

Full Length Research Paper

Quantitative analyses of phytochemical and trace elements contents of daily detox, herbal tea consumed in Nigeria

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Tea is one of the commonest drinks in most homes. Many people consume tea due to its unique taste and associated health benefits. Several medical disorders such as cancer, cardiovascular diseases and diabetes mellitus have been linked to the excessive generation of free radicals and oxidative stress. Studies conducted on Daily Detox, a tea consumed by many Nigerians have been limited to qualitative assessment of phytochemicals, but quantitative measurement of phytochemical, microelement, macro elements and heavy metal contents of the tea have not been explored. This study was designed to bridge this gap. Two packs of the daily detox made from *Agerantus conyzoides* (common name, Goat weed) and *Loranthus bengwensis* (common name, African Mistletoe) each containing 21 tea bags in dust form, supplied by the manufacturer were used for analyses. Quantitative measurements of terpenoids, trypsin inhibitors, tannin, phenol, alkaloids and carotenoids were performed using standard methods. Copper, zinc, iron, sodium, potassium, cadmium, nickel, chromium and manganese were estimated using standard methods. Quantitative values of phytochemicals obtained from the herbal tea were: Terpenoids (325.2 µg/g), trypsin inhibitors (16115.5 µg/g), tannin (39.4 µg/g), phenol (55.0 µg/g), alkaloids (1.9 µg/g), flavonoids (3.0 µg/g) and carotenoids (205.5 µg/g). Macro and micro elements measured from the herbal tea were: Copper (16.9 µg/g), zinc (82.9 µg/g), iron (2742.7 µg/g); sodium (2442.9 µg/g); potassium (22132.8 µg/g); chromium (18.9 µg/g) and manganese (340.4 µg/g). Lead was (9.9 µg/g) while nickel and cadmium levels were undetected. The metabolic roles of these chemicals are discussed in relation to their health benefits.

Key words: Herbal tea, phytochemicals, herbal medicine, traces elements.

INTRODUCTION

Tea is one of the commonest drinks in most homes. Many people consume tea due to its unique taste and

associated health benefits. Several medical disorders such as cancer, cardiovascular diseases, diabetes

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mellitus as well as other chronic and non-chronic diseases have been linked to excessive generation of free radicals thereby causing oxidative stress. Consumption of tea, especially green tea (GT) has been associated with reduced occurrence of oxidative process with all the associated medical consequences (Butt and Sultan, 2009). The health benefits derivable from tea have been attributed to its high content of polyphenols; and these have been reported to possess antioxidant, anti-inflammatory and antiviral properties (Butt and Sultan, 2009). Polyphenol present in tea has also been implicated in increasing the activities of detoxifying enzymes, stimulating immune function and decreasing platelet aggregation (Butt and Sultan, 2009; Frankel, 2008). Many plants with medicinal benefits have been identified in Nigeria and some can easily be extracted by boiling and drank as tea with a view to treating various local medical conditions.

Tea because of its desirable aroma, taste and smell is one of the commonest consumed beverages globally (Zhu, 2002). Tea leaves are good sources of minerals and trace elements such as zinc, manganese, iron, copper, magnesium, titanium, aluminium, strontium, bromine, sodium, potassium, phosphorous, iodine and fluorine (Jha and Mann, 1996; Srividhya and Raj, 2011). In addition, tea leaves or tea infusion contains tanning substances, alkaloids, carbohydrates, amino-acids, enzymes, vitamins, very little protein and aroma-forming substances as well as polyphenols (Jha and Mann, 1996; Friedman, 2007). Some of the important tea polyphenols are flavanols, predominantly catechins which effectively kill bacteria, reduce the growth of cancer suppress plaque and cavity formation and prevent excessive build-up of blood cholesterol due to their strong antioxidant activity (Oguni, 2002). It is believed that tea catechins can react with reactive oxygen species which may play central roles in carcinogenesis by terminating chain oxidative reactions (Srividhya and Raj, 2011). Other polyphenols of importance present in tea is epigallocatechin-3-gallate (EGCG), which also has significant antioxidant properties (Chandra and Mejia, 2004); cholesterol lowering effect (Maron et al., 2003), hepatoprotective effects (Hasegawa et al., 1995) and anticancer activities (Fujiki, 2005; Bettuzzi et al., 2006). The polyphenols present in tea possess antioxidative activities, useful for fighting the deleterious effect of environmental and endogenous free radicals (Ostrowska and Skrzydlewska, 2001; Srividhya and Raj, 2011).

