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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF HERBAL BLACK TEA FORMULATED USING BITTER KOLA(Garcinia kola), KOLA NUT(Cola acuminata) AND GINGER(Zingiber Officinale).

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Abstract

The aim of this work was to formulate herbal black tea using Bitter kola (*Garcinia kola*), kola nut (*Cola acuminate*) and Ginger (*Zingiber officinale*) and analyse the phytochemical, antimicrobial and microbial, properties of the formulated bagged tea. The phytochemical assessment of the formulated herbal black tea were investigated using established analytical procedures while antimicrobial activity test of the herbal tea was carried out against the bacteria isolates using disc diffusion method. The phytochemical analysis of the herbal tea revealed high concentrations of Tannin (15.00%), but low levels of Flavonoid (2.17%), Alkaloids(1.20%) and Caffeine content (1.5mg/g). The study reveals high antimicrobial activity on the clinical isolates *Staphylococcus aureus* (25 mm), *Streptococcus spp* (20 mm) and *Escherichia coli* (15 mm) respectively. Furthermore, five of the herbal tea was assayed for microbial quality assessment, the result showed ranged from (zero) 0 to 1.0 X 10^5 (cfu/g) for total aerobic count, and total fungi count ranged (zero) 0 to 1.1 X 10^5 (cfu/g) while coliform count was 0 cfu/g (zero) or absent. The result of this study therefore revealed that the herbal tea formulated from ginger, kolanut and bitter kola are of good source of phytochemicals and permitted level of caffeine. The presence of this photochemical indicated the good antimicrobial activity of the herbal tea. Hence these herbal tea can also be incorporated into medicine in treatment of illness associated with these bacteria's.

Key words: Herbal tea, Phytochemicals, Caffeine, Antimicrobial, Microorganisms.

INTRODUCTION

Herbal medicine has been used to treat diseases for thousands of years, and in recent decades, several studies have confirmed that herbal medicine has the characteristics of multi-ingredient, multi-target, and multi-pathway (El-Tantawy and Temraz, 2018). Herbal tea is believed to be the second most commonly consumed beverage, tea not only brings relaxation and enjoyment to people's lives but also treats and prevents some diseases (Kishi *et al*., 2010).

Herbal tea is prepared from the leaves, flowers, and fruits of herbal medicines (Zhao *et al*., 2013). Herbal teas are actually mixtures of several ingredients, and are more accurately known as 'tisanes.' Tisanes are made from combinations of dried leaves, seeds, grasses, nuts, barks, fruits, flowers, or other botanical elements that give them their taste and provide the benefits of herbal teas (Chandini, 2014). Similar to green tea, these raw materials are often packed in tea bags, and users only need to brew them in boiling water. As the traditional and fashionable beverage, many different herbal teas have been consumed worldwide. Bioactive compounds contained in herbal teas are variable according to different species and phenolic compounds, flavonoids, coumarins, alkaloids are common and significant (Ajuwon *et al*., 2018).

Herbal teas can be made with fresh or dried flowers, leaves, seeds or roots, generally known to posses medicinal values by pouring boiling water over the plant parts and letting them steep for a few minutes, in Nigeria, leaves such as bitter leaf (Okafor *et al*., 2009).

Bitter Kola (*Garcinia* kola) is popular in Southern Nigeria, the plant is extensively used in herbal medicine and as food. It prevails as a multipurpose tree crop in the home gardens of Southern Nigeria (Nzegbule and Mbakwe, 2001). The seeds of *Garcinia* kola is chewed as an aphrodisiac or used to cure cough, dysentery, or chest colds (Irvinee, 1961). It could serve as raw material for pharmaceutical industries (Iwu, 1989). The seeds prevent or relieve colic disorders or cure head or chest colds, suppressed cough and is often used in the treatment of Cirrhosis and hepatitis (inflammation of the liver) (Ogu and Agu, 1995).

