Phytochemical, Antioxidant And Physicochemical Evaluation Of Holoptelea Integrifolia (Roxb.) Seed Oil

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ABSTRACT
Holoptelea integrifolia (Roxb.) from the ulmaceae family, a useful medicinal plant possess number of traditional medicinal uses related to its potential phytochemical constituents and antioxidant activity. Folk use various parts of this plant like leaves, barks and seeds to treat different disease conditions. These ailments include inflammation, rheumatism, skin and eye infections and urinary tract disorders like poly urea. The current work was planned to explore the bioactive phytoconstituents present in the seed oil of this plant and its antioxidant activity using a DPPH-Radical Scavenging Assay (DPPH-RSA) . The DPPH-RSA assessed that the seed oil had significant antioxidant activity. Moreover, the detection of phenolic and flavonoid content further highlights the antioxidant potential of the seeds oil. Phytochemical screening was conducted using three spectroscopic techniques i.e., Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography Flame Ionization Detection (GC-FID), and Gas Chromatography Mass Spectrometry (GCMS). The FTIR spectra revealed characteristic functional groups, including aliphatic chains, esters, aldehydes, and phenols. In GC-FID analysis, thirteen fatty acids were identified. While the GCMS identified 15 compounds, including alkanes, aldehydes, alcohols, carboxylic acids, and phenolic compounds. Notable bioactive constituents include palmitic acid, octadec-9-enoic acid, and (E)-2-Octenal, known for their medicinal properties. Key physicochemical properties, including iodine value and saponification value, were also analyzed.
These results underscore that the seed oil of this plant is a potential candidate for further investigations and can be a rich source of bioactive constituents for the development of ethno medicine.

1. Introduction

Since ancient times, plant have been served as a rich source of medicinal compounds. They have formed the foundation of various traditional medicine systems including Ayurveda, Unani, Siddha, Chinese, and so far. Additionally, plant-derived compounds have proven effective as antimicrobial agents, offering an affordable and low-risks of side effects [1]. According to recent reports shared by the World Health Organization (WHO), nearly 80% of the population globally based on herbal remedies for common healthcare needs. In this regard, numerous developing countries have increased their focus on ethnomedical documentation and scientific validation of medicinal plants. With an estimated 250,000 to 500,000 plant species existing worldwide, their potential for drug discovery remains vast [2].

Pakistan has approximately 6000 wild plant species [3, 40] among which about 400-600 are recognized for their medicinal value. Traditional medicine, particularly the Unani system, serves as the primary healthcare source for about 80% of Pakistan's rural population, with plant-based remedies forming its core. This heavy reliance on herbal medicine underscores the significant potential of plants as reservoirs of bioactive compounds that could lead to novel treatments for various diseases [40].
In this regard, H. integrifolia undergone the phytochemical screening. Native to the Pacific Islands [4], this plant exhibits remarkable therapeutic properties, including significant anti-inflammatory and analgesic effects. Traditional medicine systems have used it to treat diverse conditions such as inflammatory disorders, gastrointestinal ailments (gastritis, dyspepsia, colic), parasitic infections, wound healing, and chronic diseases including leprosy, diabetes, and rheumatism [5]. The plant's bark and leaves demonstrate multiple bioactive properties, functioning as a bitter tonic, astringent, digestive aid, anthelmintic, and remedy for rheumatic conditions [6]. Indigenous communities traditionally prepare medicinal extracts by boiling the mucilaginous bark, with the resulting juice applied topically to reduce rheumatic swelling [7]. Decoction of the bark is used as oxytocic agent during pregnancy. Paste of the stem bark is topically used in the treatment of lymphadenitis, febrile conditions, and dermatophytosis. Leaf decoction regulates lipid metabolism and treats dermatological conditions (eczema, tinea, cutaneous disorders) [8]. Seeds are used topically for tinea treatment, while dried fruits address polyuria and urinary tract disorders [9].
Plants contain a large number of phytochemicals like tannins, proteins, carbohydrates, terpenoids, sterols, saponins, and alkaloids [10]. Similarly, H. integrifolia also consists of cardiac glycosides, coumarins, flavonoids, phenols, and quinines [11]. Holoptelin-A, Holoptelin-B, epifriedlin, friedlin, β-sitosterol, β-D-glucose, 2-aminonaphthoquinone, β-amyrin, stigmasterol, hederagenin, 1-hexacosanol, octacosanol, and α-amyrin isolated from different parts of this plant has exhibit proven bioactivity [12]. Betulinic acid has high biological potential, such as inhibitors of HIV-1 entry, HIV-protease, or reverse transcriptase (RT), whereas betulin has serious anticancer effects on adenocarcinoma, cervix carcinoma, hepatoma, and breast cancer [13].
The ongoing work was conducted to assess the antioxidant activity of Holoptelea integrifolia seeds oil using a DPPH-Radical Scavenging Assay, while also exploring its phytochemical profile. The investigation involved the finding of the total phenolic and flavonoid contents, alongside the identification of specific molecules through different analytical techniques like Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography-Flame Ionization Detection (GC-FID), and Gas Chromatography-Mass Spectrometry (GC-MS). Additionally, the physicochemical properties of the oil, including iodine and saponification values, were also assessed. To provide deeper insights into the traditional therapeutic uses of the oil, a comparative analysis was performed, correlating its phytochemical profile with previously reported pharmacological activities.

