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Nutritional compositions and antimicrobial sensitivities of Aqueous extracts of *Zingiber officinale* ROSC and *Allium sativum* L on oral microorganisms.

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ABSTRACT: Phytochemical properties of the extracts of ginger (*Zingiber officinale*) and bulb of garlic (*Allium sativum*) indicate the presence of saponins, tannins, alkaloids, flavonoid and phytic acids. Extracts of *Z. officinale* and *A. sativum* were more effective in the treatment of oral pathogens when compared to antifungi (Ketoconazole) and antibacterial (ciprofloxacin) drugs used as positive controls. *Z. officinale* extract had highest zones of inhibition of 21.60±1.16 mm and 26.00±1.03 mm against fungi and bacteria at 100 mg/ml respectively as compared to ketoconazole with zone of inhibition of 14.18±1.01 mm and ciprofloxacin with zone of inhibition of 14.17±0.22 mm. *A. sativum* extract also showed better zone of inhibition of 27.60±1.07 mm and 24.30±0.33 mm against fungi and bacteria isolates respectively at 100 mg/ml compared to positive control antibiotics of ketoconazole with highest zone of inhibition of 14.00±0.51 mm and ciprofloxacin with highest zone of inhibition of 14.17±0.42 mm respectively. The proximate composition revealed that ginger and garlic can be ranked as rich in carbohydrate due to their high caloric content. Micronutrient analysis revealed that *Z. officinale* had higher composition of calcium, magnesium, sodium and potassium than *A. sativum*. Nitrogen composition in *A. sativum* (2.24 mg/g) was higher than *Z. officinale* (2.28 mg/g). The extracts of *Z. officinale* and *A. sativum* possess antimicrobial activities against oral pathogens and contain appreciable amount of nutrients, vitamins and minerals which contribute to the nutrient and energy requirement of man when the plant is taken for curative purposes in certain diseased conditions.

Keywords: Mineral, Antimicrobial, Sensitivity, Extract, Ginger, Garlic

Introduction

The increasing reliance on drugs from natural sources has led to the extraction and development of several drugs and chemotherapeutic agent from traditional herbs and they are present in abundance in the tropic (Falodun *et al.*, 2006). Many food present have antibiotics function that are often unknown to the eater and these foods limit the growth of bacteria in their body. Some of these foods are green tea and ginger (Horiba *et al.*, 1991; White, 2007). Ginger, a common substance found increasingly in the diets of the global population, has known antimicrobial effects and is commonly used together in teas (Sebiomo *et al.*, 2011). Ginger has been used in centuries to fight infection. Its components are active against a form of diarrhea which is the leading cause of infant death in developing countries (Sebiomo *et al.*, 2011).

Allium sativum and *Zingiber officinale* rhizomes are rich sources of phytochemicals, viz: alkaloids, saponins, flavonoids, terpenes and steroids. These drugs are widely used in the treatment of different ailments in the India system of medicine. Ginger mainly contain up to 3% of volatile oil, a mixture of 24 constituents containing monoterpenoids fraction (β -phelladrene, cineol and citral) and sesquiterpenoids (β -sesquiphelladrene, bisabolene and farnasene) with zingiberene (Rohini *et al.*, 2011). Ginger has been mixed with other plant extracts and synergistic action of the phytochemicals has been observed (Reddy and Seetharam, 2009). Trikatu churna is an equiproportions of powdered fruits of *Allium sativum*, *Piper longum* and rhizome of *Zingiber officinale* and it has potent antimicrobial activity. In disease of microbial origin, the plants function as a result of antimicrobial activity against the causative agents (Sofowora, 1993).

Study conducted showed that ginger's constituents acted as strong antioxidant and effective antimicrobial agent that could heal sores and wounds of internal organs such as stomach and liver. In this relation, Mahadi *et al.* (2005) pointed out that the primary factor associated with gastritis and peptic ulcer diseases was the gram negative bacterium, *Helicobacter pylori*. According to Nanjundaiah *et al.* (2009) the aqueous ginger extract was able to protect the gastric mucosa from stress induced by mucosal lesion and inhibited the growth of *Helicobacter pylori*.

