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HPLC-MS Analysis of Phenolic Compounds in Defatted Flour of *Sesamum indicum* L. Accessions+

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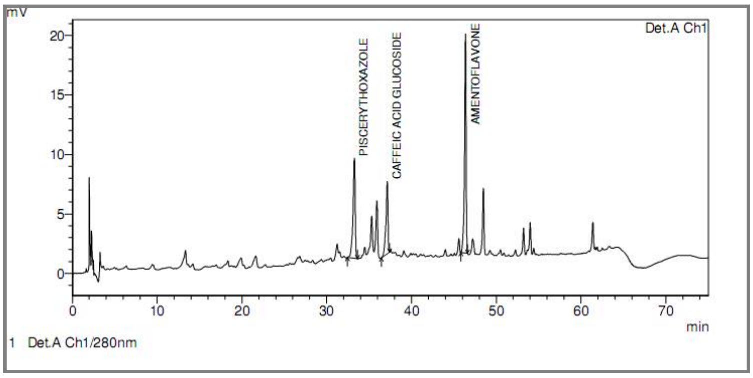
ABSTRACT

To characterize the phenolic compounds in thirteen accessions of Sesamum indicum L. GT-2, HIMA, RT-46, Nirmala, RT-54, JLT-7, MT-75, RT-46, Gouri, YLM-66, YLM-17, YLM-11 and Madhavi. There was significant variation among the thirteen accessions. Based on HPLC-MS studies along with authentic standards three phenolics were identified as Piscerythoxazole, Caffeic acid glucoside and Amentoflavone. The Piserythoxazole was reported to be high in all twelve accessions except in RT-54. Amentoflavone was also reported in all thirteen accessions and highest in Nirmala, Madhavi, Gouri and YLM-17. Caffeic acid glucoside was found in twelve accessions and it was absent in YLM-66. The Significant variation in phenolic compounds among the thirteen Sesamum indicum L accessions include in this study will help in screening of accessions for enhanced secondary metabolite production through in-vitro cultures.

Graphical Abstract



Chromatogram of the methanolic crude extract of defatted sesame flour.

Keywords: *Sesamum indicum* L, Piscerythoxazole, Caffeic acid glucoside, Amentoflavone, HPLC-MS.

INTRODUCTION

Phenolic compounds are large and diverse group of secondary metabolites in plants which have been shown lot of impact on human health by decreasing the levels of free radicals in the organism. Phenolic compounds are having one or more aromatic rings with one or more hydroxyl groups. Various phenolic are natural antioxidants which are found in various plant parts such as fruits, leaves, seeds and plant products like oils [1]. Plant phenolics are also involved in defense against UV radiation or aggression by plant pathogens and predators and to colors. They are found in all plant organs and part of human diet. *Sesamum indicum* L is an important crop and a good source of edible oil. It is composed of 45-50% lipids, 5-6% ash, 4-5% fiber and 15-20% proteins. Sesame seed is also used in baked goods and confectionery products [2]. It is observed that sesame oil is stable against oxidative changes due to presence of lignans like Sesmins, Sesamolins, Sesminol, Sesmol and Alpha tocopherol [3, 4]. The defatted sesame meal (DSM) has been reported to be more stable against oxidative changes [5]. There are reports of the presence of antioxidant compounds in the seed coat [6] and is confirmed the presence of flavonoids in *Sesamum indicum* L [7-11].

There has been no systematic study on natural phenolic variability among different *Sesamum indicum* L accessions. *Sesamum* breeding studies have mainly emphasized on producing high quality seed for food industry. In order to incorporate active constituent in the functional food it is important to understand the variation in the content of physiologically active constituent. This information is of great value to agronomists and plant breeders.

The objective of the present study is to determine the distribution and variation of phenolic compounds among the thirteen accessions of *Sesamum indicum* L with diverse genetic background. This information will help in selection of high phenolic accession through disease resistance in field and for secondary metabolite production by *in-vitro* culture.

MATERIALS AND METHODS

Preparation of plant material: The *Sesamum indicum* L accession GT-2, HIMA, RT-46, Nirmala, RT-54, JLT-7, MT-75 and RT-46 are collected from Jawaharlal Nehru Agriculture University, Jabalpur. Gouri, YLM-66, YLM-17, YLM-11 and Madhavi from Regional Agricultural Research Station, Yellamanchalli, Andhra Pradesh. The seeds of thirteen accessions are planted in Rabi and Kharif seasons 2012. The disease resistant seeds were harvested and each seed sample are ground separately and defatted with n-hexane to obtain defatted sesame flour (DSF) and stored at -20°C until further use.

