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# Phytochemical screening and physicochemical analysis of oil extracted from seeds of *Bombax ceiba* and determination of antioxidant activity

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**Abstract**: *Bombax ceiba*, an ethnomedically useful plant belonging to the Bombacaceae family, is traditionally used to treat various ailments. With the increase of interest in herbal remedies globally, it is imperative to scientifically validate the phytochemical profiling to ensure therapeutic utility and safety. The present study was designed to comprehensively analyze the phytochemical composition of *Bombax ceiba* seeds oil to provide evidence for its medicinal uses. The recommended standard Soxhlet extraction method was used to isolate the oil from the seeds. Its chemical profile, physicochemical parameters, and antioxidant potential were characterized. The GC-MS analysis revealed the presence of 31 diverse phytoconstituents including vital terpenoids, ketones, esters, alcohols, aliphatic acids, and other compounds in minor quantities which are known to possess wide pharmaceutical applications. The key unsaturated fatty acids identified with nutritional and therapeutic benefits were oleic, linoleic, palmitoleic, arachidonic, and docosahexaenoic acids. The high iodine value of 67.832g I/100g indicates a high degree of unsaturation. Although the DPPH assay showed minimal antioxidant activity, the myriad of bioactive components confers significant pharmacological utility to *Bombax ceiba* seeds oil. By providing in-depth phytochemical insights, this research work validates this oil's traditional and other medicinal uses, which can be further explored for newer ethnomedicine development.

**Keywords**: Antioxidant activity, phytochemical screening, iodine value, medicinal plants, saponification value.

## INTRODUCTION

*Bombax ceiba* is a kind of tree that belongs to the family Bombacaceae. The term "silk cotton" is often used to describe this material. Warm forested regions in India, China, and Pakistan are ideal for this plant's development. In Pakistan, it is present in northern places like Hazara, and even in Sindh province. Large trees often have a straight, unbranched trunk and a grey outer bark covered in rough, tiny prickles that become less noticeable with age (Wang *et al.*, 2023; Shah *et al.*, 2018). This plant is very important ethno medicinally. Several studies have been conducted on different parts of this plant like bark, flowers, gum, and leaves to validate its medicinal uses.

Panwar *et al*. (2020) stated the gum properties of *Bombax ceiba* species. The author cited that the purified mineral matter found in gum contains a larger number of tannins and catechol tannins. The gum can be used against infectious and other practical diseases (Panwar *et al.*, 2020). Ghazanfar *et al*. (2022) did an analytical survey of cellulose raw material and found more than 60% of cellulose and 9% lignin presence in *Bombax ceiba* (Ghazanfar *et al.*, 2022). Shukla *et al.* (2020) identified and isolated the crystalline compound namely lupeol from the bark of the plant and formed an acetyl derivative (Shukla *et al.*, 2020). Chaudhary & Tawar isolated the ceibanaphthaquinone and lupeol from the bark of the stem of *Bombax* (Chaudhary and Tawar, 2019). Emmanuel and Esther (2022) isolated the hemigossypolon-6-methylether and hemigossypol from the root bark of the plant and further reported the isolation of naphthol ether, dimethyl naphthol hemigossypol and naphthol (Emmanuel and Esther, 2022). The structures were established based on spectral and chemical properties which are nearly related to gossypol. The methanolic extract of fresh petals of *Bombax* contains two anthocyanin glycosides A and B (Huang *et al.*, 2023). Anthocyanin A was identified to be pelargonidin 5-beta-D-glucopyranoside and B was cyanidin 7-methylether-3-beta-glucopyaramoside. Trees of *Bombax ceiba* are useful for different types of medicinal purposes like gum used in dysentery, leukorrhea, diarrhea, and menorrhagia. Its powdered spike paste is used for acne. Roots are used as restorative, aphrodisiac, astringent, and alterative. Juice extract from roots is used for lowering fever or antipyretic. The top of the root is used as a demulcent, diuretic, tonic, and aphrodisiac. Roots can also be used for the treatment of dysentery, gonorrhea, and impotence. They are also used in rheumatic swelling. The bark is used as a demulcent, tonic and diuretic and is also used in inflammation. Chop leaves paste is used for skin treatment. Flowers are diuretic and laxative. Fruit can be used in ulceration, kidney problems and also use in snake bites. Seeds produce pale yellow oil which can be used for edible purposes and for soap making and as an illuminant. This article provides an overview of the phytochemical

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screening and physicochemical properties of seeds oil of *Bombax ceiba* which are first time determined by GC-MS and also evaluate the antioxidant activity. To support its application in traditional medicine, GC-MS identified a variety of phytochemicals in this extract. It is important to note, that the present study showed that this oil possessed minimal antioxidant properties, despite its traditional use as an antioxidant. Henceforth, it was urged to further extend out GC-MS as well as antioxidant evaluation on the extract.

