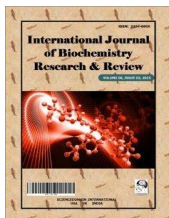

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Phytochemical Profile and Free Radical Scavenging Activities of Methanol Extract of Green Pea

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Authors' contributions

This work was carried out in collaboration between all authors. Author NCC designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author RNO Performed statistical analysis, managed the analyses of the study and wrote the final draft. Author SH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The legume, Green pea (*Pisum sativum*) is usually consumed as a vegetable. Phytochemical screening of its methanol extract showed that it contains terpenoids, resins, cardiac glycosides, steroids, saponins, phenols and flavonoids. Quantitative phytochemical estimation in (20/mg) revealed that it contains Saponins 13.4±0.11, Flavonoids 10.4±0.089, Phenols 9.8±0.063 and steroids 7.2±0.075. DPPH radical scavenging activities of methanol extract of green pea was expressed as the percentage inhibition of DPPH at varying concentrations of the extract. It was found to be proportional to the concentration of the plant extract; at 5 mg/ml (57.1%), 2 mg/ml (41.7%), 1 mg/ml (31%), 0.5 mg/ml (26.4%), 0.1 mg/ml (21.3%), and 0.05 mg/ml (16.9%). Thus, *Pisum sativum*, is a herb with some nutritional and pharmacologically important secondary metabolites with potent antioxidant, anticancer, anti-inflammatory, anti-lipidemic and growth enhancement effects.

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1. INTRODUCTION

Our environment is replete with rich flora and biodiversity of plants, some of which have been used traditionally in the treatment of myriad human diseases and disorders. These medicinal plants possess secondary metabolites with proven antioxidant, anti-diabetic, antimalarial and anti-nociceptive potentials to mention a few [1]. Some of these medicinal plants and their products have also been used as nutraceuticals.

Nutraceutical is a term derived from “nutrition” and “pharmaceutics.” The term is applied to products that are isolated from herbs, dietary supplements (nutrients) and specific diets. It also applies to processed foods such as cereals, soups and beverages that are used as medicine [2]. Nutraceuticals are essentially prophylactic or preventive in nature; they confer protection against illness and disease conditions.

Herbal bioactives, an important category of nutraceuticals, are commonly used by people who seek “out of the box” conventional health care. Herbs harbor a wide variety of active phytochemicals including the flavonoids, terpenoids, lignans, sulfides, polyphenols, carotenoids, coumarins, saponins, plant sterols, uramins and phthalates [3,4]. Green pea (*Pisum sativum*) is one of such medicinal plants; it belongs to the family (fabaceae); genus (pisum); and species (*P. sativum*). *P. sativum* is an herbaceous annual plant, with a climbing hollow stem growing up to 2–3 m long. Leaves are alternate, pinnately compound, and consist of 2–3 pairs of 1.5–8 cm long large leaf-like stipules. Flowers have five green fused sepals and five white to reddish-purple petals of different sizes. Fruit grows into a pod, 2.5–10 cm long that often has a rough inner membrane [5]. Traditionally, seeds of *P. sativum* are used as nutrient, appetizer, astringent, refrigerant and laxative. It has also been used in treating wrinkled skin, diabetes, acne, phlegm and intestinal inflammation [6]. Antioxidant, antimicrobial and hypoglycemic activities of the seeds have been reported [7].

This study was undertaken to profile the phytochemical content (Qualitative and quantitative) of methanol extract of *Pisum sativum* as well as its free radical scavenging activities as a prelude to its potential as a nutraceutical. To the best of our knowledge, no such research has been carried out on the plant.

2. MATERIALS AND METHODS

Fresh seeds of green pea (*Pisum sativum)* were used for the study.

A fresh batch of the green pea fruit was bought at the Keffi main market, Keffi, Nasarawa state, Nigeria. It was identified at the Department of Plant Science and Biotechnology, Nasarawa State University.

2.1 Sample Preparation

The sample was washed under clean running tap water to reduce surface contaminants and air-dried under the shed to avoid loss of volatile compounds. The sample was pulverized using mortar and pestle to obtain a large surface area for better solubilization of the phytochemicals in the solvent. The powdered sample was stored in airtight glass container protected from sunlight until required for analysis.

