

**Phytochemical profile and free radical scavenging activities of
methanol extract of green pea**

ABSTRACT

The legume, Green pea (*Pisum sativum*) is usually consumed as a vegetable. Phytochemicals screening of its methanol extract shows that it contains terpenoids, resins, cardiac glycosides, steroids, saponins, phenols and flavonoids. These compounds possess some important biologic health benefits such as antioxidant, anticancer, anti-inflammatory, hypocholesteromic and growth enhancement effect. Quantitative phytochemicals screening of its methanol extract revealed the percentage contents of some its phytochemical; Saponin 13.4 ± 0.11 , Flavonoids 10.4 ± 0.089 , Phenols 9.8 ± 0.063 and steroids 7.2 ± 0.075 . It's DPPH radical scavenging activities was expressed as the percentage inhibition of DPPH at varying concentration of the extract. It was found to be proportional to the concentration of the plant extract; at 5mg/ml (57.1%), 2mg/ml (41.7%), 1mg/ml (31%), 0.5mg/ml (26.4%), 0.1mg/ml (21.3%), and 0.05mg/ml (16.9%). Thus, the green pea (*Pisum sativum*), is a herb with some nutritional and pharmacologically important secondary metabolites with proven medicinal properties

Key word: Green pea, phytochemical, free radicals, nutraceuticals

22 INTRODUCTION

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

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

33 Herbal bioactives, an important category of nutraceuticals, are commonly used by people who
34 seek conventional health care. Herbs harbor a wide variety of active phytochemicals including
35 the flavonoids, terpenoids, lignans, sulfides, polyphenols, carotenoids, coumarins, saponins,
36 plant sterols, uramins and phthalates [3, 4]. Green pea (*Pisum sativum*) is one of such medicinal
37 plants; it belongs to the family (fabaceae); genus (pisum); and species (P. sativum). *P. sativum* is
38 an herbaceous annual plant, with a climbing hollow stem growing up to 2–3 m long. Leaves are
39 alternate, pinnately compound, and consist of 2–3 pairs of 1.5–8 cm long large leaf-like stipules.
40 Flowers have five green fused sepals and five white to reddish-purple petals of different sizes.
41 Fruit grows into a pod, 2.5–10 cm long that often has a rough inner membrane [5]. Traditionally,
42 seeds of *P.sativum* are used as nutrient, appetizer, refrigerant, laxative, astringent and also used

in treating wrinkled skin, diabetes, acne, phlegm and intestinal inflammation [6]. Antioxidant, antimicrobial and hypoglycemic activities of seeds also have been reported [7].

This study was undertaken to profile the phytochemical content (Qualitative and quantitative) of *Pisum sativum* as well as its free radical scavenging activities as a prelude to its potential as a nutraceutical.

Materials and Method

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

A 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.

Sample Preparation

The sample was washed under clean running tap water to reduce surface contaminants and air-dried under the shed to remove water and 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.

Phytochemical Extraction

The extraction procedure adopted was maceration involving cold extraction. 80% methanol was used for the extraction.

The extraction mixture was prepared by mixing a substantial amount of the powdered peas with 80% methanol in an air-tight glass container in the ratio of 2:10 (W/V). The mixture was allowed

to soak for 7 days. The mixture was filtered via a funnel plugged with cotton wool and the sample filtrate collected into a clean glass beaker and the residue was discarded. The filtrate obtained was concentrated by evaporating the methanol portion of the extract on water bath, eventually recovering the phytochemicals concentrate.

Qualitative phytochemical screening

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

Test for Tannins:

About 0.5g of extract was stirred with about 10ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2ml of the filtrate. The occurrence of a blue –black, or blue-green precipitate indicated the presence of tannins.

Test for Steroids and Triterpenoids:

About 0.2g of crude extract was mixed with 2ml 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 yellow colored layer indicated the presence of triterpenoids.

Test for Glycosides:

About 5ml H_2SO_4 was added to each of the test extracts in a separate test tube. The mixture was heated in 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.

Test for Anthraquinones:

About 0.2g of extract was shaken with 10ml 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.

Test for saponins:

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

Test for Phenols

To about 0.5g of the 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 extract was added to equal volume of FeCl_3 solution. The formation of a deep bluish green precipitate indicated the presence of phenols.

Test for Alkaloids

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

109 **Test for Cardenolides**

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

112 **Test for Terpenoids**

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

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118 **Test for Carbohydrate (Fehling's Test for Reducing Sugar):-**

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

122 **Test for Flavonoids**

123 A small quantity of the test extract was dissolved in dilute NaOH. A yellow solution that turns
124 colorless on addition of concentrated HCl indicated the presence of flavonoids.

