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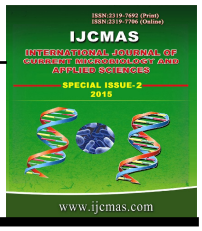

Original Research Article

Comparative Qualitative Phytochemical analysis of *Sesamum indicum* L.

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A B S T R A C T

Sesamum indicum L. (Pedaliaceae) is one of the oldest condiments and economically important oilseed crop, containing 40-60-% oil and medicinally important due to presence of wide range of secondary metabolites. Sesame oil shows highest resistance to rancidity and can be used as substitute for olive oil. In ethno-medicine oil is used for treatment of hair, skin, teeth, bone and lung problems. Oil is used in products like hair oils, perfumes, cosmetics like skin conditioning agents and moisturizers. It has pharmaceutical uses. In this study, presence of 8 primary metabolites and 16 secondary metabolites was seen in white and black sesame seeds. Qualitative phytochemical analysis was carried out using standard methods involving use of reagents detecting presence of particular metabolite. Preliminary phytochemical investigation of white and black sesame seeds showed the presence of primary metabolites like proteins, fats, volatile oils, carbohydrates and secondary metabolites like alkaloids and flavonoids. Besides these metabolites the white sesame showed presence of phlobatannins, coumarins, leucoanthocyanins, where as black sesame showed anthraquinones and emodins. Further study of pharmacological activities like antioxidant activity and antibacterial activity will lead to enhanced application of sesame in health care and other industries.

Keywords

Sesame seeds, Phytochemical analysis, Alkaloids, Flavonoids

Introduction

Since ancient times, researchers have been exploring nature in search of new drugs. Useful products can be derived from any part of the plant like bark, leaves, flowers, seeds etc. Plant products have been part of phytomedicines since times immemorial. Due to this a large number of medicinal plants with curative properties have been utilized. For primary healthcare, around 80% of world's population relies on traditional medicines, involving plant extracts. In traditional systems of Unani, Ayurveda, Homeopathy, and Siddha, almost 90% of prescriptions were based on drugs obtained from plants. Drugs from the plant sources are easily available, are less expensive, safe, and efficient and rarely have side effects. Knowledge of chemical

constituents of the secondary metabolities in plants is desirable because such information will be of value for the synthesis of complex chemical substances. A complete and detailed study of secondary metabolites of medicinal plants found in India needs to be done as they are responsible for the medicinal activity of plants (Savithramma *et al.,* 2011)

Plants may be regarded as vast libraries of small molecules, secondary metabolites, organic compounds which have considerable structural diversity which would otherwise probably be unavailable in a synthetic chemical laboratory. Plants have developed defences such as chemical defenses over millions of years against environmental threats such as UV radiations, reactive oxygen species and microbial attacks. Therefore, phytochemicals are less toxic and biologically active. There is a demand for plant drugs throughout the world. They are widely used in human therapy, veterinary, agriculture, scientific research and countless other areas. However, safety, efficacy and other properties of such compounds need to be thoroughly tested. This would ensure reliability and repeatability of pharmacological and clinical research to understand their bioactivity and to enhance the product quality control (Alphonso and Saraf, 2012)

The present study deals with one such medicinal plant which is more prominently used as an oil crop. *Sesamum indicum* L. belongs to Pedaliaceae family (Hasan *et al.,* 2013). It is one of the oldest and world's most important oil seed crop. It is cultivated in tropics and temperate zones throughout the world. The largest commercial producers of sesame are India, China and Mexico (Morris, 2002). Sesame seeds have 40-60% of oil, which makes it an economically important oil crop (Hasan, 2013). Sesame ranks ninth among the top thirteen oilseed crops which make up 90% of the world, in production of edible oil (Saha *et al.,* 2014). Sesame oil is important in nutritional, medicinal and industrial uses. In European countries it is used as a substitute for olive oil (Anilkumar *et al.,* 2010). Industrial use of sesame is in products like perfumes, cosmetics, hair oils, soaps etc. Sesame oil has many pharmaceutical uses like oleaginous vehicle for drugs, solvent for intramuscular injections etc. (Anilkumar *et al.,* 2010). It is an important part of Ayurvedic, Chinese and Tibetian traditional medicinal systems (Reshma *et al.,* 2012).

