



PHYSICOCHEMICAL CHARACTERIZATION OF *SESBANIA* *BISPINOSA* SEEDS OIL

¹*Zafar Iqbal, ²Razia Kalsoom, ¹M. Usman Sabri, ¹Mubeen Akhtar, ³Kashfa Rauf,
³Nadia Arshad and ³Iqra Zafar

¹Applied Chemistry Research Chemistry Research Centre, PCSIR Laboratories Complex,
Lahore.

²PCSIR Laboratories, Islamabad.

³Department of Chemistry, University of Education Township, Lahore.

Article Received on 06/10/2023

Article Revised on 27/10/2023

Article Accepted on 17/11/2023



***Corresponding Author**

Zafar Iqbal

Applied Chemistry
Research Chemistry
Research Centre, PCSIR
Laboratories Complex,
Lahore.

ABSTRACT

Oil was extracted from the seeds of *Sesbania bispinosa* by soxhlet apparatus using *n*-hexane as a solvent. The oil yield was 6.0%. The fatty acid composition of seed oil was analyzed by Gas Chromatography coupled with Flame Ionization Detector. The investigation showed that the seedoil contained linolenic acid as major fatty acid as it contributes 44.55% to total fatty acids. Physicochemical parameters of oil were Acid Value 2.8, Free Fatty Acids 1.4, Peroxide Value 1.8, Iodine Value 130, Saponification Value 115, Unsaponifiable Matter 1.23 and Refractive Index Value 1.4678. It was concluded that

Sesbania bispinosa seed oil is useful in cosmetics and soap industry.

KEYWORDS: The investigation showed that the seedoil contained linolenic acid as major fatty acid as it contributes 44.55% to total fatty acids.

INTRODUCTION

Oils obtained from different seeds contain different nutritional composition. In order to evaluate different characteristics of seed oil, the study of their individual constituents is compulsory (Michael et al., 2014). The shelf life of oils depends on the method of handling, storing and processing (Amoo et al., 2006). Oils are currently used as renewable energy

sources for the biodiesel manufacturing, in addition to their usage as food, which emphasizes the importance of characterization and quality analysis of plant oils (Shimamoto et al., 2015). The physical and chemical behavior of the fat and oil is affected by type of fatty acid at each position (Devi & Khatkar, 2016). The overall performance and stability and functional properties of commercial fats and oils depend upon relative proportions of every triacylglycerol in the fat and oil (Deman & Beers, 1987). The fatty acid profiles gives information about nutritional quality and performance of raw material for industrial uses. The chemical composition and structural features decides the functionality and significance of fats and oils. (Belton, 2000). According to clinical studies, intake of trans-fatty acids (TFA) causes heart problems (Tarrago et al., 2006).

Fats and oils are used not only for foods but also in the formation of soaps, detergents, varnishes, printers inks, leather goods etc. glycerin which is a by-product of soap industry is used in the formation of nitroglycerine and some other explosives (Harding, 1942).

Maximum species of *Sesbania bispinosa* are fragile and grow in agricultural field, wastelands and wet ditches along roads. The habitats of *Sesbania* species include marshes and riverine jungles, wet margins of ponds and canals and sometimes forests and grasslands, seasonally flooded valley bottoms and riverbanks (Ndungu et al., 1994). It's a low-growing, erect annual shrub with thick stems. The height of this shrub can reach up to 1-2 meter and above. The stems yield a strong, long-lasting fiber that is utilized in the paper industry (Pugalenti et al., 2004). In South Africa, it is planted as a green manure (providing 150 kg N /hec).

It is stable at higher temperatures ranges i.e., 36-44°C and high soil alkalinity ranges of pH i.e 10 (Khan & Zaki, 2019). It grows well in un-irrigated and waterlogged conditions (Anita et al., 2009). In tropical and subtropical regions there are 50 natural species of the genus *Sesbania* (Swami et al., 2012). *Sesbania bispinosa* is a reportedly common *Sesbania* weed in various tropical countries (Kamoshita et al., 2014). The plant contains anti-oxidant compounds therefore precisely used in cosmetics and pharmaceutical industries. (Larkem et al., 2021).

