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RESEARCH ARTICLE

Physicochemical Properties, Fatty Acid Profile and Estimation of Phospholipids by HPLC-ELSD Method in Seeds of *Abelmoschus esculentus* and *Benincasa hispida*

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Abstract

Background: *Abelmoschus esculentus* (Ae) and *Benincasa hispida* (Bh) are herbal plants has embarked its benefits in ayurveda medicine since history. Chemical compounds present in these two plant parts have numerous health benefits. **Aim:** In the present study seeds of the selected plants Ae and Bh are evaluated for its physicochemical, pharmacognostical, and analyzing fatty acid profile along with estimating important phospholipids such as Phosphatidyl Choline (PC) and Phosphatidyl Ethanolamine (PE) by HPLC-ELSD method. **Methods:** PC and PE are phospholipids which are the important components of cell membrane. PC and PE are involved in cell signaling, brain development, signal transduction, cell to cell communication and used for the synthesis of acetylcholine, which is a neurotransmitter. Hence, estimating PC and PE in the seeds of Ae and Bh will serve as an important data, and which will act as a source of memory enhancing components. Total lipids were extracted from the seeds using chloroform and methanol. The extracted lipids were subjected to column chromatography to estimate PC and PE. Using seed powder physicochemical and pharmacognostical values were evaluated. **Results:** Current study results show optimized physicochemical and pharmacognostical values. Fatty acids such as linoleic, palmitic and oleic acids make up the higher percentage. Importantly we could be able to estimate PC and PE by proposed HPLC-ELSD method, in Ae account for 24.6 % (PC) and 75.4% (PE), but unfortunately, we could not be able to estimate phospholipids in Bh by proposed method. **Conclusion:** The obtained results will serve as a parameter for the standardization of seeds and fixed oil obtained from seeds, with the estimation of phospholipids, which can be utilized as a potential source in the treatment of dementia, Alzheimer's disease, Parkinson disease *etc*.

Keywords: *Abelmoschus esculentus, Benincasa hispida*, Dementia, Fatty Acid, Phosphatidylcholine, Phosphatidylethanolamine

Abbreviations: Ae-*Abelmoschus esculentus;* Bh-*Benincasa hispida;* ELSD-Evaporative Light Scattering Detector; FID-Flame Ionization Detector; FAMEs-Fatty Acid Methyl Esters; GC-Gas Chromatography; HPLC-High Performance Liquid Chromatography; HCL-Hydrochloric acid; KOH-Potassium Hydroxide; LOD-Loss On Drying; PC-Phosphatidylcholine; PE-Phosphatidylethanolamine; SAP-Saponification

1. Introduction

The medicinal values of plants and various parts have been described in literature since many years. Literature gives data of usage of these herbal medicines in various diseases. Compared to synthetic drugs, plant based natural drugs are used on a large scale due to their safety and efficacy. Different parts of plants consist of enormous chemical components which are beneficial to human health. Nutritional components play a vital role in maintaining and promoting health. However, plants are not only consumed in various delicacies but also have popularity for nutraceutical and pharmaceutical purposes. Among the various

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medicinal plants, *A. esculentus* and *B. hispida* are the important herbal plants used to treat many diseases.

Abelmoschus esculentus (Figure 1) belongs to the family *Malvaceae* and is also called as Okra, Bendi and Lady's finger and its scientific classification is given in Table 1. Ae fruits, leaves, seeds and roots are commonly used, and it is a popular health food due to its high vitamin C, fiber, folate and antioxidant contents. It is a good source of inorganic elements such as potassium and calcium. Phytochemicals such as flavonoids, phenols, saponins, carbohydrates, alkaloids, terpenoids, steroids and myricetin are present and may contribute to its hepatoprotective, diuretic, anti-oxidant and anti-proliferative activities. Ae seed contains moisture, lipid, proteins, starch, resins, gums, a-sitosterol, a-D-glucoside, and volatile oil.