Research by Moore and Pizza (1992) had revealed that garlic stimulates the activity of the defensive cells of the body such as the lymphocytes and macrophages. These blood cells protect us from pathogens. They are also able to destroy cancerous cells in the initial stage of cancer formation. Garlic is currently used with some degree of success, as a complement in the treatment of AIDS. It is also active against ascariis and certain oxyuroids which are the most frequent types of intestinal parasites (Willis, 1973). It has been proved by Sofowora (1993) that garlic prevents malignant tumors, especially digestive cancers.

There has been a shift from the prescription of antibiotics to the use of medicinal plant (Borris, 1996). Many plant extracts have been shown to possess antimicrobial properties. For example, aqueous and alcohol extracts of *Ocimum sanctum* and *Ocimum gratissimum* were highly toxic against fungi after 15 days culture (Amadioha, 2000).

Some extracts of garlic, onion and ginger have been reported to inhibit the growth of *Escherichia coli*, *Salmonella typhosa*, *Shigella dysenteriae* and *Staphylococcus aureus*. Flavones, flavonoids and flavonols are chemical compounds active against microorganisms and they are synthesized by plant in response to microbial infection (Dixon *et al.*, 1983).

Allium sativum, commonly known as garlic is a species in the onion family Alliaceae and belongs to the plant order liliales (Onyeagba *et al.*, 2004). Therapeutical applications of garlic have been known for many ages. The plants are broadly used as antibiotics and are effective against diabetes, arteriosclerosis and cancer (Chiba *et al.*, 1998). This plant is also known to reduce blood plasma cholesterol and blood pressure. It also inhibits platelet mass formation (Mayeux *et al.*, 1997).

Zingiber officinale is a perennial plant that grows to a height of 2 to 3 feet from the underground rhizome (ginger) which is the most important part of the plant for human consumption. The erect leaf aerial stem grows up to approximately 1 meter in height and has purple flowers. Its root is used as spice in cooking throughout the world (O'Hara *et al.*, 1998). The plant produces an orchid like flower with petals that are greenish yellow and streaked with purple color. Ginger is cultivated in areas of abundant rainfall. *Zingiber officinale* is native to southern Asia and it is cultivated in tropical areas such as Jamaica, China, Nigeria and Haiti. It is an important spice crop in India and is mainly cultivated in Kerala, Karnataka, Tamil Nadu and Eastern states. The plant belong to the domain Eukarya, Kingdom- plantae, phylum- Magnoliophyta, Class- Liliopsida, Order- Zingiberales, Family- Zingiberaceae, Genus- *Zingiber*, Specy- *Zingiber officinale* (White, 2007). Ginger is the rhizome of *Zingiber officinale* and is the part of the plant meant for consumption. Its characteristic odour and flavour is caused by a mixture of zingerone, shogaol and gingerols, volatile oils that compose 1 to 3 percent of the weight of fresh ginger (Jolad *et al.*, 2005).

Many microorganisms have developed resistance to antibiotics. There is therefore need to search for new and additional alternative antimicrobial agent such as various plants extracts which contain bioactive phytochemical constituents and to evaluate the antimicrobial activity and estimate proximate and micronutrient compositions of ginger (*Zingiber officinale*) and bulb of garlic (*Allium sativa*).

Materials and Methods

Collection and Preparation of Plant Material

Ginger and garlic were purchased from Oba and Uselu Market in Benin City, Edo State, Nigeria. The plants were identified by Prof. Idu of the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria. The rhizomes were washed to remove soil and then peeled, washed, sliced and dried. The dried materials were powdered. Fifty grammes each of the powdered ginger and garlic were weighed into separate bottle and 500 ml of distilled water was added to each bottle. The plant materials were soaked in distilled water for 48hrs and then filtered. The respective filtrate was concentrated using water bath at 75 °C to get the crude extract from which different concentrations were prepared. All extracts were stored at 4 °C when not in use.

