



RESEARCH ARTICLE

Morphometric and phytochemical analysis of *Ximenia americana* L. fruits harvested from Amrabad Tiger Reserve Forest of India

Jadala Shankaraswamy* & Vankadavath Nagaraju

Department of Fruit Science, College of Horticulture, Mojerla, Sri Konda Laxman Telangana Horticultural University, Wanaparthy 509 382, Telangana, India

*Correspondence email - shankara.swamy@gmail.com

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Abstract

The present investigation involved phytochemical screening of wild plum to evaluate its phytoconstituents. The fruits exhibited a total phenolic content of 1.24 mg/100 g (as gallic acid equivalent) and a total flavonoid content of 79.27 mg/100 g (as quercetin equivalent). The average fruit weight was 7.12 g, ranging from 6.55 to 7.83 g. The weights of the peel, pulp, pit and seed were 3.51 g, 1.31 g, 0.30 g and 0.92 g respectively. The average fruit length was 23.26 mm, while pulp thickness and peel thickness measured 0.21 mm and 2.40 mm respectively. Pit thickness was recorded at 0.51 mm. The equatorial, polar, apical and basal diameters of fruits were 20.72 mm, 21.52 mm, 16.83 mm and 17.92 mm respectively. The pulp color parameters included lightness (L^*) value of 44.40, a^* value of 22.93 and b^* value of 36.91. The leaf area was calculated to be 8.99 cm², with a length of 3.28 cm and a width of 3.36 cm, resulting in a leaf length-to-width ratio of 0.97. Fruit extract concentration of 10, 30 and 50 µg showed free radical scavenging activities of 2.82 %, 4.08 % and 5.38 %. About 38 secondary metabolites were identified in methanolic extract of fruits, including phenols, alkaloids, glycosides, flavonoids, lignans, terpenoids, naphthoquinones, saponins, hydrolyzable tannins and steroids. Hepato-protective and antiviral properties, as well as neuro-protective properties, were demonstrated by the methyl ester 3,4-di-*o*-caffeoylquinic acid, iridoid glycosides like 6-*o*-trans-feruloylgenipin gentiobioside, 10-(6-*o*-trans sinapoylglucopyranosyl) gardendiol, as well as a neo chlorogenic acid 3-caffeoyl quinic acid, respectively. Understanding these bioactive benefits highlights the potential for commercial cultivation of wild plum, moving beyond traditional wild collection to meet the requirements of the country.

Keywords: bioactive compounds; phytochemical screening; secondary metabolites; wild plum

Introduction

Ximenia americana L. (Oleaceae) is known as tallow nut, sour plum and wild plum. It has both medicinal and economic uses. It is a small tree or semi-scandent shrub that usually grows to a height of 2-7 m (1). It is considered as a wild edible plant with diverse practical applications, including its use as food, a source of essential oils, medicine and in various industrial products (2). Its fruits and leaves have been extensively used in folk medicine since historical period (3). In particular, the leaves and twigs have been used in folk remedies to treat fever and cold (4-6). It is used traditionally in the treatment of a range of ailments, including digestive system infections, injuries and sexually transmitted infections (7). It is also known for its use in the preparation of poison antidotes, eye lotions and laxatives. Furthermore, the roots have been reported for their efficacy in treatment of puffiness, headaches, haemorrhoids, leprosy, sleeping sickness and guinea worm attack (8).

A study that screened 67 crude ethanol extracts from fifty plants (belonging to 31 families) traditionally used in North Cote-d'Ivoire for bacterial infections, included *X. americana*,

which demonstrated *in vitro* activity against gram positive (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Streptococcus pyogenes*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria (9). The extract exhibited significant antibacterial properties, highlighting its potential in clinical settings for combating bacterial infections. Additionally, *X. americana* is believed to contain a variety of bioactive substances with antiviral, antifungal, anticancer, antiparasitic, antiallergic and antibacterial effects (1) and it has also shown promising antioxidant activity (10). Investigating the composition of these bioactive agents is therefore of considerable interest.