Sesame seeds contain many phytochemically important compounds like flavonoids, phenolic acids, alkaloids, tannins, saponins, steroids, terpenoids and minerals like calcium, iron, magnesium, manganese, copper, zinc, phosphorus. Sesame has compounds like sesamin, sesaminol, gamma tocopherol, cephalin and lecithin. These compounds impart many of the pharmacological activities like antioxidant, antibacterial, cardio tonic, antidiabetic, hypocholesterolemic, antitumor, antiulcer, antiinflammatory and analgesic to sesame (Anilkumar *et al.,* 2010). In ethnomedicine sesame oil is used in hair oils and to treat other skin problems. It is also used to treat teeth, bone and lung problems (Patil *et al.,* 2008; Anilkumar *et al.,* 2010). Compounds such as hydroxysesamine (Hasan *et al.,* 2001) anthrasesamones A, B, C (Furumoto *et al.,* 2003), D and E (Furumoto *et al.,* 2006) are present in the roots of *Sesamum indicum*. Aqueous extract of leaves has shown presence of phenolic group consisting compounds such as sesamol and sesamin (Shittu *et al.,* 2007). The present study deals with comparison of secondary metabolites present in black and white sesame seeds.

The study mainly deals with detailed study of total alkaloids and flavonoids.

Material and Method

a. Qualitative estimation of secondary metabolites in white and black sesame varieties-

White and black sesame seeds; local varieties, were procured from local market of Pune.

Method of extraction - 10 gm seeds of each of the selected varieties were finely powdered and soaked for 24 hrs minimum in 50 ml of Methanol, Chloroform and distilled water each. Next day, all the extracts were filtered using vacuum pump through Whatmann filter paper no. 1 (70 mm). Methanol and Chloroform residues were taken to dryness and then the residues were dissolved in 2N HCl. Alongside; the filtered methanol and water extracts were stored for further use.

Qualitative phytochemical tests were performed as per standard methods. The tests are as per the following table.

Test for Starch – To the test solution iodine solution was added. Development of blue colour indicates presence of starch.

Tests for Mucilage

i. To the test solution iodine solution and few drops of conc. H_2SO_4 were added. Development of violet colour indicates presence of mucilage.

ii. Black and white sesame seeds were soaked in cold water overnight. Swelling of seeds indicates presence of mucilage.

Tests for proteins

i. To the test solution 40% NaOH and 2% $CuSO_4$ were added. Development of violet colour indicates presence of proteins.
ii. To the test solution conc. H_2SO_4 was added. Formation of white ppt indicated presence of proteins.

Tests for fats/ fixed oils

i. To the test solution chloroform was added. Solubility of the sample is an indicator of presence of fats.
ii. To the test solution 90% alcohol was added. Insolubility of the extract is an indicator of presence of fats.

Test for volatile oils- To the test solution 90% of alcohol was added. Solubility of the extract is an indicator of presence of volatile oils.

Test for carbohydrates – To the test solution Molisch Reagent was added and conc. H_2SO_4 was added from sides. Formation of a purple ring at the junction indicates the presence of carbohydrates.

Test for amino acids- To the test solution 5% $FeCl_3$ was added and the solution was boiled. Formation of purple/ bluish colour indicates presence of amino acids.

Test for reducing sugars- To the test solution Fehling's Reagent was added and the extract was boiled. Formation of brick rep ppt indicates the presence of reducing sugars.

Tests for alkaloids

i. To the test solution Wagner's Reagent was added. Formation of reddish brown ppt indicates presence of alkaloids.

ii. To the test solution iodine solution was added. Development of brownish ppt indicates presence of alkaloids.

iii. To the test solution Mayer's Reagent was added. Formation of creamish yellow ppt indicates presence of alkaloids.

iv. Hager's test was performed on the test solution. Picric acid was added to the test solution. Formation of yellow ppt indicates presence of alkaloids.

v. To the test solution Dragendorff's reagent was added. Development of red/brown ppt indicates presence of alkaloids.

Tests for tannins

i. To the test solution 5% $FeCl_3$ was added. Formation of bluish black/ green ppt indicates presence of tannins.

ii. To the test solution 1% lead acetate was added. Development of yellow ppt indicates presence of tannins.

