PHYTOCHEMICAL ASSESSMENT OF EXTRACTS OF SOME SELECTED SPICES FROM ANAMBRA STATE, NIGERIA

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Abstract

Spices are plant parts or organs including fruits, seeds, roots and rhizomes, and other plant parts used for flavoring or coloring of food. The phytochemical assessment of some spices from Anambra State including Capsicum annuum L., Piper nigrumL., Xylopia aethiopica (Dunal) A. Rich. (Fruits), and Myristica fragrans Hoult. (Seeds) was conducted using standard procedures.The research mas motivated by the fact that these spices are greatly and frequently used in various dishes in Anambra State. The results indicated that the spices possessed anthraquinones, alkaloids, saponins, terpenoids, tannins, flavonoids, phenols, and cardiac glycosides in varying concentrations. Alkaloid was the highest with the value of 28.70% ± 0.2 while saponin followed having the value of 28.23% ± 0.3.The least was cardiac glycoside valued as 0.02 µg/100g ± 0.005. The presence of these bioactive compounds in the study spices confirms their use in traditional medicine. The rich contents of these phytonutrients in spices studied could be harnessed in pharmaceutical industry for drug formulation.

Keywords: *Spices, Phytochemistry, Assessment, Pharmaceuticals, Anambra.*

Introduction

Plants are really of immense importance to the planet earth and for all living things in it. Plants supply food to humans, provide many vital products for human use including firewood, timber, fossil fuels, fibers, medicines, dyes, pesticides, rubber, spices, and so on. A spice is a seed, fruit, root, bark, or other plant substances primarily used for flavoring or coloring of food (O'Connell, 2016).Plants from which spices are obtained are the spice plants. Therefore spice plants are those plants which seeds, fruits arils (such as mace), rhizomes and roots, barks, flower buds or stigmas are used to add flavor and aroma to dishes. The spice plants include turmeric and ginger (roots and rhizomes), mustard, nutmeg (seeds), black pepper, chili pepper, Ethiopian pepper, cayenne pepper etc.(fruits), true cinnamon, and cassia (barks), cloves (flower buds), saffron (stigmas) etc (Duke, 2003).

Seed spices or spice seeds are the tiny aromatic fruits and oil-bearing seeds of herbaceous plants (Gopal, 2018). Some common spice of fruits/ seeds in Anambra State are used for flavoring of dishes like soups, stews, and goat head ("Isiewu"), pepper soups of meat and fish and popular delicacy "Nkwobi" (Igbo). Some of these spice fruits /seeds include chili pepper (*Capsicum annuum*), black pepper (*Piper nigrum*), and Ethiopian pepper (*Xylopia aethiopica* (fruits) and nutmeg *Myristica*

fragrans (seeds).*Capsicum annuum* L. (Syn. *Capsicum abyssinicum*A. Rich.), has various common names including sweet pepper, cayenne pepper, chili pepper etc. Chili pepper is an evergreen perennial herb belonging to the family Solanaceae growing up to 1m tall and with local names "ose" (Igbo), "Atawewe" (Yoruba), and"Tatashi" (Hausa). The fruit is aberry that may be green, yellow, orange or red when ripe (Plate 1A). *Capsicum annuum* has numerous medicinal uses including use in the treatment of cold stage offevers, varicose veins, asthma, digestive problems, sprains, neuralgia, stiff joints, rheumatism, bronchitis, chest colds with coughs, heart arrhythmias, arthritis etc (Badia *et al.*, 2017) and with anti-haemorrhoidal, antirheumatic, antiseptic, diaphoretic, digestive, irritant, rubefacient, and tonic effects (Saleh *et al.*, 2018).

Myristica fragrans Houtt. (syn. *M. moschata* L.), commonly known as Nutmeg tree, is an evergreen tree growing up to 5-15m tall, and belongs to the family Myristicaceae. The fruits are smooth, yellow, ovoid or pear shaped, each bearing a purple brown shiny seeds (Badia *et al*., 2017) (Plate1B) used as a spice. Nutmeg has many medicinal potentials and reported to be used in the treatment of stomach ulcers, rheumatism, dysentery, diarrhea, vomiting, indigestion, tooth ache, insomnia, abdominal pains including labor pains, and premature ejaculation and with diuretic, anti-tumor, anti-inflammatory, antithrombotic, emenogogue, astringent, carminative and aphrodisiac properties (Takooree *et al*., 2019).