Regarding its utilization and cultivation, indigenous communities in the Amrabad Tiger Reserve Forest, India, possess extensive traditional knowledge concerning the use and management of *X. americana*. However, overharvesting and the conversion of marginal lands for agriculture currently pose significant threats to the species, contributing to habitat degradation and potential extinction. This wild plum is distributed throughout the Amrabad Tiger Reserve and exhibits wide genetic variability and a rich nutritional composition,

which are essential for understanding the available germplasm for conservation and sustainable utilisation of the species. Accordingly, this study aimed to evaluate the physical parameters total phenolic content, phytochemical profile, flavonoid content, antioxidant activity and metabolite composition of fruits and seeds of wild plums collected from the Amrabad Tiger Reserve Forest in India.

Materials and Methods

The present research on wild plum (*X. americana*) was conducted at the College of Horticulture, Mojerla, Sri Konda Laxman Telangana Horticultural University, Wanaparthy, Telangana, India, during 2024-25. Fruits and seeds were collected from the region of the Amrabad Tiger Reserve Forest of India.

Physical characteristics of fruits

The physical characteristics of *X. americana* fruits such as seed pit, pulp, peel, seed, weight of fruit, length of fruit, pulp, peel and skin thickness were measured using an electronic weighing balance and Vernier calliper. Using a hunter lab colour meter (Chroma Meter CR-410, Konica Minolta), fruit chromacity was evaluated (11).

Extract preparation

The dried plant material (10 g) was placed in a beaker, followed by the addition of 75 mL of 95 % ethanol. The mixture was kept at 40 °C for ten min. This extraction process was performed three times. The solvent was then evaporated using a rotary evaporator and the resulting dried extract was used for subsequent analysis (12).

Determination of total phenolic content

50 mg of extract were dissolved in 5 mL of methanol. After sonication at 40 °C for 45 min, the solution mixture was centrifuged for 10 min at 1000 ×g. Then, the supernatant was collected and 0.2 mL of this sample was added with 0.6 mL of distilled water and 0.2 mL of Folin-Ciocalteus' phenol reagent (1:1). The mixture was incubated at ambient temperature for five min. Subsequently, 1 mL of 8 % (w/v) sodium carbonate solution was added, followed by addition of distilled water to make volume up to 3 mL. Again, the mixture was incubated at ambient condition in dark for 30 min. After centrifugation, absorbance was measured using a UV-Vis spectrophotometry at 765 nm. The total phenolic content was calculated utilizing a standard gallic acid curve and expressed as gallic acid equivalents (GAE) /g of dry plant material (13).

Determination of total flavonoids content

50 mg of extract were dissolved in 5 mL of methanol. After sonication at 40 °C for 45 min, the solution was centrifuged for 10 min at 1000 ×g and the supernatant were collected. A stock solution of quercetin was prepared by dissolving 5.0 mg of quercetin in 1 mL of methanol. To 0.6 mL of either the diluted extract or quercetin standard solution, 0.6 mL of 2 % (w/v) aluminium chloride solution was added. The mixture was incubated at ambient temperature for 60 min and absorbance was measured at 420 nm using a UV-Vis spectrophotometry (13).

Determination of the antioxidant activity

The ferric reducing antioxidant power (FRAP) assay was

performed (14). For the 1,1-diphenyl-2-picrylhydrazil (DPPH) assay, 50 mL of sample were mixed with 2 mL of a 0.06 mmol/L methanolic DPPH solution. The mixture was incubated at ambient temperature in dark 60 min. Ascorbic acid was used as a reference standard. UV-Vis spectrophotometry was used to measure the absorbance at 517 nm.

Secondary metabolite screening by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

For LC-MS/MS analysis, the sample was diluted tenfold in methanol and filtered through a 0.22 µm membrane filter. A 10 µL volume of the filtered sample was injected. Chromatographic separation was carried out at 25.0 ± 0.1 °C using a Shimadzu UHPLC system (Kyoto, Japan) fitted with a Shim-pack XR-ODS column (75 x 3.0 mm, 2.2 µm). The mobile phase consisted of acetonitrile and 0.1 % formic acid in water, filtered through cellulose nitrate membrane (47 mm diameter, 0.45 µm pore size; Sartorius, Goettingen, Germany). After gradient separation, the column was equilibrated to the original solvent composition for five min.

The flow rate was set at 0.5 mL/min. An LC-MS/MS System (LCMS 8040; Triple Quadrupole Shimadzu Corporation, Kyoto, Japan) was used. Ionization was performed via electrospray ionization (Positive / Negative). The ion spray voltage was set at +4.5Kv and -3.5 kV. The CDL temperature was set at 250 °C, block temperature at 400 °C and detector voltage at 1.3 kV. Nebulizer gas flow was maintained at 2 L/min and drying gas flow at 15 L/min (15).