Preparation of Different Concentrations of the Extracts

Following the method of Ekwenye and Elegalam (2005), concentration of 100 mg/ml of the extract was prepared by dissolving 0.1 g of the extract in 1ml of sterile distilled water. Then concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25 mg/ml were prepared from the stock concentration (100 mg/ml) by double dilution procedure.

Determination of Phytochemical Constituents of Garlic and Ginger

The phytochemical screening of garlic and ginger extracts were carried out to determine the presence of the following compounds; Saponins, tannins, phenols, cardiac glycoside, Anthracene, flavonoids and alkaloids using the standard procedures described by Young *et al.* (2005); Trease and Evans (1989) and Edeoga *et al.* (2005)

Antimicrobial Susceptibility Testing

Source of microorganisms

The microorganisms utilized in this study were obtained from the University of Benin Teaching Hospital. Both bacteria and fungi were sub cultured aseptically onto nutrient agar and potato dextrose agar media. Bacteria organisms include *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* and *Staphylococcus aureus* while fungi include *Rhizopus oryzae*, *Aspergillus flavus* and *Aspergillus niger*

Standardization of Inoculum

The standardization of the inoculum was carried out following the method of Chapin and Lauderdale (2003). The optical density (OD) of the prepared Mcfarland standard at a wavelength of 625 nm was measured and recorded. The acceptable range for a Mcfarland 0.5 standard is 0.08 to 0.10 OD. All inocula were standardized to 0.5 Macfarland (1×10^8 cell suspension), measuring the turbidity of the inoculum using Spectrophotometer at 625 nm.

Microbial inoculum preparation

The inocula were prepared by inoculating the test organisms in nutrient broth and incubating them for 24 hours at 37°C. After incubation, one milliliter of the culture were inoculated into nutrient agar at 45°C using a pasteur pipette.

Antimicrobial Assay

Antimicrobial activity was evaluated by the method of Eloff (1998) by noting the zone of inhibition against the test organisms. Two colonies of a 24 hrs plate culture of each organism were transferred aseptically into 10 ml sterile normal saline in a test tube and mixed thoroughly for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried nutrient agar and potato dextrose agar plates for bacteria and fungi respectively.

Three (3) adequately spaced wells of diameter 4 mm per plate were made on the culture agar surface respectively using sterile metal cupborer. 0.2 ml of each extract and control were put in each hole under aseptic condition with the aid of pipette pump, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. Conventional antibiotics were used as positive controls for bacteria and fungi respectively; distilled water was used as the negative control. The plates were then incubated at 37°C for 24 hours for the bacteria strains and at 28°C for 72 hours for fungal isolates. The zones of inhibition were measured and recorded after incubation. Zones of inhibition around the wells indicated antimicrobial activity of the extracts against the test organisms. The diameters of these zones were measured diagonally in millimeter with ruler and then mean value for each organism from the triplicate cultured plates was recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The Nutrient agar was prepared and sterilized, then poured into sterile petri dishes and allowed to solidify. The surface of the medium was inoculated with the test isolates. The discs soaked in different concentrations of the extract were placed on the surface of the seeded nutrient agar. The plates were incubated at 37°C for 24 hours, after which they were examined for the presence of growth inhibition. The MIC was taken as the lowest concentration that prevented the growth of the test microorganisms.

Minimum Bactericidal Concentrations (MBC) and Minimum Fungicidal Concentrations (MFC)

A loopful of the content of each plate in the MIC determination above, which did not show any visible growth after the period of incubation was streaked onto freshly prepared Nutrient agar, to determine their MBC and then incubated at 37°C for 24 hours after which it was observed for visible growth. The lowest concentration of the subculture with no growth was considered as minimum bactericidal concentration.

Antibiotic sensitivity disc

Two sensitivity discs a broad spectrum antibiotic (ciprofloxacin) for bacteria and ketoconazole for fungi were used. Sterile distilled water was used as negative control for all the test organisms.

