and herb extracts against different bacteria. However, difficulties arise in comparing the results due to different
methodologies used including solvents, concentrations, microbial strains and antimicrobial test methods[7]. In the present study, the aqueous extracts were found to be susceptible to gram–positive S. aureus and gram–negative E. coli. Usually, gram–positive bacteria are more sensitive to extracts of spice and herbs than are the gram–negative bacteria because of the differences in the cell envelope structure of the bacteria[56]. The gram–positive bacteria do not have an outer membrane and a particular periplasmic space that is present in the gram–negative bacteria. Antibacterial substances readily break the bacterial cell wall and cytoplasmic membrane of gram positive bacteria, resulting in the cytoplasm’s leakage and coagulation. Whereas in the gram–positive bacteria, the outer membrane has a hydrophilic surface which is rich in lipopolysaccharide molecules, and it provides protection against many antibiotic molecules. Moreover the enzymes in the periplasmic space have the ability to break molecules from the outside[3]. In our study all the six spice extracts produced inhibitory zones against both S. aureus and E. coli and is in accordance with other similar studies. In addition, many of these spices have shown to have antimicrobial properties against several pathogenic microorganisms[3]. Garlic, in particular has been shown to be active against numerous bacteria, viruses (herpes simplex, HIV and influenza and the fungi Aspergillus luchuensis, Aspergillus flavus, Rhizopus stolonifer, Mucor sp. and Scopulariopsis sp. [1,57-61]. Different solvent extracts of coriander have also proven to have effective antibacterial activities against a number of bacteria including Bacillus cereus, Bacillus subtilis, Pseudomonas auruginosa, Pseudomonas spp., Shigella dysentiriae, Salmonella typhi, Staphylococcus coagulase, Streptococcus fecaelis[62-66]. A strong correlation was observed between the antibacterial activities of the spices and total phenolic content.

We have discussed the in vitro bioactivity and phytochemicals of six spices commonly used in Mauritian dishes. Differences between this study and others might exist due to different varieties, growing conditions, and processing conditions amongst others.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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