Piper nigrum L., commonly known variously as black pepper, white pepper, green pepper, Ashanti pepper, Benin pepper, West African pepper etc., is a climbing shrub of the family Piperaceae producing a cluster of woody stems up to 10m tall, though more commonly 3-4m tall in cultivation and the stems attach themselves to other vegetation by means of adventitious roots (Chevallier, 1996). The fruits (Plate1C), commonly known as pepper corn, are dried and used as a spice. Black pepper is locally called "Uziza" (Igbo), "Iyere" (Yoruba) and "Masoro" (Hausa). Black pepper is used in the treatment of a number of diseases such as sunburn, constipation, insomnia, and toothaches, food poisoning, cholera, dysentery, rheumatism, diarrhea, vomiting, sinusitis, epilepsy and skin inflammation (Takooree*etal*., 2019).

Xylopiaa ethiopica (Dunal) A. Rich with common names Negro pepper, African pepper, Guinea pepper, Ethiopian pepper or spice tree, is locally called "Uda" (Igbo), "Eeru", "Eru" or "Erunje" (Yoruba) and "Chimba" or "Kimbaal" (Hausa). It is an evergreen aromatic tree, in the family Annonaceae. The fruit appears like a legume (Plate1D) and dried for use as spice. It has many medicinal potentials including use in the treatment of cough, stomachache, dizziness, bronchitis, dysentery, headache, neuralgia, female sterility, biliousness and skin infections and as a carminative and purgative (Tegang and Ngoune, 2018). Therefore, the aim of this study was to assess the phytochemical constituents of the fruits /seeds of these four spice plants common in Anambra State.

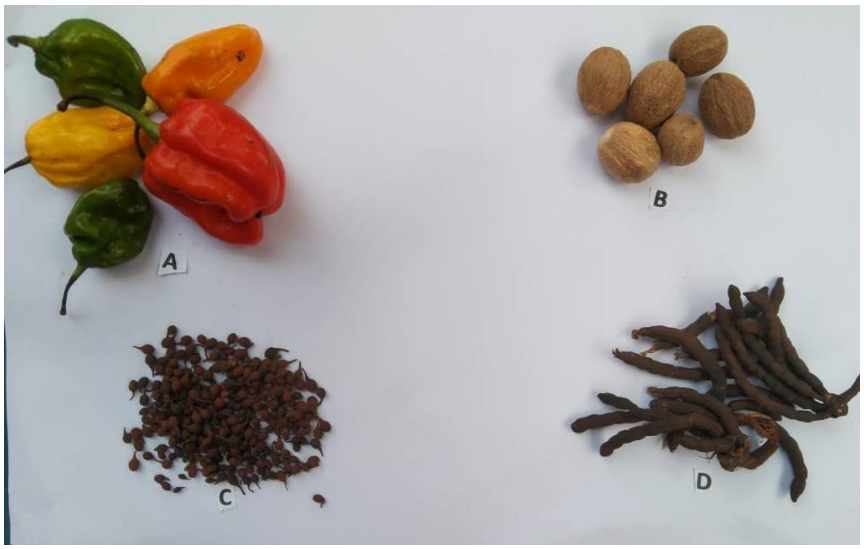

Plate 1: The study spices (A) Fruits of *Capsicum annuum* (B) Seeds of *Myristica fragrans*(C) Fruits of *Piper nigrum*(D) Fruits of *Xylopia aethiopica.*

Materials

Collection of plant materials

The fruits/seeds of *Capsicum annuum, Myristicaf ragrans, Piper nigrum,* and *Xylopia aethiopica* (fig.1) were purchased from various major markets in Anambra State, Nigeria including Eke Ekwulobia,Afo Ufuma,Agulu market,Atani market,Eke Awka etc.

Methods

Preparation of Plant Extracts

The fruit/seed samples of the spices collected were thoroughly washed with water, dried in an oven at 60^0C for 2hrs, and ground into fine powder using the Thomas milling machine. The ethanolic extracts of the samples were prepared using soxlet apparatus at 55-85^0C for 8-10hrs. The crude extracts obtained were filtered through Whatman No.42 filter paper. The the extracts were properly stored until required for phytochemical assessment. The phytochemical assessment of the study spices was conducted at the Science Laboratory of Federal Polytechnic Oko.

Qualitative Phytochemical Screening

The qualitative phytochemical screening of the samples was conducted to identity the constituent phytochemicals of the various extracts of the study spices following the methods of Harborne (2016).

Test for Anthraquinones

About 2 ml of the extracts of each study spice was put in a test tube and 4ml of benzene added and the mixture shaken. The mixture was then filtered and 2ml 0110% ammonia solution was added and shaken. The occurrence of pink color in the lower portion confirmed the presence of anthraquinones

Test for Alkaloids

Two milliliters solution of each of the spice extract and 0.2ml of dilute HCl were put in a test tube and then 1ml of Hager's reagent was added. The appearance of yellow crystalline precipitate was indicative of the presence of alkaloids.

