Journal of Pharmaceutical, Chemical and Biological Sciences
ISSN: 2348-7658
Impact Factor (SJIF): 2.092
March-May 2015; 3(1):84-90
Available online at http://www.jpcbs.info

Original Research Article

Evaluation of Phytochemical Constituents and Fatty acid Content in *Sesamum indicum L*.

Vijay.R[1], Sharmila K.P[2], Mahesh Prasad Bekal[3], Suchetha Kumari.N[4], Pushpalatha. K.C[5*]

[1]Department of Biochemistry, Mangalore University, P.G Centre, Chikka Aluvara, Kushalnagar, India.
[2]Central Research Laboratory, K. S Hegde Medical Academy, Mangalore, India.
[3]Department of Biochemistry, Kanachur Institute of Medical Sciences, Mangalore, India.
[4]Department of Biochemistry, K S Hegde Medical Academy, Mangalore, India.
[5]Department of Microbiology, Mangalore University, P.G Centre, Chikka Aluvara, Kushalnagar, India.

***Corresponding Author; Email:** kcpushpalatha@yahoo.com

Received: 23 April 2015 Revised: 01 May 2015 Accepted: 04 May 2015

ABSTRACT

Sesamum indicum L. (Black Sesame) seed is one of the most important oil seed crops in the world. It has been cultivated for centuries, particularly in Asia and Africa. Folk knowledge suggests black sesame is an indispensable ingredient in a therapeutic tea to stroke victims. The aim of this study is to analysis the phytochemical, fatty acid pattern of *S. indicum* seed. Black Sesame seeds showed the presence of Terpenoids, Tannins, Cardiac Glycosides and Saponifible lipids. The seed samples of *S. indicum* were extracted using petroleum ether at 60-80^0 C. The extract was subjected to Soxhlet and Leaching extraction for the isolation of fatty acid content. The Oily extract were purified and analysed by GC-FID. The fatty acid content extracted using Soxhlet extract method, showed high yield when compared to leaching extract. Soxhlet extract showed high amount of Linoleic acid compare to leaching extract. The Seeds studied here can be seen as a potential source of useful drugs. It also justifies the folklore medicinal uses and the claims about the therapeutic values of this seed extracts as curative agent. ω-6 and ω-3 polyunsaturated C20 fatty acids represent important components of the human diet. A more regular consumption and an accordingly sustainable source of these compounds are highly desirable.

Keyword: *Sesamum indicum L.; p*hytochemical profile; fatty acids; GC-FID

INTRODUCTION

Plants and plant products are widely used in the traditional and folk medicines throughout the world. Similarly, plants form the basis of some important drugs that have been frequently used in Ayurveda, Siddha and Unani system of medicines. Natural products are the sources of

synthetic and traditional herbal medicine. They are still practised as the primary health care system in many parts of the world [1].In recent years, secondary plant phytochemicals, previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [2].

Sesamum indicum L. (Black Sesame) seed is one of the most important oil seed crops in the world [3] and is also known as sesamum, gingelly, beniseed, sim-sim, and till. It has been cultivated for centuries, particularly in Asia and Africa, for its high edible oil and protein [4]. It is also considered to be a beneficial food to health [5].

Folk knowledge suggests black sesame is an indispensable ingredient in a therapeutic tea to stroke victims. Antioxidants substances like sesamol, sesamolin and sesaminol can be find in *S. indicum* [6].

According to Encyclopedia Britannica "sesame, also called Benne, erect, annual plant (*S. indicum*) of numerous types and varieties belonging to the family *Pedaliaceae,* cultivated since antiquity for its seeds, which are used as food and flavouring and from which a prized oil is extracted"[7].

Kiran and Asad [8] showed that seeds and oil of *S. indicum* have considerable healing activity when administered orally or topic. Furthermore, black sesame seeds are more potential antioxidant than cream sesame seeds [9] and the black sesame seed coat has the greater relative antioxidant potential [10]. Figueiredo and Modesto Filho [11] showed that defatted flour of *S. indicum* contributes positively to the rate of glycemic control and weight in patients with diabetes mellitus. Important compound in sesame seed is the sesamolin. Kang et al [12] show that sesamolin and his metabolites have an important contribution as antioxidants in seed and oil from sesame. Moreover, Kim et al observed that extracts of black sesame possesses phenolic compounds with radical scavenging ability related with inducing colon cancer cell death [13].

