

Original Research Article

Synergistic Effect of Some Essential Oil Extracted From Three Nigeria Spices Against Some Pathogenic Organisms

ABSTRACT

Introduction: Essential oils of plants and their other products from secondary metabolism have had a great usage in folk medicine, food flavoring, fragrance, and pharmaceutical industries.

Aims: This study sought to evaluate the phytochemical properties of the oil extracted from the medicinal plants such as; clove, turmeric and black pepper, as well as to determine the antimicrobial effect of essential oil of clove against selected pathogens.

Methodology: Qualitative phytochemical screening of the oil extracted from the plant samples (clove, turmeric and black pepper) was determined using standard method, oil was extracted from the medicinal plant samples, and antimicrobial susceptibility test was performed with the oil extracts in various concentrations against the test organisms.

Results: The qualitative phytochemical screening revealed the presence of alkaloids, phenols, and saponins in clove, turmeric and black pepper oil samples; however, tannins, glycosides, and flavonoids are present in clove oil, and saponins, flavonoids, and terpenoids were abundantly present in turmeric oil sample. The synergistic antimicrobial susceptibility assay of clove with turmeric and black pepper oil is more pronounced against the test bacterial with the highest zone of inhibition of 46.00 mm against *Escherichia coli*. The combination of essential oil of clove and black pepper oil inhibited *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Candida albicans* and *Streptococcus* spp., except *Pseudomonas aeruginosa* which shows antagonistic effect.

Conclusion: Spices such as clove, turmeric and black pepper have exhibited significant antimicrobial activities against intestinal bacteria like *S. aureus*, *Klebsiella* spp., *E. coli*, *C. albicans*, *P. aeruginosa* and *Streptococcus* spp. Therefore, these spices could be used to decrease the possibility of food poisoning, to increase the food safety and shelf-life of products, and to treat some infectious diseases.

Keywords: Bacteria, Medicinal plants, Phytochemical, Spices, Synergy

1. INTRODUCTION

Essential oils of plants and their other products from secondary metabolism have had a great usage in folk medicine, food flavouring, fragrance, and pharmaceutical industries. These essential oils contain a variety of volatile molecules such as terpenes, terpenoids and phenol derived aromatic and aliphatic compounds, which might have bactericidal, antiviral, and fungicidal consequences. Terpenoids are the primary constituents of the essential oils responsible for the aroma and flavor [1]. These essential oils contain a variety of volatile

molecules such as terpenes, terpenoids and phenol derived aromatic and aliphatic compounds, which might have bactericidal, antiviral, and fungicidal consequences.

Clove (*Syzygium aromaticum* L) is one of the most valuable spices that have been used from centuries as food preservative and for many medicinal purposes. *Syzygium aromaticum* flower bud have many medicinal proprieties like antimicrobial, antifungal general stimulating, hypertensive aphrodisiac, light stomachic, carminative and anesthetic [2]. The oil of cloves has been used in a variety of health conditions including indigestion, generalized stress, parasitic infestations, cough, toothaches, headache, and blood impurities [2].

Turmeric contains phenolic compounds called curcuminoids that are responsible for the yellow color of the plant, and particularly of its rhizome. In fact, it is the curcuminoids that possess all the bio-protective properties of turmeric [3]. More than 100 components have been isolated from turmeric. The main component of the root is a volatile oil which is responsible for the aroma of turmeric. Volatile oil isolated from *C. longa* had been reported to have antibacterial and antifungal activities [4].

Consequently, natural antimicrobials, such as chili peppers, are receiving a good deal of attention for a number of microorganism-control issues. Recent reports state that the *Capsicum* genus, among other plant genera, is a good source of antimicrobial and antifungal compounds [5]. The aim of this study is to evaluate the phytochemical properties of clove, turmeric and black pepper oil, as well as to determine the antimicrobial effect of essential oil of clove against selected pathogens.

2. MATERIAL AND METHODS

2.1 Collection of samples

The clove (*Syzygium aromaticum*), turmeric (*Curcuma longa*), and black pepper (*Piper nigrum*) samples were collected fresh from a nearby market in Ado-Ekiti. They were washed to remove debris and stones. The plants were air dried at room temperature at 31 °C for 28 days (until when completely dry). The medicinal plants were powdered using an electric blender.

