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Fatty acid composition and bioactivity of *Sesbania sesban* seed oil

S Singh[a,c], B N Singh[b, c] & M Srivastava[a,c,*,†]

[a]Phytochemistry Division, [b]Pharmacology Division, CSIR- National Botanical Research Institute, Lucknow 226 001, Uttar Pradesh, India

[c]Academy of Scientific and Innovative Research (AcSIR), Ghaziabad 201 002, Uttar Pradesh, India

E-mail: [†]m.srivastava@nbri.res.in

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Fatty acid composition and bioactivity of *Sesbania sesban* seed oils were analyzed. Seeds were milled by a domestic mixer at medium speed to obtain mixture containing germ and endosperm with seed coat. Germ (42.48%), Crude endosperm (26.22%) and seed coat (11.33%) were separated and whole seed along with seed parts subjected to extraction process by hot and cold method with petroleum ether. Saturated and unsaturated fatty acids in seed oils were determined and analyzed using Gas-liquid chromatography in which palmitic acid was found in endosperm oil (50.51%) and seed coat oil (30.65%) by cold extraction. Oleic acid was found in seed oil (48.22%) and in germ oil (46.87%) extracted by cold but lower in oils obtained by hot method. Linoleic acid content was found higher in oils extracted by hot extraction. Antioxidant activity was also done of oil from seed and their parts by different methods in which maximum inhibition shown by seed oil 55.45% at 100 µg/mL and minimum in endosperm oil 0.37% at all concentration from hot and cold method, which was significantly different at $p<0.05$ in the same column.The antimicrobial activity of seed oil was determined by disc diffusion method and the seed coat and whole seed extracted by cold extraction showed significant anti-bacterial activity. Studies reveal the potentiality of seed oil as an alternative source as/in edibles and also prospects the analysed oils of seed parts with useful bioactive markers for pharmaceuticals, nutraceuticals and cosmetics.

Keywords: Bioactivity, Extraction, GLC, Oil, Seed, *Sesbania*

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Medicinal plants have been used therapeutically from time immemorial as an important aspect of various traditional medicine system. *Sesbania sesban Linn*., commonly known as 'Egyptian *Sesban*' Jayanti, Jait belonging to *Fabaceae* family is traditionally important plant with various biological activities. Six species of genus *Sesbania* is commonly found to be grown in tropical region of India. Traditionally *S. sesban* seed powder is used as an antifertility agent which changed the ovarian normal function, uterine structure and prevents implantation, thus control the fertility of female[1,2]. *Sesbania* leaves, flowers, pods and seeds are sources of animal feed and also for human food[3]. The seed of *S. sesban* contain various phytochemicals viz., saponin (stigmasta-galactopyranoside), oleanolic acid, galactomannan, glucuronic acid, flavanoid glycoside, amino acids, fatty acids, vitamin-E, vitamin-A, vitamin-C and known for various activities i.e., molluscicidal, spermicidal, bactericidal, cardiac depressant and hypoglycemic activity[4]. Plant seeds are useful sources of nutritional, industrial and pharmaceutical significance[5] and belong to minor seed oil. It is not compulsory that oil obtained from plant sources are useful for all purpose because oil from different sources differs in their composition. There are two types of fatty acids that exist, saturated and unsaturated but in recent years people in practice have focused on the use of unsaturated (18:2 and 18:3) and monounsaturated (C18:1) in diet. Monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) especially omega-3 fatty acid alpha-linolenic acid and omega-6 fatty acids such as linoleic acid and arachidonic acids have widespread beneficial effects on health and prevention in many chronic disease, however, linolenic and linoleic acid present in dietary supplements are important for growth of infants and children[6-9]. The high percentage of linoleic acid with even high oleic content would increase clinical application of oil[10]. The stability of the oil is an important parameter for human consumption and detected by oleic/linoleic ratio[11]. The oils which are

*Corresponding author

having higher percentage of oleic acid and lower linoleic acid content would be more preferable for better stability[12]. *Sesbania* seed belongs to Leguminosae, dicotyledonous with its outer part called seed coat, middle endosperm and innermost yellow coloured germ. The germ is a good source of oil, protein whereas endosperm also a source of oil, protein and other secondary metabolites. The oil obtained from hot and cold extractions were analyzed by Gas liquid chromatography. The study revealed the alternative source of monounsaturated & polyunsaturated fatty acids from underutilized leguminous plants. Fatty acid chemical compositional analysis of part specific *S. sesban* seed oil and bioactivity analysis was not earlier reported, thus, the studies describe estimation of fatty acids of oil obtained from different parts of *S. sesban* seed along with antioxidant and antimicrobial activity.