2. Materials and Methods

2.1. Chemicals

n-Hexane, methanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), butylated hydroxytoluene (BHT), Folin-Ciocalteu reagent, gallic acid, ethanol, sodium carbonate, sodium nitrite, aluminum chloride, sodium

hydroxide, catechin, potassium bromide (KBr), helium gas, nitrogen gas, methyl esters of fatty acid (Supelco Sigma Aldrich Cooperation). All the solvents and chemicals were of analytical grade.

2.2. Collection of seeds

Seeds of H. integrifolia were taken from the local market in Faisalabad, Pakistan. They were then submitted to the herbarium of the Department of Pharmacognosy at Government College University Faisalabad with a specimen voucher number HI-106.

2.3. Isolation of Oil/ Oil extraction

The oil was extracted from 90g of H. integrifolia seeds in a Soxhlet extractor with 500 ml n-Hexane as the solvent, maintained at a boiling point range of 60-70 ºC for 6 hours. After extraction, the oil was concentrated and the solvent was evaporate at room temperature over 24 hours and subsequently, stored at 4 ºC until further use.

2.4. Determination of Antioxidant activity

The DPPH-Radical Scavenging Assay (DPPH-RSA) of H. integrifolia seed oil was carried out according to the method described by Gulcin and Alwasel [14]. 0.5ml seed oil of different concentrations (50 -300µg/ml) were added to 3ml of a 0.03% methanol solution of DPPH. The mixture was left for 30 min at room temperature in the dark and the color changes from violet to yellow were recorded at 517 mm absorbance using a UV-Vis spectrophotometer. The results were compared to the standard antioxidant, BHT (butylated hydroxytoluene). The following equation calculated the percentage inhibition:

% DPPH activity = $[(A_0 - A) / A_0] \times 100$

A_0 = absorbance of DPPH solution with methanol

A = absorbance of DPPH solution with seeds oil

The experiment was repeated three times and the results were given as to the average values with standard deviation (S.D).

2.5. Total Phenol Content Determination

The total phenolic content was find using the Folin-Ciocalteu reagent therewith gallic acid as the reference standard. Different concentrations of gallic acid were prepared in a 50% ethanol solution. 1 ml aliquot of the seed oil (at 1 mg/ml concentration) was mixed with 1 ml of Folin-Ciocalteu reagent. After a 5-minute incubation, 10 ml of 7% sodium carbonate solution and 13 ml of distilled water were added to the mixture, which was then thoroughly mixed. The mixture was placed in the dark for 60 minutes to develop color. Absorbance was calculated at 750 nm, and the results were expressed as milligrams of gallic acid equivalent (mg GAE) per gram of the dried sample [15].

2.6. Total Flavonoid Content Determination

Total flavonoid content in the seed oil was assessed using a specific analytical method [16]. First of all, 0.50 ml of the seed oil was added to2 ml of distilled water and 0.15 ml of 5% $NaNO_3$ solution and kept for 6 minutes. After this, 0.15 ml of 10% $AlCl_3$ solution was added, followed by an additional 6 minutes of incubation. Afterward, a 4% sodium hydroxide solution was introduced, and the mixture was diluted to a total volume of 5 ml with methanol, followed by thorough mixing. The absorbance of the resulting solution was measured at 510 nm after a 15 min incubation. Based on the catechin calibration curve, the total flavonoid content was calculated and expressed as milligrams of catechin equivalent (mg CE) per gram.

Fourier Transform Infrared Spectrophotometric (FTIR) analysis of Holoptelea integrifolia seed oil

Fourier Transform Infrared Spectrophotometric (FTIR) analysis was performed for the identification of functional groups present in the seeds oil. The wavelength of light absorbed by the oil was the characteristic of the chemical bonds present in it. These chemical bonds were determined by interpreting the infrared absorption spectrum [17]. FTIR analysis of the seeds oil was done by using the potassium bromide (KBr) pellet (FTIR grade) method as discussed by Vanitha, Kalimuthu [18] in a 1:100 ratio and the spectrum was obtained by using a Jasco FT/IR-6300 Fourier transform infrared spectrometer

fitted with JASCO IRT-7000 Intron Infrared Microscope using transmittance mode operating at a −1 resolution of 4 cm (JASCO, Tokyo, Japan).

