PLANT SCIENCE TODAY
Vol x(x): xx–xx
https://doi.org/10.14719/pst.9430

eISSN 2348-1900

RESEARCH ARTICLE

Chemical composition, antibacterial and antioxidant potential of *Aristolochia indica* L. fruit extract

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Received: 14 May 2025; Accepted: 06 October 2025; Available online: Version 1.0: 06 November 2025

Cite this article: Rajneesh K, Pooja S. Chemical composition, antibacterial and antioxidant potential of *Aristolochia indica* L. fruit extract . Plant Science Today (Early Access). https:/doi.org/10.14719/pst.9430

Abstract

Aristolochia indica L. belongs to family Aristolochiaceae is a traditional medicinal plant in Indian subcontinent used for the treatment of various diseases ailment. In the present study the antibacterial, antioxidant, chemical profiling of methanolic and hexanoic extract of *Aristolochia indica* fruit (AIF) were investigated. In- vitro antibacterial assay (Disc diffusion and microbroth dilution method) was performed against *Salmonella enterica* serovar Typhimurium (ATCC 14028), *Escherichia coli* (ATCC 25922) and *Bacillus cereus* (ATCC 11778). Methanolic extract showed the MIC value of 1700 µg/mL, 1800 µg/mL, 1000 µg/mL and hexanoic extract were 1900 µg/mL, 1500 µg/mL, 1200 µg/mL respectively against *Salmonella enterica* serovar Typhimurium (ATCC 14028), *Escherichia coli* (ATCC 25922) and *Bacillus cereus* (ATCC 11778). *In vitro* radical scavenging activity was estimated by DPPH assay; methanolic and hexane extract exhibited showed IC_{50} value of 430.5 ± 27.36 µg/mL, 559.2± 8.75 µg/mL with respect to control 3.8 ± 0.24 µg/mL(Ascorbic acid). Liquid Chromatography/Mass Spectrometry (LC/MS) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis was done for chemical components characterization. LC/MS revealed the presence of quercetin, kaempferide, linoleic acid, chlorogenic acid and other bioactive compounds. GC-MS chemical composition revealed the presence of maltol (7.42 %), pyranone (9.02 %), o-coumaric acid (4.34 %), α-monoacetin (6.05 %), mome inositol (9.29 %), tetracontane (27.49 %), campesterin (3.56 %), stigmasterol (3.64 %) and β -sitosterol (13.79 %). The phytochemical analysis based on GC-MS and LC-MS confirmed that the absence of aristolochic acid in *Aristolochia indica* fruit extracts, compound known to have nephrotoxic effect. The findings indicate that the plant's fruit could be used as a rich source of antioxidants, antibacterial agents and can be used in pharmaceutics and therapeutics industries.

Keywords: antibacterial; antioxidants; *Aristolochia indica*; GC-MS; LC-MS

Introduction

Plants are natural marvel essential for life on Earth, rich in vital components for health and medicine (1). Plants products have proven potential to treat bacterial infections, chronic diseases, food spoilage and as a preservative purpose (2). Medicinal plants and their products have provided the vitality to therapeutics for thousands of years to humanity. Traditional medicine systems, i.e., Unani, Siddha and Ayurveda are oldest prophylactic systems. This traditional system derived the therapeutic potential from plants. Approx 50% of medicament currently used in acute and chronic disease ailments are plant based natural products or their derivatives. According to World Health Organization (WHO) 80% of the population in developing countries depends on medication from plant sources (3).

The genus *Aristolochia*, part of the Aristolochiaceae family, has around 550 species. It is extensively utilized in traditional medicine for numerous therapeutic applications. *Aristolochia* species are well-documented for their pharmacological activities, including anticancer, antibacterial, antioxidant, antivenom, anti-inflammatory, anti-parasitic and anti-malarial effects (4). *Aristolochia indica* L. is a prominent plant of the Aristolochiaceae family, widely referred to as Indian Birthwort and locally known in Hindi as "Ishwarmul." It is a perennial climbing plant extensively found throughout the Indian subcontinent and utilized in ancient medical practices. *A. indica* is used in treatment of snake bites, inflammation, microbial infection, skin diseases, leprosy, cholera, ulcer and fever (5).

Bacterial foodborne diseases significantly is a major cause of human morbidity and mortality, posing serious public health challenge worldwide. Pathogenic food borne bacteria which transferred to human population through food contamination are *Bacillus cereus, Bacillus subtilis, Salmonella enterica, Escherichia coli, Listeria monocytogenes, Vibrio vulnificus* and *Shigella flexneri*. Antibiotics have been widely used for treatment; however, the emergence of antimicrobial resistance has become a critical global challenge (6).

Current study was done to investigate the antioxidant, antibacterial activity against the three foodborne bacteria *Salmonella* Typhimurium, *Escherichia coli* and *Bacillus cereus* and to investigate the phytochemical components of *Aristolochia indica* fruit extract using Liquid Chromatography/Mass Spectrometry (LC/MS) and Gas Chromatography-Mass Spectrometry (GC-MS).

Materials and Methods

Plant material collection and plant authentication

Aristolochia indica fruit samples were collected from Kushmi forest, Gorakhpur Uttar Pradesh, India (approx. location is 26° 46'25.4"N 83°26'46.5"E) during November 2022. Fruits were brought to the lab washed several times and cleaned with running tap water. Fruits were shaded dry for 10- 15 days and grinded by using grinder to make coarse powder for further analysis. Plant specimens were deposited at Botanical Survey of India (BSI) Central Regional Centre (CRC) Allahabad, Prayagraj Uttar Pradesh India for identification and confirmed with reference letter no- BSI/CRC/Tech./2023-24/267.

Preparation of extracts

Maceration process was done in different solvent to prepare extract. 5 g of fruits powder sample was mixed with 50 mL of methanol and hexane in a closed vessel, stirred with magnetic stirrer for 24 hr at room temperature for preparation of *Aristolochia indica* fruit methanolic (AIFM) and *Aristolochia indica* fruit hexane (AIFH) extract. After extraction solvent was evaporated and crude extract was stored in sterile vial at 4 ±1 °C for further use.

Bacterial strain cultures and storage

Salmonella enterica serovar Typhimurium (ATCC 14028), *E. coli* (ATCC 25922) and *Bacillus cereus* (ATCC 11778) were procured from Hi Media. Bacterial cultures were revived on Nutrient Agar Media (NAM), prepared slants maintained and stored at 4±1 °C until use.

Antibacterial assay

Kirby-Bauer disc diffusion method (7)was performed for the preliminary investigation of antibacterial potency. 20 mL of molten Muller Hinton Agar (Hi Media) was poured into 90 mm sterile Petri dish. On solid agar media 100 µL of 0.5 McFarland adjusted bacterial inoculum ($1X10^8$ CFU/mL) was inoculated for lawn culture. Sterile 6 mm Whatman filter paper no.1 discs were impregnated with 5-10 µL extract and was subjected to dry and placed on inoculated agar plates and incubated at 35±1 ^{0}C for 24 hrs. Zone of Inhibition (ZOI) was measured by antibiotic zone scale (Hi Media) in mm. Experiments were done in triplicates.

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) determination

MIC is the minimum concentration at which growth was not seen. MIC was evaluated by microbroth dilution method according to CLSI guidelines (8). For microbroth dilution desired plant extract concentrations were serially two-fold diluted in Muller Hinton broth (MHB) in 96 microtiter plate. 0.5 McFarland bacterial adjusted suspension containing 10^6- 10^8 colony forming unit per millilitre was added to each well except negative control. One positive and one negative control was also maintained during the experiment. Microtiter plates were incubated at 37±1 ^{0}C for 24 hr. INT (Iodonitrotetrazolium chloride) was used as growth indicator. MBC was determined by plating the 10 µL from microbroth dilution aliquot to Nutrient Agar Medium (NAM) plates and incubated for 37±1 ^{0}C for 24 hr. MBC was defined as the lowest concentration on which no further growth was seen. All the experiments were performed in triplicate.

Antioxidant assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was carried out to determine the antioxidant property of extract in previous studies (9) with some modifications. 0.1 mM DPPH solution was prepared in methanol and placed in the dark for 2 hr before use. Stock solutions of extract and standard were prepared. 3 mL of DPPH solution was mixed with extract to make concentration 100-500 µg/mL and incubated at room temperature for 30 min in dark. Absorbance was taken at 575 nm, Methanol+DPPH used as blank and methanol alone used as negative control. All the experiments were carried out in triplicate.

