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Phytochemical & Antioxidant activity of underutilized legume *Vicia faba* seeds and formulation of its fortified biscuits

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Abstract
The dried seeds of broad beans (*Vicia faba* L.) were investigated for different characteristics such as phytochemical analysis, antioxidant and antimicrobial activity to understand its health benefits. A thorough characterization of secondary metabolites including crude alkaloids, saponins, total flavonoids and total phenolics content was carried out. Antioxidant activities were also evaluated for the dried seeds through ABTS scavenging activity and FRAP method. Dried *Vicia faba* L *(faba*) seeds showed good phenolics content and an excellent antioxidant activity. The extract did not show antimicrobial activity against all the three gram positive and one gram negative test bacterial pathogens. GC/MS analysis showed the presence of α-tocopherol, phytol, phytosterol, stigmasterol, campesterol and fatty acids, which have different therapeutic uses. *faba* fortified biscuits were formulated to study the nutritional profile. Nutritional analysis of the biscuits demonstrated the potential of *faba* seeds as an alternate source of energy, good source of protein and dietary fibers and minerals like Phosphorus, Calcium, Magnesium and Iron followed by the other nutrients. This study demonstrates that broad beans have an excellent potential to be employed as a nutraceutical, providing high nutritional value and remarkable health promoting factors.

Keywords: *faba* bean, Phytochemicals, Antioxidant, Antimicrobial, GC/MS, Nutritional.

1. Introduction
In recent decades, consumption of fruits and vegetables has attracted huge attention since many epidemiological and biochemical studies have consistently demonstrated a clear and significant positive alliance between regular intake of these natural food products and reduced rates of heart diseases, common cancers, aging and other degenerative diseases [1]. The protection that fruits and vegetables provide against these maladies has been attributed to the presence of several antioxidants, especially antioxidative vitamins, including α- tocopherol, ascorbic acid (vitamin C), and provitamin A. Several recent studies on these compounds seem to indicate that phenolic substances are the main phytochemicals with antioxidant properties found in higher plants [2, 3]. These non-nutrient metabolites, when compared to pharmaceuticals, have a low potency, but being a part of regular diet in considerable amounts, they apparently provide long-term health profiting effects. The bioactivity of these compounds has been shown to be correlated to their antioxidant properties. Studies depict that antioxidant components have the potential to lower the risk of several diseases [4]. The antioxidant activity of polyphenols has been of special interest because they have many significant biological properties and are beneficial for health [5, 6]. Also, natural antioxidants, of late, have been the major area of focus for researchers [7]. In view of the growing interest in these compounds, there is a need to identify and quantify these important compounds in the underutilized crops in India to evaluate their potential nutritional and health benefits.

India, is a country blessed with nature's immense treasures of fruits and vegetables, many of which still remain unexplored and are restricted for regional use only. Broad beans are one such example. It is an annual legume botanically known as *Vicia faba* (L.), also known as Bakala in Hindi. It is widely cultivated in most countries of the world and is an important part of the traditional Mediterranean, Chinese, English, Middle Eastern, African and South American diets [8]. These plants are rigid, erect and 0.5–1.8 m tall. Their leaves are 10–25 cm long, pinnate and of a distinct grey-green color with 1–2.5 cm long flowers of five petals. Its fruit is a broad, leathery pod, green maturing to blackish-brown. One pod contains 3–8 seeds; being round

to oval in shape and 5–10 mm diameter in the wild plant. *faba* beans have several culinary uses, particularly in the regions of Romans and ancient Greeks. The beans can be fried, and then spiced to a crunchy snack. These are popular in countries like China, Malaysia, Colombia, Mexico and Thailand [9]. In India, it is endemic to some of the eastern states, for example, in Manipur, it is locally known as "Hawai-Amubi" and is famous for its role as an ingredient in dishes like Kangsoi and Eromba and in Tamil Nadu, it is known as avarakkai. Also, *faba* beans exhibit several medicinal properties, acting as anti-hypertensive, diuretic, anti-diabetic, etc. Apart from being used as food for humans and livestock, they play a critical role in some agricultural systems due to their ability to fix atmospheric nitrogen under a broad spectrum of environmental conditions [10].

