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LC-ESI-QTOF-MS/MS characterization of Untargeted Phenolic Screening in Wild Edible Plants: *Alternanthera sessilis* and *Alternanthera tenella* from Rampachodavaram, Andhra Pradesh, India.

M Sabitha[1&2], M Sridevi[3], P Sreevani[4], K Gayathri[5], G Raman[5&6], R Lohitha Kavya[6]*

[1]TDR-HUB, Andhra University, Visakhapatnam -530003, AP
[2]Department of Botany, S K V T Government Degree College, Rajamahendravaram -533103, AP
[3*]Department of Botany, S K R Government Degree College, Rajamahendravaram -533103, AP
[4]Department of Botany, Government Degree College, Vizianagaram Town, AP
[5]School of Life and Health Sciences, Adikavi Nannaya University, Rajamahendravaram, AP
[6]Central Instrumentation Laboratory (CIL), DST-FIST, Government College (A), Rajamahendravaram, AP

***Corresponding Author:** Prof. P. Sreevani
*Email: srvani6@gmail.com

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Abstract:
Alternanthera sessilis and *Alternanthera tenella* are two edible plants that are frequently encountered in the tribal region of Rampachodavaram, Andhra Pradesh, India. These plants offer many nutritional benefits and have positive impacts on health. The primary aim of this study is to assess the phenolic composition of both plants by extracting methanol from the whole plant using a cold maceration method. The extract of *Alternanthera sessilis* demonstrated the highest concentration of polyphenols, measuring 61.45 mg GAE/g of dry extract weight. Both extracts shown efficient free radical scavenging action on DPPH. However, the *Alternanthera tenella* extract had the lowest determined IC_{50} value of 8.53 µg mL^{-1}. LC-ESI-QTOF-MS/MS analysis detected a total of 24 phenolic compounds, which were further grouped into their respective classes such as phenolic acids (6), flavonoids (9) including flavonols and flavones, diterpenoids (2), coumarins (1), saponins (1), alkaloids (3) and glycosides (1). Notably, the *Alternanthera sessilis* extract exhibited the highest proportion of Betaine, a compound belonging to the betalanins class commonly found in plants of the Amaranthaceae family.

Keywords: *Alternanthera sessilis, Alternanthera tenella,* Wild food plants, Untargeted Phenolic profiling, LC-MS/MS.

1. INTRODUCTION:

Wild Food Plants (WFP) thrive in their native environments without any human intervention or cultivation. The WFPs serve as a vital food source for several indigenous populations and provide a significant supply of essential micronutrients worldwide. The use of these traditional food practices as a cultural custom persists to this day, and there has been a growing global interest in finding their ethnomedicinal properties in recent years. WFPs provide as a nutritious alternative to staple foods in times of drought and seasonal food scarcity (Thomas 2020; R.P. et al. 2021).

Plant secondary metabolites, primarily polyphenols, are synthesised through the shikimic acid and phenyl propanoid metabolic pathways. They are synthesized as a defence mechanism against pathogenic organisms, herbivores, and adverse environmental circumstances. The polyphenols found in wild food plants (WFPs) are effective antioxidants that can eliminate oxidative stress in different ailments in humans and hence decrease cellular damage (Shahidi and Ambigaipalan 2015). Plants generate a vast array of secondary metabolites or bioactive substances. It is believed that the plant kingdom produces about 600,000 of these molecules, which possess various pharmacological effects. However, many of these compounds still remain unidentified. Polyphenols, also known as phenolic compounds (PCs), are widely distributed secondary metabolites among the plant kingdom. Polyphenols are chemical compounds with a low molecular weight that consist of an aromatic ring (such as benzene or phenol) with one or more hydroxyl groups attached to it. They are classified into different categories and subcategories according to their chemical structure, the number of phenol rings, the arrangement of functional groups, or the carbon skeleton. Plant-derived polyphenolic compounds, commonly known as bioflavonoids, are being progressively utilised as therapeutic pharmacological agents for the treatment of diseases with diverse causes (Sergio et al. 2020; Ozturk et al. 2022).

Alternanthera sessilis and *Alternanthera tenella* belongs to Amaranthaceaes family are commonly used WFPs by the indigenous people of Rampachodavaram, Alluri Seetharamaraju District, Andhra Pradesh, India.

Leaves of *Alternanthera sessilis* are abundant in protein, making them a popular choice for consumption as a raw, leafy vegetable in several South Asian countries. The leaves and roots of this plant are extensively used in ayurveda medicine for the treatment of both eye and digestive ailments. The plant is commonly referred to as 'sessile joyweed' or 'dwarf copperleaf'. This is an enduring herbaceous plant commonly discovered in and in close proximity to bodies of water such as ponds, canals, and reservoirs. An infusion of *A. sessilis* is advised as an herbal treatment for wounds, flatulence, nausea, vomiting, cough, bronchitis, diarrhoea, dysentery, and diabetes (Kanagarasu et al. 2017).

Alternanthera tenella, a member of the Amaranthaceae family, is a captivating herbaceous plant that is commonly studied for its pharmacological and phytochemical properties. It has been widely used in traditional medical systems in India since many centuries. This medication contains empirical evidence for its effectiveness in treating infection, edoema, headache, fever, and symptoms associated with inflammation. Research has documented the antioxidant, anti-inflammatory, and immunomodulatory effects of *A. tenella* extracts (Guerra et al. 2003; Deladino et al. 2017).