Tests for steroids

i. Salkowski test was performed on the test solution by adding chloroform and conc. H_2SO_4. Development of red colour after adding chloroform and greenish yellow fluorescence after adding conc. H_2SO_4 indicates presence of steroids.

ii. To the test solution H_2SO_4 and ethanol were added. Development of violet/blue/green colour indicates presence of steroids.

iii. To the test solution H_2SO_4 and chloroform were added. Formation of red colour in lower layer indicates presence of steroids.

Tests for flavonoids

i. To the test solution chloroform, conc. HCl and a few Mg turnings were added. Development of pink colour indicates presence of flavonoids.

ii. To the test solution increasing amount of 2N NaOH was added. Development of yellow colour that disappears on adding an acid indicates presence of flavonoids.

iii. To the test solution NaOH and HCl were added. Formation of yellow/ orange colour indicates presence of flavonoids.

iv. To the test solution 1% lead acetate was added. Development of white ppt indicates presence of flavonoids.

v. To the test solution conc. H_2SO_4 was added. Formation of orange colour indicates presence of flavonoids.

Tests for saponins – The test solution was shaken vigorously and the boiled. Frothing indicates presence of saponins.

Tests for anthraquinones

i. To the test solution chloroform was added; this solution was shaken and then filtered. 10% ammonia was added to this filtrate. Formation of red or pinkish colour in ammonia layer indicates presence of anthraquinones.

ii. To the test solution benzene and 10% ammonia was added. Development of pink/red/violet colour indicates presence of anthraquinones.

Test for phlobatannin – To the test solution HCl was added and the solution was then boiled. Formation of red ppt indicates presence of phlobatannins.

Tests for anthocyanins

i. To the test solution HCl and then 10% ammonia were added. Development of pinkish red/ bluish violet colour indicates presence of anthocyanins.

ii. To the test solution 2M NaOH was added. Formation of blue green colour indicates presence of anthocyanins.

Test for coumarins – To the test solution 10% NaOH was added. Development of yellow colour indicates presence of coumarins.

Test for terpenoids – To the test solution conc. H_2SO_4 and chloroform were added. Development of yellow colour in lower layer indicates presence of terpenoids.

Tests for cardiac glycosides

i. To the test solution conc. H_2SO_4 and chloroform were added. Formation of brown ring at the junction indicates presence of cardiac glycosides.

ii. To the test solution chloroform and acetic acid were added. Formation of violet/ blue/ green colour indicates presence of cardiac glycosides.

Test for emodins - To the test solution benzene and 10% ammonia was added. Development of red colour indicates presence of emodins.

Test for glycosides – To the test solution acetic acid was added, cooled and then dilute H_2SO_4 was added. Formation of bluish green colour indicates presence of glycosides.

Tests for phenols

i. To the test solution Na nitrite was added and the solution was boiled. Dilute H_2SO_4 and excess dilute NaOH were added to it. Development of red/ green/ blue colour indicates presence of phenols.
ii. To the test solution 1% $FeCl_3$ was added. Development of deep blue/ black colour indicates presence of phenols.

Test for leucoanthocyanin

To the test solution isoamyl alcohol was added. Formation of red colour in organic layer indicates presence of leucoanthocyanins.

Tests for resins

i. To the test solution acetone water was added. Development of turbidity is the indicator of presence of resins.

ii. To the test solution Cu in acetic acid was added. Development of dark blue colour is the indicator of presence of resins.

b. Quantitative estimation of alkaloids by non – spectrophotometric method

Material: Black and white sesame seeds were collected from local market.

Method: 2 gms each of powdered seed samples were taken in bumper tubes. 10 ml of 10% acetic acid in ethanol was added in each tube. Extracts were put on shaker for 24 hours at 110 rpm, 30 ^{o}C. The upper layer from each bumper tube was transferred to a new tube. Its volume was reduced to quarter of the original volume on a hot water bath at

70^{o}C. These reduced extracts were transferred to pre-weighed 2 ml Eppendorff tubes. Liquor ammonia was added drop wise to the extracts till white precipitate formed. A short spin was given to the extracts, supernatant was discarded and the pellet was washed with 1% liquor ammonia. Again, a short spin was given and the supernatant was discarded. The tubes were weighed along with the pellet. The weight of the pellet was thus calculated (Sani *et al.,* 2013; Harborne, 2008)