ω 6- and ω 3-polyunsaturated C20 fatty acids represent important components of the human diet. A more regular consumption and an accordingly sustainable source of these compounds are highly desirable. The main aim of this study was to analyze the fatty acid patterns of black sesame seed.

MATERIALS AND METHODS

Collection of Seeds

Black Sesame Seed

The seeds were brought from the local market of Mangalore in the sealed, airtight bags in the month of July 2013 for analysis.

Extraction

Preparation of Aqueous extract

Seeds were dried in an incubator for 2 days at 40^0C, crushed in an electric grinder, and then pulverized. Out of this powder, 100g of ground sample was taken in 1000 mL of distilled water. The sample suspension was centrifuged at 10,000 rpm for 15 mins at 10^0 C and the supernatant was filtered through 0.45 µm Millipore filter and the filtrates were used for assessing the antioxidant activity and phytochemical screening of seed extract. The filtrates stored at -20°C till analysis.

Leaching Extraction (LE): 10 g of seeds were immersed in 60 mL of petroleum ether in a flask for 12 h, and the extract was obtained by filtration. Then the residue was re-extracted for twice more with fresh solvent. Finally the combined extract was evaporated at 40^0 C under reduced pressure to constant weight and the crude oil was obtained.

Soxhlet Extraction (SE): 10 g of seeds were extracted with 150 mL of petroleum ether (60-90^0) in Soxhlet Extraction at 80^0 C for 6 h, and petroleum ether was removed under reduced pressure with a rotary vacuum evaporator. [14]

Qualitative phytochemical screening

The extracts were screened for phytochemical properties using standard methods [15].

Test for Alkaloids

Dragendorff's Test: Drug solution + Dragen droff's reagent (Potassium Bismuth Iodide), formation of Orangish red colour.

Mayer's Test: Drug solution + few drops of Mayer's reagent (K_2HgI_4), formation of creamy-white precipitant.

Wagner's Test: Drug solution + few drops of Wagner's reagent (dilute Iodine solution), formulation of reddish-brown precipitate.

Test for Flavonoids

Alkaline reagent Test: Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Test for Glycosides

Anthraquinone glycosides

Borntragor's Test: To 1 gm of extract add 5-10 ml of dilute HCl boil on water bath for 10 minutes and filter. Filtrate was extracted with CCl_4/benzene and add equal amount of ammonia solution to filtrate and shake. Formation of pink or red colour in ammonical layer due to presence of anthraquinone moiety.

Cardiac glycosides

Keller Killiani Test: Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

Test for Saponins

Foam Test: Test solution was mixed with water and shaken and observed for the formation of froth, which is stable for 15 minutes for a positive result.

Test for Proteins

Ninhydrin Test: Test solution when boiled with 0.2% solution of Ninhydrin, would result in the formation of purple color suggesting the presence of free amino acids.

Biuret test: To the aqueous solution of protein in hot water, few drops of Biuret reagent (KOH, $CuSO_4$ and sodium potassium tartarate) is added, which turns blue reagent to violet. It is usually done by adding few drops of 0.5% $CuSO_4$ solution to the alkaline aqueous protein solution. At least one peptide linkage is necessary for this test; individual amino acids do not produce violet colour.

Test for Carbohydrate

Fehling solution test: It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium Tartarate. Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath. Formation of reddish brown coloured precipitate due to formation of cuprous oxide indicates presence of reducing sugar.

Benedict's test: Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Terpenoids

Salkowski Test: The extract was mixed with 2ml of chloroform and concentrate H_2SO_4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of terpenoids.

Test for Tannins

Lead Acetate Test: 1ml of the different filtrate was added with three drops of lead sub acetate

solution. A cream gelatinous precipitation indicates positive test for Tannins.

Ferric Chloride Test: 1ml each of filtrate is diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black color indicates the presence of Tannins.

Test for Steroids

2ml of acetic anhydride was added to extract 2ml of H_2SO_4. The color changed from violet to blue or green in some samples indicated the presence of steroids.

Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate.

