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Article

Qualitative and Quantitative Screening of Turmeric, Garlic, Ginger and Clove Blend

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Abstract: Natural plant or plant products has been an important part of traditional medicine. The objective of the study was to investigate the phytochemical constituents of Turmeric, Garlic, Ginger and clove blend. The phytochemical screening for various phytochemical constituents was conducted using laboratory method. The result showed that the quantitative value of combination turmeric, garlic, ginger and clove blend using alcohol were for alkaloids (0.5205%), saponin (0.2770%), tannin (0.0375%), phenol (0.2300%), flavonoids (0.0038%), steroids (0.0077%), terpene (0.0058%) and glycoside (0.1335%). respectively while the extractive value. This study concluded that the combination of turmeric, garlic, ginger and clove blend at equal proportion contain this phytochemical (alkaloids, saponin, tannin, phenol, flavonoids, steroids, terpene and glycoside).

Keywords: Phytochemical, Turmeric, Garlic, Ginger and Clove.

1. Introduction

Phytochemicals are non-nutritive bioactive component that are primarily responsible for preventing disease and they act as antioxidants, enzymes stimulant, anti-bacterial agents, anti-cancer agents (Akinmoladun *et al.,* 2007). Most plants that contain the higher percentage of these

phytochemicals are often referred to as medicinal plants. Medicinal plants have been known to contain some organic compounds having definite physiological action on the human body (Yadav and Agarwala, 2011). For couples of decades, serious attentions have been paid to the use of traditional medicines and plant drugs against numerous diseases (Satya *et al.,* 2013; Kala, 2006) since they are safer with little or no side effect (Abu-Rabia, 2005; Parvath and Brindha, 2003).

Turmeric (*Curcuma longa*) is an ancient Indian spice and a traditional remedy that has been used as medicine, condiment and flavour. Turmeric is the rootstalk of a tropical plant in ginger family and it is botanically known as Curcumin longa (Chan *et al.,* 2009). The main component of the spice is a substance called curcumin which has potential healing properties as a result of its powerful anti-inflammatory and antioxidant properties. The presence of curcumin in turmeric has made it useful in preventing and curing some inflammatory conditions such as tendonitis and arthritis, disinfecting cuts and burns, preventing prostrate and breast cancer and stop the growth of existing ones, reducing the risk of childhood leukaemia and it is a natural liver detoxifier (Nagpal and Sood, 2013). The root has been in used for thousands of years in India and China as a spice, and medicine for conditions including heartburn, diarrhoea, stomach bloating, colds, fibromyalgia and depression. It is sometimes applied on the skin for ringworm and infected woods as it is said to have anti-bacterial properties. It is the spices responsible for the yellow colour of curry. Turmeric volatile oil functions as an external antibiotic, preventing bacterial infection in wounds (Chattopadhyay *et al.*, 2004) can be also used in powdered form to encapsulation and preparing highly beneficial organic turmeric health tablets.

Garlic is one of the oldest known food flavoring and seasoning plant that managed to infuse itself into culinary tradition of many civilizations across the world. The key active ingredient in garlic is the plant chemical, allicin, which rapidly decompose to several volatile organosulphur compounds with bioactivities (Chang and Cheong, 2008). Garlic are used for consumption (raw or cooked) or for medicinal purposes. They have a characteristic pungent, spicy flavor that mellows and sweetens considerably with cooking as reported by (*Katzer et al., 2009).* Other parts of the garlic plant are also edible. The leaves and flowers (bulbils) on the head (spathe) are sometimes eaten. They are milder in flavor than the bulbs and are most often consumed while immature and still tender. Immature garlic is sometimes pulled, rather like a scallion, and sold as "green garlic" (Thompson *et al.,* 1995) When green garlic is allowed to grow past the "scallion" stage, but not permitted to fully mature, it may produce a garlic "round", a bulb like a boiling onion, but not separated into cloves like a mature bulb (Thompson *et al.,* 1995). It imparts a garlic flavor and aroma in food, minus the spiciness. Green garlic is often chopped and stir-fried or cooked in soup or hotpot in Southeast Asian. Garlic has also been used as a spice, natural growth promoters and a native medicine for many years. It has been indicated to possess

antibiotics, antibacterial, antifungal, antiparasitic, antiviral, antioxidant, anti-cholesteremic, anticancerous and vasodilator characteristics (Demir *et al.,* 2003; Khan *et al.,* 2007; Hanieh *et al.,* 2010).

