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RESEARCH ARTICLES

Antioxidant profile, and fatty acids analysis of leaves of medicinal plant *Piper chaba* H. (Chuijhal): a promising source of antioxidant and fatty acids

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Abstract
Piper chaba Hunter belonging to the Piperaceae family, is mainly used as a spice in Bangladesh and West Bengal in India. It is an ethnobotanical-supportive plant. No biological and fatty acid composition study on its' leaves' extracts has been found previously. The present study is on the antioxidant activity and fatty acid analysis of *P. chaba* leaves. The methanol extract of the leaves and its' different soluble fractionates i.e. n-hexane, dichloromethane, chloroform, ethyl acetate, and aqueous were subjected to screening for antioxidant activity. The highest free radical scavenging activity by the DPPH assay method was shown by dichloromethane fractionate (radical scavenger) with an IC_{50} value of 8.84 µg/mL which is close to the IC_{50} value of the standard. Chloroform fractionates showed a significant capacity of 216 mg/g (expressed as ascorbic acid equivalents) by the Phosphomolybdenum assay method. The analysis of fatty acids content showed that the leaves contain the highest proportion of caprylic acid (40.31%) and the lowest proportion of lauric acid and linolenic acid (0.39%) as bound form and the highest proportion of caprylic acid (34.43%) and the lowest proportion of linolenic acid (1.08%) as free form. A good number of unsaturated fatty acids such as palmitoleic, oleic, linolenic, and erucic acids were present in the leaves of this plant. About 24.02% palmitoleic acid was found as the free form which indicated that the antioxidant activity of leaves was significant as per the obtained result. This study recommends using the leaves as a potent source of antioxidants and fatty acids. Extraction of bioactive compounds using methanol extract from the leaves will open the root to new drug discovery as it is a great source of alkaloids, flavonoids, terpenes, etc.

Keywords *Piper chaba*, fatty acids · Antioxidant · Radical scavenger · Alkaloids · Ethnobotanical

Introduction

The production of free radicals and lipid peroxidation are two primary causes of illness and aging in humans and animals (Oskoueian et al. 2011). An antioxidant is a chemical compound that is very helpful because it can stop or slow the production of free radicals and lipid peroxidation in the bodies of humans and animals. It aids in delaying the aging process and protects against various diseases linked to the heart, kidneys, lungs, circulatory system, muscles, and brain (Karimi et al. 2013).

Typically, fatty acid molecules are bound to other molecules, such as sugars, glycerol, or phosphate head groups, to create lipids. Lipids are essential components of cell structures, such as cell membranes, which are phospholipids' primary constituents, and energy reserves, which are frequently composed of lipids. Free fatty acids, which have a wide range of powerful biological actions, are created when fatty acids are liberated from lipids, usually by the activity of enzymes (Desbois and Smith 2010). Free fatty acid biological activities play a part in the host's defense mechanisms against potentially harmful or opportunistic bacteria. Fatty acids are frequently recognized as the active components in traditional and herbal remedies (McGaw et al. 2002; Yff et al. 2002).

There is a great emphasis on discovering biologically active natural products from higher plants that are better than synthetic chemicals and are much safer, from a health

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Fig. 1 Leaves and stem of *P. chaba* HUNTER

Fig. 2 *P. chaba* stem used in cooking fish

and environmental point of view (Balunas and Kinghorn 2005). Plants are an essential source of chemical compounds. Plant-based treatment is becoming more popular with patients and physicians due to its potent medicinal effects, lower cost, and fewer side effects than modern allopathic medicines (Dias et al. 2012; Popović et al. 2016). So considering the beneficial effects of plants, it is necessary to isolate the molecules or bioactive agents that have been considered potential prototypes for the design and development of novel classes of drugs. Besides, almost 80% of available drugs are either directly derived from nature or their modified analogs. Thus, the importance of natural product chemistry is increasing day by day (Alam et al. 2021).

Piper chaba Hunter (*P. chaba* H) is a flowering vine in the family of Piperaceae native to south and southeast Asia. *P. chaba* H is called Chuijhal or Choijhal (Islam et al. 2020) in the Khulna-Jessore region of Bangladesh, Tripura (India), and West Bengal (India). In Bangladesh, the use of Choijhal is unique, because the twigs, stems, or roots of *P. chaba* H are used as a spice. It is a relatively expensive spice in Bangladesh, and the roots are usually more expensive than the stems because of their stronger aroma.

