Int. J. Chem. Sci.: 14(4), 2016, 2658-2672
ISSN 0972-768X
www.sadgurupublications.com

RECOVERY OF POLYPHENOLS FROM THE PODS OF GREEN GRAM AND BLACK GRAM AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITY

S. ARUN, S. DEEPAKRAJ, V. VADIVE* and P. BRINDHA

Centre for Advanced Research in Indian System of Medicine (CARISM), School of Chemical and Biotechnology, SASTRA University, Tirumalaisamudram, THANJAVUR (T.N.) INDIA

ABSTRACT

Black gram (*Vigna mungo* L. Hepper) and green gram (*Vigna radiata* L. R. Wilczek) are annual herbs growing in India and contributes about 76 and 87% of total production, respectively. Black gram and green gram plays an important role in human diet as major source of vegetable proteins. Even though the nutritional value of both the legume grains were proven earlier, the health promoting compounds present in their byproducts (pods) are not yet revealed. Hence, in the present study we have focused on recovery of polyphenol compounds from black gram and green gram pods using different treatments and evaluated their antioxidant potential using *in vitro* models. Different extraction conditions such as solvent (ethanol) extraction with stirring, without stirring, acid hydrolysis and alkali hydrolysis at different timings were optimized. For black gram, acid hydrolysis is found to be more effective in achieving maximum yield of polyphenols (806.75 mg GAE/100 g sample) whereas alkali hydrolysis (843 mg GAE/100 g sample) was effective in the case of green gram. Extracts were analyzed for antioxidant activity, which showed higher DPPH radical scavenging action for black gram than green gram. Because, during purification in column chromatography ethyl acetate fraction of black gram showed higher concentration of total phenols. The individual phenol responsible for higher antioxidant activity of black gram pod was identified as quercetin by LC-MS method. Thus, the black gram pods could be explored as a natural source of antioxidant for the development of nutraceuticals in food industries.

Key words: Black gram, Green gram, Pod extract, Antioxidant, Polyphenols.

INTRODUCTION

Food spoilage can be defined as the disagreeable change in a food's normal state. It can be detected by smell, taste, touch, or sight. Food spoilage is mainly caused due to lipid peroxidation and microbial contamination. Oxidative spoilage is the chief cause of quality loss in fats and fatty portion of foods. When lipid oxidizes, short chain carbon compounds

*Author for correspondence; E-mail: vadivel@carism.sastra.edu

are formed; these compounds have strong odour, flavor and undesirable taste[1]. Lipid peroxidation is a process generated naturally in small amounts in the food matrix, mainly by the effect of several reactive oxygen species (hydroxyl radical, and hydrogen peroxide etc.). These reactive oxygen species readily attack the polyunsaturated fatty acid of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and end products of such lipid peroxidation reactions are especially dangerous for the viability of cells, even tissues.

Synthetic antioxidants include butylated hydoxy toluene (BHT), butylated hydroxyl anisole (BHA) and propyl gallate are added to food to prevent undesirable deterioration. However, synthetic antioxidants cause several health problems including cancer and hence there is a need for alternative natural food antioxidants. Naturally occurring antioxidants include retinols (vitamin A), tocopherols (vitamin E) and ascorbic acid (vitamin C), which play a significant role in the prevention of food spoilage. However, due their negligible content and low antioxidant capacity it is insufficient to preserve the foods in long term storage. Hence the alternative choice could be the polyphenolic compound, which is extracted from the cost-effective source like agro-food byproducts and applied in food preservation. In this context, we have chosen the black gram pod and green gram pod as a natural source of antioxidants and in the present study, different conditions were optimized for the recovery of polyphenols from the above mentioned samples to apply them in food preservation.

The genus *Vigna* is a large leguminous taxon comprising 104 described species distributed in tropical and subtropical regions of Asia, Africa, America and Australia[2]. Several wild *Vigna* species are cultivated as ground cover or harvested as supplementary food[3,4]. Black gram (*Vigna mungo L.,* also known as urad) plant is an erect, sub-erect or trailing, densely hairy, annual herb. The plant grows 30-100 cm with large hairy leaves. The branched taproot produces smooth and rounded nodules. The pods are narrow, cylindrical and 6 cm long. Black gram is mainly grown in South and Southeast Asian countries, including Afganistan, Bangladesh, India, Pakistan, Myanmar and Thailand[5]. Currently cultivable variety of black gram in India is believed to be domesticated from its wild progenitor[6]. Early finds of black gram were from Gujarat and the Northern Peninsula in India, where wild black gram population persists[7]. In India, Andhra Pradesh occupied 555 thousand hectares of area and the largest producer of black gram accounting for 30% (390 thousand tones) of the total production, followed by Maharashtra, 574 thousand hectares with production 205 thousand tones (16%). The area under black gram in Uttar Pradesh was 385 thousand hectares with production 163 thousand tones (13%), whereas in Tamil Nadu, the area and production was 276 thousand hectares and 127 thousand tones (10%),

respectively. Similarly, in Madhya Pradesh, the area under the crop was 420 thousand hectares with the production of 106 thousand tones (8%). These five major states in India together contribute about 76% of total production[8].