2.7. Gas Chromatography Flame Ionization Detection (GC-FID) & Gas Chromatography Mass Spectroscopic (GCMS) analysis of Holoptelea integrifolia oil

Preliminary analysis of seeds oil was carried out using Gas Chromatography Flame Ionization Detection (GC-FID) on a Shimadzu GC-17 A (Shimadzu Corporation, Kyoto, Japan), outfitted with a SPB-5VR capillary column (30 m x 0.25mm ID and 0.25µm df). Helium gas was employed as a carrier gas at a flow rate of 1ml/min. Initially, 70 ºC temperature was given for 5 min and then increased to 260 ºC at a rate of 5 ºC/min, and finally remained constant at this temperature for next 20 min. The splitting ratio of the injector was maintained at 1:30 at the temperature of 280 ºC. For Gas Chromatography Mass Spectroscopic (GCMS) analysis, Hewlett-Packard 5890 (Bunker Lake Blvd, Ramsey, MN) Gas Chromatograph fitted with Jeol JMS-600 Mass Spectrometer was used, equipped with ZB-5MSVR capillary column (30 m x 0.25mm ID and 0.25 µm df), similar methodological conditions were maintained as mentioned in GC-FID. The electron ionization (EI) ion source was maintained at 70 eV and 250ºC. The electronic mass spectral survey (NIST 2005) and Electron impact mass spectrometry (EIMS) data published in the literature were evaluated against the experimental mass spectra (Moir and Thomson 1973) to identify the compounds. Reported retention indices (RI) data allowed for the confirmation of the compound elution order [19]. The elements were quantified using the area normalization approach.

2.8. Analysis of fatty acid, saponification value, and iodine value

Standard IUPAC Method 2.301, as discussed by Mamadaliev, Kushiev [20] was used to obtain fatty acid methyl esters (FAMEs). Perkin Elmer gas chromatograph model Clarus 500 equipped with a polar capillary column Rtx-2330 (60.0m, 0.25mm 0.20 lµm film thickness) and fitted with flame ionization detector was used to evaluate the chemical composition of fatty acid methyl esters. Nitrogen gas was used as carrier gas at a flow rate of 2.5 mL/min. Other conditions were, 70 ºC initial temperature for 5 min, then changed to 10ºC/min to 180ºC, then at 3ºC/min to a final temperature of 220ºC where it was held for 8 min; injector temperature 250ºC; detector temperature 275ºC. 0.30µL of sample was injected; (split less). The absolute and relative retention times of the obtained fatty acid methyl esters were assessed against those of standard fatty acid methyl esters bought from Supelco Sigma Aldrich Cooperation for identification. The manufacturer of the gas chromatograph provided a built-in data program for the quantification of identified compounds. The analyses were carried out three times. The GCMS analysis was carried out on an HP 5890 gas chromatograph fitted with an HP-5 capillary column (30.0 m, 0.22 mm, 0.25µ; film thickness) and a Jeol Mass Spectrometer HX-110 from Tokyo, Japan. The experimental conditions were as: 270ºC Injector temperature; oven temperature from 70 to 260ºC at 8 ºC/min, 2 min was initial hold time; carrier gas helium at 1.98mL min^{-1} flow rate; injection volume 0.30µL; split ratio, 1:50. The determination of the components was done through computational correlation with the commercially available mass spectral reference library of the National Institute of Standards and Technology (NIST 2005). The iodine value and saponification value were also calculated to determine the degree of unsaturation and the number of acidic groups present in the molecule, respectively. The factor was measured by taking 0.1N KI solution as standard.

2.9.Statistical analysis

Data was analyzed by using Microsoft Excel version 2016 and GraphPad Prism 8. All experiments for antioxidant activity were repeated 3 times and the results were shown as mean ± SD.

3. Results

Soxhlet extractor was used for oil extraction by using n-hexane. The apparatus was run for 6 hours to extract the oil completely. 90 g of the powdered sample gave 7.33g oil after the evaporation of the extra solvent by placing it for 24 hours at room temperature.

3.1. Antioxidant activity of Holoptelea integrifolia

Figure 1 shows the DPPH-Radical Scavenging Assay. As the seed oil concentration increased, the color changed from violet to yellow at 517 nm. At 300 µg/ml, the oil's highest DPPH-RSA activity (90.94%

± 1.25%) was noted. Butylated hydroxytoluene (BHT) was used as a standard, and it showed 93.17% ± 1.52% activity.