It is an ethnobotanical-supportive plant, which contains a lot of chemical compounds. Piper species have demonstrated the potential for antidiabetic, antihypertensive, immunoprotective, neuroprotective, and anticarcinogenic activities (Biswas et al. 2022; Haq et al. 2021 Singh and Shukla 2024 Islam et al. 2020). The ingestion of piper species is used to cure a wide range of illnesses, including fever, headache, diarrhea, rheumatism, boils, scabies, and stomach problems (Tsai et al. 2005; Chakraborty and Shah 2011; Sharkar et al. 2013; Umoh et al. 2013; Aziz et al. 2015) (Figs. 1 and 2).

The plant's stem bark contains unique carbamide piperine dimer and alkaloids, which have antimicrobial and pharmacological properties (Rukachaisirikul et al. 2002 Rahman et al., 2005). Piperine shows anti-inflammatory activity. It produces a burning sensation because it activates the TRPV1 receptor (Dong et al. 2019; Correa et al. 2010; Chen et al. 2013).

The previous study shows that compounds isolated from the roots and fruits (Morikawa et al. 2004; Naz et al. 2009 Buagaew and Poomipark 2020) of *P. chaba* H. exhibit pharmacological activities against different health disorders. The 80% aqueous acetone extract of the fruit of *P. chaba* has hepatoprotective effects on D-galactosamine (D-GalN/lipopolysaccharide-induced liver injury in mice (Matsuda et al. 2008, 2009 Morikawa 2010).

Alkaloid Chingchengenamide: A was isolated from the leaves of the plant for the first time (Shandhi et al. 2020). Further study will ensure the isolation of more biologically active compounds from the leaves of the plant. From this research, an evaluation of the antioxidant profile and fatty acids content of the leaves has been performed, which may consider this plant as potential therapeutics in human health.

Materials and methods

Plant source

The leaves of the plant *P. chaba* H (Locally known as Chuijhal) have been collected from the northern part of Bangladesh (Sindurmoti union, Rajarhat, Kurigram). The Department of Botany, University of Dhaka confirmed the taxonomy of the plant (Fig. 3).

Solvent extraction

The air-dried fresh leaves (~500 g) of *P. chaba* were exhaustively extracted with Methanol (MeOH) at room temperature. A solid residue (11.11 g) was obtained after the removal of the solvent using a rotary vacuum evaporator and fridge dryer. The dried MeOH extract was then suspended in H_2O and partitioned by separating the funnel successively

Fig. 3 Front and backside view of *P. chaba* leaves

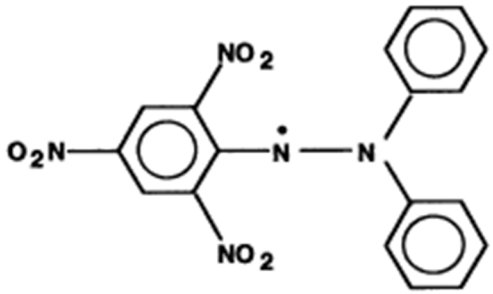


Fig. 4 DPPH• (free radical)

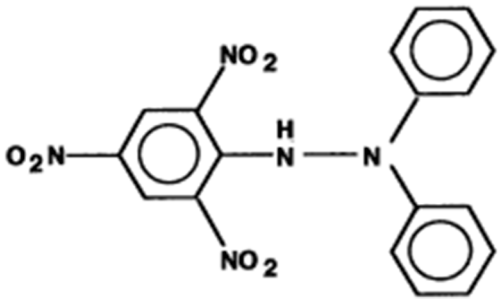


Fig. 5 DPPH-H (non-radical)

with *n*-hexane (HEX), dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EAC), and MeOH by VanWagenen method (VanWagenen et al. 1993). The fractionates were then evaporated to dryness (Rolta et al. 2020, 2022). The amount of different fractionates was *n*-hexane (2.86 g), DCM (4.0 g), ethyl acetate (0.64 g), methanol (2.7 g), and aqueous (2.4 g).