Metabolomics play a key role in identifying the novel bioactive compounds in plants. Purification and identification of bioactive phytoconstituents involves the use of toxic solvents, difficult in extraction process and cost-effective. Recently, there has been a growing trend in using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to analyse and identify the polyphenol-rich extracts. Furthermore, LC-MS/MS methods successfully reduced noise and enhanced sensitivity by utilising the multiple reaction monitoring (MRM) scan mode (Shah et al. 2023). In recent years, there has been extensive use of high-resolution LC-MS and LC-MS/MS techniques combined with multivariate statistics to achieve "metabolomic profiling" of plant meals for human nutrition. The metabolomics-based approaches, both targeted and untargeted, necessitate minimal sample preparation and provide a comprehensive analysis of the polyphenol composition of a given matrix (Manickam et al. 2023).

The present study aims to investigate the untargeted metabolomics of two WFPs (*Alternanthera sessilis* and *Alternanthera tenella*) using High resolution Orbitrap Liquid Mass spectroscopy combined with Electrospray Ionization Mass spectroscopy/Mass spectroscopy (ESI-MS/MS).

2. METHODOLOGY:

2.1. Sample Collection and Processing: The two wild plant species *Alternanthera sessilis* and *Alternanthera tenella* were collected from Rampachodavaram, Alluri Seetharamaraju District, Andhra Pradesh, India and the species were confirmed through literature. The whole plant of two species were washed thoroughly and shade dried at room temperature. The dried plants were pulverized into fine powder using electronic blender.

2.2. Cold Maceration: This method was adopted from *Alara et al. 2021*. The 50 g of both pulverized plant powders were dispersed separately into 200 mL of 80% methanol and kept on orbital shaker incubator for 24 h at 25°C. The obtained reaction mixtures were filtered and concentrated with rotary evaporator at 35°C and final yields were labelled as *Alternanthera sessilis* extract (ASE) and *Alternanthera tenella* extract (ATE) stored at until further analysis. The percentage (%) of yield was estimated as below

$$\%\ of\ yield = \frac{Dry\ weight\ of\ the\ extract}{Dry\ weight\ of\ the\ plant\ material} \times 100 \qquad Eqs\ldots 1$$

2.3. Qualitative Phytochemical Screening:

Using standard protocols, a preliminary phytochemical screening of the ASE and ATE extract was conducted to determine whether secondary metabolites, including flavonoids, phenols, tannins, alkaloids (Mayer's and Wagner's), terpenoids, anthraquinones, saponins, quinones, coumarins, glycosides, steroids and reducing sugars were present or absent (Dubale et al. 2023).

2.4. Total Polyphenol Content (TPC) estimation:

Using the Folin-Ciocalteu (FC) reagent technique, TPC was ascertained. By dissolving 1 mg mL^{-1} of dried ASE and ATE extract in methanol and filtering through Whatman No. 1 filter paper, the TPC was calculated. Test tubes were filled with 0.02 mL of extract or standard solution and 1 mL of FC reagent (1:10 v/v). This solution was mixed with 1 millilitre of 7.5% sodium carbonate and incubated for a maximum of two hours. With a blank devoid of extract, the absorbance was measured at a wavelength of 765 nm using a UV-Vis Spectrophotometer (Shimadzu UV 2600). The results were reported as gallic acid equivalents (GAE/g) in milligrammes per gramme of dry weight extract (Singla et al. 2022b).

2.5. 2,2-diphenyl picryl hydrazyl (DPPH) radical scavenging activity:

The DPPH free radical scavenging properties of ASE and ATE were evaluated using spectrophotometer method. Briefly, various concentrations of ASE and ATE (25-100 µg mL^{-1}) were added to the 3mM DPPH reagent (Absorbance adjusted to 0.600-0.650 at 517 nm wavelength). The reaction mixtures were incubated in dark for 2 hrs at room temperature to achieve complete reduction of DPPH. After incubation, the reaction mixtures were centrifuged at 5000 rpm for 5 minutes and the absorbance of supernatant was read at 517 nm. DPPH with methanol and Vitamin C were used as control and standard respectively (Chaves et al. 2020). The % of scavenging activity and (Inhibitory concentration) IC_{50} values were calculated as follows:

$$\%\ of\ Scavenging\ activity = \frac{C_0 - C_1}{C_0} \qquad \text{Eqs...2}$$

C_0 = Absorbance of control
C_1 = Absorbance of sample at each concentration
The linear regression curve was used to determine IC_{50} value $IC_{50} = (0.5 - b)/a$ Eqs...3

2.6. Inhibition of Albumin Denaturation:

This assay was performed according to the *Nahari et al. 2022* and Bovine Serum Albumin (BSA) as a protein source. Various concentrations of ASE and ATE (25–100 µg mL^{-1}) were incubated with 1% BSA in phosphate buffer solution pH 6.4 at 37°C for 20 mins. Then, these reaction mixtures were transferred to boiling water bath for 5 mins. After this treatment, the reaction mixtures were cooled down to room temperature and absorbance was read at 660 nm. Aspirin was used as standard drug. The % of inhibition of albumin denaturation was estimated as follows:

$$\%\ of\ inhibition\ of\ albumin\ denaturation = \frac{C_0 - C_1}{C_0} \qquad \text{Eqs...4}$$

C_0 = Absorbance of control
C_1 = Absorbance of sample at each concentration
The IC_{50} value was determined according to the Eqs….3.