Test for Saponins

About 10ml of each spice extract was mixed with 5ml of distilled water and shaken vigorously thereby forming a stable persistent froth. The froth was subsequently mixed with 3 drops of olive oil and shaken vigorously again and the formation of emulsion suggested the occurrence of saponins.

Test for Terpenoids

About 2 ml of each of the extract of the spice samples was put into a test tube and 2ml of chloroform added. Subsequently, 5ml of conc. H2S04 was carefully added to form a layer. The appearance of reddish brown color at the interface indicated the presence of terpenoids.

Test for Tannins

Five percent (5%) of $Fecl_3$ in 90% alcohol was prepared and a few drops were added to 3ml of extract of each spice sample. The occurrence of brown-green color was indicative of the presence of tannins.

Test for Flavonoids

Five milligrams (5mg) of each of the spice extract was added to 5ml of methanol (95% v/v). It was then treated with few drops of conc. HCl and 0.5g of magnesium metal. The appearance of pink color indicated the presence of flavonoids.

Test for Phenols

Twenty milligrams (20mg) of each spice extract was put into a test tube and subsequently treated with few drops of $Fecl_3$. The development of deep blue coloration confirmed the presence of phenols.

Test for Cardiac Glycosides

In a test tube containing about 2 mg of each of the extract of spice samples was added 2ml of glacial acetic acid containing 1 drop of $Fecl_3$ solution. Also added into the mixture was 1ml of conc. H_2So4. The appearance of a brown ring at the interface was an indication of the presence of cardiac glycoside.

Quantitative Phytochemical Assessment

The quantitative phytochemical assessment of the powdered and extracts of spice samples was conducted to estimate the crude contents of the secondary metabolites in the spice samples.

Assessment of Anthraquinones

Into a flask was added 50mg of each of the spice extract (W1) and 30ml of distilled water added and the solution properly mixed, weighed and refluxed for 15min on a water bath. The flask with its content was allowed to cool, weighed, adjusted to the original weight with distilled water and the mixture centrifuged at 4000rpm for 10min and filtered. Then, 20ml of the supernatant liquid was transferred to a separatory funnel and acidified with 2M HCl. Subsequently, 15ml of chloroform was added, the mixture extracted and chloroform layer discarded. The aqueous layer was separated and 0.10g of $NaCO_3$ was added and the mixture shaken for 3min and centrifuged at 4000rpm for another 10min. Then, 10ml of the supernatant liquid was transferred to a 100ml flask. Furthermore, 20ml of solution of 10.5% w/w ferric chloride hexahydrate was added and mixed. The mixture was refluxed on a boiling water bath for 20min. Then 1ml of conc. HCl was added and the mixture heated for 20min, with frequent shaking in order to dissolve the precipitate. Again, the mixture was cooled,

transferred to a separatory funnel and shaken with 25ml diethyl ether. The process was repeated until anthraquinones were exhaustively extracted, tested by the Bomtrager's reaction. The diethyl ether extracts were combined and washed with 15ml distilled water twice. The combined diethyl ether was then transferred to a 100ml volumetric flask and adjusted to volume. Then 25ml of the solution was evaporated to dryness. The residue (Wf) was dissolved with 10ml of 0.5% (w/w) magnesium acetate in ethanol yielding a red solution. The UV absorbance was measured at 515nm. The percentage of total anthraquinones extracted was determined with the expression:

$$\% \text{ Total Anthraquinones} = \frac{W_1 - W_f}{W_1} \times \frac{100}{1}$$

Assessment of Alkaloids

About 50mg each of the extract spice samples was taken into a beaker and a total of 20ml of 20% acetic acid was added; the beaker covered and allowed to stand for 4hrs. The mixture was later filtered and the volume reduced to one quarter in a boiling water bath. Then conc. ammonium hydroxide (NHOH) was added to the solution drop wise until the precipitation was complete. The whole solution was allowed to settle and the precipitate collected by filtration and weighed. The percentage of total alkaloid content was calculated thus:

$$\% \text{ Total Alkaloids} - \frac{\text{weight of residue}}{\text{weight of sample used}} \times \frac{100}{1}$$