Ginger (Zingiber officinale) is a flowering plant whose rhizome, ginger root or simply ginger, is widely used as a spice or a folk medicine. It is an herbaceous perennial which grows annual stems about a meter tall bearing narrow green leaves and yellow flowers. Ginger is cultivated in the tropics and it requires warm and humid climate flourishing in a well-drained friable soil, though it can also be grown in a light soil rich in humus. It has variety of names from different continents and countries and such names are Zingiberis rhizome, Shen jiany, Cochin, Asia ginger, Africa ginger and Jamaican ginger. In addition to its food usage, ginger root has been found in aiding in lowering the cholesterol level, pain relief from arthritis, digestive issue, expectorant and gesture-intestinal stimulation (Wikipedia, 2018). Ginger is the underground rhizome of the ginger plant with firm striated texture. The flesh of the ginger rhizome can be yellow, white or red in colour depending upon the variety. It is covered with a brownish skin that may either be thick or thin and it is consumed as delicacy, medicine or spice. The characteristics odour and flavour of ginger is caused by a mixture of zingerone, shogaol and gingerol (Kikuzaki and Nakatani, 2006; Haksar *et al.,* 2006). Ginger produces clusters of white and pink flower buds that bloom into yellow flower and due to its aesthetic appeal and the adaption of the plant to warm climates, ginger is often used as landscaping around subtropical homes. It is a perennial reed-like plant with annual leafy stems about a meter (3 or 4 feet) tall (Lee and Shibamato, 2002).It was found that ginger contained 1.5% - 3% essential oil, 2-12% fixed oil, 40-70% starch, 6-20% protein, 3-8% fibre, 8% ash 9-12% water, pungent principles, other saccharides, cellulose, colouring matter and trace minerals (Peter, 2000).

Clove in the third century BC, Chinese emperors of the Han Dynasty required those who addressed him to chew cloves to freshen their breath, *Andaya et al., (1993)* and they had reached the Roman world by the first century AD, where they were described by Pliny the Elder (*Lape et al., 2010).* Eugenol composes 72–90% of the essential oil extracted from cloves, and is the compound most responsible for clove aroma *Kamatou et al., (2012)*. Cloves are used in the cuisine of Asian, African, Mediterranean and the Near and Middle East countries, lending flavor to meats, curries, and marinades, as well as fruit such as apples, pears or rhubarb. Cloves may be used to give aromatic and flavor qualities to hot beverages, often combined with other ingredients such as lemon and sugar. They are a common element in spice blends such as pumpkin pie spice and speculoos spices. In Mexican cuisine, cloves are best known as clavos de color, and often accompany cumin and cinnamon Dorenburg *et al.,* (2003). They are also used in Peruvian cuisine, in a wide variety of dishes such as carapulcra and arroz con leche. A major component of clove taste is imparted by the chemical eugenol, *Kamatou et al., (2012)* and the quantity of the spice required is typically small.

The usefulness of turmeric , garlic, ginger and clove the phytochemicals they contained are well documented but no research work has been carried out to check the phytochemical present in the combination of turmeric, garlic, ginger and clove in terms of the qualitative and quantitative analysis. Therefore, the focus of this research is to qualitatively identify and quantify the phytochemical constituents of turmeric, garlic, ginger and clove

2. Materials and Methods

2.1. Source and Preparation of Plant Materials (Turmeric, Garlic, Ginger and Clove)

Fresh Turmeric, Garlic, Ginger and Clove were purchased from Bode Market, Ibadan, Oyo State. Turmeric and Ginger were washed using clean water to remove the dirt. They were poured into clean basket to drain off water. Each ingredient was sliced into flakes in order to increase the surface area to aid drying. Thereafter, they were separately air dried until each weight remained constant. The test ingredients were then reduced into lentil-size part with the aid of mortar and pestle and milled into fine powdery form with the use of electric blender. Turmeric, garlic, ginger and clove were mixed at equal proportion. Thereafter it was sieved and stored in air tight container until use.