The cola nut fruit is shaped like a capsule and is comprised of fleshy, irregularly shaped seeds which are pink, red or white when fresh, and become brown and hard once they are dried (Henry *et al*., 2014). The seeds are called nuts because of their bitter and astringent taste (Adam *et al*., 2011). The plant's rich history of traditional use paved the way for cytotoxic and anti-microbial screens (Lowe *et al*., 2001). whereas the dried nuts are used as beverages and as pharmaceutical agents in Europe and North America (Ayodele, 1995). Cola nut has a bitter taste and high caffeine content (Benjamin et al., 1991). Small doses are used to treat migraine, motion and morning sickness, in addition, it has been used to relieve inflammation disorders such as rheumatism and gout and has been administered to treat pneumonia and typhoid fever when great nervous irritability was present and Kola nut is also used to treat diarrhoea and has been used as a diuretic (Henry *et al*., 2014).

Ginger (*Zingiber officinale*) rhizome is a widely known used spice often due to it medicinal and culinary purposes in globally for its ethno medicinal and nutritious value. The plant is also rich with high phytochemical constituents (Grzanna *et al*., 2005). The plant is mostly often investigated for antimicrobial, anticancer, antioxidant, antidiabetic, nephroprotective, hepato-protective, larvicidal, analgesic, anti-inflammatory and immunomodulatory activities (Kankanam *et al*., 2020). The oil from ginger is believed to be very medicinal. The major active ingredients in ginger oil are reported to be the sesquiterpenes, which include bisapolene, zingiberol and zingiberene (Connell and Sutherland, 1969)

The aim of this work is to processing bitter kola (*Garcinia kola*), kola nut (*Cola acuminate*) and Ginger (*Zingiber officinale*) into black teas is to process them into improve tatste, preserve the products and to achieve long stable shelf life, thus helping to increase food security, create employment opportunities and generate income. Further compare the microbial, antimicrobial and phytochemical properties of the packed or bagged tea.

MATERIALS AND METHOD

Sample collection

Fresh seed of *Garcinia kola*, kola nut and Rhizome of ginger were purchased from relief market in Onitsha Anambra state, Nigeria. Package in a sterile polyethene transported to Biology laboratory in Science Laboratory Technology for identification

Method of producing Black Tea

Gabriel and Nkemakonam (2015) method was employed in black tea production with numerous modifications. 20 kg of the sample each including the seed of kola nut, seed of *garcinia kola* and rhizome of ginger were weighed out respectively, they were washed, sorted and outer skin peeled. The samples were evenly grounded adding little water for ease grinding to get powered form using manual corona plate. The ground powered samples were thinly spread on jute bags and spinkled with water. Withered under shade for 3h. The samples were allowed to lose about 60% of their moisture in order to make them pliable for rolling. This was done by weighing at intervals of 30 min. The withered seeds were rolled using a manual corona plate mill. Rolling was done to disrupt the sample cells and expose them to some of the essential oils and juice necessary for fermentation. After rolling, the samples were thinly spread on trays and left at room temperature for 3 h for fermentation to take place. The colour of the samples darkened as a result of fermentation/oxidation. The fermented sample were dried in a hot air oven at the temperature of 50°C for 4 h, cooled and coarsely milled using a manual corona plate mill in order to obtain a coarse product, which was sieved using a standard testing sieve No. 20 and At 5g per tea bag, the tea were bag using a tea bag and was read for use.