Assessment of Saponins

About 500ml of each of the spice extract was mixed with 250ml of vanillin reagent and 250ml of distilled water added. Then 2.5ml of 75% H_2S04 was added and mixed properly. The solution was kept in a water bath at 60^0C for 10min. After the time interval, the solution was cooled in ice cold water and the absorbance readat 544nm using a spectrophotometer. The saponin values were expressed as diosgenin equivalent (mg/g extract) derived from a standard curve. Thepercentagesaponin content was determined by the expression:

$$\% \text{ Total saponins} = \frac{W_2 - W_1}{W_1} \times \frac{100}{1}$$

Assessment Terpenoids

About 500ml of each of the extract of the spice sample was taken into a beaker and soaked in 9ml of methanol for 24hr. The extract of each spice sample was filtered and extraction repeated with 10ml of petroleum ether using a separatory funnel. The ether extract was separated into pre-weighed glass vials and allowed for complete drying (Wf). The ether was evaporated and the percentage yield of total terpenoid content was determined thus:

$$\% \text{ Total terpenoids} = \frac{W_1 - W_f}{W_1} \times \frac{100}{1}$$

Assessment of Tannins

In this procedure, 500ml of each spice extract was taken into a test tube and treated with 100mg of polypyrrolidone and 500ml of distilled water. The mixture was incubated at 40^0C for 4hr and later centrifuged at 500rpm for 5min and then 20ml of the supernatant was taken. The supernatant had only simple phenols free of tannins and the tannins would have been precipitated along with the polyvinyl polypyrrolidone. The phenolic content of the supernatant was measured at 725nm and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannin content of the extract was calculated as follows:

Tannins (mgCAE/g Extract) =Total phenolics (mgGAE/g extract)-free phenolics (mgGAE/g extract).

Assessment of Flavonoids

Five hundred milliliter (500ml) of each spice extract was diluted with 200ml of distilled water followed by the addition of 150ml sodium nitrate solution. The mixture was incubated for 5min and then 150ml of 10% aluminum chloride solution was added and allowed to stand for 6min. Then, 2ml of 4% sodium dioxide was added and made upto 5ml with distilled water. Subsequently, the mixture was shaken properly and left for 15min at room temperature and the absorbance was measured at 510min. Total flavonoid content was expressed as rutin equivalent of mg/g extract on a dry weight basis using the standard curve. The percentage content was calculated as follows:

$$\frac{W_1 - W_2}{W_1} \times \frac{100}{1}$$

Assessment of Phenols

The total phenol content of each of the spice samples was determined by the use of Folin-Cicalteau reagent. In this method, 20ml of each spice extract was taken into a test tube and made up to 1ml with distilled water. Then 500ml of dilute Folin's phenol reagent was added. The mixture was shaken properly and incubated in dark condition for 40min for development of color. At the endof incubation period, the absorbance was measured at 725nm. A calibration curve of Gallic acid was constructed and linearity was obtained in the range of 10-15mg/ml. The total phenolic contents in the extracts were expressed as mg Gallic acid equivalent (mgGAE/g extract) by using the standard curve.

Assessment of Cardiac Glycosides

Cardiac glycoside content of each spice sample was assessed using Baljet's reagent. In this process, 50mg of the extrct of each sample was soaked in 10ml of 70% ethanol for 2hr and then filtered. The extract obtained was then purified using lead acetate and Na_2HP0_4, solution then, the freshly prepared Baljet's reagent was added to the solution. The difference between the intensity of colors of the experimental and blank (Baljet's reagent and distilled water) samples gave the absorbance and it was proportional to the concentration of cardiac glycosides in micrograms (mg).

Results

The results of the qualitetive phytochemical screening of the spice extracts of *Capsicum annuum, Myristica fragrans,Piper nigrum,* and *Xylopia aethiopica* indicated the presence of anthraquinones, alkaloids, saponins, terpenoids, tannins, flavonoids phenols,and cardiac glycosides.Quantitatively,the alkaloid was highest with the value of 28.70% ± 0.2 while saponin followed by having the value of 28.23% ± 0.3.The least was cardia glycoside valued as 0.021µg/100g ±0.005 (Table 1).

Table 1: Phytochemical composition of ethanolic extracts of some selected spices from Anambra State, Nigeria.

S/N	Spice plant	Ant (%)	Alk (%)	Sap (%)	Terp (%)	Fla (%)	Tan mg/100g)	Phen (mg/100g)	Card Gly (ug/100g)
1	*Capsicum annuum*	22.08± 0.01	14.02± 0.5	24.62± 0.4	16.91± 0.5	17.58± 0.3	21.524± 0.3	12.62± 0.3	0.24± 0.001
2	*Myristica fragrans*	11.93± 0.5	28.70± 0.2	28.23± 0.3	25.04± 0.4	25.77± 0.4	24.14± 0.5	23.67± 0.1	0.035± 0.002
3	*Piper nigrum*	18.34± 0.04	16.01± 0.1	15.20± 0.01	11.78± 0.4	15.44± 0.4	14.46± 0.5	21.20± 0.2	0.029± 0.004
4	*Xylopia aethiopica*	12.55± 0.01	18.25± 0.4	17.47± 0.3	19.33± 0.04	19.14± 0.2	16.26± 0.4	26.71± 0.5	0.021± 0.005