USDA Nutrient Database [11] provides complete nutritional information of *faba* beans proving them to be a rich source of energy (341Kcal), protein, dietary fiber and minerals such as Phosphorus, Iron, Calcium and Potassium; all of which are necessary for growth and development of the human body. It is known to be a unique legume because of its enormously rich nutrient content, mainly, being an excellent source of protein, carbohydrates, dietary fibre, minerals and secondary metabolites (phenolics and levo dihydroxy phenylalanine (l-DOPA) [12]. l-DOPA is used in therapy for the treatment of Parkinson's disease and therefore *faba* beans are being used as a treatment for the same. Despite such a broad range of beneficial applications, in India it is still categorized as minor, underexploited and underutilized crop. The reasons for this can be lack of popularity among local communities, lack of information on nutritional composition and the lack of promotional campaigns for these beans all over India. Very little data can be retrieved pertaining to the antioxidant potential and the phytochemicals present in the *faba* beans in India. Thus, delving deep into the bioactivities of the *faba* beans can lead to a better understanding of its potential as a nutraceutical.

The objective of the present study was, therefore, to evaluate the chemical and phytochemical constituents, secondary metabolites and antioxidant potential of the *faba* seeds (dried) and to study its nutraceutical potential as fortified biscuits.

2. Materials and methods

2.1 Sample collection and extraction

Dried *Vicia faba* seeds were requested and taken from Dr. A K Singh, Sr. Scientist (Agronomy) - Division of Land & Water Management, Indian Council of Agricultural Research-Research Complex for Eastern Region, Bihar. The seeds were first grounded into powdered flour and stored in a closed container at room temperature for future nutritional profiling. 50 g of the *faba* bean flour was then mixed with 200 ml of methanol. The mixture was incubated for 24 h and then filtered. The solvent was evaporated under vaccum and resulting extracts were stored at 4 ºC.

2.2 Phytochemical analysis

The total Phenolics content of the dried *faba* seeds was estimated by Folin-Ciocalteu reagent method [13]. The absorbance of the standard (gallic acid) and the extract of *Vicia faba* seeds was measured spectrophotometrically at 765 nm against DMSO blank. The results were expressed as gallic acid equivalents (GAE, µg/mg of weight of extract).

The total Flavonoids content was determined using Aluminium chloride colorimetric method [14]. The optical density for the standard (Catechin) and the sample extract was measured at 765 nm against DMSO blank , the total flavonoid content was expressed in µg of Catechin equivalents per mg of weight of extracts (CE, µg/mg of weight of extract).

Concentration of crude alkaloids and saponins was also estimated using the methods already described by Harborne [15] and Obadoni & Ochuko [16] respectively. The results were calculated in %.

2.3 GC/ MS Profiling

Secondary metabolites were identified by analyzing 1µl of a mix solvent extract of *faba* by using GC/MS analysis. Helium was used as carrier gas. An Agilent 6890 GC with 5975B mass spectrometric detector (MSD) was used in the scan mode (m/z 35-1050) for all samples. Screening of volatiles and semi volatiles were performed using the automatic RTL screener software in combination with the Agilent NIST'05 library. Transfer line temperature was set to 300 °C, solvent delay was 3 min, ion source and quadruple temperature were 230 °C and 150 °C, respectively [17]. The detected compounds have been identified by NIST'05 mass spectrum library and more than 90% matching value were reported.

2.4 Determination of antioxidant activity

The total antioxidant capacity of the extract was determined using ABTS radical scavenging activity and FRAP assay.

2.4.1 ABTS radicals are generated through a chemical oxidation reaction with potassium persulfate. The ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) method used was previously described by Re *et al.* [18]. The $ABTS^{+}$ radical cation was produced by mixing ABTS and potassium persulfate, the mixture was then kept in the dark at room temperature for 16 h. For the analysis, the reagent was diluted in ethanol until the absorption at 734 nm was 0.7± 0.02. A 20 µl of extract was mixed with 980 µl of ABTS reagent. The absorption was measured in a Hewlett-Packard spectrophotometer after 6 min of the addition of the sample. Each determination was performed in triplicates. The percentage of radical scavenging activity was calculated using the formula by using Ascorbic acid as a control:

Scavenging activity (%) = (1-Absorbance sample/ Absorbance control) x 100

2.4.2 The FRAP (Ferric reducing antioxidant power**)** assay was based upon the methodology of Benzie and Strain. [19] The FRAP reagent consisted of TPTZ in 40 mM HCl, $FeCl_3$ and sodium acetate buffer (pH 3.6). FRAP reagent was freshly prepared. A 100 µl of extract solution containing 0.1 mg extracts was mixed with 900 µl of FRAP reagent. The mixture was allowed to stand at 37 °C for 4 min, the absorbance at 593 nm was then determined against blank. BHT was used as calibration standard. FRAP values were calculated as mg of BHT equivalents/g extract from three determinations and were averaged.