Proximate and mineral analysis

The methods described in Association of Official Agricultural Chemist (AOAC) (2002) were used to analyze the proximate composition of the spices for protein, fat, fibre, ash and moisture while carbohydrate was calculated by subtracting the sum of the values of the other nutrients from 100. The mineral analysis was carried out as described by Okalebo *et al.* (2002) using Atomic Absorption Spectrophotometer (Pye Unicam Sp9, Cambridge, UK.). The minerals determined were sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), nitrogen (N), cobalt (Co), cadmium (Cd) and lead (Pb).

Statistical analysis

The experiment was set up in triplicates and result represents mean \pm standard error. The statistical tools used in this study include one way analysis of variance (ANOVA) and student T Test. All statistical analysis was performed using the statistical package for the social sciences (SPSS) version 14.

Results

The result in Table 1 below represents the phytochemical screening of *Zingiber officinale* and *Allium sativum*. Phytochemicals present in the plant extracts include tannins, saponins, alkaloids, flavonoids, cardiac glycoside, anthracene and phytic acids.

Table 1: Determination of the qualitative phytochemical screening of *Zingiber officinale* and *Allium sativum*

Parameters	<i>Zingiber officinale</i>	<i>Allium sativum</i>
Tannins	+	+
Saponins	+	+
Alkaloid	+	+
Flavonoid	+	+
Phytic acids	+	+
Cardiac glycoside	+	+
Anthraquinones	+	+

+ = Present

Table 2 presents the measurement of zone of inhibition of ginger (*Zingiber officinale*) extract on selected oral microorganisms (fungi). *A. niger* showed highest sensitivity of 21.60±1.16 mm at 100 mg/ml and least sensitivity of 13.60±0.81 mm at 3.125 mg/ml, *A. flavus* had highest sensitivity of 19.60±1.23 mm at 100mg/ml and least sensitivity of 13.00±0.58 mm at 3.125mg/ml while *R. oryzae*, a fungus, recorded highest sensitivity of 18.60±1.40 mm at 100 mg/ml and least sensitivity of 11.00±0.52 mm at 3.125 mg/ml.

Table 3 indicates measurement of zone of inhibition of garlic (*Allium sativum*) extracts on selected oral microorganisms (fungi) with *A. niger* having highest sensitivity of 27.60±1.07 mm at 100 mg/ml and least sensitivity of 12.60±0.68 mm at 3.125 mg/ml, *A. flavus* with highest sensitivity of 31.00±1.30 mm at 100 mg/ml and least sensitivity of 14.00±0.51 mm at 3.125 mg/ml while *R. oryzae* showed highest sensitivity of 25.60±1.30 mm at 100 mg/ml and least sensitivity of 13.20±0.86 mm at 3.125 mg/ml.

Table 4 presents the measurement of zone of inhibition of garlic (*Allium sativum*) extracts on selected oral microorganisms (bacteria). *Staphylococcus epidermidis* recorded highest sensitivity of 21.00±1.21 mm at 100 mg/ml concentration while the lowest sensitivity was recorded as 12.00±0.00 mm at 3.125mg/ml concentration, all organisms including *E. coli*, *P. mirabilis*, *K. pneumoniae* and *S. aureus* had highest inhibition zones at 100mg/ml and least inhibition zone at 3.125 mg/ml respectively.

Table 5 below is the measurement of zone of inhibition of the effect of ginger (*Zingiber officinale*) extracts on selected oral bacteria with *S. epidermidis* recording the highest sensitivity of 33.00±0.24 mm at 100 mg/ml concentration while *K. pneumoniae* recorded the lowest sensitivity of 10.60±0.00 mm at 3.125mg/ml concentration. All organisms had progressive increase in their zones of inhibition as the concentration of the extract increases.

The result in table 6 presents the proximate composition of *Zingiber officinale* and *Allium sativum* samples. Ginger had higher moisture (0.04%), ash (3.00%) , lipid (12.00%) and crude fibre (0.78%) contents compared to garlic which had 0.03 % (moisture) , 2.00 % (ash), 1.00% (lipid) and 0% (crude fibre) respectively. Garlic showed higher crude protein (17.50%) and Carbohydrate (77.47%) contents compared to ginger which had 14.00% crude protein and 70.18% carbohydrate.