In Pakistan and India it is commonly known as jantar and dhaincha. Manila agath and new dhaincha are the trivial names of *Sesbania bispinosa* in Nepal. People in Thailand called it as girkhe dhaichaa. 'Sano' is a nickname given by Africans. In Orissa it is recognized as Banicha. Indonesians use Kayu ambun for it (Grosvenor et al., 1995). *Sesbania aculeate* and *Coronella aculeate* are synonyms of *Sesbania bispinosa* (Misra & Siddiqi, 2005). The

morphology of *Sesbania bispinosa* under scanning electron microscope (SEM) showed that the seeds are cylindrical to cylindrical-oblong in shape and seed length varies from 2 to 5 mm. Seed color of *Sesbania bispinosa* is olive-green, pale brown or greenish-black (Chanda et al., 2018).

The current study was designed to extract oil contents from the seeds of *sesbania bispinosa* and their physicochemical studies.

MATERIALS AND METHODS

Oil Extraction

Seeds of *Sesbania bispinosa* were collected, washed, spread on the paper and allowed to dry under fan. After drying, the seeds were transfer to domestic grinder and were ground to powder. In soxhlet extractor about 100 g of powdered seeds were extracted with 0.5 L of *n*-hexane until the green color of seeds faded and the colorless hexane returned to the round bottom flask. For the entire duration *n*-hexane condensation speed was fixed. After extraction the distillation recovers the hexane from the oil. After that the pure solvent was collected in an extractor and remove cautiously. The extracted oil was then dried using anhydrous Na₂SO₄ (Parveen & Rauf, 2008). Oil % yield was calculated using formula. (Pant et al., 2011; Warra et al., 2011).

Estimation of fatty acids of *Sesbania bispinosa* seed oil

Esterification of the extracted oil was carried out by following the procedure of Saeed et al., 2017. Briefly, 2mL of oil was taken in a test tube and 1mL of boron trifluoride was added along with 5mL of methanol. It was tightened and heated on water bath for four hours. Then layer was separated by using 10 mL *n*-hexane three times by using separating funnel. Excess *n*-hexane got evaporated by placing the mixture on hot plate which leaves the esterified fatty acids behind. Then these esterified FAs were analyzed using Gas Chromatography combined with flame ionized detector to evaluate the fatty acids composition (Hartman, 1973).

Saponification Value and Unsaponifiable Matter Determination

Accurately 2.0 g of oil was weighed in a pre-weighted 250-mL flask. 25 mL 0.5N alc. KOH was added. The solution was gently and steadily boiled in an air condenser for about 30 min until saponified. the inside of the condenser was washed with a small amount of distilled water when flask and condenser got cooled. In this about 1mL of phenolphthalein indicator was added and titrated with 0.5 N HCl until the pink color just disappeared. The burette

reading was noted. blank determinations were prepared and conducted instantaneously with the sample. (Neagu *et al.*, 2013) After the titration for the determination of saponification value, the neutralized liquid was transferred quantitatively into a separating funnel and 50 mL of water was added to wash the flask. Extraction was repeated three times with 50 mL diethyl ether while still warm. Took another separating funnel and all the ether extracts were added combined into this. Washed energetically with 20 mL of water and then discard the water.

20 mL of aqueous 0.5 M potassium hydroxide was also used to wash the ether extract. Took 150 mL clean dry beaker, weigh it and pour the extract in to it. The extract was evaporated on a waterbath. 2 – 3 mL of acetone was added and was again heated on a boiling water bath. It was extra dried to obtain the persistent weight and then add 2 mL of diethyl ether to dissolve. 10 mL neutralized ethanol was added and titrated with 0.1 N alc. KOH (Michael *et al.*, 2014). The experiment was repeated three times to get the accurate results.

Refractive Index of *Sesbania bispinosa* seed oil was determined by Automatic Digital Refractometer RX-7000 α at temperature 40 °C. Peroxide value, iodine value, acid value and free fatty acids of oil sample were determined by titration (Godswill *et al.*, 2018).