LC/MS profiling

Waters Alliance e2695/HPLC-TQD Mass spectrometer was used for LC/MS analysis for the chemical components of samples. Samples were introduced on a C-18 column (SUNFIRE C18, 250 X 2.1, 2.6µm). Acetonitrile and 0.1 % formic acid in water are used as solvents. Flow rate was 1.5 (mL/min), column temperature was 30 °C and maximum pressure was 300 bar. Spectra were recorded in negative and positive ionization mode between m/z 150 and 2000. Compounds were identified using Online Data Bank: Mass Bank, http://spectra.psc.riken.jp/menta.cgi/respect/index,

GC-MS analysis

GC-MS based chemical composition analysis was carried out using GCMS-QP2010 Ultra Mass Spectrometer (Shimadzu Corporation). Samples were separated on Rxi-5Sil MS (Restek Corporation) capillary column, length (30m ×0.25 mm i.d.×0.25 µm film thickness). The sample was injected using AOC-20i+s autoinjector. The injection port was set at 260 °C in splitless mode. Oven temperature started at 70 °C (hold time 2 min) and followed to 300 °C (hold time 17 min). The ion source and interface temperature were kept at 220° C and 280 °C. Total running time was 40 min, scan speed of 3333 and total Ion Chromatogram (TIC) were acquired in the scan range of 40 to 600 m/z. Chemical components identification were based on comparing their mass spectra search of NIST (National Institute of Standards and Technology) and Wiley library.

Statistical analysis

Data are represented as mean ± standard error and one way ANOVA was carried out on SPSS 16.0 software and IC_{50} was calculated using Graph Pad Prism-8 (GraphPad Software, San Diego, CA, USA).

Results

Antibacterial activity

AIFM extract exhibited zone of inhibition 11±0.57 mm, 10.33±0.27 mm and 12.66 ±0.72 mm against *Salmonella* typhimurium, *E. coli* and *B. cereus.* While AIFH extract demonstrated the zone of inhibition 10.33±0.33 mm, 11.33±0.27 mm, 13.33 ± 0.72 mm against *Salmonella* typhimurium, *E. coli* and *B. cereus* (Table 1). The MIC of AIFM extract were 1700 µg/mL, 1800 µg/mL, 1000 µg/mL and AIFH were 1900 µg/mL, 1500 µg/mL, 1200 µg/mL against *Salmonella* typhimurium, *Escherichia coli* and *Bacillus cereus* (Table 2). MBC of the AIFM were 1900 µg/mL, 2100 µg/mL and 1300 µg/mL and AIFH were 2150 µg/mL, 1750 µg/mL and 1400 µg/mL against *Salmonella* typhimurium, *E. coli* and *B. cereus* (Table 3).

Table 1. Zone of Inhibition (ZOI) of AI fruit extract and cefotaxime (Standard)

Bacteria	AIFM ZOI in mm	AIFH ZOI in mm	Cefotaxime ZOI in mm (30 µg)
Salmonella Typhimurium	11±0.57	10.33±0.33	31.66±0.33
Escherichia coli	10.33±0.33	11.33±0.27	33.33±0.66
Bacillus cereus	12.66±0.88	13.33±0.88	40.66±0.66

Table 2. MIC of AI fruit extract

Plant extract	*Salmonella* Typhimurium	*Escherichia coli*	*Bacillus cereus*
AIFM µg/mL	1700	1800	1000
AIFH µg/mL	1900	1500	1200

Table 3. MBC of AI fruit extract

Plant extract	*Salmonella* Typhimurium	*Escherichia coli*	*Bacillus cereus*
AIFM µg/mL	1900	2100	1300
AIFH µg/mL	2150	1750	1400

Antioxidant activity

Antioxidant activity evaluation revealed that the AIFM and AIFH extract showed IC_{50} value of 430.5 ± 27.36 µg/mL, 559.2 ± 8.75 µg/mL and 3.846 ± 0.24 µg/mL.

Chemical constituents

LC/MS analysis of AIFM and AIFH extracts revealed the presence of linoleic acid, γ- linolenic acid, kaempferide, xanthosine, chlorogenic acid, acacetin, quercetin, 1-Isothiocyanato-7-(methylsulfinyl)-heptane, canthaxanthin, scoulerine and nordihydrocapsaicin. Table 4-7 shows the list of compounds detected in LC/MS analysis. Fig. 1-4 shows the LC chromatogram. Fig. 5 shows the bioactive compounds structure detected in LC/MS analysis. GC-MS chemical composition analysis of AIFM extract showed the presence of total 47 chemical compounds. Maltol (7.42 %), pyranone (9.02 %), o-coumaric acid (4.34 %), α-monoacetin (6.05 %), mome inositol (9.29 %), palmitinic acid (4.07 %), oleic acid, methyl ester (5.17 %), oleic acid (5.75 %), are the major chemical compounds (Table 8 and Fig. 6). On the other hand, GC-MS analysis of AIFH confirmed total 40 chemical compounds. Behenic alcohol (3.53 %), tetracontane (27.49 %), campesterin (3.56 %), stigmasterol (3.64 %) and β -sitosterol (13.79 %) are major chemical constituents (Table 9 and Fig. 7).

Table 4. LC/MS of AIFM and list of compounds in positive mode

R. Time	Score	Suspected Compounds	Ion	Formula	Exact Mass	Observed Mass	Mass Diff.
1.21	0.894	L-Histidine	[M+H]+	$C_6H_9N_3O_2$	155.15	156.4011	-1.2511
1.31	0.931	3-Indolylacetonitrile	Positive	$C_{10}H_8N_2$	156.068	156.2901	-0.2221
2.27	0.936	Sinapoyl malate	Positive	$C_{15}H_{16}O_9$	340.079	342.6259	-2.5469
2.71	0.906	Sinapoyl malate	Positive	$C_{15}H_{16}O_9$	340.079	342.6999	-2.6209
3.12	0.956	Sinapoyl malate	Positive	$C_{15}H_{16}O_9$	340.079	342.6259	-2.5469
7.49	0.946	3,4-Dihydroxy-L-phenylalanine	Positive	$C_9H_{11}NO_4$	197.068	197.5090	-0.441
8.07	0.738	Adenosine-3',5'-cyclicmonophosphate	[M+H]+	$C_{10}H_{12}N_5O_6P$	329.21	329.5646	-0.3546
8.14	0.738	Adenosine-3',5'-cyclicmonophosphate	Positive	$C_{10}H_{12}N_5O_6P$	329.052	329.5646	-0.5126
8.21	0.705	Adenosine-3',5'-cyclicmonophosphate	Positive	$C_{10}H_{12}N_5O_6P$	329.052	329.5276	-0.4756
8.28	0.699	Adenosine-3',5'-cyclicmonophosphate	Positive	$C_{10}H_{12}N_5O_6P$	329.052	329.5646	-0.5126
8.38	0.784	Adenosine-3',5'-cyclicmonophosphate	Positive	$C_{10}H_{12}N_5O_6P$	329.052	329.5276	-0.4756
8.65	0.689	Adenosine-3',5'-cyclicmonophosphate	Positive	$C_{10}H_{12}N_5O_6P$	329.052	329.5646	-0.5126
16.47	0.625	1-Isothiocyanato-7-(methylsulfinyl)-heptane	Positive	$C_9H_{17}NOS_2$	219.075	219.5614	-0.4864
16.61	0.887	trans-Zeatin-riboside	Positive	$C_{15}H_{21}N_5O_5$	351.154	353.5781	-2.4241
16.84	0.645	5-Aminoimidazole-4 carboxamide-1-beta-D-ribofuranosyl 5'-monophosphate	Positive	$C_9H_{15}N_4O_8P$	338.062	337.6308	0.4312
17.50	0.862	Linoleic acid	Positive	$C_{18}H_{32}O_2$	280.24	279.6876	0.5524
19.51	0.922	N-Stearoyl-D-erythro-Sphingosine	Positive	$C_{36}H_{71}NO_3$	565.543	566.9243	-1.3813
23.43	0.61	Adenosine-3',5'-cyclicmonophosphate	[M+H]+	$C_{10}H_{12}N_5O_6P$	329.21	329.6016	-0.3916
26.54	0.71	Safranine	Positive	$C_{20}H_{19}N_4$	315.16	313.7283	1.4317
26.88	0.926	S-Lactoylglutathione	Positive	$C_{13}H_{21}N_3O_8S$	379.104	379.7376	-0.6336
32.20	0.886	Zeatin-9-glucoside	Positive	$C_{16}H_{23}N_5O_6$	381.164	381.7726	-0.6086