Sample preparation: The samples are prepared from 100 mg of DSF in 5 mL of 70% methanol (HPLC Grade, Merck, Germany) and shaken for 15 min at room temperature. The samples are centrifuged at 3000 rpm for 5 min, the supernatant is filtered through filter paper. The pellet is reextracted with 5 mL of 70% methanol and finally rinsed with 5 mL of 100% methanol. All the three supernatant are pooled together before removal of methanol under vacuum using a Rota–evaporator at 70°C and again eluted with 4 mL of 100% HPLC grade methanol and Stored at 4°C prior to analysis.

Analysis of Phenolics: 1 mL of the extracted samples are analyzed by HPLC on a reverse phase C-18 column at ambient temperatures, mobile phase methanol/0.5% formic acid with a linear gradient from 0-100% methanol, flow rate is 2 mL per minute, cut of wave length is 280 nm, injected sample is 20microlitre, run time of the chromatogram is 50 min. Each sample is injected and eluted with the said mobile phase for 50 min and they were compared with standard compounds. In order to characterize the above compounds presenting in DSF LC-MS Spectroscopy is adopted.

Mass spectroscopy of defatted sesame flour: LC-MS was conducted utilizing the same mobile phase adopting ESI method. The ISV is kept at 5500 with negative polarity, optimum heater, NEB, CAD and CE etc. Scale mode range is between100-1000D and spectra are recorded as centroid.

RESULTS AND DISCUSSION

All samples are extracted and analyzed in triplicate. Content of investigated phenolic compounds in crude extracts are given in table 1.

Table 1. Identification of 3 major peaks isolated from defatted sesame flour extracts at 280 nm.

Phenolic (identification)	Rt-min	UV max	MS(m/z)(M-H)	Peak number
Piscerythoxazole	32.0	280	377	1
Amentoflavone	45.9	280	539	2
Caffeic acid glucoside	37.5	280	341	3

Material with high phenolic content from *Sesamum indicum* L is studied and the chromatogram was recorded and presented in figure1.

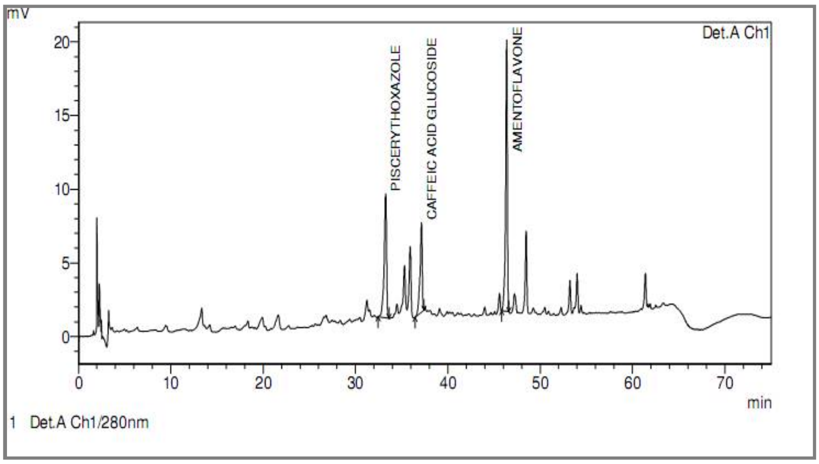


Figure1. Chromatogram of the methanolic crude extract of defatted sesame flour.

The Peaks are eluted by RP-HPLC were further analyzed LC-ESI-MS. In ESI-MS three peaks of m/z were identified. The three identified peaks are m/e 377.41, 341.14 and 539.144 which correspond to Piscerythoxazole, Caffeic acid glucoside and Amentoflavone figure 2.

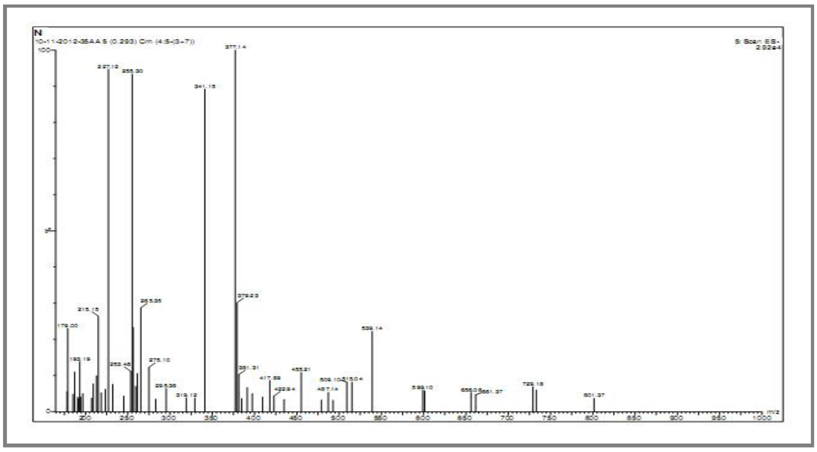


Figure 2. LC-MS Spectra of methanolic crude extract of defatted sesame flour.

