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. Medicinal plants have been used in healthcare since time immemorial and studies have been carried out globally to verify their efficacy and some of the findings have led to the production of plant-based medicines.
Aims: This study sought to evaluate the phytochemical properties of medicinal plants such as; clove, turmeric and black pepper oil, 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 *E. coli*, *S. aureus*, *K. pneumoniae*, *Candida albicans* and *Streptococcus* spp., except *P. 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, Escherichia coli, Candida albicans, Pseudomonas 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.

Comment [S1]: Please change it to This study sought to evaluate the phytochemical properties of the oil extracted from the medicinal plants such as; clove, turmeric and black pepper ,

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

1. INTRODUCTION

Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw material for many herbal industries [1]. Experts turned their concentration back towards obtaining advantages from medicinal plants after observing more side effects of synthetic drugs compared to their benefits [2]. It is estimated that about 35,000 to 70,000 plants species are used as medicinal plants out of 422127 reported worldwide plant species [3].

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. Essential oils are aromatic volatile oily hydrophobic liquid concentrates that are extracted from plant material, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits, roots and whole plant. These essential oils are highly complex mixtures of 20 to 60 volatile compounds, albeit some may contain more than 100 different components. 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 [4].

Pharmaceutical companies are much interested in traditional herbal medicine which otherwise would cost them a lot of money on screening. Plant-derived drugs based on traditional knowledge have benefited the pharmaceutical companies greatly, and indigenous knowledge of plants has played a significant role in drug development. Out of 10,000 molecules only one will emerge as a new drug. It nearly needs 15 years to be experienced and it is very expensive as it costs 800 million dollar for a single drug to be developed. This interest by companies from the more developed countries has led them to the knowledge of the indigenous peoples of the less developed countries, and converted medicinal herbs into pharmaceutical products without providing any payment to the providers of the knowledge. [2].

The development of drugs of plant origin involves botanical identification of the plant, cultivation and post-harvest procedures, extraction procedures, standardization of extracts and pharmaceutical formulation. This means that development of herbal-based medicines is in the hands of personnel from different specialties. The production of drugs from plants needs the cooperation of a big team of horticulturists, botanists, ecologists, taxonomists, phyto-chemists, pharmacists, pharmacologists, pharmaceutical specialists, marketing and distribution specialists, etc. Nowadays, the developed countries are turning towards the use of traditional medicinal systems that involve the use of herbal drugs and remedies. Most dermaceuticals are derived from algal extracts that are rich in minerals and the vitamin B group. Skincare products such as skin creams, skin tonics, etc. derived from medicinal plants are grouped together as dermaceuticals. Amongst the poor, cures and drugs, derived from plants, constitute the main source of healthcare products [5].

Higher aromatics plants have traditionally been used in folk medicine; antimicrobial properties of these plants are well documented against bacteria, fungi and yeasts. Most of the medicinal properties of these plants are directly correlated with the essential oils produced by these plants. Essential oils and extracts of these plants are able to control microorganisms related to skin diseases, dental caries and food spoilage. Essential oils are aromatic volatile oily hydrophobic liquid concentrates that are extracted from plant material, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits, roots and whole plant compounds, albeit some may contain more than 100 different components [5]. 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. The aim of this study is to evaluate the phytochemical properties of clove, turmeric and black pepper oil, as we as to determine the antimicrobial effect of essential oil of clove against selected pathogens.

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2. MATERIAL AND METHODS

2.1 Collection of samples

The clove, turmeric and black pepper samples were collected fresh from a nearby mark*et.* in Ado-Ekiti. They were washed to remove debris and stones. The plants were air dried at room temperature at 31^{0}c for 28days(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 Antimicrobial susceptibility testing

The method employed by Ajibade *et al.* [6] 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 10^3. The 10^3 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^{o}C for 18-24 hr and observed for growth and the diameter of the zones of inhibition was measured in millimeter using a metre-rule.

2.5 Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined by micro-dilution techniques, the oil were prepare with concentration ranged from 4 to 28 mg/mL. The minimum inhibitory concentration value was interpreted as the highest dilution of the sample which showed clear zone all test were performed in triplicate.

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2.6 Minimum bactericidal concentration (MBC)

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a bacterium over a fixed, somewhat extended period, such as 18 hours or 24 hours, under a specific s*et.* of conditions. It can be determined from the broth dilution of MIC tests by sub-culturing to agar plates that do not contain the test agent. The MBC is identified by determining the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by a pre-determined reduction such as ≥99.9%.

