

Quantification of Chemical Constituents in Dry Leaf Extracts of Selected Mangrove Plant Species in Godavari Region of Andhra Pradesh, India

D.Sravani^{1*}, P.V.S.Machiraju², S.Satya veni³

^{1*}Research Scholar, Department of Chemistry, JNTU University, Kakinada-533003, India

¹Department of H&BS, Aditya College of Engineering and Technology, Surampalem-533437, India

²Department of Chemistry, Pragati Engineering College, Surampalem-533437, India

³Department of Chemistry, JNTU University Kakinada-533003, India

Abstract:

Acanthus and *Excoeceria* genus belonging to the *Acanthaceae* and *Euphorbiaceae* families has been reported to be rich source of micronutrients, minerals, trace elements and antinutrients with various bioactivities. *Acanthus ilicifolius* and *Excoeceria agallocha* are Mangrove plant species are located in Coringa region near estuaries of East Godavari region in Andhra Pradesh state of India. The Plant leaves are rich source of nutrients, antinutrients and are used as traditional medicine for treatment of arthritis, skin, bacterial and fungal diseases. In the present research study the *Acanthus* and *Excoeceria* plant leaves are collected for quantifying the chemical constituents like micronutrients, minerals, trace elements and antinutrients in dry leaf extracts from solvents methanol, hexane and ethyl acetate. Microwave digestion has been employed to collect the plant extract from the solvents. ICP-OES technique has been used to find the concentration of micronutrients, minerals, trace elements. Titrimetry has been used for quantification of Antinutrients. The research study results confirmed the presence of micronutrients, minerals, trace elements and are present in significant quantities and hence can be used in making formulations by the pharmaceutical personnel in the preparation of medicines.

Keywords: Mangrove, Extract, Mineral, Trace element, Antinutrient Classification numbers

1. Introduction

Mangroves are located in the East and West coastal regions of Southern India. It is one of the second largest Mangroves after Sundarbans. Coringa mangroves of Andhra Pradesh are one of the critical habitats in India. Coringa mangroves receive fresh water from the Gaderu and Coringa rivers, Gautami-Godavari River and Nectar Rivers of Kakinada bay. In Coringa Mangroves, a total of 35 mangroves species in existence and *Excoeceria* especially are dominant. The species of *Acanthus ilicifolius* and *Excoeceria.agallocha* which are medicinally highly potential were selected for study.

Mangrove forests act as source as unique biologically significant ecosystem and become most productive ecosystems in the world and can benefit both coastal and marine ecosystems and human society [1-2]. They safeguard coastal region from the damaging effects of erosion and act as breeding and nursery grounds for a number of

marine fauna species.[3]. In tropical coastal locations, mangrove ecosystems are crucial to the biogeochemistry of heavy metal pollutants [4]. Due to their toxicity, endurance and problems with bioaccumulation caused by their prolonged residence in food chains and heavy metals are one of the most dangerous pollutants in the natural environment [5]. Mangrove species have been used in numerous studies as trustworthy bio-indicators for heavy metal contamination and pollution [6–9]. As part of our research study of *A.ilicifolius* and *E.agallocha* plant leaves were collected and the dry leaf extracts are collected from solvents and characterized for micronutrients, minerals, and trace elements, antinutrients for quantification.

2. Materials and Methods

2.1. General

ICP-OES Technique is hyphenated technique is employed for quantification of micronutrients, minerals, and trace elements. The extraction of dry

plant leaves process from the solvent like methanol, hexane and ethyl acetate by soxhlet apparatus is utilized.