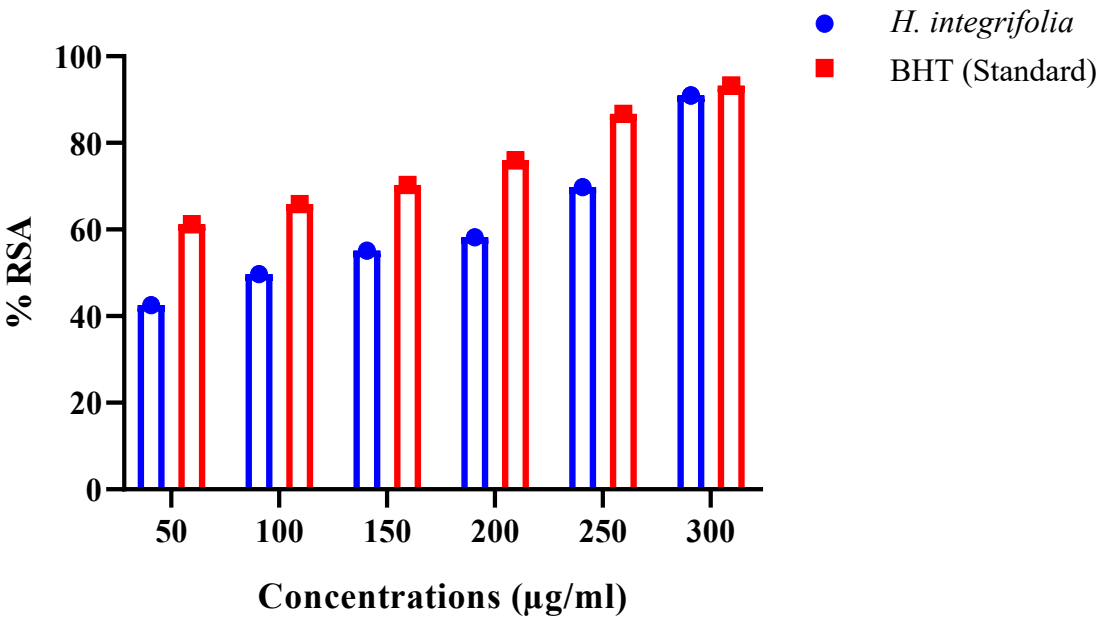

Figure 1: DPPH scavenging activity of H. integrifolia seeds extract

3.2. Total phenolic content

The total phenolic content of the seeds oil of H. integrifolia seed oil by Folin-Ciocalteu reagent method was develop to be 34.23 ± 0.26 mg of GAE per gram of the oil. A standard calibration curve was made using different concentrations of gallic acid for the determination of total phenolic content (Fig.2A).

3.3. Total flavonoid content

The total flavonoid content of the crude extract of H. integrifolia seed oil was calculated 77.68 ± 0.17 mg of CE per gram of the oil (Fig.2B).

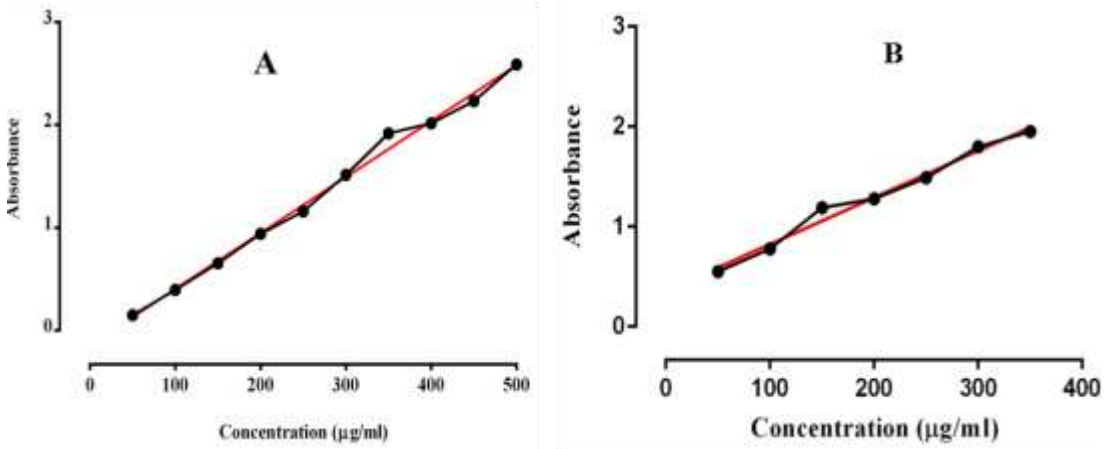

Figure 2: The standard curves of standards (A) Gallic acid and (B) Catechin to calculate the total phenolic content and total flavonoid content respectively by using spectrophotometric method.

3.4. FTIR analysis of Holoptelea integrifolia

Figure 3 depicts the FTIR spectrum of the n-hexane seed oil of Holoptelea integrifolia. The FTIR analysis revealed that it contains a series of functional groups, including aliphatic compounds with the highest peak values of 2921.51 and 2852.83, followed by esters and aldehydes with a 1743.32 peak value, carboxylic acids with a 1372.05 peak value, alcohols and phenols with a 1236.13 peak value, ethers with a 1159.59, and vinyl compounds with 994.34. The lowest peak value of 723.94 was found for sulfonyl chlorides as illustrated in Table 1.