Methodology

Materials

Seeds were purchased from the Lucknow market, India. Specimen samples were prepared, authenticated and deposited in CSIR-NBRI, herbarium, Lucknow (LWG No- 107999).

Standards were purchased from Sigma Aldrich. All the solvents and chemicals (AR grade) were obtained from Merck, Pvt Ltd.

Five strains of bacteria as test organism viz., *Pseudomonas aeruginosa* MMTC 737, *Streptococcus mutans* MTCC 233, *E.coli* MTCC 724, *Streptococcus aureus* MTCC 97 and *Candida albicans* ATCC14054 were procured from Institute Microbial Technology, Chandigarh, India. Inoculums were prepared by transferring the test organism into sterile tubes containing 10 mL of nutrient broth by an aseptic loop and then incubated for 48 h at 36-37°C. The concentrations of inoculums were determined by measuring transmittance (20%) at 600 nm.

Seperation

Seeds of *Sesbania sesban* were separated by dry method with the help of domestic grinder at medium speed to obtain mixture of germ and endosperm. The germ was separated by several times sieving and grinding from mixture with the help of sieve no. 40 mesh size. Endosperm with seed coat was soaked with ethanol: distilled water (1:1), kept for 2 h and separated by grinding and sieving with sieve no. 40 mesh size.

Extraction of oil

Separated parts of seeds (20 g each) were extracted from cold and hot method (soxhlet apparatus)[13] with 250 mL petroleum ether 40-60°C. The oil obtained from extraction was dried over anhydrous sodium sulfate and the solvent removed by vacuum at 40°C to obtain fatty oil.

Isolation of free fatty acids

The oil (150 mg) was refluxed with 0.5 N methanolic sodium hydroxide solution (15 mL) for half an hour at 40°C. The reflux mixture was then transferred to separating funnel in which distilled water (5 mL), petroleum ether (10 mL) and concentrated (1 mL) HCl was added three times, separated petroleum ether layers and then washed with water two times to obtain free fatty acids.

GLC-FID analysis

The free fatty acids were refluxed by adding 10 mL methanol and 2 drops of H_2SO_4 for 3 h. The petroleum ether extract was combined and washed with water two times, dried on anhydrous sodium sulfate[14]. Fatty acid composition of the oils of all separated parts of seed were determined through Gas-liquid chromatography by Hewlett Packard, HP 6890 series, GC system fitted with flame ionization detector fused silica capillary column (BP 21: 30 m length, 0.30 mm i.d., 0.50 µm film thickness), oven temperature 90-240°C, total time 65 min to run . The samples were identified by standard methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linoleate, methyl arachidonate, methyl eicosanoids from Sigma Aldrich. Peak areas were measured and the GC data reported are given in area percentage.

Antioxidant activity

Antioxidant activity of seed oil was determined by using DPPH radical with a method described[15]. About 1 mL of 0.1 mM DPPH was prepared in methanol and 1.0 mL of extract in methanol containing 0.02-0.1 mg of the extract. The reaction mixture was vortexed thoroughly and left in dark for 30 min and the absorbance was taken at 517 nm of wavelength using Ascorbic acid as reference standard. Different extracts were able to reduce stable free radical DPPH to the yellow-colored 1, 1-diphenyl-2-picrylhydrazyl. The radical scavenging activity was then calculated as per the following equation.

% of radical scavenging activity

=Abs (control)-abs(sample)/abs(control)×100

Antimicrobial activity

The antimicrobial activity of oils extracted by hot and cold methods from different parts of the seed viz., seed coat, germ, endosperm along with whole seed were determined at a concentration of 25 mg/mL against a gram-positive and gram-negative strains by disc diffusion method as compared[16] to standard antibiotic, *Streptomycin* (10 µg/mL). Agar plates were inoculated with 0.1 mL of standardized inoculums and spread properly. Various test samples of *Sesbania sesban* seed oil (25 mg/disc) and *Streptomycin* (10 µg/disc) were placed on agar surface. Plates were allowed to stand at room temperature for 30 min. Inoculated petri-dishes were incubated at 37ºC, overnight and the antibacterial activity was evaluated by recording the zone of inhibition in millimeter (mm) against the tested strain.