Figure 1. *Abelmoschus esculentus.*

Abelmoschus esculentus possesses various biological activities such as antispasmodic, diaphoretic, diuretic, demulcent, emollient, vulnerary and as a stimulant. The roots juice used externally to treat boils, cuts, and wounds in Nepal. The seeds have been traditionally used for neurodegenerative disorders, anti-hysteric, rheumatism, diuretic, nervous debility and cystitis[1,2].

Table 1. Scientific classification of *A. esculentus* and *B. hispida*

Botanical Name	*A. esculentus*	*B. hispida*
Synonym	*Hibiscus esculentus*	*Cucurbita hispida*, Ash gourd,
Kingdom	*Plantae*	*Plantae*
Subkingdom	*Tracheobionta*	*Tracheobionta*
Division	*Magnoliophyta*	*Magnoliophyta*
Class	*Magnoliopsida*	*Equisetopsida*
Order	*Malvales*	*Cucurbitales*
Family	*Malvaceae*	*Cucurbitaceae*
Genus	*Abelmoschus medik*	*Benincasa savi*

Benincasa hispida (Figure 2) is commonly called as ash gourd or wax gourd belongs to the family *Cucurbitaceae*. Scientific classification is given in Table 1. In *ayurveda* is known as *kushmanda* and helps to manage blood disorders, *vata* and *pitta*. It is cool in nature that helps to decrease *pitta* in the body. It is widely used in India as a vegetable, recognised for its numerous health benefits. Leaves, seeds, pulp, fruit and fruit rind are used. Chemically *B. hispida* is composed of flavonoids, polysaccharides, volatile oils, protein, carotenes, vitamins and minerals. Seeds of the fruit contains fiber, saponin, urea, citrulline, fatty acids, steroids, triterpenoids, minerals, water-soluble polysaccharides, sugars, and organic acids including malic acid and citric acid, as well as vitamin C, thiamine, riboflavin, niacin, and vitamin E[3]. Phytochemical composition of *B. hispida* includes water, proteins, carbohydrates, fat, fibers and ash[4]. It is rich in antioxidant[5] and proved its effectiveness in many diseases. It acts as anti-inflammatory, anti-microbial, anti-bacterial, anti-fungal, anthelmintic and also as antilarval[6-9]. It also proved it effect as an anti-cancer agent, useful in treating metabolic diseases such and diabetes and obesity. Several studies showed effects of *B. hispida* is useful in treating neurological disorders[10,11].



Figure 2. *Benincasa hispida.*

To rule out memory related problems, many compounds are investigated for its potency and efficacy. Phospholipids play a major role in treating neurological disorders. Phosphatidylcholine and phosphatidylethanolamine are the important component of cell membrane. PC and PE

are involved in cell signalling, brain development, signal transduction, cell to cell communication and used for the synthesis of acetylcholine, which is a neurotransmitter. In our present research, seeds of *A. esculentus* and *B. hispida* are selected, and analysed for its physicochemical, pharmacognostical, fatty acid composition and estimation of important phospholipids such as phosphatidylcholine and phosphatidylethanolamine. Goal is to obtain the informative profile about the chemical nature of the Ae and Bh, seed oil. The results obtained will serve as parameters for the standardization of seeds and fixed oil obtained from seeds, Furthermore, results will show its potential role as a nutraceutical and economical utilisation of Ae and Bh seeds as a new source of edible oils and may be utilized for the treatment of Alzheimer's disease, dementia and Parkinson disease *etc.*

2. Materials and Methods

Abelmoschus esculentus and *B. hispida* seeds were provided as a gift sample by JSS Ayurveda Medical College and Hospital, Mysuru, Karnataka.

Standards used for phospholipids identification, phosphatidylcholine and phosphatidylethanolamine were purchased from Sigma Aldrich. Chemicals used for estimation were available in laboratory. Schematic representation of the method followed is shown below in the Figure 3.

