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Antioxidant Potential of Wild Edible Fruits Consumed by Tribals of Western Ghats Region in Kerala, India and Identification of Compounds by LC-MS Profiling

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Abstract

Wild edible plants hold significant ethnobotanical and nutraceutical importance, especially among tribal communities that rely on them for nutrition and traditional medicine. This study evaluated the antioxidant potential of three wild edible fruits such as *Alangium salvifolium* subsp. *hexapetalum* (Alangiaceae), *Ardisa elliptica* (Myrsinaceae), and *Solanum nigrum* (Solanaceae), collected from the Western Ghats region of Wayanad, Kerala, India. Methanolic extracts of the fruits were analyzed using DPPH, FRAP, and ABTS antioxidant assays. Among the studied fruits, *Alangium salvifolium* subsp. *hexapetalum* exhibited the highest antioxidant activity with the lowest IC₅₀ values: 209.43 µg/mL (DPPH), 4.32 µg/mL (FRAP), and 29.44 µg/mL (ABTS). Comparatively, *Ardisa elliptica* recorded IC₅₀ values of 329.57 µg/mL (DPPH), 17.04 µg/mL (FRAP), and 49.65 µg/mL (ABTS), while *Solanum nigrum* showed the lowest activity with IC₅₀ values of 765.35 µg/mL (DPPH), 34.49 µg/mL (FRAP), and 109.73 µg/mL (ABTS). Given its superior antioxidant capacity, *Alangium salvifolium* subsp. *hexapetalum* was further analyzed using High Resolution-Liquid Chromatograph Mass Spectrometry (HR-LCMS), leading to the identification of bioactive compounds potentially responsible for its antioxidant effects. These findings highlight the nutraceutical potential of wild edible fruits and underscore the importance of preserving indigenous knowledge regarding their use.

Keywords: Wild edible plants; Antioxidant activity; Wild plants; Kerala; Spectrometry; LC-MS Profiling

Introduction

The importance of wild edible plants is well-known to the research world. Tribal communities depend on these wild edible plants to balance their nutrition. Also, these plants are utilised by them to cure many ailments, even though they are not aware of the biochemistry behind it. Thus, it is important to explore the nutraceutical potential of wild edible plants and to disseminate its importance so as to preserve these indigenous knowledges.

According to Yesodharan and Sujana (2007), 83 species of wild edible plants are used by the tribes in Parambikulam Wildlife Sanctuary, Palakkad district, Kerala, India. 55 distinct species of wild edible plants are used by the Kadar tribes in the Vazhachal Forest Division of Thrissur, Kerala.



According to Chaithanya et al. (2015), this comprises 23 species that are eaten as fruits, 12 as edible rhizomes and tubers, 12 as leafy vegetables, 8 as seeds, and 3 as shoots and bark. Majority of the wild edible plants consumed by tribal communities belongs to fruit category. This study focuses on the antioxidant capacity of wild edible fruits of *Alangium salvifolium* subsp. *hexapetalum* Wang. (alangiaceae), *Ardisia elliptica* Thunberg. (myrsinaceae) and *Solanum nigrum* L. (Solanaceae). The antioxidant activity of these fruits was determined by three assays viz., DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay, FRAP (Ferric Reducing Antioxidant Power), and ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay. The components of wild edible fruit with highest antioxidant activity among these three fruits were identified by HR-LCMS (High Resolution- Liquid Chromatograph Mass Spectrometry) method.

Materials and methods

The fruits were collected from the Western Ghats region in Wayanad district of Kerala, India. These were washed to remove the dirt and dried in shade under room temperature. Then the dried fruits were powdered finely. This was extracted with methanol in Soxhlet apparatus and evaporated the solvent to get concentrated extract. This extract was subjected to antioxidant assays.

Antioxidant activity was determined using DPPH, FRAP and ABTS assays. DPPH radical scavenging assay was performed based on the modified procedure described by John et al. (2012). The methanolic extract of sample was taken for the assay and 5 dilutions were prepared. To this, 0.2 ml of DPPH reagent was added and kept for 30 minutes in dark condition. Absorbance was measured at 517 nm against methanol as blank whereas the control used was the mixture of methanol and DPPH reagent. The percentage DPPH inhibition was calculated using the equation given below and that was plotted against concentration from which the IC₅₀ value was calculated.

$$\% \text{ DPPH inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

The antioxidant activity was also determined by finding out the ferric reducing antioxidant power of the samples. FRAP reagent is the mixture of TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution, ferric chloride solution and 300 mM acetate buffer in 1:1:10 ratio. It is prepared and kept in water bath at 37°C for at least 10 minutes. Samples and standards (ascorbic acid) at various dilutions were prepared and the control used was methanol. To these, 3 ml of FRAP reagent was added and after 4 minutes, absorbance was recorded at 593 nm (Benzie and Strain 1996). The IC₅₀ value was determined from the graph of concentration plotted against FRAP value (equation given below).

