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Original Research Article

A comparative study on the extracts from the fruits of *Ficus auriculata* L.: GC-MS profiling, phytochemical composition, biological activities and *in-silico* ADMET study

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ABSTRACT

Ficus auriculata L., commonly known as, "elephant ear fig" is a species of fig tree in Moraceae family and globally found in tropical and subtropical forests. The present comparative study investigated the GC-MS analysis, phytochemical composition, *in vitro* antioxidant assays and antidiabetic activity of methanol and hexane extracts from the fruits of *Ficus auriculata* which was collected from two different agro-climatic conditions in Uttarakhand, namely Almora (Hill region) and Haldwani (Tarai region). The GC-MS analysis of Almora unripe hexane fruit extract (AUFHE) and Haldwani unripe hexane fruit extract (HUFHE) gave rise to the characterization of two chemical profiles composed of 37 and 40 bioactive compounds with γ-sitosterol (15.46% and13.44%) as the most abundant component, respectively. Moreover, in Almora unripe methanol fruit extract (AUFME) and Haldwani unripe methanol fruit extract (HUFME), 24 and 23 bioactive compounds were characterized among which linoleic acid (71.41%) and hexadecadienoate (26.42%) were the most prevalent compounds, respectively. In view of the obtained results, HUFME exhibited prominent total phenolic, flavonoid and tannin contents. AUFME also showed potent antioxidant activity when using DPPH (2,2-diphenylpicrylhydrazyl) radical scavenging activity assay (IC_{50} =447.45 ± 0.53 µg/mL), whereas strong metal chelation assay was found for HUFHE (IC_{50}= 502.07 ± 2.50 µg/mL). Furthermore, AUFME and HUFME displayed potent anti-diabetic activity. In addition, ADMET study predicted that *F. auriculata* could be considered an effective bioactive source of phytoconstituents for various biological efficacies. The observed pharmacological properties could be attributed to the presence of polyphenols, flavonoids and fatty acids in *F. auriculata* fruit.

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1. Introduction

The use of medicinal plants is widely spread all over the world. Due to the therapeutic and healing properties of the medicinal plants, they have been widely used since ancient times (Mohammadhosseini et al., 2019; Mohammadhosseini et al., 2021; Agrawal and Jain, 2023; Anish et al., 2023). In India, forests constitute multitude aromatic and medicinal plants which are used as raw materials for the production of commercial drugs and also perfumery products. As per WHO recommendation (2004), around 80% of the world's population relies on the potential use of medicinal plants and herbs. Approximately, 21,000 varieties of plants are used in a wide range of medicinal disciplines. Currently, cancer, non-communicable diseases, and mental health disorders like Alzheimer's and Parkinson's diseases are common challenges for the human health. Foodborne illnesses, which are a major health concern, are often the result of pathogenic bacteria transmitted by humans (Mehra et al., 2022). Phytonutrients such as alkaloids, anthocyanins, betacyanins, anthraquinones, coumarins, flavonoids, saponins, tannins, terpenoids, triterpenoids, glycosides, phenol, steroids, proteins, and vitamin C in herbal medicines have numerous health benefits. According to Bahl et al. (2022), plants are capable of synthesizing a vast range of secondary metabolites with therapeutic potential to cope with diseases caused by oxidative stress. *Ficus auriculata* L., a member of the Moraceae family, is known for its diverse traditional medicinal applications that set it apart from other plant species. The *Ficus* plants are primarily found in temperate, tropical, and subtropical regions at altitudes ranging from 1800 to 2600 meters. These species are native to Asia, particularly in countries such as India, Nepal, Bhutan, Pakistan, China, Malaysia, Thailand, Myanmar, and Vietnam and are also frequently used in the treatment of diarrhoea and dysentery (El-Fishawy et al., 2011).

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F. auriculata fruit is very tasty and serves as a rich source of antioxidants. In fact, diverse *Ficus* species contain high levels of polyphenolic compounds and flavonoids, which are responsible for their remarkable antidiabetic and antioxidant properties that help in the prevention and treatment of various oxidative stress-related diseases, *e.g.*, neurodegenerative and hepatic diseases (Paasyeva et al., 2020).

Various health advantages, including antimicrobial, anticancer, and anti-inflammatory properties have been linked to the majority of *Ficus* species. The impact of a methanol extract from *F. auriculata* leaves on blood clotting was examined, along with the potential antimicrobial effects of the fruits, leaves, and bark of this herbal plant (Tamta et al., 2021). The synthesized silver nanoparticles from the leaves of *F. auriculata* displayed antioxidant property (Mehra and Tandon, 2021).

In each society, a significant portion of the population is impacted by chronic inflammatory diseases. In today's society, there is a growing emphasis on health, leading many individuals to turn to herbal remedies for treating inflammation. It has been documented that *F. auriculata* fruits have anti-inflammatory and antidiabetic properties (Walia et al., 2022), suggesting that their bioactive components could be utilized in creating new culinary and medicinal products. Researchers have found that *F. auriculata* possesses various medicinal properties (Mehra and Tandon, 2021).

Nowadays, there is a significant focus on using *in silico* methods to predict the toxicity and drug likeliness of chemical compounds in the early stages of drug discovery. It is advised to assess the bioavailability of a compound before proceeding to clinical trials to avoid potential failures in the initial stages of drug development (Jia C et al., 2020). The ADMET study uses computational modeling to predict the toxicity and pharmacokinetics of a large database of compounds, both *in vivo* and *in vitro*, by improving their chemical characteristics (Davis et al., 2004). The current study aimed to assess the organic extracts of *F. auriculata* fruits collected from two distinct climatic regions in India (Almora and Haldwani). The goal was to identify the bioactive compounds in their chemical profiles using GC-MS, quantify phytoconstituents, and evaluate their antioxidant and antidiabetic properties. Additionally, an *in silico* ADMET study was conducted to predict the drug likeliness and pharmacokinetics of the bioactive compounds found in the methanol and hexane extracts of *F. auriculata*.

2. Experimental

2.1. Materials and methods

The unripe fruits of *F. auriculata* L. were collected from two different altitudes of Uttarakhand, namely Almora (latitude-N 29° 30.89652′ and longitude-E 79° 59.9289′) and Haldwani (latitude-N 29° 13.09584′ and longitude-E 79° 30.77862′). The collection was carried out in the months of June and July and the collected samples were identified by a plant taxonomist (Dr. D.S. Rawat) from Department of Biological Science, Govind Ballabh Pant University of Agriculture and Technology, India.

2.1.1. Drying and extraction

After sampling, the unripe fruits of *F. auriculata* were washed thoroughly with water to get rid of dust and other impurities. After washing off, the fruits were chopped into small pieces and left to dry at room temperature for two weeks. The moisture content in the plant materials was removed and the dried samples were grounded to fine powder. The powder material was extracted with successive soxhlet method using methanol and hexane solvents, respectively.

2.1.2. Soxhlet extraction

The unripe fruits of *F. auriculata* were first processed in a grinder to get a powder of fine and homogenous size. The thimble of filter paper was first filled with a powder and then placed inside the soxhlet apparatus, where the extraction was carried out using successive portions of hexane and methanol. The temperature was kept constant at 60 °C, and each extraction lasted about 9-10 hours. Then, the powder from the thimble was utilized for the successive extraction using methanol after the hexane extract had been collected. The same procedure has been followed for methanol. The obtained extracts were subsequently evaporated in a water bath at 40 °C, dried in a vacuum oven at 40 °C, and the yield value(%) was then determined for each extract. Then, the dried extracts were subjected to phytochemical screening and biological activity.