Total alkaloids content was calculated as follows:

$$\text{Total alkaloids} = \frac{\text{Weight of residue}}{\text{Weight of sample}}$$

Alkaloids are present in beniseeds and absent in oil (Njoku *et al.,* 2010) Sesame seeds contain 6.28 + 4.19 % alkaloids (Mbaebie *et al.,* 2010). Alkaloids are present in brown seed oil (Warra *et al.,* 2012).The second most abundant phytochemical in the *Sesamum indicum* seed oil is alkaloid with a concentration of 132.80±0.15 mg/g. Alkaloids have been used as central nervous system stimulants, topical anesthetics in ophthalmology, powerful pain relievers, anti-puretic action, among other uses. This indicates that the white *Sesamum indicum* seed oil can be used as a component of topical or systemic pain relievers (Sani *et al.,* 2013) Alkaloids are present in aqueous and ethonalic extracts of seeds of sesame. (Sireesha *et al.,* 2013). Alkaloids are present in khali of black sesame (Yadav *et al.,* 2014).

Flavonoids are absent in beniseeds and oil. (Njoku *et al.,* 2010) Sesame seeds contain 16.12 + 10.7 % flavonoids (Mbaebie *et al.,* 2010) Flavonoids are present in brown seed oil (Warra *et al.,* 2012). The concentration of flavonoids is 59.20±0.15 mg/g. The presence of flavonoids indicates that the oil will be good for the management of cardiovascular diseases and oxidative stress because flavonoids and phenols are biological antioxidants. The presence of flavonoids in *Sesamum indicum* seed oil accounts for its antioxidant property. The presence of flavonoids in *Sesamum indicum* seed oil also accounts for its use in inhibiting the replication of human colon cancer cells. Flavonoids also provide protection against these diseases by contributing to the total antioxidant defense system of the human body. The natural *Sesamum indicum* seed oil has high stability due to the presence of high levels of these natural antioxidants (Sani *et al.,* 2013) Flavonoids are present in aqueous and ethonalic extracts of seeds of sesame. (Sireesha *et al.,* 2013)

Phlobatannins are absent in white sesame oil (Sani *et al.,* 2013) Phlobatannins are absent in khali of black sesame (Yadav *et al.,* 2014). Coumarins are present in khali of black sesame. Coumarins enhance immune system by increasing body strength and so are valuable as dietary suppliments (Yadav *et al.,* 2014). Anthocyanins and leucoanthocyanins are absent in khali of black sesame (Yadav *et al.,* 2014).

Anthraquinone derivatives have antibacterial, antiviral, antifungal and other biological activities. They are also used in field of dyestuff, papermaking, medicines, agriculture related chemicals, etc. (Alphonso *et al.,* 2012). Anthraquinones are present in white sesame oil (Sani *et al.,* 2013) Anthraquinones are absent in khali of black sesame (Yadav *et al.,* 2014).

Preliminary phytochemical testing of white and black sesame seeds showed the presence of primary metabolites such as proteins, fats, volatile oils, carbohydrates and secondary metabolites.

Table.1 Qualitative phytochemical analysis of extracts of white and black sesame seeds

No	Compound	Black Sesame				White Sesame			
		ME*	WE*	MR*	CR*	ME	WE	MR	CR
1.	Starch	-	-	-	-	-	-	-	-
2.	Mucilage	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
3.	Proteins	+	-	+	+	+	+	+	+
		-	-	-	-	-	-	-	-
4.	Fats/FixedOils	+	-	-	-	+	+	+	+
		-	-	-	-	-	-	-	-
5.	Volatile oils	+	+	+	+	+	+	+	+
6.	Alkaloids	-	+	-	-	-	+	-	+
		-	+	+	-	-	-	-	-
		-	+	+	-	-	+	-	+
		+	-	-	-	-	+	-	+
		+	+	-	-	+	+	-	-
7.	Tannins	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
8.	Carbohydrates	+	+	+	+	+	+	-	-
9.	Amino acids	-	-	-	-	-	-	-	-
10.	Steroids	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
11.	Flavonoids	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
		-	-	-	-	-	-	+	-
		+	+	+	+	+	+	+	+
		+	-	+	-	-	-	-	-
12.	Saponnins	-	-	-	-	-	-	-	-
13.	Anthraquinone	-	-	-	-	-	-	-	-
		+	-	-	-	-	-	-	-
14.	Phlobatannin	-	-	-	-	+	-	+	-
15.	Anthocyanin	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
16.	Coumarins	-	-	-	-	+	+	+	+
17.	Terpenoid	-	-	-	-	-	-	-	-
18.	Cardiac glycoside	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
19.	Emodin	+	-	-	-	-	-	-	-
20.	Glycosides	-	-	-	-	-	-	-	-
21.	Phenols	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-
22.	Leucoanthocyanin	-	-	-	-	-	-	+	-
23.	Resin	-	-	-	-	-	+	-	-
		-	-	-	-	-	-	-	-
24.	Reducing sugar	-	-	-	-	-	-	-	-