## MATERIALS AND METHODS

***Seeds collection and chemicals used***

Seeds of the *Bombax ceiba* were collected from the University of Karachi. The collected seeds were submitted with a specimen voucher number 105 E to the herbarium of the Department of Pharmacognosy, University of Karachi. 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). 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).

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

***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.

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 fatty acids, iodine value, and SAP value of the B. ceiba seeds 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 $BF_3$-MeOH complex. Ten milliliters 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 (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 *Bombax ceiba* 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 37ºC 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.

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

## STATISTICAL ANALYSIS

The statistical analysis was performed using Microsoft® Office version 2013 using the Excel sheet. Data was added to the sheet columns and statistical analysis was performed.

## RESULTS

The pale yellowish oil of *Bombax ceiba* 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***

*Bombax ceiba* seeds oil underwent phytochemical analysis using gas chromatography-mass spectrometry (GC-MS). GC-MS analysis identified fatty acids and 31 chemical compounds comprising 100% composition of the oil. The significant phytochemical classes/groups found were fatty acids (25.80%), terpenoids (20.30%), ketones (14.50%), esters (14.20%), alcohols (9.70%), aliphatic acids (5.80%) and other compounds (9.70%) as shown in fig. 1. 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) (43.71%), stearic acid (C18:0) (15.71%), eicosanoic acid (C20:0) (2.84%), and behenic acid (C22:0) (1.67%) as shown in fig. 2. While the major fatty acids (unsaturated) found were linoleic acid (C18:2) (3.08%), arachidonic acid (C20:4) (0.37%), palmitoleic acid (C16:1) (2.72%), oleic acid (C18:1) (1.91%), and decosahexaenoic acid (C22:6) (0.737%) as shown in fig. 3.

Major terpenoids found were sesquiterpenoids, lupeol, Stigmastan-3,5-diene, and Olean-12-en-3-one. Isoamyl methyl ketone, 2-decanone, and 7-pentadecanone were common ketones present in the oil (table 2). The main esters were octyl ester, ethyl hexyl ester, and octyl alcohol ester. Important alcohols and aliphatic acids found were 1-heptanol, nonanoic acid and decanoic acid respectively (fig. 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 *Bombax ceiba* seeds oil. This confers a broad-spectrum pharmacological profile to the oil.

***Physicochemical parameters***

The iodine value of oil was calculated as 67.832 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 Bombax 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 *Bombax ceiba* 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 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 *Bombax ceiba* seeds oil as mentioned in table 4.