The medicinal value of herbal tea depends on their phytochemical constituents. Phytochemical analysis of herbal tea showed that anti diarrhoea herbal tea contained tannin, saponin, flavonoid and phenols; anti-diabetic herbal tea contained alkaloids, volatile oils, flavonoids and phenols while slimming tea contained alkaloids, saponin, flavonoids and phenols, but devoid of volatile oils and tannins (Omogbai and Ikenebomeh, 2013).

Description of medicinal plants contained in Daily Detox

Daily Detox is made basically from two popular medicinal plants. These are: *Agerantus conyzoides* (Common name: Goat weed,) and *Loranthus bengwensis* (Common name: African Mistletoe).

Agerantus conyzoides

This plant is commonly called Goat weed. It belongs to the family, Asteraceae. The genus 'Ageratum' is derived from the Greek words 'ageras' which signifies the non-aging characteristics of the plant. It is an annual herb which has been widely used in herbal medicine in both tropical and sub-tropical regions of the world (Brojendro et al., 2013). Phytochemical analysis of the medicinal plant showed that it contained monoterpenes, sesquiterpenes, benzofuran, chromene, chromone, coumarin, flavonoids, alkaloids, tri-terpenes and steroids (Ekundayo et al., 1988; Adebayo et al., 2013; Singh et al., 2013). As a widely grown medicinal plant, it has been used locally for the treatment of various ailments and medical conditions which include wound dressing, skin diseases, diarrhoea, dysentery, rheumatic fever, sleeping sickness, toothache, gynaecological disorders amongst others (Brojendro et al., 2013). Furthermore, the plant has also been shown to possess pharmacological properties which include analgesic, antimicrobial, anti-inflammatory, anti malaria and anti-cancer properties (Brojendro et al., 2013).

Loranthus bengwensis

This is commonly called African Mistletoe. It belongs to the Loranthaceae family. Report has shown that the plant is specific to Africa and is often grown within the tropical regions (Ahmed and Mohammad, 2014). The plant is usually a host to other trees (Becker, 1998) and its chemical composition varies depending on the tree that hosts its growth (Osadolor, et al, 2014). Phytochemicals which have been identified in the plant include flavonoids, tannins, saponin, lectins (carbohydrate binding protein), polypeptides, polysaccharides and tri-terpenes. Pharmacological properties have been reported to include anti-cancer, anti-inflammatory and anti-diabetic (Becker, 1998; Ahmed and Mohammad, 2014).

While daily detox is currently consumed by many Nigerians, none of its acclaimed benefits have been evaluated using randomized clinical trial. Studies evaluating the quantitative and qualitative contents of phytochemicals, macronutrients, trace elements and heavy metal of this tea are scarce. Few published studies evaluating its medicinal constituents have been limited to

qualitative analysis, but limited information on quantitative measurement of its phytochemicals, heavy metals, macro- and micro-elements. This study was designed to bridge this gap. The information generated from this study could be helpful in developing protocol for clinical studies

MATERIALS AND METHODS

Selection of product

Herbal tea (Daily Detox) was supplied by the manufacturer of the product, Heritage Herbal Medicine Ventures Limited, Lagos, Nigeria. The product had neither been listed nor registered by the National Agency for Food Drug Administration and Control (NAFDAC) and none of its medical claims have been evaluated using randomized clinical trial. Two packs of the product, each containing 21 tea bags in dust form were supplied and used for the analysis.

Laboratory analysis

Laboratory analysis was performed at Jagee Laboratory, a laboratory accredited by the Nigerian Institute of Science Laboratory Technology, Ibadan, Nigeria. All reagents used for the analysis were of the analytical grade. The following parameters were quantitatively measured.

Terpenoids

Terpenoids was measured by the method described by Ferguson (1956). One gram (1 g) of tea dust was weighed and put into a conical flask containing 10 ml of petroleum ether. The mixture was left for 15 min followed by intermittent shaking. At the end of 15 min, it was filtered and the resulting absorbance was measured at 420 nm using UV-visible spectrophotometer. The intensity of colour developed is directly proportional to the concentration of terpenoids present in the herbal tea.

