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RESEARCH ARTICLE
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IDENTIFICATION OF SIGNIFICANT CHEMICAL AND BIOACTIVE MARKERS OF *SESBANIA SESBAN* (L.) MERR. SEED OIL BY PHYTOCHEMICAL AND PHYSICOCHEMICAL ANALYSIS

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Abstract:
Chemical and physicochemical characterization of *Sesbania sesban* seed oil was carried out. Oil was subjected to chemical analysis by GC-FID and GC-MS. Saturated and unsaturated fatty acids were identified Palmitic acid was found in (57.24%), Stearic acid (22.8 %) eicosanoic acid (4.5 %) and linoleic acid (4.4%). Thirteen (13) compounds belonging to diverse class were identified. Presence of 2-methylbutanal, 2-amylfuran, Pentanoic acid, Hexanoic acid, Decanal, Eucalyptol, Vitamin-D, Palmitic acid, Oleic acid, Plant steroids and Alpha Pinene were detected. This justified the use of oil as folk medicine. These constituents collectively are responsible for Antimicrobial, anti-inflammatory, analgesic, antidepressant, anti-anxiolytic, anti-parkinsonian, anti-glaucoma, muscle relaxant, antihypertensive, diuretic, anti-ulcer, anti-ageing, and anticancer activities of this extremely important medicinal oil. Iodine value was found 10.730 for Sesbania sesban oil while SAP value was found 366.207 with total fat 51.220 %, saturated (47.055%) and unsaturated (4.163%). Antioxidant activity was also done of oil from seed by DPPH assay and found insignificant. Further research is required to determine the specific dose and safety profile of recommended dose for given medical condition.

Key words: Seed oil, Bioactive, GC-MS, Antioxidant, Iodine Value, Fatty Acids, Phytochemical, Physicochemical.

Introduction:

The therapeutic value of plants is hidden in some specific chemical contents or group of compounds that causes a definite physiological action in the human body. These chemical constituents are termed

as secondary metabolites. The most important chemical substances of plants are steroids, alkaloids, terpenoids, flavonoids, tannins and phenolic compounds (1).

S. sesban is commonly known as Sesban, River bean, common, Egyptian rattle in English. It is also known by different local names such as Rivierboontjie in African, Sesaban in Arabic, Jainti or Jayant in Bengali, Dien-dien in Vietnamese, Janti, Jayanti or Puri in Indonesian, Añil francés, Tamarindillo in Spanish (2) (3)(4).

Sesbania sesban Linn. is a soft, slightly woody, 1-6 m tall perennial nitrogen fixing small tree. It possess compound leaves which are 12-18 cm long consist of 6-27 pairs of leaflets. The flowers are yellow in colour with brown or purple streaks on the corolla. Pods of the plant are straight or slightly curved and are upto 30 cm long & 5 mm wide containing 10 to 15 seeds inside.

Fig-1: Seeds of *Sesbania sesban* (L.) Merr.

The plant is widely utilize all over the world as an astringent, carminative, anthelmintic, anti-inflammatory, antifertility, antimicrobial, demulcent and purgative. It is also can be given as a curative agent against fever, ulcers etc (5).

Different parts of the plants like seeds, barks and leaves are used in folk medicine. Seeds are utilized for the treatment of diarrhea, to reduce enlargement of spleen, immoderate menstrual flow and in skin disorders. Leaves are used in the conditions like in inflammatory rheumatic swelling and are also used as an Anthelmintic agent. (6)(7) The flower petals of *S. sesban* may be of great importance & natural source of antioxidants. Antioxidants provide protection to the cells against the formation of free radicals by scavenging them and slow down the progress of many degenerative & chronic disease conditions like cardiovascular diseases and cancer. This antioxidant nature of the plant is due to the existence of saponins and flavonoids which designate the plant potentially applicable not only in pharma but in food industry as well. (8) (9).