2.2. Plant material

In Coringa Mangroves 35 plant species belonging to 24 families are in existence. Out of these two plant

species *A.ilicifolius*, *E.agallocha* which are enriched with high medicinal value and nutritional value are selected for the present research study. The photographs of the plant species are presented in the figures-(1)



Figure 1 The plant photographs of *A.ilicifolius* *E.agallocha*

2.2.Extraction and isolation

The leaves of *A.ilicifolius*, *E.agallocha* were washed with distilled water and dried under shade. The dried leaves were grinded to powder and stored at 4°C for analysis and dissolved in various solvents like methanol, ethyl acetate and hexane and are extracted by soxhlet process.10 mL of HNO₃ and 5 mL of H₂O₂ were added to the extract and digested in a microwave for analysis of micronutrients, minerals, and trace elements. The microwave digested plant extract is diluted with distilled water further and analysed by ICP-OES technique for determining the concentration of metal ions (micronutrients, minerals and trace elements).

2.3. Quantification of Nutrients

2.3.1. Micronutrient analysis:

2g of finely powdered *A.ilicifolius*, *E.agallocha* leaves were digested by using 30% H₂O₂ and concentrated HNO₃. and kept overnight and processed for Micro-oven acid digestion and analysed with ICP-OES technique for measuring the concentrations of micronutrients such as B,Al,Mn,Mo,Ni and Co.

2.3.2. Mineral analysis:

2g of finely powdered of *A.ilicifolius*, *E.agallocha* leaves were digested by using 30% H₂O₂ and concentrated HNO₃ and kept overnight and processed for Micro-oven acid digestion and analysed with ICP-OES technique for estimation of mineral such as K, Mg, Fe, Cu and Zn.

2.3.3. Trace Elemental analysis:

2g of finely powdered *A.ilicifolius*, *E.agallocha* leaves were digested using 30% H₂O₂ and concentrated HNO₃ and kept overnight and processed for Micro-oven acid digestion and then analysed with ICP-OES technique to quantify trace elements such as Pb, Cr and Hg.

2.4. Quantification of Antinutrients

2.4.1 .Phytic acid content:

2g of plant extract is taken in 250 mL conical flask and 100 mL of 2% HCl were added and kept for 3hrs and filtered.50 mL filtrate is diluted further and 10 mL of 0.3% ammonium thiocyanate were added and titrated against standard Ferrous Chloride (0.00195g) solution till it changes to yellow colour (end point) and noted as titre value as V₂ mL [10].The % of phytic acid can be calculated by using the relation.

$$\% \text{ Phytic acid} = Y \times 1.19 \times 100 \text{ where } Y = V_2 \times 0.00195g$$

2.4.2. Vitamin C content:

20 mL of plant extracts were diluted with water and 1mL of starch solution was added and is titrated against 0.05N triiodide solution till blue black colour is obtained at end point was noted. The volume of triiodide is noted as V₁ and the Normality of the solution of triiodide is determined for quantification of vitamin C is quantified by using the relation.

$$N_5 \times V_5 = N_1 \times V_1$$

$$\text{Vitamin C} = \frac{\text{Normality of triiodide} \times 88 \times 100}{N_s}$$

N_s=Normality of sample

1000 Vs =volume of sample

N_i=Normality of triiodide

V_i=volume of triiodide

2.4.3. Total Oxalate content:

1g of powdered plant leaves is taken in 100 mL conical flask and 75mL of 3M H₂SO₄ is added and stirred for 1hr with magnetic stirrer and filtered by employing whatmann no 1 filter paper and 25 mL filtrate is heated to 50-60°C and titrated against 0.05M KMnO₄ solution and till it changes colourless to pink colour.[11-12].The quantity of oxalate content has been calculated depending on the fact that for 1mL of 0.05M KMnO₄ contain 2.2mg of oxalate content is present[13].

3. Results and Discussion

3.1. Quantification of Micronutrients of *A.ilicifolius* leaf extract

Higher quantity of Boron is found to be present in *A.ilicifolius* collected from ethyl acetate (11.24

ppm) compared to the extracts collected from hexane (5.77 ppm) and methanol (4.28 ppm).Higher quantity of Aluminium is found to be present in *A.ilicifolius* leaf extract collected from hexane (17.43 ppm) compared to the extracts from ethyl acetate (10.82 ppm) and methanol (5.02 ppm) respectively. Higher quantity of manganese is found to be present in *A.ilicifolius* leaf extract obtained from hexane (2.03 ppm) whereas the ethyl acetate and methanol as solvent Mn was found to be in less quantities (1.52 ppm) and (1.18 ppm) respectively.