Statistical analysis

All analyses were carried out in triplicate**.** Data are expressed as means ± standard deviation. Tukey's test and one way ANOVA was used to differentiate the means of different test samples at $p<0.05$.

Results and Discussion

Oil and Fatty acids

A fixed oil extracted from separated parts of seed, seed coat, germ and endosperm varied in color viz., light yellow, greenish, dark yellow and creamish yellow. The maximum extractable oil was obtained from germ (9.54%) and minimum in the seed coat (0.655%) which possessed a characteristic odor. The percentage yield of seed oils are presented in Table 1. Seed is palmitic, linoleic and oleic acid rich, although myristic acid also found in appreciable amount (7.29%). Seed coat is palmitic, linoleic and arachidic acid rich. The oil analysis of germ, endosperm, seed coat including whole seed reveals that each has different fatty acids composition, thus oil value is different from each other signifying its varied applications in commercial seeds. Oil of *S. sesban* seed is light yellow, but it was unstable and changed to light greenish in the course of time at room temperature in 6 months. Fatty acid compositional analysis reveals that saturated fatty acids in whole seed, seed coat, germ and endosperm oils by cold extraction varies as 11.53%, 37.81%, 12.03%, 50.51% respectively while in the same oils obtained with hot extraction, the saturated fatty acids varies differentially as 21.65%, 40.25%, 20.35% and 41.38% respectively. Seed is estimated to have 22% palmitic acid, but its seed coat and endosperm have enough palmitic acid upto 40%, comparable to palm oil, which reveals its potentiality. MUFA (monounsaturated fatty acid) was shown maximum 48.87% from cold extraction in germ while minimum in seed coat 1.076% from hot extraction. Oleic acid (omega-9) is the only one monounsaturated fatty acid present in animal and vegetable oils. The germ of seed species have enough oleic acid content ie. 46.87% and 48.22% as presented in Table 2 as

Table 1 — Determination of oil contents in *Sesbania sesban* seed and their parts by different extraction methods

Material	% Yield	% Oil (hot)	% Oil (cold)	Colour
Seed coat	11.33	0.627	0.655	Greenish (light yellow)
Germ	44.45	9.544	7.918	Dark yellow
Endosperm	26.22	0.52	0.312	Creamish light yellow
Seed	-	4.86	2.73	Light yellow

Table 2 — Fatty acid composition of oil from *Sesbania sesban* seed and their parts by different extraction methods

Composition	Seed coat (hot)	Seed coat (cold)	Germ (hot)	Germ (cold)	Endosperm (hot)	Endosperm (cold)	Seed (hot)	Seed (cold)
Myristic acid	nil	1.025	nil	nil	0.961	nil	7.29	nil
Palmitic acid	40.25	36.56	20.35	nil	40.42	50.51	22.07	11.58
Linoleic acid	21.13	20.14	-	nil	11.11	24.84	18.86	5.77
Linolenic acid	0.720	nil	22.13	9.12	21.07	nil	nil	18.57
Oleic acid	1.08	1.29	5.97	46.87	2.41	nil	18.86	48.22
Arachidic acid	2.80	nil	nil	2.73	1.00	nil	1.52	3.31
Behenic acid	0.87	nil	1.22	nil	3.16	nil	1.29	nil
Unknown	2.10	nil	2.35	nil	1.67	nil	0.823	1.17
Unknown	1.23	nil	0.74	nil	1.83	nil	0.71	1.05
Unknown	nil	nil	0.82	nil	1.60	nil	0.74	nil
Unknown	nil	nil	nil	nil	1.43	nil	nil	nil
Saturated fatty acids	40.25	37.59	20.35	nil	41.38	50.11	29.37	11.58
Monounsaturated fatty acid	4.745	1.29	7.193	49.60	3.41	nil	20.38	51.53
Polyunsaturated fatty acid	21.85	20.14	22.13	9.123	32.18	24.84	18.86	24.34

