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PHYTOCHEMICAL SCREENING, ANTIOXIDANT PROPERTIES AND ANTIMICROBIAL ACTIVITIES OF GINGER AND SWEET ORANGE PEELS ESSENTIAL OILS.

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Abstract

Essential oils are highly concentrated substances obtained from vegetable raw materials and are complex mixtures whose composition may include hydrocarbons, alcohols, esters and aldehydes. These oils are often used for their flavour and their therapeutic or odiferous properties. The study was carried out to determine the phytochemical constituents, antioxidant properties and antimicrobial activities of essential oil from Ginger (*Zingiber officinale*) and sweet orange (*Citrus sinenesis*) peels. Fresh ginger rhizomes and sweet orange peels were hydro-distilled to get the essential oils which were screened for the presence of phytochemicals and their effect on 1,1-Diphenyl-2-picryl-hydrazyl radical (DPPH) was used to determine their free radical scavenging activity. Total alkaloids and total terpenoids were quantitatively estimated. The oils were also evaluated for antimicrobial activity against fish pathogenic bacteria by disc diffusion method. Antibacterial activity was determined against four different fish pathogens *Vibrio sp, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus* sp. *Bacillus* sp. Phytochemical screening of the ginger and sweet orange peel essential oils showed the presence of alkaloids, terpenoids, cardiac glycosides, coumarin, anthraquinones and vitamin C which are useful substances that have medicinal and physiological activities. They did not contain saponin, tannins, steroids, phenols and flavonoids. Concentrations of the essential oil required for 50% inhibition of the DPPH radical scavenging effect (IC50) were recorded as 200 µg/ml, 400 µg/ml, 600 µg/ml, 800 µg/ml and 1000µg/ml for ginger and sweet orange peel essential oil. Antioxidant screening of the ginger and sweet orange peels essential oils DPPH was positive indicating the presence of free radical scavenging molecules and antioxidant potency of the essential oils. The disc diffusion results indicated that essential oil of *Zingiber officinale* and *Citrus sinensis* peel significantly inhibited the growth of *Vibrio sp, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus sp*. The inhibition of the test isolates was dependent on the concentration of the solvent used. The phytochemical analysis of ginger and sweet orange peel essential oil revealed the presence of phytochemical constituents which conferred antimicrobial property on the oils.

Keywords: Essential oil, Sweet orange peel, Ginger, Phytochemical, Antioxidant, DPPH, Antibacterial.

1.0 Introduction

Phytochemicals are a large group of plant-derived compounds that are commonly found

in fruits, vegetables, beans, cereals and plant-based beverages such as tea and wine (Arts and Hollman 2005). Based on their chemical structure, phytochemicals can principally be categorized into alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils (Chakraborty *et al*., 2013). Phytochemicals in general are commonly recognized as safe for animals, consumers and the environment (Liu *et al*., 2011). Essential oils (EOs) obtained from plants are a complex mixture of some compounds such as hydrocarbons, alcohols, esters, aldehydes and have been reported to be effective against fish pathogens (Gattuso *et al*., 2009; Mahmoodi *et al.*, 2012; Ekici *et al*., 2011). They contain a variety of volatile molecules such as terpenes and terpenoids, phenol-derived aromatic components and aliphatic components, and are used as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and local anesthetic remedies *(*Bakkali *et al*., 2008). These products have great potential to be used in aquaculture systems due to several beneficial biological effects reported in aquatic species, such as growth promotion, appetite stimulation, immune-modulatory and antioxidant effects as well as anti-parasitic, antibacterial, anesthetic and anti-stress activities (Harikrishnan *et al.*, 2011).