Gas Chromatography - Mass Spectrometry (GC-MS) analysis

Phytochemicals analysis of *X. americana* fruit was performed using GC-MS (16). Column of Rtx-5MS (5 % diphenyl/95 % dimethyl poly siloxane) (30 m in length × 0.25 mm in diameter × 0.25 µm in thickness of film) was used. Analysis was performed by using Scion 436-GC Bruker. Flow rate of carrier gas was set as 1 mL per min. A TQ quadrupole mass spectrometer was used as a detector. The volume of a sample injected was set as 1 µL. The following was the programming for the oven temperature: Hold at 110 °C for 3.50 min, then raise to 200 °C without holding at rate of 10° C per min. Temperature was then raised to 280 °C at a rate of 5 °C per min. After that, sample was kept for 12 min. A temperature of 280 °C was established for the injector. The total running time of GC was 40-50 min. NIST Version-2011 served as the library for the MS software. A temperature of 290 °C was selected for the inlet line. 250 °C was selected as the source temperature. 70 eV was selected as the electron energy. At 50-500 amu, the mass scan (m/z) was carried out. A solvent delay of 0-3.5 min was used.

Results and Discussions

Compositional analysis

The physical parameters of fruits, seeds and leaves of *X. americana* collected from the Amrabad Tiger Reserve Forest region in India are shown in Table 1-3. The colour parameters of fruit peel and pulp are depicted in Table 4. The fruits exhibited a total phenolic content of 1.24 mg /100 g (expressed as gallic acid equivalent) and a total flavonoid content of 79.27 mg /100 g (expressed as quercetin equivalent). These values slightly differ from those reported for *X. americana* fruits

Table 3. Physical parameters of seeds of *X. americana* L. harvested from the region of the Amrabad Tiger Reserve Forest in India.

	Seed parameters					
	Weight (g)	E.D. (mm)	P.D. (mm)	B.D. (mm)	A.D. (mm)	Seed length (mm)
Minimum	0.8580	8.1800	12.0200	8.0200	7.3200	13.2600
Maximum	0.9840	9.7700	13.5600	8.9600	8.9300	14.8900
Range	0.1260	1.5900	1.5400	0.9400	1.6100	1.6300
Median	0.9295	9.2600	12.9400	8.4400	7.8600	14.0600
Mean	0.9200	9.1780	12.7300	8.4610	7.8900	14.0660
Variance (n-1)	0.0019	0.1983	0.2837	0.1263	0.1822	0.2836
Standard deviation (n-1)	0.0439	0.4453	0.5326	0.3554	0.4268	0.5326
Variation coefficient	0.0453	0.0460	0.0397	0.0398	0.0513	0.0359
Skewness (Pearson)	-0.0787	-0.9738	-0.2009	0.2008	1.3339	-0.0293
Kurtosis (Pearson)	-1.3981	0.6085	-1.1921	-1.3726	1.8229	-1.0740
Standard error of the mean	0.0139	0.1408	0.1684	0.1124	0.1350	0.1684

E.D.: Equatorial Diameter; P.D.: Polar Diameter; B.D.: Basal Diameter; A.D.: Apical Diameter

Table 4. Colour parameters of the peel and pulp of the fruits of *X. americana* L. harvested from the region of the Amrabad Tiger Reserve Forest in India.

	Peel colour			Pulp colour		
	L*	a*	b*	L*	a*	b*
Minimum	51.0900	17.4600	22.1600	32.6800	17.8000	28.5400
Maximum	56.9400	22.2600	34.8700	53.6400	31.8200	44.3800
Range	5.8500	4.8000	12.7100	20.9600	14.0200	15.8400
Median	55.0950	20.2500	29.1850	47.3600	21.7300	38.1150
Mean	54.6170	19.9580	28.7340	44.4080	22.9330	36.9190
Variance (n-1)	3.9428	2.9668	15.9383	57.4744	23.8067	29.8054
Standard deviation (n-1)	1.9856	1.7224	3.9923	7.5812	4.8792	5.4594
Variation coefficient	0.0345	0.0819	0.1318	0.1620	0.2018	0.1403
Skewness (Pearson)	-0.5488	-0.1603	-0.3763	-0.5988	0.8977	-0.3919
Kurtosis (Pearson)	-0.9138	-1.4493	-0.5760	-1.1079	-0.4996	-1.2187
Standard error of the mean	0.6279	0.5447	1.2625	2.3974	1.5429	1.7264

