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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF SOME SPICES AGAINST SELECTED FOODBORNE PATHOGENS

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ABSTRACT

Spices have been defined as fruit, root, bark, or other plant substance primarily used for flavoring, coloring or preserving food. The aim of this study was to evaluate the phytochemicals and antimicrobial properties of extracts of some spices that are commonly used for the production of ready meals against selected foodborne pathogens. Phytochemical analysis of ethanol and aqueous extracts of the spices were carried out using standard technique. Antimicrobial activity of the extracts was carried out using agar well diffusion technique. Results of the phytochemical analysis revealed that extract from the spices contained bioactive compounds such as flavonoids, phenols, alkaloid, tannins, steroids, saponins, terpenoids, phytates. The spice extracts of cumin, turmeric, cinnamon, nutmeg and coriander were tested for its antimicrobial activity against the following foodborne pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Enterobacter*. The results revealed that the ethanol extracts of different spices (MIC values of 20-80 µl/ml) have higher antimicrobial activities on all test organisms (range of inhibition 09-25 mm) as compared to aqueous extracts of spices in same concentration. Conclusively, the spices were potential source of phytochemical which can be used as natural supplements in the diet in order to encourage healthy way of living and for medicinal purposes.

Keywords: Spices, Extract, Phytochemical, Antimicrobial

1 INTRODUCTION

Spices and aromatic vegetable materials have long been used in food not only for their flavor, fragrance qualities and appetizing effects but also for their preservative and medicinal properties. [1]. Since ancient times, spices have been used to heighten the flavour and taste of cooked food [2]. According to the World Health Organization, a medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi synthesis [3]. Some spices are known to have antibacterial and antioxidant effects. They aid in the prevention and treatment of a range of diseases such as cancer, ageing, and metabolic, neurological, cardiovascular, and inflammatory disorders [4]. Their role in food safety and preservation has also been established (5). Since the ancient times, they have been used for preventing food spoilage and deterioration and also for extending the shelf life of foods [6]. Antibacterial agents, including food preservatives and organic acids, have been used to inhibit foodborne bacteria and extend shelf life of processed foods. Many naturally occurring compounds found in edible and medicinal plants, herbs and spices have been shown to possess antimicrobial function and could serve as a source of antimicrobial agents against food pathogens.[7] While certain spices are essential in the food industry, many are also useful for repressing irreversible infections and inhibit pathogens, particularly in traditional healthcare [6]. In Nigeria and other parts of Sub-Saharan Africa, indigenous spices are widely used as culinary ingredients in traditional dishes and soups and are also valued in traditional medicine for their perceived health benefits [8]. These spices are also said to be therapeutically useful in the management of stomach ache, leprosy, cough, and loss of appetite, rheumatoid pain, convulsion and inflammation [9]. Phytochemicals are naturally occurring plant-derived compounds, many of which function as secondary metabolites and contribute to plant defense and other physiological processes. [10]. Phytochemicals are divided into two groups; primary and secondary compounds. These classes are according to their functions in plant metabolism. Amino acids, sugars, proteins and chlorophyll are known as primary compounds while secondary compounds consist of alkaloids, terpenoids, phenolic compounds and many more [11]. Along with their protective abilities, Spices and herbs are believed to be the natural chemical component responsible for the antimicrobial effects against some pathogens. Various studies have shown that Spices and herbs are known to be effective against gram-negative as well as gram-positive bacteria, such as *Escherichia coli*, *Salmonella*, *Staphylococcus*, and *Streptococcus* species. Therefore, screening and testing the efficacy of plants are undertaken to explore their antibacterial activity [12].

1.1 Sample collection

The spices namely cumin, turmeric, cinnamon, nutmeg and coriander were purchased from different markets located Ado-Ekiti, Nigeria.

1.2 Preparation of spice extract

Each sample was separately cleaned of dirt and dust particles and then sun dried to constant weight before rubbing and grinding to a powder using a desk top mill fitted with a 500 µm sieve. About 100g of the ground spices was then put in plastic container, sealed and stored at 4°C in a refrigerator until required for analysis.

1.3 Test Organisms

Pure cultures of *Staphylococcus aureus*, *Escherichia coli*, *Shigella dysenteriae*, *Pseudomonas aeruginosa* and *Enterobacter* were obtained from the Microbiology laboratory of Science Laboratory Technology department of Federal Polytechnic Ado-Ekiti. They were re-identified and stored at 4°C on the appropriate agar slant.