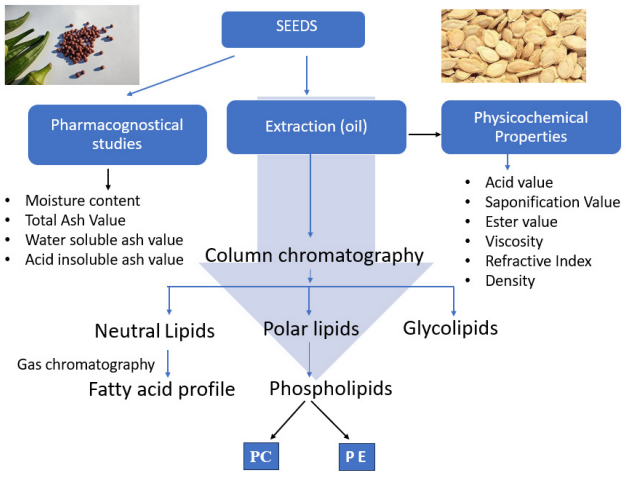


Figure 3. Schematic representation of methodology.

2.1 Pre Extraction

Seeds of *A. esculentus* and *B. hispida* were subjected to powder using mixer grinder and stored in air tight container for further studies. Seed powder was used to conduct experiments for its pharmacognostical and physicochemical evaluations.

2.2 Moisture Content Analysis

To determine moisture content, Loss on Drying (LOD) of the seed sample need to be performed. The mass upon loss on drying is measured as a percentage of moisture weight per weight. Water and volatile materials in the raw drugs are identified by the test loss on drying. Crude drugs inevitably contain moisture, which must be removed as much as possible. Procedure followed as per the guidelines provided by the Indian Pharmacopoeia[12]. The following calculation was used to compute the percentage of loss due to drying.

$$\%\text{loss on drying} = \frac{\text{loss in weight of sample} \times 100}{\text{Weight of sample}}$$

2.3 Determination of Ash Value

A crude drug's quality and purity can be assessed using its ash value, especially when it is in powdered form. When burned, crude phytopharmaceuticals typically leave an ash that is composed of sodium, potassium, calcium and magnesium silicates, carbonates, and phosphates. The amount of ash in a crude herbal drug indicates how well it was prepared. Particularly in situations when silica may be present or when the drug's calcium oxalate concentration is extremely high, a greater limit of acid- insoluble ash is applied[13].

2.3.1 Determination of Total Ash Value, Water–soluble Ash Value, Acid-insoluble Ash Value

Procedures were carried out according to the method quoted in quality control methods for medicinal plant materials by WHO[13]. Formula A, B, C mentioned below was used to determine the percentage of ash value, water-soluble and acid- insoluble ash value in relation to the air-dried sample.

A. $$\%\text{ Total ash value} = \frac{\text{weight of total ash} \times 100}{\text{Weight of crude drug taken}}$$

B. $$\% \text{ water soluble ash value} = \frac{\text{weight of total ash-weight of water insoluble ash} \times 100}{\text{Weight of crude drug taken}}$$

C. $$\% \text{ Acid insoluble ash value} = \frac{\text{weight of acid insoluble ash} \times 100}{\text{Weight of crude drug taken}}$$

2.4 Total Lipid Extraction

The seed powder was extracted with a mixture of chloroform and methanol in 2:1 (vol/vol) ratio. The mixture was magnetically stirred for about 4 hours and filtered to separate the seed material. The filtrate was washed in water and the organic phase was separated, and it was dried on anhydrous sodium sulphate. Solvent was evaporated to obtain the lipid extract (oil) which was stored in a refrigerator until further analysis[14]. Schematic representation of the total lipid extract is shown in the Figure 4.

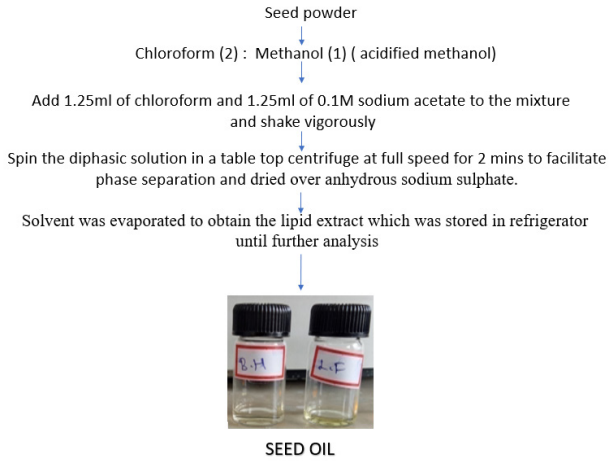


Figure 4. Schematic representation of total lipid extraction.

