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PHYTOCHEMICAL AND PHYSICOCHEMICAL INVESTIGATION OF MIRACULOUS OIL FROM SEEDS OF *ADENANTHERA PAVONINA* L.

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ABSTRACT

Adenanthera pavonina a perennial species of family Leguminosae is commonly known as red-bead tree. It is a medicinal plant traditionally used to treat various health conditions. The plant is reported to have a wide range of biological activities including astringent, anti-hemorrhagic, anti-diarrheal, anti-hematuria, anti-inflammatory, anti-rheumatic, anti-gout and anti-oxidant activities. Seeds are anticephalgic and also used for the treatment of paralysis and blood pressure. The Gas Chromatography Mass Spectroscopic (GCMS) and Gas Chromatographic Flame Ionization Detector (GCFID) spectra of plant oil indicated the presence of valuable constituents. The compounds identified by GCMS include 2-amylfuran, Pentanoic acid, Hexanoic acid, Decane, 2,4,Decadienal, C_{10} H_{18} O, Palmitic Acid, Octadec-9-enoic acid, and Stigmasta 3,5-diene-7-one.The unsaturated fatty acid content was found to be 44.628 % while unsaturated fatty acid were identified to be 2.53 % including other fatty acid as 52.502%. Main fatty acid identified were palmitic acid (60.999%), Linolenic acid (26.507%) Stearic acid (24.634%), Behenic acid (28.203%) and Decosahexonic acid (15.031%). The anti-oxidant activity and physicochemical analysis of oil including iodine value sap value was also performed to assess the bioactivity and stability characteristics of fixed oil. Iodine value was found to be 53.444 indicating low degree of saturation while Sap value was found out 283. The antioxidant potential of oil was found to be 77.187 %. Hence the current findings justified the traditional uses of this significant medicinal plant species.

Key Words: Seed, Oil, Phytochemical, Antioxidant, Fatty Acids, GCMS, Iodine Value

INTRODUCTION

Adenanthra pavonina L. is a small without armed tree. Bioactive secondary metabolites can be isolated from medicinal plants as antimicrobial agents. Based on ethno pharmacological relevance, *Adenanthera pavonina L.* is recognized as a plant with good medicinal values and forms the integral part systems of traditional medicine (Abdu, K., & Adamu, M. 2020). It belongs to Fabaceae. It is an Indian medicinal plant. (Arzumand Ara M. A., 2010). Adenanthera pavonina, popularly known as red-bead tree, is a medicinal plant traditionally used for the treatment of several diseases. *A. pavonina* Linn is a deciduous tree, 18-25 m tall, bole erect and 62 cm in diameter. The plant is reported to have a wide range of biological activities, such as astringent and styptic (used in diarrhoea, stomach haemorrhage, haematuria) and anti-inflammatory (in rheumatic, gout), antioxidant and hepatoprotective action. Seeds are anticephalgic and also used for the treatment of paralysis and blood pressure . (Mujahid, M., Ansari, V. A., Sirbaiya, A. K., Kumar, R., & Usmani, A. (2016)). The scientific name is derived from the combination of two Greek words Aden,"a gland," and anthera," anther" (Md. Mujahid V. A., 2016).*A.pavonina* commonly known as red wood and red-bread tree. It is 18-25 m tall, erect and 60cm in diameter (Md. Mujahid V. A., 2016).A. pavonina is also known as food tree as its seeds and young leaves are cooked as well as eaten by people. (Md. mujahid, 2016).This plant has been used in traditional medicine for the treatments of boil, asthma, gout, diarrhea, inflammations, rheumatism, ulcers and tumors and as a tonic. The seeds of this plant have been found to be very effective for the treatment of cardiovascular diseases during pregnancy. Phytochemical studies on this plant showed the presence of various secondary metabolites including mainly flavonoids, steroids, saponins and triterpenoids. (Arzumand Ara M. A., 2010). Preliminary Phytochemical screening of this plant has indicated the presence of valuable compounds. These diverse phytoconstituents include *O*-acetyl ethanol amine 1 octacosanol, galactitol, 2,4-dihydroxybenzoic acid, ampelopsin, butein, dihydrorobinetin, robinetin, echinocystic, acid oleanolic, acid, steroids daucosterol, βsitosterol, stigmasterol, amino acids, peptides ,2-amino-4-ethylidenepentanedioic acid ,γ-methyleneglutamine, *O*-acetyl ethanolamine and *H*-imidazole (Bisby F (1994).Study showed that *A.pavonina* seed extract have the potential to cause a blood cholesterol lowering effect. (Vettae Gounder Maruthappan, 2010). *A.pavonina* seeds usually contained 17-20% oil. (Rizwan K. Soomro, 2010) . Due to its antioxidant properties, *A. pavonina* oil could be explored as a natural ingredient in food products, cosmetics, or nutraceuticals aimed at combating oxidative stress. (Silva, I. K., & Soysa, P. (2011)