Table 5. LC/MS list of compounds of AIFM in negative mode

R. Time	Score	Suspected Compounds	Ion	Formula	Exact Mass	Observed Mass	Mass Diff.
25.91	0.966	Acacetin	Negative	$C_{16}H_{12}O_5$	284.068	281.7596	2.308356
17.61	0.968	Kaempferide	Negative	$C_{16}H_{12}O_6$	300.063	295.8199	4.2431
20.38	0.922	gamma-Linolenic acid	[M-H]-	$C_{18}H_{30}O_2$	278.43	277.6895	0.7405
22.66	0.973	gamma-Linolenic acid	[M-H]-	$C_{18}H_{30}O_2$	278.43	279.7616	-1.3316
25.33	0.97	2'-Deoxyinosine	Negative	$C_{10}H_{12}N_4O_4$	252.085	255.7111	-3.6261
26.42	0.961	Acacetin	Negative	$C_{16}H_{12}O_5$	284.068	281.7596	2.3084

Table 6. List of compounds of AIFH in positive mode

R. Time	Score	Suspected Compounds	Ion	Formula	Exact Mass	Observed Mass	Mass Diff.
13.94	0.936	Linoleic acid	Positive	$C_{18}H_{32}O_2$	280.24	281.5376	-1.2976
16.43	0.978	1-Isothiocyanato-7-(methylsulfinyl)-heptane	Positive	$C_9H_{17}NOS_2$	219.075	219.5244	-0.4494
16.60	0.427	Chlorogenic acid Hemihydrate	Positive	$C_{16}H_{18}O_9$	354.095	353.5781	0.5169
16.84	0.731	trans-Zeatin-riboside	Positive	$C_{15}H_{21}N_5O_5$	351.154	355.6131	-4.4591
17.18	0.466	Chlorogenic acid Hemihydrate	Positive	$C_{16}H_{18}O_9$	354.095	355.6871	-1.5921
17.35	0.444	Chlorogenic acid Hemihydrate	Positive	$C_{16}H_{18}O_9$	354.095	353.5781	0.5169
17.46	0.692	But-3-enylglucosinolate	Positive	$C_{11}H_{19}NO_9S_2$	373.05	337.5568	35.4932
17.70	0.847	alpha-D-Glucose-1,6-diphosphate potassium salt hydrate	[M+H]+	$C_6H_{14}O_{12}P_2$	340.12	337.7047	2.4153
17.94	0.758	nordihydrocapsaicin	Positive	$C_{17}H_{27}NO_3$	293.199	294.5249	-1.3259
18.14	0.594	5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranosyl 5'-monophosphate	Positive	$C_9H_{15}N_4O_8P$	338.062	337.5938	0.4682
18.38	0.643	Scoulerin	Positive	$C_{19}H_{21}NO_4$	327.147	323.6815	3.4655
19.06	0.667	3-Hydroxy-DL-kynurenine	[M+H]+	$C_{10}H_{12}N_2O_4$	224.21	224.5565	-0.3465
19.51	0.456	Canthaxanthin	Positive	$C_{40}H_{52}O_2$	564.396	566.9614	-2.5654
21.01	0.722	Quercetin	[M+H]+	$C_{15}H_{10}O_7$	302.25	301.5920	0.658
23.43	0.594	Adenosine-3',5'-cyclicmonophosphate	[M+H]+	$C1_0H_{12}N_5O_6P$	329.21	329.6386	-0.4286
23.67	0.539	Adenosine-3',5'-cyclicmonophosphate	[M+H]+	$C_{10}H_{12}N_5O_6P$	329.21	329.6386	-0.4286
26.47	0.74	trans-Zeatin-riboside	Positive	$C_{15}H_{21}N_5O_5$	351.154	353.7631	-2.6091
26.88	0.933	S-Lactoylglutathione	Positive	$C_{13}H_{21}N_3O_8S$	379.104	379.8116	-0.7076
32.17	0.956	Zeatin-9-glucoside	Positive	$C_{16}H_{23}N_5O_6$	381.164	381.7726	-0.6086

Table 7. List of compounds of AIFH in negative mode

R. Time	Score	Suspected Compounds	Ion	Formula	Exact Mass	Observed Mass	Mass Diff.
20.41	0.948	gamma-Linolenic acid	[M-H]-	$C_{18}H_{30}O_2$	278.43	277.7635	0.6665
22.66	0.965	gamma-Linolenic acid	[M-H]-	$C_{18}H_{30}O_2$	278.43	279.7616	-1.3316
25.32	0.974	2'-Deoxyinosine	Negative	$C_{10}H_{12}N_4O_4$	252.085	255.7481	-3.6631
25.39	0.969	2'-Deoxyinosine	Negative	$C_{10}H_{12}N_4O_4$	252.085	255.7111	-3.6261
25.91	0.968	Acacetin	Negative	$C_{16}H_{12}O_5$	284.068	281.7596	2.3084
26.52	0.939	gamma-Linolenic acid	[M-H]-	$C_{18}H_{30}O_2$	278.43	281.7966	-3.3666
31.78	0.961	Xanthosine	Negative	$C_{10}H_{12}N_4O_6$	284.075	283.7947	0.2803

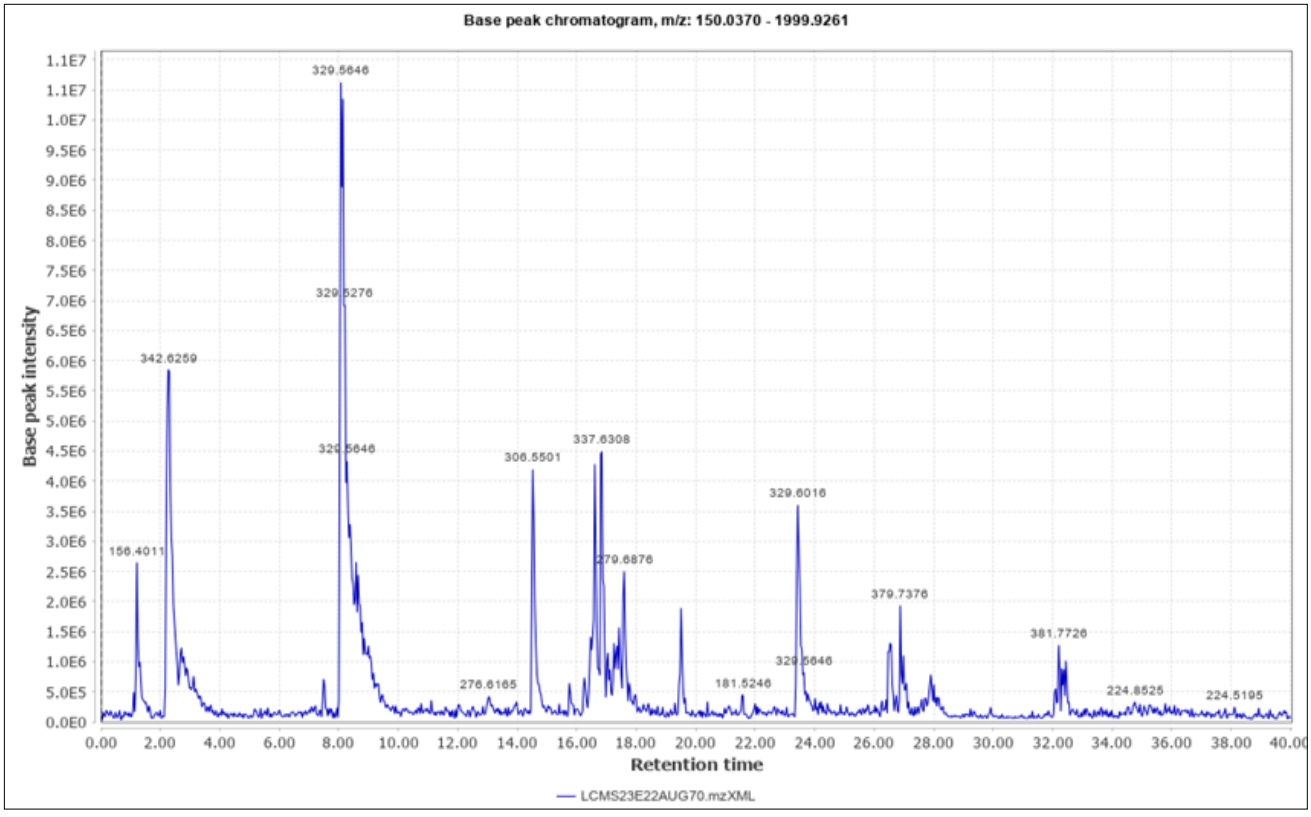

Fig. 1. AIFM (methanolic extract) total ion chromatogram (Positive mode).