The presence of oleanolic acid (10) and kaempferol Trisaccharide (11) was observed in investigating phytochemical properties of S. sesban. Indole acetic acid has also been obtained from the nodules of the roots of *Sesbania sesban* (12)

Different antinutritional constituents are found in the seeds of S. sesban like saponins, tannins & trypsin inhibitors. These compounds are the big problems when the seed is given to the animals for their feed. In the phytochemical study of the seeds of sesban, presence of oleanolic acid, stigmastane-5.24(28)-diene-3β-O-β-D-galactopyranoside and galactomannan were observed. (14) (15). Gas Liquid Chromatography analysis of seed oil has also been performed which shows the presence of linoleic acid, arachidic acid, myristic acid, palmitic acid, and oleic acid (16).

MATERIALS AND METHODS

Seeds collection and chemicals used:

Seeds of the *Sesbania Sesban* were collected from the Tehsil Shakar Gharah. The collected seeds were submitted with a specimen voucher number105 E to the Herbarium of the Department of

Pharmacognosy, Faculty of Pharmaceutical sciences Government college University Faisalabad. 2-diphenyl-1-picrylhydazyl (DPPH), ethanol, dimethyl sulfoxide-2 (DMSO 100%), n-acetyl cysteine, n-hexane, and gallic acid were the chemicals used for analysis (Merk, Germany).

Extraction and calculation of percentage oil content:

The extraction of oil from the seeds was accomplished through the utilization of the standard Soxhlet extraction apparatus (Konte®, USA). In short, 70g powdered seeds were introduced to 150cm^3 of n-hexane which served as an extractor and then placed in a porous thimble for the duration of 6 hours. The oil was subsequently obtained by subjecting the solvent to reduced pressure and temperature and then refluxing at 70^{o}C to eliminate any excess solvent further from the extracted oil. Following this, oil was stored at 4^{o}C for subsequent physicochemical analyses. The extracted oil then underwent a process in which it was placed in a measuring cylinder positioned over a water bath at 70^{o}C for almost 30 minutes. This was done to guarantee that the solvent was completely evaporated. Finally, the volume of oil was measured and the percentage of oil content was calculated using Eq. 1 (17).

Gas chromatography-mass spectroscopy (GC-MS) analysis of Sesbania Sesban seeds oil:

In this study, the equipment utilized was a Shimadzu GC-17 (Kyoto, Japan) fitted with an SPB-5VR capillary column containing 5% phenyl-methyl polysiloxane for Gas Chromatography Flame Ionization Detector (GC-FID). The column had an inner diameter of 0.25 mm and a length of 30 mm. The thickness of the HP-5MS film was 0.25µm. Helium was employed as the carrier gas, flowing at a rate of 1mL/min. 1µL of a 10% essential oil/CH_2Cl_2 (v/v) solution was injected in split mode (50:1). The injector's temperature was set to 250°C, while the detector's temperature was set to 280°C. The following temperature program was used to elute the compounds: The temperature was set at 60°C for 6 min, then it increased to 270°C at a rate of 3°C per minute, and it stayed there.

To brief, Hewlett-Packard 5890 (Bunker Lake Blvd, Ramsey, MN) Gas Chromatograph equipped with a ZB-5MSVR capillary column (30m x 0.25mm ID and 0.25m df) was utilized for Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Analytical conditions were maintained in line with those for GC-MS. The ionization voltage was set at 70 eV to speed up the ionization process. The ion source temperature was kept at 230°C and the electron multiplier voltage was adjusted to 900 V (18).