Discussion

The results of the phytochemical analysis indicate that tannins, alkaloids, saponins, flavanoids and phytic acids were present in the extracts of ginger (*Zingiber officinale*) and garlic (*Allium sativum*). The presence of these chemical components in these plants indicated that the plants have some medicinal potential. This is probably due to the fact that each of the components identified has record of one therapeutic usage or another. For instance, plants rich in saponins are known to be immune boosting and have anti-inflammatory properties (Kenner and Requena, 1996). Similarly, plants with tannins have antimicrobial potentials due to their basic character which allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane (Elmarie and Johan, 2001). The antibacterial activities of alkaloids and flavonoids have been reported by Adesina *et al.* (2000) and Onwuliri and Wonany (2005).

The results of the agar-well diffusion method show that the extracts of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) exhibits antimicrobial activity against the test organisms, with highest zone of inhibition recorded at the highest concentration which decreases as the concentration reduces (100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml). Among the tested microorganisms *Klebsiella pneumoniae* was the most and least sensitive to the garlic (*Allium sativum*) extract at 100 mg/ml (24.30±0.33 mm) and at 3.125 mg/ml (10.00±0.01 mm). This justifies the traditional use of water in extracting ginger (*Zingiber officinale*) and garlic (*Allium sativum*) components, to control pathogenic organisms. The sensitivity of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) to various microbes were evaluated as it demonstrated the best antimicrobial activity in the general screening at various concentrations used in this study.

Table 6: Proximate composition of *Zingiber officinale* and *Allium sativum* samples

Parameters	<i>Zingiber officinale</i> (%)	<i>Allium sativum</i> (%)
Moisture	0.04	0.03
Ash	3.00	2.00
Lipid	12.00	1.00
Crude protein	14.00	17.50
Crude fibre	0.78	0.00
Carbohydrate	70.18	77.47

Table 7 presents the micro nutrient composition of *Zingiber officinale* and *Allium sativum* samples showed that *Zingiber officinale* had higher composition of Calcium (723 mg/g), Magnesium (252.1 mg/g), Sodium (91.3mg/g) and Potassium (163.2 mg/g) compared to *Allium sativum* which had Calcium (489.0 mg/g), Magnesium (144.9 mg/g), Sodium (65.3 mg/g) and Potassium (110.4 mg/g). Nitrogen composition in *Allium sativum* was higher (2.80 mg/g) compared to *Zingiber officinale* with 2.24 mg/g.

Table 7: Micro nutrient composition of *Zingiber officinale* and *Allium sativum* samples

Parameters	<i>Zingiber officinale</i> (mg/g)	<i>Allium sativum</i> (mg/g)
Calcium	723	489
Magnesium	252.1	144.9
Sodium	91.3	65.3
Potassium	163.2	110.4
Phosphorus	-	-
Nitrogen	2.24	2.80
Lead	-	-
Cadnium	-	-
Cobalt	-	-

- = absent; n=3

Similar reports have been well documented earlier, which state that a great number of medicinal plants are less active against gram negative than gram positive organisms (Javdan and Estakhr, 2011; Hema, 2013). The inhibitory activities of the extracts live up to their potential in the treatment of microbially induced ailments or disease conditions, in line with the traditional use of plant extracts.

The antimicrobial activities of the extract of ginger (*Zingiber officinale*) and garlic (*Allium sativum*) were compared to those of conventional antibiotics (Ciprofloxacin) and antifungal drugs (Ketoconazole). It was observed that ginger extract was more effective in treating oral fungi infection compared to the antifungal drug (Ketoconazole). Ginger extract had highest zone of inhibition of 21.60 ± 1.16 mm while Ketoconazole recorded highest activity of 14.18 ± 1.01 mm. Garlic (*Allium sativum*) extract also proved more effective against oral fungi microorganisms compared to conventional antifungal (Ketoconazole) drugs used. *Allium sativum* and *Zingiber officinale* also showed more potent activity against the tested oral bacteria isolates compared to Ciprofloxacin. This is in agreement with the results of previous studies, which reported the efficiency of various solvents used for extraction of crude plants (Ezeifeke et al., 2004). The extracts demonstrated high activity in all the organisms since clear zones of inhibition were seen on the agar plate; which is in line with previous findings (Eloff et al., 2005; Manetti et al., 2007; Nwaokorie et al., 2010).