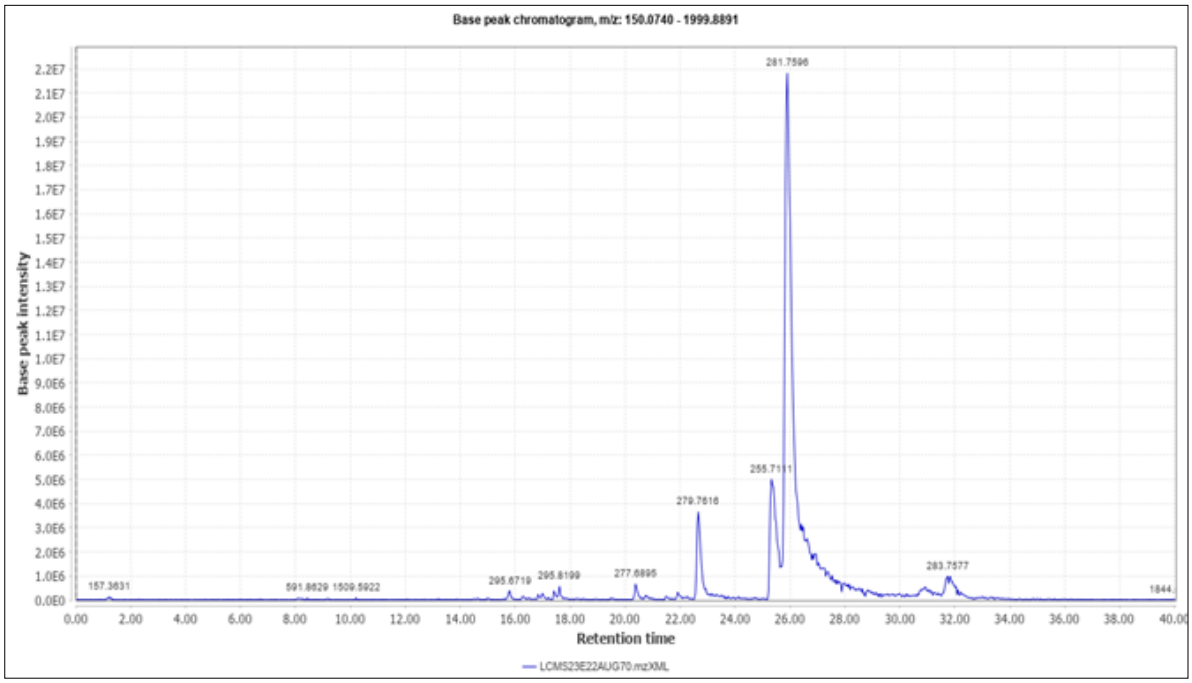

Fig. 2. AIFM extract total ion chromatogram (Negative mode).

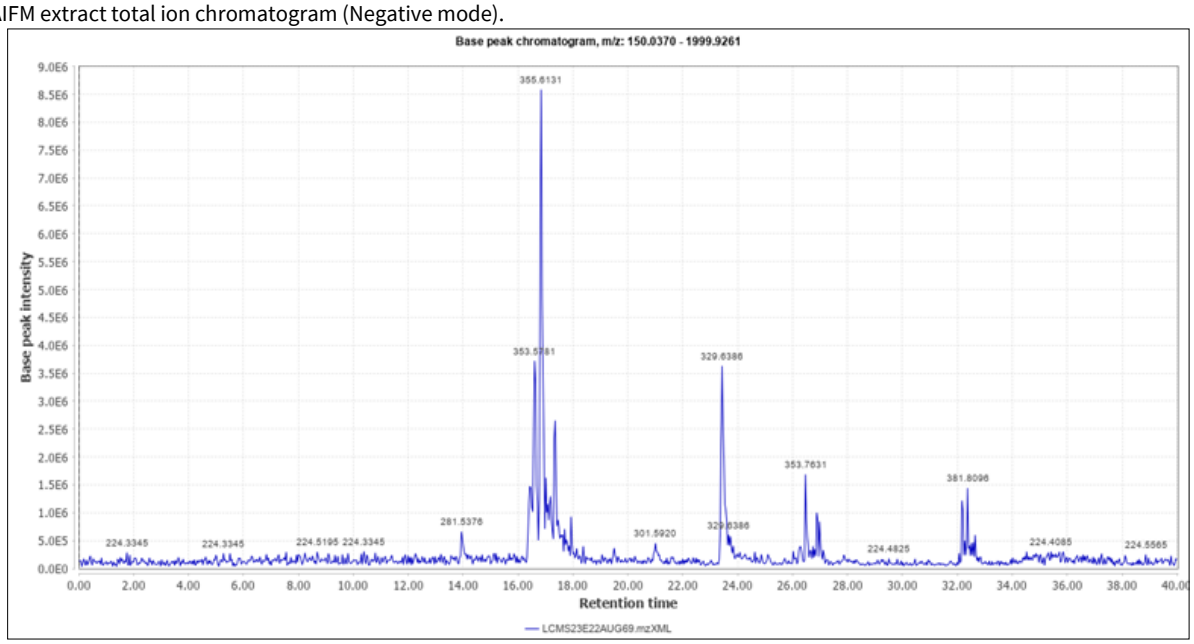

Fig. 3. AIFH extract total ion chromatogram (Positive mode).

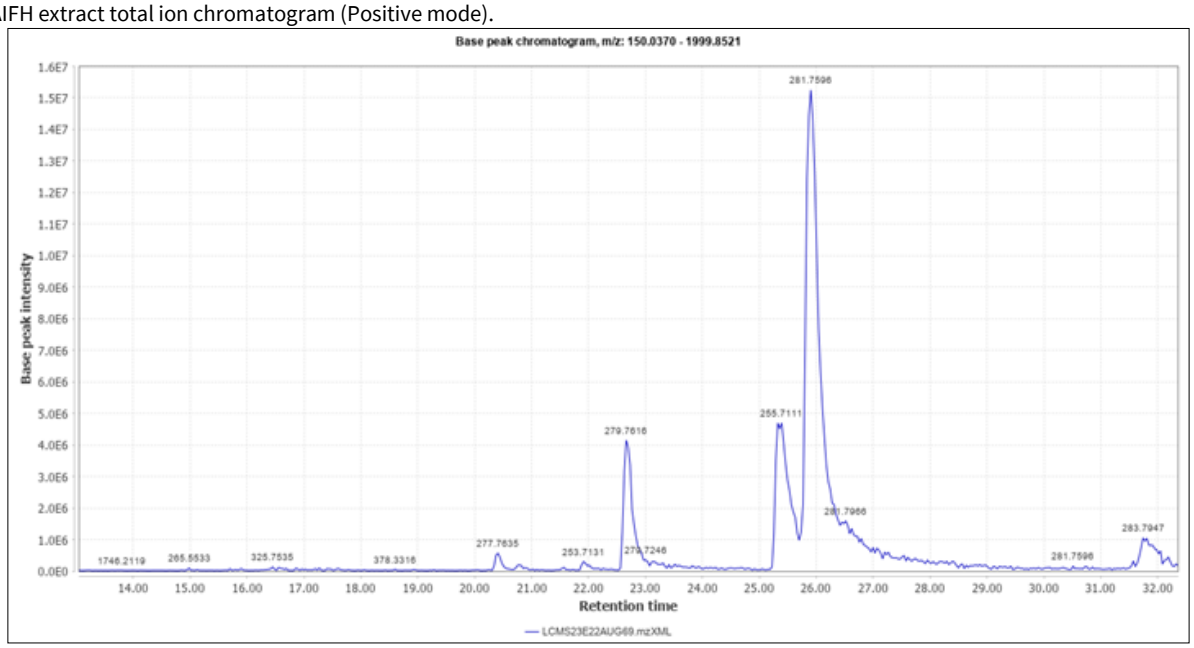

Fig. 4. AIFH total ion chromatogram (Negative mode).

