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PHYTOCHEMICAL, PHYSICOCHEMICAL AND PHARMACOLOGICAL PROFILING OF FIXED OIL EXPRESSED FROM SEEDS OF *BUTEA MONOSPERMA*

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Abstract:
Plants are one of best the source of fixed oils. Oils expressed from seeds are multipurpose. These oils exhibit plenty of therapeutic effects including antioxidant, antiinflammatory, antimicrobial and analgesic. *Butea monosperma* one of the significant source of fixed oils. It is distributed throughout Pakistan, Iran, Bangladesh, India and Africa. Traditional use of oil expressed from seeds of this marvelous plant include treatment of inflammation, rheumatism, bronchitis, diabetes, anemia, fever, gynecological disorders, and pneumonia. The plant possess antioxidant, hepatoprotective, gastro protective, wound healing, antiarthritic, larvicidal, antimalarial, antiemetic, antibacterial, antifungal, antiinflammatory, analgesic, antidiarrhoeal, antiheamolytic, diuretic, and anthelmintic activities. Scientific validation of these traditional uses is of utmost importance to gain maximum benefits and avoid any hazardous effects. Findings of this research project elaborated that this plant seed oil contains almost 32 compounds belonging to various classes. These include Isoamylene, 2-Pentene, Isoamyl methyl ketone, n-amyl methyl ketone, Methyl 2-methyl butyl ketone, 1-methyl-1-ethylcyclopentene, 5-0ctandione,Isopropyl pentyl ketone, 2-amylfuran, Hexanoic acid, 1-ethyl-1-methyl-cyclopentane, (E)-2-Octenal, Hexanoic acid ester, Sesquiterpenoid, Octyl alcohol ester ethyl hexyl ester, Octyl ester, Tridecane, 2,4,Decadienal,$C_{10}H_{18}O$,3-Decanone,4-undecanone,4-methyl-3-hetanone,2-DodecanalOctadec-9-enoic acid, Hop-22(29)-en-3β-ol, Stigmasta 3,5-diene-7-one and C_{44} H_{88} O_2 . Seven types of fatty acids including Palmitic acid (69.2%), Palmitoleic acid (2.2 %), Stearic Acid (45.8 %) Oleic Acid (83%), Linoleic acid (38.6%), Eicosanoid acid (4.5%) were identified as per by GCFID. Saturated fatty acids content was 45.206% and unsaturated content was 16.623 % Iodine value was found to be 64.669 and SAP value was 216. The antioxidant effect was

found to be 72.187%. These finding justified the traditional uses of this medicinal oil in various conditions.

Key Words: GCMS, Antioxidants, Plant, Seeds, Oil, medicinal Plant, *Butea monosperma*

Introduction:

Butea monosperma is a species of *Butea* native to tropical and sub-tropical parts of South Asia and Southeast Asia. It is also known as flame of the forest, Bengal kino, dhak, palash, and bastard teak. It is a multipurpose tree. It belongs to family Fabaceae (Germplasms resources information network).Fixed oil expressed from this important plant species needs thorough investigation. Phytochemical and physicochemical investigations can be used to set standards for pure oil identification.(Chirinos, Rosana, et al.) Due to the lack of quality standards and progressive adulteration in the natural oils, their therapeutic efficacy is continuously deteriorated. To develop quality standards and validate scientific aspects on essential oils, several chromatographic and spectroscopic techniques such as HPTLC, HPLC, NMR, LC−MS, and **GC−MS** have been termed as the choices of techniques for better exploration of metabolites, hence sustaining the authenticity of the essential oils. In this investigation, chemical profiling and quality control aspects of essential or fixed oils have been explored and compared from previously reported literature in reputed journals. Methods of chemical profiling, possible identified metabolites in essential oils, and their therapeutic applications have been described. The outcome of the investigation reveals that GC−MS/GCFID techniques are one of the most liable, economic, precise, and accurate techniques for determining the spuriousness or adulteration of oils based on their qualitative and quantitative chemical profiling studies.

Gas chromatography technique is used for both qualitative as well as quantitative analysis. It separates the components in vapors state so the compounds analyze by this technique should be convertible into gas form. GCMS can be a good candidate for the compounds that are difficult to identify from GC technique alone. (R.M. Hannan and H.H. Hill, 1991). Suitable hyphenations for GC are GC-MS and GC-Fourier transform IR. MS detector addition to GC gives it more ability to detect minor components and homologous series of compounds too (Gaines, 1999; Robert Shellie, 2004).

