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Isolation and Characterization of isolated compounds fromherbal extracts prepared using different solvents of two Indian medicinal plants *Aerva lanata*and *Curcuma caesia*

Dr.M A Naidu, Ritesh Agrawal

Faculty of Pharmacy, Mandsaur University, Mandsaur, MP, India - 485001

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ABSTRACT: The aimof this study is to characterize the isolated compounds from herbal extracts of two Indian medicinal plants namely Aerva lanata(Family: Amaranthaceae) and Curcuma caesia(Family: Zingiberaceae).For the purpose of isolating alkaloids as an active component from Aerva lanata roots and phenolic compounds from Curcuma caesia rhizomes, a basified toluene sub-fraction was optimised. Using column chromatography and preparative TLC, both were extracted. The phytochemical analysis of both the plants has been previously reported in my research publication.Using instrumental analytical techniques, the chemicals were extracted from both plants and characterised. TLC profile of successive extracts and basified fractions of Aerva lanata roots confirmed the presence of strong alkaloids at 0.45 and 0.54 Rf value (in successively basified toluene extract), while flavanoids at 0.55 Rf value were detected in the rhizomes of Curcuma caesia (in successively basified ethyl acetate extract). Both of the isolated compounds—alkaloids from Aerva lanata named IAL and flavanoids from Curcuma caesia named ICC—were identified as polyphenol derivatives and canthine-6-one derivatives, respectively, using UV-Vis, IR, 1HNMR, and mass spectrometry.According to the study mentioned above, both plants contain a variety of phytoconstituents, including carbohydrates, phenolics, alkaloids, flavonoids, and phytosterols, which may be the subject of future pharmacological and biological research.

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

INTRODUCTION

Plants have been used to treat a wide range of illnesses since the beginning of time. In pursuit of food, the first humans ate the entire plant as well as its fragments. They eventually realised how crucial certain plants and their constituent parts are to human health. The final relevance and recognition of medicinal plants can be attributed to men's never-ending quest for knowledge and their constant struggle for survival.[1-4]

Since ancient times, plants have been integral to the development of medicine. This relationship is still strong today, as seen by the common usage of plant products in traditional

medicine, ethnomedicine, and modern medical practices. In many underdeveloped countries, phytopharmaceuticals represent the mainstay of a rational health care strategy.[5,6]

The knowledge of the beneficial pharmaceuticals often vanishes without a trace since only the ignorant "medicine men" from the various tribes, who are routinely written off as quacks or cheap jacks by the educated populace, spread the word about them. The usefulness of these pharmaceuticals cannot be overstated by allopathic doctors because new research and technological advancements have not succeeded in enhancing humanity. Since nature has always had an answer, the ancient medical system in India is seeing a renaissance thanks to this recently shown interest. In order to avoid perpetuating our initial ignorance and carelessness, scientists and researchers are methodically documenting the minute study of these plants.[7,8]

Moreover, it may be argued that medicinal plants are an abundant renewable resource that, when employed prudently, not only improves quality of life but also provides reasonable, reasonably priced healthcare with few adverse effects and greatly supports the expansion and growth of their economies.[9]

Aerva lanata Juss. (Amaranthaceae), also known locally as "bui," is a prevalent weed found throughout India's fields and waste regions. It's a prostrate, erect underbrush. The herb that diureses is used to cure lithiasis. The root relieves strangury (slow, painful urine flow) and is demulcent and diuretic. The roots are used to treat headaches. The plant is considered to be a demulcent on the Malabar Coast. It is well-liked in Ceylon as a cough cure and a paediatric vermifuge.The Meena tribal people of Sawaimadhopur, Rajasthan, offer the root juice orally to patients suffering from dyspepsia, jaundice, biliousness, and liver congestion. Additionally, they offer a decoction of whole plants to treat prolonged fevers, including typhoid and pneumonia.[10, 11]