Black gram is an important food crop in India as it is the major source of vegetable protein to meet the nutritional requirements of millions of Indian[9]. Black gram seeds are mainly used as a staple food and the dehulled and split seeds (*dhal* in Hindi) are a common dish in South Asia. The black gram dhal is used in South Indian culinary preparations as it is one of the key ingredients in making the idli & dosa batter. It contains about 26% protein, which is almost three times higher than that of cereals[10]. The essential amino acid composition of black gram is tryptophan, lysine, methionine, phenylalanine, threonine, valine, leucine and isoleucine.

During milling of black gram into dhal about 25% is a by-product which comprised of hull, gram, plumulae and aleurone-rich husk fractions. After harvesting, the pods are separated in the field and the husk was removed in the milling operation and hence, both pod and husk are major byproducts from black gram production. Currently, pod and husk were used as a cattle feed or wasted and therefore, it is does not have any commercial value. Black gram husk has been used for heavy metal remediation[11]. Few oxidative and hydrolytic enzymes activities such as peroxidase, polyphenol oxidase, protease, amylase and xylanase were determined in black gram husk[12].

Total phenols and flavonoids were analyzed in black gram.[13] Sixteen isoflavonoids have been isolated from black gram such as 2-hydroxygenistein, 2-hydroxydaidzein, kievitone, dalbergioidin, cyclokievitone, 5-deoxykievitone, 2-hydroxydihydodaidzein, isoferreirin, aureole, glycinol and demethylvestitol. In addition, kievitone hydrate was isolated together with 3 noval natural isoflavonoids which were characterized as 4-O-methyl kievitone, cyclokievitone hydrate and 5-deoxykievitone hydrate[14].

Green gram is widely distributed in Asia including India, Pakistan, Bangladesh and Thailand. In India, green gram is one of the most widely cultivated pulse crops in different states like Rajestan, Punjab, Andhra Pradesh, Orissa, Madhya Pradesh, Maharashtra, Bihar, Gujarat, Karnataka and Tamil Nadu[8]. It is recently introduced in Australia, Africa and USA. It is grown over on an area of 30084 hectares with a production of 10232 tones. Maharashtra is the largest producer of Green gram accounting nearly for 23.05% of the total production followed by Karnataka (17.46%), Andhra Pradesh (17.39%), Bihar (14.69%), Rajasthan (7.50%) and Tamil Nadu (7.25%). These five major states together contribute about 87% of total production and about 84% of total area in the country[8].

Vigna radiata is an annual crop, cultivated mostly in rotation with cereals. Green gram is small annual herb growing to a height of 30-120 cm with slight tendency to twin in upper branches. The central stem is more or less erect while side branches are semi-erect. The leaves are 5-10 cm long, trifoliate with long petioles. Both the stem and leaves are covered with short hairs. Pods are linear, sometimes curved, round and slender with pubescence. This plant is fully self-fertile and self-pollinated and the seeds are small and nearly globular[15]. Green gram (*Vigna radiate*) is a food legume that is very rich in protein and essential amino acids. It is a good source of soluble carbohydrates and contains very high amount of crude fiber. It plays an important role in human diet similar to vegetables. Green gram is the most common source of edible bean sprouts. Green gram is used as a staple in china where they use the whole bean or its sprouts. In USA, they are called as bean sprouts and added to the salads and sandwiches.

Seeds of green gram are medicinally used to treat fever, obesity and other diseases. It is useful in weakness, heat disorders and skin disorders in Ayurvedic system of medicine. The flour of green gram is used as herbal soap in India. Green gram sprouts, popular in Asian cuisine are rich in vitamins and minerals. Recent research shows that green gram starch is a source of slowly digestible carbohydrate which is healthy for diabetic patients. It produces blood glycemic response in humans and modifies glucose and lipid metabolism favorably[16]. The green gram was recorded to be beneficial in the regulation of gastrointestinal upset and to moisturize the skin. High levels of proteins, amino acids, oligosaccharides, and polyphenols in green gram are thought to be the main contributors to the antioxidant, antimicrobial, anti-inflammatory, and antitumor activities of this food and are involved in the regulation of lipid metabolism.[17]