2.2 Phytochemical Extraction

The extraction procedure adopted was cold maceration using 80% methanol as the solvent. The extraction mixture was prepared by dissolving weighted amount of the powdered peas in 80% methanol in an air-tight glass container in ratio 2:10 (W/V). The mixture was allowed to soak for 7 days. The mixture was filtered with a funnel plugged with cotton wool and the sample filtrate was collected into a clean glass beaker and the residue was discarded. The filtrate obtained was concentrated on water bath to give a paste.

2.3 Qualitative Phytochemical Screening

Qualitative phytochemical screening of the plant extract was carried out according to standard procedures described by Harborne [8].

2.4 Test for Tannins

About 0.5 g of the plant extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. The occurrence of a

blue –black, or blue-green precipitate indicated the presence of tannins.

2.5 Test for Steroids and Triterpenoids

About 0.2 g of the plant extract was mixed with 2 ml of chloroform and a few drops of conc. H_2SO_4 were added. The mixture was shaken vigorously and allowed to stand for some time. Red color appeared at the lower layer indicating the presence of steroids. The formation of a yellow colored layer indicated the presence of triterpenoids.

2.6 Test for Glycosides

About 5 ml H_2SO_4 was added to the plant extract in a test tube. The mixture was heated on boiling water for 15min. Fehling solution A and B was then added and the resulting mixture was heated to boiling. A brick red precipitate indicated the presence of Glycosides.

2.7 Test for Anthraquinones

About 0.2 g of the plant extract was shaken with 10 ml of benzene and then filtered; 0.5 ml of 1% ammonia solution was then added to the filtrate and there after shaken. Appearance of a pink, red or violet color in the ammonical (lower phase) indicated the presence of free anthraquinones.

2.8 Test for Saponins

About 1 g of the plant extract was boiled with 5 ml of distilled water and filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5minutes. Frothing which persisted on warming indicated the presence of saponins.

2.9 Test for Phenols

To about 0.5 g of the plant extract was added 1% ferric (III) chloride in methanol /water (1:1). A dirty green precipitate indicated the presence of phenols. Alternatively, equal volume of the plant extract was added to equal volume of $FeCl_3$ solution. The formation of a deep bluish green precipitate indicated the presence of phenols.

2.10 Test for Alkaloids

Methanol extract of P.*sativum* was acidified with 1% HCl and was then treated with a few drops of Mayer, Wagner and Dragendroff's reagents separately in different test tubes. A creamy white (Mayer), reddish brown (Wagner) or an orange brown (Dragendroff's) precipitate indicated the presence of alkaloids.

2.11 Test for Cardenolides

About 2 ml of benzene was added to 1 ml of the plant extract. The formation of a turbid brown color indicated the presence of cardenolides.

2.12 Test for Terpenoids

About 0.5 ml of acetic anhydride was mixed with 1 ml of the plant extract and a few drops of conc. H_2SO_4 were added. A bluish green precipitate indicated the presence of terpenoids.

2.13 Test for Carbohydrate (Fehling's Test for Reducing Sugar)

About 5 ml mixture of equal volumes of Fehling's solution A and B was added to 2 ml of the plant extract in a test tube. The resultant mixture was boiled for 2 min. A brick red precipitate of copper (i) oxide indicated a positive test for reducing sugars.

2.14 Test for Flavonoids

A small quantity of the plant extract was dissolved in dilute NaOH. A yellow solution that turned colorless on addition of concentrated HCl indicated the presence of flavonoids.

2.15 Test for Cardiac Glycosides

About 0.5 g of the plant extract was dissolved in 2 ml glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 2 ml of concentrated sulphuric acid; a brown ring formation at the interphase indicated the presence of deoxy sugar characteristics of Cardiac glycosides.

2.16 Test for Phlobatannins

A few drops of 1%HCl were added to 1 ml of the plant extract and boiled. A reddish precipitate indicated the presence of phlobatannins.

2.17 Test for Resins

About 2 ml of the plant extract was mixed with equal volume of acetic anhydride solution, and then a few drops of conc. H_2SO_4 were added. A violet colouration indicated the presence of resins.