Flavonoids

Flavonoid was measured by the method described by Boham and Kocipai (1974). Ten gram (10 g) of sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight. The flavonoid was calculated as follows:

$$\text{Mg/100 g flavonoids} = \frac{\text{Weight of crucible} + \text{filtrate after drying} - \text{weight of empty crucible}}{100}$$

Trypsin inhibitor

Trypsin inhibitor was measured using the procedure described by Kakade et al. (1974). One gram of sample was mixed with 100 mL of 0.009 M HCl. The mixture was shaken at ambient temperature for 2 h and centrifuged (10000 × g, 20 min). The resulting supernatant was used for the estimation of trypsin inhibitor. The extract from each sample was diluted with distilled water to obtain a

dilution whereby 1 ml extract produced trypsin inhibition activity of between 40 and 60%. One millilitre (1 mL) of the extract was incubated with 1 mL trypsin solution at 37°C for 10 min. A 2.5 mL of pre-warmed substrate (BAPNA) was added and after exactly 10 min at 37°C the reaction was stopped with 0.5 mL of acetic acid (30%, v/v). The absorbance was measured at 410 nm against a blank using the spectrophotometer.

Tannin

This was measured using the method described by the Association Official Analytical Chemists (1990). Two hundred milligrams (200 mg) of the sample was extracted with 10 mL of 70% aqueous acetone (v/v) for 24 h at room temperature. The extracts were centrifuged at 3000 rpm for 20 min and the supernatant was analyzed for tannins. In a 10 mL test tube containing 0.5 mL Folin-Denis reagent, was added 0.5 mL of the tannins extract and 1 mL of saturated sodium carbonate solution. The volume was made up to 10 mL with distilled water. After 30 min, tannin content was measured at 760 nm with the spectrophotometer against experimental blank adjusted to zero absorbance. Tannic acid was used as a standard compound.

Phenol

Phenol was analyzed using Folin-Denis and Folin-Ciocalteu method as described by Box (1990). The method was based on the reduction of molybdc acid in the presence of phenols to a blue colour which is measured spectrophotometrically. In a test tube containing the mixture of 1 mL of organic extract, 10 mL of water (deionized) and 2 mL of Folin Denis reagent was added 2 mL of saturated sodium carbonate solution and incubated in the dark at room temperature for 1 h. The resulting colour was measured spectrophotometrically at 640 nm. The total phenolic concentration was calculated using a pre- prepared calibrated curve prepared using standard phenolic compound.

Carotenoids

Total carotenoids were estimated using the method described by Sass-Kiss et al. (2005). 10 g of sample was extracted using 20 ml of mixed extraction solvent (*hexane/acetone/ethanol*) in the ratio 2:1:1 respectively. After stirring for 30 min, the supernatant was recovered. Re-extraction was repeated for the second time using 10 ml of the extraction solvents. The mixture of the two hexane phases was used for the determination of total carotenoids by spectrophotometry at 420 nm. Concentrations of carotenoids were measured in reference to the calibration curve using β-carotene as standard and the results are expressed in µg/g.

Alkaloids

This was determined using the method described by Harborne (1973). Five gram (5 g) of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol added. The beaker was covered and allowed to stand for 4 h. It was then filtered and the extract concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (2 M) and then filtered. The residue if available is the alkaloid which is then dried and weighed.

Table 1. Quantitative values of phytochemicals obtained from Daily Detox.

Parameter	Value ($\mu\text{g/g}$)
Terpenoids	325
Trypsin Inhibitor	16115
Tannin	39
Phenol	55
Carotenoids	205
Flavonoids	2.96
Alkaloids	1.92

Table 2. Quantitative values of micro, macro elements and heavy metals measured from Daily Detox.

Element	Value ($\mu\text{g/g}$)	WHO limits ($\mu\text{g/g}$)
Copper	16.9	-
Zinc	82.87	25
Iron	2741.7	100
Sodium	2442.9	-
Potassium	22132.8	-
Lead	9.92	10
Chromium	18.9	50
Manganese	340	-
Nickel	**ND	70
Cadmium	**ND	3

** Not detectable.

Measurement of micronutrients

The mineral contents were measured using Atomic Absorption Spectrophotometry (AAS) after appropriate sample digestion had been made.