Higher quantity of Nickel is found to be present in *A.ilicifolius* leaf extract obtained from hexane (0.53 ppm) and lower quantities (0.30 ppm) are observed in methanol and ethyl acetate extracts.Equal quantities of molybdenum and cobalt are found to be present in *A.ilicifolius* leaf extract collected from methanol, hexane and ethyl acetate as a solvents observed for trace quantities (0.1)ppm.

Table 1.Micronutrients in plant leaf extracts of *A.ilicifolius* collected from various solvents methanol, hexane and ethyl acetate.

Micronutrients	AL/M ppm	AL/H ppm	AL/EA ppm	Quantification Order
B	4.28	5.77	11.24	AL/EA>AL/H>AL/M
Al	5.02	17.43	10.83	AL/H>AL/EA>AL/M
Mn	1.18	2.03	1.52	AL/H>AL/EA>AL/M
Mo	0.14	<0.10	<0.10	AL/M>AL/H=AL/EA
Ni	0.30	0.53	0.30	AL/H>AL/M=AL/EA
Co	<0.10	<0.10	<0.10	AL/M=AL/H=AL/EA

AL/M- *A.ilicifolius* leaf Methanol,AL/H- *A.ilicifolius* leaf Hexane,AL/EA- *A.ilicifolius* leaf Ethyl acetate

3.2. Quantification of Micronutrients of *E.agallocha* leaf extract

Higher quantity of Boron is found to be present in the *E.agallocha* leaf extract obtained from ethyl acetate (6.90 ppm) compared to the extracts from methanol (5.95 ppm) and hexane (5.09 ppm) respectively. Higher quantity of Aluminium is found to be present in the *E.agallocha* leaf extract collected from ethyl acetate (7.92 ppm) compared

to the extracts from methanol (6.06 ppm) and hexane (5.37 ppm) respectively. Higher quantity of manganese is found to be present in the *E.agallocha* leaf extract collected from methanol (0.55 ppm). But with less quantities in ethyl acetate and hexane as solvent extracts (0.44 ppm) and (0.42 ppm) respectively. Higher quantity of Nickel found to be present in the *E.agallocha* leaf extract collected from methanol (0.44 ppm) and less

weight as ethyl acetate and hexane as a solvents (0.29 ppm) and (0.28 ppm) respectively. Equal quantites of molybdenum and cobalt (Mo & Co) are found to be present in the *E.agallocha* leaf extract collected from methanol, hexane and ethyl

acetate and observed the Mo and Co as trace quantities (0.1) ppm each respectively. The values of Micronutrients in leaf extracts of *E.agallocha* collected from various solvents methanol, hexane and ethyl acetate are presented in the Table 2.

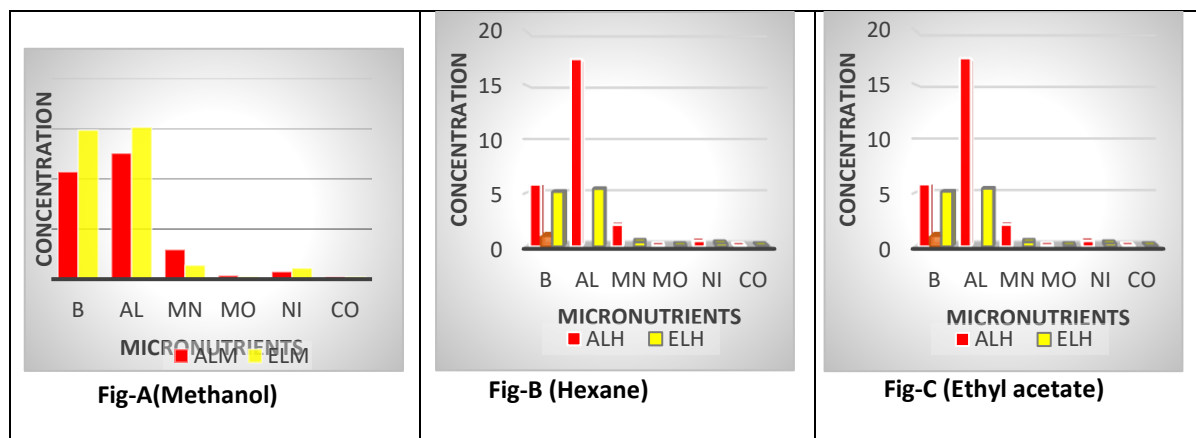
Table 2. Micronutrients in leaf extracts of E.agallocha collected from various solvents methanol, hexane

