Phytochemical analysis of herbal extracts prepared using different solvents of two Indian medicinal plants *Aerva lanata* and *Curcuma caesia*

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ABSTRACT: The objective of this study is to evaluate the pharmacognostic and phytochemical analysis of two Indian medicinal plants for antihyperlipidimic studies. In this study *Aerva lanata* belongs to family (Amaranthaceae) and *Curcuma caesia* which is a member of the Zingiberaceae family were studied. The proximate analysis , Phytochemical Screening, Isolation , Instrumental analysis of isolated compounds were performed .Results were obtained which showed the proximate analysis such as as acid insoluble ash ,water soluble ash ,alcohol soluble extractive value ,water soluble extractive value, ether soluble extractive value, loss on drying were 3.0 ,10.0 15.0, 22.0, 4.0 and 13.5 % in roots of *Aerva lanata* and 2.6, 11.2, 16.3 ,23.3, 3.1 and 14.1% in rhizomes of *Curcuma caesia.* The percentage yield were found to be highest in methanolic extract ie 11.8% in *Aerva lanata* 1.545% in water extract. Rf value of both the plants extracts were found to be 0.45 and 0.54 in successive toluene extract for alkaloid in *Aerva lanata* where as *Rf value* were 0.55 in successive ethyl acetate extract for flavanoids in *Curcuma caesia.* From the above study it was concluded that both the plants have various phytoconstituents such as a alkaloids, flavanoid, phenolics, carbohydrates and Phytosterols which can be responsible for various biological and pharmacological studies

Key words: *Aerva lanata*, *Curcuma caesia,* Phytochemicals, Isolation, Characterization

1. INTRODUCTION

Since the beginning of time, various diseases have been treated with plants. The early man went in search of sustenance and consumed both the whole plant and its pieces. They gradually came to understand the importance of some plants and their components for human health. Men's insatiable curiosity and ongoing struggle for survival led to the eventual relevance and acknowledgement of therapeutic plants..[2-4]

Plants have played a significant role in the history of medications from ancient times, and this relationship continues now with the widespread use of plant products in traditional medicine, ethnomedicine, and the arsenal of contemporary doctors. The primary component of a sensible health care programme in many developing nations is phytopharmaceuticals..[5,6]

Because only the illiterate "medicine men" from the various tribes, who are frequently dismissed as quacks or cheap jacks by the educated population, disseminate the knowledge of the valuable drugs, it frequently disappears without any record or documentation. Allopathic doctors cannot overestimate the value of these medications because new fields of research and technology have not been successful in advancing humankind. Nature has always provided a solution, and with this recently demonstrated interest, the traditional medical system in India is experiencing a revival. To prevent repeating our earliest ignorance and negligence, scientists and researchers are conducting a systematic documentation on the minute study of these plants.[7,8]

Further, it can be said that medicinal plants are a plentiful renewable resource that, when used wisely, not only enhances quality of life but also offers rational healthcare at a low cost with few side effects and significantly aids in the development and growth of their economies.[9]

Locally referred to as "bui," *Aerva lanata* Juss. (Amaranthaceae) is a common weed found in fields and waste areas all over India. It is an erect, prostrate undershrub. The diuretic plant is used to treat lithiasis. The root is demulcent, diuretic, and helpful for strangury (slow and uncomfortable urine output). The roots are applied to headache relief. On the Malabar Coast, the plant is regarded as a demulcent. In Ceylon, it is appreciated as a cough remedy and as a paediatric vermifuge. Patients with liver congestion, jaundice, biliousness, and dyspepsia are given the juice of the roots orally by the Meena tribal people of Rajasthan's Sawaimadhopur area. They also provide a whole plant decoction to treat protracted fevers like typhoid, pneumonia, and others.[10, 11]

The big genus *Curcuma caesia* is a member of the Zingiberaceae family. Curcuma caesia, a perennial herb endemic to North and Central India, has bluish-black rhizomes. Additionally, Papi Hills in the Andhra Pradesh districts of East Godavari, West Godavari, and Khammam contain sporadic black turmeric. Due to its alleged therapeutic benefits, the rhizomes of kali haldi are highly significant economically.[12] In order to treat smooth muscle relaxant action, the rhizomes are employed. Haemorrhoids, leprosy, asthma, cancer, epilepsy, fever, wound, vomiting, menstrual disorder, anthelmentic, aphrodisiac, inflammation, gonorrhoeal discharges, etc [13] . The plant is particularly auspicious in Madhya Pradesh, where it is said that those who possess it will never run out of food and cereal. The plant's rhizomes have a pleasant scent. Leucoderma, asthma, tumours, piles, bronchitis, etc. are among the conditions that are treated with *Curcuma caesia* Roxb. rhizomes.[14]