During milling, green gram by-product which is comprised of hull, plumule and husk fractions. After harvesting, the pods are separated in the field and the husk was removed in the milling operation and hence, both pod and husk are major byproducts from green gram production. Green gram husk are rich source of quality protein, which is used as a substrate for production of single cell protein[18]. Alkaline protease enzyme is commercially produced by the cultivation of *Bacillus subtilis* through solid state fermentation using agricultural wastes of low monetary value of green gram husk.[19,20] By biochemical analysis the maximum amount of protein content in green gram husk was observed (7.7 mg). It attain maximum yield of 9.2 mg in 6-10 days of fermentation, but rapidly declined to 5.4 mg of protein. The nitrogen enriched source of peptone showed high yield of 8.4 mg in 6-10 days of fermentation. The high amount of carbohydrate was found in fructose.[18]

The leaves of green gram contain the glycosides of both quercetin, kaemferol and tocotrienols[21], different types of flavonoids (195 mg catechin equiv/100 g DW), including

flavones, flavonols, flavanones, flavanonols, isoflavonoids, flavanols, and anthocyanidins. In green gram seeds, daidzein, genistein, and their 7-O-glucodides, daidzin and genistin, were reported using HPLC-immuno analysis. Quercetin-3-O-glucoside (0.49 mg/100 g DW) was detected in green gram seeds and sprouts[22]. Further, two major phenolic compounds such as vitexin and isovitexin were detected in green gram[23].

EXPERIMENTAL

Collection of samples

Black gram and greed gram pods were collected from the agriculture field in nearby areas of Kumbakonam, Thanjavur District. Pods were shade dried and grinded into fine powder (particle size 1 mm) in a Lab mill at Pharmarcy unit of CARISM, SASTRA University.

Solvent extraction

Sample (10 g) was weighed in conical flask and 50 ml of 70% ethanol was added in a ratio of 1:5 (w/v) and kept with and without stirring conditions for six hours. For stirring condition, we have used magnetic stirrer (600 rpm). The extract was collected at different timings (every 1 h) by filtration. The filtrate was further used for the analysis of total phenolic concentration and antioxidant activity.

Acid hydrolysis

For acid hydrolysis, 10 g of sample was taken in a conical flask and 100 ml of 2% HCl was added, kept without stirring at room temperature for 6 h. The acid hydrolysates were collected at different timings (every 1 h) by filtration and used for analysis of total phenolic concentration and antioxidant activity.

Alkali hydrolysis

For alkali hydrolysis, 10 g of sample was treated with 100 ml of 2% NaOH at room temperature without stirring for 6 h. At every 1 h, the alkali hydrolysates were collected by filtration and analyzed for total phenolic concentration and antioxidant activity.

Analysis of total phenols

The total phenolic concentration of solvent extract, acid and alkali hydrolysates of black gram and green gram were estimated.[24] Extracts (10 μL) were taken in a 96 micro plate well and 25 uL of Folin's Reagent followed by 230 uL of 4.4% sodium carbonate were

added. Plate was kept in a dark for 30 min and read @ 750 nm in plate reader (Make: Biotek, Model: Epoch) and data were collected. Ethanol, folin's reagent and sodium carbonate are added in a well and used as a control. Gallic acid was used as reference compound to prepare the standard curve (R2 = 0.994, Y = 0.002x + 0.182). Total phenol concentration (TPC) was calculated using the formula (Absorbance – 0.182/0.002 x Dilution factor) and expressed as mg GAE/L extract. Total phenol yield (TPY) was calculated using the formula (TPC x Volume of extract in Liter/Weight of sample in g x 100) and expressed as mg GAE/100 g sample.

Antioxidant activity

The antioxidant activity of extracts was analyzed using DPPH free radical scavenging assay.[25] Extracts (10 uL) were taken in a microplate and 200 uL of DPPH solution (5 mg/100 mL methanol) and incubated for 30 min in a dark. Then the absorbance was measured @ 515 nm in a plate reader (Make: Biotek, Model: Epoch) and the data was collected. Ethanol and DPPH solutions are added in a well and used as a blank and antioxidant activity calculated using this following formula ((blank OD-sample OD)/blank OD)*100.