Determination of fatty acids, iodine value, and SAP value of the seed oil:

The fatty acid composition was analyzed by looking at the methyl esters of the individual acids. Methyl esters of fatty acids were prepared using the AOAC method, which included the use of the BF3-MeOH complex. Ten milliliters of seed extract were put in a screw-capped glass tube and one milliliter of BF3-MeOH complex was added before being heated in a water bath at 100 degrees Celsius for one hour. Then, after it had cooled to room temperature, 1mL of deionized water and 2mL of hexane were added. Finally, the glass tube was centrifuged at a low RPM for 2 minutes to create a vortex. The solution's top layer was removed with a syringe and stored in the fridge in a hermetically sealed glass vial. After that, GC-MS analysis was performed on the FAMEs that had been synthesized. The number of acid groups and degree of unsaturation in a molecule was determined by calculating the iodine and saponification value of oil. In this study, we implemented a cutting-edge method for estimating iodine value using fatty acid methyl ester data. Capillary gas chromatography was used to determine the concentration of oil fatty acid methyl esters. The iodine value is the measure of the number of double bonds contained in the unsaturated fatty acids in a single gram of oil. Laboratory analysts often avoid the assessment process that calls for the use of dangerous chemicals. By the American Oil Chemists' Society (AOCS) technique Cd 1c-85, a methodology for calculating the iodine value of oils from their fatty acid methyl esters composition is now in use. Based on an evaluation of oils' fatty acid methyl esters, a novel procedure for determining iodine value was developed. The suggested computation methodology's effectiveness was assessed as well. When

compared to the analogous AOCS approach, the suggested computations were more in line with the Wijs method. The factor was calculated using 0.1N potassium iodide solution as the standard (19).

Antioxidant activity (DPPH-radical scavenging assay) :

The stable free radical 2, 2-dipehnyl-1-picylhydrazyl (DPPH) was used to test the antioxidant capacity of a range of *Sesbania Sesban* oil samples. This approach is easy, quick, and cheap. The stable DPPH radical is employed in this assay, which is often used to determine the antioxidant activity of various substances. The distinctive purple hue and significant absorption maximum at 517 nm of the odd electron in the DPPH free radical are noticed in this approach. The molar absorptivity of the DPPH radical at 515nm drops when the DPPH radical's odd electron pairs with hydrogen from a free radical scavenging antioxidant, changing the colour from purple to light yellow. There is a stoichiometric relationship between the number of trapped electrons and the degree of decolourization that follows. The DPPH 300mM solution was prepared using pure ethanol. Next, we dissolved test samples in DMSO (Dimethyl sulfoxide) at a concentration of 100%. Pre-readings at 515 nm were collected after 5 L of the sample was deposited in each well of the 96-well plate. The plate was covered with parafilm to prevent the solvent from evaporating, and the wells were incubated at 37^{o}C for 30 min. After that, the final absorbance was measured using a micro plate reader set at 515 nm. Only, DMSO was used in the control group (20).

Gallic acid and N-acetyl cysteine were the reference compounds for the DPPH-%RSA assay (Rabbi *et al.*, 2020). The following equations were used to calculate the percentage of Radical Scavenging Activity (%RSA) using below Eq.

$$\% \text{ RSA} = 100 - (\text{O.D of sample} / \text{O.D of control} \times 100) \quad \text{Eq.}$$

RESULTS:

The pale yellowish oil of *Sesbania Sesban* seeds was extracted by the Soxhlet extraction method employing n-hexane as solvent. The seeds yield 15.8% oil based on an initial sample of dried seeds.

Phytochemical analysis:

Sesbania Sesban seeds oil underwent phytochemical analysis using gas chromatography-mass spectrometry (GC-MS). GC-MS analysis identified fatty acids and 13 chemical compounds comprising 100% composition of the oil. The significant phytochemical classes/groups found were fatty acids (51.220 %), Saturated (47.055 %), Unsaturated (4.163%) Vitamin D, Eucalyptol, Amyl Furans and other types. All these compounds are summarized in table 1 along with their retention time and medicinal uses.

Both saturated and unsaturated fatty acids were detected in the oil by GC-MC. The key saturated fatty acids identified were palmitic acid (C16:0) (57.24%), stearic acid (C18:0) (22.8 %), eicosanoic acid (C20:0) (4.5 %) and linoleic acid (C18:2) (4.4%).