$$\text{FRAP value (\%)} = \frac{A_{\text{Sample}} - A_{\text{Control}}}{A_{\text{Standard}} - A_{\text{Control}}} \times 2$$

ABTS assay was carried out using the methanolic extract of fruit samples, according to the procedure given by Tsvetkova et al. (2023). The stock reagent was prepared by mixing 7 mM ABTS solution with equal quantity of 2.45 mM potassium persulphate solution. It was kept in dark for almost 17 hours and diluted 10 times with methanol. Thus obtained ABTS solution was added to the standard, samples and control. After 10 minutes, absorbance was recorded at 734 nm. IC₅₀ value was calculated similarly as mentioned in above methods.

$$\% \text{ Radical Scavenging Activity} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

HR-LCMS: The sample exhibiting the highest antioxidant activity was analyzed using HR-LCMS (High Resolution-Liquid Chromatographic Mass Spectrophotometer) at the SAIF Institute, IIT Bombay, India. The analysis was conducted with a Thermo Scientific Q-Exactive Plus Biopharma instrument, utilizing Thermo Scientific Xcalibur (version 4.2.28.14) for data acquisition and Compound Discoverer 3.2 SP1 for data processing. Solvent A consisted of 0.1% formic acid in Milli-Q water, while solvent D was acetonitrile.

Results and discussion

Antioxidant activity in wild edible fruits

Antioxidant activity gives an idea about the potential of that particular sample to scavenge the free radicals produced in the body. Antioxidant rich foods are important in our diet to protect the body from various diseases. Antioxidant activity is expressed by IC₅₀ value, which is the concentration at which 50 % of free radicals are inhibited. In the DPPH assay, the IC₅₀ value was lowest for *Alangium salvifolium* subsp. *hexapetalum* fruit extract (209.43 µg mL⁻¹) followed by *Ardisia elliptica* (329.57

$\mu\text{g mL}^{-1}$) and *Solanum nigrum* ($765.35 \mu\text{g mL}^{-1}$) fruit extracts. Similar trend was observed in both FRAP and ABTS assays. The IC_{50} values obtained from FRAP assay were $4.32 \mu\text{g mL}^{-1}$ for *Alangium salvifolium* subsp. *hexapetalum*, $17.04 \mu\text{g mL}^{-1}$ for *Ardisa elliptica* and $34.49 \mu\text{g mL}^{-1}$ for *Solanum nigrum* fruits whereas it was $29.44 \mu\text{g mL}^{-1}$ for *Alangium salvifolium* subsp. *hexapetalum*, $49.65 \mu\text{g mL}^{-1}$ for *Ardisa elliptica* and $109.73 \mu\text{g mL}^{-1}$ for *Solanum nigrum* fruits as per ABTS assay. The FRAP and ABTS values are pretty promising because of the low IC_{50} values ensuring its high antioxidant potential. As per Seena et al. (2020), the IC_{50} values obtained through DPPH and ABTS scavenging assays of methanolic extract of aerial parts of *Alangium salvifolium* subsp. *hexapetalum* were $175 \mu\text{g mL}^{-1}$ and $40 \mu\text{g mL}^{-1}$.

Table 1. Compounds of *Alangium salvifolium* subsp. *hexapetalum* identified by HR-LCMS in positive mode

Compounds	Retention time (minutes)	Molecular mass	Best match	Group
Quercetin	12.382	302.0427	99.9	Flavonoid
Kaempferol	12.352	286.0477	99.3	Flavonoid
Atropine	6.799	289.1678	99.1	Tropane alkaloids
Salsolinol	1.331	179.0946	98.8	Alkaloid
Hexadecanamide	22.727	255.2562	98.5	Fatty acid amide
Linoleoyl ethanolamide	21.008	323.2824	98.3	Fatty acid amide
Asparagine	1.2	132.0535	98.2	Amino acid
Betaine	1.246	117.079	98.0	Modified amino acid
Piperine	9.446	285.1365	97.8	Alkaloid
Pipecolic acid	1.322	129.0790	97.7	Carboxylic acid
Papaverine	9.034	339.1471	97.1	Alkaloid
Pyrrole-2-carboxylic acid	8.156	111.0320	96.6	Carboxylic acid
4-Methoxycinnamic acid	13.201	178.0630	91.6	Phenol
Laudanosine	11.576	357.1940	89.8	Alkaloid
8-Hydroxyquinoline	3.314	145.0528	88.2	Quinoline
4-Hydroxyindole	11.167	133.0525	87.6	Phenol