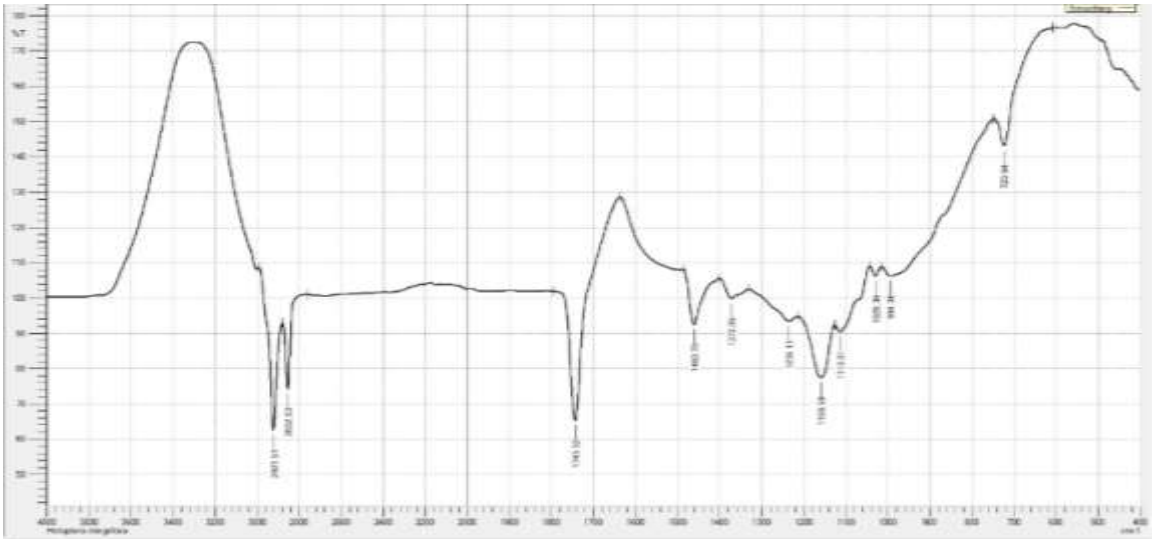

Figure 3: FTIR analysis of H. integrifolia seeds extract

Table 1: Functional groups identified in the extract by FTIR

Sr.No.	Peak values	Functional groups	Class of compounds
1.	2921.51	$-CH_3$ and $-CH_2$	Aliphatic compounds
2.	2852.83	$-CH_3$ and $-CH_2$	Aliphatic compounds
3.	1743.32	C=O and C-C-CHO	Esters, Aldehydes
4.	1460.75	$-CH_3$ and $-CH_2$	Aliphatic compounds
5.	1372.05	-OH	Carboxylic acids
6.	1236.13	-OH	Alcohols, Phenols
7.	1159.59	C-O-C	Ethers
8.	1113.81	-OH, C=O, and C-O-C	Alcohols, Esters, Ethers
9.	1029.39	-OH, and C-O-C	Alcohols, Ethers
10.	994.34	$CH=CH_2$	Vinyl compounds
11.	723.94	C-S	Sulfonyl chlorides

3.5. Phytochemical screening by GC-FID and GCMS

The Gas chromatography fitted with the Flame Ionization Detection revealed different peaks of the fatty acid methyl esters as elaborated in Figure 4. Among these fatty acids, palmitic acid has the highest quantity with 48.80% composition followed by stearic acid with 17.54%, margaric acid with 9.75%, linoleic acid with 3.44%, ecosanoic acid with 3.17%, palmitoleic acid with 3.04%, linolenic acid with 3.04%, erucic acid with 3.04%, gondoic acid with 2.21%, oleic acid with 2.13%, behenic acid with 1.86%, myristic acid with 1.71%, and decosaheanoic acid 0.28%. Both the fatty acids (saturated and unsaturated) were identified in the oil as shown in Table 2. Fatty acids have both medicinal as well as nutritional uses.

Gas Chromatography (GC) coupled with Mass Spectroscopy (MS) was used for the phytochemical screening of the oil by using solvent methanol. GCMS analysis identified 15 phytochemicals belonging to alkanes, aldehydes, alcohols, carboxylic acids, esters, phenol compounds, and branched hydrocarbons, then separated these compounds based on their mass spectra [enlisted in Table 3 along with their retention times (in increasing order) and medicinal uses that have already been reported in the literature] then we compared the spectra to identify the bioactive compounds. The alkanes include octane (4.44), decane (10.247), undecane (12.571), dodecane (14.63), tridecane (16.49), and Hexeicosane (33.875). 2-hydroxy5-methyl benzaldehyde (12.722) and (E)-2-Octenal (49.997) were the potential aldehydes found in the oil. Similarly, branched alcohol (20.193) and octyl alcohol (15.83) were also identified in the sample. Moreover, palmitic acid (28.924) and Octadec-9-enoic acid 37.267) (carboxylic acids), octyl alcohol ester ethyl hexyl ester, phenyl propanoid (15.377), and branched hydrocarbons (14.875) (branched isomer of tridecane) were also present.