2.5 Antibacterial activity

Antibacterial activities of the extract were tested by agar well diffusion method [20] against three Gram-positive bacteria and one Gram negative bacteria. Extract was reconstituted to concentrations of 80 mg/ml and 500 mg/ml. Nutrient agar was

inoculated by spreading 100 µl of the bacterial inoculums. Wells of 6 mm diameter were punched in the agar and 100 µl of extracts were loaded into the wells. The plates were incubated at 37 ^{0}C for 24 h. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition and reported in the scale of millimeter (mm).

2.6 Formulation of fortified biscuits and their nutritional profiling

Biscuits were baked through normal baking procedures by fortifying with 12% *faba* seeds flour using wheat as the main flour. Based on the appropriate storage conditions, biscuits were stored in air-tight containers and were evaluated for their nutraceutical potential and sensory characteristics. A 9 point hedonic scale was used for rating their sensory characteristics namely, colour, texture and crunchiness. Moisture content (AOAC, 1999) [21], ash content [22], protein content using kjehldahl method (AOAC, 2005 [22]), crude fat content (AOAC method Ref. 2003.06) [23] were evaluated for the fortified biscuits. Crude fiber content was estimated (Ref. 978.10 of AOAC, 2005 [22]). Mineral content by IP-OES (Ref 956.52 AOAC, 2005 [22]) was also determined. Total carbohydrates and energy content were calculated using formulae:

Total carbohydrates (% fresh weight) = 100 - moisture (%) - protein content (% fresh weight) - crude fat (% fresh weight) - ash (% fresh weight) and reported as total carbohydrates in %.

The calorific value in kilocalories (kcal) was calculated according to the system of Atwater, namely: kcal = (3.36 × % protein fresh weight) + (3.60 × % total carbohydrate fresh weight) + (8.37 × % fat).

3. Results and Discussion

3.1 Phytochemical analysis

Phytochemicals, especially phenolics are known to be major bioactive compounds for health benefits. Plant extracts containing different classes of polyphenols are very attractive in the food industry. Therefore, in this study, the total phenolics content and the total flavonoids content of the *faba* beans' extract were investigated. Folin-Ciocalteu phenol method estimated a total phenolic content of 22.415 GAE equivalents (µg GAE/mg sample). The presence of a high phenolic amount in the *faba* seeds indicates its potential as a good natural antioxidant.

It has been recognized that flavonoids show antioxidant activity [24]. The total flavonoid content of the reconstituted extract was estimated by the aluminium chloride method and the results were expressed in µg of Catechin equivalents (CE) per mg of dry weight of the extract based on the calibration curve of the standard. The total flavonoid content in the dried *faba* seeds was estimated to be 7.814 in µg of Catechin equivalents (CE) /mg.

Crude alkaloids and saponins in the *faba* seeds extract were estimated to be 2.99% and 0.002% respectively. Their presence in moderate amount in *faba* seeds divulges its therapeutic significance. These alkaloids in a diet consisting of *faba* seeds may prove helpful for healing of wounds, varicose ulcers, hemorrhoids, frost-bite and burn in herbal medicine. Saponins, on the other hand, are anti-nutritional factors and can reduce the uptake of certain nutrients, including cholesterol and glucose at the gut through intra lumenal physicochemical interaction (Okwu & Okwu) [25]. Saponin was observed in negligible amounts in the sample.

Table 1: Phytochemical analysis of *faba* seeds

sample	Total phenolic (µg GAE/ mg).	Total flavonoid (µg CE/mg)	Crude alkaloids (g/100 g)	Saponins (g/100 g)
Methanolic extract of dried *faba* seeds	22.415	7.814	2.99	0.002

3.2 Characterization of GC/MS Analysis

GC-MS results revealed the presence of most common fatty acids and other phytochemicals (Table 2) which are essentially used to regulate various functions like immune response, blood pressure, lipid levels and inflammation response to injuries. The results showed several phytosterols with a higher value of stigmasterol followed by campesterol and other useful compounds like tocopherols and phytol (Figure 1). The presence of plant sterols in a *faba* rich diet will balance the cholesterol level in the body. Stigmasterol, one of the analyzed compounds, possesses excellent antioxidant hypoglycemic and thyroid inhibiting properties. Campesterol is a valuable element in controlling cholesterol and lowering the risk of heart diseases. Tocopherol helps the body to fight chronic nervous disorders by resisting oxidization in blood and Phytol is a precursor for Vitamin E and vitamin K. These health promoting compounds, thus help to describe the merits of *faba* seeds as a functional food and a health enhancer.