2.2 Extraction of the three oils (Clove, Black pepper, Turmeric)

The grinded plant materials were placed in the extraction thimble. The weighed amount was placed in an extraction chamber which is suspended above the flask containing the solvent n-hexane and below a condenser. The flask is heated and the n-hexane evaporate is moved into the condenser where it convert into a liquid that trickled into the extraction chamber containing the plant materials. The extraction is designed so that when then solvent surrounding the sample exceeded a certain level it overflow and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the n-hexane extract is remove and n-hexane is evaporated by using rotator evaporator.

2.3 Phytochemical screening

Qualitative phytochemical screening was carried out on the crude extracts of the samples; this include, Test for alkaloids, cardiac glycosides, flavonoids, phlobatannins, saponins (foam test), sterols, tannins, terpenoids, quinines, and oxalate.

2.4 Preparation of media

The freshly prepared and autoclaved Media according to manufacturer's description was poured into the sterilized McCartney bottle after cooling it to 45°C and it was allow to solidify. A loop full of pathogenic organisms was inoculate into the solidify medium and incubated at 37°C for 24hours to obtain microbial growth [6].

2.4.1 Preparation of culture broth

Culture Broth was prepared according to manufacturer's description and inoculated with the pathogenic organism, and incubated at 37°C for 24hours to obtain microbial growth.

2.5 Antimicrobial susceptibility testing

The method employed by Ajibade *et al.* [7] was used. Different concentrations (0.2g, 0.4g, 0.6g and 0.8g,) of each extract was weighed and dissolved separately in 2ml of distilled water. These extracts were incorporated into sterilized paper disks made from Whatman No. 1 filter paper. The medium used (Mueller Hinton agar) was prepared according to the manufacturer specifications; the broth culture of each organism was serially diluted to 10³. The 10³ dilution were spread onto the prepare agar plates using sterilized cotton swabs. The disks with the different concentrations were placed on the inoculated plates, incubated at 37°C for 18-24 hr and observed for growth and the diameter of the zones of inhibition was measured in millimeter using a meter-rule.

2.6 Antimicrobial sensitivity test

A broth culture of each organism was prepared and a sterile cotton swab was inserted into the broth culture to pick the isolates, then the inoculums on the swab stick was transferred into a fresh plate of nutrient agar. Each of the organism was classified as either Gram positive or Gram negative and the sensitivity disc of each was placed on each plate containing the isolated organism. The plates were incubated inverted at 37 °C for 18 – 24 hours according to the method of [8].

3. RESULTS AND DISCUSSION

3.1 Results

The phytochemical analysis revealed that alkaloids, cardiac glycosides, tannins, phenols, glycosides, reducing sugar and saponins were all present in clove oil. Tannins, glycosides, steroids and cardiac glycosides were not detected in black pepper and turmeric. The results showed that saponins, flavonoids and terpenoids were present in a significantly high concentration in turmeric. Alkaloids, phenols, saponins were detected in all the samples. However, only phenols, saponins and alkaloids were found in black pepper as steroids were not detected in all the samples (Table 1).

From Table 2 it can be seen that the combination of the essential oil of Clove with turmeric and black pepper led to a synergistic effect against *Klebsiella*, *Staphylococcus aureus*, *Candida albicans* and *Streptococcus spp.* An antagonistic effect was observed in the other tested bacteria *Pseudomonas aeruginosa*. An additive effect was observed in combination of C/T/B at synergistic effect in *E. coli* with (46.00mm), when the combination of essential oils of Clove and black pepper was applied.

Figure 1 show that essential oils of clove exhibited low activity against most of the bacteria with a zone of inhibition of 10.00 mm. And clove showed activity against the entire test

organism used which *P. aeruginosa* have the highest minimum inhibitory concentration with (70.00 mm).