2.5 Determination of Acid Value and Saponification Value

As per the guidelines mentioned in Indian pharmacopoeia, ash value and saponification value were determined.

2.6 Ester Value

The ester value is the quantity potassium hydroxide needed to saponify 1.0g of the substance's esters in mg. The difference between the saponification and the acid value represents the ester value.

Ester value = saponification value – acid value

2.7 Refractive Index

The ratio of the sin of the angle of incidence to the sin of the angle of refraction of a light beam travelling from air into the substance is known as the refractive index (n) of that substance with respect to air. It changes depending on the wavelength of the light used to measure it.

Unless otherwise stated in the specific monograph, the refractive index, n^{20}_{D} determined at 20°±0.5° in relation to the sodium D-line's wavelength (λ=589.3nm). Due to the fact that the temperature has considerable effects on the refractive index, the temperature needs to be carefully regulated and maintained. For most of the refractive index measurements, the Abbe refractometer is practical, but alternative refractometers with similar or higher precision may be employed[12]. First turn on the light and calibrate the instrument using water. Adjust the refractometer scale knob to get the clear interface between the illuminated and dark regions. Note down the index of refraction using telescope scale. Repeated thrice to get the concordant value.

2.8 Viscosity

The temperature of the substance being examined must be precisely regulated since even tiny temperature variations can result noticeable changes in viscosity. The temperature should be maintained to within ±0.1°C for usual pharmaceutical purposes.

Spindle viscometer: The spindle viscometer rotates a spindle that is submerged in the liquid to measure viscosity. Utilising conversion factors from the scale reading at a specific rotational speed, one can directly derive relative values of viscosity or apparent viscosity. To measure viscosity, first the spindle was cleaned, then attached to the instrument. Spindle should rotate in the oil sample until constant reading displaced on the viscometer. Repeat the same way for three times and to find out the average value.

2.9 Density

A substance's density, or more specifically, its volumetric mass density is, its mass per unit volume. Density is most frequently represented by the symbol D; however Latin letter D may also be used. Density is mathematically, defined as mass divided by volume[12]. Measure the volume of the oil in millilitres and weigh

the oil in grams. Now divide the mass in grams by the volume in millilitres to arrive at the density of oil. Repeat 3 times in a same way.

2.10 Column Chromatography to Determine Composition of Lipid Classes in the Extracted Lipid Sample

Column chromatography was performed following a reported protocol. By using silica gel column chromatography, the extracted lipids were segregated into neutral lipids, glycolipids and phospholipids. The sample was loaded on to silica gel bed and was successively eluted with the solvents chloroform to obtain neutral lipid, acetone to obtain glycolipid and methanol to obtain the phospholipid fractions[15,16].

2.11 Gas Chromatography to Determine Fatty Acid Composition of Extracted Lipid

Fatty acid composition by gas chromatography: Gas Chromatography procedure (GC) was used to identify the lipids fatty acid content. According to published research, the lipid sample was transformed into fatty acid methyl esters using the methanol-sulphuric acid (2% v/v) reagent. An Agilent 6890 gas chromatography connected to a Flame Ionisation Detector (FID) outfitted with a DB 225 capillary column (30m × 0.25 mm × 0.25 µm) (J and W scientific, USA) was used to conduct the GC analysis of the Fatty Acid Methyl Esters (FAME). The scheduled column temperature was 2 mins at 160°C, 5 minutes at 230°C, and 20 minutes at 230°C. The split ratio was 10:1, with the injector temperature being 230°C. N_2 was used as the carrier gas, with a flow rate of 1ml/min. The flow rates of nitrogen and air were 300 ml/min and 30 ml/min, respectively, and the detector temperature was 270°C. By comparing the retention durations with a combination of standard FAMEs, C4-C24 (Supelco, USA), the fatty acids were identified. Every FAME sample was examined twice, and average results are shown[17].