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

| S. No. | Name of Compounds | Retention time (min) | Medicinal uses |
|---|---|---|---|
| 1 | α- isoamylene/(z) | 3.7 | |
| 2 | 2-Pentene | 3.7 | |
| 3 | f-isoamylene | 3.7 | |
| 4 | Isoamyl methyl ketone | 7.226 | Antifungal and larvicidal activities (Hanif *et al.*, 2022) |
| 5 | n-amyl methyl ketone | | |
| 6 | 2-methyl butyl ketone | | |
| 7 | 1-methyl-1-ethyl cyclopentene | 9.118 | |
| 8 | 1-Hetanol | 9.554 | Antioxidant and anti-inflammatory activities |
| 9 | 2-amyl furan | 10.032 | Antimicrobial and antioxidant properties |
| 10 | Hexanoic acid | 11.128 | Antimicrobial and antioxidant effects (Hemeg *et al.*, 2020) |
| 11 | Hexanoic acid ester | 11.744 | Antimicrobial and anti-inflammatory effects (Hemeg *et al.*, 2020) |
| 12 | Octyl ester acid | 12.012 | Emollient in cosmetics (Ortega-Requena *et al.*, 2024) |
| 13 | Unsaturated fatty alcohol | 13.85 | Antioxidant effects (Callau *et al.*, 2020) |
| 14 | Methyl ketone | 14.483 | |
| 15 | 2-Decanone | | Anticancer, antioxidant and mosquito repellent (Nahar *et al.*, 2021) |
| 16 | Sesquiterpenoid | 14.741 | Anti-inflammatory, antioxidant and anticancer activities (Diniz do Nascimento *et al.*, 2020) |
| 17 | Octyl alcohol ester | 15.541 | Emollient, antibacterial and antifungal (Handayani *et al.*, 2021) |
| 18 | Ethyl hexyl ester | 15.826 | Emollient and skin conditioning agent (Carson and Gallagher, 2020) |
| 19 | Octyl ester | 15.91 | Emollient property (Vilas Boas and de Castro, 2022) |
| 20 | Nonanoic acid | 16.439 | Antimicrobial effects (Shahrivari *et al.*, 2024) |
| 21 | 3-decanone | 17.541 | Anticancer, anti-inflammatory and larvicidal effects (Ashenagar and Amini, 2021) |
| 22 | 2-Dodecanal | 17.631 | Antioxidant and anti-inflammatory activities (N S Subir Ranjan and Bhadra, 2020) |
| 23 | Decanoic acid | 17.842 | Antifungal, antioxidant and hypocholesterolemia effects (Shen *et al.*, 2021) |
| 24 | $C_6 H_{10} O_3$ | 18.152 | |
| 25 | 7-Pentadecanone | 22.316 | Antifungal, larvicidal, and mosquito repellent (Hanif *et al.*, 2022) |
| 26 | Olean-12-en-3-one | 37.267 | Cytotoxic and apoptotic effects in cancer cells (Tang *et al.*, 2022) |
| 27 | Palmitic Acid | 28.924 | Antioxidant, anti-inflammatory and antimicrobial (Krishnaveni *et al.*, 2022) |
| 28 | Octadec-9-enoic acid | 51.193 | Wound healing, antioxidant, hypocholesterolemia (Santa-María *et al.*, 2023) |
| 29 | 3,5-Stigmastadien-7-one | 57.423 | Anticancer and anti-inflammatory effects (Carvalho *et al.*, 2023) |
| 30 | Lupeol | 34.42 | Anti-inflammatory, antioxidant, antimicrobial and anticancer activities (Omujal *et al.*, 2020) |
| 31 | Stigmastan-3,5-diene | 63.348 | |

**Table 2**: Fatty acids identified by GC-MS in seeds oil of *B. ceiba*

| Peak | Name | RT (min) | Medicinal Uses |
|---|---|---|---|
| 1 | C16:0 Palmitic Acid ($C_{16}H_{32}O_2$) | 28.557 | Cholesterol regulation (Murru *et al.*, 2022) Skin health (Dudau *et al.*, 2021) The energy source (Krishnaveni *et al.*, 2022) |
| 2 | C16:1 Palmitoleic Acid ($C_{16}H_{30}O_2$) | 28.802 | Eye health (Huang *et al.*, 2020) Cancer preventive, anti-inflammatory and metabolic regulator (Fauziah *et al.*, 2022) |
| 3 | C18:0 Stearic Acid ($C_{16}H_{30}O_2$) | 30.775 | Controlled-released drug discovery system Wound healing (Ye *et al.*, 2023) |
| 4 | C18:1 Oleic Acid ($C_{18}H_{34}O_2$) | 31.527 | Anti-inflammatory, wound healing, cancer preventive, cardiovascular health, immunomodulator (Singh *et al.*, 2020) |
| 5 | C18:2 Linoleic Acid ($C_{18}H_{32}O_2$) | 32.497 | Nervous system health, atherosclerosis, immunomodulation (Marangoni *et al.*, 2020) |
| 6 | C20:0 Eicosanoic Acid ($C_{20}H_{40}O_2$) | 33.251 | Anti-elastase, anti-oxidant, anti-urease (Zekeya *et al.*, 2022) |
| 7 | C 22:0 Behenic Acid ($C_{22}H_{44}O_2$) | 35.536 | Anti-inflammatory, anti-oxidant, antimicrobial (Alqahtani *et al.*, 2019) |
| 8 | C20:4 Arachidonic Acid ($C_{20}H_{32}O_2$) | 36.802 | Infant nutrition and skin health (Sambra *et al.*, 2021) |
| 9 | C22:6 Docosahexaenoic Acid ($C_{22}H_{32}O_2$) | 39.244 | Nervous system health, cardiovascular diseases, anti-inflammatory and cancer prevention (Watanabe and Tatsuno, 2021) |