Micronutrients	EL/M ppm	EL/H ppm	EL/EA ppm	Quantification Order
B	5.95	5.09	6.90	EL/EA>EL/M>EL/H
Al	6.06	5.37	7.92	EL/EA>EL/M>EL/H
Mn	0.55	0.42	0.44	EL/M>EL/EA>EL/H
Mo	<0.10	<0.10	<0.10	EL/M=EL/H=EL/EA
Ni	0.44	0.28	0.29	EL/M>EL/EA>EL/H
Co	<0.10	<0.10	<0.10	EL/M=EL/H=EL/EA

and ethyl acetate.

EL/M-Excoeceria.agallocha leaf in Methanol,EL/H-E.agallocha leaf in Hexane,EL/EA-E.agallocha in Ethyl acetate

The Graphical representation of Micronutrients of *A.ilicifolius* and *E.agallocha* plant species are presented in Figures- A, B, C.



AL/M- *A.ilicifolius* leaf in Methanol, **AL/H-** *A.ilicifolius* leaf in Hexane,**AL/EA-** *A.ilicifolius* leaf in Ethyl acetate,**EL/M-** *E.agallocha* leaf in Methanol,**EL/H-** *E.agallocha* leaf in Hexane,**EL/EA-** *E.agallocha* leaf in Ethyl acetate

3.3.Quantification of Minerals from *A.ilicifolius* leaf extract

Higher concentration of Mg (820.2 ppm) is found to be present in hexane solvent collected from *A.ilicifolius* leaf extract compared to the extracts from methanol (187.3 ppm) and ethyl acetate (235.5 ppm) respectively. Higher quantity of Fe (46.52 ppm) is found to be present in hexane solvent collected from *A.ilicifolius* leaf extract compared to the extracts from methanol (6.94

ppm) and ethyl acetate (0.94 ppm) respectively. Higher quantity of K (0.21 ppm) is found to be present in hexane solvent collected from the *A.ilicifolius* leaf extract compared to the extracts from ethyl acetate (0.17 ppm) and methanol (0.15 ppm) respectively. Higher quantity of Cu (1.92 ppm) is found to be present in ethyl acetate solvent extracted from

A.ilicifolius leaf extract compared to extracts from hexane (0.96 ppm) and methanol (0.59 ppm) respectively. Higher quantity of Zn (11.71 ppm) is found to be present in hexane solvent extracted from *A.ilicifolius* leaf extract compared to the extracts from ethyl acetate (2.33 ppm) and methanol (1.77 ppm) respectively.