MATERIALS AND METHODS

Seeds collection and chemicals used

Seeds of the *Adenanthra pavonina L.* 0.5 Kg were purchased. Authentication and identification of the seeds were carried out at Department of Pharmacognosy, Government College University Faisalabad Pakistan (GCUF) a specimen voucher number 102 A was submitted to the herbarium.

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). Briefly, a sample of 100 g powdered seeds were loaded to

200cm^3 of n-hexane which served as an extractor and was placed in a porous thimble for six hour the duration. The oil was obtained under reduced pressure and temperature 70^{o}C and then refluxing excess solvent was hence removed. After this, sample was stored at 4^{o}C and then physicochemical analyses was performed. 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 half hour. This was done to guarantee the complete evaporation of solvent. The volume of oil was measured and the percentage of oil content was calculated using Eq.1

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

GC-MS analysis of *Adenanthra pavonina* L. 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). Inner diameter of column was 0.25 mm and its length was 30 mm. The thickness of the HP-5MS film was 0.25µm. Helium gas was used as the carrier gas, flow rate was kept at 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 maintained at 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 (Kubeczka, 2020).

Determination of Physicochemical parameters of the *Adenanthra pavonina* L. Seeds oil

Analysis of the fatty acid content was conducted 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 BF_3-MeOH complex. 10ml of seed extract were put in a screw-capped glass tube and one milliliter 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, 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. 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 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 (Minelli *et al.*, 2023).

Antioxidant activity

The stable free radical 2, 2-dipehnyl-1-picylhydrazyl (DPPH) was applied to test the antioxidant activity of *Adenanthra pavonina L.* Oil samples. This approach is rationale in regard to economy and ease. The stable DPPH radical was used in this assay, which is commonly used assay. The prominent purple color hue and significant absorption maximum at 517 nm of the odd electron in the DPPH free radical were observed 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 decolorization 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 microplate reader set at 515 nm. Only, DMSO was used in the control group (Gulcin and Alwasel, 2023). 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 Eq. 2.

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

RESULTS

Fragrant pale yellow color oil of *Adenanthra pavonina L.* Seeds was extracted by the Soxhlet extraction method using n-hexane as solvent. The seeds yield 12.8% oil based on an initial sample of seeds.

Phytochemical analysis

Adenanthra pavonina L. Seeds oil underwent phytochemical analysis using gas chromatography-mass spectrometry (GC-MS). GC-MS analysis identified fatty acids and nine (09) chemical compounds. The compounds identified by GCMS include 2-amylfuran, Pentanoic acid, Hexanoic acid, Decane, 2,4,Decadienal, C_{10} H_{18} O, Palmitic Acid, Octadec-9-enoic acid, and Stigmasta 3,5-diene-7-one. The unsaturated fatty acid content was found to be 44.628 while unsaturated fatty acid were identified to be 2.53 including other fatty acid as 52.502. Main fatty acid identified were palmitic acid (60.999%), Linolenic acid (26.507%) Stearic acid (24.634%), Behenic acid (28.203%) and Decosahexonic acid (15.031%).Thus, GC-MS analysis demonstrated the presence of a range of phytochemicals in *Adenanthra pavonina L.* seeds oil. This confers a broad-spectrum pharmacological actions to the oil.