Some medicinal plants have been used empirically in antidiabetic and antihyperlipidemic remedies and have been reported to be helpful in diabetes worldwide. The primary mechanisms through which plants have antihyperglycemic activity are their capacity to boost insulin secretion, restrict glucose absorption

from the intestine, or facilitate metabolites in insulin-dependent activities. Although there are more than 400 plant species with hypoglycemic activity documented in literature, finding new antidiabetic drugs from natural plants is still appealing because they contain compounds that have different and safe effects on diabetes mellitus. The majority of plants include compounds that are widely suggested to have antidiabetic effects, including glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc.[15] The objective of this study is to evaluate the pharmacognostic and phytochemical analysis of two Indian medicinal plants for antihyperlipidimic studies

2. MATERIAL AND METHODS:

2.1 Collection and identification of plant material

The herbal medicinal plants and their parts such as roots and rhizomes of *Aerva lanata* and *Curcuma caesia* respectively were collected from Bhimbetka Bhojpur, Bhopal, and Madhya Pradesh. The selected plants parts were further authenticated by expert botanist Department of Botany, Barkatullah University, Bhopal (MP). The plant specimens were compared with voucher specimen (voucher specimen No. BU /BOT/03/69/805)

2.2 Proximate Analysis

Proximate analysis of powdered plant materials (Roots of *Aerva lanata* and rhizomes of *Curcuma caesia*) were carried out as per WHO guidelines i.e. loss on drying, ash value, extractive value and in organic elements like heavy metals by using standard method.[16]

2.3 Phytochemical Screening

Preparation of extracts:

The fresh parts of selected materials such as roots of *Aerva lanata* and rhizomes of *Curcuma caesia* were shade-dried, cut into pieces, coarsely powdered and successively extracted separately using various solvents in increasing order of polarity (Pet. Ether < Toluene < Chloroform < Ethyl Acetate < Methanol < Water). The colour, consistency and percentage yield of different extracts were noted and the extracts were preserved under vacuum till further processed.17

Preliminary chemical tests

Preliminary chemical tests were carried out for differents extract to identify different phyto-constituents presence.Such as Flavonoids ,alkaloids ,phytosterols tannins, triterpenoids, saponins, and cardiac glycosides were performed using standard methods.18,19

2.4 Isolation of Phytoconstituents from the selected fractions of both plants:

The basified Toluene sub-fraction was optimized for isolation of alkaloids from roots of *Aerva lanata* and Ethyl Acetate extract was optimized for isolation of phenolic compounds from rhizomes of *Curcuma caesia*, isolated using preparative TLC method for both the plants.[20]

2.4.1 Preparation of Alkaloid fraction from roots of *Aerva lanata*:

Procedure-1:

a) Powdered root basified with 10 % NH_3

b) Extracted with $CHCl_3$ and concentrated.

c) Extracted with equal volumes of dil. HCl and pH adjusted to 10 with NH_3.

d) Extracted with $CHCl_3$

i) $CHCl_3$ fraction at pH 10

ii) Aqueous acidic layer pH adjusted to 11

↓

Extracted with $CHCl_3$ → Aqueous acidic layer pH Adjusted to 12

↓

$CHCl_3$ fraction at pH 11

↓

Extracted with $CHCl_3$

↓

$CHCl_3$ fraction at pH 12

Procedure 2:

a) Hydro alcoholic solution of dried MeOH crude extract was prepared in the ratios of 1:1, 1:2 and 2:1.

b) Filtrate was adjusted to pH 8 with alcoholic KOH.

c) Successive solvent extraction.

i) n-hexane fraction

ii) Petroleum ether fraction

iii) Toluene fraction

iv) $CHCl_3$ fraction

v) Ethyl acetate fraction

vi) Acetone fraction

2.4.2 Preparation of Phenolic fraction from rhizomes of *Curcuma caesia*

Procedure:

a) Dried rhizomes powder of *Curcuma caesia* was subjected to successive solvent extraction using soxhlation technique.
b) The phenolic fraction (flavonoids) was isolated from the ethyl acetate extract of plant.