compared to commercially available olive oil with reported oleic acid 55.80%[17]. Polyunsaturated fatty acids (PUFA) present in oils from whole seed, seed coat, germ and endosperm by hot extraction varies as 18.68%, 21.85%, 22.13% and 31.18%, respectively while in oils of same materials by cold extraction PUFA varies as 24.33%, 31.10%, 16.71% and 39.42%, respectively. Palmitic acid is the most common saturated fatty acid found in animals, planta and microorganism[18] and consumption of palmitic acid increases the risk of cardiovascular diseases[19]. Palmitic acid detected in seed oil by hot and cold extraction method varies as 22.07% and 11.58%, respectively, lower than the palmitic acid content 26.19% of *Brachystegia eurycoma* seeds oil[20]. Linoleic is the essential fatty acids present in seed oils extracted by hot and cold extraction that varies as 18.86% and 5.77%, respectively. Linolenic acid was absent in seed oil obtained from hot extraction but was detected in cold extracted oils i.e 18.57%. This signifies the better stability of seed oil extracted by cold than hot method. Linolenic acid was also present in germ oil obtained from hot and cold extraction 22.13% and 9.23%, respectively. Linoleic acid is an omega-6 fatty acid which acts as metabolic precursor for the group of biologically essential lipids called eicosanoids. Eicosanoids include leucotrienes, lipoxins, prostaglandins and thromboxanes which play significant role in blood clotting, immunity and inflammations[21]. The oil obtained from cold method was found to be most stable among all with maximum stability index , Oleic/Linoleic acid ratio 9.6. Hence, seed oil obtained from cold method may be stored for a longer period. The oil possessed oleic acid more than 36% is considered for oil industries, cosmetics, pharmaceuticals and other household products.

Antioxidant activity

Antioxidants increased the attention of nutritionists and medicinal researchers for their potential effects in the prevention of chronic and degenerative disease[22] and on interaction with DPPH either transfer an electron to DPPH, thus neutralizing its free radical character[23]. Results of antioxidant activity of oil obtained from separated parts of seed was shown in Table 3 and ascorbic acid used as positive control. Maximum inhibition 55.45% at 100 µg/mL was shown by seed oil from hot method and minimum in endosperm oil from hot and cold method, which was significantly different at $p<0.05$ in the same column. Germ oil from hot extraction was shown to have maximum inhibition 41.9% at 100 µg/mL, significantly different in the same column at $p<0.05$. Minimum inhibition found in endosperm oil from both methods, not significantly different at $p<0.05$.

Antimicrobial activity

The antibacterial activity revealed that the fixed oil obtained from different extraction viz., hot and cold methods has a varying degree of a zone of inhibition against different strains. But there is no activity found against fungal strain, *Candida albicans*. Seed coat oil obtained by cold extraction showed a maximum zone of inhibition viz. 14 mm, 14 mm, and 15 mm against *Streptococcus mutans*, *Staphylococcus aureus*, and *E. coli*., respectively while no inhibition was found against *Pseudomonas aeruginosa* in all oil. The seed coat oil obtained by hot extraction also showed significant activity viz., 12.5 mm, 12 mm and 14 mm against different strain i.e., *S. mutans*, *S. aureus* & *E. coli*, respectively. The seed oil obtained by both hot and cold extraction also showed significant activity against different strains, but in comparison oil obtained by cold extraction showed a maximum zone

Table 3 — Antioxidant activity of oil from *Sesbania sesban* seed and their parts by different extraction methods

Sample	Concentrations (µg/mL)				
	20	40	60	80	100
Seed coat(hot)	1.37±0.01 ^{g}	2.37±0.005 ^{g}	3.37±0.0006 ^{g}	4.4±0.01 ^{g}	5.4±0.01 ^{f}
Seed coat(cold)	3.63±0.01 ^{f}	5.07±0.001 ^{f}	5.57±0.001 ^{e}	5.69±0.001 ^{e}	5.73±0.01 ^{e}
Germ(hot)	15.18±0.00^{d}	15.84±0.01 ^{d}	18.07±0.001 ^{d}	30.69±0.01 ^{d}	41.9±0.01 ^{c}
Germ(cold)	27.43±0.00^{b}	29.95±0.07 ^{b}	30.86±0.0006 ^{b}	31.06±0.01 ^{c}	31.31±0.01 ^{d}
Endosperm(hot)	0.37±0.006 ^{h}	0.37±0.005 ^{h}	0.37±0.005 ^{h}	0.37±0.01 ^{h}	0.37±0.01 ^{g}
Endosperm(cold)	0.37±0.001 ^{h}	0.37±0.01 ^{h}	0.37±0.001 ^{h}	0.37±0.01 ^{h}	0.37±0.01 ^{g}
Seed(hot)	25.99±0.00^{c}	28.01±0.001 ^{c}	29.45±0.001 ^{c}	51.46±0.01^{b}	55.45±0.00^{b}
Seed(cold)	5.20±0.01 ^{e}	5.20±0.001 ^{e}	5.24±0.01^{f}	5.57±0.001^{f}	5.70±0.006 ^{e}
Ascorbic acid	96.28±0.03 ^{a}	96.72±0.022 ^{a}	96.92±0.021^{a}	96.88±0.02^{a}	96.88±0.02^{a}

