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HPLC analysis and cell surface receptor binding activities of the crude aqueous and methanolic extract of *Sesamum indicum*

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PEER REVIEW

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Comments

In this study the authors performed the HPLC analysis of aqueous extract and methanol extract and showed that the aqueous extract may contain caffeine, cetirizine or its derivatives like molecules, and methanolic extract may contain Loratadine or its derivatives like molecules and 3 unidentified compounds. The results are interesting and suggestive of the utility of the compounds in therapeutics as well as new drug discovery.
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ABSTRACT

Objective: To identify the possible functional molecules for therapeutic uses by screening the crude aqueous and methanolic extracts derived from sesame seeds (*Sesamum indicum*) *in vitro*.
Methods: High performance liquid chromatography was used to scan the functional molecules present in the extracts.
Results: The crude aqueous extracts showed the possibilities to present caffeine and cetirizine or its derivatives like molecules. On the other hand, the crude methanolic extract may contain Loratadine or its derivatives like molecules. Both type of extracts showed hemagglutination inhibition activities in all types of human blood samples tested. However, they showed stronger binding with AB+ blood group than those of A+ and B+ blood.
Conclusions: Sesame seeds may be considered as a functional food.

KEYWORDS

Sesamum indicum, Hemagglutination, High performance liquid chromatography, Thin-layer chromatography

1. Introduction

Seasame seeds possess hypoglycaemic, anticoagulant, antioxidant, antifungal, hepatoprotective and wound healing activities. It is also used to increase fertility, as external poultice, emmenagogue, lactagogue, diuretic, tonic and demulcent[1-3]. Many phytochemical investigations have been done on the chemical constituents of the seeds of *Sesamum indicum (S. indicum)*. The chemical constituents include lignans and lignan glycosides, sterols and phenolic acids from the seeds. The seeds also contain beta-sitosterol, stigmasterol, sesamolin, sesamin, ferulic acid, sigmasterol-3-O-β-D-glucoside, verbascoside, rhamnetin, miquelianin, kaempferol-3-O-β-D-glucuronide *etc*. Lignan concentrations in sesame seeds (29 331 mg/100 g, mainly pinoresinol and lariciresinol) were relatively high[4-6]. Sesame seeds are a very good source of copper, and calcium. Just a quarter-cup of sesame seeds supply 74.0% of the daily value for copper, 31.6% of the magnesium, and 35.1% of the daily value for calcium. It is also high in protein, phosphorus, iron and magnesium. The

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seeds also have a good amount of manganese, zinc, vitamin B1, tryptophan and dietary fibers[7,8]. Sesame seeds (*S. indicum* L.) are widely used as dietary supplements. The plant is widely cultivated in Asian and African countries. The oil from the seed contains various phytochemical compounds that display medicinal properties. Jeng and Hou reported that health benefits of sesame seeds may be attributed to its lignans, especially sesamin[9]. Sesamin affects lipid metabolism, contributes to reduced incidence of tumorigenesis, and has the ability to protect neuronal cells against oxidative stress. The preventive ability of lignans on bone loss was also reported[10].

Here we tried to find out the presence of other chemical compounds in sesame seeds through high performance liquid chromatography (HPLC) method. We also tried to find the receptor binding activities of the crude extracts with human red blood cells.

2. Materials and methods

2.1. Materials

S. indicum was purchased from local supermarket in Dhaka, Bangladesh. Reagent grade hydrochloric acid, dibasic potassium phosphate, orthophosphoric acid and HPLC grade acetonitrile and methanol were obtained from Merck, Germany. The caffeine, cetrizine HCl, cetrizine impurity B adn Loratadine were collected from Square Pharmaceuticals Ltd. Dhaka, Bangladesh.

2.2. Thin layer chromatography (TLC) analysis

Firstly, the solvent system (Ethyl acetate:ethanol:water = 8:1.2:0.8) was prepared. The spots were for methanolic and aqueous extracts of sesame seeds, loratidine, and caffeine were used as standards. After spotting the respective TLC plate was exposed to the solvent system by dipping the plate into the solvent at one end. The tank should then be closed and the solvent was allowed to run. Upon completion of TLC, the plates were exposed under UV light for caffeine detection and charred with 10% sulphuric acid solution, dried and then heated to 80–90 °C for charring purpose for Loratadine detection.

