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PHARMACOGNOSTICAL, PHYSICO-CHEMICAL AND HPTLC PROFILE OF SHALGUM SEED (*BRASSICA RAPA* L.)

A. Jaculin Raiza*, R. Murugeswaran, S. Mageswari, P. Meera Devi Sri, Rampratap Meena and N. Zaheer Ahmed

Regional Research Institute of Unani Medicine, Royapuram, Chennai, India CCRUM, M/O Ayush Govt. of India, New Delhi.

***Corresponding Author: A. Jaculin Raiza**
Regional Research Institute of Unani Medicine, Royapuram, Chennai, India CCRUM, M/O Ayush Govt. of India, New Delhi.
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ABSTRACT
The main objective of the present study was carried out to determine the pharmacognostical, phytochemical, physicochemical and HPTLC characteristics of Unani single drug shalgum seeds (*Brassica rapa* L.) The phytochemical screening reveals the presence of alkaloids, flavonoids, tannins, glycosides and phenolic compounds in alcohol, chloroform and aqueous extracts. The physicochemical data showed that the drug contained foreign matter, moisture, total ash, acid-insoluble ash, extraction values, pH and volatile oil contents. The finger print analysis was carried out using High Performance Thin Layer Chromatography (HPTLC) methods for the separation of the active constituents in extracts. Alcohol and chloroform extractives of seeds showed various spots at 254 and 366nm (UV region) and revealed the presence of peaks with R_f values. In pharmacognostical studies the macroscopic, microscopic and powder features of shalgum seeds were carried out. The seeds were also analyzed for microbial load and for specific pathogenic bacteria as per WHO norms respectively.

KEYWORDS: Shalgum; *Brassica rapa* L.; HPTLC; Phyto-chemical screening, Physicochemical parameters; Pharmacognosy;

INTRODUCTION
Traditional herbal plants are kingdom of potential drugs and there has been increasing the awareness about the importance of medicinal values. Plants which are used as drugs should be generally are less expensive, easily available, safe, efficient, less toxic and have no side effects. In this basis, many researchers are focused in traditional medicinal plants, fruits, roots and seeds containing primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds are terpenoids, alkaloids, flavonoids, tannins and phenolic compounds (Krishnaiah D, 2007). This secondary metabolite emits antioxidant characteristics and is potentially used to prevent human diseases. The characteristic feature of an antioxidant is ability to scavenge the free radicals due to their redox hydrogen donators and singlet oxygen quencher (Wu YY, 2011). These free radicals are produced by our body and to stabilize the body's natural function, but the excess amount could cause the cell and tissue damage (Sen S, 2010). It can also cause oxidative damage to proteins, lipids and DNA and chronic diseases such as cancer, diabetes, aging and other degenerative diseases in humans (Aiyegoro OA, 2010). Due to these huge remedies, the rich antioxidant plant materials are used to cure various important pharmacological activities such as anti-inflammatory, anti-viral, anti-cancer, anti-microbial, ant-pyretic, anti-fungal activities (Mahato SB, 1997; Abdul W, 2013).

Brassica rapa L. belongs to Brassicaceae family, also known as mustard family. It is commonly called as turnip and shalgum. It is usually grown in regions that experience temperate climates. It is known in the Unani and Arab traditional medicine for its use in chronic gastritis, constipation, cholecystitis, cholecystolithiasis and in liver disease (Pithford P, 2002). The root part is used to cure common cold and seed help to treat skin cancer and breast tumors (Ahmadvand S, 2008). *Brassica rapa* L. contains a variety of antioxidants such as glucosinolates, phenylpropanoids, flavonoids, phenolics and organic acids (Fernandes F, 2007). The seeds of *Brassica rapa* L. are one of the healthiest vegetable oils for human consumption. Moreover very few reports have been performed on *Brassica rapa* L. Khalil *et al.*, reported a chemical composition and antimicrobial activity of essential oils and extracts of two varieties of *Brassica rapa* L. root and leaves in Fars-Iran (Khalil B, 2017). Mukhlesur *et al.*, described an

independent assortment of seed color and hairy leaf genes in *Brassica rapa* L. (Mukhlesur R, 2014). H. Bagheri *et al.*, discussed an identification of seed- related QTL in *Brassica rapa* L. (Bagheri H, 2013). Amiramoha *et al.*, reported an investigation of antimicrobial and antioxidant activities *of Brassica rapa* L. (Amira M, 2014). Moreover, the free radicals in the shalgum seeds can be naturally scavenged by the antioxidant and its uses shown in Figure 1. In this communication, the present research investigated the pharmacognostical, physicochemical screening and HPTLC finger print region of *Brassica rapa* L. (shalgum) seeds. HPTLC based methods could be considered as an important tool in routine drug analysis because of its simplicity and reliability which can be used for identification and authentication.

Figure 1: Graphical image of free radicals scavenged by antioxidant contained in shalgum seeds and its uses.

MATERIALS AND METHODS

Chemicals and Plant Materials

Fresh shalgum seeds were purchased in local market from Chennai, Tamil Nadu, India. All the chemicals were purchased from Merck Pvt. Ltd., Chennai. The chemicals used were of analytical grade. De-ionized water from a Milli-Q-ultrapure water system (Millipore Billerica, MA) was used throughout the experiments.

Phyto-chemical Screening

Phyto-chemical screening of the shalgum seeds as shown in Table 1, were carried out using standard methods (WHO, 2011). The freshly prepared crude extract of the drug is quantitatively tested for the presence of chemical constituents. This screening method was performed using following tests. The presence of alkaloids was confirmed by the Wagner's and Dragendorff's method. For carbohydrate (Anthrone and Fehling's test), proteins (Biuret's test), Triterpenoids (Liebermann-Burchard's test), steroids (Salkowski test), flavonoids (Shinoda's test), phenol (Liebermann test), Tannin (Ferric chloride test) and amino acids (Ninhydrin test) respectively.

Physico-chemical Screening

Physico-chemical screening was carried out under following parameters such as foreign matter (%), loss on drying (%), total ash (%) at 450^0C, acid-insoluble ash (%) at 550^0C, pH, volatile oil content (%), aqueous, alcohol and hexane extractive matter (%) were carried out as per IPC approved standard methods (WHO, 2011).