L*: Represents lightness, ranging from 0 (black) to 100 (white); a*: Represents the red-green axis, with positive values indicating red and negative values indicating green; b* represents the yellow-blue axis, with positive values indicating yellow and negative values indicating blue.

harvested in other geographical location (17). Such variations may be attributed to the variations in genetic factors of different strains, level of maturity and cultivation conditions (18). This revealed that the location in which the fruit is cultivated play a key role in determining nutrient content of *X. americana* fruits.

Physical characterization

The physical characterization of *X. americana* fruits and seeds revealed significant biometric insights. The average fruit weight was 7.12 g, ranging from 6.55 to 7.83 g, with a positively skewed distribution (skewness: 0.30) and light-tailed distribution (kurtosis: 0.77), which exceeds the values reported in earlier studies (19, 20). The weights of the peel, pulp, pit and seed weight were 3.51 g, 1.31 g, 0.30 g and 0.92 g respectively. Except for pit weight, which showed slight negative skewness (-0.078), all others were positively skewed.

The average fruit length was 23.26 mm (skewness: 0.17). Pulp and peel thicknesses were 4.79 mm and 2.40 mm respectively while the skin was 0.21 mm thick. Pit thickness averaged 0.51 mm (kurtosis: 0.73). The average diameters measured were equatorial 20.72 mm, polar 21.52 mm, apical 16.83 mm and basal diameters 17.92 mm, all showing positively skewed distribution.

Pulp color analysis indicated pulp lightness (L*) at 44.40, a* (red-green axis) at 22.93 and b* (yellow-blue axis) at 36.91. Peel color measurements followed similar trends, with slight variations. These findings emphasize variability and unique physical properties of *X. americana* fruits, contributing valuable data for their characterization and potential applications.

The leaf parameters of *X. americana* revealed an average area of 8.99 cm², with a mean length of 3.28 cm and width of 3.36 cm. The average perimeter was 13.47 cm, showing a moderate range of values. The leaf length-to-width ratio of 0.97 indicates an ovate leaf shape. There is slight variability in these measurements across different samples, suggesting some level of phenotypic variation within the population. These parameters were consistent with the typical leaf structure of the species, providing insights into its overall morphology and adaptation.

Antioxidant activity

According to earlier reports, *X. americana* fruits from Caatinga woods in the Brazilian county of Mossoro contain high levels of phenolic compounds, flavonoids and antioxidant enzymes like catalase, ascorbate peroxidase and superoxide dismutase (21). As a result, fruit is regarded as a strong antioxidant source, which is thought to play an essential role in health promotion on grounds of close relationships between oxidative stress and disease development (22-25).

The antioxidant properties of *X. americana* fruits collected from region of Amrabad Tiger Reserve Forest in India were studied. One stable free radical is DPPH which could be estimated to evaluate fruit extracts' ability to scavenge free radicals (26). Results showed that quantity of flesh and seed extract in both fruit components showed a substantial impact on scavenging of free radicals. Stigmasterol and 4,4-dimethylcyclohex-2-en-1-ol might be the key compounds involved in the antioxidant, anti-inflammatory and antidiabetic activity of *X. americana* via inhibition of the enzymes involved in oxidative stress, inflammation and diabetes (27).

Furthermore, consistent with the findings of previous studies on fruits of *X. americana* harvested from other countries, the fruits of *X. americana* harvested in the region of the Amrabad Tiger Reserve Forest in India showed strong antioxidant activity. DPPH assay illustrated that free radical scavenging activity of *X. americana* varied with concentration of fruit extract (Fig. 1). The scavenging activity was higher at concentration of 30 $\mu\text{g/ml}$ followed by 50 $\mu\text{g/ml}$. The antioxidant activity of fruit extract was further confirmed by using FRAP assay, which showed that it was 4.63 μM ferric equivalent.