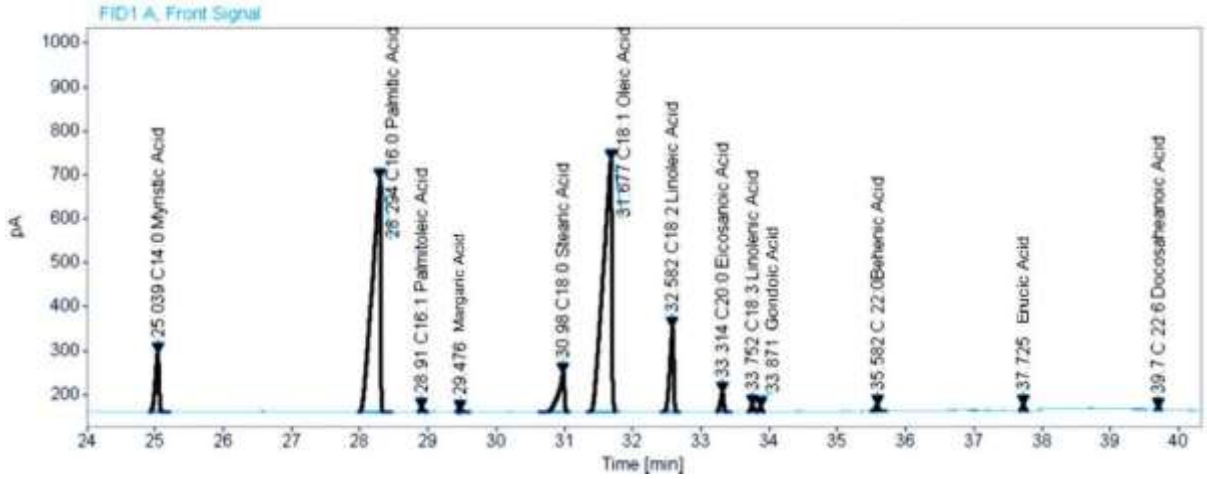

Figure 4: GC-FID chromatogram of Holoptelea integrifolia seeds oil

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

Peak#	Name	RT [min]	Height	Area	% Composition
1	C14:0 **Myristic Acid**	25.039	0.41	1.53	1.71
2	C16:0 **Palmitic Acid**	28.294	14.53	43.71	48.80
3	C16:1 **Palmitoleic Acid**	28.91	0.83	2.72	3.04

4	C17:0 **Margaric Acid**	29.476	2.66	8.73	9.75
5	C18:0 **Stearic Acid**	30.98	5.47	15.71	17.54
6	C18:1 **Oleic Acid**	31.677	0.7	1.91	2.13
7	C18:2 **Linoleic Acid**	32.582	0.9	3.08	3.44
8	C20:0 **Eicosanoic Acid**	33.314	0.96	2.84	3.17
9	C18:3 **Linolenic Acid**	33.752	0.8	2.72	3.04
10	C20:1 **Gondoic Acid**	33.871	0.62	1.98	2.21
11	C 22:0 **Behenic Acid**	35.582	0.72	1.67	1.86
12	C22:1 **Erucic Acid**	37.725	0.8	2.72	3.04
13	C22:6 **Decosaheanoic Acid**	39.7	0.74	0.25	0.28
				Total	**100.00**

Table 3: Phytoconstituents identified by GCMS of H. Integrifolia (Roxb.)

Sr. No.	Constituent	Retention time	Medicinal use
1.	Octane	4.44	Derivatives used in dental preparation [21]
2.	Decane	10.247	Derivatives used make Psych sedative agents [22]
3.	Undecane isomer	11.925	Neuroprotective [23]

4.	Undecane	12.571	Neuroprotective [23]
5.	2-hydroxy5-methyl benzaldehyde	12.722	Antifungal activity [24]
6.	Dodecane	14.63	Solvent, distillation chaser, scintillator component, diluent for tributyl phosphate (TBP) [25]
7.	Branched isomer of tridecane	14.875	Solvent, Rubber Industry (HMDB) Wishart Research Group, University of Alberta
8.	Phenyl propanoid	15.377	Antioxidant [26]
9.	Octyl alcohol ester ethyl hexyl ester	15.83	Used as cosmetics and pharmaceutical products and for the food industry [27]
10.	Tridecane	16.49	Solvent, Rubber Industry (HMDB) Wishart Research Group, University of Alberta
11.	Branched Alcohol	20.193	Antiseptic, Disinfectant [28]
12.	Palmitic Acid	28.924	The sodium salt of palmitic acid has a wide use in foodstuffs and organic products (US SAS standards)
13.	Hexeicosane	33.875	It has a role as a pheromone, a plant metabolite (US National Library of Medicines)
14.	Octadec-9-enoic acid	37.267	It is used commercially in the preparation of oleates and lotions and as a pharmaceutical solvent. (Stedman)
15.	(E)-2-Octenal	49.997	It has a role as an antifungal agent and a volatile oil component [29]

3.6. Saponification value

Identified the peaks on the chromatogram and determined the peak boundaries then calculated the area (The chromatogram will provide the area under the peaks). Take the area twice and then take the average. We have the known mass of all the fatty acids present in Holoptelea integrifolia. Then by using the formula **Mass×Area/100**, we calculate the sap value. The sap value of Holoptelea integrifolia was **199.759** as depicted in Table 4. The saponification value was also calculated to determine the degree of unsaturation and the number of acid groups.