HPTLC Studies

Extract 2 g of sample with 20 ml of respective solvents (chloroform and alcohol) is reflux on a water bath for 30 min and filtered by Whatman filterpaper no. 1. Then concentrate the extract to 5 ml and carry out the experiment in thin layer chromatography.

Chromatogram was developed on 5 x 10 cm aluminium TLC plate precoated with a 0.2 mm layer silica gel. Application of each extract was carried out using spray techniques in CAMAG HPTLC system. Apply the extract (10 µl each) on TLC plate. Develop the plate using Toluene: Ethyl acetate: Formic acid (9: 1: 0.1) as mobile phase. Densitometry scanning of the plate was performed with a CAMAG TLC scanner.

Pharmacognostical Studies

Botanical identification of the shalgum seeds was carried out using available literature (Brandis D, 1988; Kritikar KR and Basu BD, 1998). The pharmacognostical studies such as macroscopical, microscopical and powder microscopy were carried out using standard method (Johansen DA, 1940). Free hand sections and different magnification of the seeds were taken photographs using digital SLR microscope attached with camera.

Quality Control

The determination of microbiological contamination as established for microbial load viz total bacterial count, total yeast count and specific pathagons like *Escherichia coli, Salmonella species, Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively, were carried out. The methodology applied for the studies were followed as per approved WHO guidelines (WHO 1998).

RESULTS AND DISCUSSIONS

The safety and quality of the herbal medicine have become important of their incredible increase in the usage of medicinal plants globally. Hence in the present study, attempts are made to determine the chemical standards of shalgum seeds used in the Unani system of medicine which finds use in the treatment of various diseases.

Phytochemical Screening

Table 1 Shows the screening of aqueous, alcohol and chloroform extracts of shalgum seeds based on phyto-chemical tests. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. Terpenoids are used to cure various diseases including cancer. Flavonoids are water-soluble antioxidant and free radical

scavenger, which prevent oxidative cell damage and have powerful anticancer activity. Tannins have eminent stringent properties and proteins contain nutritional power for building blocks of human's life. Alkaloids used to reduce appetite and diuretic problems. Steroids help in regulating the immune response and carbohydrates have vast therapeutic efficacy as they are found in almost every medicinal plants (Venkataramaiah C, 2013).

Table 1: Preliminary Phytochemical Screening of Shalgum Seeds.

Phytochemical test	Alcohol Extract	Chloroform Extract	Aqueous Extract
Alkaloids			
a) Wagner's test	+ve	-ve	+ve
b) Dragendroff's test	+ve	-ve	+ve
Flavonoids			
Shinoda's test	+ve	+ve	-ve
Carbohydrates			
Fehling's test	+ve	-ve	+ve
Proteins			
Biurett test	-ve	-ve	-ve
Triterpenoids			
Liebermann-Burchards test	+ve	-ve	-ve
Steroids			
Salkowski reaction	-ve	+ve	-ve
Tannins	+ve	-ve	+ve
Amino acids	-ve	-ve	-ve
Phenols	-ve	-ve	-ve

Physicochemical Screening

Physicochemical constants determined for the seed of shalgum revealed the genuineness and purity of the drug. The purity of the drug i.e. the presence or absence of foreign inorganic matter can be indicated by the ash values. Total ash and acid-insoluble ash contents are important indices to illustrate the quality as well as purity of herbal medicine. Total ash content alone is not sufficient to reflect the quality of herbal medicine, since the plant materials often contain considerable levels of physiological ash, calcium oxalate in particular. Thus, the acid insoluble ash content is another index to illustrate the quality of herbal medicine (Rao Y, 2009). Figure 2 shows the percentage of total ash and acid insoluble ash present in the shalgum seeds. Estimation of extractive values describes the amount of bioactive constituents is present in the shalgum seeds when extracted with various solvents such as water, hexane and alcohol. Figure 3 shows the percentage of extractive values of shalgum seeds in the respective solvents were determined. Moreover the percentage of moisture content, foreign matter and volatile oil content of shalgum seeds were shown in Figure 4. Hence the pH of the seed is 5.1; it reveals that the seed is in acidic condition.

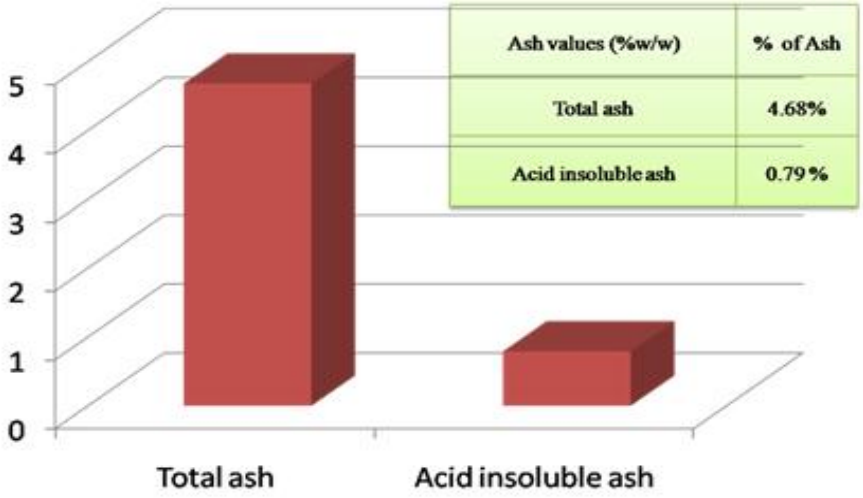


Figure 2: Ash value (%) of shalgum seed.