Table 3. Mineral content in leaf extracts of *A.ilicifolius* from various solvents methanol, hexane and ethyl acetate

Minerals	AL/M ppm	AL/H ppm	AL/EA ppm	Quantification Order
K	0.15	0.21	0.17	AL/H>AL/EA>AL/M
Mg	155.7	820.2	235.5	AL/H>AL/EA>AL/M
Fe	6.94	46.52	0.94	AL/H>AL/M>AL/EA
Cu	0.59	0.96	1.92	AL/EA>AL/H>AL/M
Zn	1.77	11.71	2.33	AL/H>AL/EA>AL/M

AL/M- *A.ilicifolius* leaf from Methanol, **AL/H-** *A.ilicifolius* leaf from Hexane, **AL/EA-** *A.ilicifolius* leaf from Ethyl acetate

3.4. Quantification of Minerals of *E.agallocha* leaf extract

Higher Quantity of Mg (925.7 ppm) is found to be present in hexane solvent extracted from the *E.agallocha* leaf extract compared to the extracts from methanol (72.71 ppm) and ethyl acetate (64.58 ppm) respectively. Higher Quantity of Fe (8.37 ppm) is found to be present in methanol solvent extracted from the *E.agallocha* leaf extract compared to the Fe extracted from ethyl acetate (8.23 ppm) and hexane (7.49 ppm) respectively.

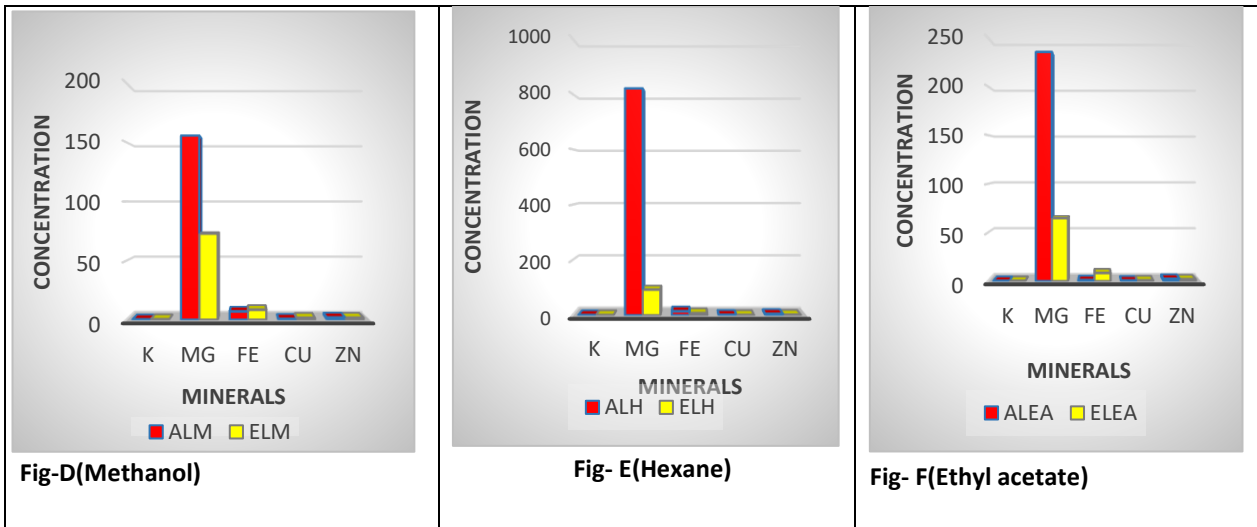
Higher Quantity of K (0.24 ppm) is found to be present in methanol solvent extracted from *E.agallocha* leaf extract compared to the extracts from hexane (0.22 ppm) and ethyl acetate (0.15 ppm) respectively. Higher Quantity of Cu (1.54 ppm) is found to be present in methanol solvent extracted from *E.agallocha* leaf extract compared to the extracts from hexane (0.85 ppm) and ethyl acetate (0.73 ppm) respectively.

Higher Quantity of Zn (1.91 ppm) is found to be present in ethyl acetate solvent extracted from *E.agallocha* leaf extract compared to the extracts from methanol and hexane was found to contain Zn (1.77 ppm) and (1.70 ppm) respectively.