2.4.3 Instrumental analysis of isolated compounds from both plant parts:

Isolated compounds were characterized using UV, IR and LC-MS spectral data.

2.5 Total Phenolic Content

By measuring the intensity of the produced blue hue, the total phenolic content was estimated using the Folin Ciocalteu procedure.[22] 0.5ml of plant extract dissolved in methanol (200g/ml) was combined with 2.5ml of 10 fold diluted Folin Ciocalteu reagent and 2ml sodium carbonate. After 30 min incubation in dark with steady shaking. The absorbance was measured at 760 nm against a gallic acid standard solution. The total phenolic content (TPC) of the various plant extracts was calculated as the average of three independent assays and reported as mg gallic acid equivalent/g dry weight extract (mg GAE /g extract).

2.6 Total Flavonoid Content

The total flavonoid concentration was determined by aluminium chloride colorimetric method.[23]The hydroxyl groups in flavonoids combine with aluminium chloride to form a compound ($AlCl_3$). A pink tint was created by the reaction with sodium nitrite. 250 litres of plant extract in methanol (500 g/ml) were mixed with 75 litres of NaNO2 (5%), and 1.3 litres of distilled water. After 5 minutes, 150 litres of 10% $AlCl_3$ were added. After 6 minutes, the reaction mixture was diluted with 275 *l* distilled H_2O and 0.5ml of 1M NaOH was added. The absorbance at 510 nm was measured after 15 minutes and compared to a reference rutin solution. All studies were done in triplicate and the total flavonoid content (TFC) was represented as mg rutin equivalent per gram extract (mg RE /g extract).

2.7 Preparation of solutions:

Bromocresol green solution was prepared by heating 69.8 mg bromocresol green with 3 ml of 2N NaOH and 5 ml distilled water until completely dissolved and the solution was diluted to 1000 ml with

distilled water. Phosphate buffer solution (pH 4.7) was prepared by adjusting the pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) to 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water). Caffeine standard solution was made by dissolving 1mg pure atropine (Sigma Chemical, Bangalore) in 10 ml distilled water.

3. RESULTS

3.1 Proximate Analysis:

Proximate analysis of powdered plant materials (roots of *Aerva lanata* and rhizomes of *Curcuma caesia*) were carried out as per WHO guidelines and readings were noted. (Table 1 & 2)

Table 1: Proximate analysis of roots of *Aerva lanata* and rhizomes of *Curcuma caesia*

S. No.	PARAMETERS	ROOT (%w/w)*	Rhizomes (%w/w)*
1	Total ash	14.0	12.0
2	Acid insoluble ash	3.0	2.6
3	Water soluble ash	10.0	11.2
4	Alcohol soluble extractive value	15.0	16.3
5	Water soluble extractive value	22.0	23.3
6	Ether soluble extractive value	4.0	3.1
7	Loss on Drying	13.5	14.1

*averages of three determinations, the values are expressed as percentage of air dried material.