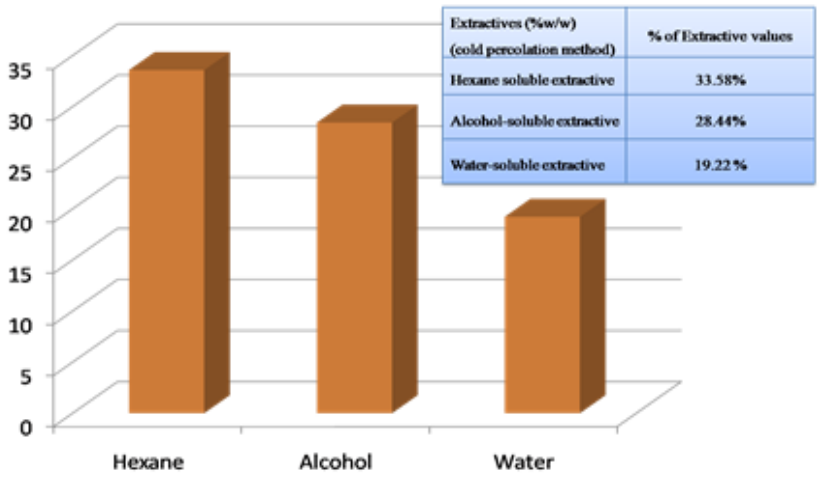


Figure 3: Extractive value (%) of shalgum seed.

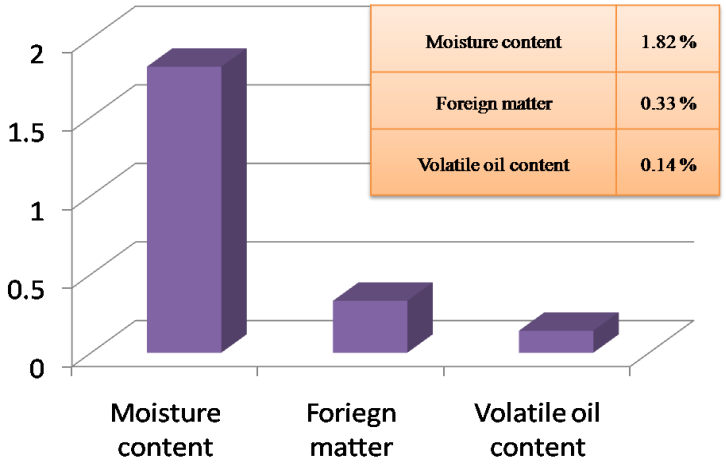


Figure 4: Moisture content, foreign matter and Volatile oil content (%) of shalgum seed.

HPTLC Studies

Standardization and quality control of herbal drugs is very difficult due to group of phyto-constituents and are subjected to variations. Hence, these methodologies that can create a finger print of each extract in large collections would be useful to detect stability of the same extract over time. HPTLC based methods could be considered as a good alternatives, as they are being explored as an important tool in routine drug analysis. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phases. This reduces the time and cost of analysis. In addition, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environmental pollution. HPTLC also facilitates repeated detection of chromatogram with same or different parameters (Leena S, 2014).

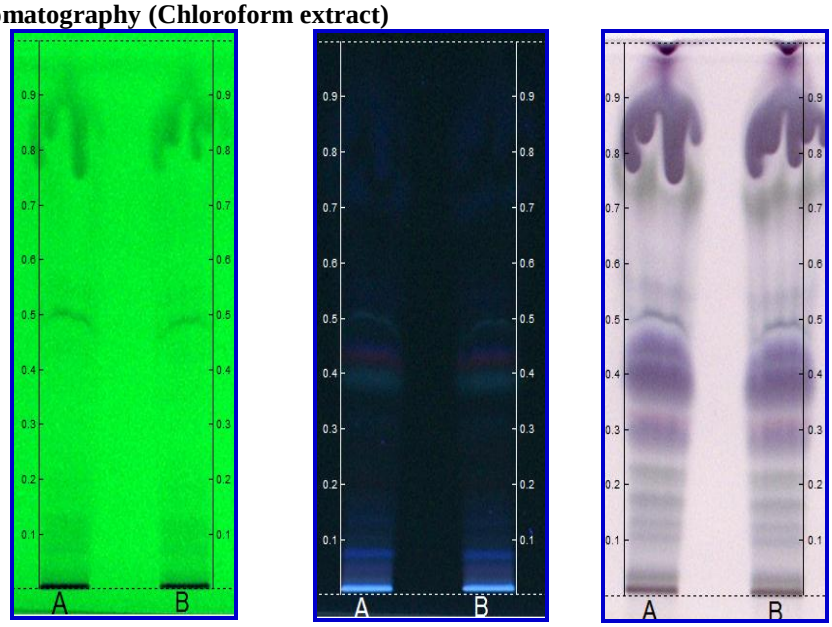
This study revealed that the shalgum seed showed best results in Toluene: Ethyl acetate: Formic acid (9: 1: 0.1) solvent system for chloroform and alcohol extract. For chloroform extract, after the development the plate was allowed to dry in air and examined under UV (254 nm), it shows major spots at R_f 0.90, 0.68, 0.48, 0.33, 0.13 and 0.07 (Green). Under UV (366 nm), it shows major spots at R_f 0.88, 0.72, 0.52 (Blue), 0.48 (Grey), 0.45 (Violet), 0.43 (Red), 0.39, 0.32 (Grey), 0.21 (Red), 0.16, 0.13 (Grey), 0.08 (Fluorescent blue), 0.05 (Blue). Dip the plate in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots at R_f 0.96 (Violet), 0.88 (Dark violet), 0.72, 0.53, 0.48 (Grey), 0.44, 0.38 (Violet), 0.31 (Brown), 0.28, 0.22, 0.18, 0.12 and 0.08 (Grey) (Figure 5).

For alcohol extract, UV (254 nm), it shows major spots at R_f 0.93, 0.58, 0.49, 0.19, 0.15, 0.12 and 0.05 (Green). Under UV (366 nm), it shows major spots at R_f 0.48 (Red), 0.45 (Grey), 0.38, 0.19, 0.15 (Blue), 0.12 (Fluorescent blue) and 0.05 (Violet). After a dip the plate in vanillin-sulphuric acid reagent it shows major spots at R_f 0.92 (Dark violet), 0.82, 0.62, 0.58 (Grey), 0.52

(Violet), 0.45, 0.36 (Dark violet), 0.28, 0.27, 0.22, 0.18, 0.16 (Grey) and 0.05 (Dark grey) (Figure 6).