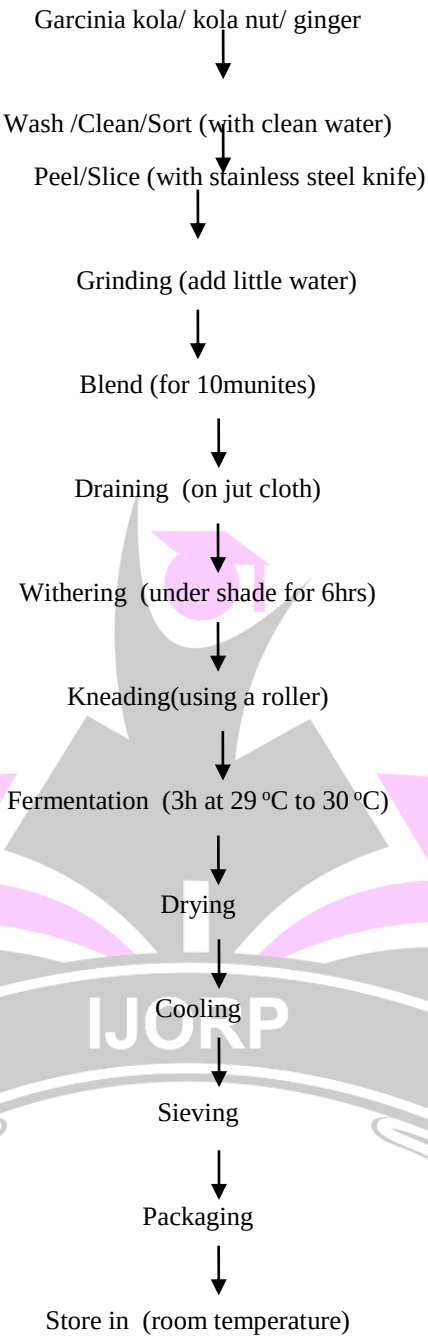

Fig 3: Flow chart Production of black herbal tea from *Garcinia* kola, kola nut and ginger rhizomes

Determination of Caffeine Content

Caffeine content was determined according to Yalwa and Bello, (2017) methods. 10g of each of the dried bagged tea samples was placed into a 250ml round bottom flask and 150ml de-ionised water was added to each. The mouth of each flask was connected to a refluxing system. Each of the flasks was placed into a heating mantle with a regulated temperature. As soon as the content begins to boil the tap of water was opened to allow draining the water out of the condenser and the sets were allowed to reflux for one hour. The refluxing system was turned off and allowed to cool for about thirty minutes. After cooling, the refluxed was sieved out of grated tea samples (with 0.1mm and 0.2 mm sieve) into a 250ml beaker. The residues were discarded and the filtrate was retained and placed in ice block for 15 minutes. Then 100ml of the filtrate were placed into a 500ml separating funnel and 100ml of chloroform added gradually. The corked separating funnel was shaken until the chloroform, water interface was established. After two hours a clear solution was formed into which caffeine dissolved in

chloroform. The caffeine chloroform solution was then transferred into a 250ml beaker and the chloroform evaporated over a water bath (Esther *et al*., 2005) leaving yellowish white caffeine crystals. The crude caffeine obtained was purified by recrystallization in toluene. In this 5ml of toluene was added onto the crude caffeine crystals in a 50ml beaker and heated on hotplate for the caffeine to dissolve. When the crude caffeine dissolved, the beaker was removed from the hotplate, 10ml of petroleum ether was added and the caffeine allowed to crystallise (Okoli *et al*., 2012). The weight of the resultant pure caffeine crystals, now white was taken on a Mettler electric balance. The final product was confirmed as caffeine by thin layer chromatography (TLC) test and melting point determination (Yalwa and Bello, 2017).

Determination of alkaloids

A volume of 200 ml of 10% acetic acid in ethanol was added to 5 g of the powered herbal teas. This was covered with a watch glass and allowed to stand for 4 h. It was then filtered and the filtrate was concentrated to ¼ of the original volume on a water bath. Concentrated ammonium hydroxide was added drop wise for complete precipitation and the solution was allowed to settle. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. Finally, the residue was dried and weighed (Edeoga *et al*., 2005 and Aluko *et al*., 2012).

Determination of flavonoids

Ten grams of the powdered sample was extracted repeatedly with 100 ml of 80% aqueous methanol. The solution was filtered and the filtrate was evaporated to dryness over a hot water bath (Obadoni and Ochuko, 2001 and Aluko *et al*., 2012).