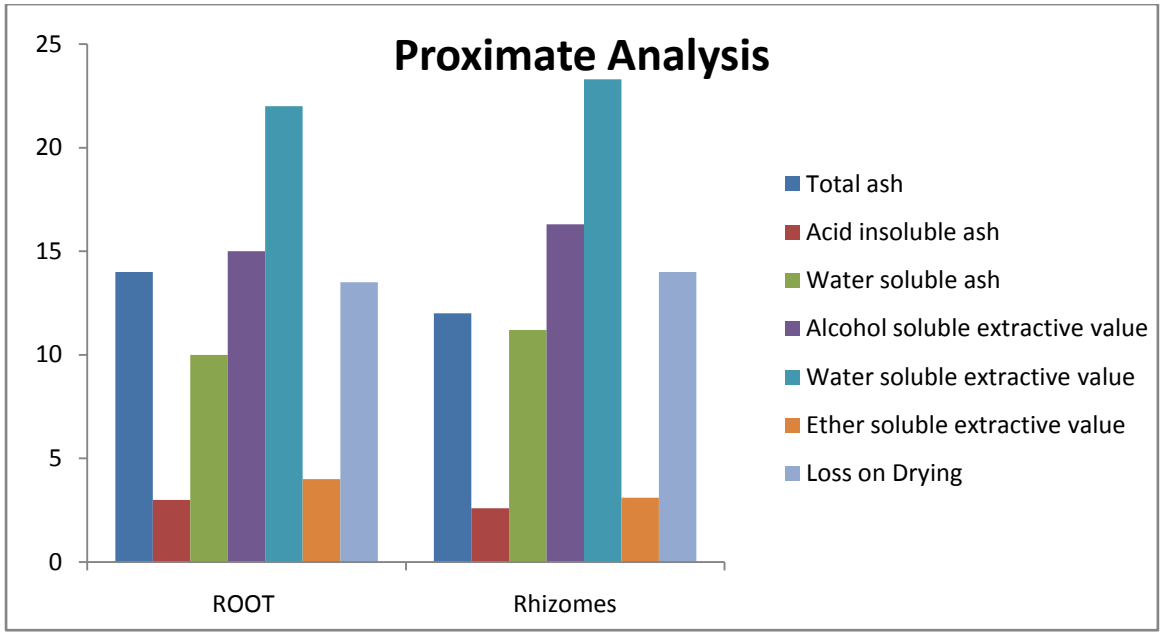


Figure 1: Proximate analysis of roots of *Aerva lanata* and rhizomes of *Curcuma caesia*

Table 2: Inorganic elements and heavy metal in *Aerva lanata* and *Curcuma caesia*

S. No.	ELEMENTS/ METAL	Roots of *Aerva lanata* (in ppm)	Rhizomes of *Curcuma caesia* (in ppm)
1	Zinc	19.75	18.3
2	Iron	179.7	196.35
3	Manganese	23.04	21.8
4	Magnesium	14.87	19.65
5	Copper	9.38	8.39
6	Cadmium	bdl	bdl

bdl = below detectable limit

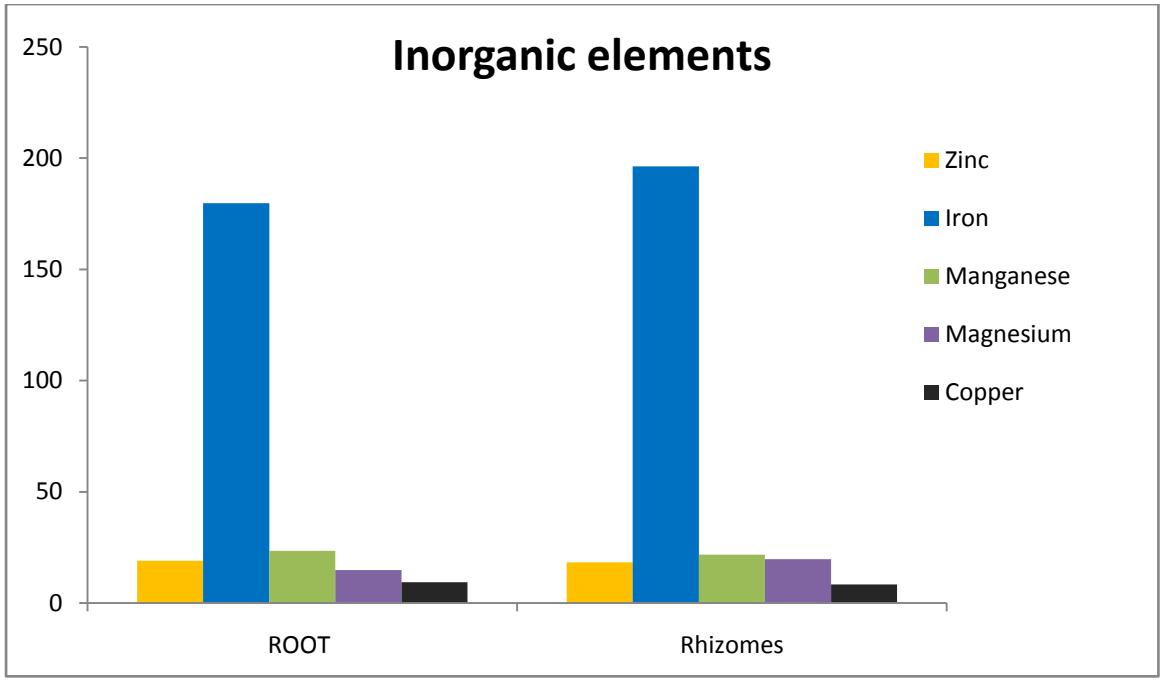


Figure 2: Inorganic elements and heavy metal in *Aerva lanata* and *Curcuma caesia*

3.2 Successive Solvent Extractions and Qualitative Chemical Tests:

The percentage yield, color and consistency of successive extracts obtained were recorded (Table 3) and then the extracts were subjected to various qualitative chemical tests, the result are as shown in Table 5 and Table 6.