Ginger (*Zingiber officinale*) rhizome (ginger root) is widely used as a spice or condiment and a potent medicinal plant according to the "folk medicines" of cultures worldwide. Ginger contains several compounds such as gingerol, gingerdiol, and gingerdione that possess anti-inflammatory, anti-diabetic, anti-cancer, chemopreventive, and chemotherapeutic effects (Kikuzaki and Nakatani, 1996). Some studies have demonstrated the effect of ginger or its compounds on growth performance, antioxidant status, immune system and serum metabolites in fish species (Talpur *et al*., 2013) Ginger (*Zingiber officinale*) (*Zingiberaceae*) is a medicinal plant whose rhizome is known for its immuno-stimulant properties (Ali *et al.*, 2008). Recent studies have shown the beneficial effects of dietary ginger on growth, immune responses and disease resistance of fishes (Dugenci *et al.,* 2003; Talpur *et al*., 2013). However, these studies used only dried and powdered ginger, with few studies on the essential oil.

Sweet orange (*Citrus sinensis*) belongs to the *Citrus* genus, taxa of flowering plants in the family *Rutaceae* (Okunowo *et al*., 2013). In Nigeria and other parts of the world, *Citrus sinensis* (sweet orange) are cheaply available and thus serves as a major source of vitamins in diets. Orange fruit and its juice have several beneficial, nutritive and health properties (Okwu and Emenike, 2006). The sweet orange peel is a primary by-product produced by the fruit processing industries, and it accounts for approximately 45% of the total bulk (Farhat *et al*., 2011) and is usually discarded as waste. Sweet orange peel essential oil exhibit anti-inflammatory, antitumor, antioxidant, and antimicrobial activities due to the presence of rich cardiac glycosides, coumarins, β- and ɤ-sitosterols, vitamins, and volatile compounds (Gao *et al*., 2006; Liu *et al.,* 2012). However, there is a paucity of information on the phytochemical constituent and antimicrobial properties of sweet orange peel essential oil on fish pathogens. This study aimed to extract, determine the phytochemical constituent, antioxidant properties and antimicrobial effects of ginger and sweet orange peels essential oil on selected fish pathogenic isolates.

2.0. Materials and Methods

2.1.0. Collection and preparation of plant extract

Ginger rhizomes were purchased at the Bodija market in Ibadan, Nigeria and sweet orange peels were obtained from fruit vendors at Agbowo area, Ibadan. The ginger rhizome and sweet orange were identified and authenticated at the herbarium unit, Department of Botany, University of Ibadan, Ibadan, Nigeria.

2.1.1. Essential oil extraction

The essential oils (EOs) used in the present study were extracted from ginger (*Zingiber officinale*) rhizome, and sweet orange (*Citrus*

sinensis) peel by hydrodistillation using a Clevenger-type apparatus (AOAC 1980). The plant materials were cleaned, washed with water, cut into small pieces and blended in a mixer. They were then immersed in 500mL water in a 2-litre round-bottom flask and after adding water to immerse the peel/rhizomes, the heating mantle was activated. After 2 hours of extraction, the essential oil was collected, placed in amber glass containers and stored in a refrigerator before use. This study was carried out at Department of Pharmaceutical Chemistry, University of Ibadan, Ibadan, Nigeria.

2.2.0. Phytochemical screening

Phytochemical screening was performed using standard procedures (Sofowora, 1993 and Trease *et al*, 1989).

2.2.1. Qualitative analysis of phytochemical constituents

Test for anthraquinones

0.5g of each of the essential oil was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for terpenoids (Salkowski test)

To 0.5 g each of the oil was added to 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish-brown colouration of the interface indicates the presence of terpenoids.

Test for flavonoids

A few drops of 1% NH_3 solution were added to a portion of an aqueous filtrate of the oil in a test tube. Yellow coloration is observed if flavonoid compounds are present.

Test for saponins

0.5 g of the oil was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for tannins

About 0.5 g of the oil was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration

Test for alkaloids

0.5 g of oil was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)

To 0.5 g of oil diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides, indicating the presence of the cardiac glycoside constituent.