Physicochemical parameters findings

The iodine value of oil was calculated as 53.44 gI/100 g which indicated a high degree of unsaturation due to the presence of high content of unsaturated fatty acids in it. The saponification value 382.968 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 *Adenanthra pavonina L.* seeds oil as identified in the GC-MS.

Antioxidant activity results

The antioxidant activity of oil was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The oil exhibited only 77.187% radical scavenging activity at the tested concentration.

Table 1: Chemical components identified in seeds oil by GC-MS

Retention time	Compounds	Uses
10.079	2-amylfuran	Antimicrobial
10.52	Pentanoic acid	Antidepressant Jayaraj, Richard L., et al.(2020)
11.255	Hexanoic acid	Antioxidant Hammoudi, A., Zatla, A. T., & El Amine Dib, M. (2023)
12.702	Decane	NarcoticFrancke, W., & Schulz, S. (2010)
16.898	2,4,Decadienal ,	Anticancer,Nematicidal Caboni, Pierluigi, et al.2012
17.373	C_{10} H_{18} O	Antiinflammatory ,anti-pain
29.072	Palmitic Acid	Raises LDL Music, Janet, et al.
37.37	Octadec-9-enoic acid	anti-inflammatory, antioxidant, anti-cancer, and anti-diabetic properties Habtemariam, S. (2019).
63.41	Stigmasta 3,5-diene-7-one	anti-inflammatory and antioxidant properties Arthritis.

Table 2: Fatty acids identified by GC-MS in seeds oil.

Peak	Name	Medicinal Uses
1	C16:0 Palmitic Acid ($C_{16}H_{32}O_2$)	Cholesterol regulation (Murru *et al.*, 2022) Skin health (Dudau *et al.*, 2021) The energy source
2	C16:1 Palmitoleic Acid ($C_{16}H_{30}O_2$)	Eye health (Huang *et al.*, 2020) Cancer preventive, anti-inflammatory and metabolic regulator (Fauziah *et al.*, 2022)
3	C18:0 Stearic Acid ($C_{16}H_{30}O_2$)	Controlled-released drug discovery system Wound healing
4	C18:1 Oleic Acid ($C_{18}H_{34}O_2$)	Anti-inflammatory, wound healing, cancer preventive, cardiovascular health, immunomodulator
5	C18:2 Linoleic Acid ($C_{18}H_{32}O_2$)	Nervous system health, atherosclerosis, immunomodulation
6	C20:0 Eicosanoic Acid ($C_{20}H_{40}O_2$)	Anti-elastase, anti-oxidant, anti-urease (Zekeya *et al.*, 2022)
7	C 22:0 Behenic Acid ($C_{22}H_{44}O_2$)	Anti-inflammatory, anti-oxidant, antimicrobial
8	C20:4 Arachidonic Acid ($C_{20}H_{32}O_2$)	Infant nutrition and skin health (Sambra *et al.*, 2021)
9	C22:6 Docosahexaenoic Acid ($C_{22}H_{32}O_2$)	Nervous system health, cardiovascular diseases, anti-inflammatory and cancer prevention (Watanabe and Tatsuno, 2021)