Antioxidants inhibit or delay the oxidation process of oxidants. In many diseased conditions there is oxidative stress. To overcome these conditions antioxidants are usually used (Amorati, 2013). Vegetables oils contain polyphenols that are usually responsible for antioxidants (M.Silvia Taga, 1984). Presence of antioxidants in vegetables oils protects them from rancidity or deterioration (Ghulam Shabir, 2011).

In the present study we concentrate on GCMS analysis of seed oils of Butea monosperma. Study also inspects the presence of antioxidant activity in oils extracted from the seeds of Butea monosperma.

MATERIALS AND METHODS

Collection of Plant Material:

The seeds (1kg) of dhak tree were collected from Punjab province of Pakistan, the Narowal district. Sample specimen was allotted voucher Number # (5A) and submitted to Seed collection center department of Pharmacognosy Government college University Faisalabad (GCUF) Pakistan.

Seeds of *Butea monosperma*

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 70g powdered seeds was introduced to 150cm^3 of n-hexane which served as an extractor and was 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 (Nikita and Shweta, 2020).

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

Gas chromatography-mass spectroscopy (GC-MS) analysis of B. ceiba 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.

Methodology was adopted from already established studies. Briefly 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 taken for (GC-MS) analysis. Parameters were set for optimum outcomes. Conditions were maintained in line with those for GC-MS. The ionization was done at set voltage at 70 eV to speed up the ionization process. Source temperature was maintained at 230°C and the electron multiplier voltage was set to 900 V (Kubeczka, 2020).

Determination of fatty acids, iodine value, and SAP value of the seeds oil

Analysis of fatty acid composition was done by investigating analyzed the methyl esters of the acids. These were prepared using the AOAC method, BF3-MeOH complex was included. 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. 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 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 (Minelli *et al.*, 2023).

The stable free radical 2, 2-dipehnyl-1-picylhydrazyl (DPPH) was used to test the antioxidant capacity of a range of *Butea monosperma* 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 stochiometric 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 micro plate 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.

% RSA = 100- (O.D of sample/ O.D of control×100) … Eq. 2

In vitro Antioxidant study:

DPPH-Radical Scavenging Assay:

The evaluation of the antioxidant capacity of different samples involves the use of the free radical, 2, 2-Dipehnyl-1-picylhydrazyl (DPPH). It is a rapid, simple and inexpensive method and is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of different samples by utilizing the stable 2, 2-diphenyl-1-picrylhydazyl (DPPH) radical .The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to pale yellow as the molar absorptivity of DPPH radical at 515nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from

a free radical scavenging antioxidant to form the reduced DPPH. The resulting decolourization is stoichiometric with respect to number of electron captured.

Ash content:

A crucible is used to contain 1 g of Butea monosperma Seeds fine powder, which is thereafter subjected to combustion within a muffle furnace operating at a temperature of 500 ^{o}C for 2 hours. The sample is cooled at room temperature within a desiccator, and the ash content is then measured using the following Equation.

Ash content = Ash weight Dried/ sample weight × 100

Moisture content:

The quantification of moisture content two crucibles were used to hold separate 1 gm samples of *Butea monosperma* Seeds. These samples were then subjected to a temperature of 100 ^{o}C in a muffle furnace for 6 hours. After the allotted time, the crucibles were allowed to cool down before being weighed. The subsequent Equation (13) is employed for the estimation of the moisture content.

Moisture Content = Sample weight after dried /Taken sample weight × 100

Proximate analysis:

The proximate components of the Seed of *Butea monosperma*. Each finding collected was on a dry basis and represented as a percentage. The levels of crude fiber and ash content were found to be increased. Protein content study is fundamental to the identification of varieties. *Butea monosperma* Seeds with a considerable carbohydrate content exhibit greater advantage.

Results:

Identification of Compounds from GCMS spectral Analysis from Butea monosperma seeds oil.