Table 4. Details of Minerals in leaf extracts of *A.ilicifolius* collected from solvents methanol, hexane and ethyl acetate

Minerals	EL/M ppm	EL/H ppm	EL/EA ppm	Quantification Order
K	0.24	0.22	0.15	EL/M>EL/H>EL/EA
Mg	72.71	925.7	64.58	EL/H>EL/M>EL/EA
Fe	8.37	7.49	8.23	EL/M>EL/EA>EL/H
Cu	1.54	0.85	0.73	EL/M>EL/H>EL/EA
Zn	1.65	1.70	1.91	EL/EA>EL/H>EL/M

EL/M-*E.agallocha* leaf in Methanol,EL/H-*E.agallocha* leaf in Hexane,EL/EA-*E.agallocha* leaf in Ethyl acetate
The Graphical representation of mineral content of plant species are observed in Figures D, E, F respectively.



AL/M- *A.ilicifolius* leaf in Methanol,AL/H-*A.ilicifolius* leaf in Hexane,AL/EA- *A.ilicifolius* leaf in Ethyl acetate,EL/M-*E.agallocha* leaf in Methanol,EL/H-*E.agallocha* leaf in Hexane,EL/EA-*E.agallocha* leaf in Ethyl acetate

3.5. Quantification of Trace elements in *A.ilicifolius* leaf extract

Higher quantity of Pb (0.89 ppm) is found to be present in hexane solvent extracted from the *A.ilicifolius* leaf extract compared to the Pb extracts from methanol (0.53 ppm) and ethyl acetate (1.22

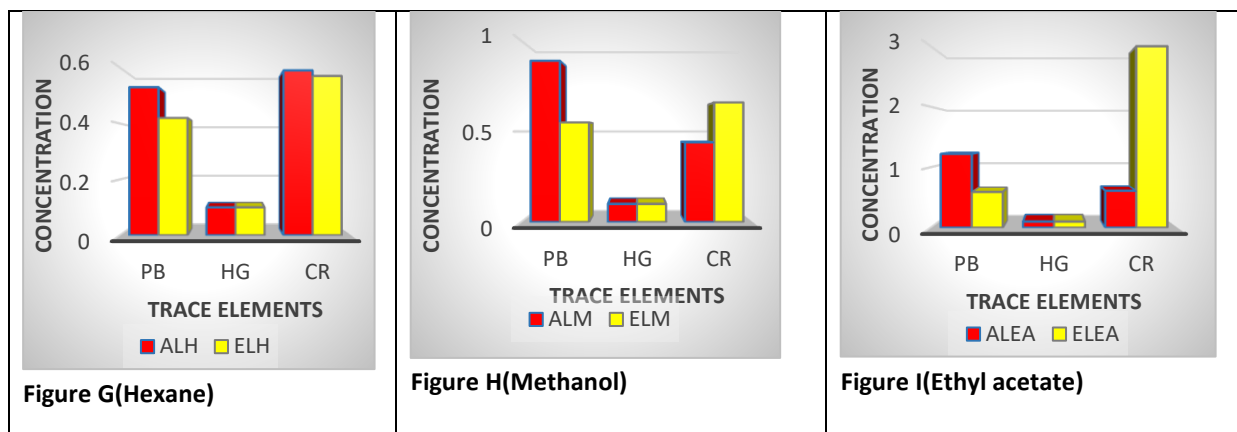
ppm) respectively. Equal quantites of Hg (0.1 ppm) is found to be present in methanol, hexane and ethyl acetate solvent extracts from *A.ilicifolius* leaf. Higher quantity of Cr (0.44 ppm) is found to be present in methanol solvent extracted from the *A.ilicifolius* leaf extract compared to the extracts from ethyl acetate (0.59 ppm) and hexane (0.74 ppm) respectively. The analytical data is represented in the table 5 and the graphical representation is presented the figures G,H,I respectively.