125 **Test for Cardiac Glycosides**

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

130 **Test for Phlobatannins**

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

133 **Test for Resins**

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

136 **Test for Balsams.**

137 About 3 drops of alcoholic FeCl_3 was added to 4ml of the extract which was then warmed. A
138 dark green colouration indicated the presence of balsams.

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140 **Test for Volatile oils.**

141 A small quantity of the sample was shaken with dil. NaOH 0.1M HCl. A white precipitate is
142 formed with volatile oils.

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147 **Quantitative phytochemical screening**

148 The **Determination of Saponins Content**

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

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

159 $\text{Saponin (g/100g)} = \text{weight of untreated sample} / \text{weight of treated sample} \times 100$

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161 **Determination of Flavonoid Content**

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

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

168 $\text{Flavonoids (g/100g)} = \text{weight of untreated sample} / \text{weight of treated sample} \times 100.$

169 **Determination of Phenols Content**

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

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

177 **Determination of Steroids Content**

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

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

183 The radical scavenging activity of the green pea extract against DPPH was determined by UV-
184 visible spectrophotometer at 517nm. Radical scavenging activity was measured by a slightly
185 modified method previously described by Ayoola *et al.*, [12]. The following concentrations of
186 the extracts were prepared, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg/ml in methanol (Analar
187 grade). 1ml of the extract was placed in a test tube and 3ml of methanol was added, followed by
188 0.5ml of 1mM DPPH in methanol and thereafter the decrease in absorption was measured on a

UV-visible spectrophotometer 10minutes 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:

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

RESULTS

At the end of the phytochemicals screening, Table 4.1 shows that Steroids, Triterpenoids, Saponins, Phenols, Terpenoids, Flavonoids, Cardiac, Glycosides, and Resins were present in the methanol extract of *P.sativum*. While Table 4.2 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

Table 4.1: Result for the qualitative phytochemicals screening

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

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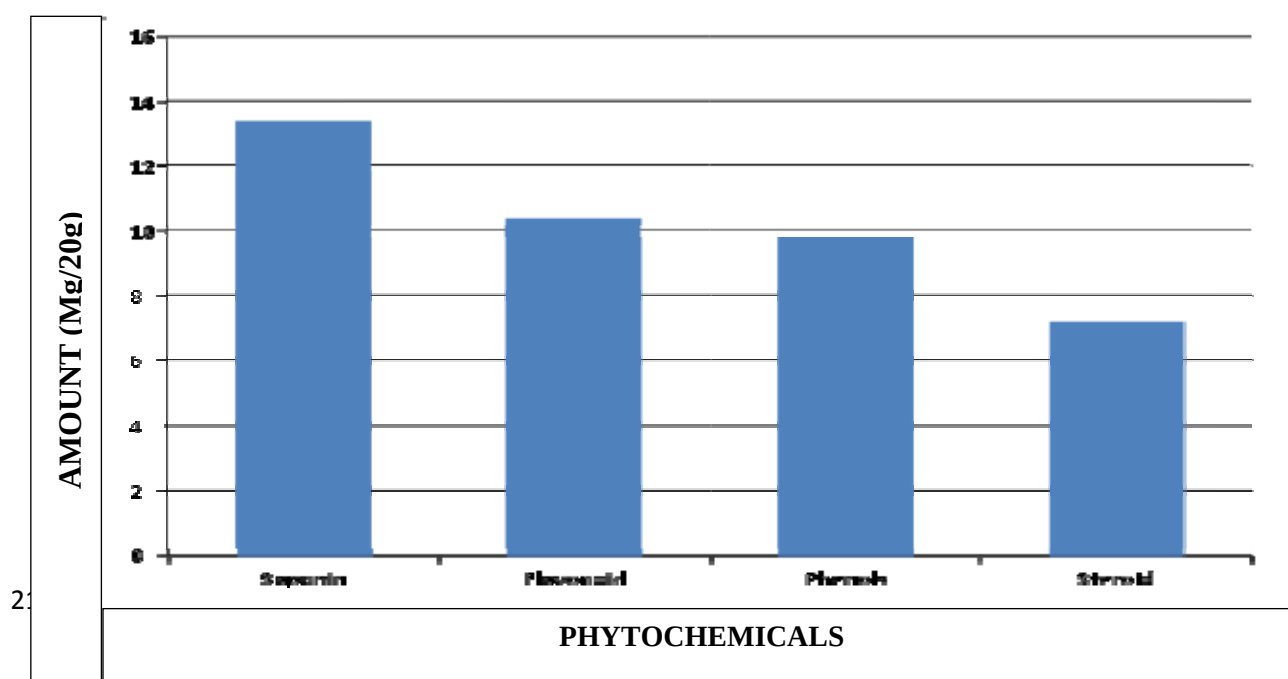
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216 **Table 4.2 shows the result of quantitative phytochemical estimation**

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

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218 **Figure 4.1: Percentage content of the quantified phytochemicals**



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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 test sample and the absorption of the control as measured at 517nm 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 figure 4.2 below.

