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Research Article

Phytochemicals and Antioxidant Properties of Five Wild Edible Plants Consumed by Pregnant Women in Buikwe District, Uganda

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ABSTRACT

Exaggerated oxidative stress during pregnancy results in spontaneous abortions, recurrent pregnancy loss, preeclampsia and intrauterine growth restriction. This condition can be minimized by boosting the antioxidant defense mechanism of the pregnant woman to balance with the generated reactive oxygen species. Plants are a good natural source of antioxidants. This study investigated the phytochemicals and antioxidant properties of *Solanum anguivii* L., *Cleome gynandra* L., *Afromomum angustifolium* K. Schum, *Solanum nigrum* Pax. Ex Dunal and *Physalis angulata* L. These wild edible plants are consumed by the pregnant women of Buikwe district. Using standard procedures, preliminary phytochemical analyses were carried out on concentrated freeze dried methanolic plant extracts. The antioxidant activity of the plant extracts was determined using DPPH radical scavenging assay. All plant species tested positive for presence of reducing compounds, alkaloids, flavonoids, coumarins, sterols and triterpenes. Anthracenosides were absent in all the plant extracts. The percentage free radical scavenging activity of all the species was above 80%. *S. nigrum* leaves had the highest free radical scavenging activity with an AAE = 20.87±0.005 mg/ g. and a percentage scavenging activity of 97.4% at 40 µl concentration. *S. nigrum* leaves also showed the highest amount of antioxidant intake (2087 mg) per 100 g of edible portion. The analysed wild edible plant species have good free radical scavenging activity thus can be explored as natural sources of antioxidants by the pregnant women in this area.

Keywords: phytochemicals, antioxidants, wild edible plants, pregnancy

INTRODUCTION

During pregnancy, 30,000 kcal (336 MJ) are required to produce a baby, increase the size of the placenta and reproductive organs, provide energy for newly formed tissues and create additional fat stores in the mother[1]. These increased energy demands result in greater oxygen use, which causes the over-production of oxygen byproducts (called free radicals) that can be harmful to the mother and her baby[2]. If there is an imbalance between generated reactive oxygen species (ROS) and the level of antioxidant defense system, it results in oxidative stress[3]. Oxidative stress is generated during normal placental development; however, when supply of antioxidant micronutrients is limited, exaggerated oxidative stress within both the placenta and maternal circulation occurs, resulting in adverse pregnancy outcomes. The adverse pregnancy outcomes include; spontaneous abortions, recurrent pregnancy loss (RPL), pregnancy-induced hypertension - PIH (preeclampsia), and intrauterine growth restriction (IUGR)[4]. Therefore, antioxidants protect the pregnant woman from the effects of excessive oxidative stress. Plants contain a number of compounds (phytochemicals) which are responsible for the biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism[5]. Phytochemicals are compounds that occur naturally in plants and are reported to possess properties of biological significance[6]. Phenols, flavonoids, tannins and saponins are the phytochemicals majorly responsible for the antioxidant properties of plants [7,8] therefore, good for controlling oxidative stress related disorders[9]. Antioxidants are more effective when taken in their natural state other than when isolated from a food. Synthetic antioxidants are available on market but these have been proven to be highly toxic. Actually, some supplements have been reported to increases cancer risks. For example, Vitamin A (beta-carotene) has been associated with a reduced risk of certain cancers, but an increase in others e.g. lung cancer in smokers if Vitamin A is purified from food stuffs[10]. This justifies the search for natural antioxidants which is gaining much importance due to their presumed safety and therapeutic value. This study investigated the phytochemicals and antioxidant properties of five wild edible plants (*Solanum anguivii* L., *Cleome gynandra* L., *Afromomum angustifolium* K. Schum, *Solanum nigrum* Pax. Ex Dunal and *Physalis angulata* L. which are consumed by the pregnant women in Buikwe district, Uganda.

MATERIALS AND METHODS

Collection and Identification of the Plant species

During an ethnobotanical survey[11], pregnant women in Buikwe district were interviewed on which wild edible plants they consumed. Through focus group discussions, pair wise ranking was used to prioritise the five most pre

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ferred species. These species were locally identified by the respondents in the field. They were scientifically identified according to the Flora of Tropical East Africa from the Makerere University Herbarium. These species are; *Solanum anguivii* L., *Cleome gynandra* L., *Afromomum angustifolium* K. Schum, *Solanum nigrum* Pax. Ex Dunal and *Physalis angulata* L.

