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RESEARCH ARTICLE
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PHYTOCHEMICAL, PHYSICOCHEMICAL AND ANTIOXIDANT SCREENING OF OIL EXPRESSED FROM SEEDS OF ADANSONIA DIGITATA L. A VALUABLE PHARMACEUTICAL, NUTRACEUTICAL AND COSMECEUTICAL PLANT.

Ijaz Ali[1], Muhammad Muzammil Ramzan[1],Muhammad Abdul Haq Khan[1], Irfana Lalarukh[2*],Haseeb Ahmad Saeed[3], Umbreen Farrukh[4], Summan[1], Muhammad Umar[1,] Syed Faizan Abbas Naqvi[5], Syeda Faiqa Ijaz[5], Hassan Farooq Ahmad Khan[5],Umar Farooq[6]

[1]Department of Pharmacognosy, Faculty of Pharmaceutical Sciences Government College University Faisalabad, Punjab Pakistan
[2*]Department of Botany Government College University Faisalabad, Punjab Pakistan
[3]Department of Pharmacy, Sahara Medical College Narowal Punjab Pakistan
[4]Department of Pharmacognosy, Faculty of Pharmaceutical sciences, Dow college of Pharmacy, Dow University of Health sciences, Karachi, Pakistan
[5]Department of Pharmacy, University of Lahore, Lahore Punjab, Pakistan.
[6]Department of Pharmacy, COMSATS University Abbottabad-Pakistan

***Corresponding Author:** Irfana Lalarukh
*Email**:** dr.lalarukh.i@gcwuf.edu.pk

Abstract:
Human beings have been gaining benefits from plants since prehistoric times. Plants bestow us with many ways. In recent years, extensive research on plants has been carried out, including pharmaceutical, nutraceutical, and cosmetic aspects. Hence, collaborative and multidisciplinary efforts are needed. Exploration of phytochemical and physicochemical parameters of a plant species enhances its better understanding. This research is aimed at investigation of oil expressed from seeds of Adansonia digitata L., a multi-purpose tree. Phytochemical, physicochemical, and bioactive investigations have been carried out. Such investigations can help to evaluate the traditional claims and folkloric use of baobab seed oil. Oil is traditionally used for cosmetic purposes. It is applied for skin health. It is also used for treatment of hair fall and for growing healthy and long hair. The kernels are rich in fat. The whole seeds and the kernels have relatively high lipid content. Oleic acid was present with the highest area percentage (115.4), following linoleic acids (79.5) as major fatty acids, whereas others include palmitic acid (33.4) and stearic acid (14.75). Eight compounds belonging to various chemical classes were screened. These compounds exhibit significant pharmaceutical, nutraceutical, and cosmeceutical values. The antioxidant effect of the seed content was found to be very high (91.187%). These findings give a clear indication of the use of this oil as multipurpose oil.

Keywords: Pharmaceuticals, Cosmeceuticals, Nutraceuticals, Herbs, Phytochemical, Physiochemical, Antioxidant, GCMS, Baobab, Seeds, oil

Introduction:

Adansonia digitata L. is commonly known as Baobab. It belongs to the family Malvaceae. It is a long-lived tree, having lived for several centuries. Baobab is a copious tree having a trunk of approx. 10 m in diameter and growing up to 25 m in height. Its leaves may have a diameter of up to 20 cm. Its fruit is enclosed in a dry shell that is 20–30 cm in length and up to 10 cm in diameter (Donatien Kaboré, 2011). It is native to Africa. Most of the plant parts of baobab are comestible and are also used for various medicinal purposes. Baobab seeds can be used as thickening agents as well as flavouring agents in soups. It can tolerate long durations of hot as well as very dry weather. Several plant parts have antioxidant activity (Jitin Rahul, 2015). Juices obtained from the pulp of baobab fruit are high in phosphorus, calcium, magnesium, iron, and zinc and also contain copper. Fruit pulp is also rich in vitamin C and pectin. Its bark can be used for making hats, mats, and ropes (John Saka, 2007; J. Gebauer, 2002; Salisu Abubakar, 2015). Its fruit pulp is high in carbohydrate and is recommended to pregnant women (Emmy De Caluwé, 2010). It has antipyretic, antimicrobial, analgesic, insecticidal, and antioxidant activities. It also has antiviral activity against the herpes simplex and polio viruses. Its bark extract has hypoglycemic effects. It also showed anti-diabetic and hypolipidimic effects in type I diabetes. (G.P.P. Kamatou, 2011). Its seed oils were used in various conditions, such as hair dandruff, muscle spasms, varicose veins, and wounds, for topical application (Zahrau Bamalli, 2014). Plant seed oil is extensively used for cosmetic purposes. It is the best antioxidant and is applied for skin health. It is also used for treatment of hair fall and for growing healthy and long hair.