2.2. Qualitative Determinations of the phytochemical composition of turmeric, garlic, ginger and clove (Blend)

Qualitative screening of phytochemicals in turmeric, garlic, ginger and clove blend was carried out at the chemistry laboratory by the methods described by (Mittal *et al.*, 2014 and Bharadwaj *et al.*, 2019)

2.2.1. Test for alkaloid

1ml of extract of the blend was mixed with 1ml of Wagner's reagent (iodine in KI). The appearance of reddish brown precipitate indicates the presence of alkaloids

2.2.2. Test for saponin

About 0.2 g of plant extract was shaken with 4 mL of distilled water and then heated to boil on a water bath. Appearance of creamy miss of small bubbles (Frothing) shows the presence of saponin.

2.2.3. Test for tannin

0.5 g of plant extract was mixed with 2mL of water and heated on water bath. The mixture was filtered and 1mL of 10% FeCl3 solution was added to the filtrate. A blue-black solution indicates the presence of tannin.

2.2.4. Test for phenol

About 0.5 g of plant extract was added to 1 mL of 10% FeCl3 solution. A deep bluish green colouration was an indication for the presence of phenol.

2.2.5. Test for flavonoid

5 mL of distilled water and about 0.2 g of plant extract were mixed thoroughly. And 1 mL of 1% AlCl3 solution was added and shaken. A light yellow precipitate indicates the presence of flavonoids.

2.2.6. Test for steroids (Salkowski test)

0.2 g of plant extract and 2 mL of chloroform were added together, 2 mL of concentrated sulphuric acid was added to form a layer. The formation of a violet/blue/green/reddish-brown ring at the interface indicates the presence of steroidal ring.

2.2.7. Test for terpenoids

1ml of extracted clove sample was mixed in 500 µl of chloroform. Add 500 µl of conc. H2SO4 to form a layer. A raddish brown precipitate colour at the interface formed indicates the presence of terpenoid

2.2.8. Test for glycoside

0.2 g of plant extract and 2.5 mL of dilute sulphuric acid were mixed together and boiled for 15 minutes, cooled and neutralized with 5 mL each of Fehling solution A and B. The formation of brick red precipitate confirmed glycoside.

2.3. Quantitative Determinations of the Phytochemical Composition of Turmeric, Garlic Ginger and Clove (Blend)

The four phytochemical blend used in this study (turmeric, garlic, ginger and clove) were analysed at chemistry laboratory to check the quantitative of the constituent present in each test ingredient.

2.3.1. Alkaloids content of the blend

2g of finely ground sample (turmeric, garlic, ginger and clove) were weighed into a 100ml beaker and 20mls of 80% absolute alcohol added to give a smooth paste in a distillation and titrimetric procedure. The blend was transferred to a 250ml flask and more alcohol added to make up to 100ml and 1g magnesium oxide added. The blend was digested in a boiling water bath for 1.5hrs under a reflux air condenser with occasional shaking. The blend was filtered while hot through a small buchner funnel. The residue was returned to the flask and redigested for 30min with 50ml alcohol after which the alcohol

was evaporated, adding hot water to replace the alcohol lost. When all the alcohol has been removed, 3 drops of 10% HCL was added. The whole solution was later transferred into a 250ml volumetric flask 5ml of zinc acetate solution and 5ml of potassium ferrocyanide solution was added, thoroughly mixed to give a homogenous solution. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10ml of the filterate was transferred into a separatory funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10ml hot distilled water and transferred into a kjeldahl tube with the addition of 0.20g sucrose and 10ml Conc.H2SO4 and 0.02g selenium for digestion to a colorless solution to determine %N by Kjeldahl distillation method. %Nitrogen got is converted to % total alkaloid by multiplying by a factor of 3.26 i.e % Total alkaloid = %N X 3.26. (Harborne *et al.,* 1973 and Trease 1989)