1.4 Antimicrobial activity of Spices

The antibacterial activities of the extracts were tested against the selected bacterial strains. Agar well diffusion method was used for screening for antimicrobial activity. Seven wells were made on each Mueller Hinton agar plate seeded with the test organism using a 6-mm cork borer. Six of the wells contained the extracts while the seventh well (made at the center of the plate) contained ethanol as a control. 20mL of sterilized agar medium was poured into each sterile Petri plates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5cm was made in the medium by using a sterile cork borer, 150 µL of each ethanol and aqueous extracts were transferred into separate wells. After these, plates were incubated at 37°C for 24-48 hours. After incubation period, the results were observed and measure the diameter of inhibition zone around the each well [13]

1.5 Antimicrobial Activity

The in vitro screening for antimicrobial study was carried out using selected pathogens which includes gram negative bacteria and gram positive bacteria. The antibacterial screening of the extracts were carried out by determining the zone of inhibition using well diffusion method. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37°C for 24 hr and were referred to as

seeded broth. The extracts were prepared by reconstituting with methanol. The test microorganisms were seeded into respective medium by spread plate method 10 μ l (10 cells/ml) with the 24 hr cultures of bacteria growth in nutrient broth. One ml of this was used in flooding over nutrient agar plates in the well diffusion method of the in vitro antimicrobial sensitivity test. The plates were left for 5 mins after which they were dried at 37^o C for 1 hr. Wells were bored in the plate using a sterile cork borer. One hundred microlitres of the various spices' extract were put inside the wells. Methanol were put inside the well in separate petridishes to serve as negative control while Chloramphenicol (1mg/ml) was used as positive control in the separate petridishes. The plates were left free for 1 hour after which there were incubated at 37^oC for 24 hrs and were examined for zones of inhibition. The diameter of inhibition zones were measured in mm and the results were recorded. Inhibition zones with diameter less than 12mm were considered as having no antimicrobial activity. Diameters between 12 and 16mm were considered moderately active and with >16mm were considered highly active [14].

1.6 Phytochemical Screening

The essential oils of each spice were subjected to qualitative and quantitative phytochemical analysis to detect the presence of alkaloids, flavonoids, tannins, glycosides, saponins, steroids, and terpenoids, phenolics, phytate, oxalate using standard procedures described by [15].

2 RESULTS

Table 1: Antimicrobial activity of Ethanolic and Aqueous extract of Cumin against selected foodborne pathogens (μ l/ml)

	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Isolates	0.2	0.2	0.4	0.4	0.6	0.6	0.8	0.8
<i>Staphylococcus aureus</i>	14	15	16	16	20	18	25	20
<i>Escherichia coli</i>	10	12	15	17	17	19	20	22
<i>Shigella dysenteriae</i>	10	11	13	13	15	15	20	16
<i>Pseudomonas aeruginosa</i>	10	12	11	14	13	16	16	19
<i>Enterobacter</i>	10	10	13	13	15	15	20	19

Table 2: Antimicrobial activity of Ethanolic and Aqueous extract of Turmeric against selected foodborne pathogens (μ l/ml)

	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Isolates	0.2	0.2	0.4	0.4	0.6	0.6	0.8	0.8
<i>Staphylococcus aureus</i>	12	11	14	13	16	16	19	19
<i>Escherichia coli</i>	11	11	14	13	15	16	19	19
<i>Shigella dysenteriae</i>	15	11	19	13	20	16	25	21
<i>Pseudomonas aeruginosa</i>	11	10	14	12	17	16	20	19
<i>Enterobacter</i>	10	10	14	12	17	15	20	18

Table 3: Antimicrobial activity of Ethanolic and Aqueous extract of Cinnamon against selected foodborne pathogens (µl/ml)

	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Isolates	0.2	0.2	0.4	0.4	0.6	0.6	0.8	0.8
<i>Staphylococcus aureus</i>	11	13	14	17	16	20	19	22
<i>Escherichia coli</i>	10	10	14	12	19	14	21	16
<i>Shigella dysenteriae</i>	12	10	15	13	17	15	22	20
<i>Pseudomonas aeruginosa</i>	11	11	14	13	15	15	22	18
<i>Enterobacter</i>	09	09	11	13	13	15	17	20

Table 4: Antimicrobial activity of Ethanolic and Aqueous extract of Nutmeg against selected foodborne pathogens (µl/ml)