HPTLC finger print regions and densitometric chromatogram of the samples of the single drug scanned at 254 and 366 nm. In both chloroform and alcohol extracts a sharp and symmetrical peaks were obtained it has been shown in Figure 7-10. Densitometry is an instrumental technique that is more accurate than visual. In this method the resolved spots are scanned and their densities were determined with a densitometer. Figure 11-14 shows the densitometric chromatogram of both chloroform and alcohol extracts at two different wavelength of 254 and 366nm.

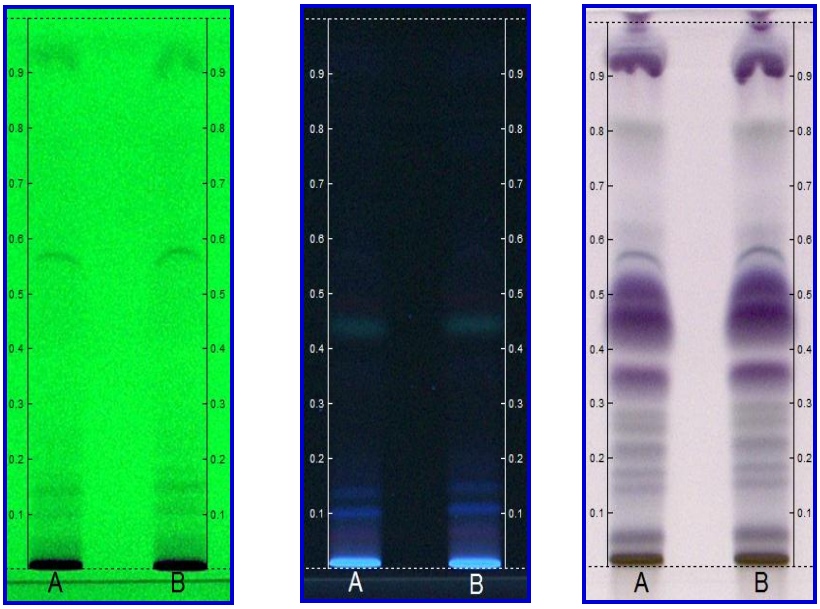
Thin Layer Chromatography (Chloroform extract)



UV – 254 nm **UV – 366 nm** **V - S Reagent**

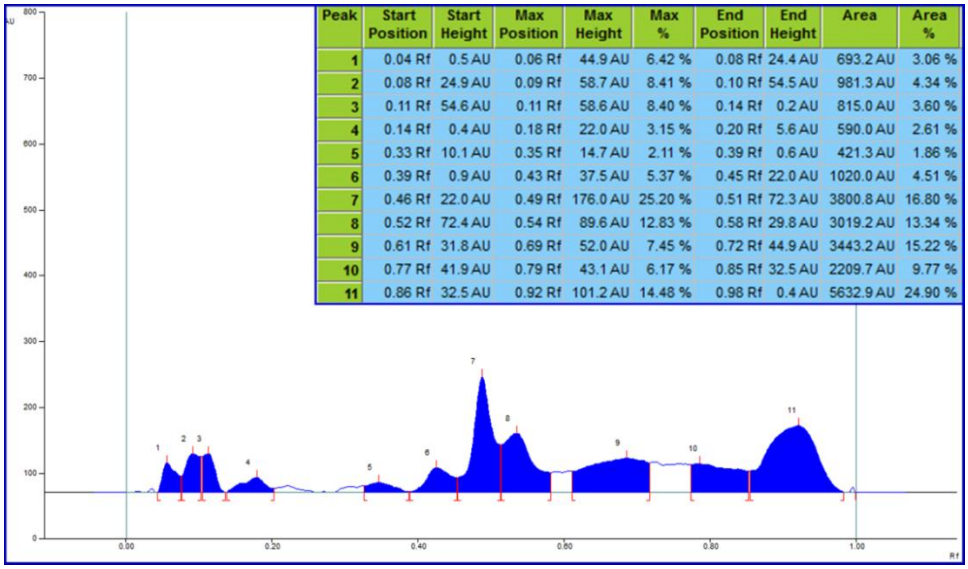
Figure 5: Toluene : Ethyl acetate : Formic acid (9: 1: 0.1) 2 µl
Track 1. Batch - I; Track 2. Batch - II

Thin Layer Chromatography (Alcohol extract)



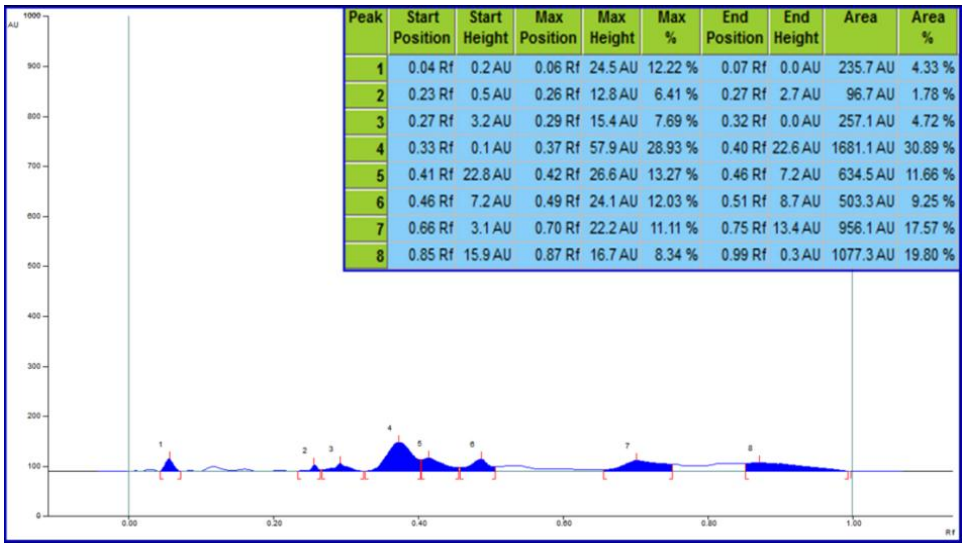
UV – 254 nm **UV – 366 nm** **V - S Reagent**

Figure 6: Toluene : Ethyl acetate : Formic acid (9: 1: 0.1) 5 µl
Track 1. Batch - I; Track 2. Batch - II