Lipid peroxidation inhibition assay

This assay is normally conducted to determine the ability of extract to inhibit peroxidation in food lipid samples.[26] The sesame seed is one of the common sources of food lipids in India. Dried seeds of black sesame (*Sesame indicum* L.) were collected and grinded well. The powdered sample was weighed (20 g) and taken in conical flask and mixed with 40 mL of hexane. The contents were stirred for 30 minutes to extract the lipids and then filtered through filter paper. The polyphenol extract (2.5 mg/2.5 mL) was added and boiled for 1 h. In control, same conditions were followed without adding extract, whereas the blank was not heated. To measure peroxidation value, 30 ml of acetic acid/chloroform (3:2 ratio) and 0.5 ml of saturated potassium iodide were added to the contents. After 2 minutes, 30 ml of distilled water was added followed by 0.5 ml of starch. Finally the solution was titrated against 0.01 M sodium thiosulfate. Based on the volume of sodium thiosulfate consumed, peroxide values was calculated using the formula (PV = Abs x 0.01/Amount of sample). Further, percentage of inhibition of lipid peroxidation was calculated using the formula LPI% = (PV control – PV extract)/PV control x 100.

Purification and LC-MS analysis

The samples (50 g each) were extracted with 500 mL of 70% ethanol and kept for 5 h at room temperature and filtered using filter paper. Later on, the extract was evaporated

and dry extracts were obtained. Slurry was prepared with dried extracts of black gram and green gram using silica gel. Column with 60 cm length and 2.5 cm diameter was packed with silica gel and vacuum pump was applied to speed-up the elution process. Firstly, hexane was used to form a silica gel bed in ¾ of column length and washed with hexane (100 mL) for two times. Then 2 g of slurry was loaded above the silica bed and plugged with cotton. The extract was eluted with hexane, chloroform, ethyl acetate, methanol, ethanol and water one by one. The eluted compounds from above mentioned solvents were collected separately in conical flasks and analyzed for total phenolic concentration. Based on the higher phenolic concentration, black gram pod extract (Ethy acetate fraction) was selected and re-suspended in ethanol and submitted for LC-MS analysis to identify the individual phenolic compounds.

UHPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with Column: Shim-pack XR-ODS III (100 x 2 mm, 2.2 µm particle size), Column temp. 40ºC. The mobile phase consisted of (A) 0.1% formic acid in water and (B) Acetonitrile. Both mobile phases were filtered through a cellulose nitrate filter, diameter 47 mm, pore size 0.45 µm (Sartorius, Goettingen, Germany). After the gradient separation, the column was re-equilibrated for 5 min using the initial solvent composition. The flow rate was set to 1 mL/min. Samples were kept in amber vials at 4°C in the autosampler, and the injection volume was 5 µL. The separation was performed at 25.0 ± 0.1°C. LC-MS/MS System (Make: Shimadzu Corporation, Kyoto, Japan, Model: LCMS 8040, Triple Quadrupole) Ionization: ESI (Negative mode), Ion spray voltage: + 4.5 kV/ − 3.5 kV, MRM: 427 MRM transitions (2 MRMs/compound) Dwell time 5 msec./Pause time 1 msec. Ambient CDL Temperature: 250ºC, Block Temperature: 400ºC, Detector voltage: 1.3 kv, Nebulizer Gas flow: 1.5 L/min, Drying gas: 10 L/min Detection. The resulting chromatogram and mass specra details are given below.

RESULTS AND DISCUSSION

Total phenolic content

Polyphenols are naturally occurring compound found largely in the plant materials. Polyphenols are secondary metabolite of plants, which involved in defense against UV radiation and contribute to oxidative stability, color, odor, bitterness. The results of recovery of polyphenols from the black gram pod by using four different conditions at every one hour time intervals was shown in Table 1. The data reveals that solvent extraction without agitation shows maximum yield of total phenols at 5th hour (90.50 mg GAE/100 g of sample) whereas in solvent without agitation is maximum at 6th hour (25.00 mg GAE/100 g of sample). Acid hydrolysis and alkali hydrolysis have maximum yield at 5th and 6th hour with

806.75 and 125.00 mg GAE/100 g of sample, respectively. Among the four different conditions acid hydrolysis exhibited highest recovery of polyphenols (806.75 mg GAE/100 g of sample).

Table 1: Recovery of phenolic compounds from black gram pods during different conditions at different time intervals

Extraction time (h)	Total phenolic content (mg GAE/100 g sample)			
	Solvent without agitation	Solvent with agitation	Acid hydrolysis	Alkali hydrolysis
1	25.25 ± 4.60	1.25 ± 1.06	681.00 ± 92.63	12.25 ± 1.06
2	41.75 ± 5.30	10.50 ± 4.24	668.50 ± 26.87	14.50 ± 4.24
3	71.00 ± 9.90	20.75 ± 0.35	662.00 ± 234.05	20.75 ± 0.35
4	66.25 ± 3.18	8.25 ± 10.25	708.00 ± 53.74	82.25 ± 10.25
5	90.50 ± 7.78	15.25 ± 3.89	806.75 ± 60.46	115.25 ± 3.89
6	63.75 ± 8.84	25.00 ± 9.90	753.75 ± 123.39	125.00 ± 9.90

Polyphenol is a kind of phytochemical that protects cells and body system against the damage caused by the free radicals. The results of recovery of polyphenols from green gram pods with four different conditions were given in Table 2.