3.3 Determination of antioxidant activity

3.3.1 ABTS Scavenging assay

The total antioxidant activity of *faba* dried seeds was evaluated in accordance with the decolorization of ABTS to its radical cation ABTS+ as percentage inhibition using the % radical scavenging formula. The ABTS radical scavenging activity (%) of the extract compared to ascorbic acid is shown in the graph. IC_{50} value for the extract is 0.97 mg/ml as estimated from the following graph. The results revealed excellent potential of *faba* seeds as an antioxidant sample (Table 3).

3.3.2 Ferric reducing antioxidant power (FRAP) assay

The principle of this method relies on the reduction of ferric tripyridyl-s-triazine complex to a coloured ferrous complex form in the presence of antioxidants. Antioxidants in the samples reduce ferric TPTZ complex to form blue colored complex which can be measured at a wavelength of 593 nm. The intensity of the colour is related to the amount of

antioxidant reductants in the samples. The activity was obtained to be 13.95 µg BHT equivalents (BE)/mg of sample (Table 3).

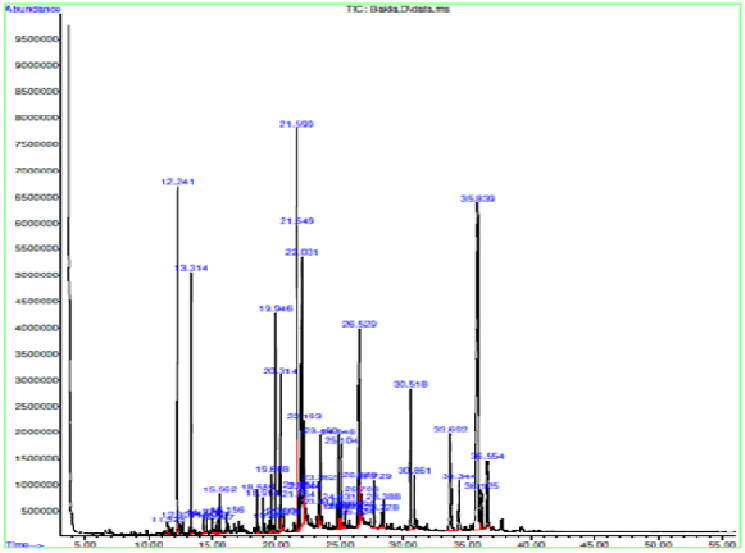

Fig 1: GC-MS chromatogram of *Vicia faba* extract.

Table 2: Secondary metabolites in *Vicia faba* by GC-MS screening

Compound name	CAS#	RT	% Area
Propanal, 2-methyl-3-phenyl-	1000131-87-6	11.521	0.20
Benzene, 1-methoxy-4-(1-propenyl)-	000104-46-1	12.239	5.08
Benzenemethanol, 4-(1-methylethyl)	000536-60-7	12.306	0.13
3-Allyl-6-methoxyphenol	000501-19-9	13.315	2.83
Caryophyllene	000087-44-5	14.269	0.31
2H-Benzopyran-2-one	000091-64-5	14.504	0.24
Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl	000644-30-4	15.031	0.20
Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	000495-61-4	15.357	0.19
Phenol, 2-methoxy-4-(1-propenyl)-,Acetate	000093-29-8	15.558	0.57
2-Hydroxy-2-(4-methoxy-phenyl)-N-methyl-acetamide	087920-02-3	16.153	0.29
Isopropyl Myristate	000110-27-0	18.564	0.61
Pentadecane	000629-62-9	19.663	0.63
Hexadecanoic acid, methyl ester	000112-39-0	19.944	2.68
n-Hexadecanoic acid	000057-10-3	20.314	3.17
Hexadecanoic acid, ethyl ester	000628-97-7	20.605	0.17
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	000112-63-0	21.603	5.78
9-Octadecenoic acid, methyl ester, (E)-	001937-62-8	21.648	4.23
Phytol	000150-86-7	21.749	0.41
Octadecanoic acid, methyl ester	000112-61-8	21.850	0.46
9-Octadecenoic acid, (E)-	000112-79-8	22.030	11.41
9,12-Octadecadienoic acid (Z,Z)-	000060-33-3	22.198	1.74
8-Octadecenoic acid, methyl ester (E)-	026528-50-7	22.243	0.57
Cyclotetracosane	000297-03-0	24.934	0.46
Eicosane	000112-95-8	24.979	0.32
9-Tricosene, (Z)-	027519-02-4	26.404	0.28
9-Octadecenoic acid (Z)-, 2,3-dihy droxypropyl ester	000111-03-5	26.527	4.63
Stigmasterol, 22,23-dihydro-	1000214-20-7	35.836	24.55
Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	056362-45-9	36.127	1.83
beta.-Amyrin	000559-70-6	36.553	3.32
Campesterol	000474-62-4	33.694	4.59
Gamma- Tocopherol	007616-22-0	30.520	4.09