2.3.2. Saponin content of the blend

The Spectrophotometric method of Brunner (1984) was used for Saponin Analysis. 1g of finely ground sample was weighed into a 250ml beaker and 100ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter the mixture was filtered through a whatman No1 filter paper into a 100ml beaker and 20ml of 40% saturated solution of maganesium carbonate was added. The mixture obtained with saturated MgCO3 was again filtered through a Whatman No1 filter paper to obtain a clear colorless solution. 1ml of the colorless solution, was pipetted into 50ml volumetric flask and 2ml of 5% FeCL3 solution was added and made up to mark with distilled water. It was allowed to stand for 30min for blood red color to develop. 0-10ppm standard Saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2ml of 5% FeCL3 solution as done for 1ml sample above. The absorbance of the sample as well as standard saponin solutions were read after color development in a Jenway V6300 Spectrophotometer at a wavelength of 380min. (Harborne *et al.,* 1973 and Trease 1989).

$$\%\text{Saponin} = \frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{Wt\ of\ sample \times 10000}$$

2.3.3. Tannin content of the blend

0.20g of the blend was measured into a 50ml beaker 20ml of 50% methanol was added and covered with parafilm and placed in a water bath at 77-80^{o}C for 1 hour. It was shaking thoroughly to ensure a uniform mixing. The extract was quantitatively filtered using a double layered What man No 41 filter paper into a 100ml volumetric flask, 20ml water added, 2.5ml folin-Denis reagent and 10ml of 17% na2Co3 were added and mixed properly. The mixture was made up to mark with water mixed well and allow to stand for 20min. the bluish –green color will develop at the end of range 0-10ppm were treated similarly as 1ml sample above. The absorbance of the Tannic acid standard solutions as well as

samples was read after color development on a spectronic 21D spectrophotometer at a wavelength of 760nm. % Tannin was calculated using the formula. (Harborne *et al.*, 1973 and Trease 1989).

$$\% \text{ TANNIN} = \frac{\text{Absorbance of sample} \times \text{average gradient factor} \times \text{dilution factor}}{Wt\ of\ sample \times 10000}$$

2.3.4. Phenol content of the blend

0.20g of sample was weighed into a 50ml beaker, 20ml of acetone was added and homogenize properly for 1hr to prevent lumping. The mixture was filtered through a Whatman No.1 filter paper into a 100ml Volumetric Flask using acetone to rinse and made up to mark with distilled water with thorough mixing.1ml of sample extract was pipetted into 50ml Volumetric flask, 20ml water added, 3ml of phosphomolybdic acid added followed by the addition of 5ml of 23% NaCO3 and mixed thoroughly, made up to mark with distilled water and allowed to stand for 10min to develop bluish-green colour. Standard Phenol of concentration range 0-10mg/ml were prepared from 100mg/l stock Phenol solution from Sigma-Aldrich chemicals, USA. The absorbances of sample as well as that of standard concentrations of Phenol were read on a Digital Spectrophotometer at a wavelength of 510nm. (Harborne *et al.*, 1973 and Trease 1989). The percentage Phenol is calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{Wt.of\ sample \times 10000}$$

2.3.5. Flavonoids content of the blend

0.50g of finely ground sample was weighed into a 100ml beaker and 80ml of 95% Ethanol added and stirred with a glass rod to prevent lumping. The mixture was filtered through a Whatman No.1. filter into a 100ml volumetric flask and made up to mark with Ethanol. 1ml of the extract was pipetted into 50ml volumetric flask, four drops of Conc. HCL added via a dropping pipette after which 0.5g of magnesium turnings added to develop a magenta red coloration. Standard flavonoid solution of range 0-5ppm were prepared from 100ppm stock solution and treated in a similar way with HCL and magnesium turnings like sample. The absorbance of magenta red coloration of sample and standard solutions were read on a digital Jenway V6300 Spectrophotometer at a wavelength of 520nm according to (Allen *et al.* 1992) The percentage flavonoid is calculated using the formula.

$$\frac{\text{Absorbance of sample} \times \text{average gradient factor} \times \text{dilution factor}}{Wt.of\ sample \times 10000}$$