Sample Preparation and Extraction

The plant samples were washed under running tap water to remove the surface pollutants. The leaves were spread on clean surface and air dried for 14 days. The fruits were sliced into small pieces and put in an oven (Sanyo OMT oven, UK) at 40ºC to dry for seven days. Using a grinder, the dried plant samples were ground to fine powder. The powders were extracted for 24 hours with 20 mL of methanol at room temperature. After filtration through Whatman No 0.45 µm, the resulting solutions were evaporated under vacuum at 60°C by Buchi Rotavapor R-200 to dryness. The residues were weighed and preserved for phytochemical and antioxidant analyses. The chemicals used as solvents and reagents were of analytical grade.

Qualitative Phytochemical Analyses

Using standard procedures[12,13], phytochemical analyses were carried out on concentrated freeze dried methanol extracts. The extracts were qualitatively screened for presence of alkaloids, flavonoids, tannins, saponins, phenolic compounds, sterols, reducing sugars, volatile oils, coumarins, anthracenocides and terpenes.

Determination of Volatile oils

The ether extract was placed in a flask and evaporated to dryness. When the residue acquired a pleasant odour, it was dissolved in small amounts of alcohol by repeated elution. One part of the alcoholic solution was then evaporated to dryness. The residue produced a characteristically pleasant odour which was confirming presence of volatile oils.

Determination of Saponins

Saponins occur widely in plants, they are characterized by their foaming power in aqueous solution. A dilute solution of the methanolic extract (2ml) was placed in a test tube and shaken for 15 minutes. Formation of a form (soapy like) column of about 2cm above liquid level in the test tube indicated the presence of saponins.

Determination of Reducing compounds

The methanolic extract (1ml) was diluted with water (2ml). Fehling's solutions I (1ml) and Fehling's solution II (1ml) were added to the solution and heated in water bath at 90ºC. Formation of a brick-red precipitate denoted the presence of reducing compounds.

Determination of Tannins

One milliliter of aqueous extract was added to 2ml of water. Three drops of ferric chloride were added to the solution. The occurrence of a blackish blue color or green blackish color indicated the presence of gallic tannins or catechol tannins respectively.

Determination of Alkaloids

The presence of alkaloids in plant material is shown by precipitation of alkaloid salts and reaction with Mayer's reagent (potassium tetra-iodo mercurate solution). To aqueous extract (15ml) was dissolved in 10% v/v Hydrochloric acid (10ml) and the alkaloids were precipitated from the aqueous solution as bases with the help of 10 % v/v ammonia solution (10ml) and extracted with ether (15ml). The ether solution was evaporated to dryness and to it, added hydrochloric acid solution (1.5ml). The acidic solution was divided into three portions each 0.5ml. To one of three acidic solutions was added 2-3 drops of Mayer's reagents. Formation of opalescence or a yellowish-white precipitate confirmed the presence of alkaloids.

Determination of Terpenoids

Five milliliters of the extract were mixed with 2ml of chloroform. Three milliliters of concentrated sulphuric acid were added to form a layer. A red to purple color formation on the interface indicated presence of terpenoids.

Determination of Sterols

An extract was prepared using 1g of powdered sample and 20 ml of ether macerating for 24 hours using a water bath. The extract obtained was used to detect sterols. Ten milliliters of ether macerate were evaporated to dryness. One milliliter of chloroform was added to the residue. The solution obtained was divided into two test tubes. One to 2ml of concentrated sulphuric acid was placed in the bottom of one of the test tubes the other acting as a control. The formation of a brownish-red or violet ring where the two phases meet indicated the presence of sterols.

Determination of Phenols

Phenolic compounds in the plant extracts were detected by Folin Ciocalteu reagent. Two milliliters of the extract were mixed with a few drops of diluted Folin Ciocalteu reagent and aqueous sodium carbonate solution. The mixture was allowed to stand for 10 minutes. Formation of grey colour indicated the presence of phenolic groups.