Table 3: Extractive values obtained from *Aerva lanata* and *Curcuma caesia*

S. No.	Solvent	% Yield	
		Aerva lanata (roots)	***Curcuma caesia*** (rhizomes)
1.	Pet. Ether	0.49 %	0.32 %
2.	Toluene	0.38 %	0.29 %
3.	Chloroform	0.50 %	0.41 %
4.	Ethyl acetate	0.45 %	2.49 %
5.	Methanol	11.8 %	1.01 %
6.	Water	4.236 %	1.545 %

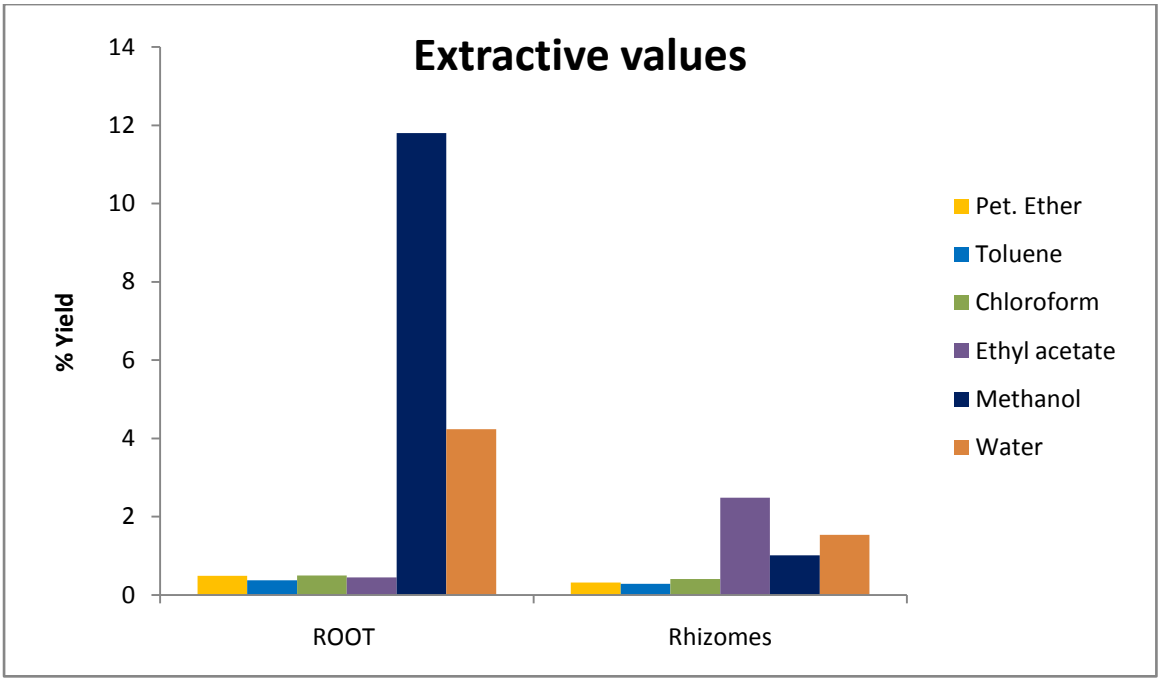


Figure 3: Extractive values obtained from *Aerva lanata* and *Curcuma caesia*

Table 4: Colour and consistency *of Aerva lanata* and *Curcuma caesia*

S. No.	Solvent	Colour and consistency	
		Aerva lanata (roots)	***Curcuma caesia*** (rhizomes)
1.	Pet. ether	Yellowish green sticky & solid	Green sticky & semi-solid
2.	Toluene	Green sticky & semi-solid	Green sticky & semi-solid
3.	Chloroform	Greenish brown sticky & solid	Greenish brown sticky & semi-solid
4.	Ethyl acetate	Greenish brown sticky & semi-solid	Greenish brown sticky & semi-solid
5.	Methanol	Brownish red sticky semi- solid & sugary	Brownish red sticky & semi- solid
6.	Water	Brown, sticky & solid	Brown, sticky & solid

