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Full Length Research Paper

Phytochemistry and *in vitro* anti-oxidant activities of *Stellaria media*, *Cajanus cajan* and *Tetracera potatoria* methanolic extracts

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Phytochemical screening and determination of antioxidant activities of methanolic extracts of leaf of *Stellaria media*, *Cajanus cajan* and root of *Tetracera potatoria* were conducted. Phytoconstituents screened for included alkaloid, saponins, tannins, phlobatannins, flavonoids, anthraquinones and cardiac glycosides. Free radicals scavenging capacity of the extract of the three plants were assessed *in vitro* by 1,1 diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays, while inhibition of free radicals generation was assessed by ability of the extracts to inhibit lipid peroxides formation. The antioxidant activities of the extracts were dose dependent. The highest percentage inhibition of DPPH by *T. potatoria*, *S. media* and *C. cajan* was 82, 76 and 57%, respectively, as against 76% for ascorbic acid. Percentage ferric ions reduction in FRAP assay was 104, 79 and 26% for *T. potatoria*, *S. media* and *C. cajan* as against 100% for Gallic acid, the standard compound. The standard drugs however appeared more potent than the crude extracts because the bioactive compounds have not been isolated or purified. Lipid peroxidation assay showed that *T. potatoria* inhibited lipid peroxides formation by 54%. It was concluded that the abundance of flavonoids, tannins and phlobatannins in the three plants account for their high antioxidant activities which probably explains their ethnomedical applications against inflammatory conditions. It is believed that isolation of the specific bioactive principles will serve to improve the free radicals scavenging potency of the compounds.

Key words: Free radicals, phytoconstituents, *Stellaria media*, *Cajanus cajan*, *Tetracera potatoria*, extracts.

INTRODUCTION

Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage (De Diego-Otero et al., 2009). All forms of life maintain a constant reducing environment within their cells which is preserved by enzymes through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cells including proteins and DNAs (Gems and Partridge, 2008). It was estimated that there are more than ten thousand oxidative assaults to a DNA per cell per day in humans (Ames et al., 1993). However, the body is availed with both endogenous (catalase, superoxide dismutase, glutathione peroxidase/reductase) and exogenous (vitamins C and E, – carotene, uric acid) defense systems against free radicals generated within it. These systems are however not sufficient in certain situations in which production of free radicals significantly increases (Mondon et al., 1999).

The beneficial effects of phytochemicals in this direction are associated with a number of their biological activities including antioxidant and free radical scavenging properties (Ramchoun et al., 2009). Most of the protective effects of plants on living cells have been attributed to their non-nutrient constituents such as

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carotenoid, flavonoids, isoflavonoid and phenolic acids (Badmus et al., 2010). Different phytochemicals have been shown to possess a range of activities which help in protecting against lipid oxidation and by extension chronic conditions such as cancer, osteomyelitis or cardiovascular conditions (Hollman and Katan, 1997; Liu, 2003; Jayaprakasam et al., 2003; Arun and Nalini, 2007). *Stellaria media* of the plant family *Caryophyllaceae*, is used as tonic, diuretic, demulcent, expectorant, and laxative (Haragan, 1991). It is often recommended for asthma, bronchitis, or congestion. It is also said to help control obesity and is an ingredient in some herbal weight loss preparations (Whitson, 1996). *Cajanus cajan* or pigeon pea is a perennial member of the family *Fabaceae*. The leaves are used as a weak decoction for the treatment of measles, catarrh and hepatitis. An aqueous infusion of the seeds sometimes mixed with the leaves is dispensed for the management of sickle-cell anaemia (Akinsulie et al., 2005). The seed extract has been shown to possess hypoglycaemic and antimicrobial activities (Iwu, 1993). DukerEshun et al. (2004) demonstrated its activity against the chloroquine-sensitive *Plasmodium falciparum* strain 3D7. *Tetracera potatoria* is of the family of *Dilleniaceae*. It is a climber, found in most parts of the world. It is used in the treatment of jaundice and haemorrhoids among the Baka Pygmies of Cameroon (Betti, 2004). It is also used in the traditional treatment of inflammatory skin infection and ulcer. Adesanwo et al. (2002) has described the anti-ulcerogenic effect of this plant. The role of oxidative stress has been basically incriminated in the pathogenesis of inflammatory disorders in the body (Kunchandy and Rao, 1990; Joe and Lokesh, 1994; Reddy and Lokesh, 1994). The three plants in this present study are commonly used for treatment of inflammatory conditions in the folkloric medicine. This study was designed to investigate the anti-oxidant and free radicals scavenging capacities of the three plants.