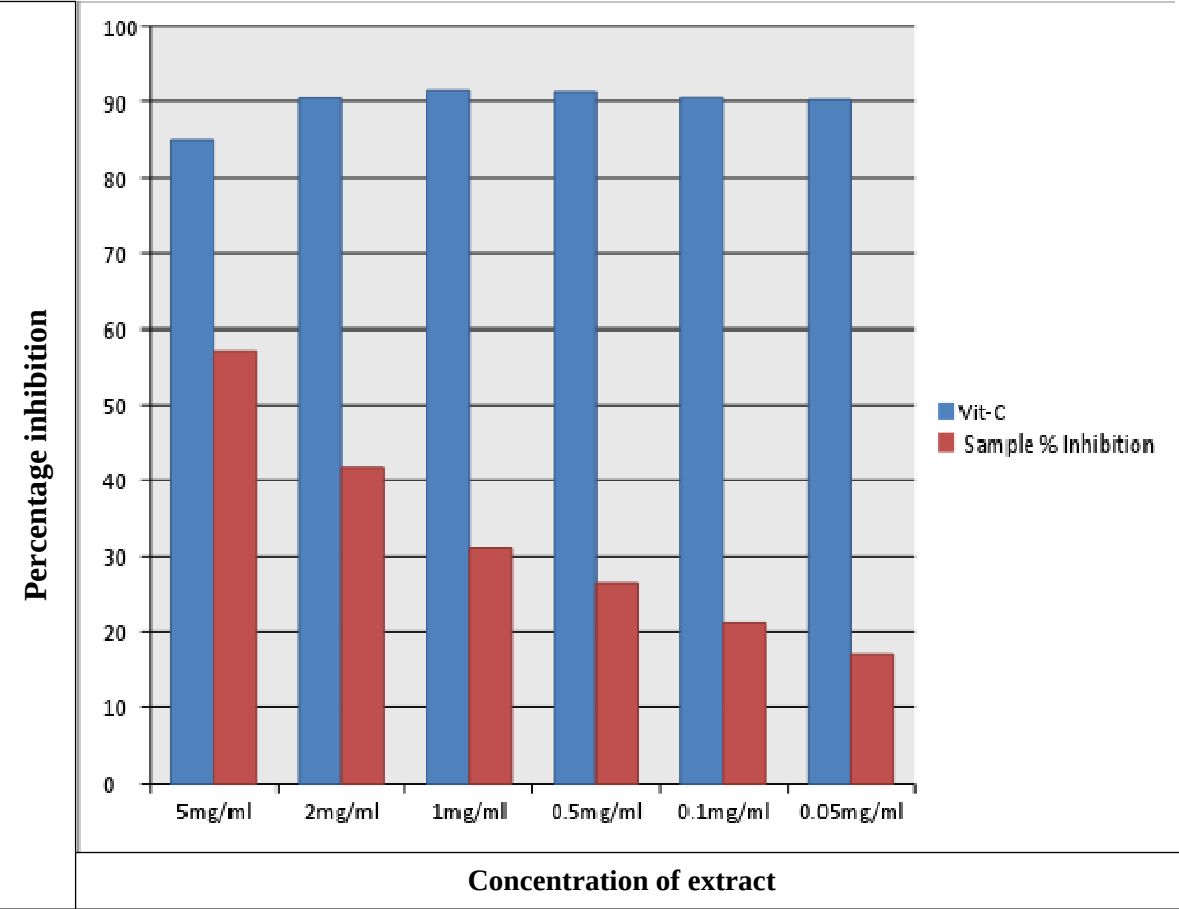


Figure 4.2: % radical scavenging activity of the extract

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].

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 effect 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 therapeutic 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 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 concentration 5mg/ml (57.1%) and the least at concentration 0.05mg/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 517nm 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 represent a potential source of new nutraceuticals for the prevention of disease conditions.

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 treatment of disease condition.