Table 2: Recovery of phenolic compounds from green gram pods during different conditions at different time intervals

Extraction time (h)	Total phenolic content (mg GAE/100 g sample)			
	Solvent without agitation	Solvent with agitation	Acid hydrolysis	Alkali hydrolysis
1	0	0	456.00 ± 144.25	557.75 ± 34.29
2	0	0	514.00 ± 85.56	753.75 ± 24.40
3	10.50 ± 4.95	4.75 ± 1.06	481.25± 9.55	771.25± 26.52
4	15.50 ± 6.36	0	570.75± 49.55	484.00± 72.83
5	18.75 ± 3.89	0.50 ± 21.92	489.00± 120.92	843.00± 106.07
6	39.00 ± 10.60	28.25 ± 17.45	521.75± 92.98	722.25± 73.19

It reveals that at 6th hour solvent extraction without agitation shows the maximum yield (39.00 mg GAE/100 g of sample) where as with agitation, maximum yield was 28.25 mg GAE/100 g of sample. In acid hydrolysis polyphenols yield was maximum of 570.75 mg GAE/100 g of sample at a 4th hour where as in alkali hydrolysis maximum yield was at 5th hour (843.00 mg GAE/100 g of sample). When compared to other treatments, alkali hydrolysis recorded a maximum yield of polyphenols at 5th hour (843.00 mg GAE/100 g of sample) in green gram pod.

Antioxidant activity

Antioxidant is a molecule that could be used to terminate the chain reaction which produce free radicals and cause damage to the cellular system. Antioxidant activity of black gram pods with four different conditions was shown in the Fig. 1. The result revealed that the solvent without stirring shows the 50% inhibition at 4th hr whereas solvent extraction with stirring exhibited the maximum inhibition of 90% from 1 to 5th hr. Both acid and alkali hydrolysis showed lower level of antioxidant effect of 40% and 20%, respectively, which may be due to denaturation of phenols under acid and alkaline conditions of the medium. Since there is a maximum activity observed in solvent extraction with stirring, it could be used as antioxidant in food industrial applications to prevent the formation of free radicals and subsequent oxidative spoilage.

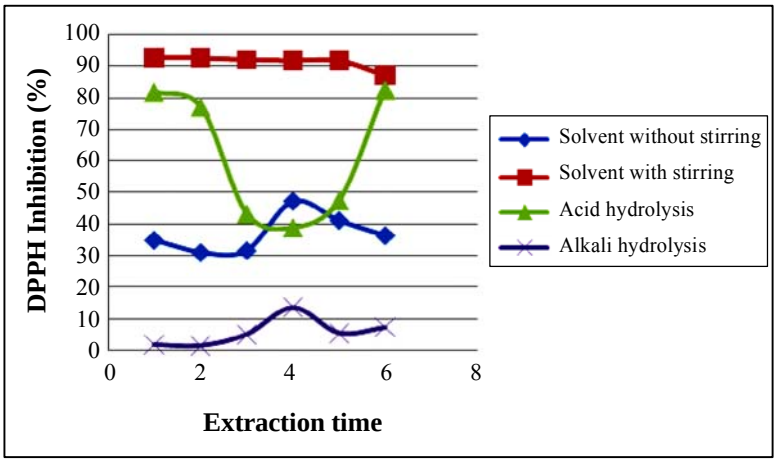


Fig. 1: Effect of different extraction conditions on the antioxidant activity of black gram pods

The antioxidant inhibits the oxidation reaction that happens in any food sample and results in food spoilage. The antioxidant activity of green gram pods with four different conditions was given in Fig. 2. In general, the extract of green gram pod obtained from four

different treatments exhibited poor antioxidant activity. This reveals that the solvent extraction without agitation recorded the maximum activity of 5% at 4th hr whereas in solvent with agitation, acid hydrolysis and alkali hydrolysis shows the maximum antioxidant activity of 25% inhibition at 3rd hr. It means that the polyphenols obtained from green gram pod by employing different treatments resulted in recovery of high amount of polyphenols, but their antioxidant activity was poor.