Test for Saponifible Lipids

Approximately 0.5 ml of a extract was added to 0.5 ml of 3 M NaOH. After the sample is heated for 15 minutes, 10 ml of water is added and the solution shaken vigorously. The formation of bubbles (which indicate that soap was formed).

Test for Iodine

It is specific for polysacchrides. Few drops of Iodine solution was added to aqueous solution of extract. Formation of blue colour, which disappears on heating and reappears on cooling, indicates the presence of starch.

Methylation

Samples (100 mg) were dissolved in 5 mL 0.5 % (w/v) sodium hydroxide in methanol in a 25 mL flask with a ground-glass stopper and boiled under reflux for 45 min. Then, 2.0 mL boron trifluoride-ether solution was added and the solution boiled for 5 min. After this, 5 mL hexane was added and the solution boiled for 5 min. The solution was cooled and then a quantity of saturated sodium chloride was added and shaken for about 10 s. After this, 1.0 mL of the upper phase was collected and 1mL Iso-octane solution was diluted sample solution.

Gas chromatography- FAME Analysis

GC conditions:

GC analysis was performed on an HP 5890 Gas Chromatograph equipped with FID. Compounds were separated on 30 m × 0.25 mm i.d. DB-23 capillary column. The initial temperature of the polar column was 120 ºC then it was programmed to 190ºC at 2 ºC·min, and maintained at 190 ºC for 8 min. Split injection was conducted with a split ratio of 100:1, nitrogen was used as a carrier gas at a flow-rate of 1.00 mL/min and the injector temperature was 250 ºC and the detector temperature was 250 ºC. Palmitic acid methyl ester, oleic acid methyl ester and linoleic acid methyl ester were identified by co-injection of reference standards and comparison of their retention times.

RESULTS

The curative properties of seeds are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc. The aqueous extracts of Black Sesame seeds showed the presences of Terpenoids, Tannins, Cardiac Glycosides and Saponifible lipids.

The results of preliminary phytochemical analysis are shown in Table 1.

Sesamum indicum L Seed subjected to soxhlet and leaching extraction the oil yield obtained was high in soxhlet extraction compared to Leaching Showed in Table 2.

Palmitic acid, Stearic acid, oleic acid and Linoleic acid (w-6) were found in both the extracts. α – linolenic acid was found in Soxhlet extract in very less amount. Fatty acid Composition of Black Sesame showed in Table 3, Figure 1 and 2 showed the Chromatogram of Black Sesame Soxhlet and leaching extracts.

DISCUSSION

In this work we have determined the phytochemical profile and fatty acid content of black sesame seeds.

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development.

Table 1: Qualitative Analysis of the Phytochemical of Black Sesame Aqueous Extracts.

Chemical Tests	Black Sesame Seed
Test for Alkaloids	
1. Dragendorff's Test:	-
2. Mayer's Test:	-
3. Wagner's Test:	-
Test for Flavonoids:	
1. Alkaline reagent Test	-
Test for Glycosides:	
Anthraquinone glycosides	
1.Borntragor's Test	-
Cardiac glycosides	
1.Keller Killiani Test	+
Test for Saponins:	
1.Foam Test	-
Test for Proteins:	
1.Ninhydrin Test	-
2. Biuret test	-
Test for Carbohydrate	
1. Fehling's solution test	-
2. Benedict's test	-
Test for Terpenoids:	
1. Salkowski Test	+
Test for Tannins:	
1.Lead Acetate Test	+
2. Ferric Chloride Test	+
Test for Steroids	-
Test for Quinones	-
Test for Saponifible Lipids	+
Test for Iodine	-

Table 2: Oil Yields of Black Sesame by different methods 1) Soxhlet extraction 2) Leaching method.

Sl.No	Method	Time (hours)	Oil Yields (%)
1	Soxhlet extract	6	34
2	Leaching extract	12	18

Table 3 : Fatty acid Composition of Black Sesame.