REFERENCES

1. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: A review. *J Ethnopharmacol.* 2011; 133:289-302.
2. Kalra EK. Nutraceutical – Definition and introduction. *AAPS Pharm Sci.* 2003; 5:E25.
3. Craig, WJ. Health-promoting properties of common herbs. *American J. Nutr.* 1999; 70(suppl): 491S-499S.
4. Dureja H, Kaushik D, Kumar V. Developments in nutraceuticals. *Ind. J. Pharmacol* 2003; 35: 363-372.
5. Angels MB, Joan V. Ethnobotany of Montseny biosphere reserve (Catalonia,Iberian peninsula): plants used in veterinary medicine. *J Ethnopharmacol.* 2007; 110(1):130- 47.
6. Alasalvar C, Shahidi F, Quantick P. Food and health applications of marine nutraceuticals: a review. In: Alasalvar C, Taylor D, editors. *Sea-foods Quality,technology and nutraceutical applications.* New York: Springer-Verlag 2002; 175-204.
7. Aima R, Azra K. Isolation and characterization of peptide(s) from *Pisum sativum* having antimicrobial activity against various bacteria. *Pak J Bot.* 2011; 43(6):2971-8.
8. Harborne JB. *Phytochemical Methods; A guide to Modern Techniques of Plant Analysis*, 1998; Chapman and Hall Ltd., London, Great Britain.
9. Obadoni B, Ochuko P. Phytochemical studies comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria, *Global J. Pure Appl. Sci.*,2001; **8**:203-208.
- 10.Boham BA, Kocipai-Abyazan R. Flavonoids and condensed tannin from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pacific Sci.* 1994; **48**:458-463.

- 320
- 321 11.Harborne JB. (1973). Phytochemical methods, London.Chapman and Hall,
322 Ltd. 1973; 49-188.
- 323
- 324 12.Ayoola GA, Sofidiya T, Odukoya O, Coker HAB. Phytochemical screening
325 and free radical scavenging activity of some Nigerian medicinal plants. J.
326 Pharm. Sci. & Pharm. Pract. 2006; 8: 133-136.
- 327
- 328 13.Singh R. Medicinal Plants: A Review. Journal of Plant Sciences. Special
329 Issue: 2015; Vol. 3, No. 1-1, 50-55. doi: 10.11648/j.jps.s.2015030101.18.
- 330
- 331 14.Van Vuuren SF. Antimicrobial activity of South African medicinal plants. J
332 Ethnopharmacol. 2008;119:462-472
- 333
- 334 15.Kasbia GS. Functional foods and nutraceuticals in the management of
335 obesity. Nutrition and Food Science, 2005; 35: 344-351.
- 336 16.Lifongo LL, Simoben CV, Ntie-Kang F, Babiaka SB, Judson PN. A
337 bioactivity versus ethnobotanical survey of medicinal plants from Nigeria,
338 West Africa. Nat Prod Bioprospect. 2014;4:119.
- 339
- 340 17.Nasir B, Fatima H, Ahmed M, Haq IU. Recent trends and methods in
341 antimicrobial drug discovery from plant sources. Austin J Microbiol.
342 2015;1:1002.
- 343 18.Marinangeli CP, Jones PJ . Whole and fractionated yellow pea flours reduce
344 fasting insulin and insulin resistance in hypercholesterolaemic and
345 overweight human subjects. Br J Nutr 2011; 105, 110-117.
- 346
- 347 19.Abete I, Parra D, Martinez JA. Legume-, fish-, or high-protein-based
348 hypocaloric diets: effects on weight loss and mitochondrial oxidation in
349 obese men. J Med Food 2009; 12, 100-108.

- 20.Duenas M, Estrella I , Hernandez T . Occurrence of phenolic compounds in the seed coat and the cotyledon of peas (*Pisum sativum*). Eur Food Res Tech 2004; 219,116-123.
- 21.Dahl WJ, Whiting SJ, Healey AD. Increased stool frequency occurs when finely processed pea hull fiber is added to usual foods consumed by elderly residents in long-term care J Am Diet Assoc. 2003;103(9):1199-202
- 22.Tahiya, HA, Amira HS, Qasim A. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. *Journal of King Saud University Science*, 2014; 26(3):237-243. doi: 10.1016.
- 23.Amrita S, Gaire BP, Sunar CB. Phytochemical screening and analysis of antibacterial and antioxidant activity of *Ficus auriculata*, lour. stem bark. *A Thesis for Bachelor's Degree. School of Health and Allied Sciences. Pokhara University, NEPAL.* 2009.

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