The baobab tree is a native African tree. Its name is also mentioned in some ancient African poetry and folklore. It is a majestic tree that has a lifespan of about 6000 years. It is believed that spirits dwell on the inside of this tree. Besides being a wonderful moisturizer, baobab oil may be used as a natural skin cleanser for clearer skin. Because oil dissolves oil, cleaning with baobab won't leave your skin oily or greasy. Baobab hot oil treatments help to restore dry and dull hair. Please see our step-by-step instructions at the bottom of the page to create your own hot oil treatment. Baobab oil also makes a wonderful hair and scalp moisturizer that absorbs deeply into the hair follicles. It helps to treat dandruff and itchy scalp when the scalp is overly dry. Regular treatments of baobab oil give the hair a healthy shine, prevent dryness, and make thin hair. Gas chromatography has been used for qualitative as well as quantitative analysis for many years. It separates the components in gas form, so the compounds analyzed by this technique should be convertible into vapors. Gas chromatography coupled with mass spectroscopy (GCMS) can be a good candidate for the compounds that are difficult to identify accurately with the with the GC technique alone. (R.M. Hannan and H.H. Hill, 1991). Hyphenated techniques offer two-dimensional separations. Suitable hyphenations are GC-MS and GC-Fourier transform IR. GCMS hyphenation gives us three-dimensional separations of mixtures. MS detector addition to GC gives it more ability to detect minor components as well as homologous series of compounds. (Gaines, 1999; Robert Shellie, 2004) Antioxidants are the compounds that suppress the oxidation process. There are many pathological conditions in which oxidative stress gets increased, so antioxidants can be helpful in treating them. (Amorati, 2013). Many vegetables and fruits contain polyphenols that act as natural antioxidants (M.Silvia Taga, 1984). In the present communication, we report the GC/MS analysis of the seed oil of Adansonia digitata and also determine the presence or absence of antioxidant activity in the oil obtained from its seeds.

MATERIALS AND METHODS:

Seeds collection:

Seeds of the Adansonia digitata were purchased from Jhang Bazar Faisalabad. The collected seeds were authenticated by Dr. Irfana Lalarukh, Department of Botany, GCWU Faisalabad, and submitted with a specimen voucher number 10A to the herbarium of the Department of Pharmacognosy, Government College University Faisalabad. Chemicals used for this investigation were purchased from Merck Germany. Commonly used chemicals include 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 of Oil:

The extraction of oil from the seeds was accomplished through the utilization of the standard Soxhlet extraction apparatus (Konte®, USA) in the Pharmacognosy lab of Government College University Faisalabad. Baobab seeds 100 g (powdered) were loaded with 150 cm3 of n-hexane, which served as an extractor, and were placed in a porous thimble for the duration of 6 hours. Solvent was subjected to reduced pressure and temperature, and then refluxing was carried out at 70 oC to minimize any excess solvent further from the extracted oil. Following this, oil was stored at 4 oC 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 oC 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 (UL Msalilwa).

$$\%\,\text{oil content} = \frac{\text{Weight of oil}}{\text{Weight of a sample}} \times 100 \quad \ldots \text{Eq-1}$$

19.01 ml of golden yellow oil from Adansonia digitata seeds was extracted by the Soxhlet extraction method employing n-hexane as solvent. The seeds yield 19% oil based on an initial sample of seeds.

Gas chromatography-mass spectroscopy (GC-MS):

The instrument used was a Shimadzu GC-17 (Kyoto, Japan) fitted with an SPB-5VR capillary column containing 5% phenyl-methyl polysiloxane for the Gas Chromatography Flame Ionisation Detector (GC-FID). Inner diameter of column 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 1 mL/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 study was performed at 60°C for 6 min, then it was increased to 270°C at a rate of 3°C per minute. To brief, a Hewlett-Packard 5890 (Bunker Lake Blvd., Ramsey, MN) gas chromatograph equipped with a ZB-5MSVR capillary column (30 m x 0.25 mm ID and 0.25 m df) was utilised for gas chromatography-mass spectrometry (GC-MS) analysis. The ionisation voltage was kept at 70 eV to speed up the ionisation process. The source temperature was maintained at 230 °C, and the electron multiplier voltage was adjusted to 900 volts (Kubeczka, 2020).

Physicochemical Analysis:

The fatty acid composition analysis was conducted by methyl esters of the individual acids. Methyl esters of fatty acids were prepared using the AOAC method, which included the use of the BF_3-MeOH complex. Ten millilitres of seed extract were put in a screw-capped glass tube, and one millilitre of BF_3-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, 1 mL of deionised water and 2 mL 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. Table 2 lists the nine fatty acids found in the seeds' oil, including their retention times, chemical structures, and therapeutic applications. Four of these acids are saturated, while the other five are unsaturated. The number of acid groups and degree of unsaturation in a molecule were determined by calculating the iodine and saponification values 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 gramme 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 (Minelli et al., 2023).