2.18 Test for Balsams

About 3 drops of alcoholic $FeCl_3$ was added to 4 ml of the plant extract which was then warmed, a dark green colouration indicated the presence of balsams.

2.19 Test for Volatile Oils

A small quantity of the plant extract was shaken with dil. NaOH and 0.1M HCl. The formation of a white precipitate indicated the presence of volatile oils.

2.20 Quantitative Phytochemical Screening

2.20.1 The determination of saponins content

This was carried out according to the method described by Obadoni and Ochuko [9].

About 5 g of the plant extract was weighed, and dispersed in 100 mL of 20% ethanol. The suspension was heated over a hot water bath for 4hrs with continuous stirring at about 55°C. The filtrate and residue were re-extracted with another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated over a water bath. After evaporation, the samples were dried in the oven to a constant weight.

Saponin (g/100 g) = weight of untreated sample / weight of treated sample x100.

2.20.2 Determination of flavonoid content

This was carried out according to the method described by Boham and Kocipai-Abyazan [10].

About 5 g of plant extract was weighed in a 250 ml titration flask, and 100 mL of 80% aqueous methanol was added at room temperature and shaken for 4hr on an electric shaker. The entire solution was filtered with Whatman filter paper no. 42 and again, this process was repeated. The filtrate as a whole was later transferred into a crucible and evaporated to dryness over a water bath and weighed.

Flavonoids (g/100 g) = weight of untreated sample / weight of treated sample x100.

2.20.3 Determination of phenols content

About 2g of the plant extract was defatted with 1 ml of diethyl ether using a soxhlet apparatus for 2h. The fat free extract was boiled with 50 ml of ether for the extraction of the phenolic components for 15 minutes. About 5 ml of the extract was pipetted into 5 ml flask and then 10ml distilled water was added. About 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol was also added.

The sample was made up to mark and left to react for 30 min for colour development, this was measured at 505 nm.

2.20.4 Determination of steroids content

About 1 g of the plant extract was weighed into conical flask and 20 ml of ethanolic sodium hydroxide was added followed by 1 ml of tetrazolium in methanolic hydroxide and 1 ml of tetra-methyl ammonium hydroxide. It was allowed to react for 90 min, the absorbance was measured at 525 nm, using ethanolic sitosterol as standard, Harborne [11].

2.20.5 Free radical scavenging activity of green peas (*Pisum sativum*)

The radical scavenging activity of the plant extract against DPPH was determined by UV-visible spectrophotometer at 517 nm. Radical scavenging activity was measured by a slightly modified method previously described by Ayoola et al*,* [12]. The following concentrations of the extract was prepared; 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml in methanol (Analar grade). 1 ml of the extract was placed in a test tube and 3 ml of methanol was added, followed by 0.5 ml of 1 mM DPPH in methanol and thereafter the decrease in absorption was measured on a UV-visible spectrophotometer 10 minutes later. A blank/control solution was prepared containing the same amount of methanol and DPPH. The actual decrease in absorption was measured against that of the control and the percentage inhibition was calculated. All tests and analysis were done in duplicates and the results obtained were averaged. The radical scavenging activity (RSA) was calculated as the percentage

inhibition of DPPH discoloration using the equation below:

$$\% \text{ inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100\%$$

3. RESULTS

At the end of the phytochemical screening, Table 1 shows that Steroids, Triterpenoids, Saponins, Phenols, Terpenoids, Flavonoids, Cardiac, Glycosides, and Resins were present in the methanol extract of P.*sativum*. While Table 2 shows the quantitative estimation of the phytochemicals as follows: Saponin 13.4 ± 0.1; Flavonoid 10.4 ± 0.089; Phenols 9.8 ± 0.063; and Steroid 7.2 ± 0.075.

3.1 Radical Scavenging Assay

In analyzing the antioxidant capacity of the green peas, different concentration of the methanolic extract was used in a DPPH radical scavenging assay. The actual decrease in absorption induced by the plant extract and the absorption of the control as measured at 517 nm wavelength, were used to obtain the % inhibition of the plant extract and eventually compared to that of a vitamin C standard. The result of the experiment are shown in Fig. 2.

4. DISCUSSION

All human populations have relied on the use of medicinal plants at one point or the other in their development [13]. The developing nations of the world still depend heavily on medicinal plants for treatment of various disease conditions .This is not surprising given the rich flora biodiversity of our biosphere [14].