2.7. Screening of phenolic compounds using LC-ESI-QTOF-MS/MS:

The phenolic metabolites of *Alternanthera sessilis* and *Alternanthera tenella*, which are native to India, were identified and characterised using the LC-ESI-QTOF-MS/MS and MS/MS techniques. In this experiment, a Synergi 4 µm Hydro-reversed phase (RP 80 Å) LC column (250 × 4.6 mm) was coupled to a C18 ODS (4.0 × 2.0 mm) guard column. Unknown phenolic metabolites from native Australian plants were identified using the Agilent 6520 Accurate Mass QTOF LC-MS/MS coupled with an Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) for Metabolites 2022, 12, 1016 4 of 21. To put it briefly, a gradient of 10 min (10–20 % B), 10–20 min (20–25% B), 20–30 min (25–30% B), and 30–40 min (30–45%) was applied to an aliquot of 10 µL from each plant extract. In brief, 10 µL of each plant extract aliquot was injected using the gradient below at a flow rate of 0.6 mL/min: Ten minutes (10–20% B), ten minutes (20–25% B), twenty minutes (25–30%), thirty minutes (30–45% B), forty minutes (45–60%), fifty minutes (60–90%), sixty-seven minutes (90–100%), sixty-seven minutes (100–10%), and sixty-seven minutes (10% B) of mobile phase A (0.1% LC-MS grade formic acid in Milli-Q water) and mobile phase B (0.1% LC-MS grade formic acid in acetonitrile) were completed. Agilent Mass Hunter Workstation Software Quality Analysis (version B.06.00) was used for the identification and characterization of phenolic metabolites with the help of the Personal Compounds Database and Library (PCDL) for metabolites, PubChem (https://pubchem.ncbi.nlm.nih.gov, accessed on April 2024), Human Metabolome Database (https://hmdb.ca, accessed on April 2024), and FooDB (https://foodb.ca), accessed on April 2024 while offline versions of GNPS, NIST, and MassBank libraries and databases were also used in this experiment to match the MS/MS spectra of phenolic metabolites.

3. Result and Discussion:

There are roughly 3000 consumable plant varieties known to man, with just 30 crops accounting for more than 90% of global calorie intake and only 120 crops being commercially significant on a national basis. In India, there are 1532 edible wild food species, the majority of which come from the Western Ghats and the Himalayas. Similarly, in the Eastern Ghats region, several tribes eat wild plants. Andhra Pradesh's tribal people have extensive knowledge on how to use wild plants as part of the food (Reddy et al. 2007).

3.1. Qualitative phytochemical screening:

In the present study two important WFPs *Alternanthera sessilis* and *Alternanthera tenella* were collected from tribal region, Rampachodavaram, Andhra Pradesh. The table 1 represents the qualitative phytochemical investigation of both ASE and ATE. The sign (+) indicates the presence and sign (-) indicates the absence of phytochemicals. The similar studies supporting the presence of various phytochemicals in both plants (Kanagarasu et al. 2017; Singla et al. 2022a).

Table 1. Qualitative phytochemical screening of ASE and ATE.

S.No	Phytochemical constituents	ASE	ATE
1	Tannins	+	+
2	Alkaloids (Mayer's test)	+	-
3	Alkaloids (Wagner's test)	-	+
4	Saponins	+	-
5	Cardiac glycosides	-	+
6	Steroids	+	+
7	Terpenoids	+	+
8	Flavonoids	+	+
9	Flavonoids (Shinoda's test)	+	+
10	Phlobatannins	-	-
11	Anthraquinones	-	+
12	Reducing sugars	-	-
13	Phenols	+	+

Key (+) = Positive, (-) = Negative.

3.2. Estimation of Total Polyphenol Content (TPC):

The health effects of polyphenols, which are present in many vegetable and herbal medications, have been the subject of much research in recent decades. Preventive effects of food extracts, polyphenol-rich extracts, and single compounds on metabolic syndromes, such as hyperglycemia, hyperlipidemia, hypertension, neurodegenerative disorders, and various malignancies, have also been confirmed by certain pre-clinical, clinical, and population investigations (Zhang et al. 2021). In the present study both ASE and ATE were subjected to estimation of TPC using FC reagent method and quantity of TPC was estimated by mg gallic acid equivalent (GAE)/g dry extract weight represented in table 2. *Deladino et al* estimated TPC in leaves of *Alternanthera tenella* and found 23.95 mg (Deladino et al. 2017) and Aryal et al revealed TPC of 292.65 mg (Aryal et al. 2019) which is higher than the present study. The difference in their quantity based on several factors such as geographical location, soil profile and effect of environmental and biological factors (Oracz and Nebesny 2019).

Table 2: Total polyphenol content (TPC) of ASE and ATE.

No.	Sample	TPC (mg GAE/g dry extract wt)
1.	ASE	61.45 mg
2.	ATE	45.87 mg

ASE - *Alternanthera sessilis* extract, ATE- *Alternanthera tenella* extract.

3.3. DPPH free radical scavenging activity:

1,1-diphenyl-2-picrylhydrazyl, also known as 2,2-diphenyl-1-picrylhydrazyl, is a free radical that can receive hydrogen from antioxidants. The compound is now commonly employed in the DPPH assay to measure the antioxidant activity of fruits, medicinal herbs, and other biological substrates (Singh et al. 2021). In the present study, antioxidant properties of ASE and ATE were reported using DPPH assay and concentration dependent % of scavenging activity is represented in Fig.1. Both the samples showed similar % of activity 84.84 and 84.53 % for ASE and ATE respectively at maximum concentration of 100 µg mL^{-1}. The inhibitory concentration 50 (IC_{50}) value represents the amount of plant extract/antioxidant compound required to scavenge the 50% of DPPH free radicals in 1 mL of solution. The ASE and ATE showed IC_{50} values of 55.95 and 8.53 µg mL^{-1} respectively. The previous studies on antioxidant properties of both plants are represented in table 3.