MATERIALS AND METHODS

The plant materials and extraction

The leaves of *S. media*, *C. cajan* and the root of *T. potatoria* were purchased from a local herb market, and were authenticated by Mr. Ademoriyo of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The leaves and roots were dried under shade and powdered. 500 g of the vegetal powder was dissolved in 1 L of methanol for 72 h. The extracts obtained were concentrated in rotary vacuum evaporator at 40℃.

Phytochemical analysis

Phytochemical screening for major constituents was undertaken using standard qualitative methods as described by Sofowora (1993). The test for tannins was carried out by subjecting 3 g of each plant extract in 6 ml of distilled water, filtered and ferric chloride reagents added to the filtrate. For cardiac glycosides, Killer-Kiliani test (Trease and Evans, 1989) was adopted (0.5 g of extract was added to 2 ml acetic anhydrate plus H_2SO_4). The test for alkaloids was carried out by subjecting 0.5 g aqueous extract in 5 ml 1% HCl, boiled, filtered and Mayer's reagent added (Harborne, 1998; Trease and Evans, 1989). The extract was subjected to frothing test for the identification of saponin. Haemolysis test was further performed on the frothed extracts in water to remove false positive results (Sofowora, 1993). The extract was also tested for free glycoside bound anthraquinones (Sofowora, 1993). Five grams of extract was added to 10 ml benzene, filtered and ammonia solution added. The presence of flavonoids was determined using 1% aluminum chloride solution in methanol concentrated HCl, magnesium turnins, and potassium hydroxide solution (Kapoor, 1969; Earnsworth et al., 1974). To 1 mg of the extract, 2 ml of 25% ammonia solution was added and stirred. A cherish-red solution indicates the presence of emodols (aglycones of anthracenosides in oxidized form) (Francis et al., 2009).

DPPH radical assay

The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method of Braca et al. (2001). Methanolic extracts of the plants (0.1 ml) at different concentrations were added to 3 ml of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percent inhibition activity was calculated as [(Ao – Ae)/Ao] x 100, where Ao = absorbance without extract; and Ae = absorbance with extract. The IC_{50} value of the extract was compared with that of the vitamin C.

Ferric reducing antioxidant power (FRAP)

The FRAP assay used antioxidants in the redox linked colorimetric method with absorbance measured with a spectrophotometer (Benzie and Strain, 1999). A 300 nmol/l acetate buffer of pH 3.6 (3.1 g of sodium acetate + 16 ml of glacial acetic acid made up to one litre with distilled water, 10 nmol/L 1,2,4,6-tri 2-pyridyl 1,3,5-triazine, 98% (Sigma Aldrich), 3.1 mg/ml in 40 mmol/HCl) and 20 mmol/l of ferric chloride were mixed together in the ratio of 10:1:1, respectively to give the FRAP working reagent.

50 µl aliquot of extract or standard was added to 150 µl of FRAP reagent in a semi-micro plastic cuvette and was run in triplicate. Absorbance measurement was taken at 593 nm (A_{593}) exactly 10 min after mixing, using 50 µl of water as the reference. 50 mls of standard [iron (III) sulfate, 1 mM] was added to 1.5 mls of FRAP reagent to standardize the preparations. All measurement was taken at room temperature with the extracts protected from direct sunlight. FRAP values of the extracts were determined from a calibration curve of ferrous sulfate solutions at concentrations of 125, 250 and 500 M, expressed as M of ferric iron reduced per gram of dried sample. The antioxidant activity was measured by its ability to reduce the Fe^{3+}/ferricyanide complex by forming ferrous products. Fe^{2+} can be monitored by measuring the formation of Perlûs Prussian blue at 593nm. Increased absorbance at 593 nm indicates a stronger reducing power. Different concentrations of the extracts were used while Gallic acid served as reference antioxidant compound (Kruawan and kangsadalampai, 2006).

Lipid peroxidation assay

A modified thiobarbituric acid reactive species (TBARS) assay (Ohkowa et al., 1979) was used to measure the lipid peroxide formed using egg yolk homogenate as lipid rich media (Ruberto et al., 2000). Egg homogenate (0.5 ml of 10%, v/v) and 0.1 ml of extracts were added to a test tube and made up to 1 ml with

distilled water; 0.05 ml of $FeSO_4$ (0.07 M) was added to induce lipid peroxidation and the mixture incubated for 30 min. Then, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added and the resulting mixture was vortexed and then heated at 95℃ for 60 mi n. After cooling, 5.0 ml of butan-1-ol were added to each tube and centrifuged at 3000 rpm for 10 min.