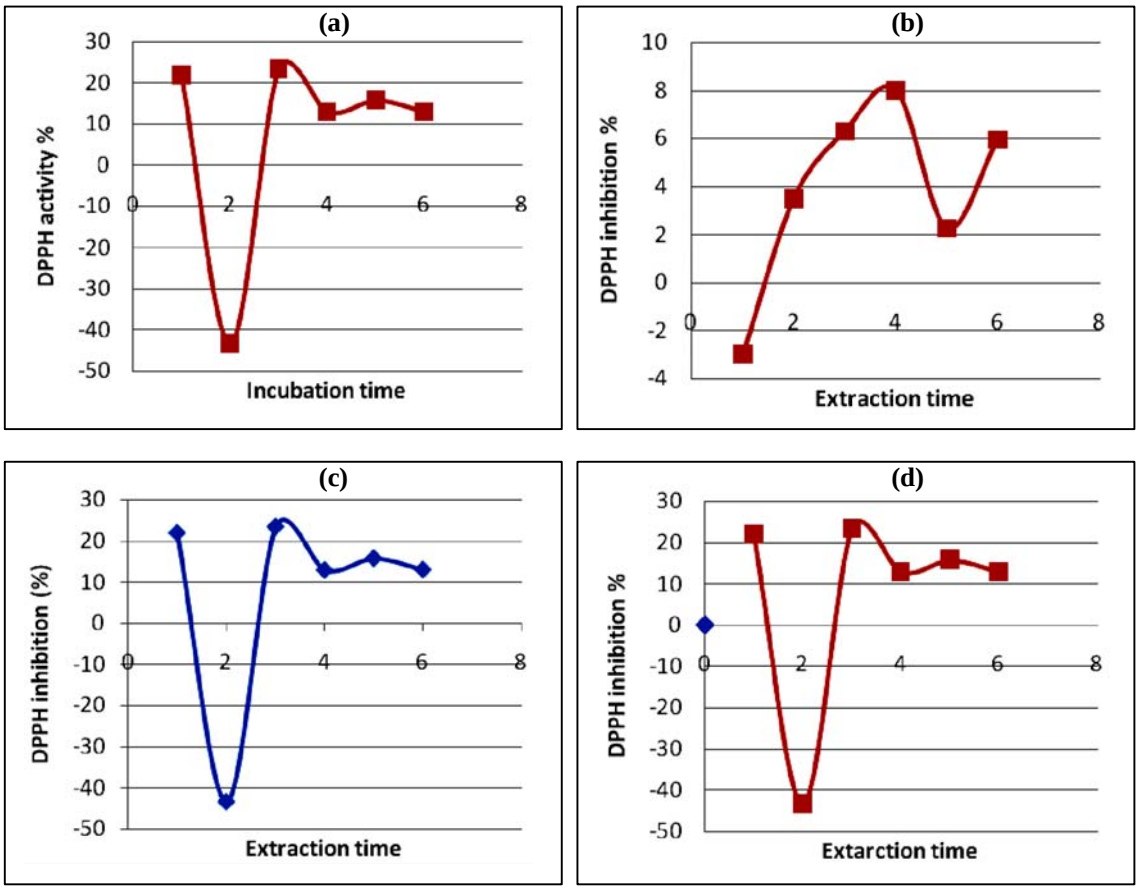


Fig. 2: Effect of different extraction conditions on the antioxidant activity of green gram pods (A: solvent with agitation, B: solvent without agitation, C: acid hydrolysis, D: alkali hydrolysis)

Lipid peroxidation inhibition

Peroxide value will increase with the increasing rancidity of the fat or oil. In the above mentioned Table 3, peroxide value of sesame seed oil treated with black gram pod

extract was shown. The result reveals that peroxide value of un-heated sesame seed oil was very low (0.00001), which indicates there is very low level of lipid peroxidation taken place but, when heating the oil for 1 h at 100 C, high level of lipid peroxidation was occurred with the peroxide value of 0.00010. Black gram pod extracts treated sesame seed oil has showed peroxide value of 0.00010, which indicates that lipid peroxide inhibition value was increased with 60%. It depicts that polyphenol extract of black gram pod strongly controlled the lipid oxidation in sesame oil sample.

Table 3: Peroxide value of sesame seed oil and lipid peroxidation inhibition capacity of black gram pod extract

S. No.	Sample	Peroxide value	Lipid peroxide inhibition (%)
1	Control (without extract)	0.00025	25
2	Black gram extract treated	0.00010	60
3	Blank (Un-heated)	0.00001	--

Peroxide value is commonly used to determine the rancidity of the fat or oil when subject to oxidation. In the above mentioned Table 4, peroxide value of sesame seed oil treated with green gram pod extract was shown. The result reveals that peroxide value of un-heated sesame seed oil was 0.00001, which indicates there is very low level of lipid peroxidation taken place. But, when heating the oil for 1 h at 100 C, high level of lipid peroxidation was occurred with the peroxide value of 0.00025. Green gram pod extract treated sesame seed oil has showed peroxide value of 0.00020, which indicates that peroxide value was decreased by 5%. It reveals that polyphenol extract of green gram pod mildly controlled the lipid oxidation in sesame oil sample.