RESULTS AND DISCUSSION

The data obtained from this study are shown in Tables 1 and 2. Quantitative concentration of various phytochemicals obtained from the herbal tea were: Terpenoids, 325.2 $\mu\text{g/g}$; trypsin inhibitors, 16115.5 $\mu\text{g/g}$; tannin, 39.4 $\mu\text{g/g}$; phenol, 55.0 $\mu\text{g/g}$; alkaloids, 1.9 $\mu\text{g/g}$; flavonoids, 3.0 $\mu\text{g/g}$ and carotenoids, 205.5 $\mu\text{g/g}$. The concentration of macro and micro elements measured from the herbal tea were: Copper, 16.9 $\mu\text{g/g}$; zinc, 82.9 $\mu\text{g/g}$; iron, 2742.7 $\mu\text{g/g}$; sodium, 2442.9 $\mu\text{g/g}$; potassium, 22132.8 $\mu\text{g/g}$ and manganese 340.4 $\mu\text{g/g}$. The concentrations of lead and chromium were 9.9 and 18.9 $\mu\text{g/g}$, respectively. Nickel and cadmium were undetected.

Generally, herbal tea had been reported to contain different phytochemicals including flavonoids, catechins, alkaloids, saponins, terpenoids, carotenoids, phenols and tannins (Sharma et al., 2011; Ikenebomeh, 2013). From

this study, it was evident that Daily Detox contained different concentrations of flavonoids, terpenoids, carotenoids, alkaloids, phenols and tannins. Phytochemicals identified from the studied herbal tea was consistent with those previously reported in herbal tea (Sharma et al., 2011; Omogbai and Ikenebomeh, 2013). This implies that the investigated herbal tea could be a very good source for these phytochemicals.

The level of flavonoids detected in the herbal tea was 3.0 $\mu\text{g/g}$. Flavonoids have been reported to be one of the major constituents of herbal tea (Ikenebomeh, 2013). Flavonoids are vital in the scavenging of oxygen-derived free radicals. *In vitro* studies have shown that flavonoids possess anti-inflammatory, anti-allergic, anti-viral, and anti-carcinogenic properties (Middleton, 1998). Flavonoids prevent injury from free radicals by reacting with the reactive oxygen species thus stabilizing the reactive oxygen species, scavenging superoxides and peroxy nitrites, inhibiting oxidation of low density lipoprotein (LDL) thereby protecting against atherosclerosis (Ikenebomeh, 2013).

The level of carotenoids in the herbal tea was 205.5 $\mu\text{g/g}$. Carotenoids are important constituents of most herbal tea. They are precursors of vitamin A. Consuming

diet rich in carotenoids has been epidemiologically correlated with a lower risk for several diseases (Faulks and Southon, 2005). Increased carotenoid intakes have been linked with reduced risk to cancer and cardiovascular disease. This justifies the health benefits derived from consumption of tea.

One the major phytochemicals which is rarely quantitated in herbal tea is trypsin inhibitor (TI). From this study, 16115.5 $\mu\text{g/g}$ of TI was quantitated in the herbal tea. The concentration of TI contained in the herbal tea was higher than all the other phytochemicals combined. There could be possible antagonistic effects of TI on other phytochemicals when this tea is consumed especially on a regular basis. This could limit the beneficial effects of the phytochemicals due to antagonistic effects of TI on metabolic process. This hypothesis could be elucidated using further research. Furthermore, although, published studies reporting the acceptable level of TI are scanty, yet in the researchers' opinion, this value was considered to be excessively high. Trypsin inhibitor has been reported to inhibit the physiological activities of trypsin, a proteolytic enzyme, which is physiologically essential in the digestion of proteins (Hama and Khalid, 2007; Gu et al., 2014). Study conducted on animal models have shown that TI induced oxidative stress, limited the activities of both enzymatic and non-enzymatic antioxidants, increased the formation of lipid peroxidation and generation of free radicals (Gu et al., 2014).

The herbal tea, Daily Detox, contained various levels of essential micronutrients. The human body requires both metallic and non-metallic mineral elements within certain permissible limits for growth and good health. Many of these elements play a very important function in the metabolic processes and in the general wellbeing of humans.