Table 1. Result for the qualitative phytochemicals screening

Parameters	Content
Tannins	_
Steroids	+
Triterpenoids	+
Glycosides	_
Antraquinones	_
Saponins	+
Phenols	+
Alkaloids	_
Cardenolides	_
Terpenoids	+
Carbohydrate	_
Flavonoids	+
Cardiac Glycosides	+
Pholobatannins	_
Resins	+
Balsams	_
Volatile oils	_

Table 2. Shows the result of quantitative phytochemical estimation

Phytochemical	mg/20 g
Saponin	13.4 ± 0.1
Flavonoid	10.4 ± 0.089
Phenols	9.8 ± 0.063
Steroids	7.2 ± 0.075

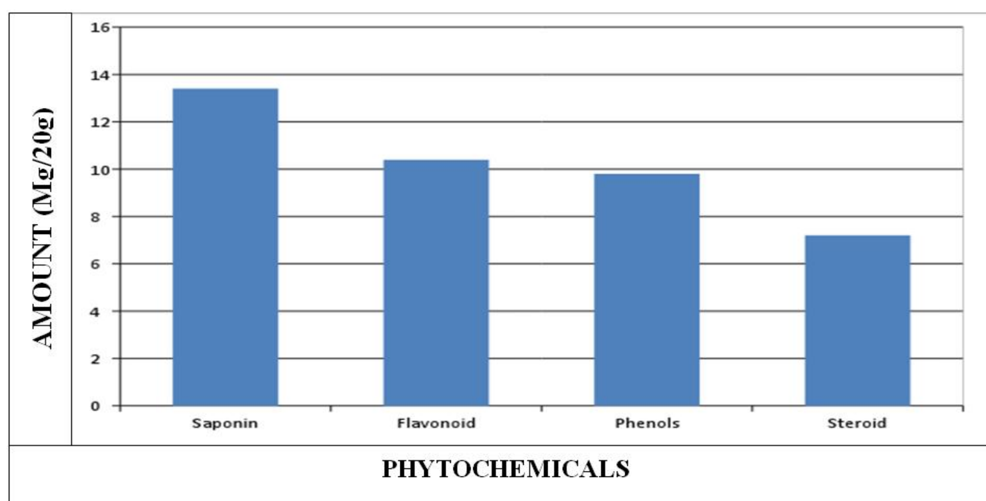

Fig. 1. Percentage content of the quantified phytochemicals

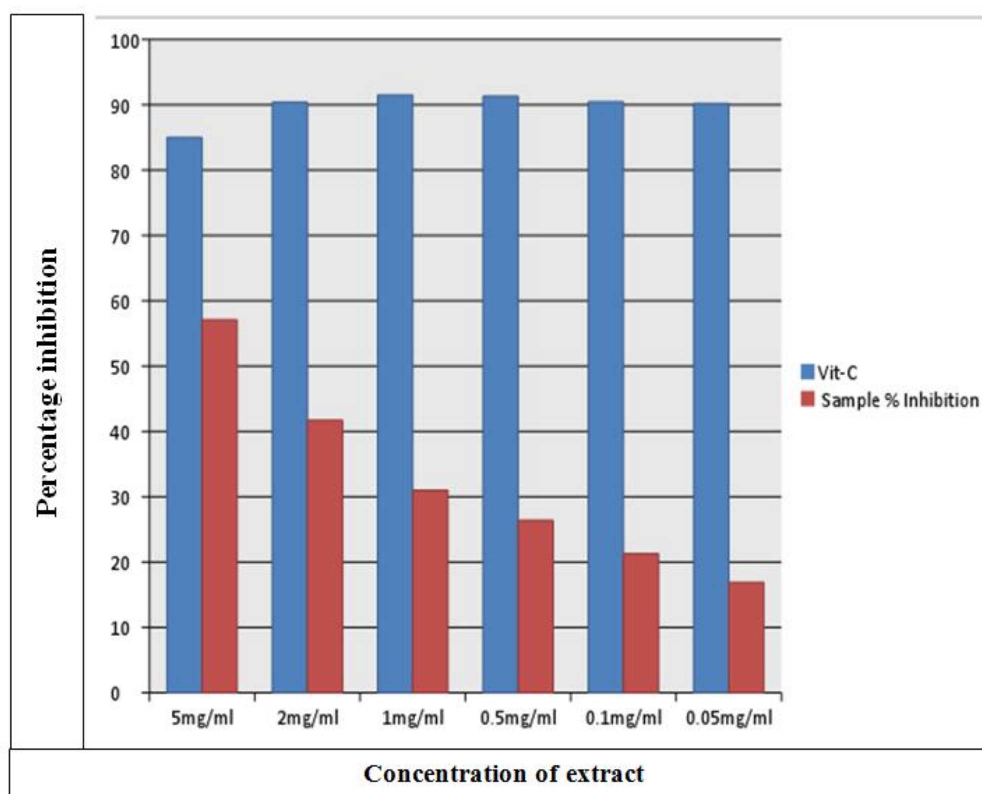

Fig. 2. % radical scavenging activity of the extract

Medicinal plants have been used in the treatment of myriad disease conditions that have plagued human populations. They have been used as anti-diabetics, anti-malaria, anti-nociceptive, antioxidant, anti-lipidemic etc [1].

These medicinal plants also form a major category of Nutraceutical. Nutraceutical is an emerging concept in the prophylaxis and prevention of diseases [15].

Nutraceutical is a combination of nutrients and pharmaceuticals. It is a new concept whereby nutrients/bioactive herbal component are used in the prevention of diseases [16].

Nutraceuticals and medicinal plants are desired for their efficacy, affordability and minimal (if any) side effects compared to the conventional drugs [17].

The therapeutic properties of these medicinal plants are tied to their content of phytochemicals. These phytochemicals have proven to be therapeutically relevant to our health [1].

Green pea, (*Pisum sativum*) is a delicacy consumed as a vegetable. It is a normal component of our diet e.g salads and vegetarian meals, because of its content of starch, protein, fiber and other nutrients. It has been reported to possess anti-diabetic [18], anti-obesity [19], and anti-oxidant [20]. It has also been reported to positively modulate cardiovascular, gastro-intestinal functions and homeostasis [21].