Free radical scavenging assay

The free radical scavenging activities of the MeOH extract and different soluble fractionates on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams et al. 1995. 2.0 ml of a methanol solution of the extract at different concentrations was mixed with 2.0 ml of a DPPH methanol solution (0.1mM). The antioxidant potential was assayed from the bleaching of purple-colored methanol solution of DPPH radical by the plant extract as compared to that of ascorbic acid by UV spectrophotometer (Figs. 4 and 5).

Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$\mathbf{(I\%) = (1 - A_{sample}/A_{blank}) \; X \; 100}.$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material), sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Phosphomolybdenum assay method

The total antioxidant capacity of the MeOH extract and different soluble fractionates were evaluated by the phosphomolybdenum assay method which is based on the reduction of Mo (VI) to Mo (V) and the subsequent formation of a green phosphate-Mo (V) complex in acidic condition. The 0.3 ml of each sample was allowed to mix with 3.0 mL of the reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4, 4 mM ammonium molybdate). This reaction mixture was incubated at 95°C for 90 min. After letting the solution cool back to room temperature, the absorbance was measured at 695 nm using a spectrophotometer against a blank solution. The total antioxidant capacity was determined and expressed as mg ascorbic acid equivalents per gram of dry sample using the equation obtained from a standard ascorbic acid calibration curve ($y = 0.0084x - 0.0141$, $R^2 = 0.9939$).

Analysis of fatty acids through GC-FID

Fatty acid methyl esters (FAMEs) were prepared from the n-hexane soluble fractionate according to the method described by Savage et al. (1997). n-hexane soluble fractionate (0.431 g) of leaves was dissolved in n-hexane (50 mL) and extracted with 5% sodium bicarbonate solution (25mL× 2). The mixture was taken in a separatory funnel and shaken vigorously and allowed to stand overnight. Two layers were obtained. The lower layer (aqueous) was separated and taken to analyze free fatty acid (FFA). The upper layer was separated and taken to analyze bound fatty acids. The FAMEs were determined by GC (Shimadzu 9 A, Column-BP-50, Detector-FID, 105 °C–5 °C/min-150 °C–2 °C/min-280) according to the method reported by Azadmard-Damirchi and Dutta (2006). The FAMEs were analyzed by comparison of their retention times with standard FAMEs and the peak areas are reported as a percentage of the total fatty acids (Figs. 6, 7 and 8).

Fig. 6 Separation

Fig. 7 Distillation

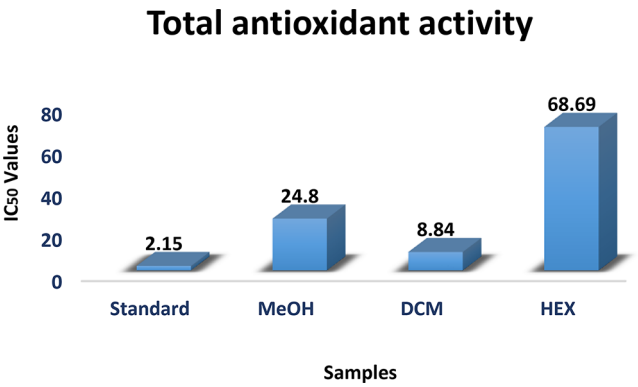


Fig. 9 IC_{50} values of the standard, MeOH extract, and different fractionates of *P. chaba* leaves

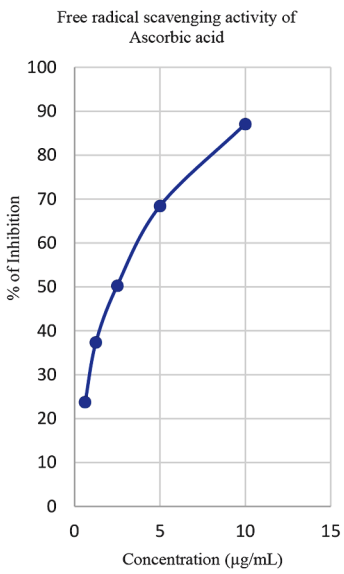
Result and discussion

Free radical scavenging assay

The antioxidant activity of IC_{50} values in the DPPH method differed in MeOH extract and different fractionates ranging from (8.84 µg/mL) to (68.69 µg/mL). In this investigation, DCM showed the highest free radical scavenging activity with an IC_{50} value of 8.84 µg/mL as compared to Ascorbic acid at 2.15 µg/mL (Fig. 9). At the same time, the MeOH extract and n-hexane fractionate also exhibited antioxidant potential having IC_{50} values of 24.80, and 68.69 µg/mL respectively.