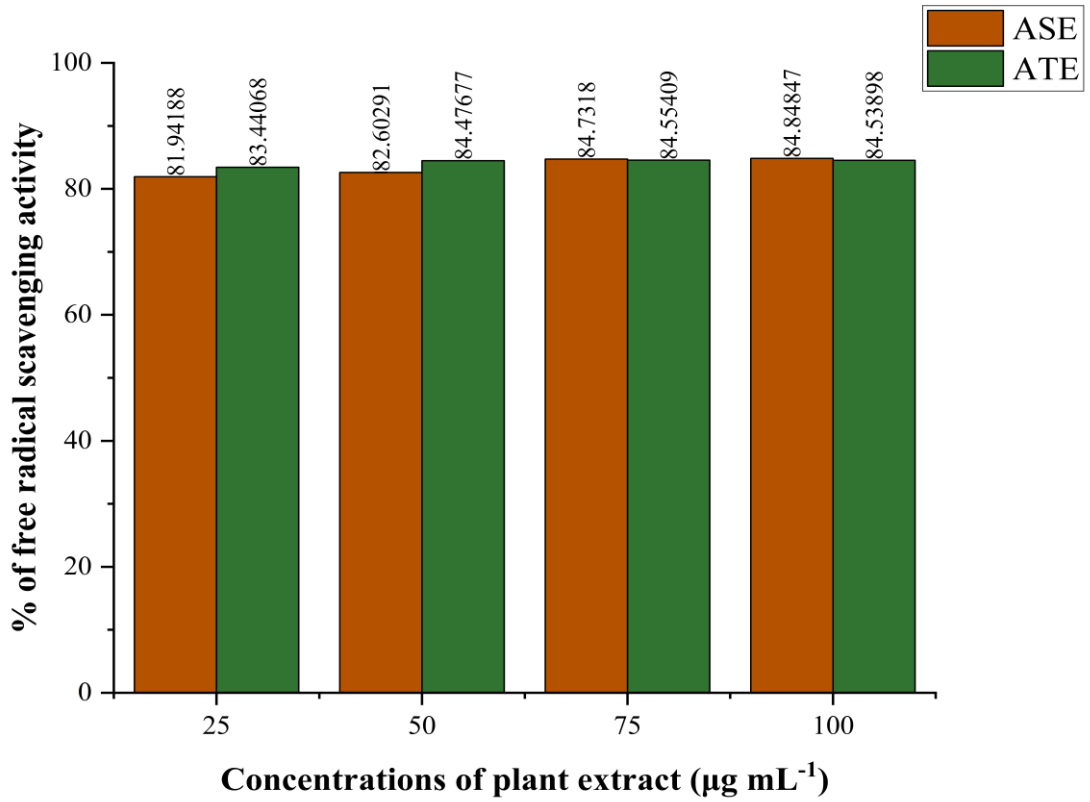

Figure 1: DPPH free radical scavenging activity of ASE and ATE.

Table 3. Comparison of DPPH activity with previous studies

S.No	Plant and/or extract	IC_{50} value µg mL^{-1}	Reported by
1	*Alternanthera sessilis* ethanolic extract	82.6	*Yap et al. 2019*
2	*Alternanthera sessilis* methanolic extract	71.10	*Pathak and Budhathoki 2020*
3	*Alternanthera tenella* ethanolic extract	190.0	*Shetty 2019*

3.4. Inhibition of Albumin Denaturation:

An intricate biological process, inflammation entails coordinated communication between various immune cells as well as a multitude of intracellular signalling chemicals. Proteins that have undergone denaturation lose their quaternary, tertiary, and secondary structures, which impairs their ability to perform biological functions. Applying external stress, such heat, or being exposed to chemicals, like organic solvents, might trigger this process (Derbel et al. 2023). Therefore, in order

to gain insight into the anti-inflammatory activity, it was determined whether or not both extracts (ASE and ATE) could inhibit heat-induced albumin denaturation. In the present study, the ASE showed 92.62 % and ATE showed 93.62 % of inhibitory activity at maximum concentration of 100 µg mL^{-1}. The calculated IC_{50} values are 63.17 and 80.22 µg mL^{-1} for ASE and ATE respectively.