2.12 HPLC- ELSD Method to Determine Phospholipid Classes

For the current study, the method done by Donato *et al* followed with slight modifications[18]. The phospholipid fractions were qualitatively analyzed by HPLC. The HPLC analysis was performed on an Agilent-1260 infinity series chromatograph equipped with a quaternary pump and an evaporative light scattering detector (1260 infinity). The temperature of the ELSD was set at 100°C and the nebulizer temperature was set at 80°C with nitrogen gas flow rate at 2.7 lit/min. The samples of phospholipids were prepared in 1mg/ml concentration and were separated on a Kimtex Hilic column (50 × 4.6 mm; Phenomenex columns) at a mobile phase flow rate of 1 ml/min. The components of phospholipids were eluted within 20 minutes using a binary gradient system mobile phase. A binary gradient mobile phase system composed of eluent A (water) and eluent B (acetonitrile) was used for elution. Initially the elution was performed with 5% water in acetonitrile which was changed to 50% water in acetonitrile in 15 min and finally restored to 5% water in acetonitrile at 20 min. The identification of phospholipids was carried out by comparing the retention times of the respective commercial standards.

3. Results

Table 2. Pharmacognostical and physicochemical analysis report

Experiment	*A. esculentus*	*B. hispida*
Total yield (%)	13	18.9
Color	Light yellow	Pale yellow
Density	90 ± 0.005	28.15 ± 0.07
Acid value	0.76 ± 0.01	1.17 ± 0.01
Sap value	164.93 ± 0.04	154.83 ±0.78
Ester value	164.17 ± 0.21	153.66 ± 0.08
Refractive index	1.463 ± 0.002	1.462 ± 0.17
Viscosity	28.15 ± 0.07	33.29 ± 0.01
Moisture content (%)	10.5 ± 0.26	5.5 ± 0.1
Total ash (%)	8.45 ± 0.01	5 ± 0.57
Water soluble ash (%)	4.47 ± 0.08	1 ± 0.5
Acid insoluble ash (%)	0.2 ± 0.1	0.66 ± 0.01

Table 3. Composition of lipid classes in the seed oil

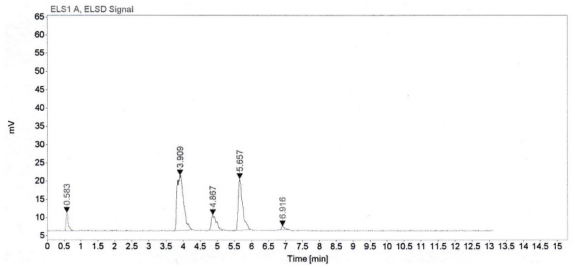
	Neutral Lipid [g, (%)]	Polar Lipid [g, (%)]
A. esculentus	4.35 (87%)	0.65 (13%)
B. hispida	100 %	0

Table 4. Fatty acid composition neutral lipids of the seed oil

Fatty Acid Compound	A. esculentus Amount (%)	*B. hispida* Amount (%)
Myristic acid (14:0)	0.35	-
Palmitic acid (16:0)	32.81	10.5
Palmitoleic acid (16:1)	0.5	-
Stearic acid (18:0)	3.96	6.3
Oleic acid (18:1)	24.77	7.3
Linoleic acid (18:2)	36.73	75.6
Linolenic acid (18:3 ALA)	0.26	.
Arachidic acid (20:0)	0.49	.
Eicosenoic acid (20:1)	0.1	.

Table 5. Phospholipid composition (%) of the polar lipid of the seed oils

Seed Oil	Phosphatidyl Choline (PC)	Phosphatidyl Ethanolamine (PE)
A. esculentus	24.6	75.4



Signal: ELS1 A, ELSD Signal

RT [min]	Type	Width [min]	Area	Height	Area%	Name
0.583	BB	0.0577	22.4595	5.0159	5.6960	
3.909	MM R	0.2182	198.7185	15.1785	50.3971	
4.867	MM R	0.1674	43.2442	4.3043	10.9672	
5.657	MM R	0.1460	123.5903	14.1060	31.3438	
6.916	MM R	0.0976	6.2931	1.0745	1.5960	
		Sum	394.3055			

Figure 5. Chromatogram of *A. esculentus* seed oil.