	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Isolates	0.2	0.2	0.4	0.4	0.6	0.6	0.8	0.8
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-
<i>Escherichia coli</i>	14	12	17	14	19	16	22	19
<i>Shigella dysenteriae</i>	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-
<i>Enterobacter</i>	10	11	11	12	12	14	14	16

Table 5: Antimicrobial activity of Ethanolic and Aqueous extract of Coriander against selected foodborne pathogens (µl/ml)

	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous
Isolates	0.2	0.2	0.4	0.4	0.6	0.6	0.8	0.8
<i>Staphylococcus aureus</i>	-	11	-	13	-	16	-	20
<i>Escherichia coli</i>	-	15	-	17	-	20	-	24
<i>Shigella dysenteriae</i>	10	10	13	14	16	15	20	20
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-
<i>Enterobacter</i>	10	12	11	14	15	16	19	18

Table 6: Phytochemical Screening

S/N	Secondary metabolites	INFERENCE				
		A	B	C	D	E
1	Alkaloid	+	+	++	++	+++
2	Tannin	+	++	+	++	+++
3	Oxalate	-	-	-	-	-
4	Saponin	+	+	+	++	+++
5	Flavonoid	+	++	+++	+++	+++
6	Steroid	+	+	+	+	+
7	Terpenoid	+	+	++	++	++
8	Phenolics	+	++	+++	+++	+++
9	Glycosides	+	+	+	+	++
10	Phytate	+	+	+	++	++

Key: +++Highly present, ++Very present, +Present, -Absent.; Keys: A= Cinnamon; B= Nutmeg; C =Turmeric.; D = Coriander; E= Cumin

Table 7: Phytochemical Constituents

S/N	Secondary metabolites	CONSTITUENTS				
		A	B	C	D	E
	(%)					
1	Alkaloid	0.9877	1.000	1.5132	1.7431	2.1412
2	Tannin	1.0002	1.4131	1.3245	1.4432	1.5722
3	Oxalate	ND	ND	ND	ND	ND
4	Saponin	0.7765	0.8773	0.7990	0.9932	1.034
5	Flavonoid (mg)	6.0041	7.1132	9.0024	10.173	14.1123
6	Steroid	0.6341	0.8823	0.9911	1.0333	1.4622
7	Terpenoid (mg/100g)	0.1467	0.6124	0.8771	1.0421	0.9934
8	Phenolics(mg)	8.743	9.1000	12.008	14.113	17.174
9	Glycosides (mg/100g)	0.4677	0.6523	0.8894	0.987	1.032
10	Phytate	0.3211	0.4550	0.741	0.6333	0.9811

Keys: A= Cinnamon; B= Nutmeg; C =Turmeric. D = Coriander; E= Cumin

3 DISCUSSION

Spices exhibit a broad spectrum of activity against various microorganisms, including dimorphic fungus and both Gram-positive and Gram-negative bacteria [16]. This study was conducted on five (5) different spices namely: Cumin, Coriander, Turmeric, Cinnamon and Nutmeg against five selected bacteria food pathogens and shows that the extracts from different spices exhibited varying degrees of antimicrobial activity against the selected foodborne pathogens. Table 1 shows the Antimicrobial effects of Cumin against selected clinical isolates at different concentrations using two different solvents (ethanol and aqueous). According to this study, Both ethanol and aqueous extract of Cumin showed a varying zones of inhibition and the highest activity against all the isolates were observed in ethanol extract at 0.8µl/ml (25 µl/ml, 20 µl/ml, 20 µl/ml) except *E.coli* and *P. aeruginosa* who had the highest activity in aqueous extract, while the lowest activity were observed in all the ethanol extract at 0.2µl/ml From this current study, the secondary metabolites present contribute significantly towards the biological activities of medicinal plants. The results in Ethanol extract from Turmeric and Cinnamon also had similar results. The highest activity were observed at dilution 0.8µl/ml (22 µl/ml, 20 µl/ml) while the lowest activity were observed in dilution 0.2µl/ml for all the isolates except the aqueous extract of turmeric which had the lowest activity against *Staph. aureus*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* at dilution 0.2µl/ml. Also, aqueous extract of cinnamon which had the highest activity against *Staphyococcus aureus* and

Enterobacter at 0.8µl/ml. and ethanol extract of cinnamon had the highest activity at 0.8 µl/ml against *E.coli*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* (21 µl/ml, 22 µl/ml and 22 µl/ml) respectively. Crude extracts have moderate inhibition effect in comparison with the standard drug against the tested bacteria and fungi due to naturally occurring combinations of phytochemical constituents. [12]