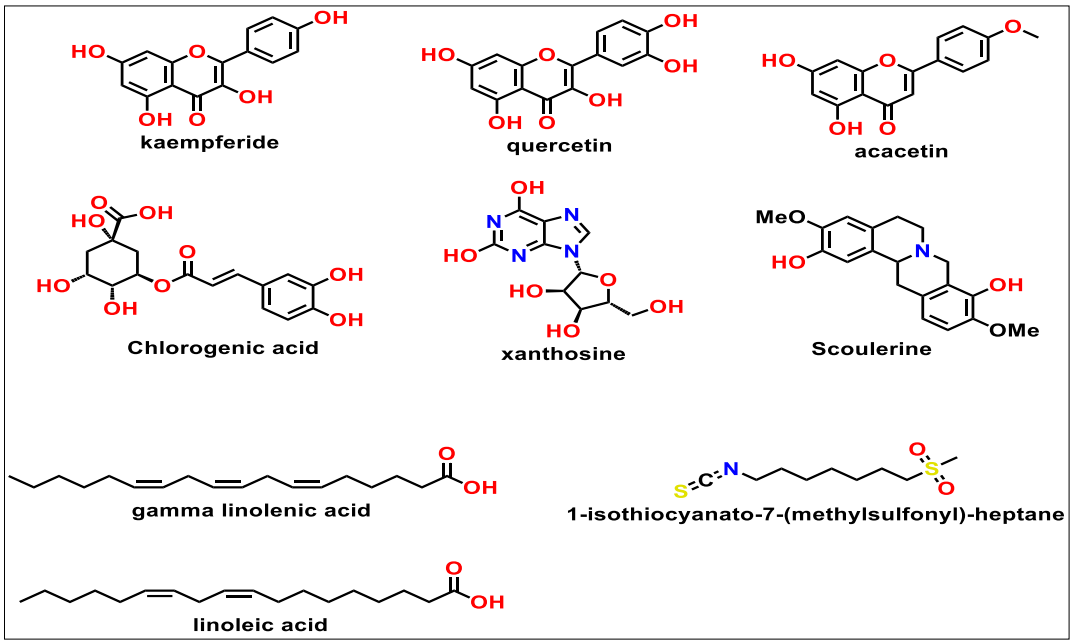

Fig. 5. Major bioactive compounds detected in AIF extract using LC/MS.

Table 8. GC-MS chemical constituents of AIFM

S.No.	R.T.	%	Compound Name
1	4.837	1.59	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
2	5.083	0.76	Pyranone 2-Hexen-4-olide
3	5.686	0.38	1,4-Dimethylpiperazine
4	5.800	0.60	Levulinic acid
5	6.344	7.42	Maltol
6	6.569	0.45	4-Methyloxazole
7	7.178	1.15	α-Acetobutyrolactone
8	7.396	9.02	Pyranone
9	8.465	4.34	o-Coumaric acid
10	8.561	1.79	5-Hydroxymethylfurfural
11	8.832	6.05	α-Monoacetin
12	9.465	3.34	6-Oxoheptanoic acid
13	9.860	0.34	2,4,4-trimethyl-3-nitroso-1,3-oxazolidine
14	10.260	0.53	Valeric acid, propyl ester
15	10.724	0.32	Oxyhydroquinone
16	11.422	1.48	Lactone G
17	11.915	2.27	Nitroisobutylglycerol
18	12.724	0.35	2,5-Pyrrolidinedione, 3-(1-aminoethylidene
19	13.606	1.93	Methyl galactoside
20	14.365	1.78	3-Deoxyhexonic acid
21	15.004	9.29	Mome inositol
22	15.326	0.30	Myristic acid
23	15.643	0.29	p-Coumaric acid
24	17.095	2.36	Methyl palmitate
25	17.442	4.07	Palmitinic acid
26	18.744	3.00	Linoleic acid, methyl ester
27	18.799	5.17	Oleic acid, methyl ester
28	18.897	3.25	(E)-Phytol
29	19.036	0.30	Stearic acid methyl ester
30	19.089	1.16	Linolic acid
31	19.156	5.75	Oleic Acid
32	19.348	0.39	Octadecanoic acid
33	20.816	1.04	Methyl arachate
34	21.907	0.18	2-(Dimethylamino)ethyl vaccenoate
35	22.325	4.02	β-Monopalmitin
36	22.455	0.15	Behenic acid methyl ester
37	22.962	0.13	1,2-Cyclohexanedicarboxylic acid, bis(2-ethylhexyl) ester
38	23.733	2.78	Glycerol oleate
39	23.904	1.96	α-Monostearin
40	24.076	1.15	Biphthalide
41	26.264	3.16	Liriodendronine
42	26.636	0.41	Hinokinin
43	26.986	0.34	Quinazolin-4(3H)-one
44	27.118	0.21	Vitamin E
45	28.285	0.49	Campesterol
46	28.577	0.38	Stigmasterol
47	29.343	2.37	β-Sitosterol
		100.00	

R.T.-Retention Time, % Percentage

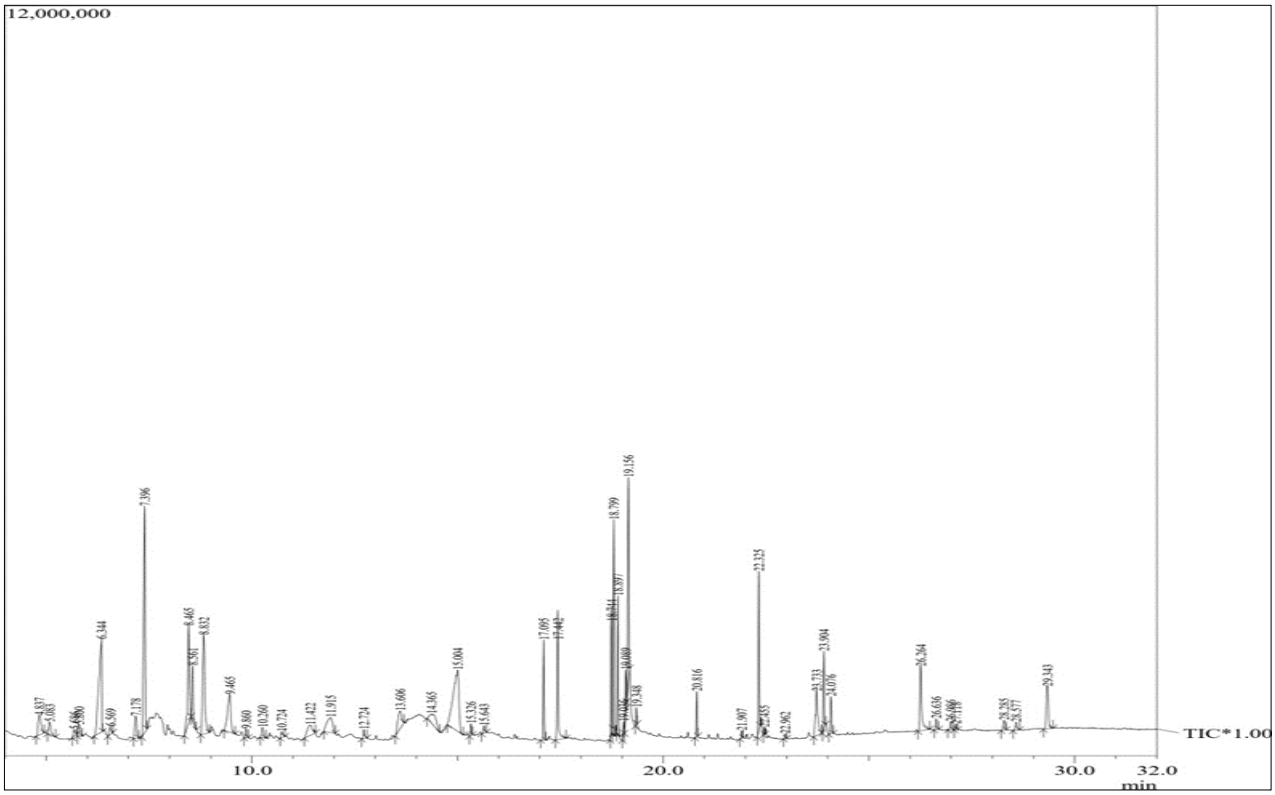

Fig. 6. AIFM GC chromatogram.