Phytochemical and secondary metabolite screening

Fruits of *X. americana* are known to contain a large diversity of bioactive agents. Phenols, alkaloids, glycosides, saponins, flavonoids and terpenoids were identified in fruits. Methyl ester 3,4-di-o-caffeoylquinic acid, iridoid glycosides like 6''-o-trans-feruloylgenipeningentiobioside, 10-(6-o-trans sinapoylglucopyranosyl) gardendiolaw well as a neo chlorogenic acid 3-caffeoyl quinic acid contain hepato-protective and anti-viral activities and neuro-protective agent for Alzheimers' disease, respectively (28).

The observation of the high complexity of chemical composition of fruits of *X. americana* has been corroborated by using GC MS/MS, in which various phytochemicals such as 5-hydroxypiperic acid, 3-o-methyl-d-glucose, 1-o-decyl- α -D-mannofuranoside, 5-hydroxymethylfurfural and 1-hexadecyl-2,3-dihydro-1H-indene were identified (Fig. 2).

About 38 secondary metabolites were identified in methanolic extract of fruit (Fig. 3 & 4). Classes of secondary metabolites that were present in the fruits of *X. americana* include alkaloids, flavonoids, lignans, terpenes, naphthoquinones, saponins, hydrolysable tannins and steroids (2). Some phenolic compounds detected in methanolic extract of fruit of *X. americana* include citric acid [m/z (negative mode): 190.95; $[\text{M-H}]^-: \text{C}_6\text{H}_7\text{O}_7^-$] and sinapic acid [m/z (negative mode): 224.9; $[\text{M-H}]^-: \text{C}_{11}\text{H}_{11}\text{O}_5^-$]. The presence of these compounds was consistent with results reported (29, 30).

In addition, different enzymes like ascorbate peroxidase, catalase and superoxide dismutase, vitamins and secondary metabolites were recorded in the skin and pulp of the fruit of *X. americana*. Owing to the relatively limited literatures on its chemical compositions and the fact that

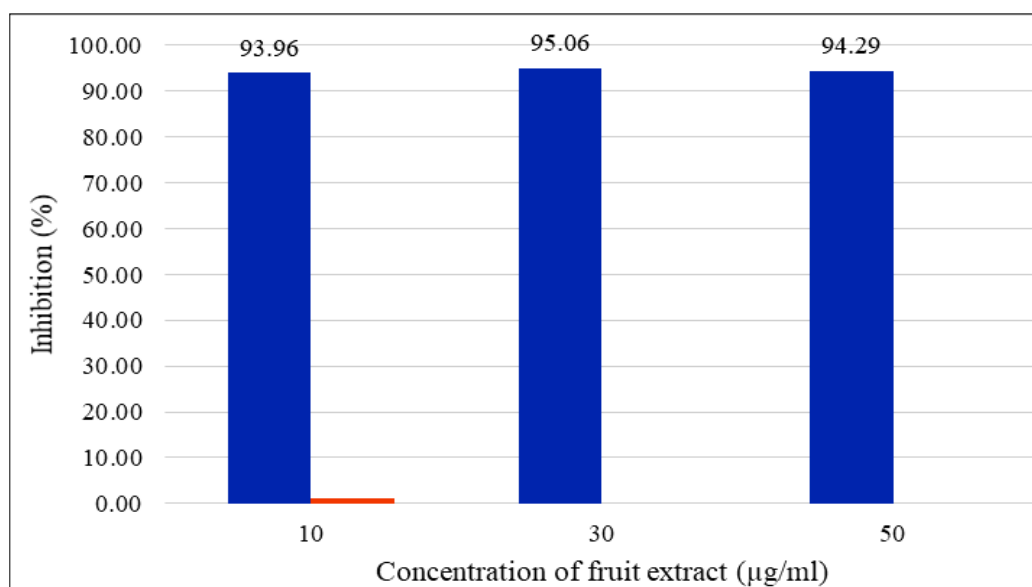


Fig. 1. DPPH - free radical scavenging activity fruit extract of *X. americana* L. harvested from the region of the Amrabad Tiger Reserve Forest in India.