2.3.6. Steroids content of the blend

0.50g of sample extract was weighed into a 100ml beaker .20ml of Chloroform-Methanol (2:1) mixture was added to dissolve the extract upon shaking for 30minutes on a shaker. The whole mixture was later filtered through a Whatman No.1 filter paper into another dry clean 100ml Conical Flask/Beaker. The resultant residue was repeatedly treated with Chloroform-Methanol mixture until free of Steroids.1ml of the filtering rate was pipetted into a 30ml test tube and 5ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37^0C-40ºC for 90minutes.It was cooled to room temperature and 10 ml of petroleum ether added followed by the addition of 5ml distilled water. This was evaporated to dryness on the water bath. 6ml of Liebermann Burchard reagent was added to the residue in dry bottle and absorbance taken at a wavelength of 620nm on a Spectronic 21D digital Spectrophotometer. Standard Steroids of concentration of 0-4mg/ml were prepared from 100mg/ml stock steroid solution and treated similarly like sample as above (Wall *et al.,* 1952). % Steroid was calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{Wt.of\ sample \times 10000}$$

2.3.7. Terpene content of the blend

0.50g of sample was weighed into a 50ml Conical Flask,20ml of 2:1 Chloroform-Methanol mixture was added, shaked thoroughly and allowed to stand for 15minutes.The mixture was later centrifuged for another 15minutes.Supernatant obtained was discarded, and the precipitate was re-washed with another 20ml chloroform-methanol mixture for re-centrifugation. The resultant precipitate was dissolved in 40ml of 10% Sodium Deodocyl Sulphate solution.1ml of 0.01M Ferric Chloride solution was added to the above at 30s interval shaked well, and allowed to stand 30minutes.Standard Terpenes of concentration range 0-5mg/ml were prepared from 100mg/l stock Terpenes solution from Sigma-Aldrich chemicals, USA. The absorbances of sample as well as that of standard concentrations of Terpenes were read on a Digital Spectrophotometer at a wavelength of 510nm. (Harborne *et al.,* 1973 and Trease 1989). The percentage Terpene is calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{Wt.of\ sample \times 10000}$$

2.3.8. Glycoside content of the blend

10ml of extract was pipette into a 250ml Conical Flask.50ml Chloroform was added and shaken on a Vortex Mixer for 1hr. The mixture was filtered into 100ml Conical flask and 10ml pyridine, 2ml of

2% sodium nitroprusside were added, shaken thoroughly for 10 minutes. 3ml of 20% NaOH was later added to develop a brownish yellow colour. Glycoside standard of concentrations which range from 0-5mg/ml were prepared from 100mg/ml stock Glycoside standard. The series of standards 0-5mg/ml were treated similarly like sample above. The absorbances of sample as well as standards were read on a Spectronic 21D Digital Spectrophotometer at a wavelength of 510nm. (Harborne *et al.,* 1973 and Trease 1989). % Glucoside was calculated using the formula:

$$\frac{\text{Absorbance of sample} \times \text{gradient factor} \times \text{dilution factor}}{Wt.of\ sample \times 10000}$$

3. Results and Discussion

Table 1 shows the qualitative and quantitative analysis of the phytochemical of the combination of turmeric, garlic, ginger and clove. The result obtained from the phytochemical study for qualitative and quantitative study is in line with the report of (Alok, 2020**,** Muhammad *et al.,* 2019, Jacob *et al.,* 2018 and Sawant *et al.,* 2013) reported that alkaloids, saponin, tannin, phenol, flavonoids, steroids, terpene and glycoside were found to be present in the entire blend (turmeric, garlic, ginger and clove).

Due to the presence of these phytochemicals in this study has proven that they can be used in the treatment of cough, asthma, anti-diarrhoeal, insecticidal, antimicrobial, antioxidant property, treatment of cardiac arrhythmia, myocardial infection and controlling blood pressure as reported by (Bharadwaj *et al.,* 2019, Han *et al.,* 2005 and Nyarko *et al.,* 1990).

Table 1: Qualitative and quantification component of turmeric-garlic-ginger-clove blend

Qualitative	**Quantitative (%)**
Alkaloids	0.5205
Saponin	0.2770
Tannin	0.0375
Phenol	0.2300
Flavonoids	0.0038
Steroids	0.0077
Terpenes	0.0058
Glycosides	0.1335

4. Conclusion

This study concluded that the combination of turmeric, garlic, ginger and clove blend at equal proportion contain this phytochemical (alkaloids, saponin, tannin, phenol, flavonoids, steroids, terpene and glycoside)

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