Table 9. GC-MS chemical constituents of AIFH

S.No.	R.T.	%	Compound Name
1	13.559	0.57	Cetane
2	15.812	0.28	Octadecane
3	16.451	0.38	Isobutyl phthalate
4	17.097	3.12	Palmitic acid, methyl ester
5	17.424	1.83	Phthalic acid, dibutyl ester
6	17.769	2.16	Ethyl palmitate
7	18.740	3.10	Methyl linoleate
8	18.800	4.33	Methyl oleate
9	18.899	1.66	Phytol
10	19.151	0.45	Petroselinic acid
11	19.355	1.53	Linoleic acid ethyl ester
12	19.412	2.18	Ethyl Oleate
13	19.645	0.61	Palmitic acid, ethyl ester
14	20.595	0.38	2-Methylhexacosane
15	20.817	2.63	Arachidic acid methyl ester
16	21.368	1.24	Arachidic acid, ethyl ester
17	22.238	0.54	Tetracontane
18	22.326	3.34	β-Monopalmitin
19	22.457	2.14	Methyl stearate
20	22.961	0.43	Ethyl palmitate
21	23.231	0.18	Tricosanoic acid, methyl ester
22	23.738	3.53	Behenic alcohol
23	23.906	1.69	α-Monostearin
24	23.975	0.55	Methyl lignocerate
25	24.080	0.21	2-Ethylhexyl 4-nitrobenzoate
26	24.599	0.44	Squalene
27	25.192	1.07	Tetratriacontyl heptafluorobutyrate
28	26.164	0.21	Phenol, 2,4,6-tris(1-phenylethyl)-
29	26.248	0.37	Liriodendromine
30	26.634	0.85	Hinokinin
31	26.790	27.49	Tetracontane
32	27.174	0.33	p-Dihydroartemisinin Oxy Methyl Benzoic Acid
33	27.754	0.34	Dotriacontane
34	28.288	3.56	Campesterin
35	28.586	3.64	Stigmasterol
36	28.909	0.77	Hexatriacontane
37	29.024	0.79	Myricyl alcohol
38	29.359	13.79	β-Sitosterol
39	31.081	4.35	Nonacosanal
40	32.169	2.96	Heptacosyl heptafluorobutyrate
		100.00	

R.T.-Retention Time, % Percentage

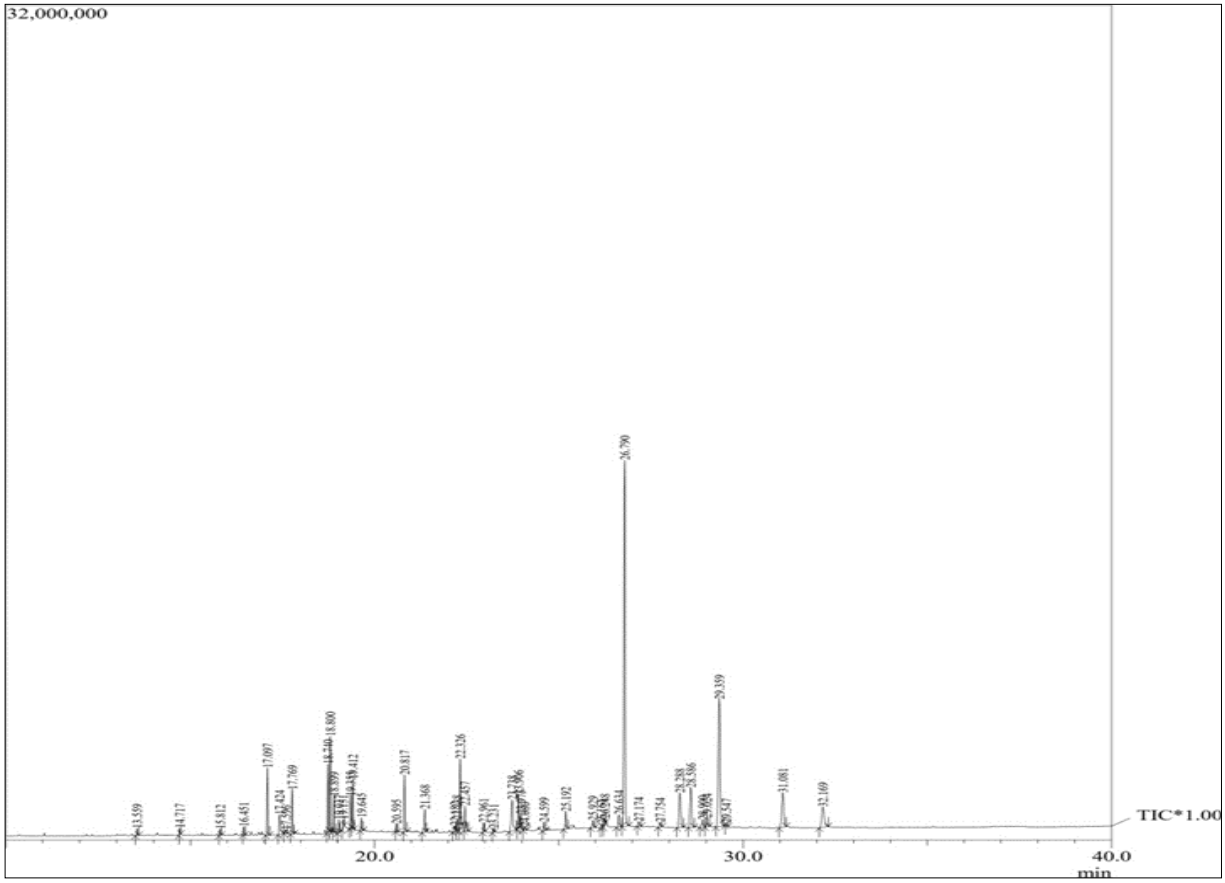

Fig. 7. AIFH GC chromatogram.

Discussion

From decades researchers have emphasized the search of plants which have an arsenal of eco-friendly antibacterial constituents. Plant's secondary metabolites such as phenolics, flavonoids, alkaloids, terpenoids received much attention for their bioactivity potential. Secondary metabolites produced by the plants have diverse bioactivity and therapeutics potential such as antimicrobial, anticancer, anti-inflammatory, anti-diabetic, analgesic, sedative, antimalarial and antioxidant (10).

In the present study *Aristolochia indica* fruit (AIF) extract was evaluated to access antibacterial, antioxidant activity and phytochemical profiling. AIFM and AIFH extract demonstrated promising antibacterial activity against three food borne pathogenic bacterium *Salmonella typhimurium, E. coli* and *B. cereus*. AIFM and AIFH showed MIC value below 2000 µg/mL. In previous studies (11) the plant extract antimicrobial cut off point stated that MIC values below 100 µg/mL is Highly active, 100 ≤ MIC ≤ 512 µg/mL is significantly active, 512 < MIC ≤ 2048 µg/mL is moderately active and if MIC is above 2048 µg/mL it is not active. According to the above antimicrobial cut-off points it is clearly indicating that AIF extracts are moderately active against the *Salmonella* typhimurium, *E. coli* and *B. cereus*. It is already established that the genus *Aristolochia* has tremendous antibacterial activity against the diverse pathogenic bacteria. *Aristolochia bracteolata* root extract showed antibacterial activity against the *Pseudomonas aeruginosa, E. coli, B. cereus* and *Staphylococcus aureus*. Ethyl acetate extract fraction was highly active with MIC value of 175-400 ppm (12). Former researchers (13) reported that *Aristolochia brevipes* root extract showed antimycobacterial potential against *Mycobacterium tuberculosis* H37Rv having MIC value 12.5 µg/mL. Previous researchers investigated that ethanolic extract of *A. indica* have antibacterial potential against multidrug-resistant bacteria *E. coli*, *Vibrio cholerae*, *P. aeruginosa* and *Klebsiella pneumoniae* in the range of MIC value 50-100 µg/mL (14). *Aristolochia tagala* Cham. hydro-alcoholic and methanolic extract demonstrated antibacterial activity against *P. aeruginosa, S. typhi* and *B. subtilis* (15).