Table 3: Iodine value of seeds oil of *Adenanthera pavonina*

Peak#	Name	Mass	Area	Mass*Area/100	Area %
1	C16:0 Palmitic Acid	256	23.828	256*23.828/100	60.999
2	C18:0 Stearic Acid	284	8.674	284*8.674/100	24.634
3	C18:1 Oleic Acid	282.46	0.34	282.46*0.34	0.960
4	C18:3 Linolenic Acid	278.43	2.082	278.43*2.082	5.796
5	C20:0 Eicosanoic Acid	310.53	3.845	310.53*3.845	11.939
6	C 22:0Behenic Acid	340.68	8.281	340.68*8.281/100	28.203
7	C22:6 Decosahexanoic Acid	328.488	4.576	328.488*4.576/100	15.031
8	Saturated Fatty Acids	44.624		SUM	133.790
9	Unsaturated F.A	2.53		SAP VALUE	382.9

Components	*Names*	*Factors*	*Areas*	*FAC*Area*
C16:!	*Palmitoleic Acid*	*0.956*	*0.000*	*0*
C18:1	*Oleic Acid*	*0.859*	*0.340*	*0.292*
C18:2	*Linoleic Acid*	*1.731*	*0.448*	*0.775*
C18:3	*Linolenic Acid*	*2.616*	*2.082*	*5.446*
C20:4		*3.201*	*8.281*	*26.507*
C22:6		*4.663*	*4.576*	*20.422*
		Iodine Value		*53.444*

Fig. 1: Percentage composition of phytochemicals of oil identified by GC-MS

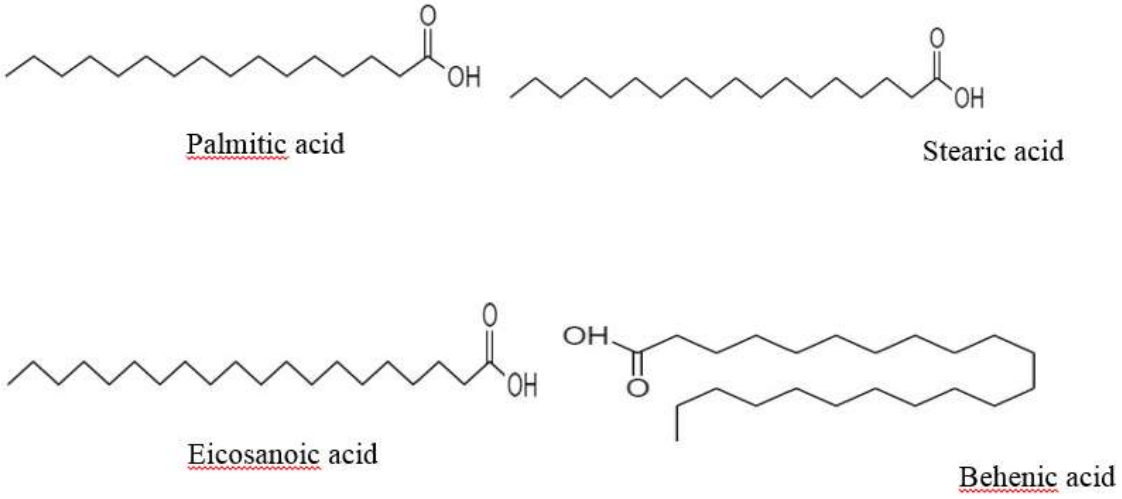

Table 4: Percentage Scavenging activity (PSA) values of *Adnanthera pavonina* seeds oil

Sample Code	IC_{50} ± SEM	PSA (%)
Sample	active	77.187
Gallic acid	23.436 ± 0.43 (µM)	93.93
N- acetylcysteine	111.44 ± 0.7 (µM)	95.95

Thus, GC-MS analysis demonstrated the presence of various bioactive phytochemicals imparting medicinal value to *Adenanthra pavonina L.* seeds oil. Key parameters like iodine and saponification values verified the unsaturated fatty acid profile of the oil.