**Table 3**: Iodine value of seeds oil of *B. ceiba*

| Composition | Name | Factor | Area | FAC × Area |
|---|---|---|---|---|
| C16:1 | Palmitoleic acid | 0.956 | 0.478 | 0.456968 |
| C18:1 | Oleic acid | 0.859 | 34.322 | 29.482598 |
| C18:2 | Linoleic acid | 1.731 | 18.696 | 32.362776 |
| C18:3 | Linolenic acid | 2.616 | 0.000 | 0 |
| C20:4 | | 3.201 | 0.700 | 2.2407 |
| C20:5 | | 4.027 | | 0 |
| C22:1 | | 0.723 | 0.000 | 0 |
| C22:5 | | 3.697 | | 0 |
| C22:6 | | 4.463 | 0.737 | 3.289231 |
| | Iodine value | | | 67.832 |

**Table 4**: Percentage Scavenging activity (PSA) values of *Bombax ceiba* seeds oil

| Sample Code | $IC_{50}$ ± SEM | PSA (%) |
|---|---|---|
| Sample | Inactive | 7.187 |
| Gallic acid | 23.436 ± 0.43 (µM) | 93.93 |
| N- acetylcysteine | 111.44 ± 0.7 (µM) | 95.95 |

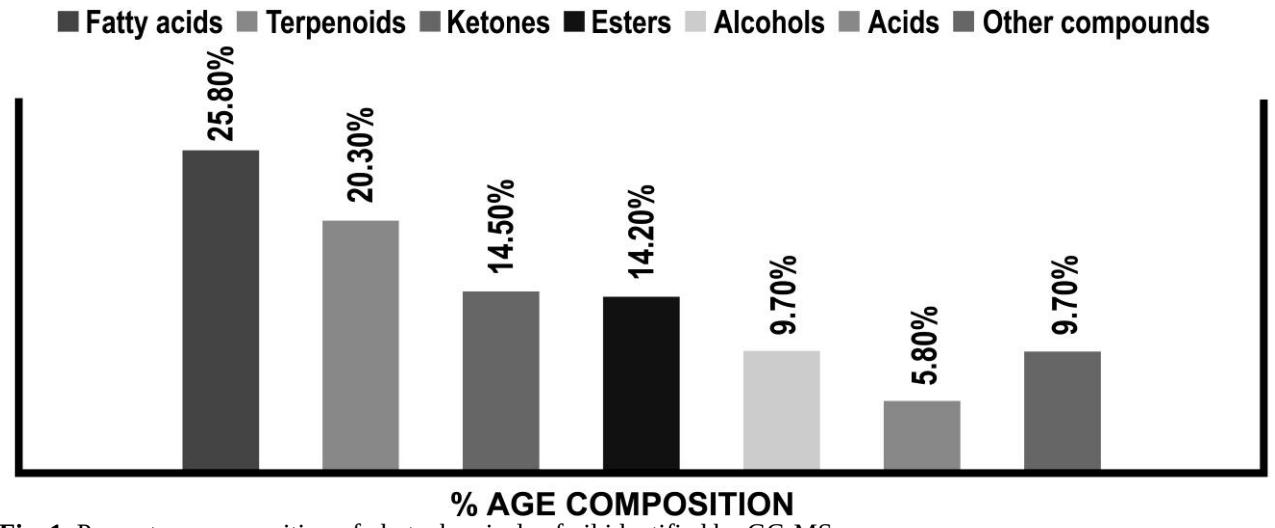


**Fig. 1**: Percentage composition of phytochemicals of oil identified by GC-MS

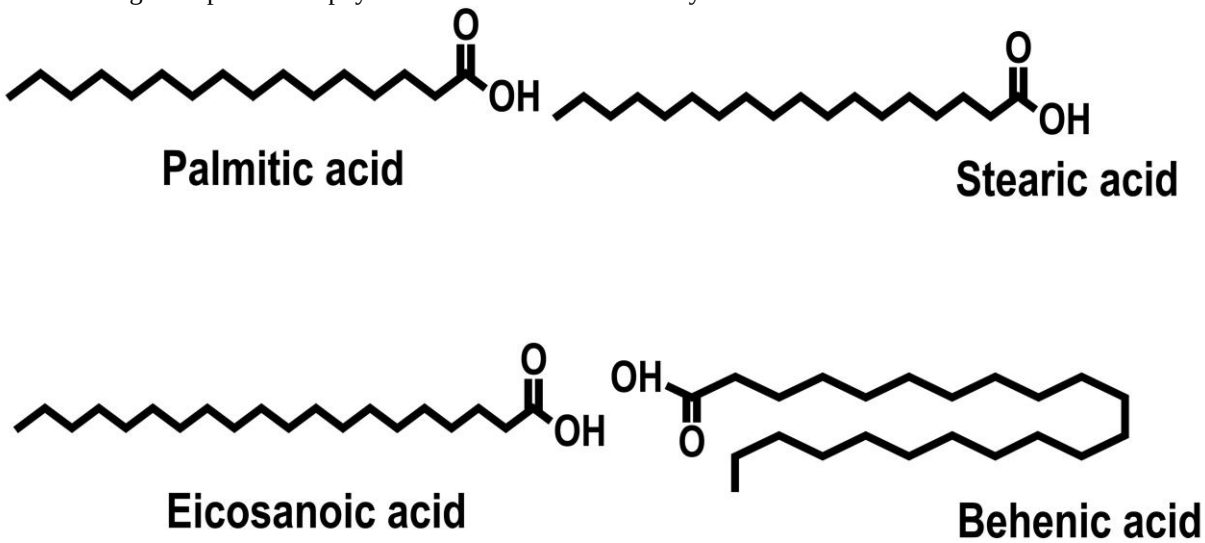


**Fig. 2**: Structures of saturated fatty acids identified by GC-MS analysis of seeds oil of *B. ceiba*

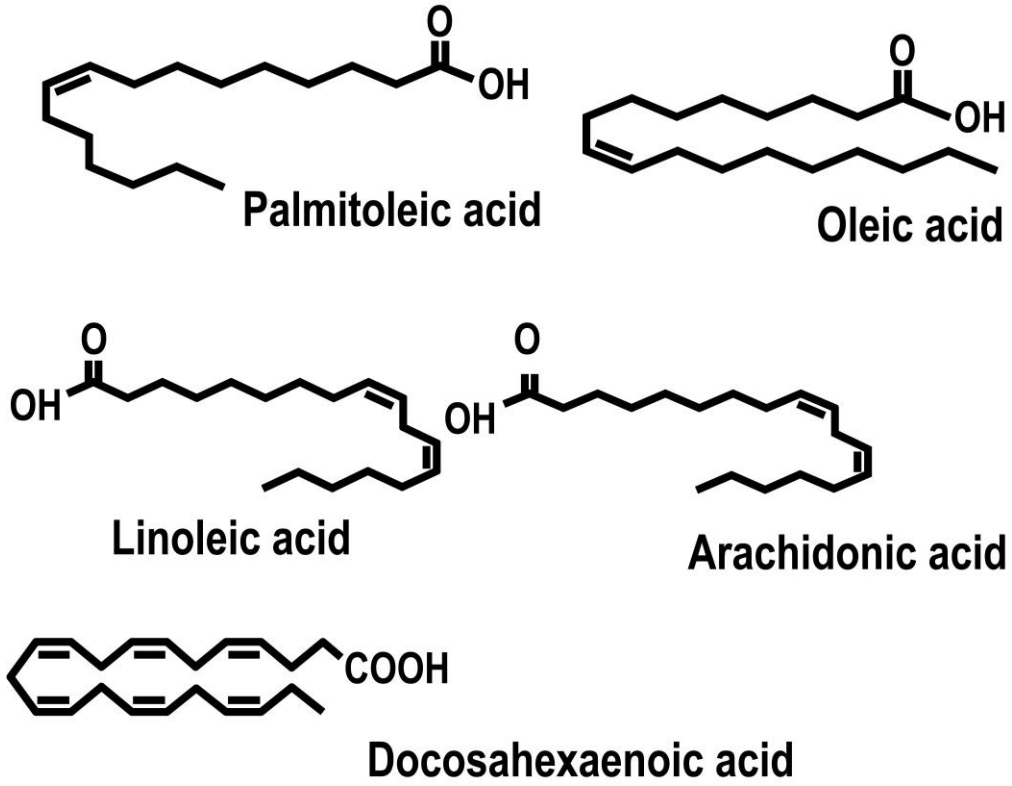


**Fig. 3**: Structures of unsaturated fatty acids identified by GC-MS analysis of seeds oil of *B. ceiba*

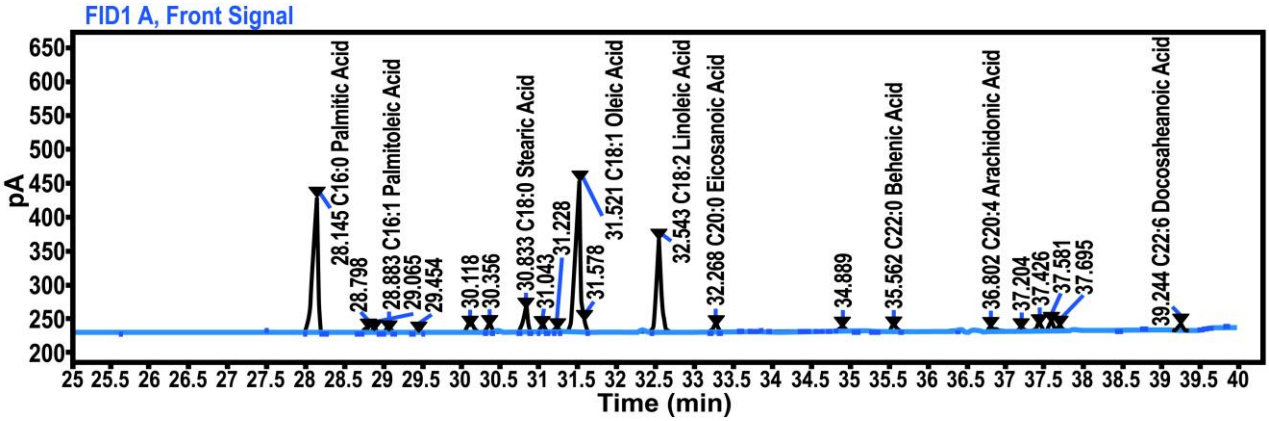


**Fig. 4**: GC-MS chromatogram of *Bombax ceiba* seeds oil

Further detailed evaluation using multiple antioxidant assays is required to confirm the antioxidant profile of Bombax oil. Thus, GC-MS analysis demonstrated the presence of various bioactive phytochemicals imparting medicinal value to *Bombax ceiba* seeds oil. Key parameters like iodine and saponification values verified the unsaturated fatty acid profile of the oil. However, DPPH radical scavenging assay revealed poor antioxidant efficacy of the oil.