Phosphomolybdenum assay method

It is determined through the phosphomolybdenum assay method (Figs. 10 and 11),



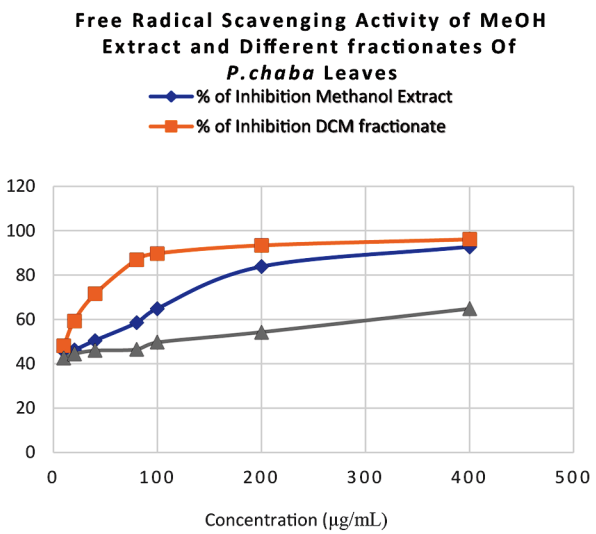


Fig. 8 Free radical scavenging activity of standard, MeOH extract, and different fractionates of *P. chaba* leaves

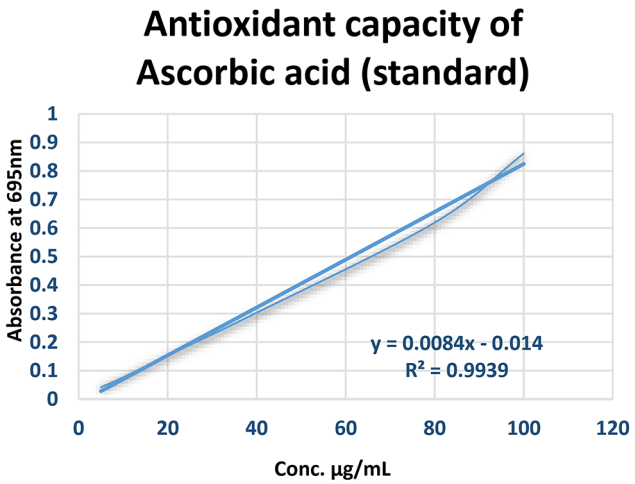


Fig. 10 Absorbance curve of ascorbic acid

The total antioxidant capacity of the $CHCl_3$ fractionate of the leaves of *P. chaba* was 216.56 mg/g (expressed as ascorbic acid equivalents), the highest antioxidant capacity compared with other fractionates. On the other hand, the Aqueous fractionate was found to show 21.44 mg/g (as ascorbic acid equivalents) which is the lowest antioxidant capacity compared with others. MeOH extract, n-hexane, DCM, and EAC fractionates showed 122.51, 146.56, 111.08, and 82.27 mg/g antioxidant capacity respectively. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with maximal absorption at 695 nm.

Fatty acids content

The prepared methyl ester of free fatty acids (FFA), Bound fatty acids (BFA) along with standard fatty acids ester samples were analyzed in GC-FID (Shimadzu 9 A, Column-BP-50, Detector-FID, 105 °C–5 °C/min-150 °C–2 °C/min-280) and their retention time was recorded. The relative percentages of the free fatty acid and bound fatty acids were calculated from the retention time. The fatty acids of the plant were converted to their methyl esters and analyzed by GC-FID. The fatty acids present in the plant were identified by comparison with the retention time of the standard samples. Their relative percentages were also determined.

The analysis of bound fatty acids showed that *P. chaba* contains the highest proportion of caprylic acid (40.31%), and the lowest proportion of lauric acid and linolenic acid (0.39%). Others, such as palmitoleic, stearic, oleic, arachidic, behenic, and erucic acids are present with intermediate percentages of 9.52, 22.10, 3.08, 0.62, 1.09, and 0.45%.

The analysis of free fatty acids showed that caprylic acid is the most abundant (34.43%) and linolenic acid is the lowest proportion (1.08%) of fatty acid present in free form in the leaves of *P. chaba* (Figs. 12 and 13).