Reactions carried out in the hydrolysed aqueous extract

Hydrolysis Procedure:

Aqueous extract (25ml) was mixed 10% v/v hydrochloric acid (15ml) and refluxed for 30 minutes. The solution was cooled and extracted with diethyl ether (36ml) in portions of 12ml each. The ether extract was dehydrated using anhydrous sodium sulphate. The hydrolysed aqueous extract was used to test for anthracenosides, coumarin derivatices, steroid glycosides, and flavanosides.

Determination of Anthracenosides

The ether extract (4ml) was concentrated to 2ml and shaken with 25% v/v ammonia solution (2ml). Formation of a cherished-red solution on top layer indicated the presence of emodols (aglycones of anthracenosides) in an oxidized form.

Determination of Coumarin derivatives

To a residue obtained by evaporating ether extract (5 mL) was added hot water (2ml) to dissolve. The solution was cooled and divided into two portions. To one tube was added10% v/v ammonium solution (0.5ml). The occurrence of a blue or green fluorescence under UV light deeper for the alkaline solution indicated the presence of coumarins and their derivatives.

Determination of flavonosides (flavone glycosides)

Table 1: Qualitative phytochemical screening of *Solanum nigrum, Physalis angulata, Cleome gynandra, Solanum anguivii* and *Afromomum angustifolium* methanol extracts

Compound	Wild Edible Plant Species				
	S. anguivii (Whole Fruits)	*A. angustifolium* (Fruit pulp)	*C. gynandra* (Leaves)	*P. angulate* (Whole Fruits)	*S. nigrum* (Leaves)
Saponins	+	-	+	+	+
Reducing compounds	+	+	+	+	+
Tannins	+	-	+	-	+
Alkaloids	+	+	+	+	+
Volatile oils	-	+	-	-	-
Phenols	+	-	+	-	+
Anthracenosides	-	-	-	-	-
Flavonoids	+	+	+	+	+
Sterols & triterpenes	+	+	+	+	+
Coumarins	+	+	+	+	+

Note: (+) Sign indicates presence of active ingredient and (-) indicates absence of active ingredient.

The residue obtained by evaporating ether extract (5ml) was heated in 50% aqueous (2ml) and metallic magnesium 0.5g) and conc. Hydrochloric acid (5 drops) and added. Formation of a red or orange solution indicated presence of flavonols or flavanones (Shibata's reaction).

Determination of Antioxidant Activity

The antioxidant activity of plant extracts was determined using DPPH free radical scavenging assay[14]. The free radical scavenging activities of the plant extracts were followed via their reaction with the stable DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical. During the DPPH assay, the antioxidants reduce the stable radical DPPH to the yellow coloured diphenyl-picrylhydazine, resulting in a colour change from purple to yellow. The absorbance decreases when DPPH is scavenged by an antioxidant through donation of hydrogen to form a stable DPPH radical. The lower the absorbance, the higher the antioxidant activity of the plant extracts.

DPPH radical scavenging assay

The free radical scavenging activity was measured by a DPPH assay[14]. Fifty microliters of various concentrations of the extracts in methanol were added to 1.950ml of a 0.025g/l methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

DPPH scavenging activity (%) = $(A_c - A_t / A_c) \times 100$

where:

A_c = 1.633 is the absorbance of the control reaction (DPPH) and

A_t is the absorbance in the presence of the sample of the extracts.

The prepared concentrations were 20, 40, 80 and 120 µl for each plant extract. Absorbances for the aforementioned concentrations were read in duplicate. Average of the absorbances was calculated and all variables entered in SPSS work sheet version16. They were then statistically analysed using one way analysis of variance (ANOVA). All data were expressed as mean ± standard error mean. The ascorbic acid methanolic solution was used as positive control.