Free radicals are formed during normal cell metabolism and generate oxidative stress. These free radicals are hydroxyl radicals, superoxide anions and dioxygen (singlet). Excessive engendering of ROS causes genotoxicity, mutation, cancer, inflammation, skin diseases, cell degeneration and damage. Plant extract has diverse groups of phytochemicals exhibiting radical-scavenging potential. They have curative and therapeutical ability (16, 17). In our investigations AIFM and AIFH extract showed IC_{50} value of 430.5 ± 27.36 µg/mL, 559.2± 8.75 µg/mL and ascorbic acid (Control) had IC_{50} value of 3.846 ± 0.24 µg/mL. *A. indica* fruit extract demonstrated dose dependent radical scavenging activity. This investigation revealed that it has radical scavenging capacity (Fig. 8 -10). DPPH and ABTS assay investigation of aqueous fraction of *Aristolochia longa* roots extract which discloses antioxidant activity with 25.40±2.40 µg/mL (IC_{50}) and 65.23±2.49 µg/mL (IC_{50}) (18). Another study examined that *Aristolochia baetica* and *Aristolochia paucinervis* showed antioxidant activity with IC_{50} value of 150 ± 8 µg/mL and 160 ± 10 µg/mL evaluated by DPPH assay (19).

Synthetic chemicals and products are confined to direct incorporation in food and industries due to harmful mammalian toxicity and environmental hazards. Phenolic, polyphenols, carotenoids, vitamin C and E are major antioxidant compounds from plants. Moreover, natural antioxidant products from plants are cheap, feasible and central attention for its bioactive components in food, cosmetics, medicine and pharma industries. Natural products also have less mammalian toxicity and environmental pollution (20).

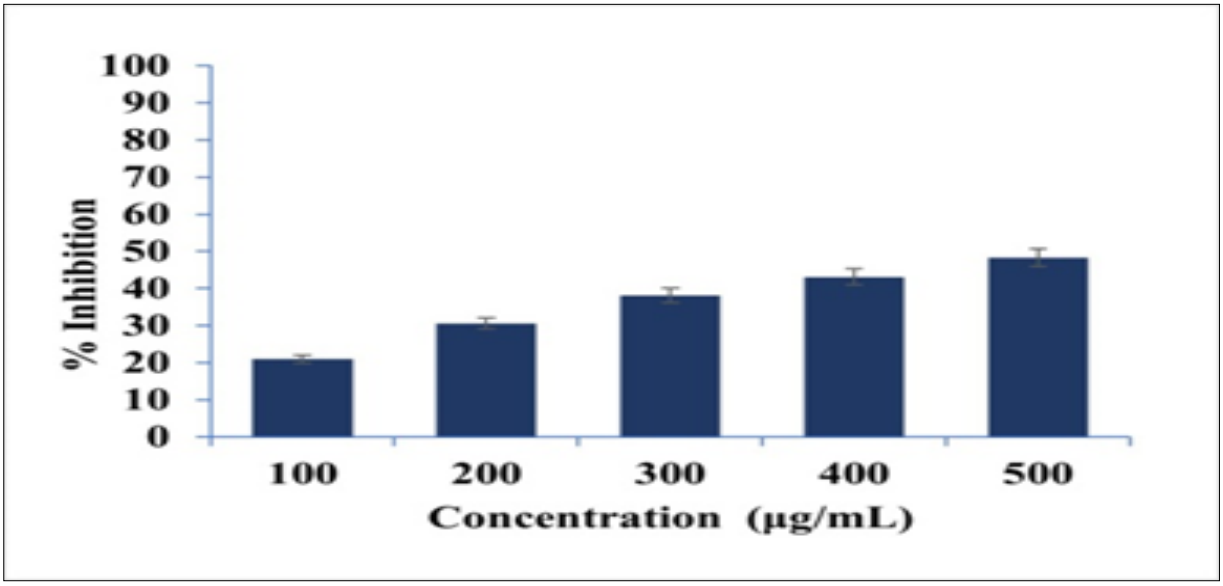

Fig. 8. DPPH analysis (AIFH).

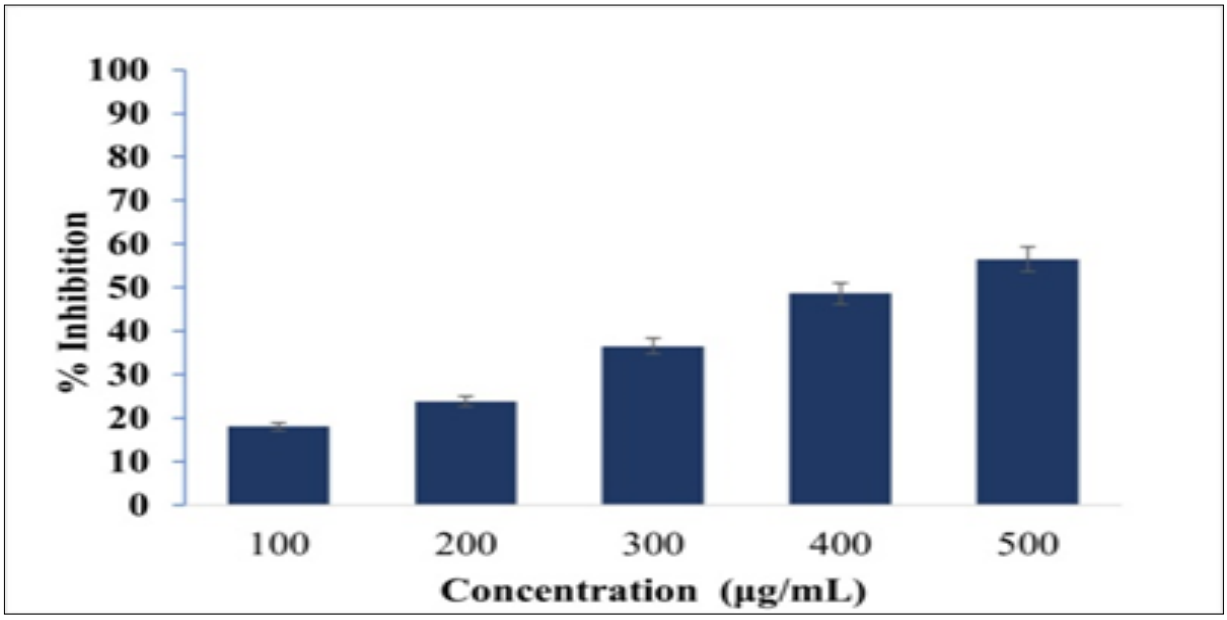

Fig. 9. DPPH analysis (AIFM).

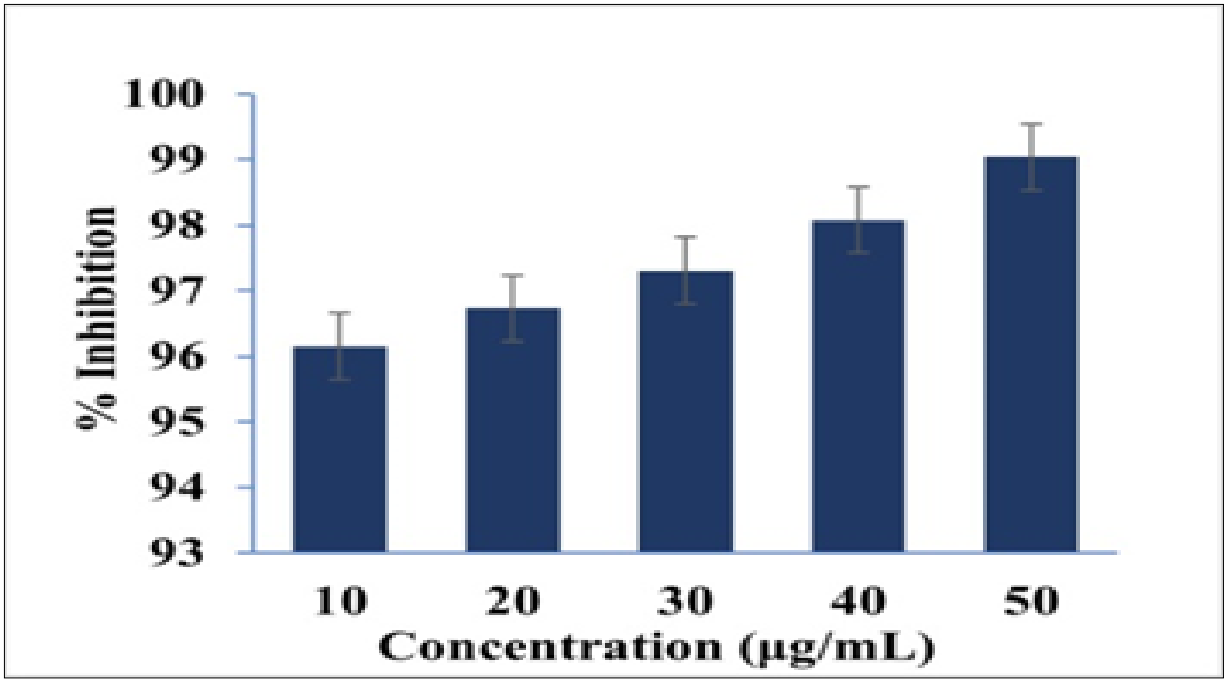

Fig. 10. DPPH radical scavenging activity of ascorbic acid.