2.1.3. GC-MS analysis

The phytoconstituents present in the hexane and methanol extracts of *F. auriculata* fruits were identified by GC-MS analysis. Gas chromatography-mass spectrometery was performed at the Advanced Instrumental Research Facility, Jawahar Lal Nehru University (JNU), New Delhi. In this relation, *F. auriculata* unripe fruits were analyzed for their chemical constituents using a combined gas chromatography (GC) HP 6890 with mass selective detector MS 5973 equipped with a split-splitless injector, an electronic pressure control, and a DB-5 silica column (30 m X 0.25 µm film thickness) (Agilent Technologies, USA). Helium was used as a carrier gas at the flow rate of 1.0 mL min^{-1}. The injector was operated at 250 °C and the oven temperature was programmed at 60 °C for 15 minutes. The detection was performed in full scan mode over the *m/z* range of 41-450. The Calibur 4.0 software was used to analyze the data, and the chromatograms were identified by matching their mass spectral fragmentation patterns which were compared to the database stored at the National Institute of Standards and Technology (NIST 2.2) library.

2.2. Quantitative phytochemical analysis

2.2.1. Determination of total phenolic content (TPC)

The total phenolic content of the methanol and hexane

extracts from the fruits of *F. auriculata* was assessed using the standard procedure given by Orphanides et al. (2013). In accordance with this method, in each test tube, 5 mL of FCR (Folin-Ciocalteu Reagent, 1 N) was added to 0.5 mL of each hexane and methanol extract of *F. auriculata* fruit. 1 mL of saturated sodium carbonate solution (1 N) was then added to the reaction mixture to neutralize it. The reaction mixture was kept at 24 °C for 35 minutes. In the next step, the reaction mixture was centrifuged at 4000 rpm for 10 minutes. Then, the absorbance was measured against the reagent blank at 725 nm. The total phenolic content (TPC) was finally quantified using a standard curve of gallic acid at different concentrations (30-150 µg) and results were recorded in terms of mg gallic acid equivalents (mg $GAEg^{-1}$).

2.2.2. Determination of total flavanoid content (TFC)

The standard procedure for the determination of total flavonoid contents was according to Orphanides et al. (2013) report. Briefly, 4 mL of distilled water and 300 µL of $NaNO_2$ (5.0%) solution were added to 1 mL of each hexane and methanol extract from the fruits of *F. auriculata*. After mixing, the solution was incubated for 5 minutes followed by the addition of 300 µL of $AlCl_3$ (10%) to the reaction medium which was allowed to stand for 1 minute. Immediately after, 2 mL of NaOH (1 M) was added to the reaction mixture followed by 2.4 mL of distilled water. All the reagents are mixed and incubated in a dark place for 15 minutes at 24 °C, centrifuged at 4000 rpm for 5 minutes and the relevant absorbance was measured at 510 while using catechin as the standard. Finally, total flavonoid content was expressed in terms of mg $CAEg^{-1}$.

2.2.3. Determination of proanthocyanidin

The evaluation of proanthocyanidin was done according to a standard method developed by Sun et al. (1998). Accordingly, 3 mL of methanol solution of vanillin was added to 1 mL of each hexane and methanol fruit extract of *F. auriculata* in a test tube followed by 1 mL of HCl. The reaction mixture was then incubated for 15 minutes and the corresponding absorbance was recorded at 500 nm while using catechin as a standard. Finally, proanthocyanidin content was expressed in terms of mg $CAEg^{-1}$.

2.2.4. Determination of orthodihydric phenol

The orthodihydric phenol content of the prepared extracts was assessed by a standard method (Mahadevan et al., 1986). In this context, hexane and methanol extracts of *F. auriculata were first* mixed with 1 mL of HCl (0.5 N) and after that, 1 mL of arnows reagent consisting of 10 g HNO_3 + 10 g of sodium molybdate dissolved in 100 mL of distilled water was added to the reaction medium. After the addition of 2 mL of NaOH (1 N) to the reaction mixture, 4.5 mL of distilled water was added. Thereafter, at 515 nm, the relevant absorbance was recorded against a reagent blank using catechol as a standard and orthodihydric phenol was expressed in terms of mg $CLEg^{-1}$.

2.2.5. Determination of total tannin

The total tannins of *F. auriculata organic extracts* were determined according to the method given by Sadasivam et al. (1992). In this regard, 0.5 mL of Folin-Denis reagent was mixed with 1 mL of each hexane and methanol extract of *F. auriculata* and 6.6 mL of distilled water. Thereafter, 1 mL of saturated sodium carbonate solution was added followed by the addition of distilled water. The reaction mixture was kept at room temperature for 30 minutes and the absorbance was measured against a reagent blank at 700 nm. Tannic acid was used as a standard and the total tannins were reported as mg tannic acid equivalents (mg TAE^{-1}).

2.3. Biological study

2.3.1. Antioxidant activitiy

2.3.1.1. DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the fruit extracts of *F. auriculata* was monitored using a previously reported procedure (Chen et al., 1995). Antioxidant activity is based on the potential of an extract to scavenge the free DPPH radicals present in the reaction medium. To determine the potential antioxidant activity of the organic extracts, 1 mL of each hexane and methanol extract of *F. auriculata* was incubated in the dark at room temperature with 5 mL of a DPPH solution (0.4 mM) for 30 minutes. The reaction mixture's absorbance was measured at 517 nm using ascorbic acid and gallic acid as standards, and a blank solution as the control.

2.3.1.2. Metal chelating method

The metal chelating behavior of the prepared organic extracts from the fruits of *F. auriculata* was monitored regarding the method developed by Hsu et al. (2003) on the basis of the chelating ability of Fe^{2+} ions of antioxidants. Accordingly, 1 mL of each hexane and methanol extract of *F. auriculata* was treated with 0.1 mL of $FeCl_3.6H_2O$ (Iron (III) chloride hexahydrate, 2 mM) reagent, followed by 0.2 mL of ferrozine solution (5 mM). A test sample with different concentrations (200-1000 µg/mL) was taken. EDTA (Ethylene diamine tetraacetate) was used as a standard and the reaction mixture was shaken vigorously and incubated at room temperature for 10 minutes. The absorbance was measured at 562 nm and Fe^{2+} chelating activity was compared with the concentration of standards.

2.4. *In vitro* antidiabetic activity

One of the goals of the current investigation was to provide the potential inhibitory evidence for *F.*

auriculata fruit extracts on alpha-amylase and alpha-glucosidase enzymes in accordance with the method proposed by Kidane et al. (2018).

2.5. Alpha-amylase inhibitory activity

The methanol and hexane extracts of *F. auriculata* having concentrations over the range 200-1000 µg/mL were placed in a test tube. A sodium phosphate buffer (0.02 M, pH-6.9) solution was added to the alpha-amylase enzyme. The reaction mixture was incubated at 25 °C for 10 minutes. A starch solution (1.0%) was then added to the sodium phosphate buffer (0.02 M). The reaction mixture was incubated again for 10 minutes at 25 °C followed by the addition of 2 mL of DNS (3,5-dinitrosalicylic acid, 40 mM) reagent for the completeness of the reaction. The mixture was finally incubated for 5 minutes in a water bath at 35 °C and subsequently cooled at room temperature. In the final step, the mixture was diluted with distilled water and the relevant absorbance was taken at 540 nm against a reagent blank while using acarbose as a standard.

2.6. Alpha-glucosidase inhibitory activity

The procedure of alpha-glucosidase inhibitory activity was initiated by incubating maltose substrate (6 mM) with 1 mL of tris buffer (pH 8.0) and various concentrations of each hexane and methanol extract from the fruits of *F. auriculata* (200-1000 µg/mL) at 35 °C. Alpha-glucosidase enzyme was added into the reaction mixture to initiate the reaction. To stop the reaction, DNS color reagent was introduced. The intensity of the color was measured by assessing the absorbance at 540 nm.

2.7. *In-silico* ADMET study

To evaluate the *in silico* ADMET study, structure of the selected compounds were identified via GC-MS and drawn using Chem draw. Firstly, the selected compounds were transformed into canonical SMILES format and their drug-like and pharmacokinetic characteristics were forecasted using ADME tool by a Swiss ADME online web server, according to the developed protocol (Daina et al., 2017). The toxicity profiling was done using ProTox-II webserver. The toxicity of the selected compounds was forecasted in the sense of different criteria, *i.e.*, oral toxicity, organ toxicity (Hepatotoxicity) and toxicological end points (Mutagenicity, cytotoxicity, immunotoxicity and carcinotoxicity).