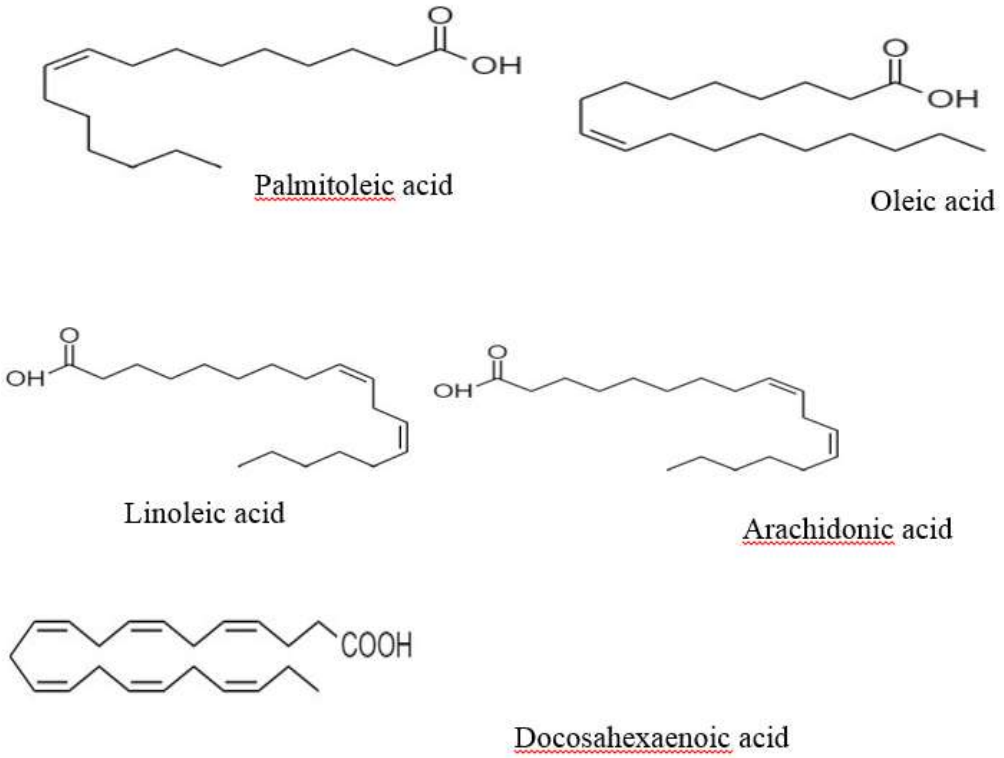

Fig. 3: Structures of unsaturated fatty acids identified by GC-MS analysis of seeds oil of *Adenanthera Pavonina*