The absorbance of the organic upper layer was measured at 532 nm. The percentage Inhibition of lipid peroxidation by the extract was calculated as [(1-E)/C] × 100 where C is the absorbance value of the fully oxidized control and E is the absorbance in presence of different extract concentrations.

RESULTS

Phytochemical study

Analysis of the plants showed that *S. media* contains phlobatannins and saponins; *C. cajan* contains tannins, phlobatannins and saponins while *T. potatoria* contains tannins, flavanoids, phlobatannins and cardiac glycosides (Table 1).

DPPH radical inhibition assay

Results in Table 2 showed that the extract of *T. potatoria* had the highest inhibition of 82% of DPPH in the solution while *S. media* inhibited 76% and *C. cajan* inhibited 58% at concentration of 500 µg/ml, respectively as against 76% for vitamin C. The Ic_{50} values were 14.0 µg/ml (vitamin C), 210.8 µg/ml (*S. media*), 270.5 µg/ml (*T. potatoria*) and 436.0 µg/ml (*C. cajan*).

Ferric reducing antioxidant power (FRAP) assay

The concentration or percentage of ferric (Fe^{3+}) ions reduced to ferrous (Fe^{2+}) ions are shown in Table 3. The extract of *T. potatoria* had the highest value of 104%; *S. media* (79.3%) and *C. cajan* (25.6%) at concentration of 500 µg/mL, respectively, using the value for Gallic acid (100%) as the reference. Lower doses of the extracts or Gallic acid followed the same pattern (Table 3).

Lipid peroxidation assay

The inhibition of lipid peroxides formation by the three extract was dose dependent. The extract of *T. potatoria* recorded 53.8% inhibition, *C. cajan* (11.6%) and *S. media* (5.0%) (Table 4).

DISCUSSION

Phytochemical analysis of the three plants indicated that *S. media* contains measurable level of phlobatannins and saponins; *C. cajan* contains tannins, phlobatannins and saponins while *T. potatoria* contains tannins, flavonoids, phlobatannins and cardiac glycosides. The biological importance of compounds such as saponins and cardiac glycosides has been well documented (Leverin and McMatron, 1999). This study is focused on the evaluation of the free radicals scavenging capacities of the extracts of the three plants. This study pointed to the fact that while the extracts may have shown low inhibition of peroxides generation, they rather have a high capacity to scavenge free radicals. Free radicals scavenging assay conducted using DPPH showed that *T. potatoria* with IC_{50} values of 270.5 µg/ml had the highest free radicals scavenging activity (82.3%) followed by *S. media* (76.3%) and *C. cajan* (57.7%) with IC_{50} values of 210.8 µg/ml and in 436.0 µg/ml, respectively.

Kamlesh et al. (2007) had described a high free radical scavenging activity for *Calendula officianalis* with yet a relatively higher IC_{50} values of 546.1, 852.8 983.8 and 1,222.5 µg/ml for its root, leaf, whole plant and stem extract than what was obtained for the plants in this study. Results from the FRAP assay also had similar pattern for *T. potatoria, S. media and C. cajan*. Comparatively, *T. potatoria* was equipotent with 5 and more potent than 35 of the 70 medicinal plants screened by Katalinic et al. (2006) for antioxidant activity using the FRAP assay. *S. media* was equipotent with 18 and more potent than 10 of the plants while *C. cajan* was equipotent with 4 and more potent than 3 of the plants. The presence of flavonoids, tannins including phlobatannins in the extracts actually account for their free radicals scavenging actions.

Flavonoids and tannins including phlobatannins have been reported to have a high value of antioxidant properties among other biological activities. Flavonoids are phenolic substances isolated from a wide range of vascular plants, with over 8000 individual compounds known.

They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellants, and for light screening (Pietta, 2000). Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antimicrobial (Cushnie and Lamb, 2005), anti-inflammatory (Yamamoto and Gaynor, 2001) and cardiovascular effects (Knekt et al., 2002; Verena et al., 2006; David et al., 2004).

However, most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals (Sellappan and Akoh, 2002; Williams et al., 2004). Most flavonoids out perform well-known antioxidants, such as ascorbate (vitamin C) and α-tocopherol (vitamin E), in *in vitro* antioxidant assays because of their strong capacity to donate electrons or hydrogen atoms (Hernandez et al., 2009). While there has been a major focus on the antioxidant properties, there is an emerging view that flavonoids and their

Table 1. Phytochemical constituents of *S. media*, *C. cajan* and *T. potatoria*.