Table 2. Compounds of *Alangium salvifolium* subsp. *hexapetalum* identified by HR-LCMS in negative mode

Compounds	Retention time (minutes)	Molecular mass	Best match	Group
Citric acid	1.33	192.0266	99.1	Organic acid
4-Oxoproline	1.72	129.0416	97.5	Amino acid
Eriodictyol	12.34	288.0638	96.7	Flavonoid
Quercetin	12.38	302.0431	96.3	Flavonoid
Luteolin	12.35	286.0482	96.2	Flavonoid
α, α -Trehalose	1.259	342.1162	84.7	Sugar
Malic acid	1.33	134.0206	75.5	Organic acid
7-Methylxanthine	1.23	166.0470	73.5	Purine derivative

Nahar et al. (2012) reported that the flowers of *Alangium salvifolium* exhibited IC_{50} value of $182.31 \pm 0.31 \mu\text{g mL}^{-1}$ in DPPH assay whereas its peel and seeds exhibited IC_{50} values of 38 and $60 \mu\text{g mL}^{-1}$ respectively (Paul et al. 2022). According to Dey et al. (2014), IC_{50} value obtained from DPPH assay of the ethanolic extract of *Ardisa elliptica* fruits was $30.75 \mu\text{g mL}^{-1}$ whereas it was $120 \pm 2.3 \mu\text{g mL}^{-1}$ in another study by Al-Abd et al. (2017). In another study, the FRAP value obtained for *Ardisa elliptica* fruit extract was $0.12 \pm 0.3 \mu\text{mol Fe (II) g}^{-1}$ (Al-Abd et al. 2017). The result of antioxidant assays (Fig 1) reveals that among the three wild edible fruits, *Alangium salvifolium* subsp. *hexapetalum* exhibited highest antioxidant activity with lowest IC_{50} values in all the three assays. This might be due to the presence of compounds contributing for antioxidant properties like phenols, flavonoids etc.

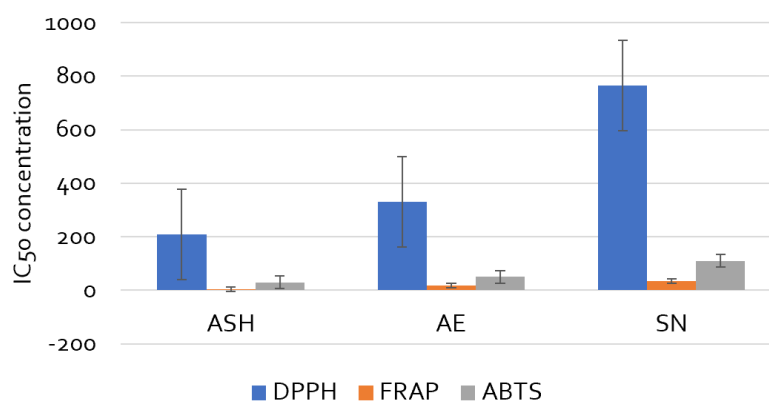


Fig. 1. Antioxidant activity in terms of IC_{50} value of the three fruits *Alangium salvifolium* subsp. *hexapetalum* (ASH), *Ardisa elliptica* (AE) and *Solanum nigrum* (SN) by DPPH, FRAP and ABTS assays

Identification of compounds of *Alangium salvifolium* subsp. *hexapetalum* through mass spectrometry

The fruits of *Alangium salvifolium* subsp. *hexapetalum* exhibited highest antioxidant activity among the three wild edible fruits. So, it was subjected to HR-LCMS to identify the compounds present in the methanolic extract of *Alangium salvifolium* subsp. *hexapetalum*. The tables 1 and 2 shows the compounds identified in the methanolic extract of *Alangium salvifolium* subsp. *hexapetalum* in both positive and negative modes.

Conclusion

The three wild edible fruits consumed by tribals of Western Ghats were found to possess potential antioxidant activities. Among the three fruits, highest antioxidant activity in all the three assays was observed for *Alangium salvifolium* subsp. *hexapetalum*. The methanolic extract of *Alangium salvifolium* subsp. *hexapetalum* was detected with the presence of various compounds like flavonoids, alkaloids, fatty acid amides, amino acids, phenols etc. Further studies are required to understand the pharmacological properties and the effects of these wild edible plants on health.

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Author Contributions

SJ: carried out research work, compiled the data and prepared the manuscript; SG: Idea and supervision, guidance, manuscript correction; ZPMB: raw material collection, supervision; AMM: supervision; AR: supported research work

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Availability of data and materials

Not applicable.

Competing interest

The authors declare no competing interests.

Ethics approval

Not applicable.



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