2.8. Statistical analysis

Correlation and PCA were performed using R Studio (Version 4.2.2, 2022-10-31) in Intel^{R} Core (TM), i5-103 5GI CPU @1.00Ghz on 64 bit windows with 8 GB RAM.

3. Results and Discussion

3.1. Percent yield

The yields of the hexane and methanol extracts from the fruits of *F. auriculata* have been presented in Table 1. As can be seen in this table, the highest yield has been obtained for the methanol extract of the AUFME sample, whereas the lower yield has been found for the hexane extract of the AUFHE sample.

3.2. GC-MS analysis

The peak areas (%) of the bioactive compounds in the hexane and methanol extracts from the fruits of *F. auriculata* using GC-MS have been shown in Table 2. As seen, a wide the range of compounds have been characterized which demonstrate significant antioxidant, antidiabetic, and various pharmacological effects.

In total, 23 phytoconstituents were observed in HUFME, accounting for 97.94% of the overall peak areas of the chemical profile comprising hexadecadienoate (26.42%), γ-sitosterol (15.57%), linoleic acid (methyl ester) (7.17%), palmitic acid (methyl ester) (5.45%) as the most abundant constituents. In AUFME, a total of 24 phytoconstituents were identified constituting 99.82% of total composition with linoleic acid (71.41%), quinic acid (kinic acid) (10.61%), and γ-sitosterol (3.33%) as the major bioactive components. In the hexane extract of HUFHE, a total of 40 phytoconstituents were identified accounting for 93.98% of the total composition and the major identified bioactive components were found to be γ-sitosterol (13.4%), lupeol (9.26%), oleic acid (methyl ester) (9.71%), whereas in AUFHE, a total of 37 phytoconstituents were identified altogether making up 97.09% of hexane extract composition and the major characterized bioactive components were β-sitosterol (15.46%), lupeol (10.36%), octacosanol (9.11%) and petroselinic acid (methyl ester) (6.64%).

The result obtained reveals that AUFME was characterized by high levels of fatty acids in decreasing order of ketones, aldehydes, esters, and alcohols, whereas phytosterols and terpenoids in AUFHE have been detected at high levels. In HUFME, fatty acids and phytosterol have been detected at high levels, whereas in HUFHE, phytosterol, fatty acids and terpenoids have been detected at high levels. Visual interpretation of GC-MS depicted metabolite profiles of both fractions varied quantitatively, and these tentative compounds were compared with the reported literature and databases. A previous study depicted that *Ficus* spp. viz. *F. auriculata* fruits have organic acids, *e.g.*, citric and quinic acids (Boelsma et al., 2001). On the other hand, stearic, oleic, linoleic, and linolenic acids were reported. In addition, steroids were identified, *i.e.*, stigmasterol in the ethanol extract of *F. auriculata*. Chemical compounds were also reported in a previous study comprising quercetin, isoquercetin, quercitrin, kaempferol, catechin, epicatechin, myricetin, vitexin, apigenin, rutin, gallic acid, vanillic acid, chlorogenic acid, and caffeic acid (Yunus et al., 2021).

Flavanols, flavanonols, flavones, flavanones, flavonolignans, anthocyanins, hydroxycinnamic acids, and derivatives were also characterized in some *Ficus* species (Elhawary et al., 2018). However, the present study revealed that the fruit extracts of *F. auriculata*

Table 1
Percent yields of hexane and methanol extracts of of *F. auriculata* fruits.

Solvent	Sample	Weight(g)	Yield(g)	Yield (%
Methanol	HUFME	206	11.303	5.48
	AUFME	222	15.28	6.88
Hexane	HUFHE	220	8.46	3.84
	AUFHE	230	5.97	2.59

HUFME-Haldwani Unripe Fruits Methanol Extract, AUFME-Almora Unripe Fruits Methanol Extract, HUFHE-Haldwani Unripe Fruits Hexane Extract AUFHE-Almora Unripe Fruits Hexane Extract.

Table 2
GC-MS analysis of hexane and methanol extracts from the fruits of *F. auriculata*.

Compounds	% Composition			
	HUFME	AUFME	HUFHE	AUFHE
Benzenedicarboxylic acid, (dioctyl ester	0.43	-	-	-
Cetane	0.81	0.93	0.32	-
Cyclopentyl propionic acid	0.65	-	-	-
Cyclopentylpropionic acid	3.59	-	-	-
Docosanoic acid(methyl ester)	0.37	-	-	-
Dodecane	3.65	0.28	-	-
Hexadecadienoate	26.42	-	-	-
Hexyloxacyclotridec-10-en-2-One	0.89	-	-	-
Hydrocinnamic acid,	1.56	-	-	-
Linoleic acid (methyl ester)	7.17	-	-	1.7
Lupeol	5.85	-	9.26	10.36
Methyl commate B	2.58	0.44	-	-
Palmitic acid(methyl ester)	5.45	0.65	2.07	1.57
Palmitoyl glycerol	1.97	-	-	
Stearic acid(methyl ester)	2.47	-	-	0.48
Stigmastenol	1	0.4	-	-
Stigmastenone	2.33	-	-	-
Stigmasterol	2.73	-	3.17	-
Tetradecane	3.52	0.57	-	-
y-sitosterol	15.57	3.33	13.44	-
α-Linolenic acid	0.79	-	-	-
α-Tocopherol	2.75	0.14	-	-
β-Amyrone	5.39		-	-
(-)-Spathulenol	-	0.12	-	-
Aromadendrane	-	0.19	-	-
Decyl acetate	-	0.19	-	-
Eicosatrienoic acid(methyl ester)	-	4.9	-	-
Ergostadienol	-	0.25	-	-
Methyl commate D	-	0.72	-	-
Neophytadiene	-	0.16	-	0.22
Octadecadienoic acid	-	71.41	-	-
Octadecane	-	0.78	-	-
Palmitic amide	-	0.25	-	-
Pluchidiol	-	0.95	-	-

Table 2 Contined

Compounds	Composition (%)			
	HUFME	AUFME	HUFHE	AUFHE
Quinic acid	-	10.61	-	-
Squalene	-	0.15	5.89	1.7
y-Gurjunenepoxide	-	0.29	-	-
β-Amyrin acetate	-	0.22	-	-
Icosane	-	-	0.3	-
Juniper camphor	-	-	0.25	-
1-Pentadecanol	-	-	0.34	-
1-Hexadecanol	-	-	0.43	-
Butylated hydroxytoluene	-	-	0.28	0.25
Ethyl tridecanoate	-	-	1.29	-
Hexadecadienoic acid, methyl ester	-	-	2.23	-
Oleic acid, methyl ester	-	-	9.71	-
Methyl stearate	-	-	0.65	-
Linolelaidic acid	-	-	1.01	-
Ethyl oleate	-	-	1.67	-
Nonane	-	-	0.24	-
1-Octadecene	-	-	0.93	-
Octadecyl 2-propyl ester	-	-	0.36	-
4,8,12,16-Tetramethylheptadecan-4-olide	-	-	0.47	-
Ethyl linalool	-	-	1.15	-
Oxalic acid	-	-	0.33	-
9-Tricosene	-	-	2.33	2.96
2-Methyl-1-hexadecanol	-	-	0.45	-
Di-N-octyl phthalate	-	-	0.38	-
1-Heneicosyl formate	-	-	1.16	-
Geranyl linalool	-	-	0.83	-
Cyclobutylcarboxylic acid	-	-	1.09	-
Amyrin, acetate	-	-	6.69	-
Lupenyl acetate	-	-	2.09	4.94
Lupenone	-	-	1.73	1.67
Nonacos-1-ene	-	-	4.11	-
Cholesterol	-	-	0.86	-
Campesterol	-	-	4.43	1.04
Sitostenone	-	-	4.73	1.35
Arachidic alcohol	-	-	7.31	-
3-Hexadecene	-	-	-	0.57
Phenol	-	-	-	3.3
Pentadecene	-	-	-	1.15
Nonadecane	-	-	-	0.23
Heptadecene	-	-	-	2
Phytone	-	-	-	0.23
O-Benzoquinone	-	-	-	0.19
Nonadecene	-	-	-	1.25
Petroselinic acid (methyl ester)	-	-	-	6.64