Phytoconstituents	*S. media*	*C. cajan*	*T. potatoria*
Tannins	Absent	Present	Present
Flavonoids	Absent	Absent	Present
Phlobatannins	Present	Present	Present
Cardiac glycosides	Absent	Absent	Present
Saponins	Present	Present	Absent
Anthraquinones	Absent	Absent	Absent
Alkaloids	Alkaloids	Absent	Absent

Table 2. Percentage inhibition of DPPH by different concentrations (µg/ml) of the extract of *S. media, C. cajan* and *T. potatoria.*

S. media extract	*C. cajan* extract	*T. potatoria* extract	Vitamin C
500 (76.3%)	500 (57.7%)	500 (82.3%)	20 (76.4%)
250 (48.95%)	250 (27.51%)	250 (56.75%)	10 (26.05%)
125 (45.15%)	125 (16.04%)	125 (26.01%)	5 (17.80%)
62.5 (44.28%)	62.5 (6.84%)	62.5 (15.67%)	2.5 (9.03%)
IC_{50} (210.8)	IC_{50} (436.0)	IC_{50} (270.5)	IC_{50} (14.0)

Table 3. Percentage reduction of ferric ions (FRAP value, µmol/L) by different concentrations (µg/ml) of the extract of *S. media, C. cajan* and *T. potatoria.*

S. media extract (%)	*C. cajan* extract (%)	*T. potatoria* extract (%)	Gallic acid (%)
500 (2465, 79.3)	500 (796, 25.6)	500 (3234, 104)	50 (3108, 100.0)
250 (1680, 54.1)	250 (464, 14.9)	250 (3100, 99.7)	25 (3094, 99.6)
125 (1064, 34.2)	125 (298, 10.0)	125 (3029, 97.5)	12.5 (2946, 94.7)

Table 4. Percentage inhibition of lipid peroxidation by different concentrations (µg/ml) of the extract of *S. media, C. cajan* and *T. potatoria.*

S. media extract (%)	*C. cajan* extract (%)	*T. potatoria* extract (%)
500 (5.0)	500 (11.6)	500 (53.8)
250 (4.1)	250 (20.4)	250 (31.45)
125 (3.2)	125 (4.5)	125 (13.3)

in vivo metabolites, do not act as conventional hydrogen-donating antioxidants but may exert modulatory actions in cells through actions at protein kinase and lipid kinase signalling pathways. Flavonoids, and more recently their metabolites, have been reported to act at phosphoinositide 3-kinase (PI 3-kinase), Akt/protein kinase B (Akt/PKB), tyrosine kinases, protein kinase C (PKC), and mitogen activated protein kinase (MAP kinase) signalling cascades. Inhibitory or stimulatory actions at these pathways are likely to affect cellular function profoundly by altering the phosphorylation state of target molecules and by modulating gene expression (Robert et al., 2004).

Tannins are plant-based polyphenols which acts like vitamins E and C (Hassig et al., 1997) but are often neglected group of natural polyphenols. The mechanisms of their antioxidative action, like free radical scavenging activity, chelation of transition metals, inhibition of prooxidative enzymes and lipid peroxidation. The mechanisms of action of antibacterial, antiviral, anticarcinogenic, cardiovascular system preventing, and anti-inflammatory effects as well as the absorption, metabolic fate and positive *in vivo* effects of tannins have been comprehensively reviewed by Koleckar et al.

(2008). Alkaloid and flavonoid content of plant materials have severally been reported to be a major antioxidant, anti-inflammatory and analgesic active principle; while tannins and phlobatannins have been reported to have wound healing properties (Okwu and Okwu, 2004; Kagbo and Ejebe, 2010).

In a nutshell, phytochemicals have thus shown great capacity at inhibiting free radical generation, act as free radical scavengers (Kunchandy and Rao, 1990; Joe and Lokesh, 1994; Reddy and Lokesh, 1994), antioxidants (Ruby et al., 1995), or inhibit lipid peroxidation (Rajakumar and Rao, 1994; Sreejayan and Rao, 1994) and oxidative DNA damage (Subramanian et al., 1994), and have become natural sources of anti-inflammatory and anticancer agents (Oyagbemi et al., 2009; Saba and Oridupa, 2010). This study has also shown that some of the reported ethno-medicinal applications of *T.* potatoria, *S. media* and *C. cajan* in respect of chronic inflammatory disorders are due to the presence of anti-oxidants principles present in them. Further studies are currently underway to isolate and characterize the active constituents responsible for the antioxidant activity.

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