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

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**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 analyse the phytochemical, fatty acid pattern of *S. indicum* seed. Black Sesame seeds showed the presence of Terpenoids, Tannins, Cardiac Glycosides and Saponifiable lipids. The seed samples of *S. indicum* were extracted using petroleum ether at 60-80°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.; phytochemical 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. Antioxidant substances like sesamol, sesamolin and sesaminol can be found 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 topical. 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°C, 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°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°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°C) in Soxhlet Extraction at 80°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 (K2HgI4), 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 CCl4/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, CuSO4 and sodium potassium tartarate) is added, which turns blue reagent to violet. It is usually done by adding few drops of 0.5% CuSO4 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 Tartrate. 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 H2SO4 (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 \( \text{H}_2\text{SO}_4 \). 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 \( \text{HCl} \) and observed for the formation of yellow colour precipitate.

**Test for Saponifiable Lipids**
Approximately 0.5 ml of a extract was added to 0.5 ml of 3 M \( \text{NaOH} \). 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 polysaccharides. 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 x 0.25 mm i.d. DB-23 capillary column. The initial temperature of the polar column was 120 °C then it was programmed to 190°C at 2 °C-min, and maintained at 190 °C 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 °C and the detector temperature was 250 °C. 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 Saponifiable 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. \( \alpha \)-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 Phytochemicals of Black Sesame Aqueous Extracts.

<table>
<thead>
<tr>
<th>Chemical Tests</th>
<th>Black Sesame Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test for Alkaloids</td>
<td></td>
</tr>
<tr>
<td>1. Dragendorff’s Test:</td>
<td>-</td>
</tr>
<tr>
<td>2. Mayer’s Test:</td>
<td>-</td>
</tr>
<tr>
<td>3. Wagner’s Test:</td>
<td>-</td>
</tr>
<tr>
<td>Test for Flavonoids:</td>
<td></td>
</tr>
<tr>
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<td>-</td>
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<td>Test for Glycosides:</td>
<td></td>
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<tr>
<td>Anthraquinone glycosides</td>
<td></td>
</tr>
<tr>
<td>1. Borntrager’s Test</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td></td>
</tr>
<tr>
<td>1. Keller Killiani Test</td>
<td>+</td>
</tr>
<tr>
<td>Test for Saponins:</td>
<td></td>
</tr>
<tr>
<td>1. Foam Test</td>
<td>-</td>
</tr>
<tr>
<td>Test for Proteins:</td>
<td></td>
</tr>
<tr>
<td>1. Ninhydrin Test</td>
<td>-</td>
</tr>
<tr>
<td>2. Biuret test</td>
<td>-</td>
</tr>
<tr>
<td>Test for Carbohydrate:</td>
<td></td>
</tr>
<tr>
<td>1. Fehling’s solution test</td>
<td>-</td>
</tr>
<tr>
<td>2. Benedict’s test</td>
<td>-</td>
</tr>
<tr>
<td>Test for Terpenoids:</td>
<td></td>
</tr>
<tr>
<td>1. Salkowski Test</td>
<td>+</td>
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<tr>
<td>Test for Tannins:</td>
<td></td>
</tr>
<tr>
<td>1. Lead Acetate Test</td>
<td>+</td>
</tr>
<tr>
<td>2. Ferric Chloride Test</td>
<td>+</td>
</tr>
<tr>
<td>Test for Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Test for Quinones</td>
<td>-</td>
</tr>
<tr>
<td>Test for Saponifiable Lipids</td>
<td>+</td>
</tr>
<tr>
<td>Test for Iodine</td>
<td>-</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Method</th>
<th>Time (hours)</th>
<th>Oil Yields (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Soxhlet extract</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>Leaching extract</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 3: Fatty acid Composition of Black Sesame.

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Fatty acids</th>
<th>Soxhlet Extract</th>
<th>Leaching extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palmitic acid</td>
<td>4.4</td>
<td>2.83</td>
</tr>
<tr>
<td>2</td>
<td>Stearic acid</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>Oleic acid</td>
<td>18.58</td>
<td>10.85</td>
</tr>
<tr>
<td>4</td>
<td>Linoleic acid (w-6)</td>
<td>21.25</td>
<td>15.62</td>
</tr>
<tr>
<td>5</td>
<td>α-linolenic acid (w-3)</td>
<td>0.3</td>
<td>-</td>
</tr>
</tbody>
</table>

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**


2. Krishnaraj AV et al. Assessment of bioactivity of Indian Medicinal plants using

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