Table 5.Details of Trace elements in leaf extracts of *A.ilicifolius* from solvents methanol,hexane and ethyl acetate

Trace elements	AL/M ppm	AL/H ppm	AL/EA ppm	Quantification Order
Pb	0.89	0.53	1.22	AL/EA>AL/M>AL/H
Hg	0.1	0.1	0.1	AL/M=AL/H=AL/EA
Cr	0.44	0.59	0.74	AL/EA>AL/H>AL/M

AL/M- *A.ilicifolius* leaf from Methanol,AL/H- *A.ilicifolius* leaf from Hexane,AL/EA- *A.ilicifolius* leaf from Ethyl acetate

The Graphical representation of trace element concentrations of *A.ilicifolius* and *E.agallocha* are presented in Figure G,H,I



AL/M- *A.ilicifolius* leaf from Methanol, **AL/H-** *A.ilicifolius* leaf from Hexane, **AL/EA-** *A.ilicifolius* leaf from Ethyl acetate, **EL/M-** *E.agallocha* leaf from Methanol, **EL/H-** *E.agallocha* leaf from Hexane, **EL/EA-** *E.agallocha* leaf from Ethyl acetate

3.6. Quantification of Trace Elements of *E.agallocha* leaf extract

Higher quantity of Pb (0.59 ppm) is found to be present in ethyl acetate solvent extracted *E.agallocha* from the leaf extract compared to the extracts from methanol (0.55 ppm) and ethyl

acetate (0.42 ppm) respectively. Equal quantities of Hg (0.1 ppm) in the leaf extract of *E.agallocha* collected from methanol, hexane and ethyl acetate Hg was found to be the trace amounts as (0.1 ppm). Higher quantity of Cr (0.66 ppm) is found to be present in methanol as a solvent extracted from *E.agallocha* compared to the extracts from hexane and ethyl acetate as (0.57 ppm) and (0.59 ppm) respectively. The values are represented in table-4 and Figures-G, H, I. respectively.

Table 6. Details of Trace elements in leaf extracts of *E.agallocha* from solvents methanol, hexane and ethyl acetate are presented

Trace elements	EL/M ppm	EL/H ppm	EL/EA ppm	Quantification Order
Pb	0.55	0.42	0.59	EL/EA>EL/M>EL/H
Hg	0.1	0.1	0.1	EL/M=EL/H=EL/EA
Cr	0.66	0.57	0.59	EL/M>EL/H>EL/EA

EL/M- *E.agallocha* leaf from Methanol, **EL/H-** *E.agallocha* leaf from Hexane, **EL/EA-** *E.agallocha* leaf from Ethyl acetate

3.7. Quantification of Antinutrients:

Antinutrients contains natural or artificial compounds that inhibit nutrients intake. Antinutrients may also act as binding agent to vitamins and minerals and inhibits their uptake. Antinutrients like total oxalate content, phytic acid and vitamin C in plant extracts were quantified and results are presented in table 6 and 7

3.7.1. Antinutrients in leaf extract of *A.ilicifolius*.

Higher quantity of Total oxalate content 49.28mg/mL is found to present in methanolic leaf

extract of *A.ilicifolius*. The values are recorded in Table 7 and the represented graphically in figure J. Lesser quantity of phytic acid 9.861% is found to present in methanolic leaf extract of *A.ilicifolius*. The values are recorded in Table 7 and the represented graphically in figure K. Lower quantity of vitamin C (0.0704mg) is found to present in methanolic leaf extract of *A.ilicifolius*. The values are recorded in Table-7 and the represented graphically in figure L.

Table 7. Details of Antinutrients in *A.ilicifolius* leaf extract collected from methanol solvent.