ME: Methanol Extract; WE: Water Extract; MR: Methanol Residue; CR: Chloroform Residue

Table.2 Determination of total alkaloid content of white and black sesame seeds by non – spectrophotometric method

Name of Sample	Residue (mg)	Sample (gm)	Total Alkaloids (mg/gm)
Black Sesame	30	2	15
White Sesame	113	2	56.5

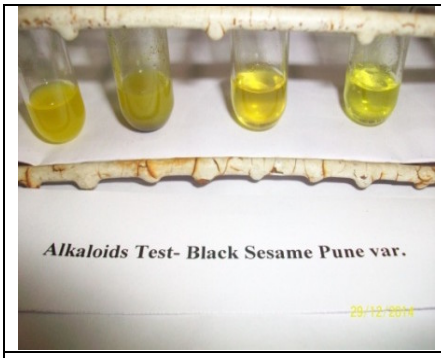

Fig.1 Yellow precipitate indicates presence of alkaloids in black sesame seeds. The extracts were in sequence of ME, WE, MR, CR. ME showed the presence of alkaloids.

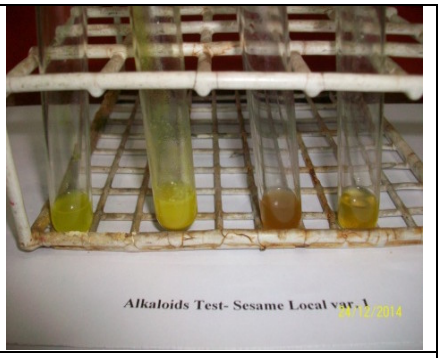

Fig.2 Yellow precipitate indicates presence of alkaloids in white sesame seeds. The extracts were in sequence of ME, WE, MR, CR. WE and CR showed the presence of alkaloids.

Fig.3 Brown precipitate indicates presence of alkaloids in black sesame seeds. The extracts were in sequence of ME, WE, MR, CR. ME and WE showed the presence of alkaloids.

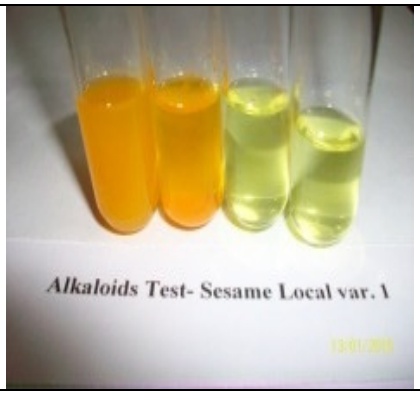

Fig.4 Brown precipitate indicates presence of alkaloids in white sesame seeds. The extracts were in sequence of ME, WE, MR, CR. ME and WE showed the presence of alkaloids.

Besides these metabolites the white sesame showed presence of phlobatannins, coumarins, leucoanthocyanins. Black sesame seeds showed presence of anthraquinones and emodins. Total alkaloids were found to be more in white sesame as compared to black sesame.

The profile of the bioactive compounds of a plant indicates its medicinal value. Antioxidant and antimicrobial properties of various plant extracts is of great interest because of their use as natural additives and replacement of synthetic ones. Preliminary qualitative tests can be of help in detection of such bioactive principles, leading to discovery of new drugs. Thus, phytochemical screening of medicinal plants is very important (Alphonso and Saraf, 2012).

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