Table 3: Determination of antioxidant activity

Antioxidant potential	Antioxidant activity
ABTS	IC_{50} = 0.97 mg/ ml
FRAP	13.45 µg BE/mg of sample.

3.4 Antibacterial Activity

Three gram positive (*Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis*) and one gram negative (*Proteus mirabilis*) test bacterial pathogens were used to evaluate the antibacterial activity of the dried *faba* seeds. Agar well diffusion method was used to assess the activity against the test organisms by measuring zone of inhibition. Inhibiting concentrations used for sample was 80 mg/ml and at a higher concentration of 500 mg/ml. Dried seeds were found to be least effective in inhibiting any of the strains. No zone of inhibition was obtained in any of the four strains. Where the *faba* beans (leaves and pods) has been previously reported for different extracts against different microbial strains [26].

3.5 Nutritional profiling and sensory evaluation of *faba* fortified biscuits: Nutritional studies of the formulated *faba* fortified biscuits have demonstrated its functionality as a nutraceutical (Table 4 & 5). The biscuits have been found to be very rich source of energy (454.46 kcal).The ash and moisture content were calculated to be 0.298 % and 2.87% respectively; which represent stable and longer shelf life of the biscuits. The total protein and crude fiber content in the biscuits were estimated to be 7.67% and 4.97% by weight, respectively. Mineral determination by ICP-OES revealed the presence of good amounts of essential minerals like calcium (478 µg/g), iron (68.70 µg/g), phosphorus (1479.9 µg/g) and magnesium (489.2 µg/g), which assure a direct correlation of *faba* seeds and good health since Iron is used against anaemia, tuberculosis and growth disorder [27]. Also, Ca and P are essential for proper growth of bones, teeth and ligaments.

Table 4: Proximate Analysis of *faba* fortified biscuits

Parameters	Results
Energy	454.46 kcal
Carbohydrate	75.75 %
Protein	7.67 %
Fat	13.42 %
Ash	0.29%
Moisture	2.87%
Dietary fibre	4.97%

Table 5: Mineral content of the fortified biscuits

Analytes	Concentration (µg/g)	Analytes	Concentration (µg/g)
Cu	5.809	Sb	0.371
Fe	68.70	As	*ND
Ca	478.0	Be	ND
Mg	489.2	Cd	0.009
Mn	5.465	Co	0.254
P	1479.9	Cr	1.169
Zn	40.52	Pb	0.102
Ti	0.236	Li	0.051
Ni	1.490	Mo	0.835

*Not detected

Three parameters, namely taste, colour and crunchiness of the biscuits were evaluated for sensory analysis to understand the consumer acceptability of the product. The average score for taste was observed to be 8, indicating 'like very much' [28]. While for colour, it was calculated to be 7 indicating 'like moderately' and for the crunchiness of the biscuits an average of 9 was evaluated which indicated 'like extremely'. These results depict a positive consumer acceptance for the *faba* fortified biscuits which provide taste along with health.

4. Conclusion

The chemical composition of the underutilized Indian broad bean seeds (*Vicia faba*) shows that it can be a potential source of nutraceuticals and functional foods. On the basis of the results presented here, the legume is a rich source of bioactive compounds and offers opportunities to develop value added products and other food applications to boost health. These results are useful to provide more value addition and usefulness from this legume. The obtained compounds have

potent antioxidant properties along with therapeutic potential and may play an important role in drug development, health supplement. The biscuits produced using *faba* bean flour showed good nutritional composition and are thus healthy to be consumed. Furthermore, the high correlation observed between the various assays employed and phenolic content is a strong indication that these phenolics (total, free and flavonoids) are among the predominant source of antioxidant activity in *faba* beans.