Table 4: Saponification value of Holoptelea integrifolia (Roxb.)

Sap Value		**Chromatograms**		**Sample**		
Composition	**Name**	**Area 1**	**Area 2**	**Mass**	**Average Area**	**Mass×Area/100**
C6:0	Caproic Acid	0	0	116.1583	0	0
C8:0	Caprylic Acid	0	0	144.21	0	0

C10:0	Capric Acid	0	0	172.26	0	0
C12:0	Lauric Acid	0	0	200.3178	0	0
C14:0	Myristic Acid	4.382	4.386	228.37	4.384	10.0117408
C16:0	Palmitic Acid	38.756	38.758	256.43	38.757	99.3845751
C16:1	Palmitoleic Acid	0.21	0.214	2544.414	0.212	5.39415768
C18:0	Stearic Acid	4.518	4.52	284.48	4.519	12.8556512
C18:1	Elaidic Acid	0	0	282.46	0	0
C18:1	Oleic Acid	42.94	42.944	282.46	42.942	121.2939732
C18:2	Linoleic Acid	6.482	6.484	280.4472	6.483	18.18139198
C18:3	Linolenic Acid	0.372	0.374	278.43	0.373	1.0385439
C20:0	Eicosanoic Acid	1.217	1.219	312.5304	1.218	3.806620272
C20:4	Arachidonic Acid	0	0	304.47	0	0
C22:0	Behenic Acid	0.366	0.368	340.58	0.367	1.2499286
C22:1	Erucic Acid	0	0	338.57	0	0
C22:6	Decosahexanoic Acid	0.074	0.076	328.488	0.075	0.246366
	Saturated Fatty Acid	**49.245**	**Total**	**6695.076**	**Sum**	**273.462949**
	Unsaturated Fatty Acids	**50.085**				
	Sap Value	**199.759**				

3.7. Iodine value

Identified peaks on chromatogram and determined the peak boundaries then calculated the area (The chromatogram will provide the area under the peaks). Take the area twice and then take the average. The factor was calculated by having 0.1N KI solution as standard. Then by using the formula Factor×Area, we calculate the iodine value. Table 5 illustrates the iodine value of each fatty acid reported in the Holoptelea integrifolia seed oil, while the cumulative value was 52.79976g. The iodine

value was also calculated to determine the degree of unsaturation and the number of acid groups as illustrated in Table 5.

Table 5: Iodine Value Holoptelea integrifolia (Roxb.)

Composition	Name	Factor	Area	Factor×Area
C16:1	Palmitoleic acid	0.956	0.212	0.202672
C18:1	Oleic acid	0.859	42.945	36.88976
C18:2	Linoleic acid	1.73	6.483	11.21559
C18:3	Linolenic acid	2.616	0.373	0.975768
C20:4	Arachidonic acid	3.201	0.367	1.174767
C20:5	Eicosapentaenoic acid	4.027	0.000	0
C22:1	Erucic acid	0.723	0.000	0
C22:5	Docosapentaenoic acid	3.697	0.000	0
C22:6	Docosahexaenoic acid	4.463	0.000	0
Iodine Value				**52.79976g**

4. Discussion

The current study analyzed the phytochemical profile and antioxidant potential of Holoptelea integrifolia seed oil, a plant known for its wide use in traditional medicine. The analysis was conducted by using different analytical techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Gas Chromatography Flame Ionization Detection (and Gas GC-FID), Chromatography Mass Spectrometry (GCMS) to comprehensively identify the existence of bioactive compounds in the seed oil. Additionally, the DPPH-Radical Scavenging Assay (DPPH-RSA) was carried out to evaluate the antioxidant activity of the oil. The results approved the presence of several medicinally valuable compounds, further supporting the traditional applications of this plant.

The antioxidant activity of H. integrifolia seed oil was analyzed using the DPPH free radical scavenging assay and a generally accepted method for assessing antioxidant potential. The results showed that the seed oil has important free radical scavenging activity, with a maximum inhibition rate of 90.94% ± 1.25% at a concentration of 300 µg/ml. This value is comparable to the standard antioxidant butylated hydroxy toluene (BHT), which showed a scavenging potential of 93.17% ± 1.52%. These findings focused on the strong antioxidant capacity of H. integrifolia seed oil, which may be assigned to the presence of phenolic compounds and fatty acids identified in the phytochemical screening.