Discussion

The results revealed that these spices are rich in active secondary metabolites that tend to confirm their usage in flavoring of foods and in traditional medicinal in view of the fact that they have been reported to possess medicinal qualities (Saleh *et al*., 2018;Tokooree *et al*., 2019). For instance, anthraquinones besides their utilization as colorants have been used for medicinal applications in the treatment of ailments like cancer diabetes, kidney and liver diseases, constipation arthritis, multiple sclerosis, and malaria and possess biological activities including antioxidant, antimicrobial,anti-inflammatory, insecticidal, emetic and as a laxative (Bolen, 2020). Alkaloids are reported to be utilized in the treatment of cancer, asthma, malaria, heart problems and diabetes (Cushnie *et al*., 2014), and with anti-inflammatory, anticancer, analgesic, antimitotic, psychotropic, antitumor, antimicrobial, and cardio-protective activities (Heinrich *et al*.,2021).Moreover, saponins have potentials in the treatment of many afflictions including cancers, diabetes, dental caries, with cholesterol lowering power and antidote against acute lead poisoning (Shi *et al*., 2004). Saponins exhibit a variety of biological activities including anti-inflammatory, antimicrobial, insecticidal, anticancer, cytotoxic, anti-irritation and antiseptic (Egbuna *et al*., 2020). On the other hand, terpenoids are claimed to be involved in the treatment of cardiovascular diseases, cancer, malaria, bronchitis, catarrh, colds, migraines, rheumatism, strong torments, pains (Bishi *et al*., 2021) and exhibit anticancer, antidiabetic, antimalarial, anti-inflammatory, antioxidant, antiseptic, astringent, diuretic, antimicrobial,antihyperglycemic, neuroprotective, immunoregulative,and antiparasitic effects (Joshee *et al*., 2019; Yang *et al*., 2020). Also tannins are used in the treatment of varicose ulcers, hemorrhoids, inflammation of gums, diarrhea, catarrh, neurodegenerative diseases, cardio-vascular diseases, stomach ulcers and certain cancers (Singh and Kumar, 2020) and show variety of biological activities such as antioxidant, antidiabetic,

astringent, immunomodulatory, anti-inflammatory, antitumor, antimicrobial, antiseptic, anti-helminthic, and anticancer properties (Ukoha *et al*., 2011).

Furthermore, many flavonoids have potential in the treatment of cancer, cardiovascular and respiratory disorders, arthritis, and early ageing (Bose *et al.*, 2018) and are shown to have antioxidant, hepatoprotective, anti-inflammatory, antimicrobial, anticancer, cardioprotective, immune systempromoting, antimutagenic, and anticarcinogenic effects (Panche *et al.*, 2016; Tungmunnithum *et al*., 2018). In like manner, phenolic compounds are reported to be used in the treatment of sore throats, cold sore, toothache, pains, cancer, mouth and throat pains, meningitis, typhoid fever, small pox, polio, removal of warts, pharyngitis and pneumonia (Jewell, 2019) and exhibit antioxidant, anticarcinogenic, antimutagenic, and anti-inflammatory effects (Kumar, 2020); Another important bioactive compounds assessed from these spices are cardiac glycosides. These compounds at therapeutic levels are used to treat congestive and arterial arrhythmia and show anticancer and antineoplastic effects (Aronson, 2016);applied to poison arrows for use in hunting and fighting (Morsy, 2017).The presence of these compounds in the study spices confirms their utilization in traditional medicine for the management of a variety of human diseases .Moreover, in view of the quantities of the bioactive compounds assayed in the spices, it is therefore recommended that these spices be harnessed in pharmaceutical formulation of drugs.

Conclusion

Spices are basically used as condiments in flavoring of variety of dishes. These spices give aroma, color, flavor, and sometimes even texture to food. The present survey showed that spices of Capsicum*annuum,Piper nigrum*, *Xylopiaaethiopica* (fruits), and *Myristicafragrans* (seeds) quantitatively contain substantial amounts of bioactive compounds. The study therefore confirms the use of these spices in traditional medicine. Inview of the relatively high amounts of bioactive compounds contained in the study spices, it is therefore recommended that these spices be harnessed in pharmaceutical industry for drug formulation. It is also recommended that further research be conducted for full isolation, identification and characterization of the various bioactive compounds assessed from each spice material.

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