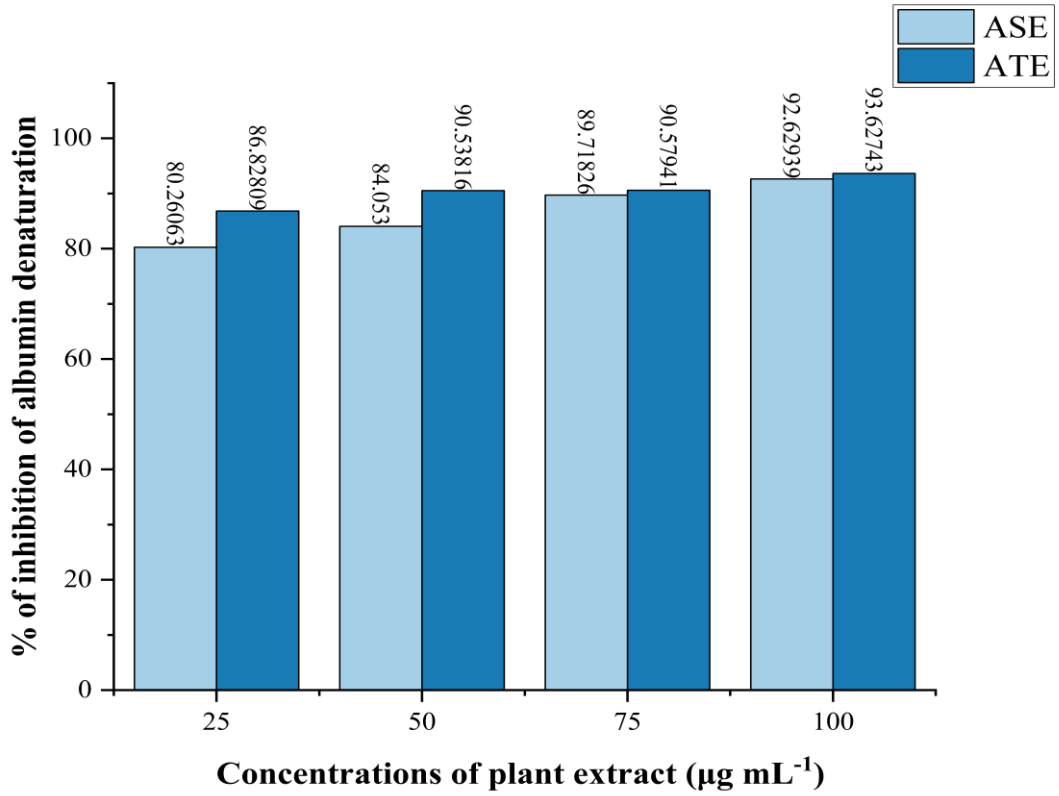

Figure 2: Inhibition of albumin denaturation activities of ASE and ATE.

3.5. Screening of phenolic compounds of ASE and ATE using LC-ESI-QTOF-MS/MS:

The untargeted screening of phenolic metabolites in ASE and ATE collected from wild region of Rampachodavaram, Andhra Pradesh, India using LC-ESI-QTOF-MS/MS and MS/MS spectra. The obtained data further compared with standard NIST libraries and existing literature. The wide range of compounds obtained from both ASE and ATE extracts, out of which the primary metabolites and fatty acids were excluded. The phenolic compounds and other secondary phytochemicals were separated into their respective classes and represented in table 4. The spectra of both samples at ESI(+) and ESI(-) were represented at Figure 3&4.