Some other compounds such as α-isoamylene, 2-amyl furan and 2-dodecanal were also identified in the oil. Thus, GC-MS analysis demonstrated the presence of a wide range of phytochemicals in *Sesbania Sesban* seeds oil. This confers a broad-spectrum pharmacological profile to the oil.

Physicochemical parameters:

The iodine value of oil was calculated as 10.730 gI/100 g which indicated a high degree of saturation due to the presence of high content of saturated fatty acids in it. The saponification value calculated for Sesbania sesban oil was also high indicating the presence of more fatty acids with longer chain lengths. The saponification value is inversely related to the average molecular weight of the fatty acids. Both iodine and saponification values confirmed the prevalence of long-chain polyunsaturated fatty acids in *Sesbania Sesban* seeds oil as identified in the GC-MS study.

Antioxidant activity:

The antioxidant activity of oil was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The oil exhibited only 7.187% radical scavenging activity at the tested concentration. This was very low as compared with the standard antioxidants gallic acid and contradictory to previous findings.

Gas Chromatography:

Gas Chromatographic studies of *Sesbania sesban* were performed and following spectrum was obtained. On the basis of spectral peaks various constituents of commercial and medicinal significance were analyzed (Table-1,2).

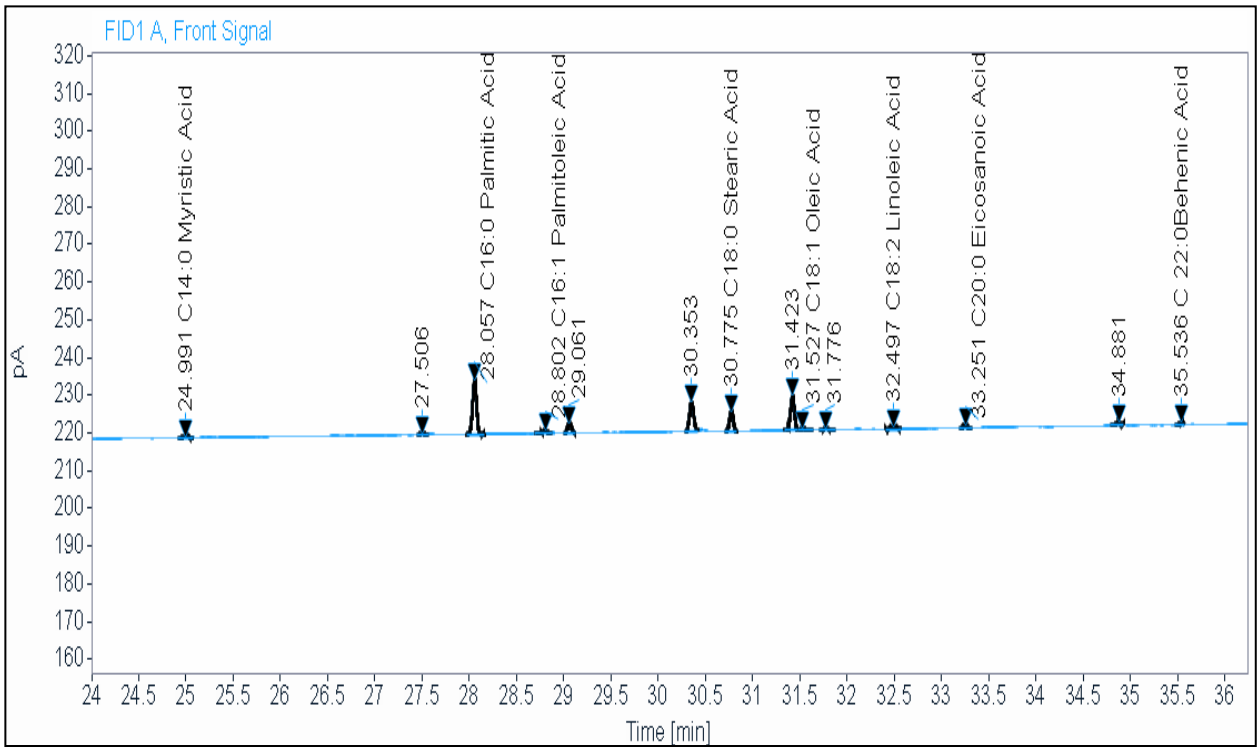

Table -1 GC-FID Results of Seed Oil from Sesbania sesban.

Peak#	Name	RT[min]	Mass (M)	Area(A)	% = M*A/100
1	C16:0 Palmitic Acid	28.057	256.00 A.M.U	32.424	57.24%
2	Stearic Acid	30.775	284.00 A.M.U	11.293	22.80%
3	C18:1 Oleic Acid	31.527	282.46 A.M.U	1.370	3.86%
4	C18:2Linoleic Acid	32.497	280.45 A.M.U	2.216	4.40 %