2.3. HPLC analysis

The aqueous extract of *S. indicum* was analyzed in HPLC of Shimadzu (Prominence), Japan in gradient mode composing mobile phase A(17% v/v of acetonitrile and 83% v/v of water, the apparent pH adjusted to 1.5 with orthophosphoric acid) and mobile phase B (35% v/v of acetonitrile and 65% v/v of water, pH adjusted to 1.5 with orthophosphoric acid) using Phenomenex Luna C18 column (4.6 mm×25 cm, 5 μm column that containing L1 packing) with column temperature 30 °C, UV detection at 230 nm, injection volume 20 μL and flow rate 1 mL per minute. The gradient elution was designed to 0–50 min, mobile phase A (100–0)% and mobile phase B (0–100)% *i.e.* linear gradient, 50–53 min, mobile phase A 0% and mobile phase B 100% *i.e.* isocratic, 53–54 min, mobile phase A (0–100)% and mobile phase B (100–0)% *i.e.* linear gradient, and finally 54–60 min, mobile phase A 100% and mobile phase B 0% *i.e.* re-equilibrium. The aqueous extract was prepared by taking 15 g powder of *S. indicum* with purified water to volume 150 mL, and then 1 mL extracts was transferred to volume upto 10 mL by mobile phase A. About 1.5 mg caffeine standard (potency-99.30%) were poured to volumetric flask for volume upto 10 mL by mobile phase A to prepare standard caffeine solution and the resolution solution contained the cetrizine HCl and cetrizine impurity B.

The methanolic extract of *S. indicum* was analyzed in HPLC of Shimadzu (Prominence), Japan to separate the mixture of compounds dissolved in methanol in isocratic mode composing mobile phase of filtered and degassed mixture of 0.01 mol/L dibasic potassium phosphate, methanol and acetonitrile through proper mixing in the proportion of 7:6:6 and adjusted to an apparent pH of 7.2 with 10% phosphoric acid solution using Hichrom C8 column (4.6 mm×15 cm, contains 5 μm packing L7) with column temperature 30 °C, UV detection at 254 nm, injection volume 15 μL and flow rate 1 mL per minute. To prepare the diluents, 100 mL of 0.05 mol/L hydrochloric acid and 20 mL of 0.6 mol/L dibasic potassium phosphate were transferred to a 250 mL volumetric flask, diluted with a mixture of methanol and acetonitrile (1:1), and mixed. The standard Loratadine solution was prepared by pouring 40 mg Loratadine into 100 mL volumetric flask and making volume up to the mark with the diluents to have a final concentration of Loratadine 0.4 mg/mL. Experimental alcoholic sample prepared by taking 10.7 mg methanolic extract (obtained from 200 mg powered *S. indicum* in 400 mL methanol, soaked for five days and filtered) was taken into 10 mL volumetric flask and made volume up to the mark with diluents. Later, 1 mL of this solution was transferred into a 100 mL volumetric flask, diluted with diluents to volume and mixed well to concentration of methanolic extract 0.0107 mg/mL.

2.4. Hemagglutination assay

Stock solution of the test sample was prepared at concentration of 5 mg/mL and each solution was serially diluted. Fresh blood from healthy person was collected only for the test of haemagglutination assay. The blood group A+, B+, AB+ were collected from healthy volunteers. Then the all bloods were centrifuged and the erythrocytes were separated. Briefly, 4% erythrocyte suspension was prepared in phosphate buffer (pH 7.4) of all blood groups. A total of 1 mL of the test sample (100 mg/mL) dilution was taken with 1 mL of 4% erythrocyte and incubated at 25 °C. After incubation, the results were noted. Smooth button formation in bottom indicated negative activity, while a rough granular deposition at bottom showed positive activity. The intensity of haemagglutination was determined from the extent of deposition.