Variations in the sensitivity of the microbial species tested on the extracts might be as a result of differences in the strains employed which quite differ from the wild strains sourced from oral samples used in this study. Wild strains of microorganisms could possess genetic capabilities that could make them adapt well to the tough environments they dwell in compared to stock cultures, which have been isolated and preserved. The antimicrobial activity of plant extracts has been linked by many researchers to be due to the presence of phytochemicals in them (Sofowora, 1993; Cowan, 1999). The antimicrobial activity of the extracts tested in vitro could be higher than they are reported if active ingredients from the extracts are isolated and tested. Ebi and Ofoefule (1997) reported that crude extracts of plant materials may contain inactive substances which may also antagonize the antimicrobial actions of one other.

The proximate analysis revealed that ginger (*Zingiber officinale*) and garlic (*Allium sativum*) can be ranked as carbohydrate rich due to their high calorie content *Zingiber officinale* (70.18 %), *Allium sativum* (77.47 %) in table 6. Though low in crude fat content, the oil could be extracted for use as essence or essential oil (Okwu and Nnamdi, 2008). The high crude protein content of garlic and ginger may be due to the presence of active proteinous metabolites such as allicin, ajoene and capsaicin. According to Dashak et al. (2001), the normal daily protein requirement for a normal adult is 45 – 50 g. Therefore, these spices could serve as supplements since they are usually combined in human main dishes. Low ash is usually an indication of low inorganic mineral content (Oloyede, 2005). However, the nutritionally important ones such as Calcium, Magnesium, Sodium and Potassium were found in relatively high amount in the two spices. The reduced levels of crude fibre obtained from the two spices posed no threat since they are not usually consumed in isolation but as adjuncts or additives to other foods. Hence, their low fibre contents many serve as a boost to the total dietary fibre of the dishes in which they are used. The reduced moisture content in the spices is an indication that their shelf life would be prolonged and that deterioration due to microbial contamination would be limited (Dashak et al., 2001). The data obtained for the proximate analysis of the spices agree with earlier reports and indicates that they contribute nutrients to the diet (Dashak and Nwanegbo, 2000; Nwinuka et al., 2005; Edeoga et al., 2005).

The mineral elements contained in these spices are very important in human nutrition. Sodium, potassium, calcium and magnesium play a central role in the normal regulation of blood pressure (Karppanen, 1994). In particular, these elements have important interrelationships in the control of arterial resistance (Altura and Altura, 1999). They also regulate the fluid balance of the body and hence, influence the cardiac output. It is increasingly being realized that a lower than normal dietary intake of Magnesium can be a strong risk factor for hypertension, cardiac arrhythmias, ischemic heart disease, atherogenesis and sudden cardiac death (Altura and Altura, 1999). Zinc and chromium are well known trace elements in diabetes as cofactors for insulin (Kimura, 1996) while calcium, magnesium and phosphorus are also essential for bone and teeth formation (Okwu, 2005). The importance of these elements cannot be overemphasized because they are required by many enzymes as co-factors (Ozcan, 2004). The non-detection of Lead (Pb), Cadmium (Cd) and Cobalt (Co) is of great advantage to consumers of these spices as these elements have been reported to be highly toxic even at low concentrations (Asaolu et al., 1997; Oloyede, 2005).

Conclusion

The present study has provided some comparative information on the proximate, mineral element and phytochemistry of garlic and ginger. There are indications that the two spices are good sources of nutrients, mineral elements and phytochemicals. Therefore, their use as nutritional supplements is highly promising and recommendable.

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