Table 2 Contined

Compounds	Composition (%)			
	HUFME	AUFME	HUFHE	AUFHE
Linoleic acid	-	-	-	0.26
Oleic acid (ethyl ester)	-	-	-	0.16
Docosanol	-	-	-	0.64
Heptacosane	-	-	-	0.69
Hexadecanol	-	-	-	0.2
Dioctyl phthalate	-	-	-	0.39
Heptacosene	-	-	-	1.62
Octacosane	-	-	-	2.48
Celidoniol	-	-	-	0.63
Dodecyl tiglate	-	-	-	0.99
Nonacosene	-	-	-	4.57
Octacosanol	-	-	-	9.11
Pentatriacontene	-	-	-	7.79
α-Tocopherol	-	-	-	1.86
β-Amyrin Acetate	-	-	-	6.44
β-Sitosterol	-	-	-	15.46

contain considerable amounts of both saturated (palmitic acid) and unsaturated fatty acids, *e.g.*, linoleic acid, oleic acid, and hexadecadienoate and lower levels of the others. AUFME is rich in unsaturated fatty acids. Saturated fats play a crucial role in cardiovascular health and play an important role in the appropriate release of insulin (Farhana et al., 2022). Moreover, unsaturated fatty acids reduce the risk of diabetes. Hexadecadienoate has antioxidant and other pharmacological roles (Dubey et al., 2020). It was previously reported that quinic acid belongs to the cyclitol class found in AUFME and that it is the major constituent of coffee and a strong antioxidant with many beneficial effects (Ma et al., 2015). Petroselinic acid plays a significant role in the food, chemical, and cosmetics industries (Uitterhaegen et al., 2016). Fatty alcohols (octacosanol) are found in AUFHE as the major constituting group. Sharma et al. (2020) revealed that lupeol has various pharmacological responses, including decreasing the levels of calcium-oxalate and having cytoprotective action against free-radical-induced damage. Phytosterols like γ-sitosterol and β-sitosterol have been characterized in AUFHE and HUFHE samples, respectively. A wide number of studies have reported remarkable pharmacological effects. β-Sitosterol, a well-known phytosterol, was used as sunscreen emulsion and anti-ageing additives in cosmetic products (Dweck, 2006). It has previously been reported that γ-sitosterol could be used in the development of protein anti-diabetic drugs (Tripathi et al., 2013). Almora fruits of *F. auriculata* contain appreciable and comparable amounts of saturated and unsaturated fatty acids, terpenoids, phytosterols, and other bioactive compounds that are found in trace quantities as compared with Haldwani fruits justifing the traditional uses of this important medicinal plant for the treatment of various diseases. Molecular docking of the identified major phytoconstituents with their receptors can be carried out in the future, which can show further potential pharmacological activities like antidiabetic, anti-inflammatory, anticancer, etc.

3.3. Quantitative phytochemical analysis

The results of the phytochemical screening of the fruit organic extracts of *F. auriculata* have been represented in Table 3. As can be seen in this table, the highest quantities of TPC, TFC, OPC, proanthocynidin and tannin were found in the methanol extract of HUFME sample. However, the least values for the TPC, TFC and OPC were observed for the hexane extract of AUFHE. The hexane extract of HUFHE sample also showed the lowest proanthocynidin and tannin contents.

3.3.1. Total phenolic contents

In the methanol extract from the fruits of *F. auriculata*, the maximum amount of total phenol content was examined in HUFME (41.95 ± 4.2 mg GAE/g) followed by AUFME (38.99 ± 2.24 mg GAE/g). Total phenolic content found in HUFHE (28.19 ± 2.82 mg GAE/g) followed by AUFHE (25.10 ± 0.14 mg GAE/g) in the hexane extract. A previous study reported that total phenol content in *Parkia roxburghii* fruits was found to be 4.13 ± 0.52 mg GAE/g which was collected from Sikkim (Pandey et al., 2018). A previous research also found that an ethanol extract of *F. auriculata* fruits contained 33.25 ± 0.94 mg

Table 3
Phytochemical composition in methanol and hexane extracts from the fruits of *F. auriculata*.

Process Co-products	TPC (mg GAE/g)	TFC (mg GAE/g)	OPC (mg CLE/g)	Proanthocynidin (mg CAE/g)	Tannin (mg TAE/g)
HUFME	41.9±4.20	23.08±0.09	50.56±2.14	27.04±0.12	8.03±0.021
AUFME	38.99±2.24	19.6±0.22	41.75±0.531	23.17±0.056	7.71±0.048
HUFHE	28.19±2.82	14.85±0.019	29±0.87	15.11±0.14	5.44±0.059
AUFHE	25.06±0.14	13.4±0.12	22.72±2.00	15.9±0.100	6.00±0.021

TP = Total phenolic content, OPC= Orthodihydric phenol cotent, GAE/g = Equivalent of gallic acid per gram, CLE/g: Equivalent of catechol, CAE= Equivalent of catechin and TAE = Equivalent of tannic acid.

GAE/g DF (Shahinuzzaman et al., 2021). The findings of the current study show more positive results in terms of phenolic content compared to earlier studies.

3.3.2. Total flavonoid content

The highest amount of total flavonoid content in the methanol extract from the fruits of *F. auriculata* was found in HUFME (23.08 ± 0.09 mg GAE/g) followed by AUFME (19.65 ± 0.224 mg GAE/g). In the hexane extract of *F. auriculata* fruits, the highest amount of total flavonoid content was obtained in HUFHE (14.85 ± 0.019 mg GAE/g) followed by AUFHE (13.4 ± 0.128 mg GAE/g).

3.3.3. Orthodihydric phenol content

In the methanol extract of *F. auriculata* fruits, the highest orthodihydric phenol content was found in HUFME (50.56 ± 2.14 mg CLE/g) followed by AUFME (41.75 ± 0.531 mg CLE/g). However, in the hexane extract of *F. auriculata* fruits, the highest amount was obtained in HUFHE (29 ± 0.87mg CLE/g) followed by AUFHE (22.72 ± 2.00 mg CLE/g).

3.3.4. Proanthocyanidin content

In the methanol extract of *F. auriculata* fruits, the highest proanthocyanidin content was found in HUFME (27.04 ± 0.12 mgCAE/g), followed by AUFME (23.17 ± 0.056 mgCAE/g). The highest amount of CAE/g was obtained in AUFHE (15.9 ± 0.100 mg CAE/g), followed by HUFHE (15.11 ± 0.14 mg CAE/g). In the current study, quantitative determination of proanthocyanidin content has been reported for the first time for *F. auriculata*. These results indicate that proanthocyanidin is a safe and a valuable natural antioxidant.

3.3.5. Tannin content

In the methanol extract of *F. auriculata* fruits, the highest tannin content was observed for HUFME (8.03 ± 0.021 mg TAE/g) followed by AUFME (7.71 ± 0.048 mg TAE/g). The hexane extract of *F. auriculata* fruits contained the most significant tannin content in AUFHE (6.00 ± 0.021 mg TAE/g), followed by HUFHE (5.44 ± 0.059 mg TAE/g). Among all the five biochemical parameters analyzed for the methanol and hexane extracts from the fruits of *F. auriculata* from Almora (Hill region) and Haldwani (Tarai region), the methanol extract was found to have the highest orthodihydric and phenolic content compared to the hexane extract. The phenolic contents were found to be moderate followed by proanthocyanidin, flavonoid and tannins. In the present study, variation in phytoconstituents could be strongly related to abiotic factors such as the climate, geographical factors, and altitude and soil type.