Myristic, palmitoleic, stearic, oleic, arachidic, behenic, and erucic acids are the other fatty acids present with an intermediate percentage of 1.41, 24.02, 4.45, 7.77, 1.72, 4.07 and 1.39%. A small amount of fatty acid was present in the n-hexane extract of the plant in a free state compared with bound fatty acid.

Conclusion

The plant *P. chaba* H is a medicinal plant with significant pharmacological activity. Leaves of this plant could be a good source of antioxidant agents. This study was not performed previously and should be carried out further to determine this plant's medicinal importance. DPPH assay method showed that DCM fractionate had the highest free radical scavenging activity with an IC_{50} value of 8.84 µg/mL which was very significant in comparison with antioxidant agent Ascorbic acid. On the other hand, the $CHCl_3$ fractionate showed a significant capacity of 216 mg/g (expressed as

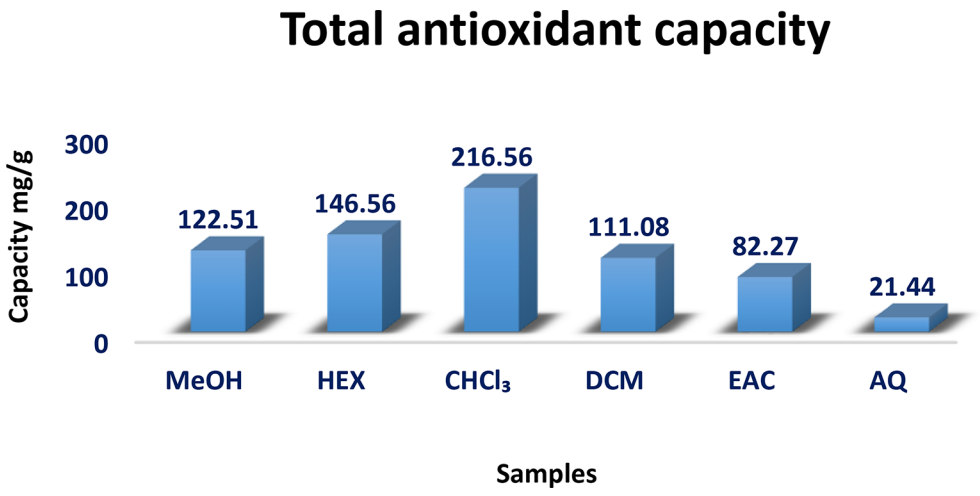


Fig. 11 Total antioxidant capacity of MeOH extract and different fractionates of *P. chaba* leaves