2.2.2. Quantitative phytochemical analysis

Alkaloids

1g each of the oil samples were prepared in a beaker and 40 ml of 10% acetic acid in ethanol was added to the oil samples. The mixture was covered and allowed to stand for 4 h. The mixture was filtered and the oil was allowed to become concentrated in a water bath until it reaches one-quarter of the original volume. Concentrated ammonium hydroxide was added until the precipitation is complete. The whole

solution was allowed to settle and the precipitate is collected and washed with dilute ammonium hydroxide and then filtered. The residue is an alkaloid, which was then dried and weighed.

Terpenoids

Total terpenoid content in ginger and sweet orange peel essential oil was determined by the method as described by Ferguson, (1956). 1g of each oil was taken in a conical flask and soaked in ethyl alcohol for one day. Then it was filtered and the filtrate was extracted with petroleum ether. The ether extract was taken as the measure of total terpenoid.

$$\text{Total terpenoid content} = \frac{(\text{Final weight of the sample} - \text{Initial weight of the extract}) \times 100}{\text{Weight of the Sample}}$$

Total phenolic content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Ebrahimzaded *et al.,* 2008a, Nabavi *et al.,* 2008a). The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ ml solutions of Gallic acid in methanol: water (50:50, w/w). Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound.

2.2.3. Determination of antioxidant activity

DPPH radical scavenging assay

Free radical scavenging activity of ginger and sweet orange peel essential oil using DPPH (1,1-Diphenyl-2-picrylhydrazyl), a stable free radical soluble in methanol, was determined by the modified method proposed by Çoruh *et al.,* (2007). DPPH has an absorption peak at 517 nm and the radical scavenging potential was identified by the degree of discolouration from purple to yellow. The reaction mixture was prepared by mixing varying concentrations of 100 µl of the extract solution in methanol with 1 ml of 100 µM freshly prepared solution of DPPH in methanol. For comparison, Ascorbic acid was taken as the standard. Control was prepared by mixing 1 ml of DPPH solution in methanol with 100 µl methanol. Incubation of the reaction mixture for 20 min at room temperature in the dark was done and the absorbance was measured against methanol which was the reference solution. Reduction in optical density on addition of oil was compared to the control and standard to estimate the antioxidant activity. The percentage of inhibition of DPPH radical was computed as follows:

$$\% \text{ of inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control (DPPH radical solution without test sample) and A_{sample} is the absorbance of the extract.Radical Scavenging activities were measured by a slightly modified method.

2.3.0 Determination of the antibacterial activity of *Ziginber officinale* and *Citrus sinenesis* peel essential oil against fish pathogenic bacteria

Fish-borne bacterial (*Staphylococcus aureus, Vibrio sp, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus sp*) used were isolated from diseased *Heterobranchus bidorsalis.* 1g of the fish gill, intestine and tissue were dissected out, blended and mixed properly in a mortar. The sample was aseptically transferred and spread over the surface of blood agar and MacConkey agar before incubation between 24 and 48 hours at 37^{o}C as described by (Slaby *et al.,* 1981). Gram staining was done to differentiate organism based on the structure of their cell walls.

The antibacterial potential of the essential oils of ginger and sweet orange peel against some fish pathogenic bacteria was done using the agar well diffusion method at the Department of Microbiology of the University of Ibadan. The isolates tested were *Staphylococcus aureus, Vibrio sp, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus*

sp. An 18-24 hr old culture of each test isolate was inoculated into 5ml normal saline in a test tube and standardized to 0.5 MacFarland. A sterile swab stick was used to apply the suspension to the surface of already prepared Nutrient Agar (NA) plates after which a sterile 8 mm cork borer was used in boring wells on the agar and a micropipette was used in dispensing 100µL of each essential oil into the labeled wells respectively. Ethanol and gentamycin discs were used as control. The antimicrobial activities were then determined by measuring the diameter of the zones of inhibition.

2.6.1. Minimum inhibitory concentration (MIC) determination. The MIC of the essential oils from sweet orange and ginger on fish pathogenic bacteria isolates was determined using a two-fold dilution method (Russel *et al.,* 1977). Sterile 8 mm cork borer was used to bore four wells onto prepared nutrient agar plates seeded with the test isolates. Different concentrations of the essential oils (100%, 50%, 25%, and 12.5%) were dispensed into each well and labelled. The preparation was left to diffuse before incubating at 37ºC for 24 h. The lowest concentration of the agent that prevented the growth of the bacteria was taken as the minimum inhibitory concentration (MIC). The zones of inhibition were measured in diameter (mm) to indicate the presence of antibacterial activity for each sample compared to the positive control.