Sl.No	Fatty acids	Soxhlet Extract	Leaching extract
1	Palmitic acid	4.4	2.83
2	Stearic acid	1.3	0.3
3	Oleic acid	18.58	10.85
4	Linoleic acid (w-6)	21.25	15.62
5	α – linolenic acid (w-3)	0.3	-

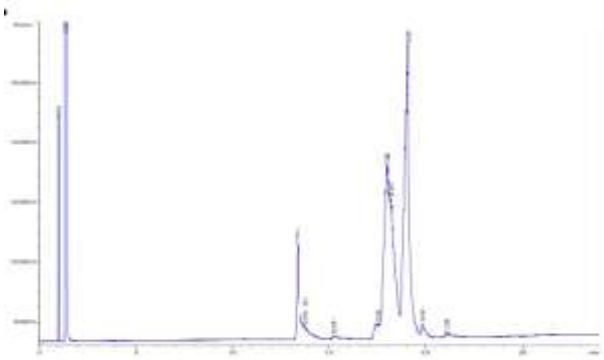

Fig.1: Chromatogram of Black Sesame Soxhlet Extract.

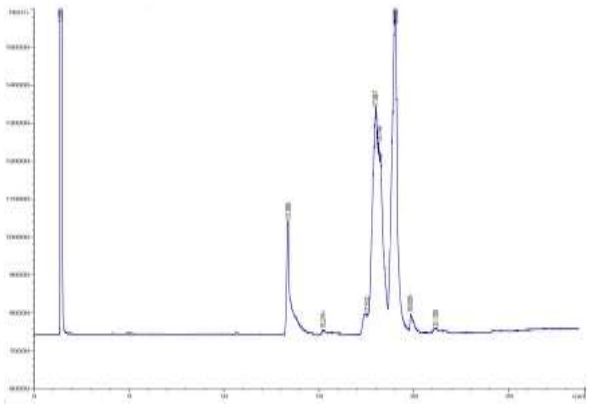

Fig. 2: Chromatogram of Black Sesame Leaching Extract.

An important part of natural products from plants, biomolecules and secondary metabolites usually exhibits some kind of biological activities. They are widely used in the human therapy, veterinary, agriculture, scientific research and in countless other areas. [16] The usefulness of plant materials medicinally is due to the presence of bioactive constituents such

as alkaloids, tannins, flavonoids and phenolic compounds. [17]

The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example, saponins have hypotensive and cardio depressant properties. [18] Glycosides are naturally cardio active drugs used in the treatment of congestive heart failure and cardiac arrhythmia. [19] The presence of Cardiac glycosides in the aqueous extract of seeds might play a protective role in the cardiac related problems.

Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have been shown to have remarkable activity in cancer prevention and treatment. [20]

The presence of terpenoids, tannins, cardiac glycosides and saponifible lipids obtained in this study showed that these seeds can be harnessed for both nutritional and medicinal purposes. The preliminary phytochemical tests are helpful in finding chemical constituents in the Seed extracts that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound.

The preliminary phytochemical tests revealed that seed contained phytoconstituents such as Cardiac glycoside, tannins, terpenoids and Saponifible lipids, which also possess antioxidant activity.

Fatty acids represent a chemically inert class of organic compounds that are easy to extract from biological material. Normally, fatty acids are acids produced in cell after catabolism break down of fat. These compounds are hydrophobic and not water soluble. They are important part of a healthy diet and body requires them for organs and tissues and utilize them in many cellular activities [21].

Petroleum ether has low polarity index of 0.1, therefore in our work we aimed to use them as extracting solvents for the fatty acid content of the leaching and soxhlet extracts of Black Sesame seeds. *Sesamum indicum L* Seed subjected to soxhlet and leaching extraction the oil yield obtained was high in soxhlet extraction compared to Leaching.

Palmitic acid, Stearic acid, oleic acid and Linoleic acid (w-6) were found in both the extracts. α – linolenic acid was found in Soxhlet extract in very less amount. Fatty acid Composition of Black Sesame showed in Table No.2. Figure 1 and 2 showed the Chromatogram of Black Sesame Soxhlet and leaching extracts.

Omega-3 and omega-6 fatty acids are consumed they are incorporated into cell membranes in all tissues of the body. Because of this fact, dietary changes in the composition of PUFAs can have profound effects on a cell function because the membrane lipids serve as a source of precursors for the synthesis of important signalling molecules involved in cell growth and development as well as modulation of inflammation.