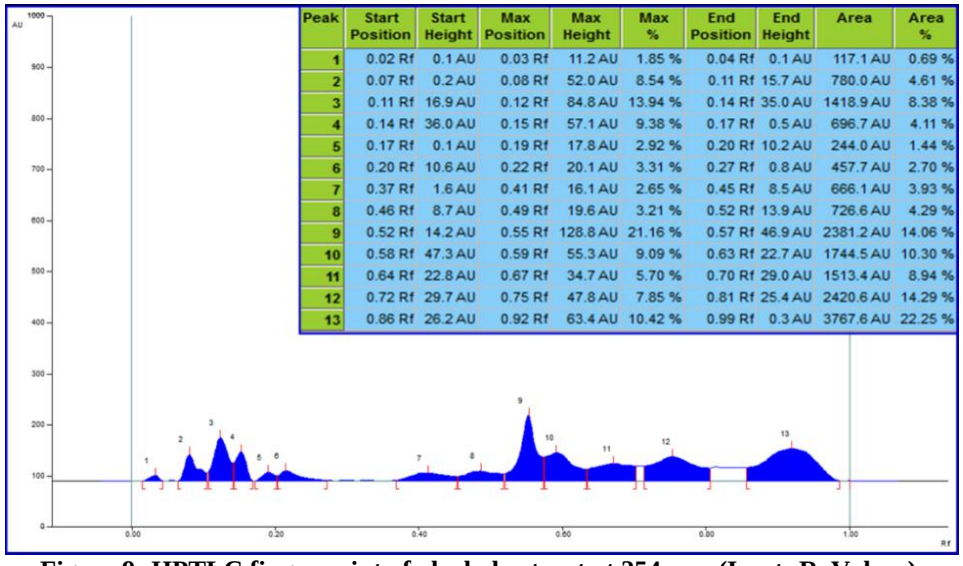
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.5 AU	0.06 Rf	44.9 AU	6.42 %	0.08 Rf	24.4 AU	693.2 AU	3.06 %
2	0.08 Rf	24.9 AU	0.09 Rf	58.7 AU	8.41 %	0.10 Rf	54.5 AU	981.3 AU	4.34 %
3	0.11 Rf	54.6 AU	0.11 Rf	58.6 AU	8.40 %	0.14 Rf	0.2 AU	815.0 AU	3.60 %
4	0.14 Rf	0.4 AU	0.18 Rf	22.0 AU	3.15 %	0.20 Rf	5.6 AU	590.0 AU	2.61 %
5	0.33 Rf	10.1 AU	0.35 Rf	14.7 AU	2.11 %	0.39 Rf	0.6 AU	421.3 AU	1.86 %
6	0.39 Rf	0.9 AU	0.43 Rf	37.5 AU	5.37 %	0.45 Rf	22.0 AU	1020.0 AU	4.51 %
7	0.46 Rf	22.0 AU	0.49 Rf	176.0 AU	25.20 %	0.51 Rf	72.3 AU	3800.8 AU	16.80 %
8	0.52 Rf	72.4 AU	0.54 Rf	89.6 AU	12.83 %	0.58 Rf	29.8 AU	3019.2 AU	13.34 %
9	0.61 Rf	31.8 AU	0.69 Rf	52.0 AU	7.45 %	0.72 Rf	44.9 AU	3443.2 AU	15.22 %
10	0.77 Rf	41.9 AU	0.79 Rf	43.1 AU	6.17 %	0.85 Rf	32.5 AU	2209.7 AU	9.77 %
11	0.86 Rf	32.5 AU	0.92 Rf	101.2 AU	14.48 %	0.98 Rf	0.4 AU	5632.9 AU	24.90 %

Figure 7: HPTLC finger print of Chloroform extract at 254 nm. (Inset: R_f values)



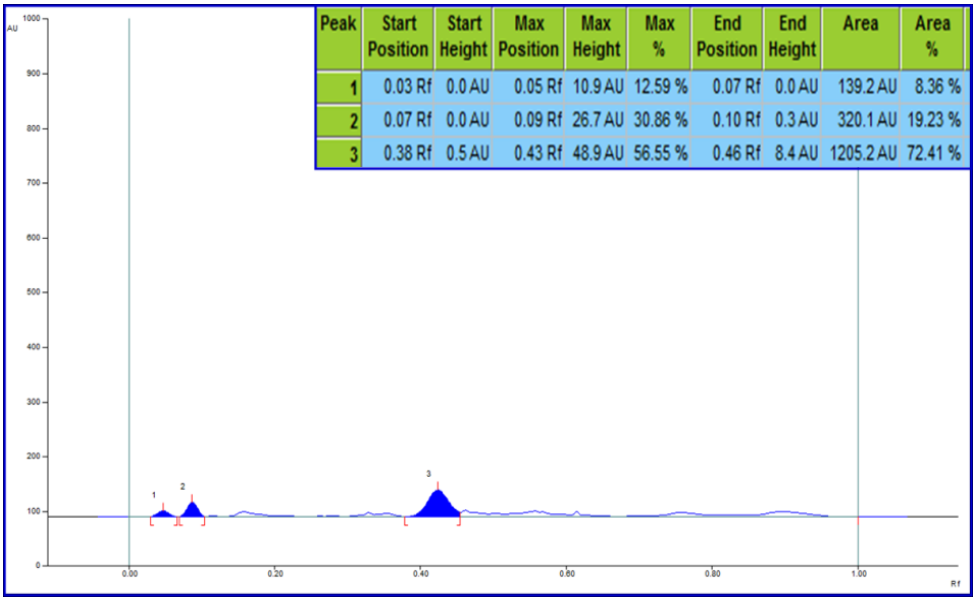
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.04 Rf	0.2 AU	0.06 Rf	24.5 AU	12.22 %	0.07 Rf	0.0 AU	235.7 AU	4.33 %
2	0.23 Rf	0.5 AU	0.26 Rf	12.8 AU	6.41 %	0.27 Rf	2.7 AU	96.7 AU	1.78 %
3	0.27 Rf	3.2 AU	0.29 Rf	15.4 AU	7.69 %	0.32 Rf	0.0 AU	257.1 AU	4.72 %
4	0.33 Rf	0.1 AU	0.37 Rf	57.9 AU	28.93 %	0.40 Rf	22.6 AU	1681.1 AU	30.89 %
5	0.41 Rf	22.8 AU	0.42 Rf	26.6 AU	13.27 %	0.46 Rf	7.2 AU	634.5 AU	11.66 %
6	0.46 Rf	7.2 AU	0.49 Rf	24.1 AU	12.03 %	0.51 Rf	8.7 AU	503.3 AU	9.25 %
7	0.66 Rf	3.1 AU	0.70 Rf	22.2 AU	11.11 %	0.75 Rf	13.4 AU	956.1 AU	17.57 %
8	0.85 Rf	15.9 AU	0.87 Rf	16.7 AU	8.34 %	0.99 Rf	0.3 AU	1077.3 AU	19.80 %