3.0. STATISTICAL ANALYSIS

All experimental results were recorded in triplicates and data obtained was expressed as means ± standard deviation (SD) using Excel Statistical Package.

4.0. RESULTS

4.1. Phytochemical screening of Ginger and Sweet Orange peel essential oils

The phytochemical screening of ginger essential oil has shown the presence of steroids, terpenoids, coumarin, anthraquinones, alkaloids, and cardiac glycosides while saponin, tannins, steroids, phenols and flavonoids were absent. Sweet orange peel essential oil showed the presence of terpenoids, coumarin, anthraquinones, alkaloids, and cardiac glycosides while the oil was negative for the presence of saponin, tannins, steroids, phenols and flavonoids. (Table 1)

Table1: Qualitative analysis on phytochemical constituents.

Phytochemicals	Ginger Essential oil	Orange Essential oil
Saponin	-ve	-ve
Tannin	-ve	-ve
Flavonoid	-ve	-ve
Steroid (Liebermann-Burchard Test)	+ve	-ve
Terpenoid (Salkowski test)	++ve	++ve
Coumarin	++ve	+ve
Anthraquinone (Borntrager's Test)	++ve	++ve
Alkaloids (Dragenduff's Test)	+ve	+ve
Cardiac Glycosides (Keller-Killiani Test)	++ve	+ve
Phenols(Keller-Killiani Test)	-ve	-ve
Vitamin C	++ve	++ve

*Presence of phytochemical constituents: +; Abundant presence of phytochemical constituent: ++; Absence of phytochemical constituents: -

4.2. Quantitative analysis

The results of quantitative analysis on two major groups of phytochemical constituents in the essential oils is summarized and shown in Table 2. Ginger oil has the highest yield of alkaloid, which is 1.05%w/w followed by orange essential oil which is 0.75%w/w. Ginger oil also produced the highest yield of terpenoid which is 9.8% w/w followed by orange oil.

Table 2. Quantitative analysis of phytochemical constituents (w/w).

Essential oil	Alkaloids (% w/w)	Terpenoids (% w/w)
Orange EO	0.75±0.07	2.2±0.14
Ginger EO	1.05±0.07	9.8±0.10

Radical scavenging (antioxidant) activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples. The DPPH radical scavenging activity of the essential oils of ginger and sweet orange peel at varying concentrations were measured along with standard ascorbic acid. The rapid screening for free radical scavenging activity showed positive activity for the oils; since they showed yellowish spot indicating the presence of antioxidant in the essential oil. It was found that the radical-scavenging activities of all the essential oils increased with increasing concentration. IC50 for DPPH radical-scavenging activity were reported in Table 3.

Table 3: % DPPH scavenging activity of the different samples against the standard

Conc. (µg/mL)	Orange EO Mean ±SD	Ginger EO Mean ±SD	Ascorbic Acid (Standard) Mean ±SD
1000.00	33.47±0.00	38.24±0.00	96.99±0.01
800.00	27.78±0.00	38.07±0.00	95.82±0.00
600.00	23.41±0.01	37.9±0.00	95.68±0.00
400.00	17.61±0.01	37.16±0.00	95.68±0.00
200.00	11.70±0.00	34.09±0.02	95.60±0.01

Determination of total phenol content

Total phenol values are expressed in terms of Gallic acid equivalent (mg/ g of dry mass), which is a common reference compound

Table 5: Total phenolic content of the different samples (in µg) at Gallic acid equivalent

Concentration(µg/mL)	Orange Essential Oil Mean±SD	Ginger Essential Oil Mean±SD
200	1.19±0.02	1.19±0.00
400	1.28±0.00	1.32±0.00
600	1.36±0.05	1.48±0.00
800	1.65±0.01	1.67±0.02
1000	1.80±0.00	1.88±0.00