Table 1: Phytochemical screening of Black pepper, clove, and turmeric oil

Parameters	Black pepper oil	Clove oil	Turmeric oil
Alkaloids	+	+	+
Phenols	+	+	+
Tannins	-	+	-
Glycosides	-	+	-
Saponins	+	+	++
Steroids	-	-	-
Flavonoids	-	+	++
Terpenoids	-	-	++
Cardiac glycosides	-	+	-
Reducing sugar	-	+	+

- = Absent

+ = Present

++ = Significantly present

Table 2: Synergistic effect and antimicrobial test on pathogen organisms

Test Organisms	Antimicrobial Test	Synergistic Effect			
	Clove Oil	C/T	C/B	C/T/B	
	0.2 mg/ml	0.4 mg/ml	0.6 mg/ml	0.8 mg/ml	
<i>Escherichia coli</i>	-	43.00	20.00	46.00	
<i>Klebsiella</i>	30.00	22.00	19.00	38.00	
<i>Pseudomonas aeruginosa</i>	-	32.00	-	21.00	
<i>Staphylococcus aureus</i>	27.00	33.00	16.00	27.00	
<i>Streptococcus</i> spp.	20.00	15.00	20.00	32.00	
<i>Candida albicans</i>	-	37.00	27.00	20.00	
Control	-	-	-	-	

C/T = Clove and turmeric

C/B = Clove and black pepper

C/T/B = Clove, turmeric and black pepper

- = Nil

Table 3: Antibiotic susceptibility test of clove essential oil, positive and negative susceptibility disc (mm)

Test organisms	Clove Oil	POSTIVE					NEGATIVE			
		CN	PEF	CPX	R		CN	CH	PEF	OFX
<i>E. coli</i> -ve	-	-	-	-	-		-	-	-	17.00
<i>Klebsiella</i> -ve	30.00	-	-	-	-		2.00	23.00	-	-
<i>P. aeruginosa</i> -ve	-	-	-	-	-		24.00	-	21.00	25.00

<i>S. aureus</i> +ve	27.00	25.00	30.00	23.00	12.00	-	-	-	-
<i>Streptococcus</i> spp. +ve	20.00	-	-	15.00	-	-	-	-	-
<i>C. albicans</i> +ve	-	-	24.00	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-

CN = Gentamycin; PEF = Pefloxacin; CPX = Ciprofloxacin; R = Rocopihin; CH = Chloramphenicol; OFX = Tarvid;
-ve = Negative; +ve = Positive; - = Nil