Antinutrients	AIL
Total oxalate content mg/ dry wt	49.28 mg/dry wt.
% Phytic acid	9.861%
Vitamin C	0.0704 mg

AIL- *A.ilicifolius* leaf extract

3.7.2. Antinutrients in leaf extract of *E.agallocha*

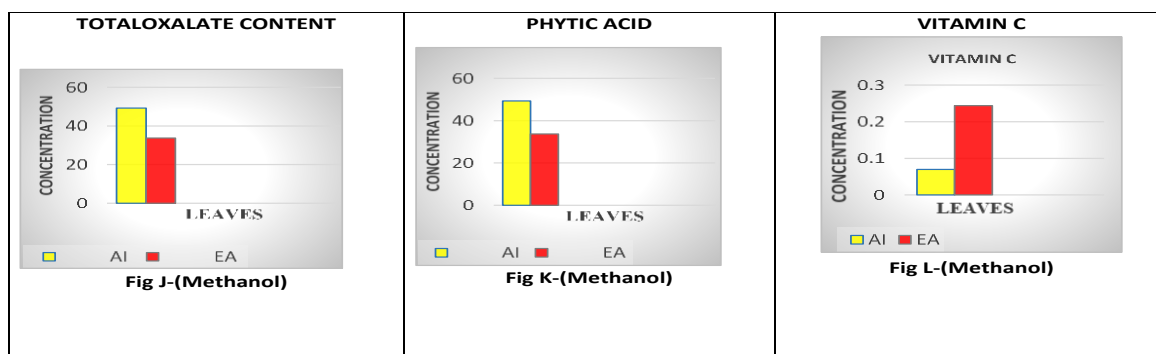
Higher quantity of Total oxalate content (33.73 mg/mL) is found to present in *E.agallocha*. The values are recorded in Table 8 and the represented graphically in figure J. Higher quantity of Phytic acid (18.56%) is found to present in methanolic leaf

extract of *E.agallocha*. The values are recorded in Table 8 and the represented graphically in figure K. Trace quantity of Vitamin C (0.2442 mg) is found to present in methanolic leaf extract of *E.agallocha* leaf extract. The values are recorded in Table-8 and the represented graphically in figure L.

Table 8.Details of Antinutrients in leaf extracts of *E.agallocha*

Antinutrients	EAL
Total oxalate content mg/ dry wt	33.73 mg/dry wt.
% Phytic acid	18.56%
Vitamin C	0.2442 mg

EAL- *E.agallocha* leaf extract



The Graphical representation of concentrations of Antinutrients in *A.ilicifolius*. and *E.agallocha* are presented in Figures J,K, and L.

EL/M-*EAgallocha* leaf from Methanol, **EL/H**-*E.agallocha* leaf Hexane, **EL/EA**-*E.agallocha* from Ethyl acetate
AIL- *A.ilicifolius* leaf from methanol **EL/M**-*EAgallocha* leaf from Methanol,

4. Conclusions

The research results indicate that the micronutrients B and Al are present with significant concentrations in the two species *A.ilicifolius* and *EAgallocha* whereas Mn concentration is more in *A.ilicifolius* species. Micronutrients Mo, Ni and Co are observed with lower concentration in all the two species. The mineral ion Mg concentration was found to more compared to K, Fe, Cu and Zinc respectively. The trace element Cr Concentration

was found to be higher in *E.agallocha* compared to the *A.ilicifolius*. The trace element Hg was found to be with same concentration in two plant species. But the trace element Pb was found to be lower in all species. The Antinutrients Total oxalate content is more in *A.ilicifolius* leaf extract compared to other Antinutrients, Phytic acid and vitamin C, while the Antinutrients, Phytic acid content is more in *E.agallocha* leaf extract compared to other Antinutrients Total oxalate content and vitamin C.

The research results confirmed the presence of micronutrients, minerals, trace elements. The plant species can be considered as potential sources for medicinal values and can be considered for use in the selected pharmaceutical preparations and also be employed in new drug design useful for the mankind.

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Credit Authorship statement. The First Corresponding author Dr.P.V.S.Machiraju of equally responsible for manuscript and responsible for plagiarism checking, Reviewing, editing and corrections process and D.Sravani responsible sample preparation, data analysis, data interpretation and editing process. S.Satyaveni is responsible for manuscript checking, reviewing and Corrections.

Declaration of completing interest. The authors declare the no financial interest or personal relationships that could have appeared with influence the work reported on this paper.

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