3.4. Biological activity

3.4.1. Antioxidant activity

The DPPH and metal chelation radical scavenging activity of the methanol extracts of *F. auriculata* fruits from two different regions, namely Almora and Haldwani, was dose-dependent at different concentrations (200-1000 g/mL). The IC_{50} values of the methanol and hexane extracts of *F. auriculata* fruits are presented in Table 4, Fig. 1 and Fig. 2.

3.4.1.1. DPPH radical scavenging activity

In the methanol extract from the fruits of *F. auriculata*, AUFME showed strong radical scavenging activity (IC_{50} = 447.45 ± 0.53 µg/mL) followed by HUFME (IC_{50} = 465.63 ± 0.56 µg/mL) compared with the standard. In the hexane extract, HUFHE exhibits strong radical scavenging activity (IC_{50} = 597.12 ± 1.66 µg/mL) followed by AUFHE ((IC_{50} = 627.07 ± 1.08 µg/mL). A previous research depicted that ethanol extract of *F. auriculata* fruit has a DPPH radical scavenging inhibition (85.20 ± 0.96%) (Shahinuzzaman et al., 2021). However, El-Fishawy et al. (2011) reported that the ethanolic fruit extract exhibited various radical scavenging activity (44.90-88.24%) over the concentration range of 2-8 mg/mL. The reported variation in the previous studies may be due to drying methods affecting the fruits' pharmacological properties (Bushra et al., 2012), as obtained from this study. In the present study, AUFME exhibited greater maximum DPPH radical scavenging activity than HUFME. As it was investigated that phenolic content was found to be maximum in HUFME, a similar finding was also reported in the extracts of a number of medicinal plants and no correlation was observed in total phenol content and antioxidant capacity (Yilmaz et al., 2009). HUFHE was found to have maximum DPPH

radical scavenging potential as compared with AUFHE due to the presence of high levels of phytochemicals. We concluded that methanol extract of Almora unripe fruits has maximum antioxidant capacity due to its maximum orthodihydric phenolic compounds. The other reason might be the presence of other bioactive compounds like fatty acids, phytosterols, etc. The results also presented the remarkable antioxidant features and polyphenolic content which make this wild fruit a better candidate for its use in various functional foods and nutraceuticals.

3.4.1.2. Metal chelating activity

In the hexane extract from the fruits of *F. auriculata*, HUFME exhibited strong metal chelating activity (IC_{50} = 505.05±3.98 µg/mL), followed by AUFME (IC_{50} = 529.48 ± 0.736 µg/mL), while AUFHE exhibited strong metal chelating activity (IC_{50} = 502.07 ± 2.50 µg/mL), followed by HUFME (IC_{50} = 522.27 ± 1.29 g/mL). The hexane extract shows greater metal chelation potential than the methanol extract. In the hexane extract of Haldwani fruits, phytoconstituents were negatively correlated with metal chelation antioxidant activity because methanol extract contains the maximum levels of phenolic compounds. The chelating capacity of the investigated extracts from *F. auriculata* fruits decreased with decreasing polarity. According to the previous data, *F. maclellandii* and *F. racemosa* ethanolic fruit extracts had comparatively lower chelating power than *F. auriculata* (Tamuly et al., 2015). It suggested that methanol extract had potent chelating power and the methanol fraction was observed to exhibit a significant capacity to chelate ferrous ions in comparison to hexane fractions. The current outline demonstrates that chelation therapy used in formulation of synthetic compounds may contain some side effects. Thus, chelation of metal ions by natural phytoconstituents in the fruits of *F. auriculata* is of therapeutic importance.

3.4.2. Antidiabetic activity

Antidiabetic potential of methanol and hexane extracts from the fruits of *F. auriculat*, in terms of alpha-amylase and alpha-glucosidase inhibitory action, was presented in Table 5.

3.4.2.1. Alpha-amylase inhibitory activity

The highest α-amylase inhibitory action was found in AUFME (IC_{50} = 240.45 ± 1.26 µg/mL) followed by HUFME (IC_{50} = 255.1 ± 1.02 µg/mL). Anjum et al. (2019) observed that the methanol fraction of *F. auriculata* fruits recorded the maximum α-amylase. *F. auriculata* fruits might be useful in treatment of Type II Diabetes mellitus. In the hexane extract, the maximum inhibitory effect was shown in AUFHE (IC_{50} = 271.4 ± 0.84 µg/mL) followed by HUFHE (IC_{50} = 290.73 ± 0.546 µg/mL). Acarbose which is widely used as an anti-diabetic remedy, showed an IC_{50} of 230.16 ± 0.032 µg/mL. The present study highlights that less significant differences were observable between IC_{50} values of all extracts which are single-fold less potent and closer to the inhibition effect of acarbose. Such α-amylase inhibitors are also known as starch blockers as they prevent or slow the digestion of starch in the body, basically by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharide sugars (Kumar et al., 2010). AUFME exhibited the maximum inhibitory effect. Linoleic acid, which is abundant in AUFME, has anti-diabetic activity by inhibiting protein tyrosine phosphatase linked to insulin resistance.

3.4.2.2. Alpha-glucosidase inhibitory activity

The highest alpha glucosidase inhibitory action of the prepared organic extracts from the fruits of *F. auriculata* was found in HUFME (IC_{50} = 245.02 ± 1.81 µg/mL) followed by AUFME (IC_{50} = 257.5 ± 1.38 µg/mL). Anjum et al. (2019) observed that methanol fraction of *F. auriculata* fruit reported the maximum α-glucosidase with an IC_{50} value 103.43 ± 0.67 g/mL, respectively. In the hexane extract, the maximum inhibitory effect was shown in AUFHE (IC_{50} = 291.87 ± 1.49 µg/mL) followed by HUFHE (IC_{50} = 316.7 ± 1.60 µg/mL). γ-Sitosterol and linoleic acid were responsible for antidiabetic activity (Tripathi et al., 2014). In the hexane extract of AUFHE, significant tannin content was found as compared with HUFHE. Polyphenols in *Ficus* contribute to the health benefits because of their antioxidant, antitumor, and antidiabetic efficacy (Shin et al., 2014) and inhibited α-amylase and α-glucosidase activity but less potent than methanol extract of fruits.

Further, the highest inhibitory activity shown by the methanol fraction of *F. auriculata* fruit was comparable to that of acarbose that may be attributed to the higher concentration of these compounds in the methanol fraction as compared to other fractions. Overall, correlation was observed and indicated the maximum number of phytochemicals and other bioactive compounds contribute to higher antioxidant and antidiabetic activity.

3.5. *In silico* ADMET analysis

The ADME (absorption, distribution, metabolism and excretion) attributes, pharmacokinetics and drug likeness of some chosen compounds are assessed using Swiss ADME online software presented in Table 6. As per rule, the molecular weight (MW) is < 500, topological surface area (TPSA) < 140, number of H-bond donors (nOHD) ≤ 5, H bond acceptors (nOHA) ≤ 5, rotatable bonds (nRB) ≤ 10, water partition coefficient (WLOGP) ≤ 15. The present analysis depicts that 6 out of 13 compounds exhibit potent drug like properties subsequently as per Egan's Lipinski's and Verber's rule. The bioavailability score in chosen compounds were observed 0.55 which predicts the effective bioactivity of compound. TPSA value less than 30 $Å^2$, predicts the good brain barrier potential. Effective bioactive nature was confirmed by bioavailability score 0.55 observed in all the compounds. P-glycoprotein (P-gp) substrate was not found in any molecule, recommending the fine intestinal absorption of compounds. The consensus Log Po/w observed in the range 3.26-13.34 for the compound exhibits good lipophilicity character. Palmitic

Table 4
DPPH and metal chelation IC50 value in methanol and hexane extracts from the fruits of *F. auriculata*.

Process Co-products	DPPH scavenging IC_{50} Mean ± SD (µg/mL)	Metal chelation IC_{50} Mean ± SD (µg/mL)
HUFME	465.6±0.560	505.05±3.98
AUFME	447.54±0.53	529.48±0.736
HUFHE	597.12±1.66	502.07±2.50
AUFHE	627.07±1.08	522.27±1.29
Ascorbic acid	193.9±1.12	-
Gallic acid	199.32±1.97	-
EDTA	-	197.3±1.04

*= Standard antioxidant, '-'= Not present, values are means of three replicates±standard deviation.