The antioxidant characteristics of H. integrifolia seed oil suggest its potential part in soothing oxidative stress-related disorders. Oxidative stress is a major of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative conditions [30]. By scavenging free radicals, the bioactive compounds in H. integrifolia can help deplete the damage caused by reactive oxygen species [31] and improve overall health outcomes.

The findings from this study are compatible with previous research on H. integrifolia, which has highlighted the plant's broad spectrum of medicinal properties. For instance, studies have described the availability of flavonoids, phenols, terpenoids, and alkaloids in the plant's bark, leaves, and seeds, which contribute to its anti-inflammatory, antioxidant, and antimicrobial activities [32]. The current study extends these findings by providing a detailed analysis of the seed oil, which has not been extensively explored in previous research.

The higher antioxidant potential of H. integrifolia seed oil suggests that it may be a more powerful source of bioactive compounds for therapeutic use, mainly in formulations aimed at combating oxidative stress.

Similarly, the traditional use of H. integrifolia in curing inflammatory conditions, skin disorders, and gastrointestinal problems is further confirmed by the identification of compounds such as palmitic acid and oleic acid, which have been associated with anti-inflammatory and skin-protective effects [33]. The strong antioxidant activity observed in this study gives additional support for the use of this plant in treating oxidative stress-related conditions, such as arthritis, diabetes, and cardiovascular diseases.

While, the presence of phenolic compounds is mainly significant, as phenolics are well known for their antioxidant properties. These compounds are efficient in neutralizing free radicals and preventing oxidative stress, which is suggested in ageing and chronic diseases such as cancer, cardiovascular disease, and diabetes [34]. Therefore, the detection of these functional groups in H. integrifolia seed oil provides a molecular basis for its traditional use in the treatment of various ailments, particularly those involving inflammation and oxidative stress.

The FTIR analysis showed a variety of functional groups, including aliphatic compounds, esters, aldehydes, carboxylic acids, alcohols, phenols, and ethers, all of which are associated with different pharmacological activities. The identification of -CH3 and -CH2 aliphatic chains suggests the availability of lipids, which are necessary for maintaining cell structure and function [35]. Furthermore, the observation of C=O and C-C-CHO bonds in esters and aldehydes suggests that these compounds are responsible for the overall bioactivity of the seed oil, mainly in wound healing and anti-inflammatory effects [36].

The GCMS analysis of H. integrifolia seed oil found 15 bioactive compounds, a variety of alkanes, aldehydes, alcohols, carboxylic acids, esters, phenolic compounds, and branched hydrocarbons. The obvious compounds identified were palmitic acid, octadec-9-enoic acid, and (E)-2-octenal, which are well-articulated for their medicinal properties. Palmitic acid, for example, is known for its antioxidant and antimicrobial characteristics and has been reported to play a vital role in regulating lipid metabolism and skin health [37]. Similarly, octadec-9-enoic acid (oleic acid) is recognized for its anti-inflammatory effects and benefits in cardiovascular health [31]. (E)-2-Octenal, an aldehyde compound, is marked for its antimicrobial activity, further supporting its inclusion in traditional treatments for infections [38].

The availability of these bioactive compounds confirms the ethnopharmacological use of H. integrifolia for curing inflammatory conditions, skin diseases, and gastrointestinal disorders. These findings synchronize with traditional reports of the plant's use in the therapy of rheumatism, leprosy, and intestinal worms [39]. Additionally, the recognition of multiple fatty acids recommends potential applications in skincare formulations and other medicinal products.

The promising results from this study enhance the need for further investigations into the bioactive compounds of H. integrifolia. Future research should focus on extracting and characterizing individual compounds responsible for the observed antioxidant activity. Additionally, in vivo studies are important to assess the curing potential of these compounds in treating specific diseases, particularly those related to oxidative stress and inflammation.

Moreover, the oil's saponification and iodine values, as well as the presence of unsaturated fatty acids, suggest that it may have applications in the cosmetics and pharmaceutical industries for the emergence of skin-care products and natural antioxidants. The use of green chemistry approaches in further refining and promoting the extraction of bioactive compounds from H. integrifolia could lead to more acceptable and efficient methods for utilizing its medicinal potential.

5. Conclusion

Holoptelea integrifolia stands out as a flexible medicinal plant, producing a wide array of chemically diverse bioactive compounds. Extensive phytochemical research conducted in recent decades has revealed substantial potential in its various plant parts, motivating further scientific exploration of this species. This study was specifically plotted to address the critical need for systematic characterization of its phytoconstituents and their correlation with observed biological activities. Such investigations may lead to the identification of novel bioactive compounds underlying its traditional medicinal uses.

As part of this research initiative, we focused on analyzing the seed oil composition, which could provide valuable insights into the plant's phytochemical profile and enhance understanding of its therapeutic significance. This approach aligns with ongoing efforts to scientifically validate and expand the medicinal applications of H. integrifolia.

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