5	C20:0 Eicosanoic Acid	33.251	320.53 A.M.U	2.042	4.50 %

Table -2: Physical Parameters of Sesbania Sesban Oil.

Sr. No	Properties	Observations
1	Density (g/cm^3)	0.8511
2	Extractive value	19.01 %
3	Color	Yellowish
4	Acid Value	0.68
5	Iodine value	10,730
6	SAP value	366.207
7	Refractive Index	1.5
8	Moisture	0.11 %

Table-2: Compounds Identified by GCMS from *Sesbania sesban* seed Oil. [21]

Sr.	Retention time in Minutes	Compound Identified	Medicinal Uses
1	4.483	2-Methylbutanal	It has a role as a volatile oil component, a plant metabolite
2	10.079	2-amylfuran	Antibacterial or antifungal or antiviral, anti- inflammatory, analgesic, antidepressant, anti-anxiolytic, anti-parkinsonian, anti-glaucoma, muscle relaxant, antihypertensive, diuretic, anti-ulcer, anti-ageing, and anticancer
3	10.52	Pentanoic acid	Anticonvulsant, Lipid regulator
4	11.255	Hexanoic acid	Anticonvulsant, Antianxiety
5	12.702	Decane	Decane is used as a solvent in organic synthesis reactions
6	15.585	Vitamin D (C_{27} $H_{44}O_3$)	maintain intracellular and extracellular calcium and phosphorous concentration within a physiological acceptable range and also used in the fortification of milk, margarines and other foods worldwide [21]
7	16.898	2,4,Decadienal	Used as an oxidation indicator for linoleic acid-rich oils. (Have toxic effects)
8	17.373	Eucalyptol (C_{10} H18 O)	It controls airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition. Eucalyptol
9	29.072	Palmitic Acid	Palmitic acid is a key ingredient in soaps and detergents (Cosmetics)
10	37.37	Oleic Acid (Octadec-9-enoic acid)	Oleic acid is a major component of many oils, including canola, peanut
11	51.15	Plasticizer	Might be mixed from storage equipment.
12	63.41	Stigmasta 3,5-diene-7-one	Stigmasta-3,5-dien-7-one is a natural steroid compound
13	7.09	Alpha Pinene	Anti-inflammatory, Antibacterial , antibiotic.