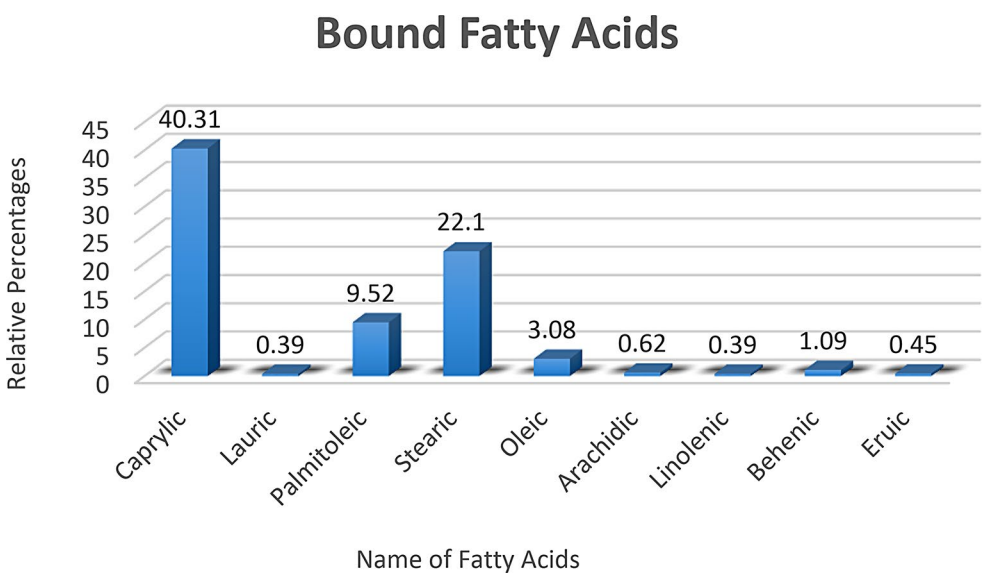


Fig. 12 Relative percentages of bound fatty acid

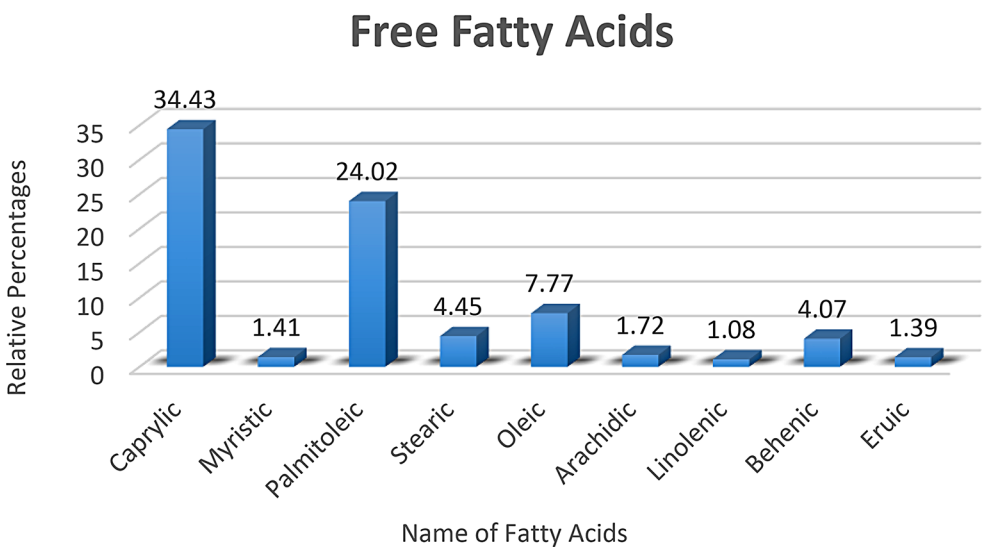


Fig. 13 Relative percentages of free fatty acid

ascorbic acid equivalents) which was higher than many antioxidant sources. *P. chaba* H leaves are a prominent source of fatty acids that have not been researched previously. It contained the highest proportion of caprylic acid (40.31%) and the lowest proportion of lauric acid and linolenic acid (0.39%) as bound forms of fatty acids. The analysis of free fatty acids showed that caprylic acid was the most abundant (34.43%) and linolenic acid was the lowest proportion (1.08%). Myristic, palmitoleic, stearic, oleic, arachidic, behenic, and erucic acids were the other fatty acids that were also present in the leaves of this plant, which are precious fatty acids for living beings. From this research it can be concluded that leaves of *P. chaba* H are a potent antioxidant source, they can be used for making antioxidant agents. The antioxidant profile of this plant is too rich, which will open the root of isolation of potent pharmacological active compounds by attending phytochemical investigation of this plant. The fatty acid content of the leaves is also significant. The leaves are a potent source of fatty acids which are very important for living beings. Many unsaturated fatty acids were found and palmitoleic was present in a good quantity, that's why the antioxidant activity of the leaves was significant. The researcher should emphasize their research interest in this potent medicinal plant. In the future, this plant will contribute a lot to the medicinal sector as a potent drug discovery agent if it is studied properly by dedicated researchers.

Abbreviations

DCM	Dichloromethane
EAC	Ethyl acetate
DPPH	2,2-diphenyl-1-picrylhydrazyl
AQ	Aqueous
HEX	n-hexane

D-GalN	D-galactosamine
MeOH	Methanol
GC-FID	Gas chromatography-flame ionization detector
BFA	Bound fatty acid
FFA	Free fatty acid
FAME	Fatty acid methyl ester

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42535-024-00898-0.

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Author contributions SPS the 1st and corresponding author has done all the lab works and writing, editing the manuscript related to the research work.

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Data availability The different sets of data that have been generated or used and examined to write the present manuscript are available from the corresponding author upon reasonable request.

Declarations

Ethical approval Not applicable.

Consent of interests No, I declare that there is no consent of interests as defined by the journal.

Competing Interests No, I declare that the author has no competing interests as defined by the journal, or other interests that might be perceived to influence the results and/or discussion reported in this paper.

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