## DISCUSSION

The fatty acids identified through the GC-MS have nutritional as well as medicinal uses. 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. as described in table 2 with their retention time. The seed oil of plants is a concentrated source of fatty acids, sterols, glycerides, tocopherols and other non-glyceride components like flavonoids, carotenoids, 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

*Bombax ceiba* 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 *Bombax ceiba* 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, glycosides, fatty acids, phytosterols, naphthoquinone, etc. in *Bombax ceiba* plant possessing anticancer, antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, analgesic, antidiabetic and wound healing properties (Emmanuel and Esther, 2022; Shukla *et al.,* 2020). Despite its traditional medicinal uses, there is limited scientific data available on the chemical profiling of Bombax 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 very low radical scavenging activity of 7.187% 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 inactive. This suggested that the *Bombax ceiba* seeds oil did not possess significant antioxidant properties. This could be attributed to the low content of antioxidant phytochemicals in the oil. Despite the minimal antioxidant activity exhibited by Bombax oil itself, previous studies indicate *Bombax ceiba* seeds are rich reservoirs of bioactive compounds with therapeutic effects. Steroidal phytoconstituents like β-sitosterol, stigmasterol and campesterol isolated from Bombax seeds extracts have shown anti-hypercholesterolemic, antidiabetic, anti-inflammatory and immunomodulating properties (Padilla *et al.,* 2021; Gupta *et al.,* 2023). Flavonoid bombaxquinone 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 (Janarny *et al.,* 2021). These earlier researches support the therapeutic potential of Bombax seeds oil irrespective lack of antioxidant effect of the oil itself. The active constituents may be concentrated in the seed coat rather than the oil-rich kernel and may not be extracted efficiently in the oil fraction. Further research on isolation of the agent responsible for antioxidant activity in Bombax 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 Bombax 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

Overall, the present study provided the detailed phytochemical composition of *Bombax ceiba* seeds oil. It contains medicinally important bioactive compounds such as fatty acids, terpenoids, alcohols and esters. However, the oil showed poor antioxidant potential. Further studies can isolate these bioactive phytochemicals and investigate their pharmacological activities. The seed oil has 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 *Bombax ceiba* seed oil for medicinal uses.

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