Table 5: Preliminary phytochemical screening of roots of *Aerva lanata*

S. No.	Phytoconstituents	Test Name	Pet. ether	Toluene	$CHCl_3$	Ethyl acetate	Methanol	Water
1	Alkaloids	Dragendorff's Test	- ve	+ ve	+ ve	+ ve	+ ve	+ ve

2	Carbohydrates	Fehling's Test	- ve	- ve	- ve	- ve	- ve	+ ve
3	Flavonoids	Schinoda test	- ve	- ve	- ve	- ve	- ve	- ve
		Alkaline reagent test	- ve	- ve	- ve	- ve	- ve	- ve
4	Proteins & Amino acids	Precipitation test	- ve	- ve	- ve	- ve	- ve	- ve
5	Phenols	Ferric chloride test	- ve	- ve	+ ve	+ ve	+ ve	+ ve
6	Diterpenes	Copper acetate test	- ve	- ve	- ve	- ve	- ve	- ve
7	Saponins	Foam test	- ve	- ve	- ve	- ve	+ ve	+ ve
8.	Phytosterols	Liebermann Burchard Test	+ ve	+ ve	- ve	- ve	- ve	- ve

Table 6: Preliminary phytochemical screening of rhizomes of *Curcuma caesia*

S. No.	**Phytoconstituents**	**Test Name**	**Pet. ether**	**Toluene**	**$CHCl_3$**	**Ethyl acetate**	**Methanol**	**Water**
1	Alkaloids	Dragendorff's Test	- ve	- ve	- ve	- ve	+ ve	- ve
2	Carbohydrates	Fehling's Test	- ve	- ve	- ve	+ ve	+ ve	- ve
3	Flavonoids	Schinoda test	- ve	- ve	- ve	+ ve	- ve	- ve
		Alkaline reagent test	- ve	- ve	- ve	+ ve	- ve	- ve
4	Proteins & Amino acids	Precipitation test	- ve	- ve	- ve	- ve	- ve	- ve
5	Phenols	Ferric chloride test	- ve	- ve	- ve	+ ve	+ ve	+ ve
6	Diterpenes	Copper acetate test	- ve	- ve	- ve	- ve	- ve	- ve
7	Saponins	Foam test	- ve	- ve	- ve	- ve	+ ve	- ve
8.	Phytosterols	Liebermann Burchard Test	+ ve	+ ve	- ve	- ve	- ve	- ve

Table 7: TLC profile of Successive Extracts:

Solvent System	**Phyto-constituents detected**	**Spraying reagents used**	**Successive Pet-ether**	**Successive Toluene**	**Successive $CHCl_3$**	**Successive EtOAc**	**Successive MeOH**

***Aerva lanata* (roots)**							
$CHCl_3$: EA (8:5)	Alkaloids	Dragendorf's	0.018	0.45, 0.54	0.2, 0.45, 0.54 Spot on the applied region	Spot on the applied region	Spot on the applied region
MeOH : H_2O (3:3)	Alkaloids	Dragendorf's	----	----	0.35, 0.82	0.82	0.82
MeOH : H_2O (3:3)	Phenolics	5% $FeCl_3$	----	----	----	0.82	0.82
***Curcuma caesia* (rhizomes)**							
EA : MeOH : H_2O (8:1.5: 0.5)	Flavanoids	Schinoda test	-------	-------	-----	0.55	Spot on the applied region
		Alkaline reagent test	-------	-------	-----	0.55	Spot on the applied region

3.3: Preparation of Fractions:

Alkaloidal fractions from roots of *Aerva lanata*:

The above results revealed the presence of alkaloids in successive extracts of root, considering the phytochemical and biological importance of alkaloids; root part was selected for further phytochemical studies. Alkaloidal fractions were prepared from root powder by different procedures.