The observed zinc content of this herbal tea was 82.9 $\mu\text{g/g}$. This is higher than WHO recommended limit of 25 $\mu\text{g/g}$ for herbal tea. From this study, it implies that the herbal tea could be a good source of zinc, but excessive consumption could be damaging to the body. Zinc is an indispensable trace element in the body (Dosa et al., 2014). It is required for numerous enzymatic and cellular processes including protein synthesis, intracellular signalling, in addition to functioning as antioxidant and anti-inflammatory agent (Shannon et al., 2011; Maret, 2013; Cruz et al., 2015; Bonaventura et al., 2015). It plays significant role in ageing (Stefanidou et al., 2006), normal growth and development, testicular maturation, neurological function, wound healing and immunocompetence (European Food Safety Authority (EFSA); European Food Safety Authority, 2006), thyroid functions and glucose metabolism (Zargar, 1998) as well as endocrine system (Mahdzadeh et al., 2014).

The level of manganese quantitated in the herbal tea was (340.4 $\mu\text{g/g}$). This implies that Daily Detox could serve as a good source of this micronutrient. There is no

formal Recommended Dietary Allowance (RDA) for manganese. However, an estimated safe and adequate dietary intake (ESADDI) of 2 to 5 mg/day for adults was established by the US National Research Council (Freeland-Graves et al., 1982), and the Scientific Committee for Food (SCF) of the European Union estimated 1 to 10 mg/day as an acceptable range of intake (Scientific Committee on Food, 1993). Manganese has been reported to play essential role in various enzymatic activities in numerous species. Insufficient intake of manganese can result in adverse effects such as impaired growth, skeletal abnormalities, reproductive deficits, ataxia of the newborn, and defects in lipid and carbohydrate metabolisms (European Food Safety Authority, 2006). On the contrary, evidence of manganese deficiencies in man is poor. A specific deficiency syndrome has not been described in humans (Scientific Committee on Food, 1993; World Health Organisation, 1996; Deepak, 2011).

The level of iron quantitated in the herbal tea was 2742.7 $\mu\text{g/g}$. This suggests that the herbal tea could be a very good source of iron. For adequate metabolic process, there should be a balance between intake of iron and those lost through normal body physiological processes.

Iron is an essential trace element that has important metabolic functions - such as oxygen transport and storage as well as many redox reactions. Insufficient intake results in deficiency leading to anaemia, adverse outcomes of pregnancy, impaired psychomotor development and cognitive performance and reduced immune function (European Food Safety Authority, 2006).

The level of sodium and potassium quantitated from the herbal tea was 2442.9 and 22132.8 $\mu\text{g/g}$, respectively. This implies that the tea could be good source of these essential elements. Sodium and potassium do play essential roles in cell metabolism and enzymatic function. However, the observed higher content of potassium over sodium in this herbal tea suggests that it could be helpful in controlling hypertension since consumption of potassium - rich food and drinks has been linked to reduction of blood pressure.

The level of copper quantitated in Daily Detox was 16.9 $\mu\text{g/g}$. Copper is an essential micronutrient which is useful for several biological functions in human (Deepak, 2011). It is a constituent of many enzymes such as tyrosinase, cytochrome oxidase, peptidyl and glycyamidating monooxidase, caeruloplasmin and other ferroxidases (Uauy et al., 1998) as well as Cu/Zn superoxide dismutase (Uauy et al., 1998).

The level of lead (9.9 $\mu\text{g/g}$) quantitated in the herbal tea was within the WHO recommended level. This implies that lead toxicity might not be a possible consequence from consuming this herbal tea. Furthermore, the undetected levels of Nickel and Cadmium are suggestive that these might not be possible causes of toxicity when

the herbal tea is consumed

Conclusion

Consumption of food and food products rich in phytochemicals have been associated with reduction in oxidative stress and its associated health disorders. Information obtained from this study showed that the herbal tea (Daily Detox) contained various arrays of phytochemicals, macro and micro elements. Premised on this, it could be a very good source for these chemicals; and could be helpful in improving general health status of consumers. The high level of trypsin inhibitor obtained in this tea calls for caution among consumers. The value of lead quantitated from the tea was within the acceptable limit recommended by WHO. The quantitated levels of cadmium and nickel suggest that these metals might not be possible causes of toxicity when the herbal tea is consumed.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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