Antioxidant activity:

The stable free radical 2, 2-dipehnyl-1-picylhydrazyl (DPPH) was used to test the antioxidant capacity of a range of Adansonia digitata 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 515 nm 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 stochiometric relationship between the number of trapped electrons and the degree of decolorisation that follows. The DPPH 300 mM 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 oC for 30 min. After that, the final absorbance was measured using a microplate reader set at 515 nm. Only DMSO was used in the control group. Reference compounds for the DPPH-% RSA assay are gallic acid and N-acetyl cysteine (Rabbi et al., 2020). Calculations were performed by using the below equation. Percentage of Radical Scavenging Activity (% RSA) using Eq. 2.

$$\% \text{ RSA} = 100 - (\text{O.D of sample} / \text{O.D of control} \times 100) \quad \ldots \text{Eq. 2}$$

Results:

Table-1 Physical Parameters of Baobab seed oil

Sr.no	Parameter	Baobab Seed Oil
1	Refractive Index	1.46 ± 0.005
2	Acid value (mg KOH/g)	0.50 ± 0.06
3	Iodine value (mgI2/100g)	90.63
4	Saponification value (mg KOH/g	208.57
5	Yellowing index	85.15 ± 0.05
6	Density	0.88
7	Viscosity	62.81
8	PH	6.18

Table-2 Chemical components identified in seeds oil by GC-MS

Sr.no	Compounds	Retention time	Uses
1	Acetylene	4.453	Pharmaceuticals , Insecticides
2	Caproic Acid	12.65	Perfumes, Milk Chocolate, Drugs
3	Nonanoic Acid	16.241	Herbicide, Cosmetics, Research

4	Palmitic Acid ME	27.096	Cosmetics, Detergents, Medical Research
5	Palmitic Acid	29.015	Soaps, Cosmetics, Food additives,
6	Octadec-9-enoic acid (Oleic acid	37.08	Antioxidant, Edible Oils, Soaps
7	(E)-2-Octenal	49.994	Flavoring, Fragrance, Antifungal
8	Ethyl 3 hydroxybutanoate	56.1	Flavoring and Fragrance in food and Cosmetics

Fig-1: Gas Chromatography Mass Spectrometric Studies

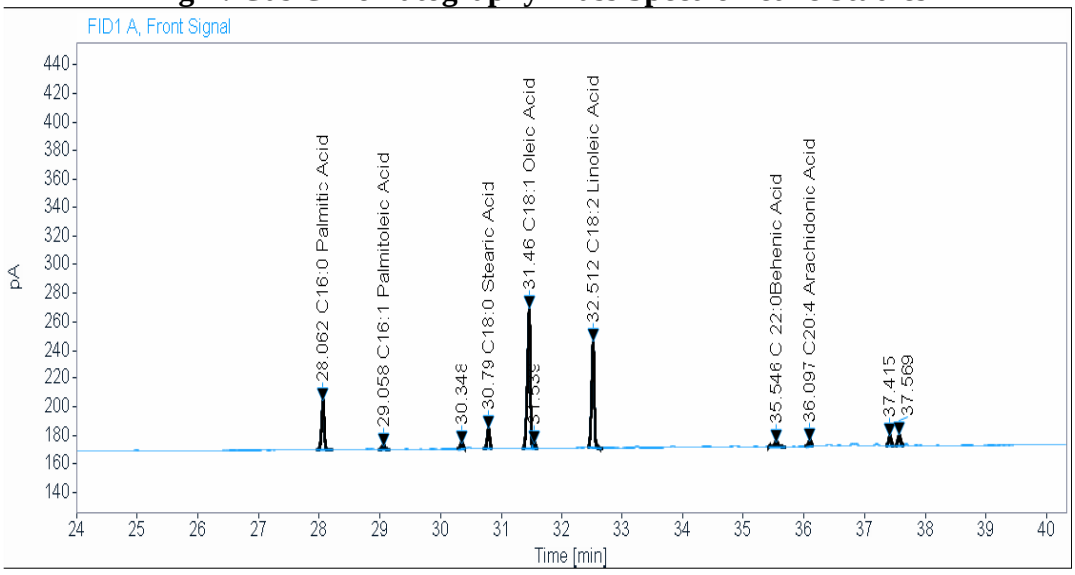

Table-3: Chemical components identified in seeds oil by GC-FID

Peaks (Significant)	Name	RT[min]	Mass (M)	Area (A)	Area% =M*A/100
1	C16:0 Palmitic Acid	28.557	256	13.04	33.3824
2	C16:1 Palmitoleic Acid	28.802	254.4	1.174	2.98
3	C18:0 Stearic Acid	30.775	284	5.196	14.75664
4	C18:1 Oleic Acid	31.527	282.46	40.854	115.39621
5	C18:2 Linoleic Acid	32.497	280.45	28.334	79.462703
6	Arachidonic Acid C 20:4	34.881	304.47	1.506	4.5853182
7	C 22:0Behenic Acid	35.536	340.58	1.676	5.7081208