CONCLUSION

The Seeds studied here can be seen as a potential source of useful drugs. It also justifies the folklore medicinal uses and the claims about the therapeutic values of this seed extracts as curative agent. ω 6- and ω 3-polyunsaturated C20 fatty acids represent important components of the human diet. Linoleic acid (w-6) and α – linolenic acid (w-3) are an essential fatty acid, plays an important role in the human diet. This study helps to differentiate fatty acid content of different extracts of Black Sesame seeds.

REFERENCES

1. Sarker SD and Nahar L. Chemistry for Pharmacy Students General, Organic and Natural Product Chemistry. John Wiley and Sons Publishers. England; 2007.p.283-359.
2. Krishnaraj AV et al. Assessment of bioactivity of Indian Medicinal plants using

Brine shrimp (*Artemia salina*) lethality assay. Int J Appl Sci Eng 2005; 2:125-34.
3. Shyu YS and Hwang LS. Antioxidative activity of the crude extract of lignan glycosides from unroasted Burma black sesame meal. Food Res Int 2002; 35: 357-365.
4. Salunkhe DK et al. Sesame. In: Van Nostrand R. (ed.) World Oil Seeds: History, Technology and Utilization. Spring: New York, 1991; 371-402.
5. Fukuda Y and Namiki M. Recent studies on sesame seed and oil. Nippon Shokuhin Kogyo Gakkaishi 1988; 35: 552-562.
6. Wichitsranoi J et al. Antihypertensive and antioxidant effects of dietary black sesame meal in pre-hypertensive humans, Nutrition Journal 2011; 10.
7. Encyclopedia Britannica, 2012. Avaliable in: http://global. britannica. com/EBchecked /topic/536043/sesame
8. Kiran K and Asad M. Wound healing activity of Sesamum indicum L seed and oil in rats. Indian Journal of Experimental Biology 2008; 46: 777-782.
9. Da Silva ER et al. Capacidade antio-xidante e composição química de grãos integrais de gergelim creme e preto. Pesquisa Agropecuária Brasileira 2011; 46 (7):736-742.
10. Shahidi F, Liyana-Pathirana CM, WALL DS. Antioxidant activity of white and black sesame seeds and their hull fractions. Food Chemistry 2006; 99: 478-483.
11. Figueiredo AS and Modesto Filho J. Efeito do uso da farinha desengordurada do Sesamum indicum L nos níveis glicêmicos em diabéticas tipo 2. Revista Brasileira de Farmacognosia 2008;18: 77-83.
12. Kang M et al. Sesamolin Inhibits Lipid Peroxidation in Rat Liver and Kidney. Journal of Nutrition 1998; 1017-1022.
13. Kim M et al. Radical scavenging activity and apoptotic effects in HT-29 human colon cancer cells of black sesame seed extract, Int J Food Science Technology 2009; 44: 2106–2112.
14. Zhou Z et al. Studies on the smashing tissue extraction and purification process of the total saponins of the basal part of stem and beard from *Panax notoginseng*. Mod Chin Med 2009; 3: 34-36.
15. Sofowora AE. Medicinal Plants and Traditional Medicines in Africa. 2nd edition. Ibadan, Nigeria: Spectrum Books; 1993, p289.
16. Kandukuri V et al. Biomolecular and phytochemical analysis of three aquatic angiosperms. African J Microb Res 2009; 3(8): 418-421.
17. Lata N, Dubey V. Preliminary phytochemical screening of Eichhornia crassipes: the world's worst aquatic weed. J Pharm Res 2010; 6: 1240-1242.
18. Olaleye MT. Cytotoxicity and antibacterial activity of methanolic extract of Hibiscus sabdariffa. J Med Plants Res 2007; 1: 9–13.
19. Brian FH, Thomas-Bigger J, Goodman G. The Pharmacological Basis of Therapeutics, New York: Macmillan; 1985, p7.
20. Aiyegoro AO and Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Com Altern Med 2010; 10:21-25.
21. Lew Mander and Liu HW. Comprehensive Natural Products II, Chemistry and Biology. Amsterdam: Elsevier; 2010, p 528.

Cite this article as:
VijayR, Sharmila KP, Mahesh Prasad Bekal, Suchetha Kumari N and Pushpalatha KC. Evaluation of Phytochemical Constituents and Fatty acid Content in *Sesamum indicum L*. J Pharm Chem Biol Sci 2015; 3(1): 84-90