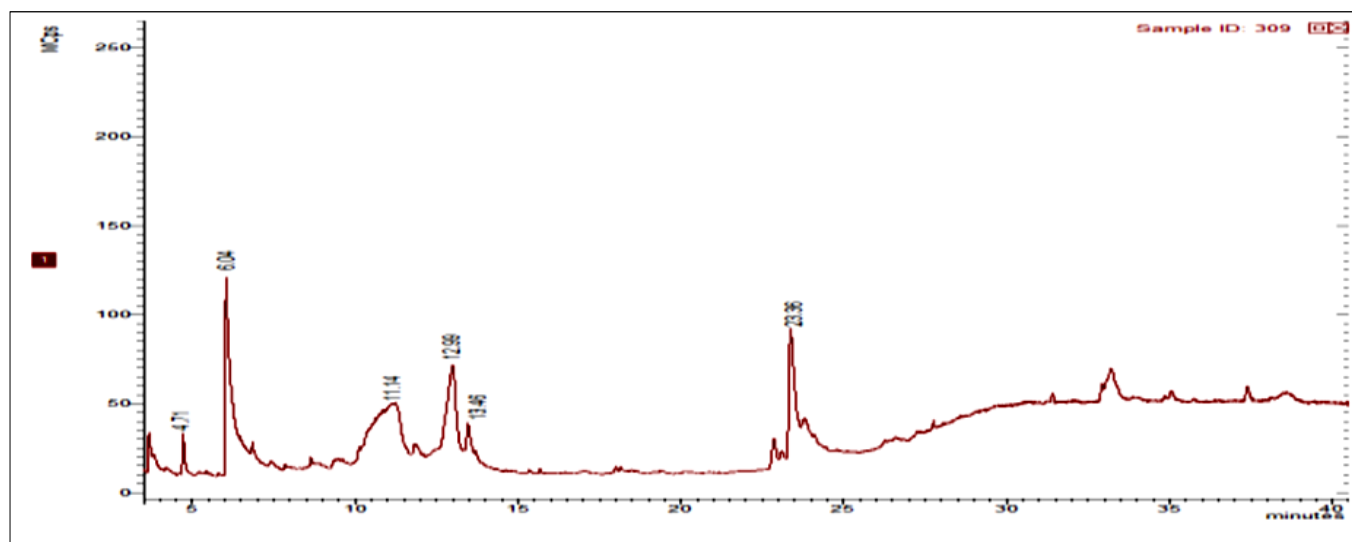


Fig. 2. GC MS/MS chromatogram of the extract of the fruits of *X. americana* L. harvested from the region of the Amrabad Tiger Reserve Forest in India.

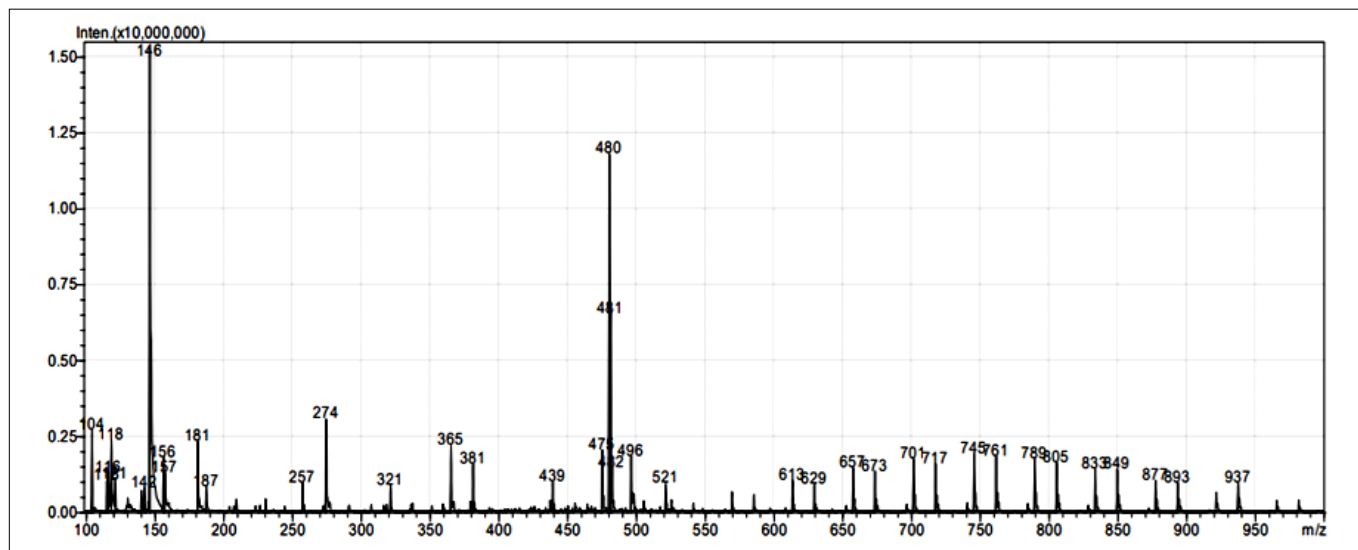


Fig. 3. Positive ionization spectrum of the extract of the fruits of *X. americana* L. harvested from the region of the Amrabad Tiger Reserve Forest in India.