Figure 8: HPTLC finger print of Chloroform extract at 366 nm. (Inset: R_f values)



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.1 AU	0.03 Rf	11.2 AU	1.85 %	0.04 Rf	0.1 AU	117.1 AU	0.69 %
2	0.07 Rf	0.2 AU	0.08 Rf	52.0 AU	8.54 %	0.11 Rf	15.7 AU	780.0 AU	4.61 %
3	0.11 Rf	16.9 AU	0.12 Rf	84.8 AU	13.94 %	0.14 Rf	35.0 AU	1418.9 AU	8.38 %
4	0.14 Rf	36.0 AU	0.15 Rf	57.1 AU	9.38 %	0.17 Rf	0.5 AU	696.7 AU	4.11 %
5	0.17 Rf	0.1 AU	0.19 Rf	17.8 AU	2.92 %	0.20 Rf	10.2 AU	244.0 AU	1.44 %
6	0.20 Rf	10.6 AU	0.22 Rf	20.1 AU	3.31 %	0.27 Rf	0.8 AU	457.7 AU	2.70 %
7	0.37 Rf	1.6 AU	0.41 Rf	16.1 AU	2.65 %	0.45 Rf	8.5 AU	666.1 AU	3.93 %
8	0.46 Rf	8.7 AU	0.49 Rf	19.6 AU	3.21 %	0.52 Rf	13.9 AU	726.6 AU	4.29 %
9	0.52 Rf	14.2 AU	0.55 Rf	128.8 AU	21.16 %	0.57 Rf	46.9 AU	2381.2 AU	14.06 %
10	0.58 Rf	47.3 AU	0.59 Rf	55.3 AU	9.09 %	0.63 Rf	22.7 AU	1744.5 AU	10.30 %
11	0.64 Rf	22.8 AU	0.67 Rf	34.7 AU	5.70 %	0.70 Rf	29.0 AU	1513.4 AU	8.94 %
12	0.72 Rf	29.7 AU	0.75 Rf	47.8 AU	7.85 %	0.81 Rf	25.4 AU	2420.6 AU	14.29 %
13	0.86 Rf	26.2 AU	0.92 Rf	63.4 AU	10.42 %	0.99 Rf	0.3 AU	3767.6 AU	22.25 %

Figure 9: HPTLC finger print of alcohol extract at 254 nm. (Inset: R_f Values).



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	0.0 AU	0.05 Rf	10.9 AU	12.59 %	0.07 Rf	0.0 AU	139.2 AU	8.36 %
2	0.07 Rf	0.0 AU	0.09 Rf	26.7 AU	30.86 %	0.10 Rf	0.3 AU	320.1 AU	19.23 %
3	0.38 Rf	0.5 AU	0.43 Rf	48.9 AU	56.55 %	0.46 Rf	8.4 AU	1205.2 AU	72.41 %

Figure 10: HPTLC finger print of alcohol extract at 366 nm. (Inset: R_f values)

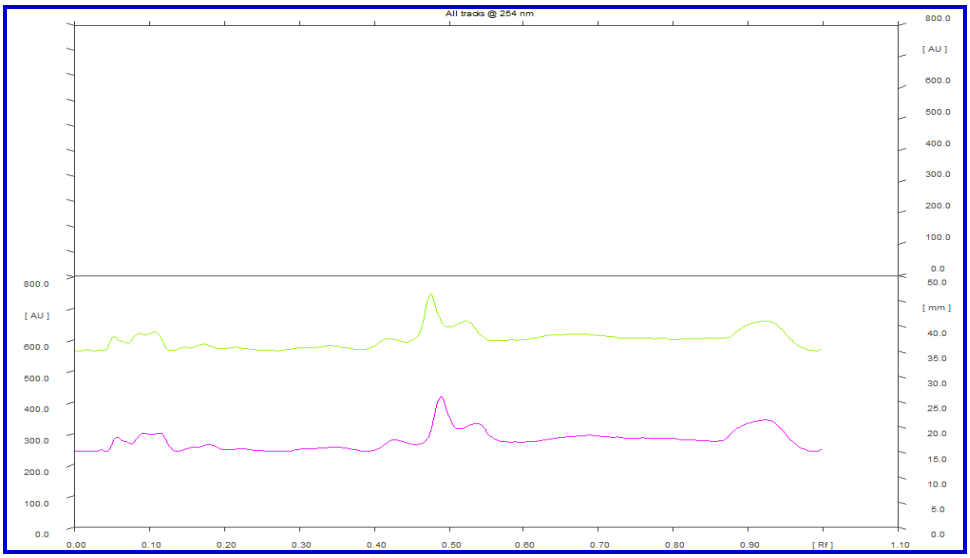


Figure 11: Densitometric chromatogram of Chloroform extract at 254 nm.