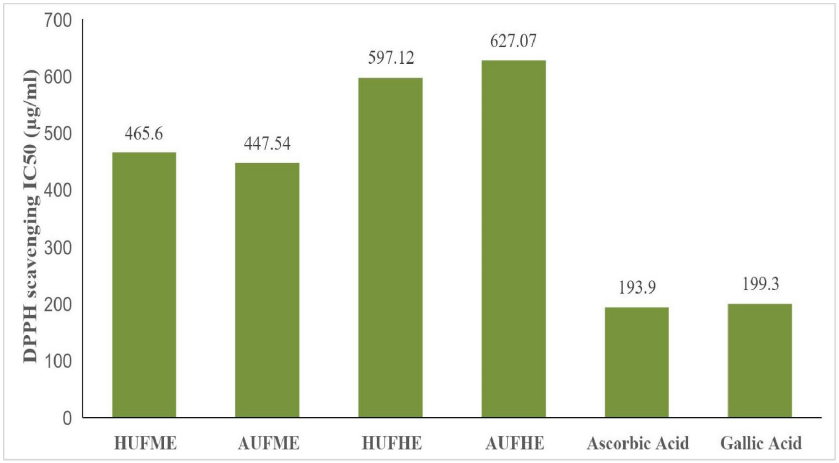

Fig. 1. DPPH activity in extracts and standards.

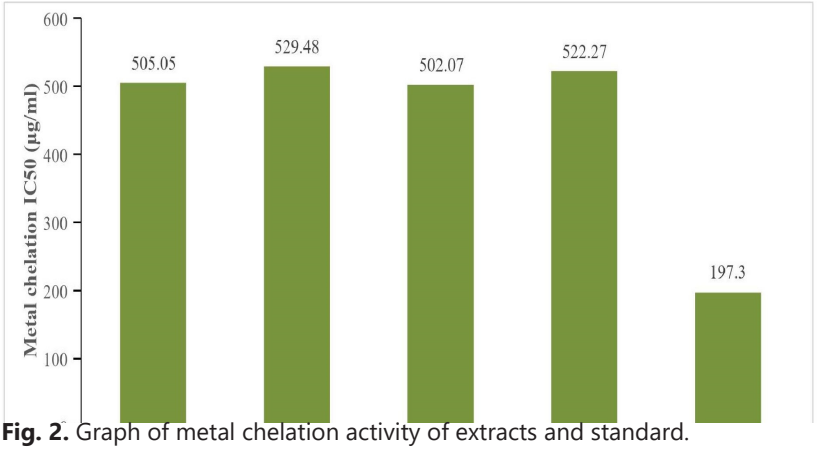

Fig. 2. Graph of metal chelation activity of extracts and standard.

amide, (-)-spathulenol, hexadecadienoate and oleic acid methyl ester demonstrated high gastrointestinal absorption power. Oleic acid methyl ester, petroselinic acid (methyl ester), lupeol, arachidic alcohol, octacosanol and pentatriacontene were forecasted to not intercross the blood-brain barrier (BBB). Some of the selected compounds mainly interact with two isoenzymes of cytochrome (CYP) family, *i.e.*, CYP1A2 and CYP2C19, suggesting their potency, although possessing least toxicity. The GI absorption and drug like qualities in selective compounds of HUFME, AUFME, HUFHE and AUFHE were presented by bioavailablity radar graph (Fig. 3) and boiled-egg prediction (Fig. 4). In the boiled-egg graph, yellow area can permeate via blood-brain

Table 5
Alpha-glucosidase and alpha-amylase inhibitory activity of methanol and hexane extracts from the fruits of *F. auriculata*.

Process Co-products	Alpha glucosidase IC_{50} Mean ± SD (µg/mL)	Alpha amylase IC_{50} value Mean ± SD (µg/mL)
HUFME	245.02±1.81	255.1±1.02
AUFME	257.54±1.38	240.45±1.26
HUFHE	317.8±1.6	290.73±0.546
AUFHE	291.87±1.49	271.46±0.84
Acarbose	230.4±0.32	230.16±0.032

'*' = Standard, Values are means of three replicates ± Standard deviation.

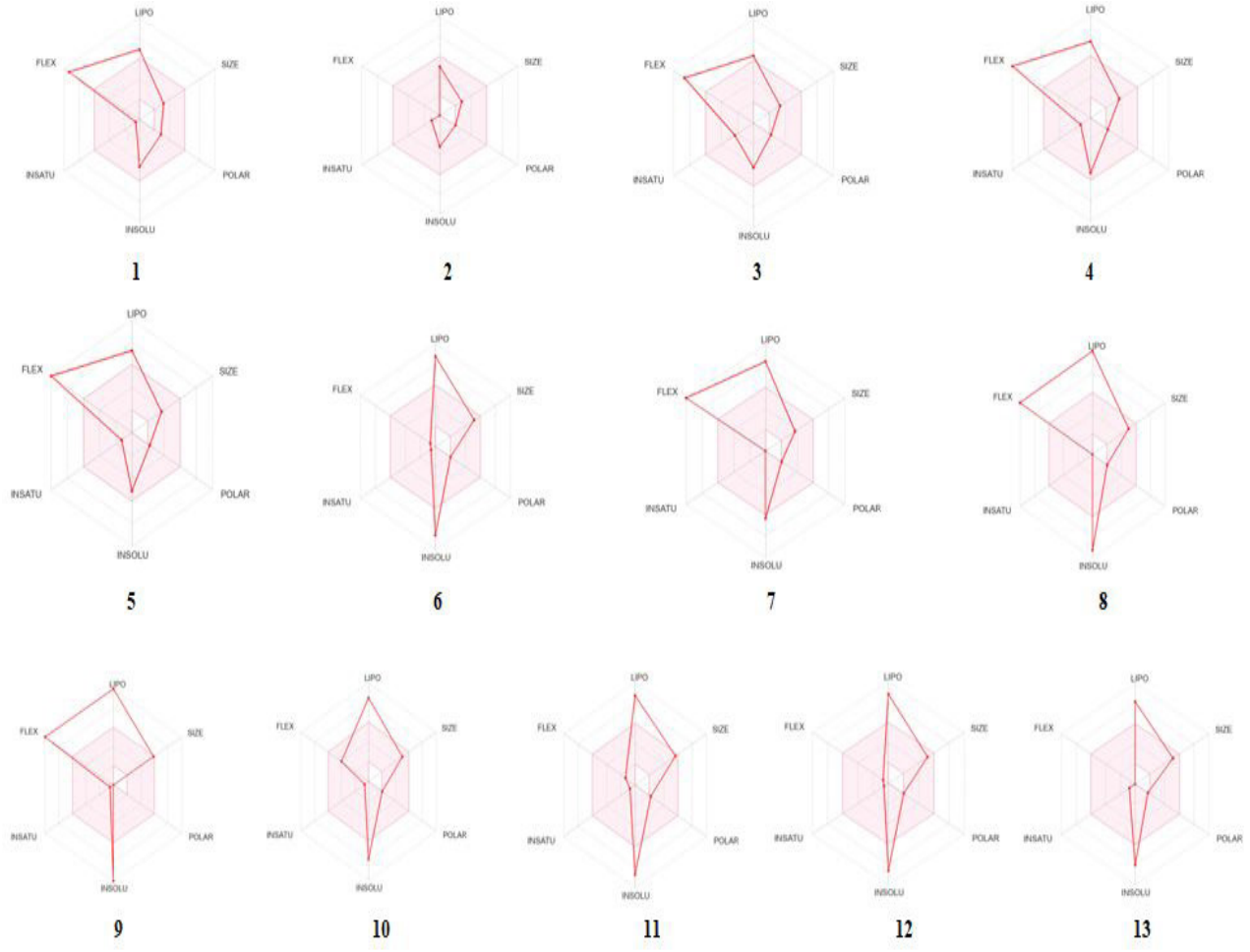

Fig. 3. Bioavailability radar graph of selected compounds (pink area demonstrated the drug likeness action of chosen compounds) 1: palmitic amide, 2: (-)-Spathulenol, 3: Hexadecadienoate, 4: Oleic acid, methyl ester, 5: Petroselinic acid (methyl ester), 6: Lupeol, 7: Arachidic alcohol, 8: Octacosanol, 9: Pentatriacontene, 10: β-Sitosterol, 11: Amyrin, acetate, 12: Triterpene lupeol, 13: β-Amyrone.