Table 4: Peroxide value of sesame seed oil and lipid peroxidation inhibition capacity of green gram pod extract

S. No.	Sample	Peroxide value	Lipid peroxide inhibition (%)
1	Control (without extract)	0.00025	25
2	Green gram extract treated	0.00020	20
3	Blank (Un-heated)	0.00001	--

Purification and LC-MS analysis

TPC for column fractions of black gram pod were shown in Fig. 3. The result reveals that total phenolic content of black gram pod extracts was purified in a column with solvents like hexane, chloroform, ethyl acetate, methanol, ethanol and water. From that chloroform, ethyl acetate and methanol extracts were given positive result for total phenols. Among the collected fractions, higher total phenolic content was registered in ethyl acetate fraction, which was used for the LC-MS analysis.

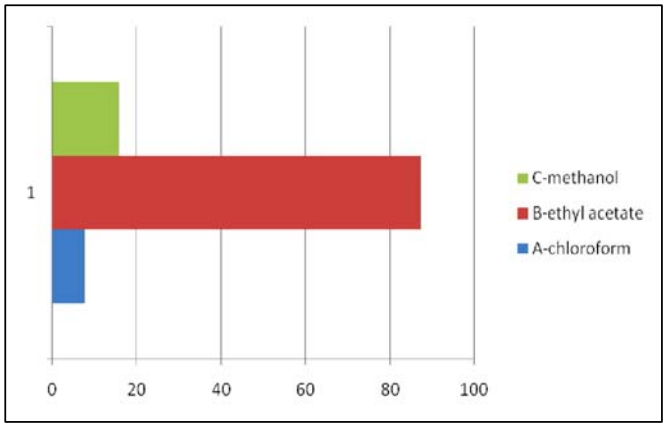
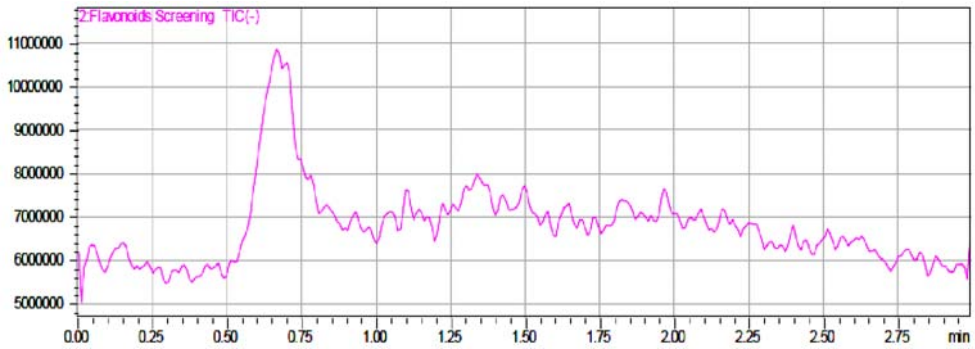


Fig. 3: Total phenolic content of column fractions of black gram pods

In LC-MS analysis, we have detected 8 different peaks, among which the peak with mass 301 was identified as quercetin based on mass data bank reference. Other peaks are inosine (mass 268), 11-Nor-8-carboxy-Delta-9-THC (mass 344) and betamethasone (mass 392).

Precursor Ion Q3 Scan – Negative ionization



Cont…

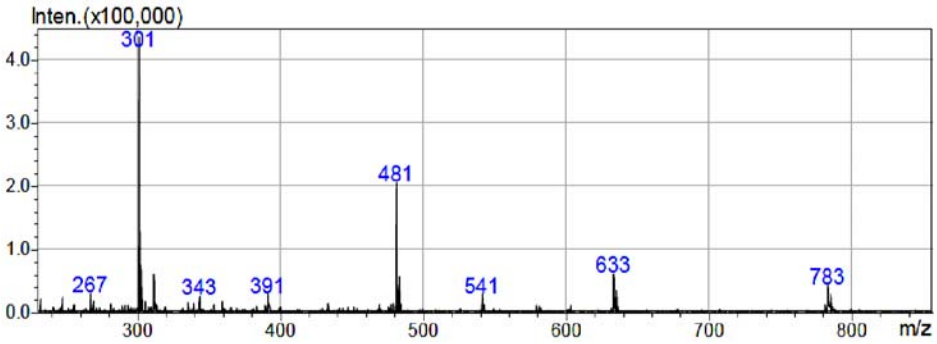


Fig. 4: LC-MS chromatogram and mass spectra results of black gram pod extract fraction