Curcuma caesia, the large genus, belongs to the Zingiberaceae family. Rhizomes of the perennial herb Curcuma caesia, which is native to North and Central India, are bluish-black in colour. Furthermore, black turmeric is occasionally found in the Papi Hills in the Andhra Pradesh districts of Khammam, West Godavari, and East Godavari. The medicinal properties of kali haldi are said to have made its rhizomes extremely valuable.[12]The rhizomes are used for their smooth muscular relaxing effects, leprosy, haemorrhoids, inflammation, gonorrhoeal discharges, fever, wounds, vomiting, menstrual disorders, anthelmentic, aphrodisiac, cancer, epilepsy, and so on.[13] Those who own the plant in Madhya Pradesh are believed to have endless supplies of food and cereal, making it very auspicious in that region.A nice aroma

emanates from the plant's rhizomes. *Curcuma caesia* Roxb. rhizomes are used to cure a variety of ailments, including bronchitis, inflammation, tumours, leucoderma, and piles.[14]

Several medicinal plants have been shown to be beneficial for diabetes globally and have been empirically employed in antidiabetic and antihyperlipidemic medicines. Plants exhibit antihyperglycemic action primarily through increasing insulin secretion, inhibiting intestinal glucose absorption, or facilitating metabolites in insulin-dependent processes. Finding novel antidiabetic medications from natural plants is still intriguing even if there are over 400 plant species with hypoglycemic activity that have been reported in the literature. This is because natural plants include chemicals that have distinct and safe effects on diabetes mellitus. Most plants include substances that are generally believed to have antidiabetic properties, such as flavonoids, glycosides, alkaloids, terpenoids, carotenoids etc.[15]This study aims to assess the phytochemical and pharmacognostic analysis of two Indian medicinal plants for research on antidiabetic properties.

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.

2.2Phytochemical Screening

Preparation of extracts:

The fresh parts of selected plants *Aerva lanata*(roots) and *Curcuma caesia*(rhizomes) were shade-dried, cut into pieces, coarsely powdered and successively extractedone by one using various solvents(Pet. Ether <Toluene < Chloroform < Ethyl Acetate < Methanol < Water) in increasing order of polarity. Different extracts' colours, consistency, and yield percentages were noted, and the extracts were vacuum-sealed until further processing.[17]

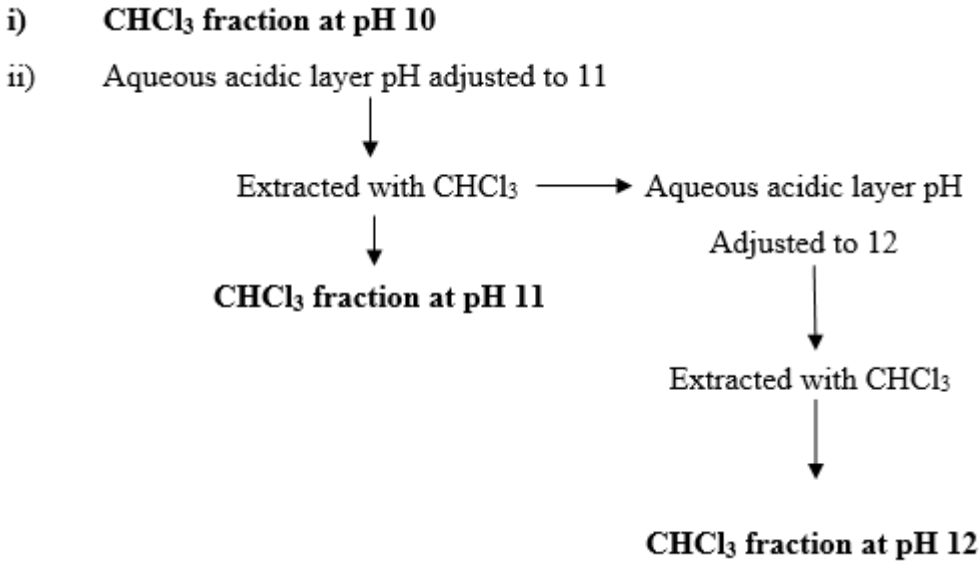
2.3Isolation of Phytoconstituents from the selected fractions of both plants:

Using a preparative TLC approach, the basified Toluene sub-fraction was utilised to isolate the alkaloids from the roots of *Aerva lanata* and the ethyl acetate extract was employed to isolate the phenolic compounds from the rhizomes of *Curcuma caesia*.[18]