4. Discussion

The extracted seed oil of *A. esculentus* and *B. hispida* was light and pale yellow in colour, and yield was about 13% and 18.9%, respectively. Physico-chemical analysis of the seeds for loss on drying, total ash value, soluble ash value, acid insoluble ash value was analysed. Later extracted seed oil was analysed for its density, acid value, sap value, ester value, refractive index and viscosity. All the values are tabulated in Table 2. By column chromatographic method, lipid classes were estimated. It was found to contain more of neutral lipids (94.6%) than glycolipids (5.4%) in *A. esculentus* seed oil, whereas in *B. hispida* seed oil only neutral lipids were found (100%). By this column chromatographic method, the polar lipids in *B. hispida* seed oil were couldn't be able to obtained. The composition of the lipid classes is tabulated in Table 3. Chromatogram of *A. esculentus* seed oil is shown in the Figure 5.

Table 4 reports the fatty acid composition of the oil. Nine fatty acids were detected in the extracted seed oil of *A. esculentus* ranging from C14:0 to C20:1 where as in *B. hispida* only 4 fatty acids were found ie., C16:0, C18:0, C18:1, C18:2. Among the fatty acids, the predominant constituent was found to be linoleic acid and palmitic acid followed by oleic acid. Compared to *A. esculentus* (36.73%), *B. hispida* (75.6%) has the highest percentage of linoleic acid. This study proves that, both the oils are rich in unsaturated fatty acids compared to saturated fatty acids. But in *B. hispida* saturated fatty acid percentage is very less (10.5%).

Fatty acid composition in this current study is like those found in earlier studies done by Ramadan *et al*[19]., Sengupta and Bhargava[20]. However, study by Sengupta and Bhargava and Sengupta *et al.*, the report found to be palmitic acid (31.26% and 32.8%) as the major fatty acid, but the current study found to contain linoleic acid (*A. esculentus* - 36.75%, *B. hispida* -75.6%) to be the major fatty acid. Remarkably, arachidic acid and eicosenoic acid were found to be present in *A. esculentus* oil, which was not estimated in the *B. hispida* oil and also in previous research studies for other oils.

Further estimation of phospholipids was done by HPLC-ELSD method. This method successfully estimated phosphatidylcholine and phosphatidylethanolamine in *A. esculentus* oil, but unfortunately this method failed to estimate in *B. hispida* oil. In *A. esculentus* oil, it contains more of phosphatidylethanolamine which is about 75.4% and phosphatidylcholine about 24.6 % (Table 5). The PC and PE plays a vital role in treating many neurological disorders like Alzheimer's disease, Parkinson's disease, dementia *etc*. PC especially has been proven to be antioxidant agent and useful in treating of neurological diseases and providing lifelong memory enhancing characteristic.

5. Conclusion

The current study gives results of pharmacognostical evaluation and physicochemical data of *A. esculentus* and *B. hispida*. Major fatty acid composition was found to be linoleic acid, palmitic acid and oleic acid. Bh has the highest percentage of linoleic acid. The composition of phospholipids gives a good data for future studies and this seed can be utilised for research purpose related to many therapeutic activities involved the role of phospholipids as mentioned in the introduction part specifically treating neurological disorders. Further research may be needed to estimate phospholipids using different method in *B. hispida* seed oil, as the current method failed to estimate it.