CONCLUSION

Optimization of recovery of polyphenolic compounds from both black gram and green gram pods was investigated in the present study. The results revealed that black gram pods yielded higher polyphenol content in acid hydrolysis whereas green gram pod in alkali hydrolysis. In both the samples, maximum antioxidant activity was shown by polyphenols obtained from solvent extraction with stirring method through DPPH assay. Black gram pod extract recorded higher level of antioxidant activity when compared to green gram pod. Even though, higher levels of polyphenol contents were noted in the acid and alkaline hydrolysates of black gram and green gram pods, their antioxidant activity was poor. This might be due to the denaturation of phenolic compounds under acid/alkaline pH of the medium. In lipid peroxidation inhibition, black gram pod extract was found to be more effective in controlling the lipid peroxidation in sesame seed oil when compared to green gram pod. So we conclude that, black gram pods could be used in an efficient way as natural and cost-effective food preservative to prevent the food spoilage. Further, toxicity of the extracts should be evaluated using suitable *in vivo* models to advocate them as food preservatives in industries.

REFERENCES

1. L. Gram, L. Ravn and M. Givskov, Int. J. Food Microbiol., **78**, 79-97 (2002).
2. E. M. Haston, G. P. Lewis and J. A. Hawkins, Am. J. Bot., **92**, 1359-1371 (2005).
3. R. Marechal, J. M. Mascherpa and F. Stainier, Boisiera., **28**, 1-273 (1978).
4. N. Tomooka, A. Kaga, D. A. Vaughan, A. K. Sharma and S. Sharma, Science Publishers, New Jersy (2006) pp. 87-126.

5. A. Kaewwongwal, A. Kongjaimun and P. Srinivas, Breed. Sci., **65**, 127-137 (2015).

6. K. P. S. Chandal, B. S. Joshi, R. K. Arora and K. C. Pant, Indian Farming., **9**, 19-22 (1978).

7. D. Q. Fuller and E. L. Harvey, Env. Archaeol., **11**, 219-246 (2006).

8. Department of Agriculture, Government of India, Post Harvest Profile of Black Gram and Green Gram, www.agmarknet.nic.in (2006).

9. S. Singh and S. B. Agarwal, Acta Physiologiae Plantarum., **33**, 2399-2411 (2011).

10. S. Gandhi, E. A. Sri Devi and L. Mullainathan, Int. J. Res. Bot., **2**, 5-8 (2012).

11. A. Saeed, M. Iqbal and W. M. Akhtar, J. Haz. Mat., **117**, 63-73 (2005).

12. C. M. Aijila and U. J. S. Prasada Rao, J. Mol. Cat., **60**, 36-44 (2009).

13. H. S. Gujral, M. Angurala, P. Sharma and J. Singh, Int. J. Food Prop., **14**, 1366-1374 (2011).

14. A. Suberri, A. Melanie, J. O. N. Eill and M. F. Roberts, Zeitschrift Fur naturforschung C., **39**, 888-893 (2014).

15. E. S. Oplinger, L. L. Hardman, A. R. Kaminski, S. M. Combs and J. D. Doll, www.hort. purdue.edu/newcrop/afcm/mung bean.html. (1990).

16. R. Randhir, Y. T. Lin and K. Shetty, Process Biochem., **39**, 637-646 (2004).

17. D. Tang, Y. Dong, H. Ren and H. Congfen, Chem Central J., **8**, 4 (2014).

18. S. Anbuselvi, S. Mahalanobis and M. Jha, Int. J. Pharm. Sci., **28**, 188-190 (2014).

19. H. Meruvu, M. Vangalapati and M. R. Renitta, Int. J. Adv. Pharm. Sci., **2**, 2-3 (2011).

20. R. S. Prakasham, C. H. Subba Rao and P. N. Sarma, Bioresource Technol., **97**, 1449-1454 (2006).

21. A. G. Krishnaa, J. V. Prabhakara and K. Aitzetmüllerb, JAOCS, **74**, 1603-1606 (1997).

22. X. Guo, T. Li, K. K. Tang and R. H. Liu, J. Agric. Food Chem., **60**, 11050-11055 (2012).

23. X. Peng, Z. Zheng, K. W. Cheng, F. Shan, G. X. Ren, F. Chen and M. Wang, Food Chem., **106**, 475-481 (2007).

24. V. L. Singleton, R. Orthofer and R. M. Lamuela-Raventos, Method. Enzymol., **299**, 152-178 (1998).

25. C. Sanchez-Moreno, J. A. Larrauri and F. A. Saura-Calixto, J. Sci. Food Agric., **76**, 270-276 (1998).

26. G. C. Yen and C. L. Hsieh, J. Agric. Food Chem., **46**, 3952-3957 (1998).

Accepted : 04.10.2016