In the current study methanolic and hexanoic extracts of *Aristolochia indica* fruits (Fig. 11) were investigated. γ-linolenic acid, acacetin, xanthosine, quercetin, chlorogenic acid hemihydrate, 1-Isothiocyanato-7-(methylsulfinyl)-heptane, scoulerine and kaempferide are bioactive components present in the fruit extract.

Genus *Aristolochia* has been reported to have important medicinal plant species and distributed to different parts of the world. They have a diverse range of phytochemicals mainly consisting of phenanthrene and isoprenoid derivatives, phenolics, polyphenols, flavonoids, alkaloids, steroids, tannins and many more (21). Aqueous methanolic extract of *A. tagala* using LC-MS/MS analysis revealed the presence of twenty-one compounds. kaempferol, aristolochic acid I, aristolactam IIIa, stigmasterol and β-sitosterol were the major compounds (22). Previous researchers investigated and identified quercetine-3-glycoside, quercetin, rutin, apigenin, apigenin 7-O glycoside, taxifolin, diosmin and neohesperidin by LC-MS analysis from *A. longa* leaves extract (23). These metabolites are accountable for the different biological and pharmacological effectiveness of the *Aristolochia* species.

There are various therapeutic potentials of reported compounds in LC-MS analysis already depicted and revealed by researchers. Gamma-linolenic acid supplements in diet impaired inflammatory responses to various diseased conditions (24). Former researchers extracted γ-linolenic acid from *Arthrospira platensis* and investigated that it had anti-inflammatory, anti-oxidative, anti-allergic potential in zebrafish (25). Xanthosine isolated from *Tribulus terrestris* L. exhibited antidiabetic activity by phosphorylating AMP-activated protein kinase and fork head box transcription factor O1 (26). Acacetin, a natural flavonoid is extensively present molecule in plant Kingdom. It is major bioactive constituent in the traditional Chinese medicine "Snow lotus". It has antidiabetic, anti-obesity, anticancer, anti-inflammatory and antimicrobial activities (27, 28). Acacetin impedes the *S. pneumoniae* bacterium pore-forming pneumolysin activity *in vivo* and *in vitro* were examined earlier (29). It inhibited sortase A (SrtA); a membrane associated cysteine transpeptidase of *S. aureus* at IC_{50} = 36.46 ± 4.69 µg/mLdose value (30). It also inhibits listeriolysin O (LLO) a virulence factor of *Listeria monocytogenes* (31). Kaempferide exhibited antibacterial activity against *S. aureus*, *Enterococcus faecalis*, *E. coli* and *P. aeruginosa* at MIC value of 23 µg/mL, 250 µg/mL, 125 µg/mL and 250 µg/mL (32). Quercetin (polyphenolic flavonoid) showed antibacterial activity against *S. aureus*, *P. aeruginosa*, *E. coli* and *Proteus vulgaris* had 20, 20, 300 and 400 mcg/mL MIC value (33). Quercetin exhibited antibacterial activity against *P. aeruginosa*, *S. enterica* serotype Typhimurium, *E. coli* and *S. aureus* evaluated (34). Chlorogenic acid (polyphenol) have already known antibacterial, antioxidant, antimutagenic, anti-inflammatory, antiviral, anti-cardiovascular, anticancer immunomodulatory and reduces neuropathic pain (35, 36). Scoulerine (isoquinoline alkaloid) had cytotoxic and antiproliferative activity against leukemic cells with IC_{50} of 2.7 to 6.5 µM by reducing mitochondrial dehydrogenases (37). 1-isothiocyanato-7-(methylsulfonyl) has anti-inflammatory potential was already reported (38). The presence of bioactive phytochemicals having a significant pharmacological literature can be co-related to the antibacterial and antioxidant potential of *Aristolochia indica* fruit extracts. These phytochemicals are responsible for bioactivity.

GC-MS chemical composition analysis of AIFM extract showed the presence of total forty-seven chemical compounds. Maltol (7.42 %), pyranone (9.02 %), o-coumaric acid (4.34 %), α-monoacetin (6.05 %), mome inositol (9.29 %), palmitinic acid (4.07 %), oleic acid, methyl ester (5.17 %), (e)-phytol (3.25 %), oleic acid (5.75 %), β-monopalmitin (4.02 %) and liriodendronine (3.16 %) are the major chemical compounds. On the other hand, GC-MS analysis of AIFH confirmed total forty chemical compounds. Palmitic acid, methyl ester (3.12 %), methyl linoleate (3.10 %), methyl oleate (4.33 %), behenic alcohol (3.04 %), tetracontane (27.49 %), campesterin (3.56 %), stigmasterol (3.64 %) and β-sitosterol (13.79 %) as major chemical composition. The presence of oxalic acid, 2-methylcyclohexyl ester,2-propenoic acid, 2-Fluoro-6-trifluoromethylbenzoic acid, phosphoric acid, butylphosphonic acid, cyclobutyl ethyl ester, octadecanoic acid and many other compounds in GC-MS analysis of aqueous extract *A. indica* was already reported (39).

Limited research has been conducted on the phytochemical properties of *A. indica* fruits. The chemical content of a plant extract is influenced by the plant sample material (parts), harvesting time, chemotype, habitat, geographical location, ambient conditions, soil type and extraction procedure (40).

Plants of family Aristolochiaceae generally contain aristolochic acids. Its exposure causes nephrotoxicity and hepatobiliary cancers due to genotoxicity (41, 42). The presence of aristolochic acids in herbal products can have negative impact and

Fig. 11. Image of *Aristolochia indica* fruit.

tend to cause aristolochic acids linked to cancers. Therefore, traditional uses of *A. indica* possess a public health threat (42, 43). But the chemical analysis using LC-MS and GC-MS of *A. indica* fruit extract in our investigation showed the absence of aristolochic acids. Therefore, the fruits of *A. indica* have arsenal of molecules and its crude drugs can further be explored for therapeutics uses. From the decades of isolation, identification and prompt therapeutics potential of biologically active molecules from plants has led to the discovery of new therapeutics with wide-ranging potential applications. Research efforts can be focused on exploring *A. indica* fruit molecules for novel biologically active compounds that could lead to new novel therapeutics molecules.

Conclusion

This study was done to evaluate the antibacterial and antioxidant potential of *A. indica* fruit extracts. *A. indica* fruit extract has promising antibacterial and antioxidant potential. LC-MS and GC-MS analysis confirmed that it has an arsenal of bioactive phytochemicals and can be harnessed for therapeutics against various disease ailments. Also, it can be safer due to the absence of aristolochic acids in the fruits. Various formulations and further studies are needed on *A. indica* fruits for isolation, purification, exploration and bioprospection of bioactive compounds and their activity on *in vivo* animal model for future use in pharma, food and drug developments industries.

Acknowledgements

We acknowledge Indian Council of Medical Research (ICMR), India for their financial support (ICMR JRF/ SRF Fellowship). Authors are thankful to Botanical Survey of India (BSI) Allahabad, Prayagraj Uttar Pradesh India for plant identification and authentication. Thanks to the Head of Department, Botany DDUGU for required lab facilities.

Authors' contributions

RK worked on, experimental, methodology, analysis and original draft writing. PS supervised, conceptualized, revised and edited the final draft. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: We declare there is no conflict of interest between the Authors.

Ethical issues: None

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