Sr.No.	Retention time	Peak	Compounds	Number
1	3.753	A	a-isoamylene	563-45-1
2	3.753	A	(z) 2-Pentene	627-20-3
3	3.753	A	(E) 2-pentene	646-04-8
4	3.753	A	f-isoamylene	563-46-2
5	7.266	C	Isoamyl methyl ketone	110-12-3
6	7.226	C	n-amyl methyl ketone	110-43-0
7	7.226	C	Methyl 2-methyl butyl ketone	105-42-0
8	9.125	E	1-methyl-1-ethylcyclopentene	16747-50-5
9	9.868	CT	5-0ctandione,Isopropyl pentyl ketone	3241-41-3, 923-28-4
10	10.076	G	2-amylfuran	3777-69-3
11	10.361	I	Pentanoic acid	109-52-4
12	10.178	J	Hexanoic acid	
13	11.439	BX	1-ethyl-1-methyl-cyclopentane	
14	11.673	K	(E)-2-Octenal	2548-87-0
15	11.754	L	Some Hexanoic acid ester	
16	14.604	Q	Isomer of 2-Decanone	
17	14.75	R	Sesquiterpenoidal	
18	15.826	T	Octyl alcohal ester ethyl hexyl ester	
19	15.916	U	Octyl ester	
20	16.459	AR	Tridecane	
21	16.831	CC	2,4,Decadienal	
22	17.35	Y	C_{10} H_{18} O	
23	17.608	Z	3-Decanone , 4-undecanone,4-methyl-3-hetanone	
24	17.631	AA	2-Dodecanal	4826-62-4

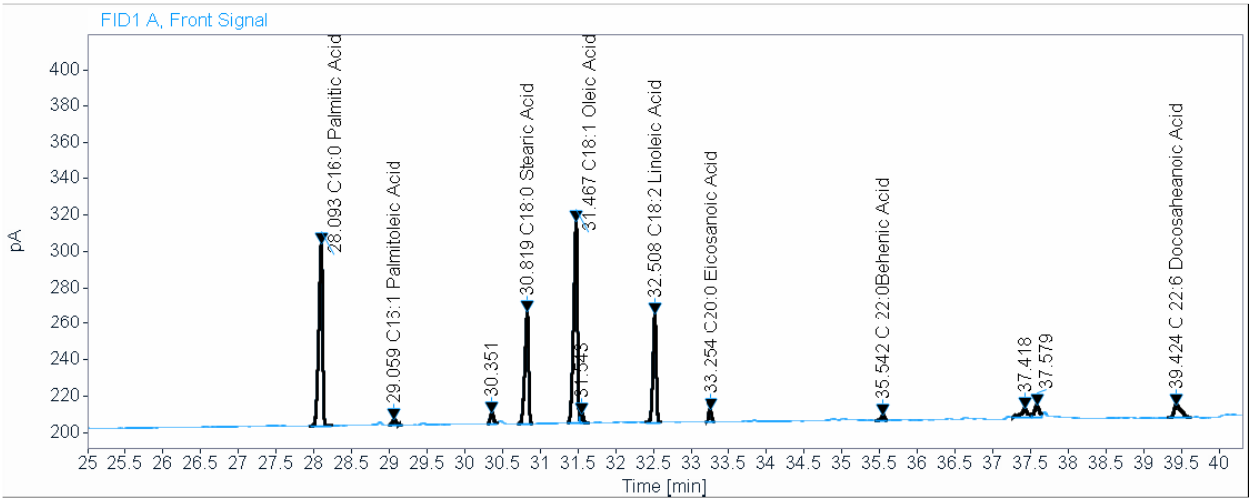
25	19.835	AD	Fatty Alcohol	
26	20.186	CF	Branched Alcohol	
27	21.282	AV	Hexadecane	
28	28.887	AH	Palmitic Acid	28.32
29	37.2	AI	Octadec-9-enoic acid	
30	63.391	BT	Hop-22(29)-en-3β-ol	58801-23-3
31	63.563	AN	Stigmasta 3,5-diene-7-one	
32	68.8	BU	C_{44} H_{88} O_2	

Phytochemical screening of *Butea monosperma* Seeds oil:

S. N.	Extract	MeOH	EtOH	AQE	EA
1	Flavonoids	+	+	+	+
2	Alkaloids	-	+	+	+
3	Terpenoids	+	+	+	+
4	Steroids	+	+	+	-
5	Phenols	+	+	+	-
6	Sterol	-	+	+	+
7	Saponins	-	+	+	+
8	Tannins	+	+	+	+
9	Glycosides	-	+	+	+
10	Carbohydrates	-	+	+	+
11	Amino Acid	+	+	+	-
12	Phlobatannins	-	-	-	-
13	Quinonones	-	-	-	-
14	Oxalates	-	-	-	-

Proximate analysis of Butea monosperma Seed:

Sr.No	Parameters	Values
1	Total Ash	7.28
2	Protein	17.42
3	Fiber	8.91
4	Fat	7.23
5	Carbohydrates	45.11
6	Moisture	88.54



Gas Chromatography Mass Spectrometric studies:

SAPONIFICATION & IODINE VALUE

SAP VALUE		Chromatograms			Sample #(Dr. Rija)1			
Compostion	Names	Area % (1)	Area % (2)	Area % (3)	Average	Mass	Area	Aass*area/100
C6:0	Caproic Acid				0.000	116	0	0
C8:0	Caprylic Acid				0.000	144.21	0	0
C10:0	Capric Acid				0.000	172.26	0	0
C12:0	Lauric Acid				0.000	200.3	0	0
C14:0	Myristic Acid				0.000	228	0	0
C16:0	Palmitic Acid	27.033			27.033	256	27.033	69.20448
C16:1	Palmitoleic Acid	0.859			0.859	254.4	0.859	2.185296
C18:0	Stearic Acid	16.118			16.118	284	16.118	45.77512
C18:1	Elaidic Acid				0.000	282.46	0	0
C18:1	Oleic Acid	29.722			29.722	282.46	29.722	83.952761
C18:2	Linoleic Acid	13.764			13.764	280.45	13.764	38.601138
C18:3	linolenic Acid				0.000	278.43	0	0
C20:0	Eicosanoic Acid	1.453			1.453	310.53	1.453	4.5120009
C22:0	Behenic Acid	0.602			0.602	340.58	0.602	2.0502916
C22:1	Erucic Acid				0.000	338	0	0
C22:6	decosahexonic	3.247			3.247	328.488	3.247	10.666005
Saturated Fatty Acid		45.206		Total	89.551		SUM	246.28109
UnSaturated Fatty Acid		14.623		Others	10.449			
SAP VALUE →						216.621		

IODINE VALUE

Compostion	Names					FACTOR	AREA	FAC*AREA
C16:1	Palmitoleic Acid					0.956	0.859	0.821204
C18:1	Oleic Acid					0.859	29.722	25.531198
C18:2	Linoleic Acid					1.731	13.764	23.825484
C18:3	linolenic Acid					2.616	0.000	0
C20:4						3.201		0
C20:5						4.027		0
C22:1						0.723	0.000	0
C22:5						3.697		0
C22:6						4.463	3.247	14.491361
Iodine Value →								64.669

The pale yellowish oil of *Butea monosperma* seeds was extracted by the Soxhlet extraction method employing n-hexane as solvent. The seeds yield 14.14% oil based on an initial sample of dried seeds.

Phytochemical analysis:

This investigation was performed using GCMS ,GCFID and general phytochemical testing. This investigation resulted in to Presence of 32 compounds by GC MS, five prominent Fatty acid by GCFID and 10 secondary metabolites including Flavonoids,alkaloids,and terpenoids. Findings of this research project elaborated that this plant seed oil contains almost 32 compounds belonging to various classes. These include Isoamylene, 2-Pentene, Isoamyl methyl ketone, n-amyl methyl ketone, Methyl 2-methyl butyl ketone, 1-methyl-1-ethylcyclopentene, 5-0ctandione,Isopropyl pentyl ketone, 2-amylfuran, Hexanoic acid, 1-ethyl-1-methyl-cyclopentane, (E)-2-Octenal, Hexanoic acid ester, Sesquiterpenoid, Octyl alcohol ester ethyl hexyl ester, Octyl ester, Tridecane,

2,4,Decadienal,$C_{10}H_{18}O$,3-Decanone,4-undecanone,4-methyl-3-hetanone,2-DodecanalOctadec-9-enoic acid, Hop-22(29)-en-3β-ol, Stigmasta 3,5-diene-7-one and C_{44} H_{88} O_2 . Seven types of fatty acids including Palmitic acid (69.2%), Palmitoleic acid (2.2 %), Stearic Acid (45.8 %) Oleic Acid (83%), Linoleic acid (38.6%), Eicosanoid acid (4.5%) were identified as per by GCFID. Saturated fatty acids content was 45.206% and unsaturated content was 16.623 % Iodine value was found to be 64.669 and SAP value was 216. The antioxidant effect was found to be 94%. These finding justified the traditional uses of this medicinal oil in various conditions.