Table 4 shows the Antimicrobial effects of Nutmeg against selected clinical isolates. It was observed that both the ethanol and aqueous extract had no activity against *Staph. aureus*, *Shigella dysenteriae* and *Pseudomonas aeruginosa* while ethanol extract of nutmeg had highest activity at dilution 0.8µl/ml. against *E. coli* (22 µl/ml) and aqueous extract at dilution 0.8µl/ml against *Enterobacter* (16µl/ml). Table 5 shows the Antimicrobial effects of Coriander against selected clinical isolates. Ethanol extract had no activity in all the dilution factors against *Staph. aureus*, *E. coli* and *Pseudomonas aeruginosa*. While the aqueous extract at dilution 0.8µl/ml had the highest activity against *Staph. aureus*, *E.coli* and *Shigella dysenteriae* (20 µl/ml, 24 µl/ml and 20 µl/ml) respectively. From this study, ethanolic extract exhibited maximum activity against the test organism better than the aqueous extract. This is similar to the result of [17] who also reported that the ethanolic extract gave the best results among all the solvent used for nine spices against *Staphylococcus aureus*

The Qualitative and Quantitative analysis of all the spices were carried out to determine the bioactive compounds. The phytochemicals analysis revealed the presence of key bioactive compounds: Alkaloids, Tannins, Oxalate, Saponins, Flavonoids, Steroids, Terpenoids, Phenolic, Glycosides and phytates. These secondary metabolites of plants serves as defense mechanism against predation by many microorganisms. The constituents are well-documented for their antimicrobial mechanisms, including disruption of microbial membranes, enzyme inhibition, and oxidative damage [17]. According to this study, all the secondary metabolites were present in all the spices except Oxalate which was not determined in all the samples. Although, Tannin, Flavonoid and phenolic are moderately present in nutmeg, In turmeric, Alkaloids and Terpenoids are very present while Flavonoid and phenolic are highly present. This is similar to the report of [18] who also reported the presence of Alkaloids and Terpenoids in the turmeric samples. In Coriander, Alkaloid, Tannin, Saponin, Terpenoid and phytate are very present while flavonoid and phenolic are highly present. However, in Cumin, Terpenoid, Glycosides and phytates are very present while Alkaloids, Tannin, Saponin, flavonoid and Phenolics are highly present. The presence of phytoconstituents such as flavonoids, saponins, tannins, and phenols in the crude extract is likely responsible for the antimicrobial properties. Herbs that have tannins as their component are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery, thus exhibiting antimicrobial activity [19].

Hence the presence of alkaloids in turmeric could account for the significant antimicrobial activity shown in this study and its use as an antimicrobial agent. Flavonoids have antimicrobial properties as well as health promoting effects [20]. Saponins are useful having antitumor, antioxidant and anti-mutagenic effects hence can lower the risk of human cancers by inhibiting cancer cells growth [21]. Tannins are important in promoting wound healing [22]. Glycosides and reducing sugar detected in this study has been reported to have antimicrobial activity

In Quantitative analysis, the highest percentage value of all the secondary metabolites were observed in sample E (Cumin) with values of (2.14, 1.57, 1.03, 14.11, 1.46, 17.17, 1.03, 0.98) for Alkaloids, Tannins, Saponins, Flavonoids, Steroids, Phenolic, Glycosides and phytates respectively, except Coriander which has the highest value in Terpenoids (1.03mg/100g).

4 CONCLUSION

The present study demonstrates that the selected spices contain natural antioxidants and phytochemicals which may be accountable for their use in treating infections, curing certain diseases and as ingredients in traditional medicine. The inclusion of these spices or their extracts in the production and development of functional foods should be encouraged. The results of this study also provide ample evidence that most of the spices were still under-utilized either as medicinal plants or flavoring agents. Based on these findings, this study suggests the need to lay emphasis on the consumption of spices as well as create more public awareness of their health benefits. From the above study, it is concluded that the presence of phytochemical constituents revealed in the ethanol crude extract of Spices could contribute for their antimicrobial activities. The crude extract was active against the tested bacteria and *Staphylococcus aureus*. These findings suggest that the selected phytoextracts possesses antimicrobial properties and could be used for bio-control of bacterial cultures. Antimicrobial activity was confirmed by the selected species and the results revealed that plant extracts varied in their efficacy for inhibiting the bacterial growth of the tested pathogens.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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