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 while turmeric showed the presence in high concentration of saponins, flavonoids and terpenoids. However, only phenols, saponins and alkaloids were found in black pepper as steroids were not detected in all the samples (Table 1).

Table 2 shows the activity of antimicrobial susceptibility against selected six test organisms which are gram positive and gram negative bacteria. The result shows that 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 to 12.00 mm). And clove showed activity against the entire test organism used which *P. aeruginosa* have the highest minimum inhibitory concentration with (70.00mm).

Figure 2 show that essential oils of clove exhibited low activity against most of the bacteria with a zone of inhibition of (10.00 to 12.00 mm). And clove showed activity against the entire test organism used which *P. aeruginosa* have the highest minimum inhibitory concentration with (70.00mm).

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
Escherichia coli	-	43.00	20.00	46.00
Klebsiella	30.00	22.00	19.00	38.00
Pseudomonas aeruginosa	-	32.00	-	21.00
Staphylococcus aureus	27.00	33.00	16.00	27.00
Streptococcus spp.	20.00	15.00	20.00	32.00
Candida albicans	-	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)

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Comment [S7]: Please check this sentence as you mentioned that phenols, saponins and alkaloids present in oil of clove , Tannins and black pepper .

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Comment [S9]: Please look again at Figure 1 and write the corrected values

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Test organisms	Clove Oil	POSTIVE CN	PEF	CPX	R	NEGATIVE CN	CH	PEF	OFX
E. coli –ve	-	-	-	-	-	-	-	-	17.00
Klebsiella -ve	30.00	-	-	-	-	2.00	23.00	-	-
P. aeruginosa -ve	-	-	-	-	-	24.00	-	21.00	25.00
S. aureus +ve	27.00	25.00	30.00	23.00	12.00	-	-	-	-
Streptococcus spp. +ve	20.00	-	-	15.00	-	-	-	-	-
C. albicans +ve	-	-	24.00	-	-	-	-	-	-
Control	-	-	-	-	-	-	-	-	-

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

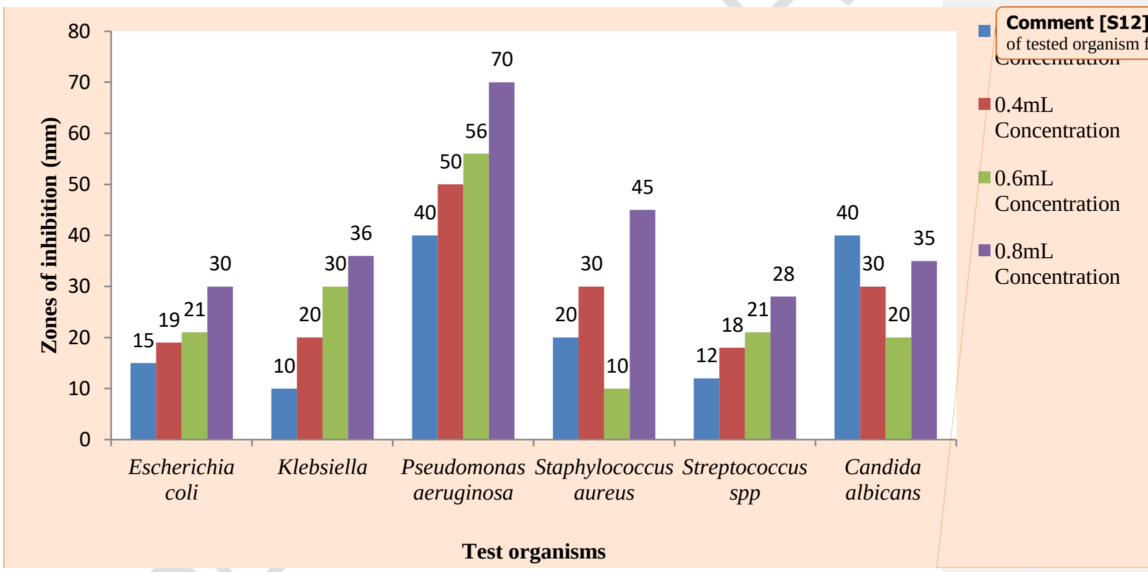

Figure 1: Minimum inhibitory concentration (MIC) for clove showing the zone of inhibition