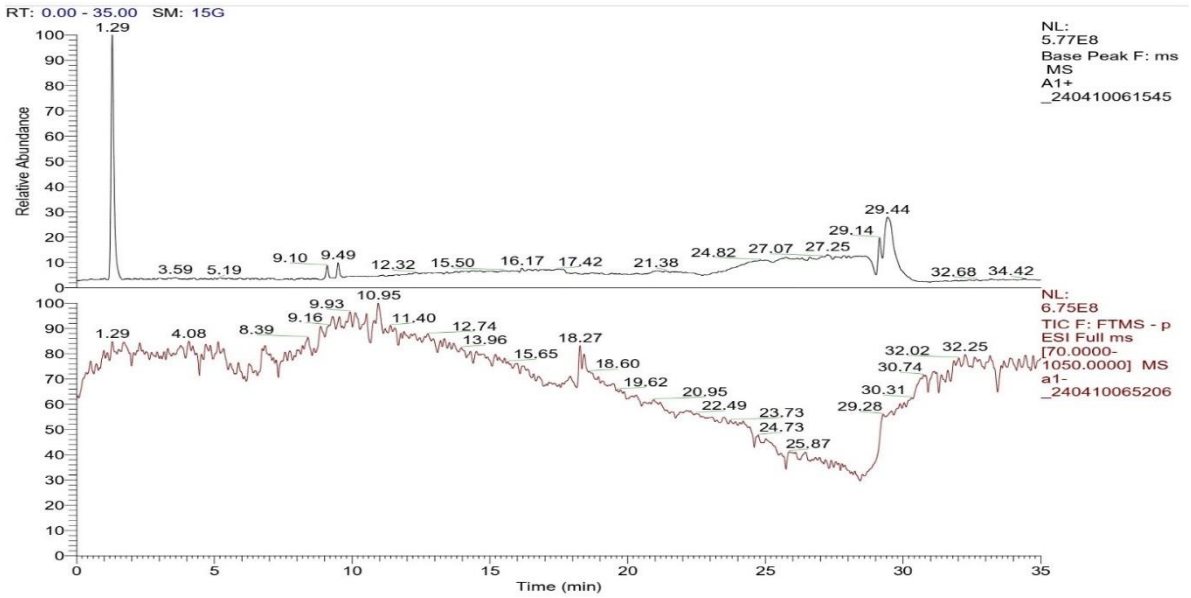

Figure 3: LC-ESI-QTOF-MS/MS chromatogram of *Alternanthera sessilis*

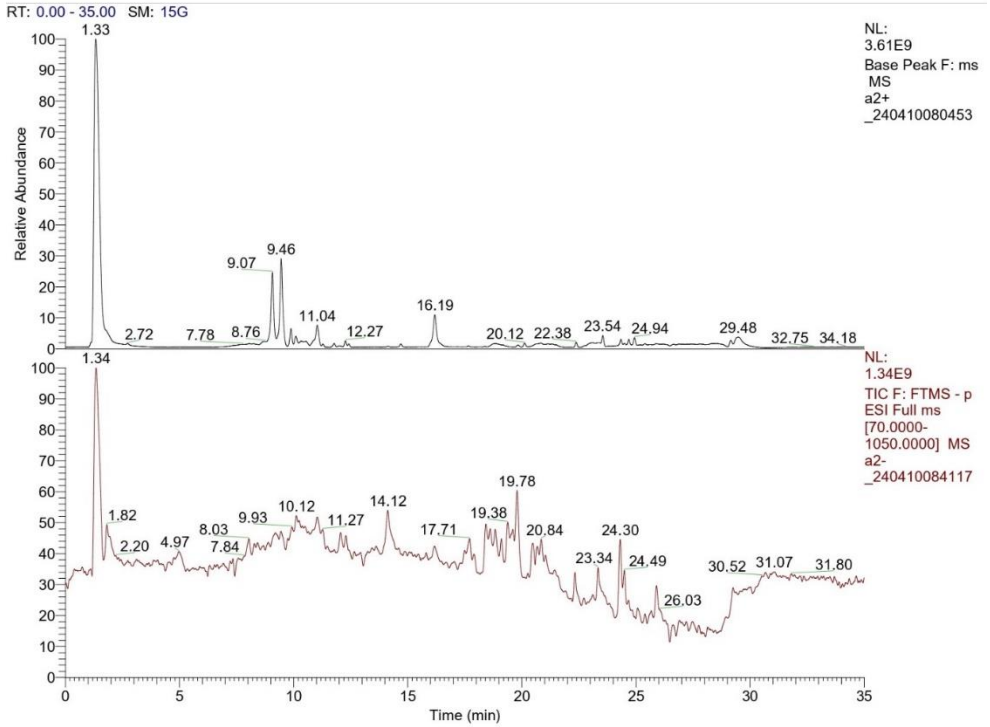

Figure 4: LC-ESI-QTOF-MS/MS chromatogram of *Alternanthera tenella*

In the present study, A total of 24 phenolic compounds were identified and classified into phenolic acids (6), Flavonoids (9) (flavonols and flavones), Diterpenoids (2), Coumarins (1), Saponins (1), Alkaloids (3), Glycosides (1) and others (1) from both ASE and ATE.

The Amaranthaceae family of plants also contains a class of pigments called betalains, which give the plant its reddish-purple colour. Two types of water-soluble nitrogen-containing pigments are known as betalains: yellow betaxanthins and reddish-violet betacyanins (Mohd Hazli et al. 2019). In the present study the ASE showed betaine (a compound belongs to betalains class) showed highest peak at $[M-H]^+$ and found at multiple retention times (from 0.09 min to 32.41 min).

Phenolic compounds (PCs) are widely present phytochemicals that can be found in various parts of plants, such as fruits and vegetables. PCs exhibit significant promise in terms of their nutritional

and medicinal capabilities (Luna-Guevara et al. 2018). A total of 6 phenolic compounds identified in both ASE and ATE which are Homogentisic acid C8 (ATE – $[M-H]^-$), Protocatechuic acid (ATE – $[M-H]^-$), Caffeic acid (ATE – $[M-H]^-$), Psoralidin (ATE – $[M-H]^+$), Butylparaben (ASE – $[M-H]^-$) and Phloroglucinol (ASE – $[M-H]^+$).

Dietary flavonoids are crucial in preventing disorders associated with oxidative stress in living organisms. Quercetin and rutin are abundant flavonoids that are commonly found in a wide range of frsuits and vegetables, including tea, coffee, and other grains. Similar to other biologically active nonnutrient components, flavonoids have been found to have both beneficial and detrimental physiological effects in humans (Trugo et al. 2003). Surprisingly no flavonoid class compound was found in ASE and 9 flavonoid class compounds only found in ATE. Kaempferol-3- Galactoside-6''-Rhamnoside-3'''- Rhamnoside (ATE – $[M-H]^-$), Quercetin-3β-D-glucoside (ATE – $[M-H]^-$), 5,6,7-trihydroxy-2-(4-methoxyphenyl)- 4H-chromen-4-one (ATE – $[M-H]^+$), Rutin (ATE – $[M-H]^-$), Isorhamnetin (ATE – $[M-H]^+$), Catechin (ATE – $[M-H]^+$), Luteolin (ATE – $[M-H]^-$), Cynaroside (ATE – $[M-H]^-$) and Tangeritin (ATE – $[M-H]^+$).

Two diterpenoid compounds were found in ASE and no such class compounds were found in ATE. Thes2-[(1S,2S,4aR,8aS)-1-hydroxy-4a-methyl-8-methylidene-1,2,3,4,5,6,7,8a octahydronaphthalen-2-yl]prop-2-enoic acid (ASE – $[M-H]^-$), and (1R,4aS,5R,8aS)-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylidene-3,4,5,7,8,8a-hexahydro-2H naphthalene-1-carboxylic acid (ASE – $[M-H]^-$) are two diterpenoids found in ASE. Diterpenoid natural products encompass a wide range of chemical variations and consist of numerous molecules that are significant in both medical and industrial applications. All diterpenoids originate from a shared foundation, (E,E,E)-geranylgeranyl diphosphate, which is transformed into various structures by a diterpene synthase (DTS) (Smanski et al. 2012).

Alkaloids are a fascinating and intricate collection of chemical substances that are synthesized through the secondary metabolism of many animals in diverse habitats. These compounds are widely found in all types of living beings across all habitats (Aniszewski 2015). A total of three alkaloids found only in ASE which are Trigonelline (ASE- $[M-H]^+$), Arecoline (ASE- $[M-H]^+$) and Nicotine (ASE- $[M-H]^+$).

Other class of phytochemicals found in ATE are Coumarins-Scopoletin (ATE- $[M-H]^+$), Saponins-Diosgenin (ATE- $[M-H]^+$) and Glycosides-Kaempferol (ATE- $[M-H]^+$). These three classes are not found in ASE.