In order to identify the structure of the compounds, we have procured the authentic sample purchased from the Sigma Aldrich; USA and Acros organics. The five authentic sample purchased are Amentoflavone, Caffeic acid glucoside and Myrcetin (Sigma Aldrich;USA) Piscerythoxazole, Sesamol(Acros organics). The above authentic are further examined on HPLC and their Rt is recorded in table 2.

Table 2. Identification of peaks from authentic standards at 280 nm

Standard Phenolic compounds	Rt-min	UV max
Piscerythoxazole	31.5	280
Amentoflavone	45.2	280
Caffeic Acid Glucoside	37.0	280

All thirteen accessions are analyzed based on intensity of peaks and area percent of peaks which are given in table 3.

Table 3. Grouping of Sesamum Indicum L accession based on concentration of Phenolic compound

S.No.	Seed color	Sesamum accession number	Phenolic compound arrangement in decreasing orderconcentration	Peak percent area (%)
1.	White	GTT-2	Piscerythoxazole/ Amentoflavone/ Caffeic acid Glucoside	54.53>39.14>6
2.	Black	YLM-66	Piscerythoxazole/ Amentoflavone/ Caffeic acid Glucoside	40.265>59>6
3.	Light brown	Nirmala	Amentoflavone/ Piscerythoxazole	51.5>3606>9.83
4.	Brownish black	Gouri	Caffeic acid glucoside Amentoflavone/ Piscerythoxazole	56.02>29.86>.14.10
5.	Browniish black	YLM-17	Amentoflavone/ Piscerythoxazole/ Caffeic acid Glucoside	49.06.>18.72<31.66
6.	Brownish black	Madhavi	Amentoflavone/ Piscerythoxazole/ Caffeic acid Glucoside	49.5>30.33>20.14
7.	White	JTS-8	Piserythoxazole / Amentoflavone/ Caffeic acid Glucoside	57.4>23.9<=19.27
8.	White	Hima	Piserythoxazole / Amentoflavone/ Caffeic acid Glucoside	58.3>18.09<=23.36
9.	White	RT-46	Piserythoxazole / Amentoflavone/ Caffeic acid Glucoside	48.6>31.87>19.87
10.	White	MT-75	Piserythoxazole / Amentoflavone/ Caffeic acid Glucoside	47.68>41.77.10.54
11.	Light Brown	RT-54	Caffeic acid Glucoside/ Amentoflavone /Piscerythoxazole	62>26>10
12.	Brownish black	YLM-11	Amentoflavone = Piscrerythoxazole/ Caffeic acid Glucoside	41.3=41.3>31
13.	Light Brown	JLT-7	Piscerythoxazole / Amentoflavone/s Caffeic acid Glucoside	60>25>14.2

The Piserythoxazole is reported to be high in all twelve accessions except in RT-54 where it is reported very low i.e., 10%. This flavonoid is known for its wide range of biological activity like treating for migraine, nervous tension, anti diabetic, anticancer and also as an insect antifeedant [**12, 13**]. Amentoflavone which has anti inflammatory, antiviral and chemo preventive activity is also reported in all thirteen accessions and highest in Nirmala, Madhavi, Gouri and YLM-17.There is a report of its presence in the roots of Sesamum indicum L [**14**]. There are reports where the flavonoids of *Sesamum indicum* L elicited Hypolipidemic and Hypoglycemic activities in rats raised the haemoglobin levels [**15, 16**]. It is reported that color intensity is good predictor of the amount of flavonoid .The dark colour seeds has higher content of flavonoids [**17**]. However, the white seeds like JTS-8, GT-2, HIMA, TKG-22, RT-46, MJS-75 had significantly higher flavonoids in our study Table.3. There are reports of presence of caffeic acid glucoside in *Sesamum indicum* L [**18**]. Similar reports are also reported in the defatted flour which shows more antioxidant activity [**6, 19**]. The phenolic Caffeic acid glucoside which act as antioxidant, anti thrombosis ,anti hypertension, antivirus and anti tumor is found in 12 accessions and was absent in YLM-66.

APPLICATION

The Significant variation in phenolic compounds in all the thirteen accessions of the *Sesamum indicum* L accessions include in this study will help in manipulating the above compounds through molecular and biotechnological approach. This will help in the development of seeds with high phenolic compounds which can be used as functional food to fight against major diseases like diabetics, cancer and cardiovascular ailment. This study also be helpful in screening of accessions of *Sesamum indicum* L for *in-vitro* cultures for enhanced secondary metabolite production.

CONCLUSION

This analytical study of phenolic compounds in defatted flour of *Sesamum indicum* L. Accessions+ provides the development of seeds with high phenolic compounds which can be used as functional food to fight against major diseases like diabetics, cancer and cardiovascular ailment. This study also be helpful in screening of accessions of *S.indicum* L. for *in-vitro* cultures for enhanced secondary metabolite production.

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