Table 6
In silico ADMET prediction of major constituents in AUFME, HUFME, AUFHE and HUFHE.

Entry	1	2	3	4	5	6	7	8	9	10	11	12	13
TPSA * (A2)	43.09	20.23	26.3	26.3	26.3	20.23	20.23	20.23	0	20.23	26.3	20.23	17.07
Consensus * Log Po/w	4.83	3.26	5.05	5.95	5.88	7.26	6.88	9.8	13.34	7.19	7.63	7.26	7.21
Mol wt (g/mol)	255.44	220.35	266.42	296.49	296.49	426.72	298.55	410.76	490.93	414.71	468.75	426.72	424.7
NRB	14	0	13	16	16	1	18	26	32	6	2	1	0
NOHA	1	1	2	2	2	1	1	1	0	1	2	1	1
NOHD	1	1	0	0	0	1	1	1	0	1	0	1	0
WLOGP	4.95	3.39	5.19	6.2	6.2	8.02	7.02	10.14	13.68	8.02	8.74	8.02	8.38
Water solubility	Poorly soluble	Soluble	Poorly soluble	Poorly soluble	Poorly soluble	Insoluble	Poorly soluble	Insoluble	Insoluble	Poorly soluble	Insoluble	Insoluble	Poorly soluble
GI absorption **	High	High	High	High	High	Low	Low	Low	Low	Low	Low	Low	Low
BBB permeant **	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No
P-gp substrate **	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No
CYP1A2 inhibitor **	Yes	No	Yes	Yes	Yes	No	Yes	No	No	No	No	No	No
CYP2C19 inhibitor **	No	Yes	No	No	No	No	No	No	No	No	No	No	No
CYP2C9 inhibitor **	No	No	Yes	No	No	No	No	No	No	No	No	No	No
CYP2D6 inhibitor **	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor **	No	No	No	No	No	No	No	No	No	No	No	No	No
Log Kp (cm/s)	-3.23	-5.44	-3.85	-2.82	-3.03	-1.9	-1.53	0.86	4.37	-2.2	-2.25	-1.9	-2.61
Lipinski violation	0	0	1	1	1	1	1	1	1	1	1	1	1
Bioavailability score ***	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55

Drug Likeliness ***, Pharmacokinetics**, Lipophilicity*, TPSA: topological polar surface area, nHA: no. of H-bond acceptor, nHD: no. of H-bond donor, nRB: no. of rotable bond, WLOGP: water partition coefficient, GI: gastrointestinal absorption, BBB: blood-brain barrier, P-gp: permeability glycoprotein, CYP: cytochrome P450, Y-Yes, No-N. Entry: 1: palmitic amide, 2: (-)-Spathulenol, 3: Hexadecadienoate, 4: Oleic acid, methyl ester, 5: Petroselinic acid (methyl ester), 6: Lupeol, 7: Arachidic alcohol, 8: Octacosanol, 9: Pentatriacontene, 10: β-Sitosterol, 11: Amyrin, acetate, 12: Triterpene lupeol, 13: β-Amyrone.

Fig. 4. Boiled-egg graph of selected phytoconstituents.

barrier (BBB) and drug-likeness of selected compounds were shown in bioavailability radar graphs by pink area.A web server ProTox II was used to predict the toxicity parameter of some chosen compounds presented in Table 7. All the selected compounds were predicted to be hepatotoxic and cytotoxic in nature except Palmitic amide. The chosen compounds were predicted not to be carcinogenic, mutagenic and immunotoxic character. The LD_{50} value was also determined to assure the safety of these chosen compounds.

3.6. PCA analysis

PCA is one of the best multivariate statistical tools to interpret the dataset having large number of dimension per observation. In the present work, PCA was used to explore the chemical profiling of compounds in which changes occurred by altitudinal variations, distinct sample can be used in PCA pattern perception. PCA demonstrated the collective contribution rate of variance of PC1 and PC2 could report for 58.1% of variance facts for the change in chemical compounds presented in Fig. 5. The compositional difference in sample was explained in terms of PC1 and PC2 shown in Fig. 6. PC1 contributed 31.7% of variance on positively linked with γ-sitosterol, palmiticamide, hexadecadienoate, α-tocopherol, arachidoic alchol, spathulenol though PC2 makes 26.4% of variance on linked with spathulenol, lupenylacetate, lupeol, pentatriacontene, octacosal and β-sitosterol.
A correlation analysis representation in Fig. 6 shows the phytochemicals and biological activity of *F. auriculata* exhibited a significant correlation exists between them.

4. Concluding remarks

The current research dealt with the GC-MS analysis, phytochemical composition, *in vitro* antioxidant properties, and antidiabetic effects of *F. auriculata* organic extracts. The plant material was collected from Almora and Haldwani in Uttarakhand, India. The GC-MS analysis resulted in identification of 37 bioactive compounds in the unripe hexane fruit extract from Almora region consisting of γ-sitosterol as the major constituent component (15.46%), and 40 bioactive compounds in the unripe hexane fruit extract from Haldwani with γ-sitosterol as the major constituent component (13.4%). Additionally, 24 bioactive compounds were found in the unripe methanol fruit extract from Almora with linoleic acid as the major

Table 7
Toxicity analysis of selected compounds from AUFME, HUFME, AUFHE and HUFHE.

Compounds	Hepatotoxicity		Carcinogenicity		Cytotoxicity		Immunotoxicity		Mutagenicity		Predicted LD_{50} (mg/kg)	Toxicity Class
	Pr	Pb	Pr	Pb	Pr	Pb	Pr	Pb	Pr	Pb		
Palmitic amide	NH	0.82	NC	0.61	NCy	0.99	NI	0.99	NM	0.72	1000	IV
(-)-Spathulenol	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Hexadecadienoate	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Oleic acid, methyl ester	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Petroselinic acid (methyl ester)	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Lupeol	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Arachidic alcohol	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Octacosanol	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Pentatriacontene	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
?-Sitosterol	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Amyrin, acetate	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
Triterpene lupeol	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV
?-Amyrone	H	0.69	NC	0.62	Cy	0.96	NI	0.97	NM	0.93	1190	IV

Pr: Prediction, Pb: Probability, H: Hepatotoxic, NH: Non-hepatotoxic, C: Carcinogenic, NC: Non-carcinogenic, NCy: Non-cytotoxic, NI: Non-immunotoxic, (Class I: if swallowed, fatal (LD50 ≤ 5), (Class II: if swallowed, fatal (5< LD50 ≤ 50), (Class III: if swallowed, toxic (50< LD50 ≤ 300), (Class IV: if swallowed, harmful (300< LD50 ≤ 2000), (Class V: if swallowed, possibly harmful (2000< LD50 ≤ 5000), (Class VI: non-toxic(LD50 >5000).