Physicochemical parameters

The iodine value of oil was calculated as 64.660 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 calculated for *B.monosperma* oil was also high indicating the presence of more fatty acids with longer chain lengths (table 3). 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 *Butea monosperma* 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 72.187% radical scavenging activity at the tested concentration. This was low as compared with the standard antioxidants gallic acid and N-acetyl cysteine which showed 94-96% scavenging activity. The IC_{50} ± SEM value of oil could not be determined as it was inactive against the DPPH radical. Therefore, the present study showed the non-significant results of antioxidant activity of the *Butea monosperma* seeds oil. Further detailed evaluation using multiple antioxidant assays is required to confirm the antioxidant profile of oil. The analysis elaborated the presence of various bioactive phytochemicals giving medicinal value to *Butea monosperma* seeds oil. Physicochemical parameters like iodine and saponification values verified the unsaturated fatty acid profile of the oil.

DISCUSSION

The fatty acids identified through this investigation possess three fold uses medicinal, nutritional and cosmeceutical. They have significant medicinal uses such as cholesterol regulation, skin health, cancer prevention, eye health, metabolic regulator, anti-inflammatory properties, in controlled-released drug discovery systems, cardioprotective properties, atherosclerosis, anti-elastase, anti-urease, antimicrobial, antioxidant activity, wound healing, etc. The seed oil of plants is a concentrated source of fatty acids, sterols, glycerides, tocopherols and other non-glyceride components like flavonoids, c etc. 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 *Butea monosperma* 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 *Butea monosperma* seeds oil using GC-MS and the free radical scavenging activity of DPPH. This plant is ethnomedicinally useful and is mostly found in the tropical areas of Southeast Asia. Different plant parts including gum, leaves, bark, flowers, fruits, roots, and seeds have traditionally been used in various ailments. Previous studies claimed the separation of various metabolites such as flavonoids, fatty acids, phytosterols, etc. in *Butea monosperma* plant possessing anticancer, antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, analgesic, antidiabetic and wound healing properties (Sutariya ,B.K). Despite its traditional medicinal uses, there is limited scientific data available on the chemical profiling of B.monosperma seeds oil for which the current study was undertaken to fulfill this gap.

Rajput (2022) had already reported 17% oil content in the seeds of this plant (Rajput, 2022). The GC-MS technique identified 31 chemical compounds comprising different phytochemical classes such as terpenoids, ketones, esters, alcohols, acids and other compounds in minor quantities. 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. The major terpenoids found in the oil were sesquiterpenoids, lupeol, Stigmastan-3,5-diene and olean-12-en-3-one. Terpenoids are known to have anti-inflammatory, anti-cancer and antioxidant activity. The significant ketones present, were isoamyl methyl ketone, 2-decanone and 7-pentadecanone possessing antimicrobial and larvicidal properties. Key esters found were octyl ester, ethyl hexyl ester and octyl alcohol ester which have emollient properties. 1-Heptanol and nonanoic acid were the major alcohols and acids found in the oil respectively. 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 67.832 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 potential of the oil was evaluated through the DPPH radical scavenging assay method. In this study, the oil showed low radical scavenging activity of 72.147% as compared to the standard antioxidants gallic acid (93.93%) and N- acetyl cysteine (95.95%). The IC_{50} ± SEM of the oil could not be calculated as it was active. This suggested that the *Butea monosperma* seeds oil possess significant antioxidant properties. B.monosperma oil itself, previous studies indicate *Butea monosperma* seeds are rich reservoirs of bioactive compounds with therapeutic effects. Steroidal phytoconstituents like β-sitosterol, stigmasterol and campesterol isolated from B.monosperma seeds extracts have shown anti-hypercholesterolemic, antidiabetic, anti-inflammatory and immunomodulating properties (Sutariya, B. K., & Saraf,). Flavonoid B.monospermaquinone from the seeds displayed potent antioxidant activity comparable to standard compounds butylated hydroxytoluene (BHT) and α-tocopherol by effectively scavenging DPPH, superoxide, and nitric oxide radicals (Sutariya, B. K., & Saraf,).Further research on isolation of the agent responsible for antioxidant activity in B.monosperma seeds will be worth pursuing for drug discovery efforts. In addition to therapeutic efficacies and antioxidant activities, previously limited toxicity studies were also conducted on rats showing B.monosperma 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). No doubt 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:

This study provides the thorough phytochemical investigation of *Butea monosperma* seeds oil. It contains medicinally important bioactive compounds such as fatty acids, terpenoids, alcohols and esters. Further investigations can be planed to isolate these bioactive phytoconstituents and investigate their pharmacological activities. The seed oil has traditional applications which need to be scientifically validated through *in vitro* as well as *in vivo* studies. In future antimicrobial, anti-inflammatory and wound-healing properties of this oil can be studied. Clinical trials should also be planed for establishing the therapeutic efficacy and safety of *Butea monosperma* seed oil for medicinal uses.

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