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

Procedure-1:

a) The powdered form of roots was basified with NH_3(10%)
b) The drug was extracted with solvent $CHCl_3$ and reduced to ¼ .
c) The pH of the extract has been adjusted upto 10 with $NH_3$by using appropriate volume of dil. HCl.
d) Extracted with $CHCl_3$



Procedure 2:

a) The preparation of hydroalcoholic solution of crude methanolic extract was performed in the ratio 1:1, 1:2 and 2:1.
b) The pH of filtrate was adjusted to 8 using alcoholic KOH.
c) Successive solvent extraction process performed.
   i) Fractions of Petroleum ether
   ii) Fractions of Toluene
   iii) Fractions of $CHCl_3$
   iv) Fraction of Ethyl acetate
   v) Fraction of Acetone

2.3.2 *Curcuma caesia*(rhizomes) phenolic fractions preparation

Procedure:

a) The powdered form of rhizomes of *Curcuma caesia* was subjected to successive solvent extraction using soxhlate apparatus.
b) The successive ethyl extract of plant was used for isolation of phenolic fraction (flavanoids).

2.3.3 Analysis of isolated compounds from both plant parts:

The compounds isolated from both the plants were characterized by UV, IR, NMR and MS spectral data.

3. RESULTS

3.1 TLC Profile of Successive Solvent Extracts:

The successive solvent extracts of both plants were subjected to various solvent systems for detection of Rf value of present active phytoconstituents.

Table 1: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

3.2:Preparation of Fractions:

3.2.1: Alkaloidal fractions from roots of *Aerva lanata*:

Considering the phytochemical and biological importance of alkaloids present in roots, various alkaloidal fractions were prepared from powdered form by two 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 Pet. ether fraction

b. Basified Toluene fraction

c. Basified Chloroform fraction

d. Basified Ethyl acetate fraction

e. Basified Acetone fraction

3.2.2: Flavonoids from rhizomes of *Curcuma caesia*

a. Ethyl acetate extract

3.3: Characterization of isolated compounds by TLC method:

3.3.1: TLC Profile of Alkaloid fractions from roots of *Aerva lanata*

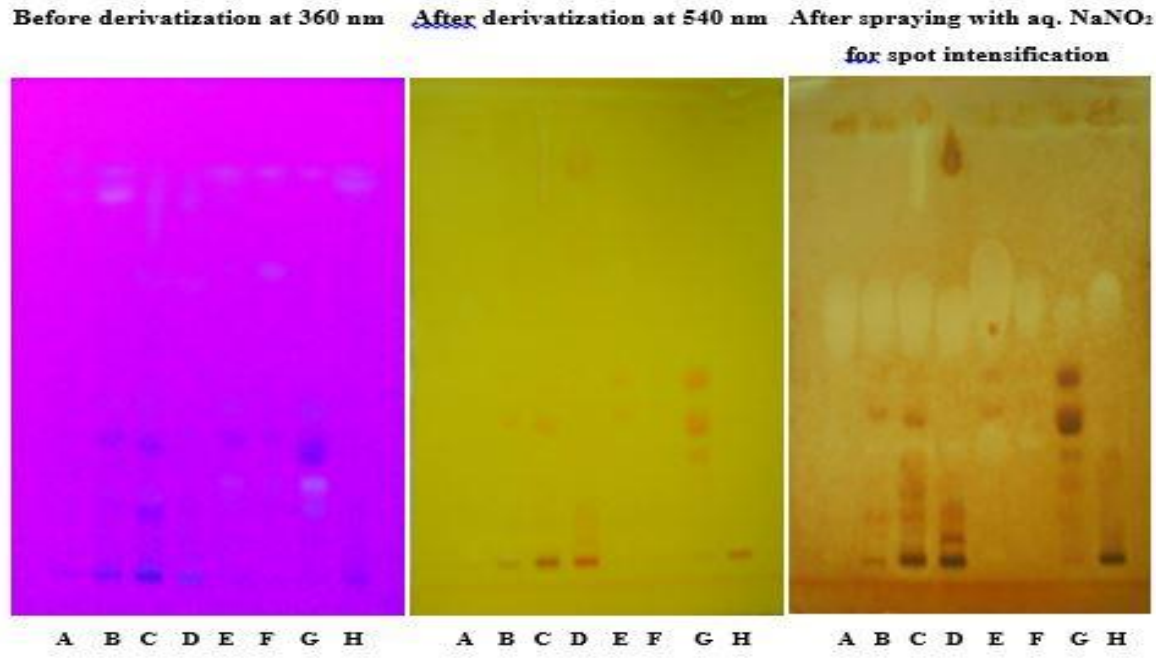
Solvent system **:** Chloroform : Ethyl Acetate (8:5)

Spraying Reagent : Dragendorff's Reagent

Table 2: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.