6. Acknowledgement

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7. Reference

1. Jain N, Jain R, Jain V, Jain S. A review on: *Abelmoschus esculentus*. Pharmacia. 2012; 1(3):1-8.
2. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. The quarterly review of biology (QRB). Stony Brook University. 1958; 33(2):156. https://doi.org/10.1086/402350
3. Al-Snafi AE. The pharmacological importance of *Benincasa hispida*- A review. Int J Pharm Sci Res. 2013; 4(12):165-170.
4. Pagare MS, Patil L, Kadam VJ. *Benincasa hispida*: A natural medicine. Res J Pharm Technol. 2011; 4:1941-1944.
5. Islam MT, Quispe C, El-Kersh DM, Shill MC, Bhardwaj K, Bhardwaj P, *et al.* A literature-based update on *Benincasa hispida* (Thunb.) Cogn.: Traditional uses, nutraceutical, and phytopharmacological profiles. Oxid Med Cell Longev. 2021; p. 1-19. https://doi.org/10.1155/2021/6349041
6. Samad NB, Debnath T, Jin HL, Lee BR, Park PJ, Lee SY, Lim BO. Antioxidant activity of *Benincasa hispida* seeds. J Food Biochem. 2013; 37:388-395. https://doi.org/10.1111/j.1745-4514.2011.00643.x
7. Gill NS, Dhiman K, Bajwa J, Sharma P, Sood S. Evaluation of free radical scavenging, anti-inflammatory and analgesic potential of *Benincasa hispida* seed extract. Int J Pharmacol. 2010; 6(5):652-657. https://doi.org/10.3923/ijp.2010.652.657
8. Qadrie ZL, Anandan R, Mushtaque M, Kumar KA, Ashraf H. Anthelmintic and anticonvulsant studies of ethanolic extract of *Benincasa hispida* seeds. Pharmacology. 2011; 2:1298-12302.
9. Ota E, Tsuchiya W, Yamazaki T, Nakamura M, Hirayama C, Konno K. Purification, cDNA cloning and recombinant protein expression of a phloem lectin-like anti-insect defense protein BPLP from the phloem exudates of the wax gourd, *Benincasa hispida*. Phytochem. 2013; 89:15-25. https://doi.org/10.1016/j.phytochem.2013.01.013
10. Aloizou AM, Siokas V, Pateraki G. Thinking outside the ischemia box: Advancements in the use of multiple sclerosis drugs in ischemic stroke. J Clin Med. 2021; 10(4):630. https://doi.org/10.3390/jcm10040630
11. Ambikar D, Mohanta G. Effect of dried fruit extract of *Benincasa hispida* on brain behavior in laboratory animals. Cell Tissue Res. 2013; 13(2):3519- 3524.
12. Govt. of India. Ministry of Health and Family welfare. Indian Pharmacopoeia Ghaziabad: The Indian pharmacopoeia commission; 2019; volume 2.
13. World health quality control methods for medicinal plant materials. World Health Organization. 1998: p. 115.
14. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957; 226(1):497-509. https://doi.org/10.1016/s0021-9258(18)64849-5
15. Rouser G, Kritchevsky G, Yamamata A. Column chromatographic and associated procedures for separation and determination of phosphatides and glycolipids. Lipid chromatographic Analysis.1967; (1):99–161.
16. Kielbowicz G, Trziszka T, Wawrzenczyk C. Separation and quantification of phospholipid and neutral lipid classes by HPLC–CAD: Application to egg yolk lipids. J Liq Chromatogr R T. 2015; 38(8):898–903. https://doi.org/10.1080/10826076.2014.991869
17. Rao KS, Chakrabarti PP, Rao BVSK, Prasad RBN. Phospholipid composition of *Jatropha curcus* seed lipids. J Am Oil Chem Soc. 2009; 86(2):197–200. https://doi.org/10.1007/s11746-008-1325-8
18. Donato P, Cacciola F, Cichello F, Russo M, Dugo P, Mondello L. Determination of phospholipids in milk samples by means of hydrophilic interaction liquid chromatography coupled to evaporative light scattering and mass spectrometry detection. J Chromatogr A. 2011; 1218(37):6476-6482. https://doi.org/10.1016/j.chroma.2011.07.036
19. Ramadan MF, Sharanabasappa G, Seetharam YN, Seshagiri M, Moersel J-T. Profile and levels of fatty acids and bioactive constituents in mahua butter from fruit-seeds of Buttercup tree [*Madhuca longifolia* (Koenig)]. Eur Food Res Technol. 2006; 222:710–718. https://doi.org/10.1007/s00217-005-0155-2
20. Sengupta, A., Bhargava, H.N. Investigation of the seed fat of Celastrus Panzculatus. J Sci Food Agric. 1970; 21:628–631. https://doi.org/10.1002/jsfa.2740211207