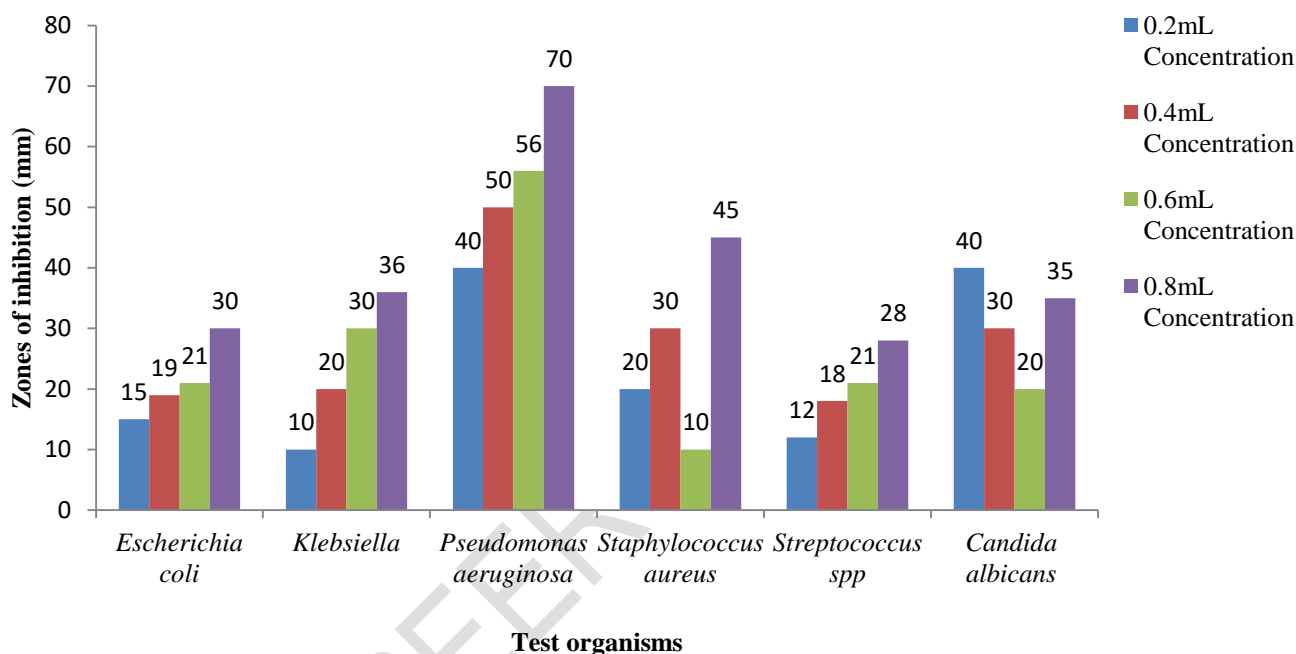


Figure 1: Minimum inhibitory concentration (MIC) for clove showing the zone of inhibition

3.1 Discussion

The qualitative phytochemical analysis of the samples showed that clove oil contained most of the tested phytochemicals, followed by turmeric contained appreciable amounts of these phytochemicals while the least was found in black pepper. Saponins protect against hypercholesterolemia and antibiotics properties [1]. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins. Phenols and tannins acts as antioxidants [9].

The results of this work summarize the antimicrobial activities of the essential oil extracts of spices (clove, turmeric and black pepper). Among the 3 spices, clove was found to be effective against tested bacteria. Essential oil of clove shown to be broad spectrum inhibiting all the bacteria tested with the highest inhibitory effects producing inhibition zones of 30mm of diameter against *Klebsiella*. The combination of some essential oil of (clove and turmeric) inhibited all the bacteria except *Streptococcus* spp. with the lowest zone of inhibition of (15.00mm). Also the combination of essential oil of clove and black pepper oil inhibited *E. coli*, *S. aureus*, *K. pneumoniae*, *Candida albicans* and *Streptococcus* spp. except *P. aeruginosa* which shows antagonistic effect. Essential oil combination of clove, turmeric and black pepper inhibited all tested organisms used that indicate synergistic effect of essential oil against selected pathogenic bacteria. Similar to the result of Cikricki and Yılmaz [10] also reported the clove oil inhibited the *L. monocytogenes* in cheese at 1% concentration.

The inhibitory activity of clove oil is due to the presence of some active components such as eugenol, also contribute similar result which shows that essential oils such as Clove (*Syzygium aromaticum*) and turmeric showed antibacterial activity against food borne pathogens and have large spectrum activity due to their composition in phenolic compound. They constitute the more potential bio preservatives of food among these plants. Essential oils of clove seem to be the most effective essential oil which is more active against the majority of bacteria and yeast which affect the quality of foods. Its activity is due to high concentration in eugenol, a phenolic component recognize to be active against more pathogens reported that clove, turmeric and garlic had the antibacterial activity against various bacterial pathogens *Staphylococcus aureus*, *E. coli*, *Listeria monocytogenes*, *Bacillus cereus* and *Campylobacter jejuni* [11].

The use of spices and herbs in enhancing flavour and aroma of food dates back for thousands of the centuries. Spices in the past decade confirm that the growth of both Gram-positive and Gram-negative foodborne bacteria can be inhibited by clove, turmeric, black pepper and other spices. Although, the primary purpose of spices is to impart flavour and piquancy to food, the medicinal, antimicrobial properties of spices have also been exploited [12].

The minimum inhibitory concentration of essential oil of clove for various bacterial pathogens is shown in figure 1. Among the bacterial pathogens tested, *Pseudomonas aeruginosa* was found to be most sensitive with a minimum inhibitory concentration of 70.00 mm, followed by *E. coli* and *Staph. aureus* with a minimum inhibitory concentration of 45.0 mm. *Streptococcus* spp. is comparatively less sensitive to essential oil of clove with a minimum inhibitory concentration of 28.0mm.

4. CONCLUSION

Spices such as clove, turmeric and black pepper have exhibited significant antimicrobial activities against pathogenic bacteria like *S. aureus*, *Klebsiella*, *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa* and *Streptococcus* spp. These spices can be considered to be useful and effective to decrease the emergence of food poisoning, as well as to increase the food safety, and in the treatment infectious diseases from these organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist. the products used for this research are commonly and predominantly use products in our area of research and country. there is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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