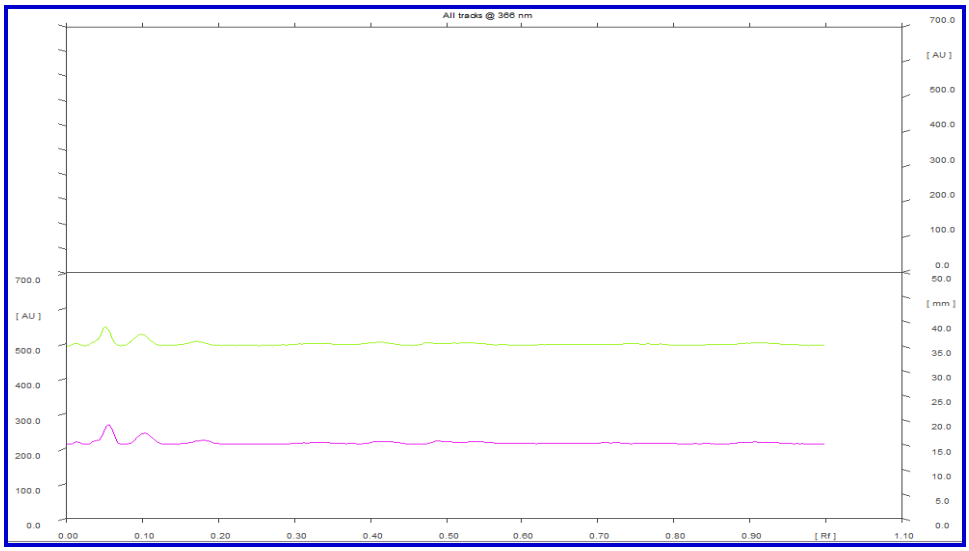


Figure 12: Densitometric chromatogram of Chloroform extract at 366 nm.

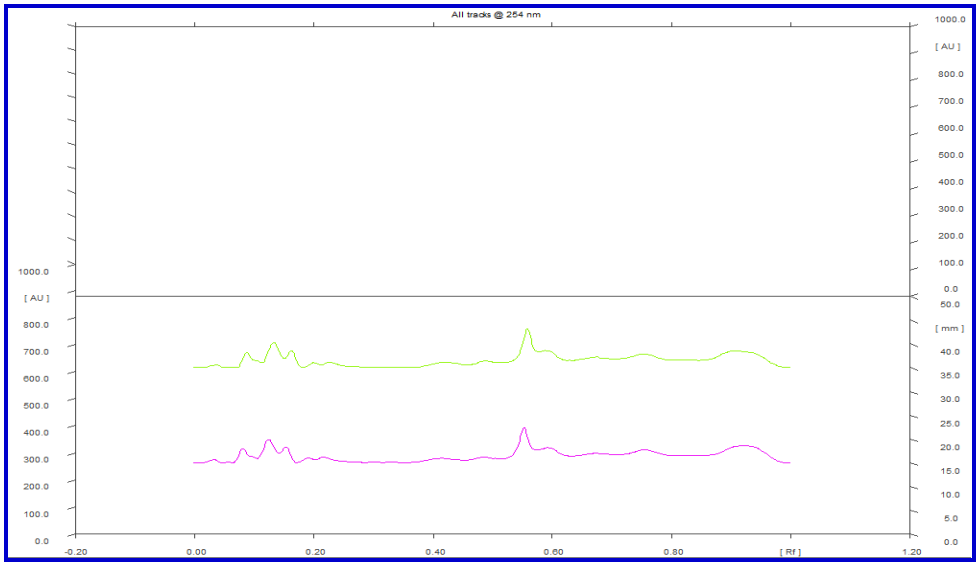


Figure 13: Densitometric chromatogram of alcohol extract at 254 nm.

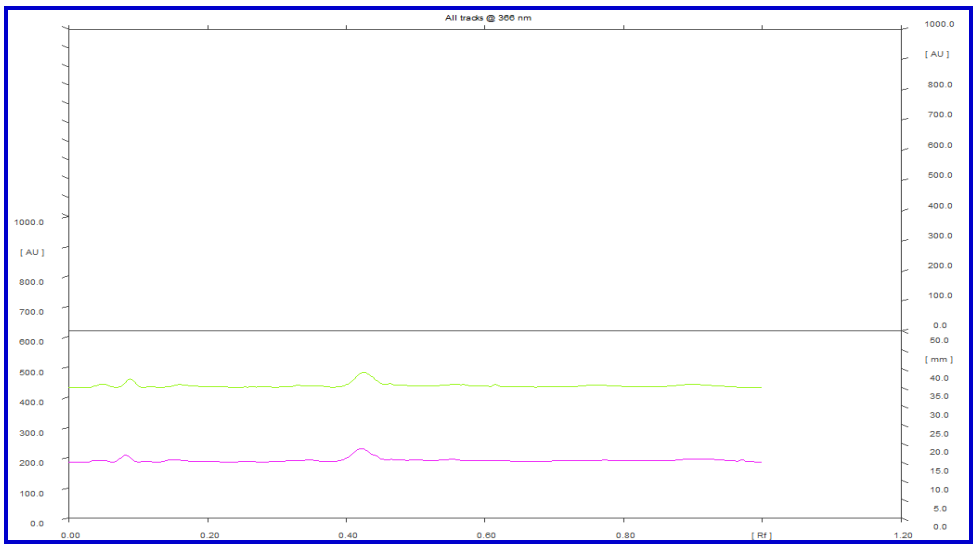


Figure 14: Densitometric chromatogram of alcohol extract at 366 nm.

Pharmacognostical Studies

Pharmacognostic studies helps to determine the substituents, adulterants, commercial varieties and any other quality control of herbal drugs. It is a simple and reliable study, helps to attain in sequence about morphological characters such as macroscopic, microscopic and powder features of herbal drugs. The macroscopic study performs the morphological description of the plant parts which are seen by naked eye or magnifying lens. Microscopic feature is the anatomical study which is done by taking appropriate section of the plant parts whereas powder feature is similar to microscopic studies except here dried powder is taken instead of section of plants. Figure 15 depicted the macroscopic, microscopic and powder features of shalgum seeds. The morphological characters of shalgum seeds were described as follows;

Macroscopic Features: Seeds small, black to brown, spherical, 1.5 to 2mm in diameter; testa thin, brittle, surface minutely reticulate; taste sharp and bitter.

Microscopic Features: T. S. of seed shows embryo and two cotyledons enclosed in a testa. The testa single layered consisting of well developed polygonal tabular cells completely filled brownish content; hypodermis 2-3 layered, consists of large empty and flattened cells, few cells contain oil globules; cotyledons two, large, consists of oval to polygonal, thin-walled parenchymatous cells containing aleurone grains and oil globules; embryo conduplicate, consists of thin-walled parenchymatous cells.