RESULTS AND DISCUSSION

Oil extraction and Fatty acids composition of *Sesbania bispinosa* seed oil

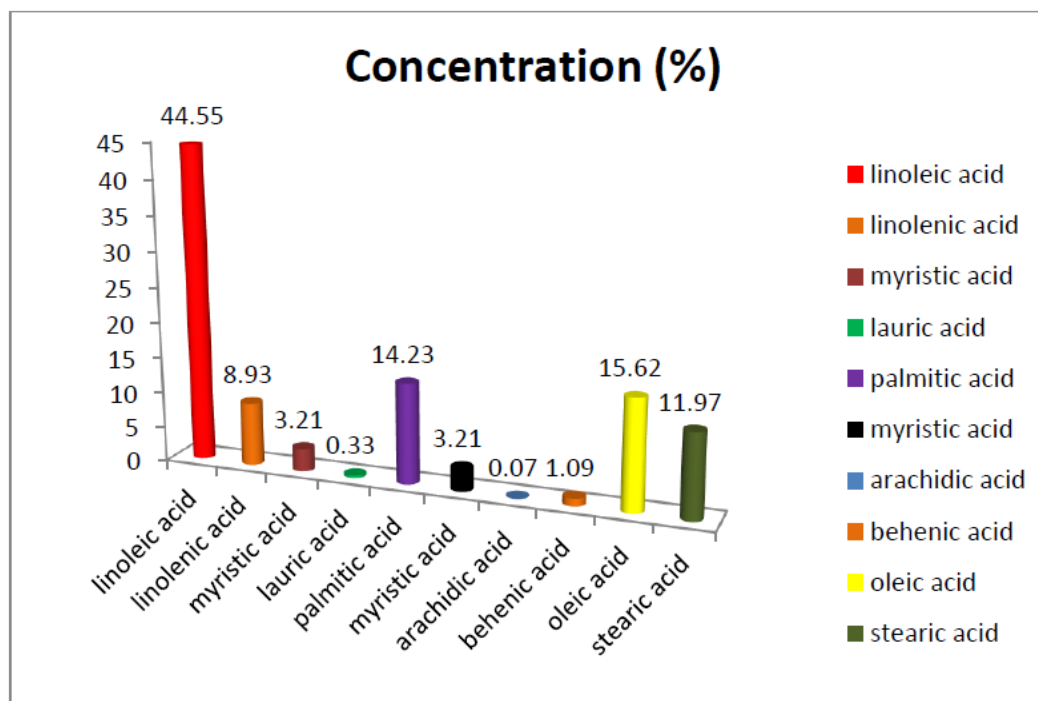
The seeds were collected from the local market in Lahore, washed, grinded and oil was extracted using soxhlet apparatus. The oil yield was 6%. *n*-hexane as a solvent was used to extract the oil. The oil of *Sesbania bispinosa* was converted into fatty acid methyl ester by Hartman's method and the FAMES were analyzed by Gas Chromatography coupled with Flame Ionized Detector (GC- FID). This analysis showed that oil of *Sesbania bispinosa* consisted of nine fatty acids.

The fatty acids consisted of carbon chains ranging from C10-C24. Three out of nine fatty acids were unsaturated FFAs (linoleic acid, linolenic acid and oleic acid) while the remaining six (lauric acid, myristic acid, palmitic acid, steric acid, behenic acid and arachidic acid) were saturated FFAs. Linoleic acid was one of the most dominant fatty acid among these 10 FFAs.

Table 1: Fatty Acid Composition (%) of Methyl Ester of *Sesbania bispinosa* Seed Oil.

Sr. No.	Retention Time	Concentration (%)	Fatty Acids	Fatty Acid Formula
1	5.89	0.33	Lauric Acid	C12:0
2	6.482	3.21	Myristic Acid	C14:0
3	8.79	14.23	Palmitic Acid	C16:0
4	9.579	11.97	Stearic Acid	C18:0
5	10.01	15.62	Oleic Acid	C18:1 9(Z)
6	10.820	44.55	Linoleic Acid	C18:2 (9(Z), 12(Z))
7	13.41	8.93	Linolenic Acid	C18:9(Z),12 (Z),15(Z)
8	15.48	0.07	Arachidic Acid	C20:0
9	16.671	1.09	Behenic Acid	C24; 0

From the tables, it is estimated that the saturated fatty acid content in *Sesbania* oil was greater than unsaturated fatty acids content that is why it imparted higher oxidative and thermal stabilities, leading to a slower deterioration rate for the lipid characteristics. Literature showed that linoleic acid has an anti-inflammatory activity. This fatty acid covered the major portion of fatty acid concentration (44.55%) in *Sesbania bispinosa* seed oil, so the oil can be used to reduce inflammation and acne.

**Figure 1: Percentage concentration of fatty acids of *Sesbania bispinosa* seed oil.**

Once the oil was extracted from seeds of *Sesbania bispinosa*, it was further tested for several parameters. The number of double bonds in a sample was measured by the iodine value. It

indicated the amount of iodine (I₂) consumed per 100 grams of sample.

The physicochemical properties of *Sesbania bispinosa* are being described in the table 2.

Table 2: Physicochemical properties of *Sesbania bispinosa* seed oil.