From **Procedure 1** three fractions were obtained:

a. $CHCl_3$ fraction at pH 10

b. $CHCl_3$ fraction at pH 11

c. $CHCl_3$ fraction at pH 12

From **Procedure 2** six basified fractions were obtained:

a. Basified n-Hexane fraction

b. Basified Pet. ether fraction

c. Basified Toluene fraction

d. Basified Chloroform fraction

e. Basified Ethyl acetate fraction

f. Basified Acetone fraction

Flavonoids from rhizomes of *Curcuma caesia*

a. Ethyl acetate extract

TLC Profile of Alkaloid Fractions from roots of *Aerva lanata*

Solvent system : Chloroform : Ethyl Acetate (8:5)

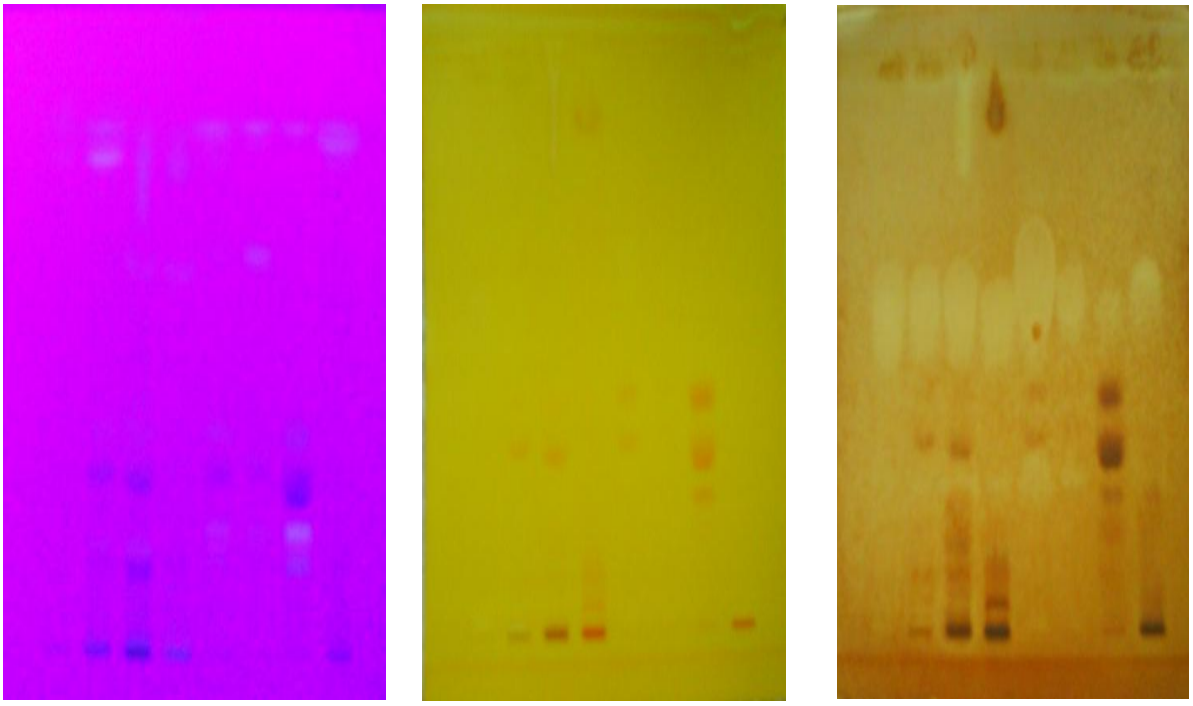
Spraying Reagent : Dragendorff's Reagent

Table 8: TLC profile of alkaloid fractions

Fractions	**Rf values**
$CHCl_3$ fraction pH 10	0.036, 0.50, 0.945
$CHCl_3$ fraction pH 11	0.036, spot on the applied region
$CHCl_3$ fraction pH 12	Slight spot on the applied region
Basified n-Hexane Fraction	0.45, 0.54
Basified Pet. ether Fraction	0.45, 0.54
Basified Toluene Fraction	0.45, 0.54, 0.30, Dark spot on the applied region
Basified $CHCl_3$ Fraction	0.45, 0.54,0.30, Dark spot on the applied region
Basified Ethyl acetate Fraction	Dark spot on the applied region
Basified Acetone Fraction	Dark spot on the applied region

The successive pet. ether, successive toluene, successive $CHCl_3$, $CHCl_3$ fraction pH 10, basified n-hexane fraction, basified pet. ether fraction, basified toluene fraction, basified $CHCl_3$ fraction were selected for alkaloids.