Powder Features: Brownish-yellow; fragments of testa with thin walled large epidermal cells in surface view, thick walled hexagonal filled with brown contents; groups of thick walled endosperm cells; thin walled

cotyledonary parenchyma cells filled with oil globules and aleurone grains.

Quality Control and Quality Assurance Parameters

The quality of herbal drug depends on several factors like environment, collection method, cultivation, harvest, post harvest processing, transport and storage practices. Inadverant contamination by microbial agent and its secondary metabolites during any of the stage can lead to deterioration in safety and quality of herbal drugs. It also raises the inferior quality of herbal product, reduces the therapeutic efficacy and can ultimately cause health hazard to consumer's inspite to cure the disease. In present study, the data of the quality control and quality assurance studies performed on shalgum seed using WHO standard methods are given Table 2. The microbial load was found within the permissible limits. The aflatoxin's toxic contamination estimation showed no detectable levels of any of the toxins in the shalgum seeds (Table 3).

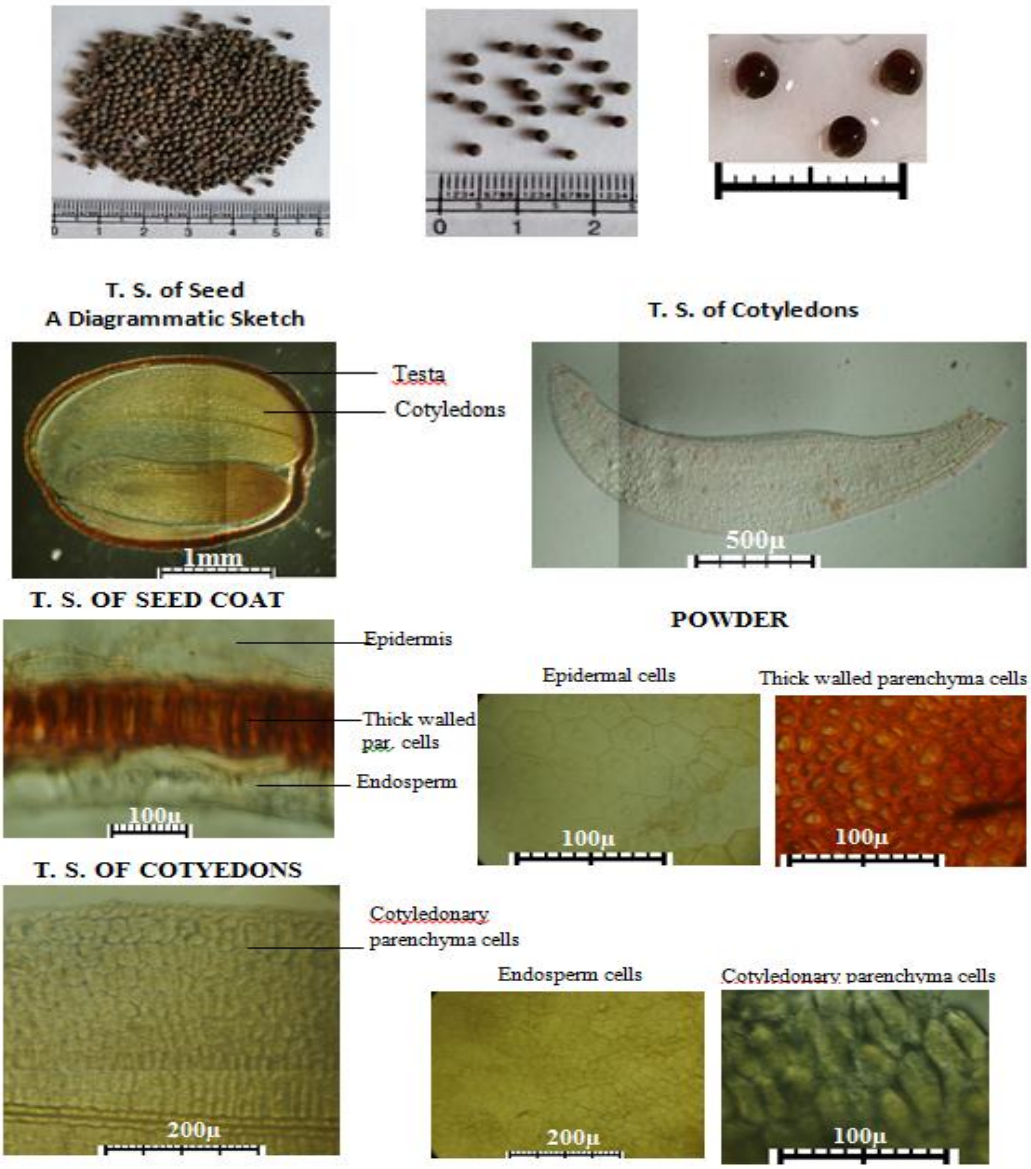


Figure 15: Depicted the macroscopic, microscopic and powder features of shalgum seeds.

Table 2: Estimation of microbial load.

Parameters analyzed	Results
Total microbial plate count	$9x10^4$
Total Yeast & Mould count	$5x10^2$
Escherichia coli	Absent
Salmonella species	Absent
Pseudomonas aeruginosa	Absent
Staphylococcus aureus	Absent

Table 3: Estimation of Aflatoxins.

Parameters analyzed	Results	WHO and API limits
B1	BDL	0.5 ppm
B2	BDL	0.1 ppm
G1	BDL	0.5 ppm
G2	BDL	0.1 ppm

BDL: Below detection limit

CONCLUSION

To afford a guideline towards authentic identification, this paper describes the morphological, anatomical and phytochemical characters of a Unani drug obtained from shalgum seeds available on the local market at Chennai. Various chemical and biological tests like phytochemical screening, physicochemical parameters, finger print profile, pharmacognostic features are reported in order to facilitate the identification of the drug respectively. The total yeast and mould count of shalgum seeds is within the permissible limits of WHO while specific pathogenic bacteria are absent. In Unani medicine, shalgum seeds used in various diseases like Sual (Cough), Laghari (Weakness), Zof-e-Ishtiha (Anorexia), Ehtebas-e-Baul (Anuria), Zof-e-Bah (Sexual debility) and Riqqat-e-Mani (Attenuated semen)

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