Table 4: LC-ESI-QTOF-MS/MS identification of phenolic compounds in ASE and ATE:

No.	Proposed compound	Molecular formula	Retention Time (RT) (min)	Mode of Ionization	Observed Molecular weight (*m/z*)	Sample(s)
Other Phenolic acids						
1.	Homogentisic acid C8	$C_8H_8O_4$	14.12	$[M-H]^-$	168.15	ATE
2.	Protocatechuic acid	$C_7H_6O_4$	17.71	$[M-H]^-$	154.12	ATE
3.	Caffeic acid	$(HO)_2C_6H_3CH{=}CHCO_2H$	19.78	$[M-H]^-$	180.16	ATE
4.	Psoralidin	$C_{20}H_{16}O_5$	9.07	$[M-H]^+$	336.09	ATE
5.	Butylparaben	$C_{11}H_{14}O_3$	10.93	$[M-H]^-$	194.09	ASE
6.	Phloroglucinol	$C_6H_6O_3$	4.60	$[M-H]^+$	126.03	ASE
Flavonoids						

7.	Kaempferol-3-Galactoside-6''-Rhamnoside-3'''-Rhamnoside	$C_{33}H_{40}O_{19}$	9.96	$[M-H]^-$	740.67	ATE
8.	Quercetin-3β-D-glucoside	$C_{12}H_{20}O_{12}$	9.93	$[M-H]^-$	464.38	ATE
9.	5,6,7-trihydroxy-2-(4-methoxyphenyl)-4H-chromen-4-one	$C_{16}H_{12}O_6$	14.38	$[M-H]^+$	302.2	ATE
Flavonols						
10.	Rutin	$C_{27}H_{30}O_{16}$	9.33	$[M-H]^-$	610.15	ATE
11.	Isorhamnetin	$C_{16}H_{12}O_7$	10.68	$[M-H]^+$	316.05	ATE
12.	Catechin	$C_{15}H_{14}O_6$	14.80	$[M-H]^+$	290.07	ATE
Flavones						
13.	Luteolin	$C_{15}H_{10}O_6$	26.03	$[M-H]^-$	286.23	ATE
14.	Cynaroside	$C_{21}H_{20}O_{11}$	31.07	$[M-H]^-$	448.37	ATE
15.	Tangeritin	$C_{20}H_{20}O_7$	16.48	$[M-H]^+$	317.72	ATE
Terpenoids (Diterpenoids)						
16.	2-[(1S,2S,4aR,8aS)-1-hydroxy-4a-methyl-8-methylidene-1,2,3,4,5,6,7,8a octahydronaphthalen-2-yl]prop-2-enoic acid	$C_{15}H_{22}O_3$	1.26	$[M-H]^-$	250.15	ASE
17.	(1R,4aS,5R,8aS)-5-(5-hydroxy-3-methylpentyl)-1,4a-dimethyl-6-methylidene-3,4,5,7,8,8a-hexahydro-2H-naphthalene-1-carboxylic acid.	$C_{20}H_{34}O_3$	1.29	$[M-H]^-$	322.5	ASE
Coumarins						
18.	Scopoletin	$C_{10}H_8O_4$	13.03	$[M-H]^+$	192.04	ATE
Saponins						
19.	Diosgenin	$C_{27}H_{42}O_3$	23.30	$[M-H]^+$	414.31	ATE
Alkaloids						
20.	Trigonelline	$C_7H_7NO_2$	1.30	$[M-H]^+$	137.14	ASE
21.	Arecoline	$C_8H_{13}NO_2$	1.39	$[M-H]^+$	155.09	ASE
22.	Nicotine	$C_{10}H_{14}N_2$	6.20	$[M-H]^+$	162.23	ASE
Glycosides						
23.	Kaempferol	$C_{27}H_{30}O_{14}$	13.72	$[M-H]^+$	286.23	ATE
Others						
24.	Betaine	$C_5H_{11}NO_2$	0.09 - 32.41	$[M-H]^+$	117.15	ASE

Conclusion:

In conclusion, the study on *Alternanthera sessilis* and *Alternanthera tenella* from Andhra Pradesh revealed valuable insights into the phytochemical composition, antioxidant properties, and total polyphenol content of these wild food plants. The findings underscore the potential health benefits and nutritional value of these plant species, highlighting their significance in traditional diets and potential applications in functional foods and nutraceuticals. Further research is warranted to explore the therapeutic potential and bioactive compounds present in these indigenous plants for human health and well-being.

Declaration

The authors declare no conflict of interest

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References:

1. Alara OR, Abdurahman NH, Ukaegbu CI (2021) Extraction of phenolic compounds: A review. Curr Res Food Sci 4:200–214. https://doi.org/https://doi.org/10.1016/j.crfs.2021.03.011
2. Aniszewski T (2015) Chapter 1 – Definition, typology, and occurrence of alkaloids. In: Aniszewski T (ed) Alkaloids (Second Edition), Second Edi. Elsevier, Boston, pp 1–97
3. Aryal S, Baniya MK, Danekhu K, et al (2019) Total Phenolic content, Flavonoid content and antioxidant potential of wild vegetables from western Nepal. Plants 8:. https://doi.org/10.3390/plants8040096
4. Chaves N, Santiago A, Alías JC (2020) Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. Antioxidants 9:. https://doi.org/10.3390/antiox9010076
5. Deladino L, Alvarez I, De Ancos B, et al (2017) Betalains and phenolic compounds of leaves and stems of Alternanthera brasiliana and Alternanthera tenella. Food Res Int 97:240–249. https://doi.org/10.1016/j.foodres.2017.04.017
6. Derbel H, Elleuch J, Mahfoudh W, et al (2023) applied sciences In Vitro Antioxidant and Anti-Inflammatory Activities of Bioactive Proteins and Peptides from Rhodomonas sp .
7. Dubale S, Kebebe D, Zeynudin A, et al (2023) Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. J Exp Pharmacol 15:51–62. https://doi.org/10.2147/JEP.S379805
8. Guerra RNM, Pereira HAW, Silveira LMS, Olea RSG (2003) Immunomodulatory properties of Alternanthera tenella Colla aqueous extracts in mice. Brazilian J Med Biol Res 36:1215–1219. https://doi.org/10.1590/S0100-879X2003000900011
9. Kanagarasu R, Bhavan PS, Rajkumar G (2017) Phytochemical Characterization of Alternanthera sessilis and Assessment of its Growth Promoting Potential on the Freshwater Prawn Macrobrachium rosenbergii. Int J Res Stud Zool 3:25–38. https://doi.org/10.20431/2454-941x.0304004
10. Luna-Guevara ML, Luna-Guevara JJ, Hernández-Carranza P, et al (2018) Chapter 3 – Phenolic Compounds: A Good Choice Against Chronic Degenerative Diseases. In: Atta-ur-Rahman (ed). Elsevier, pp 79–108
11. Manickam S, Rajagopalan VR, Kambale R, et al (2023) Plant Metabolomics: Current Initiatives

and Future Prospects. Curr Issues Mol Biol 45:8894–8906. https://doi.org/10.3390/cimb45110558