Determination of tannins

The determination of tannin was done using the method of Edeoga (2005) and Aluko *et al*., (2012) with some modifications. One gram of the herbal tea was added to 40 ml of 50% methanol. The mixture was shaken vigorously and placed in a hot water bath at 80°C for 1 h. The extract was filtered into a 100 ml volumetric flask, then 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% sodium carbonate was added and shaken vigorously. The mixture was then made up to the mark with distilled water and allowed to stand for 20 min for full color development. The absorbance was read at 760 nm on a UV Spectrophotometer and tannic acid of concentrations ranging from 0-10 ppm was prepared and used as standard, with the Spectrophotometer against experimental blank adjusted to zero absorbance.

Microbiological Analysis

Media used

Nutrient agar, and Sabourand Destrose Agar. All the media was prepared according to their manufacturer's instructions. It was autoclaved at 121^0C for 15 minutes.

Preparation of the inoculum

10g of the herbal tea sample was weighed out and homogenized in a sterile blender with 100ml sterile water. The sample suspensions were serially diluted using sterile water up to 10^{-2} dilutions and was used to inoculate on the various media.

Total viable bacteria (TVB) and the Total fungal load of the produced tea bag.

0.1 ml of each sample from the dilutions 10^2 was introduced onto the nutrient agar (NA) and Sabouraud's dextrose agar (SDA) plates, respectively, by means of spread plate technique. Plates were incubated at 37 ^{o}C for 24 hours and at 25 ^{o}C for 48 hours for the isolation and enumeration of total viable bacteria and fungi, respectively (Cappuccino and Sherman, 1996)

Antimicrobial activity of the herbal tea

Water Extraction

Ten grams (10g) portions of the powdered herbal tea bag was extracted with 100ml of warm water in a 250cm^3 conical flask, and were allowed to stay three 3hrs, the extracts were separated using the rope on the tea bag.

Determination of antimicrobial activity

Test Organism

The microorganism used were *Escherichia coli, Streptococcus* spp, *and Staphylococcus aureus.* They were obtained from Microbiology laboratory of Science Lab Tech., Federal Polytechnic Oko Anambra state, Nigeria.

Antimcrobial Activity

The antimicrobial activity was performed by disc diffusion method. The bacteria strains were grown in nutrient broth. Muller Hinton agar was the media used to study the bacteria susceptibility. The broth cultures were grown for 24 hours and serially diluted in the same broth (sterilized at 121^0C for 15 minutes) to 10^{-3}. The 24hrs broth culture contains approximately 4.0 x 10^5 Cfu/ml, 2.8 x 10^5 Cfu/ml, and 3.2 x 10^5 Cfu/ml, for *Escherichia Coli*, *Staphylococcus aureus*, and *Streptococcus* spp, respectively, as determined by plate count method. Sterile swab stick was used to inoculate the media by dipping it in the diluted culture and spreading all the surfaces of the agar plate. Sterile paper disc about 10mm diameter was soaked with the extract and allowed to dry for some minutes. Another disc was soaked in a solution containing 100µg/ml of Chloramphenicol antibiotic and placed by the side to serve as positive control. This was placed on the surface of inoculated agar plates. The plates were then incubated for 24hours at 37ºC. After incubation, the diameter zone of inhibition was measured and recorded using millimeter rule

RESULTS

Table 1. Quantitative values of phytochemicals obtained from produced Herbal Tea

Parameter	Value
Caffeine	1.5 (mg/g)
Tannin	15.0 (ug/g)
Flavonoid	2.17 (ug/g)
Alkaloid	1.20 (ug/g)

Table 2: Antimicrobial activity of the herbal tea extracts

Microorganisms	**Zones Of Inhibition (MM)**	
	Warm Water Extract of herbal tea	**Control Chloramphenicol(Antibiotics)**
Staphylococcus aureus	25 mm	45mm
Streptococcus spp	20 mm	36mm
Escherichia coli	15 mm	35mm