Values are mean±standard deviation of triplicate analysis. Different letters in the same row are significantly different ($p<0.05$) as measured by one way ANOVA Tukey's test

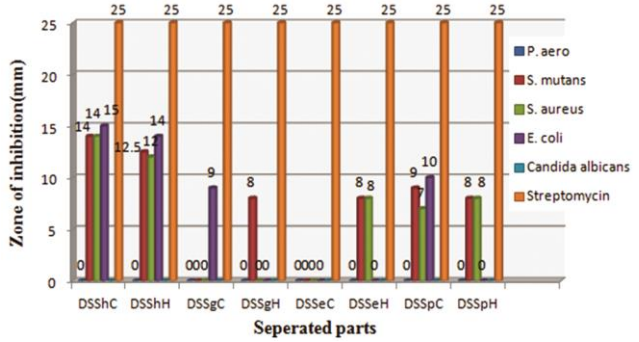

Fig. 1 — Antimicrobial activity of oil from *Sesbania sesban* seed and their parts from different methods

Abbreviation: DSShC-Seed coat oil by cold method, DSShH-Seed coat oil by hot method, DSSgC- Germ oil by cold method, DSSgH- Germ oil by hot method, DSSeC- Endosperm oil by cold method, DSSeH- Endosperm oil by hot method, DSSpC-Seed oil by cold method, DSSpH-Seed oil by hot method

of inhibition viz., 9 mm, 7 mm and 10 mm against *S. mutans*, *S. aureus* & *E. coli*, respectively, while oil obtained by hot extraction viz., 8 mm against both *S. mutans & aureus* (Fig. 1). Thus, seed coat oil showed a maximum percent inhibition up to 60%, 48% and 48% respectively as compared to Streptomycin against *E. coli*, *S. mutans* and *S. aureus* bacterial strain. Also, the oil obtained by cold extraction showed more significant inhibition in comparison to hot method. MIC value of endosperm oil obtained by cold extraction was found to be 3 mg/mL against *S. aureus* while 2 mg/mL of oil obtained by hot extraction. MIC of seed coat oil from cold extraction viz., 1.5, 1, 0.5 against *S. mutans*, *S. aureus* and *E. coli* respectively. The MIC of seed coat oil from hot extraction was found to be 2, 2, 0.5 mg/mL against *S. mutans*, *S. aureus* and *E. coli*, respectively.

Conclusion

Sesbania seed may be exploited as a source of major-minor seed oil due to the high content of oil in germ through specific separation methods other than seed, germ may be exploited for its oil potential. *Sesbania* seed oil is a good source of palmitic acid, linoleic acid and oleic acid. Oleic acid was major marker identified that is characteristic of the edible seed oil. Thus antioxidant and antibacterial, particularly of seed oil, has scope for its utilization as antibacterials for therapeutics. Studies also scope to utilize potential bioactive markers of *S. sesban* seed oil for soap, cosmetics and pharmaceutical industries.

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Conflict of interest

Authors declare no conflict of interest

Author contributions

SS. Experimental work and writing, BNS. Contributed in antimicrobial activity, MS. Experimental design, writing, editing and supervision.