This experiment was designed to profile the phytochemical content (qualitative and quantitative) of methanol extract of green pea. Qualitative phytochemical screening of its methanol extract reveals that it contains steroids, triterpenoids, saponins, phenols, flavonoids, cardiac glycosides and resins. However, quantitative estimation of these phytochemicals shows that it is abundant in saponins, flavonoids, phenols and steroids. The presence of flavonoids, saponins, phenols and aromatic compounds is an indication of a significant antioxidant property [22]; all these bioactive compounds were able to discolour DPPH solution by their hydrogen donating ability. Typical phenolics that possess antioxidant

activity have been characterized as phenolic acids and flavonoids [23]. Steroids are important molecules with hypocholesteromic effect. They inhibit the absorption of cholesterol in the intestine; legumes are rich in steroids. This accounts for their beneficial effect in regulation of blood cholesterol.

In the antioxidant assay of the green pea methanol extract, the percentage inhibition was found to be proportional to the concentration of the extract. The highest level of percentage inhibition was recorded at 5 mg/ml (57.1%) and the least at 0.05 mg/ml (16.9%) of the plant extract. The DPPH test provides information on the reactivity of the green pea extract with stable free radical and it gives a strong absorption band at 517 nm in the visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolorized as the color changes from deep violet to light yellow. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity and the degree of reduction in absorbance measurement is indicative of the radical scavenging activity (antioxidant) power of the extract [12]. From this analysis, a higher percentage of green pea extract has a good radical scavenging activity in a concentration dependent manner.

Medicinal plants with potent antioxidant and other therapeutic activities can present a potential source of new nutraceuticals for the prevention of disease conditions.

5. CONCLUSION

Phytochemical profile (qualitative and quantitative) of methanol extract of green pea revealed its high content of flavonoids, phenols, steroids, terpenoids, saponins, resins, and cardiac glycosides. These phytochemicals have been reported to possess among others, antioxidant, anti-lipidemic and have been reported to positively modulate cardiovascular functions. Thus, green pea may present a potential source of nutraceuticals for the prevention and treatment of disease conditions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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