Table 2: Percentage DPPH free radical scavenging activity

Species	Part used	Conc./ µl	Percentage Scavenging Activity
C. gynandra	Leaves	120	89
S. nigrum	Leaves	40	97.4
S. anguvii	Fruits	120	93.5
P. angulata	Fruits	120	95
A.angustifolium	Fruits	120	91

RESULTS

Qualitative Phytochemical Analyses

Preliminary qualitative phytochemical analyses of the methanolic plant extracts showed presence of alkaloids, flavonoids, tannins, saponins, phenols, sterols, reducing sugars, volatile oils, coumarins and terpenes (Table 1). All the five wild edible plant species tested positive for presence of reducing compounds, alkaloids, flavonoids, coumarins, sterols and triterpenes. Anthracenosides were absent in all the plant species. Whereas *A. angustifolium* is the only plant that showed presence of volatile oils, it is the only plant that does not contain saponins.

DPPH free radical Scavenging Assay

The antioxidant activity of the plant extracts was determined using DPPH free radical scavenging assay. The DPPH percentage scavenging activity was calculated using the concentration at which there was highest antioxidant activity (Table 2). The free radical scavenging activity of the methanolic extracts were then compared with ascorbic acid and expressed as ascorbic acid equivalents (Table 3). The percentage free radical scavenging activity of all the five wild edible plant species is above 80%. *Solanum nigrum* showed a very high scavenging activity at low concentration (40 µl) as compared to other species which showed highest activity at 120 µl. *S. nigrum* showed the highest free radical scavenging of activity of 20.87±0.005 when compared with ascorbic acid standard. *C. gynandra* had the lowest scavenging activity. There is a significant difference in the free radical scavenging activity of the methanolic plant extracts. An individual will acquire more antioxidants by taking 100g of *S. nigrum* as compared to other species. Quantity of antioxidants taken

Table 3: DPPH Free radical scavenging ability of the methanolic plant extracts and amount of antioxidant intake in mg per 100g of Edible portion

Species	Part used	Ascorbic Acid Equivalent (AAE) (mg/ g of dry material) (Mean ± SEM)	Antioxidant intake (mg per 100g) Edible portion
Cleome gynandra	Leaves	17.57±0.00	1757
Solanum nigrum	Leaves	20.87±0.005	2087
Solanum anguvii	Fruits	19.46±0.04	1946
Physalis angulata	Fruits	19.93±0.03	1993
Afromomum angustifolium	Fruits	18.51±0.01	1851

Values are considered as significant at $p<0.05$

in per 100g of *S. anguivii* and *P. angulata* is comparable therefore one can consume either of the species.

DISCUSSION

The presence of various active compounds as revealed by the phytochemical screening (Table 1) supports the resourcefulness of these wild edible plant species[15]. Knowledge on the phytochemical constituents of these wild edible plant species is very important because it gives us the lead to the discovery of new bioactive compounds. Phytochemicals have complementary and over lapping actions which include; antioxidants, modulation of detoxification enzymes, stimulation of the immune system, reduction of inflammation, modulation of steroid metabolism, antibacterial, antihelmintic and antiviral effects in humans[16]. Phenols, flavonoids and tannins are good antioxidant substances and have been reported to prevent or control oxidative stress related disorders[17,7]. Flavonoids are hydroxylated phenolic substances which are best known for their strong antioxidant properties [17,18] and hence protect the body against oxidative cell damage. Tannins have antioxidant and antimicrobial properties and are good for soothing relief, skin regeneration and anti-inflammatory[19]. The presence of tannins may account for the sharp taste of *S. nigrum*[20]. Phenols bind proteins and may lower protein digestability and quality as well as reducing the risk of heart diseases and certain types of cancer[21]. The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds in plant and fruit extracts and food materials[22]. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability[23]. The analysed wild edible plant species have good antioxidant properties because their extracts showed proton donating ability. This is confirmed by the results which show that all the five species had a percentage scavenging activity above 80% (Table 2). *S. nigrum* is the best antioxidant among the analysed species. This is because it showed highest scavenging activity at a low concentration of 40µl whereas the rest of the plant species showed highest scavenging activity at 120µl concentration. The antioxidant properties of *S. anguivii* and *P. angulata* are comparable because the values of their scavenging activity are close. This implies that one can consume either of the plants and will get the same value. These plant species are a very good natural source of antioxidants for the pregnant women in this area.

CONCLUSION

Solanum anguivii, *Cleome gynandra*, *Afromomum angustifolium*, *Solanum nigrum* and *Physalis angulata* posses several phytochemicals which account for their nutrition and health benefiting properties. Additionally, these plants have good free radical scavenging activity thus can be explored as natural sources of antioxidants.