A = Succ.Pet.ether Extract
B = Succ.Toluene Extract
C = Succ.$CHCl_3$ Extract
D = $CHCl_3$ fraction pH10
E = Basified n-Hexane Fraction
F = Basified Pet-ether Fraction
G = Basified Toluene Fraction
H = Basified $CHCl_3$ Fraction

Fig: 1 TLC profile of successive extracts and their fractions

Basified toluene fraction was further selected for isolation of alkaloid. The fraction was sub fractioned and six alkaloid bands were identified.

Solvent system **:** $CHCl_3$: Ethyl acetate (9:1)

Spraying Reagent **:** Dragendorff's reagent

SAMPLE	Rf
Basified Toluene sub fraction	0.98,0.81, 0.59, 0.40, 0.31 and 0.12

Before derivatization at 360 nm **After derivatization at 540 nm** **After spraying with 10 % aq. $NaNO_2$ for spot intensification**

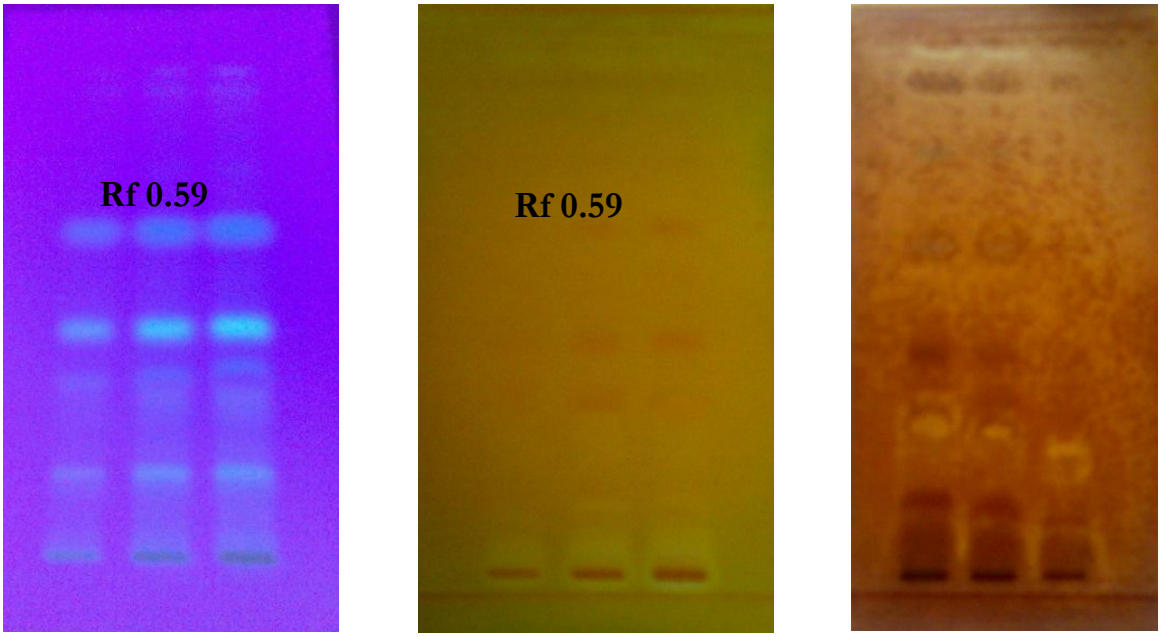


Fig: 2 Rf Value for Identification of Alkaloids

3.3.2: Characterization of Isolated Compounds:

The alkaloidal band at R_f 0.59 was targeted for isolation. The alkaloidal band was isolated by preparative TLC with mobile phase $CHCl_3$: Ethyl acetate (9:1). Alkaloid band at R_f 0.59 was denoted as IAL.

Characterization of IAL

The yield of compound was 100 mg.