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6. References

1. Steimez KA, Potter JD. Vegetables, fruits and cancer prevention: a review. Journal of the American Dietetic Association 1996; 96:1027–1039.
2. Rodríguez ICM, Segura AC, Fernández AG. Use of high-performance liquid chromatography with diode array detection coupled to electrospray-Qq-time-of-flight mass spectrometry for the direct characterization of the phenolic fraction in organic commercial juices. Journal of Chromatography a 2009; 1216:4736-4744.
3. Liazid A, Palma M, Brigui J, Barroso GC. Investigation on phenolic compounds stability during microwave-assisted extraction. Journal of Chromatography A 2007; 1140:29-34.
4. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha J.P, Pihlaja K, Kujala TS *et al.* Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural Food Chemistry 1999; 47:3954–3962.
5. Vinson JA, Jang J, Dabbagh YA, Serry MM, Cai S. Plant polyphenols exhibit lipoprotein-bound antioxidant activity using an in vitromodel for heart disease. Journal of Agricultural and Food Chemistry 1995; 43:2800–2802.
6. Middleton EJr, Kandaswami C, Theoharides T. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Journal of Pharmacological Reviews 2000; 52:673–751.
7. Lopez V, Akerreta S, Casanova E, Garcia-Mina JM, Cavero RY, Calvo MI. *In vitro* antioxidant and anti-rhizopus activities of lamiaceae herbal extracts. Plant Foods Human Nutrition 2007; 62(4):151-155.
8. Singh AK, Bhatt BP. An Introduction to Faba Bean (*Vicia faba* L.) 2013.
9. Vaughan JG, Geissler CA. The New Oxford Book of Food Plants (revised and updated edition). Oxford University Press, New York, 1997.
10. Köpke U, Nemecek T. Ecological services of faba bean. Field Crops Research 2010; 115:217–233.
11. Agricultural Research Service. United States Department of Agriculture, National Nutrient Database for Standard Reference 2013; Release 26.
12. Rabey JM, Vered Y, Shabtai H, Graff E, Korczyn AD. Improvement of Parkinsonian features correlate with high plasma levodopa values after broad bean (*Vicia faba*) consumption. Journal of Neurology Neurosurgery & Psychiatry 1992; 55:725–27.
13. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. American Journal of Ecology and Viticulture 1977; 28:49- 55.
14. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Journal of Food Chemistry 1999; 64:555-559.
15. Herborne JB. Phytochemical Methods. Chapman and Hall London 1973:110-113.
16. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences 2001; 8:203-208.
17. Adams RP. Identification of essential oils by ion trap mass spectroscopy. Academic Press: New York, 1989.
18. Re R, Pellergrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biology and Medicine 1999; 26:1231-1237.
19. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power the FRAP assay. Analytical Biochemistry 1996; 239:70-76.
20. Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* L. African Journal of Pharmacy and Pharmacology 2009; 3(2):058-062.
21. Official methods of analysis, AOAC, Association of Official Analytical Chemists. Ed 16, Washington, D.C.: AOAC, 1999.
22. Official methods of analysis, AOAC, Association of Official Analytical Chemists. Edition 18: AOAC, 2005.
23. Official methods of analysis, AOAC, Association of Official Analytical Chemists. Edition 17: AOAC, 2003.
24. Milo-Ohr, L. Nutraceuticals and functional foods. Food Technology 2004; 58:65–68.
25. Okwu DE, Okwu OME. Chemical composition of *Spondias mombin* Linn plant parts. Journal of Sustainable Agriculture and the Environment 2004; 6:140-147.
26. Chanda Sumitra, Dudhatra Sandeep, Kaneria Mital. Antioxidative and antibacterial effects of seeds and fruit rind of nutraceutical plants belonging to the Fabaceae family. Food & Function 2010; 1:308-315.
27. Claude B, Paule S. The Manual of Natural Living. Edition 1, Biddles Limited Guildford Surrey, 1979, 98.
28. Lawless HT, Heymann H. Sensory evaluation of food-Principles and practices, 1998.