Recommendations

Promoting wider consumption of wild fruits and vegetables being rich natural sources of antioxidants. Furthermore, domestication of these species should be emphasized for preservation of their germ plasm.

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REFERENCES

1. World Food Programme (WFP). Food and Nutrition Handbook. Boston, Tufts University. 2000.
2. Casanueva E, Viteri FE. Iron and oxidative stress in pregnancy. Journal of Nutrition 2003; 133(5, suppl 2): 1700S-1708S.
3. Karthikeyan J, Rani P. Enzymatic and nonenzy-matic antioxidants in selected piper Species. Indian Journal of Experimental Biology 2003; 41: 130-140
4. Webster RP, Roberts VH, Myatt L. Protein nitration in placenta – functional significance. Placenta 2008; 29: 985–994.
5. Saxena M, Saxena J, Nema R, Singh D, Gupta, A. Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry 2013; 1(6): 168
6. Geetha TS, Geetha N. Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of *Cymbopogan citratus* (DC) stapf. leaves from Kodaikanal hills, Tamilnadu. International Journal of PharmTech Research CODEN (USA) 2014; 6(2): 521 – 529.
7. Stephen UA, Abiodun F, Osahon O, Ewaen E. Phytochemical analysis and antibacterial activity of Khaya

grandifoliola stem bark. Journal of Biological Sciences 2009; 9 (1): 63-67
8. Agbor AG, Talla L, Ngogang JY. The Antidiarrhoeal activity of Alchornea cordifolia leaf extract. Phytotherapy Research. 2004; 18(11):873–876.
9. Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant flavonoids, especially tea flavonoids are powerful antioxidant using an in-vitro antioxidant model for heart disease. Journal of Agricultural Food Chemistry 1995. 43: 2800-2802.
10. http// www. betterhealth.vic.gov.au. Accessed 30th September 2015.
11. Martin GJ. 1995. Ethnobotany: A people and plants conservation manual. Chapman and Halls. London, UK. 1995.
12. Paris. Pharmacopee Africaine. Publication dividion 2 –OUA CSTR. 1985.
13. Bruneton J. Pharmacognosie, Phytochimie, Plantes medicinales. Lavoisier Technique and Documentation. Paris. 2nd Edition 1993.
14. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181: 1199- 1200.
15. Sofowora A. Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd (Pub.), Ibadan.1993.
16. Johana WL. Spiring up a vegetarian diet.Chemoprotective effect of phytochemicals. American Journal of Clinical Nutrion 2003; 78 (3): 579 - 583.
17. Awomukwu DA, Nyananyo BL, Onukwube ND, Uka CJ, Okeke CU, Ikpeama AI. Comparative Phytochemical Constituents and Pharmacognistic Importance of the Vegetative Organs of Some Phyllanthus Species in South Eastern Nigeria. International Journal of Modern Botany 2014; 4(2): 29-39. DOI: 10.5923/j.ijmb.20140402.01
18. Mensah JK, Ihenyen JO, Okhiure MO. Nutritional, phytochemical and antimicrobial properties of two wild aromatic vegetables from Edo State. Journal of Natural Products Plant Resources 2013; 3 (1): 8-14.
19. Okwu DE and Okwu MR Journal for Sustainable Agricultural Environment 2004; 6: 140-147.
20. Okwu DE, Emenike IE. Evaluation of the Phytonutrients and Vitamin content of citrus frui. ,International Journal of Molecular Medicine Advances in Science 2006; 2(1): 1-6.
21. Mibei EK. Nutritional, Phytochemical and In vitro Antimicrobial screening of some Indigenous leafy vegetables. Masters thesis (Biochemistry). Jomo Kenyatta University of Agriculture and Technology. 2011.
22. Wang SY, Stretch AW. Antioxidant capacity in cranberry is influenced by cultivar and storage temperature. Journal of Agricultural and Food Chemistry 2002; 49: 969–974.
23. Swapna PL, Kanabiran K. 2006. Antimicrobial and Phytochemicals of *Solanum trilobatum* L, African Journal of Biotechnology 2006; 5(23), 2402-2404.