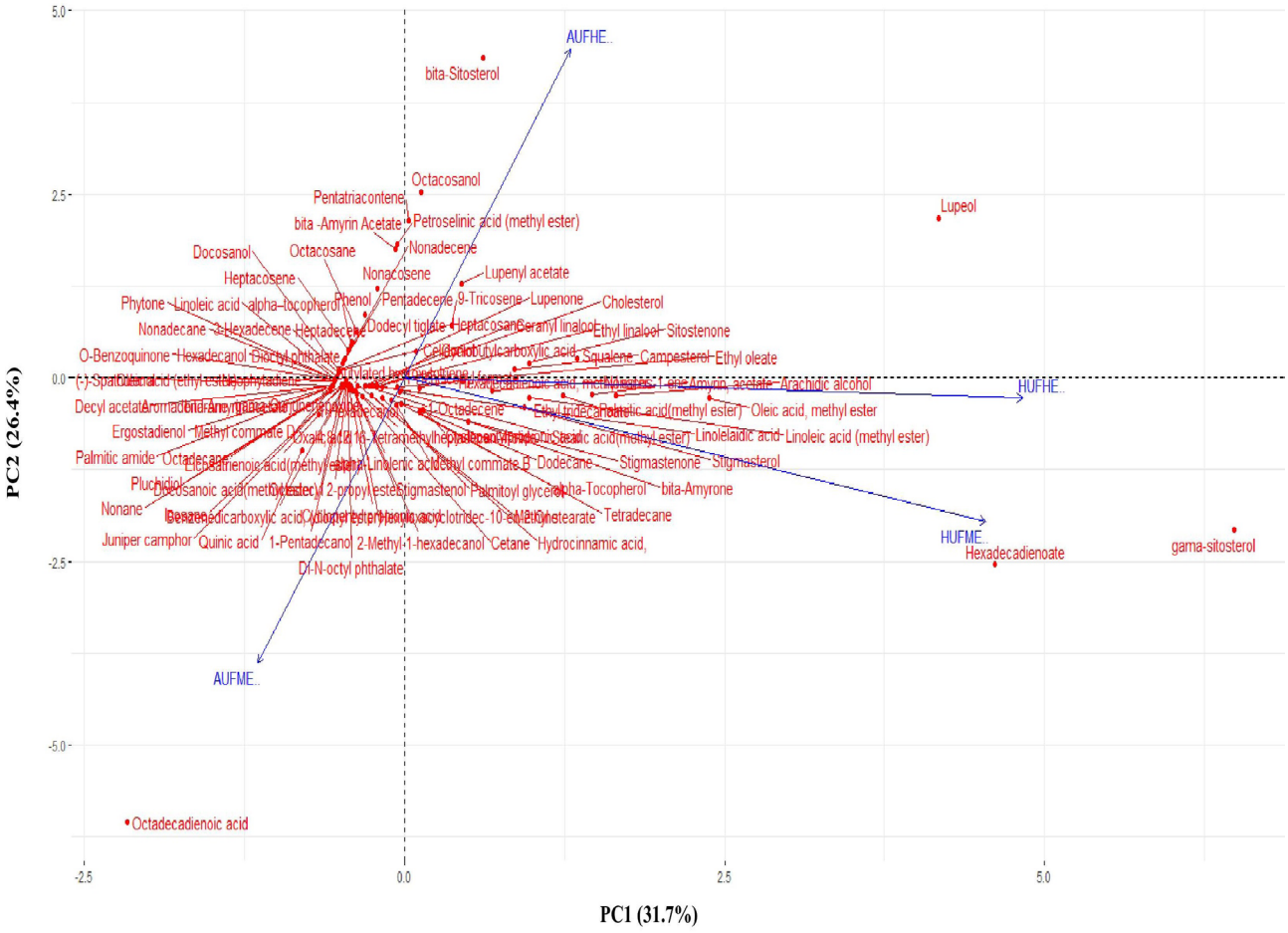

Fig. 5. Principal component analysis (PCA) of phytoconstituents observed in *F. auriculata* extract.

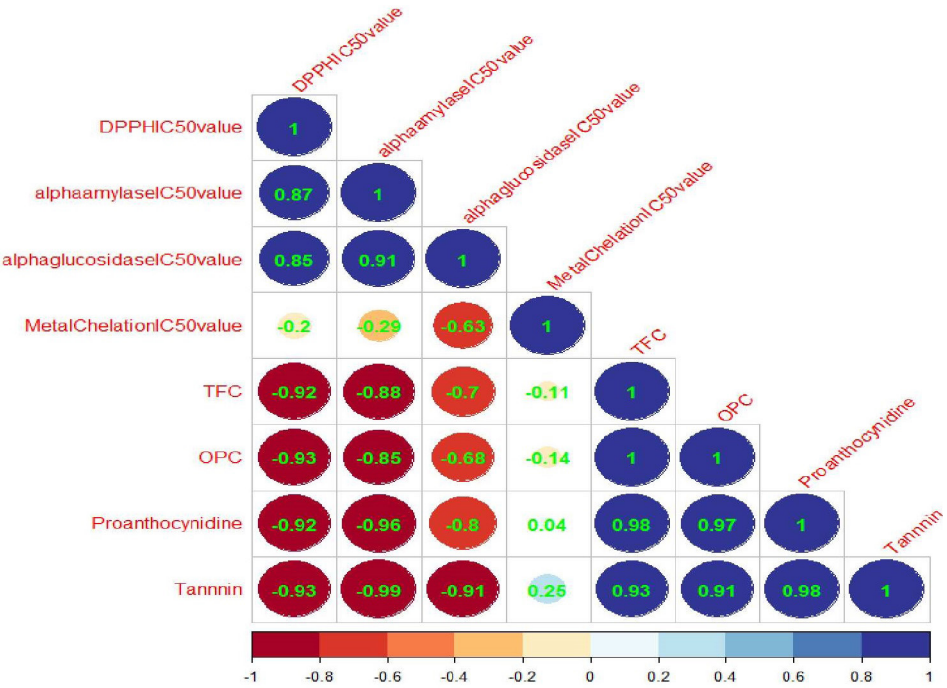

Fig. 6. A correlation study between phytochemicals and biological activity of *F. auriculata* fruit extract.

compound (71.41%), and 23 bioactive compounds were recognized in the unripe methanol fruit extract from Haldwani with hexadecadienoate (26.42%) as the dominant compound. This study revealed that the Haldwani unripe methanol fruit extract exhibited higher total phenolic, flavonoid, and tannin content, whereas the Almora unripe methanol fruit extract showed an IC_{50} of 447.45 ± 0.53 µg/mL when using DPPH radical scavenging activity assay. The Haldwani unripe hexane fruit extract displayed significant metal chelation activity (IC_{50} = 502.07 ± 2.50 µg/mL), as well. According to our findings, both Almora and Haldwani unripe methanol extracts of *F. auriculata* fruit could serve as potent antidiabetic effects. Furthermore, an ADMET study suggested that *F. auriculata* could be a valuable source of bioactive compounds with various biological benefits. The presence of polyphenols, flavonoids, and fatty acids in the fruit could also verify the relevant pharmacological properties.

It is concluded that Almora unripe fruit methanol extract has prominent biological and pharmacological potential than Haldwani unripe fruits. The fruits can be used to cure and reduce oxidative stress induced by free radicals. The antidiabetic potential focuses on the inhibitory action on α-amylase and α-glucosidase. Present study reported a potential mechanism of *F. auriculata* fruit and recommend that outcome of the fruit is due to the inhibition of digestive enzymes. However, the presence of phenols and flavonoids signifies the multiple biological efficacies of this plant. Some new bioactive compounds like quinic acid and other unsaturated fatty acids have been found, which will be researched deeply in the future along with their pharmacological activity. Natural antioxidants are extensively found in food, medicinal plants and herbs. Such natural antioxidants, commonly polyphenols, flavonoids and various other phytoconstituents, exhibits a endless biological significance and pharmacological properties and are also promising therapeutic agents, including anti-inflammatory, antidiabetic, antimicrobial and herbicidal activity. In the future, effective drugs from plant-derived components could be isolated and formulated for human ailments and cures.

Author Contribution statement

Conceptulization and design of study was done by Shishir Tandon and Viveka Nand. Literature search was performed by Shishir Tandon and Garima Tamta. The first draft of the manuscript was prepared by Garima Tamta and Nisha Mehra. *In silico* study was done by Nisha Mehra. Statistical analysis was performed by Manish Pant. Viveka Nand and Vinita Gouri gave suggestion to finalize the manuscript. All authors read and approved the final manuscript.

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Competing interests

The authors declare that there is no competing interests.

Conflict of interest

The authors declare that there is no conflict of interest.

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