Table 3. Microbial assessment obtained from produced Herbal tea

Total Viable count in cfu/g

Sample	Total Aerobic Count	Total fungi count	Coliform count
HT1	5 X 10^4	0	0
HT2	9 X 10^4	0	0
HT3	1.X 10^4	1.1 X 10^5	0
HT4	0	9 X 10^4	0
HT5	1.0 X 10^5	0	0

Key HT- Herbal Tea

DISCUSSION

The results of phytochemical analysis of the produced herbal tea using seed of *garcinia* kola, cola nut and rhizome of ginger are shown on Table 1. The tea contained tannin 15.00 ug/g, alkaloid 3.20 ug/g, flavonoid 2.17 ug/g and caffeine 1.5mg/g, respectively. Presence of phytochemicals including flavonoids, alkaloids, saponins, terpenoids, carotenoids, phenols and tannins have be reported in previous research in herbal tea (Sharma *et al*., 2011).

From this research work there is evidence that herbal tea could be a good source of plant phytochemicals such as tannin, flavonoid, and alkaloid and caffeine depending on the plant. The presence of alkaloids is an indicative that alkaloid used in medicine are morphine, caffeine and coffee; in which caffeine in tea and coffee, act as stimulate to the nervous system (Stanley *et al*., 2007). This justify the presence caffeine in the tea.

These results are similar to that reported for herbal detox by Orimadegun et al., (2018) where they reported Quantitative values of phytochemicals obtained from the herbal tea were: Tannin (39.4 µg/g, Alkaloids (1.9 µg/g) and Flavonoids (3.0 µg/g). The presence of caffeine in the herbal tea could be attributed to the presence of kolanut used in the formulation of the tea. The herbal tea showed caffeine content of 1.5 mg/g per tea bag. This reported value is conformity with the acceptable limit for caffeine in formulated beverage as classified and regulated as a food under Standard 2.6.4-Formulated Caffeinated Beverages (FCB) of the Australia New Zealand Food Standards Code. This standard specifies that energy drinks contain between 145 mg/L and 320 mg/L of caffeine; comply to labelling provisions disclosing nutrient composition including caffeine content (per serving and per 100 mL) along with daily usage; and display warnings that product is not suitable for children, pregnant, or lactating women (Food Standards, 2014).

Hence the presence of caffeine in the product considers the product not suitable for children, pregnant, or lactating women. But might serve as energy drink for these aside this restriction.

The herbal tea was evaluated for the antimicrobial activity on some selected microorganism which include *Staphylococcus aureus*, *Streptococcus* spp, and *Escherichia coli*. The hot extract of the herbal tea indicated varying zone of inhibition on the various organism respectively (*Staphylococcus aureus* 25 mm, *Streptococcus* spp 20 mm and *Escherichia coli* 15mm) this is a good indication that the herbal tea could serve as a good antibiotic against selected organism when taken. This is an indication that the herbal tea formulated could be able to heal stomach or digestive problems associated with enteric bacteria. This justifies the report of Chandini, (2014) who repoted that Herbal Teas are commonly consumed for its therapeutic and energizing properties, since it can help to induce relaxation. Hence the ability to induce relaxation may be attributed to the presence of caffeine in the tea. herbal teas can help provide cleansing properties to the body, and strengthens the immune system as well and as well possess antimicrobial properties.

The herbal tea was assayed for microbial quality assessment, the result ranged from 5 X 10^4 to 1.0 X 10^5 for total aerobic count, total fungi count ranged 9 X 10^4 to 1.1 X 10^5 and coliform count was zero or absent. The counts were minimal and are within acceptable limits and hence when brewed with hot water will be reduced to minimal. The zero level of coliform is an indication of good manufacturing practice.

The product is in conformity based on the Codex standard (CX/NEA 03/16:2002) the allowable TPC levels for fruit juices and drinks should be within 5 x10^3 to 10^4 cfu/ml (FAO, 2002). The herbal tea was also in confirmity with WHO guildline for Total aerobic bacteria 10^5 cfu/ml in herbal medicine. And as such could be produced in large quantity for market supply and profit wise. This is also a good indication of prolong shelf life when kept for longer time before consumption.

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