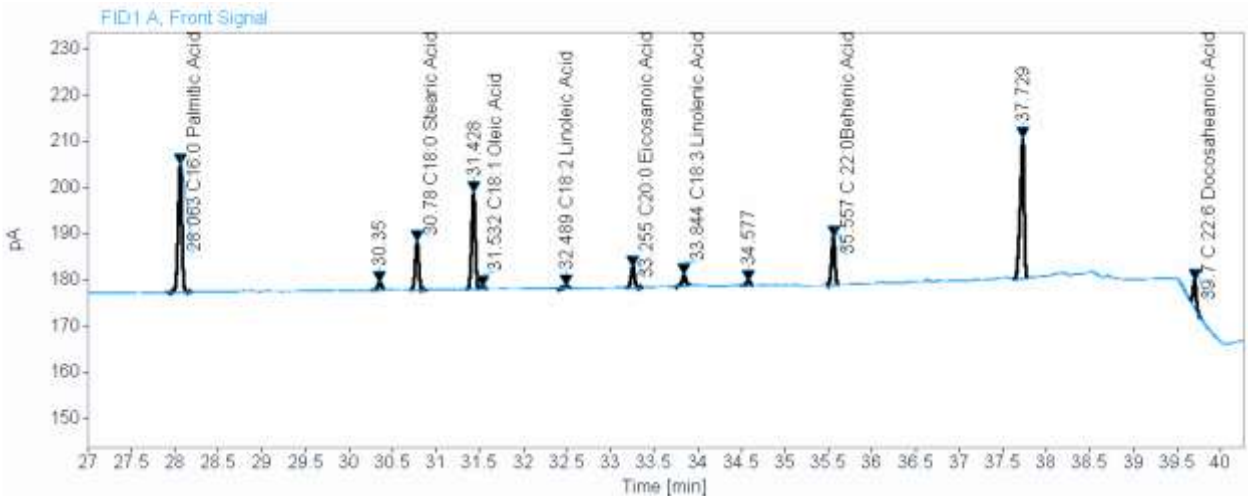

Fig. 4: GC-MS chromatogram of *Adenanthera Pavonina* seeds oil

DISCUSSION

The fatty acids identified through the GC-MS have nutritional as well as medicinal uses.

The oil is found to be rich with lipids that provide organisms with inert forms of energy used in conditions of nutrient deprivation and environmental stress.(R. Zarnowski et al 2004) They also provide an excellent "sink" to buffer the toxic effects of fatty acids and fatty alcohols. The seed oil of plants is a concentrated source of fatty acids. The phytochemical components and the physicochemical characteristics of any oil determine its applications. Literature review shows that *Adenanthra pavonina L.* seeds are rich in essential oils but its phytochemical profile and bioactivities have not been completely explored. Thus, the present study focused on looking into the complete phytochemical screening, physicochemical analysis and antioxidant potential of *Adenanthra pavonina L.* seeds oil using GC-MS and the free radical scavenging activity of DPPH. This plant is ethno medicinally useful and is mostly found in the tropical areas of Southeast Asia. Different plant parts including leaves, bark, flowers, fruits, roots, and seeds have traditionally been used in various ailments. Previous studies claimed the separation of various metabolites. in *Adenanthra pavonina L.* plant possessing various medicinal properties (Kothale, K. V., & Rothe, S. P. (2012). Despite its traditional medicinal uses, there is limited scientific data available on the chemical profiling of oil for which the current study was conducted.

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 53.444 Ig/100g 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 acids profile of the oil. The antioxidant effect of the oil was determined by DPPH radical scavenging assay method. In this study, the oil showed very low radical scavenging activity of 77.187% as compared to the standard antioxidants gallic acid (93.93%) and N- acetyl cysteine (95.95%). This suggested that the *Adenanthera pavonina L.* seeds oil possess significant antioxidant properties. Previous studies also indicated *Adenanthra pavonina L.* seeds are rich reservoirs of bioactive compounds with therapeutic effects. In addition to therapeutic efficacies and antioxidant activities, previously limited toxicity studies were also conducted on rats showing seeds extracts to be non-toxic up to 2000mg/kg acute oral dose, indicating a high margin of safety. Sub-acute 28-dayoral toxicity studies in rats showed slight elevations in serum enzymes like ALT, AST, etc. at 500-1000 mg/kg doses signifying mild hepatotoxicity which was confirmed histologically (Gulcin and Alwasel, 2023). Nevertheless, there were no adverse effects observed up to a dose of 100mg/kg, thereby establishing a preliminary level of safety. Additional studies on sub-chronic toxicity are necessary to decisively ascertain the secure and effective therapeutic dose range in humans which is only possible by knowing its phytochemical profile.

CONCLUSION

In conclusion the present study provided the detailed physicochemical and phytochemical profiling of *Adenanthra pavonina L.* Seeds oil. It contains medicinally important bioactive compounds such as fatty acids, including Pentanoic acid, Hexanoic acid and palmitic acid along with 2-amylfuran and decane. Oil exhibited significant antioxidant potential. Further studies can isolate these bioactive phytochemicals and investigate their pharmacological activities. The oil showed traditional medicinal uses which need to be scientifically validated through *in vitro* as well as *in vivo* studies. Future studies can also evaluate the antibacterial, antifungal, anti-inflammatory and wound-healing properties of this oil. Clinical trials are also required to establish the therapeutic efficacy and safety of *Adenanthra pavonina L. S*eed oil for medicinal uses.

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