12. Mohd Hazli UHA, Abdul-Aziz A, Mat-Junit S, et al (2019) Solid-liquid extraction of bioactive compounds with antioxidant potential from Alternanthera sesillis (red) and identification of the polyphenols using UHPLC-QqQ-MS/MS. Food Res Int 115:241–250. https://doi.org/10.1016/j.foodres.2018.08.094
13. Nahari MH, Ali A Al, Asiri A, et al (2022) Green Synthesis and Characterization of Iron Nanoparticles Synthesized from Aqueous Leaf Extract of Vitex leucoxylon and Its Biomedical Applications
14. Oracz J, Nebesny E (2019) Effect of roasting parameters on the physicochemical characteristics of high-molecular-weight Maillard reaction products isolated from cocoa beans of different Theobroma cacao L. groups. Eur Food Res Technol 245:111–128. https://doi.org/10.1007/s00217-018-3144-y
15. Ozturk HI, Nas H, Ekinci M, et al (2022) Antioxidant Activity, Phenolic Composition, and Hormone Content of Wild Edible Vegetables. Horticulturae 8:. https://doi.org/10.3390/horticulturae8050427
16. Pathak I, Budhathoki R (2020) Phytochemical Screening, Cytotoxic and Antioxidant Activity of Alternathera sessilis and Moringa oleifera. https://doi.org/10.3126/arj.v1i1.32456
17. R.P. H, R. SS, G. R (2021) Dependency and economic benefits of the use of wild food plants among tribal communities in Malai Madeshawara Hills wildlife sanctuary, Southern india. Futur Food J Food, Agric Soc 9:1–19. https://doi.org/10.17170/kobra-202011192211
18. Reddy KN, Pattanaik C, Reddy CS, Raju VS (2007) Traditional knowledge on wild food plants in Andhra Pradesh. Indian J Tradit Knowl 6:223–229
19. Sergio L, Boari F, Pieralice M, et al (2020) Bioactive phenolics and antioxidant capacity of some wild edible greens as affected by different cooking treatments. Foods 9:. https://doi.org/10.3390/foods9091320
20. Shah SMZ, Ramzan M, Khan MN, et al (2023) Untargeted screening of plant metabolites based on data-independent and data-dependent acquisition modes using LC-ESI-QTOF-MS: Tribulus terrestris L. as a case study. Arab J Chem 16:104978. https://doi.org/10.1016/j.arabjc.2023.104978
21. Shahidi F, Ambigaipalan P (2015) Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review. J Funct Foods 18:820–897. https://doi.org/10.1016/j.jff.2015.06.018
22. Shetty PG (2019) IN-VITRO ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF LEUCAS INDICA AND ALTERNANTHERA TENELLA . 6:418–424
23. Singh M, Patra S, Singh RK (2021) 15 - Common techniques and methods for screening of natural products for developing of anticancer drugs. In: Srivastava AK, Kannaujiya VK, Singh RK, Singh D (eds) Evolutionary Diversity as a Source for Anticancer Molecules. Academic Press, pp 323–353
24. Singla RK, Dhir V, Madaan R, et al (2022a) The Genus Alternanthera: Phytochemical and Ethnopharmacological Perspectives. Front Pharmacol 13:. https://doi.org/10.3389/fphar.2022.769111
25. Singla RK, Dhir V, Madaan R, et al (2022b) The Genus Alternanthera: Phytochemical and Ethnopharmacological Perspectives. Front Pharmacol 13:769111. https://doi.org/10.3389/fphar.2022.769111
26. Smanski MJ, Peterson RM, Shen B (2012) Chapter Eight – Platensimycin and Platencin

Biosynthesis in Streptomyces platensis, Showcasing Discovery and Characterization of Novel Bacterial Diterpene Synthases. In: Hopwood DA (ed) Natural Product Biosynthesis by Microorganisms and Plants, Part A. Academic Press, pp 163–186

27. Thomas DS (2020) Expression of Silk Sericin–Cecropin B Fusion Protein in Pichia pastoris and Cell-Free System. Biosci Biotechnol Res Commun 13:975–979. https://doi.org/10.21786/bbrc/13.2/87
28. Trugo LC, von Baer D, von Baer E (2003) LUPIN. In: Caballero B (ed) Encyclopedia of Food Sciences and Nutrition (Second Edition), Second Edi. Academic Press, Oxford, pp 3623–3629
29. Yap CH, Mat Junit S, Abdul Aziz A, Kong KW (2019) Multiple extraction conditions to produce phytochemical- and antioxidant-rich Alternanthera sessilis (red) extracts that attenuate lipid accumulation in steatotic HepG2 cells. Food Biosci 32:100489. https://doi.org/https://doi.org/10.1016/j.fbio.2019.100489
30. Zhang L, Han Z, Granato D (2021) Chapter One - Polyphenols in foods: Classification, methods of identification, and nutritional aspects in human health. In: Granato D (ed) Application of Polyphenols in Foods and Food Models. Academic Press, pp 1–33