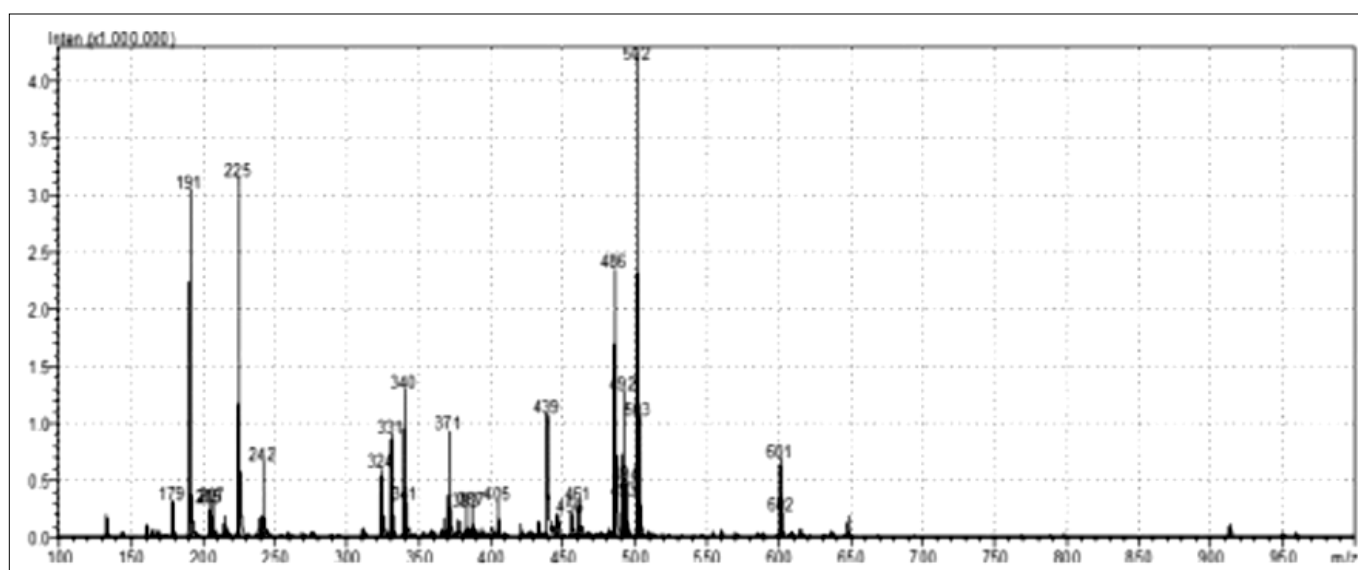


Fig. 4. Negative ionization spectrum of the extract of the fruits of *X. americana* L. harvested from the region of the Amrabad Tiger Reserve Forest in India.

almost all previous publications were focussed on the components from aqueous extract of fruit of *X. americana*, further study is required to validate the comparatively lipophilic components of extract. Based on these results, the presence of different hydrophilic bioactive compounds in fruit of *X. americana* were confirmed.

Conclusion

The fruits of *X. americana* harvested in India represents a promising source of bioactive compounds with potential applications in both food and healthcare sectors. These results will provide insight into practical utility and feasibility of cultivating this wild fruit crop in India. Owing to the fact that increased use of these fruits could promote commercial cultivation thereby reducing dependence on wild harvesting, there is a compelling need to prioritise the domestication and systematic cultivation of *X. americana*. Such efforts would not only contribute to meeting the nutritional demands of the country but also enhance opportunities for commercial production and trade.

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Authors' contributions

JS and VN carried out the treatment work and participated in interpretation of results. JS perceived the experimental design, carried out the experiment, participated in the interpretation of data, edited, corrected and reviewed the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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