References:

1. Edeoga HO, Okwu DE, Mbaebie BO, (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology, 4:685-688.*
2. Dande PR, Talekar VS, Chakraborthy GS (2010). Evaluation of crude saponins extract from leaves of *Sesbania sesban* (L.) Merr. for topical anti-inflammatory activity. Int. J. Res. Pharm. Sci.1(3):296-299.
3. Orwa C, Mutua A, Kindt R, Jamnadass R, Simons A (2009). Agroforestree Database: a tree reference and selection guide version 4.0 (http://www.worldagroforestry.org/af/treedb/)
4. Pravin G, Priti G, Shaikh A, Sindha S, Khan MS (2012). *Sesbania sesban* Linn: A Review on Its Ethnobotany, Phytochemical and Pharmacological Profile. Asian J. Biomed. Pharmaceut. Sci. 2(12):11-14.
5. Sheikh Sajid R, Pawar Vijay T and Md Rageeb Md Usman, (2012). Anti-inflammatory activity of *Sesbania sesban* (L) Merr. International Research Journal of Pharmacy, 3 (1) : 176-180.
6. Nadkarni KM, Indian Materia Medica, 3rd ed., Vol. I, Popular Prakashan, Bombay, 1130(1982).
7. Rastogi RP, Mehrotra BN, Compendium of Indian Medicinal Plants, 1st ed., Vol. II, Central Drug Research Institute, Lucknow, 62(1993).
8. Kathresh M., Suganya P., Saravanakumar M. Antioxidant effect of Sesbania sesban flower extract. International Journal of pharmaceutical Sciences,3(2), 1307-1312(2011).
9. Mani RP, Pandey A. , Shambaditya G.,Tripathi P., Kumudhavalli, Phytochemical Screening and In-vitro Evaluation of Antioxidant Activity and Antimicrobial Activity of the Leaves of Sesbania sesban (L) Merr.
11. Yeung Ming Fai, Che Chun Tao, A review of presence of Oleanolic acid in Natural Products, Sample Review for Natura Proda Medica,1-271, (2009).
12. El-Sayed, NH, A rare Kaempferol trisaccharide anti tumor principle from *S.sesban*, Pharmazie, 46: 679-680, (1991).

13. Mutluru S, and Konada VM, Bioproduction of indole acetic acid by Rhizobium strains isolated from root nodules of green manure crop, *Sesbania sesban* (L.) Merr. Iranian J Biotech, 5(3): 178-182, (2007).
14. Hossain MA, Becker K (2001). Nutritive value and antinutritional factors in different varieties of Sesban seed and their morphological fractions. Food Chem.73:421-431.
15. Das N, Chandran P, Chakraborty S (2011). Potent spermicidal effect of oleanolic acid 3-beta-D-glucuronide, an active principle isolated from the plant *Sesbania sesban* Merrill. Contracept. 83:167-175.
16. Kapoor VK, Jindal OP. A comparative study on the seed oils of *Sesbania sesban* Merr. (Yellow flower and violet flower varieties). Indian J. Pharm. Sci., 223, (1978).
17. Ullah, M. N., Ali, I., Hanif, S., Syed, M. A., Talha, M., Samran, M. A., ... & Huraira, M. (2024). Phytochemical screening and physicochemical analysis of oil extracted from seeds of Bombax ceiba and determination of antioxidant activity. *Pakistan Journal of Pharmaceutical Sciences*, *37*(4).
18. Kubeczka, K. H. (2020). History and sources of essential oil research. In *Handbook of essential oils* (pp. 3-39). CRC Press.
19. Minelli, G., D'Ambra, K., Macchioni, P., & Lo Fiego, D. P. (2023). Effects of pig dietary n-6/n-3 polyunsaturated fatty acids ratio and gender on carcass traits, fatty acid profiles, nutritional indices of lipid depots and oxidative stability of meat in medium–heavy pigs. *Foods*, *12*(22), 4106.
20. Gulcin, İ., & Alwasel, S. H. (2023). DPPH radical scavenging assay. *Processes*, *11*(8), 2248.
21. Irabor, E. E. I., Onukwugha, P. U., Eze, P. N., & Ogbeide, O. K. (2020). EXTRACTION AND CHARACTERIZATION OF FAT SOLUBLE VITAMINS FROM OIL OF Calliandrasurrinamensis SEED. *Journal of Chemical Society of Nigeria*, *45*(3).