4.3. Antibacterial Activity of Essential Oils

The in vitro antibacterial activity of each of the essential oil was done by agar well diffusion method using Nutrient agar (NA). The isolates tested were *Staphylococcus aureus, Vibrio sp, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus sp*. by agar disc diffusion method (Tables 6-9). The results showed that ginger EO at different doses had substantial inhibitory effect against all the tested bacterial strains (Table 6). Among all the tested bacteria, the ginger EO showed the strongest antibacterial activity with the highest activity observed against *Bacillus sp*. (>30mm)

Table 6. Antibacterial activity of essential oils from Ginger, Sweet Orange Peel, Ethanol and Gentamycin controls against selected fish pathogens.

Test Bacteria	Zone of inhibition (mm)			
	Ginger EO	Orange EO	Ethanol	Gentamicin
Bacillus sp.	>30	>30	8	27
Escherichia coli	7	-	15	28
Vibrio sp.	12	-	8	21
Pseudomonas aeruginosa	8	-	10	-

KEY: Positive Control: Gentamycin; Negative control: Ethanol

Table 7. Minimum inhibitory concentration (MIC) of sweet orange peel essential oil on the test bacteria

Test bacteria	Zone of inhibition (mm)			
	100%	50%	25%	12.5%
Bacillus sp.	>30	>30	>30	>30
Escherichia coli	-	26	20	15
Vibrio sp.	-	17	21	23
Pseudomonas aeruginosa	-	13	16	20

Table 8. Minimum inhibitory concentration (MIC) of Ginger essential oil on the test bacteria

Test bacteria	Zone of inhibition (mm)			
	100%	50%	25%	12.5%
Bacillus sp.	>30	>30	>30	>30
Escherichia coli	7	30	30	21
Vibrio sp.	12	26	25	24
Pseudomonas aeruginosa	8	15	18	19

5.0. Discussion

Phytochemical screening of the essential oils of ginger and sweet orange peel revealed some differences in the constituents of the two essential oils tested. Ginger essential oil tested positive for alkaloids, steroids, terpenoids, cardiac glycosides, coumarin and anthraquinones which are useful substances that have medicinal and physiological activities. It showed the absence of saponin, tannins, phenols and flavonoids. Sweet orange essential oil tested positive for alkaloids, terpenoids, cardiac glycosides, coumarin and anthraquinones. It showed the absence of saponin, tannins, phenols, steroids and flavonoids. Ginger essential oil has an abundance of coumarin and terpenoids compared to orange essential oil. Both ginger and sweet orange peel essential oil exhibited potent antioxidant activity.

5.1 Quantification of alkaloids and terpenoids:

The results of quantitative analysis on two major groups of phytochemical constituents in ginger and sweet orange peel essential oil. The essential oil of ginger has higher amount of terpenoids and alkaloids content (1.05%w/w and 9.8%w/w) while the alkaloid and terpenoid contents of sweet orange peel essential oil revealed 0.75%w/w and 2.2%w/w respectively. Both essential oils are rich in alkaloids and terpenoids which are important compounds for aquaculture, as they have antibacterial activity mediated by their functional groups that act on the outer membrane of the bacteria, altering its permeability and fluidity, as well as affecting membrane proteins and periplasmic enzymes (da Cunha *et al.,* 2018). They also have anti-inflammatory properties as they play important roles in promoting wound healing (Okwu and Josiah, 2006), strengthening the skin, increasing the concentration of antioxidants in wounds, and restoring inflamed tissues by increasing blood supply (Hawkins and Ehrlich, 2006).

The DPPH test provides information on the reactivity of various samples with a stable free radical scavenging ability and gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution changes colour from deep violet to light yellow. Substances which can perform this reaction can be considered as antioxidants and radical scavengers (Dehpour *et al*., 2009). It was found that the radical-scavenging activities of the essential oils increased with increasing concentration. IC50 for DPPH radical-scavenging activity of ginger and sweet orange peel essential oil showed considerable radical scavenging activity in a concentration dependent manner.