Phytochemical analysis:
Adansonia digitata seed oil underwent phytochemical analysis using gas chromatography-mass spectrometry (GC-MS). GC-MS analysis identified fatty acids and 8 chemical compounds comprising 100% of the of the composition of the oil. 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) (33.3%), stearic acid (C18:0) (14.71%), and behenic acid (C22:0) (5.7%). The major fatty acids (unsaturated) found were linoleic acid (C18:2) (79.6%), arachidonic acid (C20:4) (4.37%), palmitoleic acid (C16:1) (2.98%), and oleic acid (C18:1) (115.31%). Major terpenoids found were acetylene, caproic acid, nonanoic acid, octadec-9-enoic acid, palmitic acid, (E)-2-octenal, and ethyl-3-hydroxybutane. Thus, GC-MS analysis demonstrated the presence of few significant phytochemicals in Adansonia digitata seed oil.

Physicochemical parameters:
The iodine value of oil was calculated as 90.63 gI/100 g, which indicated a high degree of unsaturation due to the presence of a high content of unsaturated fatty acids in it. The saponification value calculated for oil was also high, indicating the presence of more fatty acids with longer chain lengths. There is an inverse relation between the saponification value and the average molecular weight of the fatty acids. Both iodine and saponification values confirmed the prevalence of long-chain polyunsaturated fatty acids in Adansonia digitata seed oil as identified in the GC-MS study.

Antioxidant activity:
The antioxidant activity of oil was evaluated by a 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The oil exhibited only 91.187% radical-scavenging activity at the tested concentration. Study showed the significant results of antioxidant activity of the Adansonia digitata seed oil. Further detailed evaluation using multiple antioxidant assays is required to confirm the antioxidant profile of the of the oil. Thus, GC-MS analysis demonstrated the presence of various bioactive phytochemicals imparting medicinal value to Adansonia digitata seed oil. Key parameters like iodine and saponification values verified the unsaturated fatty acid profile of the oil.

Discussion:
Screened compounds by GC-MS have nutritional, cosmeceutical, and medicinal uses. These compounds possess significant uses, including pharmaceuticals, insecticides, perfumes, milk chocolate, drugs, herbicides, cosmetics, detergents, medical research, soaps, food additives, edible oils, flavouring, fragrance, and antifungal, flavouring, and fragrance in food and cosmetics. The seed oil of plants is a concentrated source of fatty acids, sterols, glycerides, tocopherols, and other non-glyceride components like flavonoids, carotenoids, etc. that provide nutritional as well as therapeutic benefits (Szydłowska-Czerniak et al., 2022). The phytochemical components and the physicochemical characteristics of any oil determine its applications. Literature review shows that Adansonia digitata seeds are rich in essential oils. Thus, the present study focused on looking into the complete phytochemical screening, physicochemical analysis, and antioxidant potential of Adansonia digitata seed oil using GC-MS and the free radical scavenging activity of DPPH. This plant is ethnomedicinally useful and is mostly found in Africa. Both saturated and unsaturated fatty acids were found in the oil and have been reported to possess diverse medicinal properties such as antimicrobial, antioxidant, anti-inflammatory, wound healing, and cardioprotective effects. Some other compounds were also identified in the oil.

A higher iodine value increases the susceptibility of oils to oxidation but also enhances their antimicrobial potency. The iodine value of the oil was determined to be 90.63 Ig/100 g, indicating a high degree of unsaturation. The saponification value was also high, suggesting the presence of high molecular weight fatty acids. These results verified the fatty acid profile of the oil. The antioxidant potential of the oil was evaluated through the DPPH radical scavenging assay method. In this study,

the oil showed very good radical scavenging activity of (91.187%) comparable to the standard antioxidants gallic acid (93.93%) and N-acetyl cysteine (95.95). In addition to therapeutic efficacies and antioxidant activities, previously limited toxicity studies were also conducted on rats, showing Baobab seeds found to be non-toxic up to 75% to goats in cooked form, indicating a high margin of safety. (Ujor, A., Oche, E. E., & Oloche, J. J. (2020). Additional studies on subchronic toxicity are necessary to decisively ascertain the secure and effective therapeutic dose range in humans, which is only possible by knowing its phytochemical profile.

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