Before derivatization at 360 nm **After derivatization at 540 nm** **After spraying with aq. $NaNO_2$ for spot intensification**



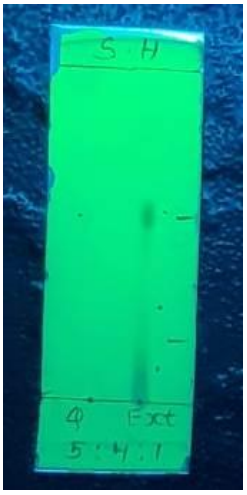
A B C D E F G H A B C D E F G H A B C D E F G H

A = Succ.Pet.ether Extract
B = Succ.Toluene Extract
C = Succ.$CHCl_3$ Extract
D = $CHCl_3$ fraction pH 10
E = Basified n-Hexane Fraction
F = Basified Pet-ether Fraction
G = Basified Toluene Fraction
H = Basified $CHCl_3$ Fraction

Fig: 4 TLC fingerprints of extracts and their fractions

TLC Profile of Flavanoid Fractions from rhizomes of *Curcuma caesia*

Solvent system **:** Ethyl Acetate : MeOH : H_2O (8:1.5:0.5)
Spraying Reagent **:** Alkaline reagent
Rf value **:** 0.55



DISCUSSION:

Proximate analysis of powdered plant materials (roots of *Aerva lanata* and rhizomes of *Curcuma caesia*) showed that the total ash vale was found to be 14.0% and 12 % respectively where as acid insoluble ash ,water soluble ash ,alcohol soluble extractive value ,water soluble extractive value, ether soluble extractive value, loss on drying were 3.0 ,10.0 15.0, 22.0, 4.0 and 13.5 % in roots of *Aerva lanata* and 2.6, 11.2, 16.3 ,23.3, 3.1 and 14.1% in rhizomes of *Curcuma caesia*

Inorganic elements and heavy metal analysis of *Aerva lanata* and *Curcuma caesia* showed that the presence of Zinc, Iron, Manganese, Magnesium and Copper were 19.75, 179.7, 23.04, 14.87 and 9.38 (ppm) in roots of *Aerva lanata* and 18.3, 196.35, 21.8 ,19.65 and 8.39 (ppm) in rhizomes of *Curcuma caesia*

The percentage yield, color and consistency of successive extracts obtained were observed in various solvents such as Pet. Ether ,Toluene, Chloroform, Ethyl acetate ,Methanol and Water were 0.49 % ,0.38 % ,0.50 %, 0.45 %, 11.8 % and 4.236 % in roots of *Aerva lanata* and 0.32 % ,0.29 %, 0.41 % ,2.49 %, 1.01 % and 1.545 % respectively in rhizomes of *Curcuma caesia*.

In preliminary phytochemical screening of both the plants Alkaloids, Carbohydrates Flavanoid, Proteins, Amino acids, Phenols, Diterpines, Saponins and Phytosterols were tested. In this analysis the results showed the presence of alkaloids in Toluene, Chloroform, Ethyl acetate, Methanol and Water. whereas carbohydrates were present in water extract only. Saponins were present in methanol and water extract. Phytosterols were present in Pet. Ether, and Toluene. Phenols were present in Chloroform, Ethyl acetate, Methanol and Water extracts. Flavanoid, amino acid, proteins and diterpines were found absent in roots of *Aerva lanata.*

In this analysis the results showed the presence of alkaloids in Methanol extract. whereas Carbohydrates were present in Ethyl acetate, Methanol extract. Flavanoid were found to be present in ethyl acetate extract only. Phenols were detected in Ethyl acetate, Methanol and Water extracts. Saponins were present in methanol extract only. Phytosterols were present in in pet. Ether and toluene extract. Amino acid, proteins and diterpines were found absent in rhizomes of *Curcuma caesia.*

Rf value of both the plants extracts were found to be 0.45 and 0.54 in successive toluene extract for alkaloid in *Aerva lanata* where as Rf value were 0.55 in successive ethyl acetate extract for flavanoids in *Curcuma caesia*

CONCLUSION: From the above study it was concluded that both the plants have various phytoconstituents such as a alkaloids, flavanoid, phenolics, carbohydrates and Phytosterols which can be responsible for various biological and pharmacological studies. In *Aerva lanata* alkaloids were isolated and flavanoids were isolated from *Curcuma caesia* which would be used in hypoglycaemic studies in combination dose. In overall both the plant have rich source of phytoconstituents which will be use in combination for antidiabetic studies.

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