5.2 Antibacterial activity of Ginger and Sweet Orange Peel essential oil

The antimicrobial activities of ginger and sweet orange peel essential oil against *Vibrio sp, Escherichia coli, Pseudomonas aeruginosa,* and *Bacillus sp*. by the disc diffusion method were shown. The results showed that ginger essential oil inhibited the growth of four fish borne bacteria tested. Ginger essential oil has the maximum zone of inhibition (>30 mm) against *Bacillus sp.* followed by *Vibrio sp*. (12mm) and *Pseudomonas aeruginosa* (18mm). The minimum was (7mm) zone inhibition produced against *Escherichia coli*. The strongest antibacterial activities were obtained by ginger essential oil with inhibition zones greater than 30 mm against *Bacillus sp*. The sweet orange peel essential oil also produces a maximum zone of inhibition (>30 mm) against *Bacillus sp* while *Vibrio sp, Escherichia coli, Pseudomonas aeruginosa* being gram negative bacteria were resistant to sweet orange peel essential oil. Gram-positive bacteria are more susceptible to the effects of EOs than Gram-negative bacteria (Trombetta *et al.,* 2005), due to significant structural differences in the cell wall of these two groups of bacteria. Gram-positive bacteria cell wall is composed of peptidoglycan, which allows hydrophobic molecules to easily penetrate the cell (Nazzaro *et al*., 2013) while gram-negative bacteria have an outer membrane in addition to the peptidoglycan allowing some hydrophobic molecules to penetrate the cell, but only through porin proteins that form water-filled channels are distributed throughout the cell wall. Therefore, Gram-negative bacteria are more resistant to essential oil than Gram-positive bacteria, due to the presence of the multi-layered structure of Gram-negative bacteria. The antibacterial effect of essential oil on gram-negative bacteria depends on their final concentration in the solution: at low concentrations, they interfere with enzymes involved in the production of energy, and they can cause protein denaturation at higher concentrations (Tiwari *et al*., 2009; Nazzaro *et al.,* 2013).

5.3 Minimum inhibitory concentration (MIC)

The ginger essential oil against *Bacillus sp.* showed higher MIC values for all the concentrations, followed by *Escherichia coli* at

50% and 25% (50 µl/mL and 25 µl/mL), *Vibrio sp*. at 50% (50 µL/mL). The sweet orange peel essential oil against *Bacillus sp*. also showed the highest MIC values at all concentrations, followed by *Escherichia coli* at 50% (50 µL/mL), *Vibrio sp*. at 12.5% (12.5 µL/mL) and *Pseudomonas aeruginosa* at 12.5% (12.5 µL/mL). MIC of both ginger and sweet orange peel essential oil on *Vibrio sp, Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus sp*. were at 12.5% (12.5µl/mL). This illustrates an increasing inhibitory effect of the ginger and sweet orange peel essential oil as the concentration decreases and suggests a dose-dependent activity. This implies that the antimicrobial activity of a substance is concentration-dependent, which is in concordance with the report of Dubey *et al*. (2010) and Oboh (1997), that antimicrobial activity is a function of the active ingredient that reaches an organism.

6.0 Conclusion

Essential oils of ginger and sweet orange peel can inhibit fish pathogenic bacteria growth due to its phytochemical constituents which include alkaloids, terpenoids, steroids, cardiac glycosides, coumarin and anthraquinones. The results of tests from various concentrations and the presence of antioxidant component of the essential oils suggest that the EO have some significant inhibitory action against pathogens. Both ginger and sweet orange peel EOs showed antibacterial activity at a low concentration of 12.5% (12.5µl/mL) against selected fish-borne pathogenic bacteria, this serves as an indication of the potential of ginger and sweet orange essential oil as a source of antibacterial substances for the development and formulation of antibiotics with broad spectrum activity and they can play a major role in enhancing the safety and quality of fish and fishery products.

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