Nature: Yellow sticky and resinous

Bluish yellow fluorescence (UV 366 nm)

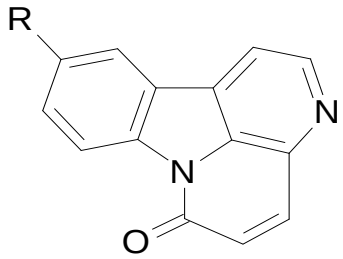
Melting point = 168-173°C

UV Spectrum: Ultraviolet absorption maxima (λmax) of isolated IAL in Ethanol obtained at 214 nm

IR (ν, cm^{-1})**:**3255-3431 (-OH str), 1207-1346 (-C-N str), 1620-1705 (-C=O str)

^{1}H-NMR(δ, ppm/ DMSO-d6): 6.9-7.4 (3H, m, Ar-H), 7.27-8.63 (2H, d, Pyr-H), 2.08 (1H, s, -CH_3), 6.51-7.58 (2H, d, -C_2H_5).

MS-(ESI) m/z (%): 275 (M^+,100).



R = $OCOCH_3$(IAL)

Possible Structure of Isolated Compound

3.3.3: 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

Fig: 2 Rf value of isolated compound in *Curcuma caesia* rhizomes

3.3.4: Characterization of Isolated Compounds:

The flavanoidal band at R_f 0.55 was targeted for isolation by preparative TLC with mobile phase Ethyl Acetate: MeOH: H_2O (8:1.5:0.5). Flavanoid band at R_f 0.55 was denoted as ICC.

Fraction ICC

The yield of compound was 95 mg.

Nature: Yellow sticky and resinous

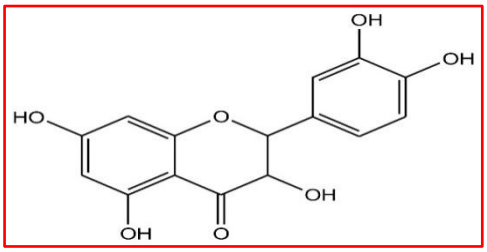
Melting Point: 317-318°C

UV Spectrum: Ultraviolet absorption maxima (λ max) of isolated **ICC** in ethanol obtained at 256 and 373nm.

IR (ν, cm^{-1})**:** 3255-3600 (-OH str), 1323 (-C-O str), 1518-1664 (-C=O str)

^{1}**H-NMR**(δ, ppm/ DMSO-d6): 5.84-6.58 (5H, m, Ar-H), 5.59-5.61 (2H, d, -CH_2-).

MS-(ESI) m/z (%): 323 (M^+,100).



Possible Structure of isolated compound ICC

DISCUSSION:

The results for ***Aerva lanata*** **roots** 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. The results for ***Curcuma caesia*** **rhizomes** showed the presence of alkaloids in Methanol extract. Whereas presence of Carbohydrates was confirmed in Ethyl acetate, Methanol extract. **Flavanoids** was present in ethyl acetate extract only. Phenols were found to present in Ethyl acetate, Methanol and Water extracts.

Rf value of both the plants extracts were found to be 0.45, 0.54& 0.59 in successive toluene extract for alkaloid in*Aerva lanata*whereas Rf value were 0.55 in successiveethyl acetate extract for flavanoids in *Curcuma caesia.*TLC profile ofbasified fractions of roots of *Aerva lanata* confirmed the presence of intense alkaloids at 0.59 whereas rhizomes of *Curcuma caesia* showed the presence of flavanoids at 0.55 Rf value. Both isolated compounds (alkaloids named as IAL from *Aerva lanata* and flavanoids named as ICC from *Curcuma caesia*) were characterized (using UV-Vis, IR, ^{1}HNMR and Mass spectrum) as canthine- 6-one and polyphenol derivatives respectively.

CONCLUSION: According to the study mentioned above, both plants contain a variety of phytoconstituents, including carbohydrates, phenolics, alkaloids, flavonoids, and phytosterols, which may be the subject of future pharmacological and biological research. Alkaloids from *Aerva lanata* and flavanoids from *Curcuma caesia* were isolated, identified and their combined dosage will be utilised in hypoglycemic research. Overall, both plants are great sources of phytoconstituents that will be combined for research on antidiabetes.

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