Sr. No.	Test parameters	Value
1	Acid value mg KOH/g	2.8
2	Free fatty acids (w/w % Oleic Acid)	1.4
3	Peroxide value	1.8
4	Iodine value (g I ₂ /100 g)	130
5	Saponification value (mg KOH/g)	195
6	Refractive index at 40 C	1.4678
7	Unsaponifiable Matter (w/w %)	1.23

The iodine value of *Sesbania bispinosa* seed oil was determined according to AOAC method. The iodine value of oil was 130. This represented the higher unsaturation in the oil which means that the unsaturated FAs are present in high quantity which showed that oils having a high degree of unsaturation and high iodine content were more sensitive to oxidative deterioration. However, Oil deterioration can be accelerated by factors such as high temperatures, light exposure, moisture and air. The peroxide value of present investigation of oil was 1.8. This showed that the oil did not oxidize under given conditions.

Acid value is an important factor which evaluates the quality and purity of oil. Acid value of the oil in this research came out as 2.8. This showed that oil was in pure form and there is no deterioration. As the oil was well preserved, there was no hydrolysis of triglycerides. The FFA value is usually determined as oleic acid by dividing the acid value by 2. The number of free fatty acids in Jantar (*Sesbania bispinosa*) seed oil was counted as 1.4.

Saponification value tells us about the amount of free fatty acids in food. number of FFAs which ultimately tells us about how much quantity of fat or oil will produce soap. In other words it tells us about the quantitative saponification reaction. The saponification value of jantar seed oil in the present case study came out as 195. Unsaponifiables are the components that cannot form soaps when an oil or fat react with alkali. The unsaponifiables were determined by applying partition law. The value was 1.23. This was due the fact that in recent oil, more fatty acids got converted into soaps. Refractive index of the oil was evaluated by Automatic Digital Refractometer RX- 7000 α . The value which was observed at 40°C was 1.4678.

CONCLUSION

The current investigation revealed that the oil yield of *Sesbania bispinosa* seeds was 6.0%. The seed oil contained nine fatty acids in which linolenic acid was major fatty acid as it contributes 44.55% to total fatty acids. Acid Value 2.8, Free Fatty Acids 1.4, Peroxide Value 1.8, Iodine Value 130, Saponification Value 115, Unsaponifiable Matter 1.23 and Refractive Index Value 1.4678 were observed for *Sesbania bispinosa* seed oil. Acid value, saponification value and free fatty acid contents revealed that the oil can be used in cosmetics and soap manufacturing.

REFERENCES

1. Ahmed, A., Howland, M. S. I., Dey, S. K., Hira, A. & Hossain, M. H. (2013). Phytochemical screening, antimicrobial and cytotoxic activity of different fraction of *Sesbania sesban* bark. *International Journal of Basic Medical Sciences and Pharmacy (IJBMSP)*, 3(1).
2. Amoo, I. A. & Asoore, F. P. (2006). Effect of processing on the nutrient composition and oil of peanut (*Arachis hypogea*) seed flour. *Journal of chemical Society of Nigeria*, 31: 1-5.
3. Anita, D. D., Sridhar, K. R. & Bhat, R. (2009). Diversity of fungi associated with mangrove legume.
4. *Sesbania bispinosa* (Jacq.) W. Wight (Fabaceae). *Livest Res Rural Dev*, 21(5): 1-15.
5. Belton, P. (2000). The functional properties of fats and oil-A richness of diversity. *Grasas y aceites*, 51(1-2): 1-5.
6. Chanda, S. C., Prodhan, A. K. M. A. & Sarwar, A. K. M. G. (2018). Morphological descriptors of seed and seedling for identification of dhaincha (*Sesbania* spp.) accessions. *Bangladesh Journal of Botany*, 47(2): 237-246.
7. Deman, J. M. & Beera, A. M. (1987). Fatcrystal networks: structure and rheological properties. *Journal of Texture Studies*, 18(4): 303-318.
8. Devi, A. & Khatkar, B. S. (2016). Physicochemical, rheological and functional properties of fats and oils in relation to cookie quality: a review. *Journal of food science and technology*, 53(10): 3633-3641.
9. Godswill, A. C., Amagwula, I. O., Victory, I. S. & Gonzaga, A. I. (2018). Effects of repeated deepfrying on refractive index and peroxide value of selected vegetable oils.
10. Grosvenor, P. W., Supriono, A. & Gray, D. O. (1995). Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2: antibacterial and antifungal activity. *Journal of*