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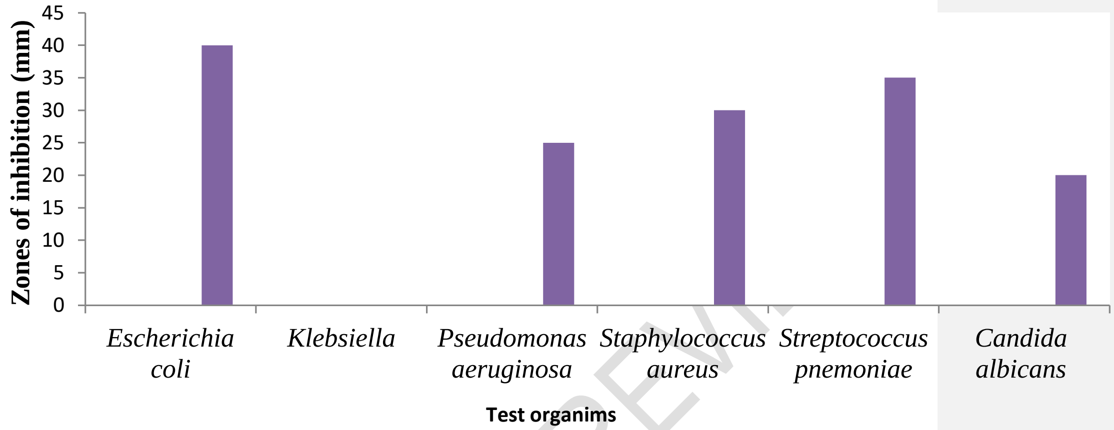

Figure 2: Minimum bactericidal concentration (MBC) for clove showing the zone of inhibition

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. For instance, Kuang, *et al.* [7] reported that alkaloids are powerful pain relievers, have an antipyretic action, a stimulating effect and can act as topical anesthetic in ophthalmology. Cardenolides have been used in the treatment of congestive heart failure [8] and cardiac arrhythmia [9].

Saponins protect against hypercholesterolemia and antibiotics properties [4]. The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins. Phenols and tannins acts as antioxidants [10]. The potential of plants as a source of drugs is still to be vastly explored. Multiple drug resistance has become a critical problem in pharmaco-therapeutics as there are increasing numbers of diseases exhibiting various levels of drug resistance, including bacterial infections. Herbal medications and phytochemical screening of various plant species for medicinal leads are now receiving much attention [11].

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The results of this work summarize the antimicrobial activities of the essential oils, 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

Comment [S14]: Please change it to oils extracts black pepper inhibited all tested organisms used that indicate synergistic effect of essential against selected pathogenic bacteria. Similar to the result of Cikricki and Yýlmaz [12] also reported the clove oil inhibited the *L. monocytogenes* in cheese at 1% concentration.

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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* [13]. Generally, the essential oils possessing the strongest antibacterial properties against food borne pathogens contain a high phytochemical constituent clove oil such as alkaloids, phenols, tannins, glycosides, saponins, flavonoids, cardiac and reducing sugar.

Spices and herbs nave been used for thousands of the centuries by many cultures to enhance the flavour and aroma of food. Early culture also reorganized the values of using spices and herbs in preventing foods and for their medical values. 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 [14]. The antimicrobial activity of spices is documented and interest continues to the present [15].

The growing concern about food safety has recently led to the development of natural antimicrobials to control food borne pathogens and spoilage bacteria. Spices are one of the most commonly used natural antimicrobial agents in foods and have been used traditionally for thousands of years by many cultures for preserving foods and as food additives to enhance aroma and flavour [14].

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 70.00mm, followed by *Escherichia coli* and *Staphylococcus aureus* with a minimum inhibitory concentration of 45.0mm. *Streptococcus* spp. is comparatively less sensitive to essential oil of garlic with a minimum inhibitory concentration of 28.0mm.

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Depending upon the minimum inhibitory concentrations two major clusters of human bacterial pathogens could be observed. The microbial inhibitory action of essential oil of clove may be due to its antimicrobial compound, allicin and the effect will be brought about by its ability to inactivate proteins and also by inhibition of RNA synthesis. Many scientists have tested the efficacy of clove preparations in inhibiting different bacterial pathogens and reported varying results depending upon the microorganisms tested in their study.

4. CONCLUSION

The antibacterial activities of commonly used spices have been summarized. Spices such as clove, turmeric and black pepper have exhibited significant antimicrobial activities against intestinal bacteria like *S. aureus*, *Klebsiella, Escherichia coli, Candida albicans,* *Pseudomonas 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. In the future, as the combinations of several spices were proven to possess higher inhibitory effects on specific bacteria than those of individual spices, the interactions of more spices should be studied and evaluated to inhibit different microorganisms in different food products. Additionally, spices could be consideration, such as how to prevent odor/flavor transferring from packages containing natural spice extracts to the packaged foods.

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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