References

1 Singh S H, Fertility Control of female through *Sesbania sesban* seeds, *J Res Edu Indian Med*, 9 (4) (1990) 27-32.

2 Saravanan K, Priya G & Renuka C, Medicinal plants with potential antifertility activity- A review of sixteen years of herbal medicine research (1994-2010), *Int J Pharm Tech Res*, 4 (1) (2012) 481-494

3 Sidhuraju P, Vijaykumari K & Janardhan K, Studies on the under under exploited legume, *Indigofera linifolia* and *Sesbania bispinosa:* nutritional and antinutritional factors, *Int J Food Sci Nutr*, 46 (1995) 195-203.

4 Mani R P, Pandey A, Goswami S, Tripathi P, Kumudhavalli V Singh A P, Phytochemical screening and in-vitro evaluation of antioxidant activity and antimicrobial activity of the Leaves of *Sesbania sesban* (L) Merr, *Free Rad Antioxidant*, 3 (1) (2011) 66-69.

5 Ramadan M F, Sharanabasappa G, Seetharam Y N, Seshagiri M & Moersel J T, Characterization of fatty acids and bioactive compounds of Kachnar (*Bauhinia purpurea L.*) seed oil, *Food Chem*, 98 (2) (2006) 359-365.

6 Kang J X, Leaf A. Protective effects of free polyunsaturated fatty acids on arrhythmias induced by lysophosphatidyl choline or palmitoylcarnitine in neonatal rat cardiac myocytes, *Eur J Pharmacol*, 297 (1996) 97-106

7 Schimidt E B, Dyerberg J, Omega-3 fatty acids, Current status in cardio-vascular medicine, *Drugs*, 47 (1994) 405-424.

8 Chan J K, Dietary alpha-linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholestrol in normolipidemic men, *Am J Clin Natur*, 53 (1991) 1230-1234.

9 Sree A, Bapuji M, Gupta J K, Siddqui Kai, Fatty acid composition of lipids of some of the marine sponges from Orrisa coast, *Ind J Pharm Sci*, 63 (2) (2001)158-161

10 Knowles PF, In proceedings of 2nd international Safflower conference, Hyderabad, 9-13th Jan, 1989.

11 Banerji R, Shukla P, Bajpai A & Dixit B S, Review Article: Omega-3 Fatty Acid, Applied Botany Abstracts, 27 (4) (2007) 315-333.

12 Muralidharu Y, Virupakshappa, Extent of variation in oil, protein and fatty acid composition of high oleic hybrids, wild species and some cultivars of sunflower, *J OT A I*, 30 (1998) 4.

13 AOAC, official methods of analysis (17th ed.), Gaithersburg, MD, USA: Association of Official Analytical Chemists, (2000).

14 Phillips BE, Smith CR, Hangemann JW, Glyceride structure of *Erlanga tomentosa* seed oil, a new source of vernolic acids, *Lipids*, 4 (1969) 473.

15 Liyana-Pathiranan C M, Shahidi F, Antioxidant activity of commercial soft and hard wheat (*Tritricum aestivum* L.) as affected by gastric pH conditions, *J Agric Food Chem*, 53 (2005) 2433-2440

16 National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement, MI00-S12 (M2). NCCLS Wayne, PA. 2002.

17 World health organization. Diet nutrition and prevention of chronic diseases. WHO Technical Report series 916, Report of a joint WHO/FAO Expert Consultation, Geneva, 2003, 88.

18 Gunstone FD, John L H & Albert J D, The lipid hand book with Cd- Rom. 3rd ed., Boca Raton: CRC press, 2007.

19 Ajayi I A, Oderinde R A, Kajogbola D O & Ukponi J U, Oil contents and fatty acid composition of some selected underutilized legumes from Nigeria, *Food Chem*, 99 (2006) 115-20.

20 Neuringer M, Connor W E, Van Petten C & Barstad L, Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys, *J Clin Invest*, 73 (1984) 272-6.

21 Bourre J M, Bonneil M, Clement M, Dumont O, Durand G, *et al*., Function of dietary polyunsaturated fatty acids in the nervous system, *Prostaglandins Leukot Essent Fatty acids*, 48 (1993) 5-15.

22 Hossain M A & Becker K, Nutritive value and anti-nutritional factors in different varieties of *Sesbania* seeds and their morphological fractions, *Food chem*, 73 (2001) 421-431.

23 Liu F, Oovi V F C & Chang S T, Free radical scavenging activity of mushroom polysaccharide extracts, *Life Sci*, 6 (1997) 763-777.