- ethnopharmacology*, 45(2): 97-111.
11. Harding, T. S. (1942). Fats and Oils in Wartime. *The Scientific Monthly*, 55(3): 273-275.
 12. HARTMAN, L. (1973). Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practices*, 22: 475-476.
 13. Hassan, L. G., Sani, N. A., Dangoggo, S. M. & Ladan, M. L. (2008). Nutritional value of bottle gourd (*Lagenaria siceraria*) seeds. *Global Journal of Pure and Applied Sciences*, 14(3): 301-306.
 14. Kamoshita, A., Araki, Y. & Nguyen, Y. T. (2014). Weed biodiversity and rice production during the irrigation rehabilitation process in Cambodia. *Agriculture, ecosystems & environment*, 194: 1-6.
 15. Khan, D. & Zaki, M. J. (2019). The stomatal types in *Sesbania bispinosa* (Jacq). WF Weight seedlings. *Int. J. Biol. Biotech*, 16(4): 1047-1061.
 16. Michael, A., Fausat, A. & Doyinsola, I. (2014). Extraction and physicochemical analysis of some selected seed oils. *International Journal of Advanced Chemistry*, 2(2): 70-73.
 17. Misra, L. & Siddiqi, S. A. (2005). Biologically activity inositol, sterols and lipid derivatives from *Sesbania bispinosa*. Mortuza, M. G., Mamun, A. & Rashid, M. H. (2009). Biochemical composition and oil characteristics of sunhemp seed, an unconventional legume in Bangladesh. *J. Agrofor. Environ*, 3(2): 35-37.
 18. Ndungu, J. N. & Boland, D. J. (1994). *Sesbania* seed collections in Southern Africa. *Agroforestry System*, 27(2): 129-143.
 19. Neagu, A. A., Nita, I., Botez, E. & Geaca, S. (2013). A physicochemical study for some edible oils properties. *Ovidius University Annals of Chemistry*, 24(2): 121-126.
 20. Onyeike, E. N. & Oguike, J. U. (2003). Influence of heat processing methods on the nutrient composition and lipid characterisation of ground nut (*Arachis hypogaea*) seed pastes. *Biokemistri*, 15(1): 34-43.
 21. Pant, K. S., Khosla, V., Kumar, D. & Gairola, S. (2006). Seed oil content variation in *Jatropha curcas* Linn. In different altitudinal ranges and site conditions in HP India. *Lyonia*, 11(2): 31-34.
 22. Parveen, H. & Rauf, A. (2008). (Z)-12-Hydroxyoctadec-9-enoic acid in *Sesbania aculeata* seed oil. *Industrial Crops and Product*, 27(1): 118-122.
 23. Pugalenti, M., Vadivel, V., Gurumoorthi, P. & Janardhanan, K. (2004). Comparative nutritional evaluation of little-known legumes, *Tamarindus indica*, *Erythrina indica* and *Sesbania bispinosa*. *Tropical and Subtropical Agroecosystems*, 4(3): 107-123.
 24. Ravulapalli, S., Kunta, R., & Ramamoorthy, M. (2019). Preparation, characterization and

- feasibility analysis of methyl ester of Sesbania seeds oil (MESSO) as alternate liquid dielectrics in distribution transformers. *RSC advances*, 9(6): 3311-3319.
25. Saeed, A., Iqbal, Z., Khalil, H. I., Hai, Z., Akram, M., Liaqat, L. & Gulzar, z. (2017). Fatty acid profile of aerial roots of ficus elastic. *World Journal of Pharmacrurgical Research*, 6(8): 54-60.
26. Shimamoto, G. G., Favaro, M. & Tubino, M. (2015). Simple methods via mid-IR or ¹H NMR spectroscopy for the determination of the iodine value of vegetable oils. *Journal of the Brazilian Chemical Society*, 26: 1431-1437.
27. Swami, C., Saini, S. & Gupta, V. B. (2012). A Study of Green Dyeing of cotton with Ethanolic Extract of *Sesbania aculeata*. *Universal Journal of Environmental Research & Technology*, 2(2).
28. Tarrago-Trani, M. T., Phillips, K. M., Lemar, L. E. & Holden, J. M. (2006). New and existing oilsand fats used in products with reduced trans-fatty acid content. *Journal of the American Dietetic Association*, 106(6): 867-880.
29. Warra, A. A., Wawata, I. G., Gunu, S. Y. & Aujara, K. M. (2011). Extraction and physiochemical analysis of some selected Northern Nigerian industrial oils. *Archives of Applied Science Research*, 3(4): 536-541.