Extraction and physiochemical evaluation of Randia dumetorumroots & leaves.

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KEYWORDS
Extraction, Physiochemical evaluation, Randia dumetorum.

ABSTRACT:
"*Randia dumetorum*, commonly known as the emetic nut or 'Mainphal,' is a medium-sized shrub native to tropical and subtropical regions of Asia. This plant, belonging to the Rubiaceae family, has been traditionally used in Ayurveda for treating ailments such as fever, gastrointestinal disorders, and skin diseases. Present paper deals with preliminary pharmacognostic and physicochemical evaluation of roots & leaves of Randia dumetorum Lamk., to establish authenticity and possibly to help to distinguish drug from other species. The study includes preparation of different extracts by successive solvent extraction for detailed analysis. Different physicochemical parameters were carried out as per WHO recommended physicochemical determinations and authentic phytochemical procedures. Preliminary qualitative chemical test for different extract shows the presence of Glycosides, Carbohydrates,Phytosterols/triterpenoids, Saponins, Fixed oils & Fats and phenolic/tannins. Also, acute toxicity studies has been carried out as per Organization for Economic co-operation and Development (OECD) guidelines.

INTRODUCTION

"*Randia dumetorum*, commonly known as the emetic nut or 'Mainphal,' is a medium-sized shrub native to tropical and subtropical regions of Asia. This plant, belonging to the Rubiaceae family, has been traditionally used in Ayurveda for treating ailments such as fever, gastrointestinal disorders, and skin diseases. Despite its widespread traditional use, there is limited scientific evidence supporting its medicinal properties.[1,2,3]

Medicinal plants have long served as a cornerstone for drug discovery, contributing bioactive compounds such as alkaloids, flavonoids, and phenolics to modern pharmacology. However, many plants with therapeutic potential, including *Randia dumetorum*, remain underexplored. To date, only a handful of studies have investigated its phytochemical profile or pharmacological activities, leaving significant gaps in our understanding of its medicinal value. [3]

This study aims to extract bioactive compounds from *Randia dumetorum*leaves and roots using standardized solvent-based techniques, perform a preliminary phytochemical screening, and evaluate their antioxidant and antimicrobial properties. By bridging the gap between traditional knowledge and scientific validation, this research seeks to contribute valuable insights into the therapeutic potential of *Randia dumetorum* and lay the groundwork for future studies. The findings could support its development into a source of natural pharmaceuticals and nutraceuticals, meeting the increasing global demand for plant-based therapeutic agents."[4,5,6]

MATERIALS AND METHODS

Plant Material

Collections and Drying

Randia Dumetorum (Roots & leaves) collected from Regional Ayurvedic Research Institute C.C.R.A.S.Ministry of AYUSH Govt.of India, Neharu garden, KothrudPune, Maharashtra, India, in the month of January-February.

Authentication

The plant, Randia Dumetorum was authenticated by Botanist of Botanical Survey of India, Pune by comparing morphological features. The herbarium of the plant specimen was deposited at Botanical Survey of India, Pune; with the Voucher specimen number MMDCS1 (Ref.No.BSI/WRC/Iden.Cer/2023/1103230002980 Dated 24/3/2023).

Preparation of Plant Material

Plant Randia Dometorum (Roots & leaves) were dried in the shade and pulverised. Each powdered component was passed through a 40# sieve and kept in an airtight container.

Pharmacognostic Study-

For plant Randia Dometorum (Roots & leaves) pharmacognostic study were carried as per below procedure and methods. [7]

Macroscopy

Organoleptic characters, extra feature and macroscopical details for fruit of plant were carried out.

Microscopy [7, 8]

Microscopical study was done as per the method described by Khandelwal, (2008). Transverse section of stem and leaf was taken, stained with phloroglucinol: Hydrochloric acid (1:1) and observed under microscope at 10X, 45X.

Evaluation of Physical Constants [9,10]

1. Determination of foreign organic matter
2. Determination of moisture content
3. Ash value
4. Determination of Total ash
5. Determination of Acid -insoluble ash
6. Determination of Water- soluble ash
7. Extractive values
8. Determination of water-soluble extractive value
9. Determination of Alcohol-soluble extractive value

Extraction:[20,21]

The leaves and roots were dried in sunlight separately and reduced to a coarse powder. Then the powder was subjected to Soxhlet extraction with methanol for 72 hours at a temperature of 50-60ºC. The extract was concentrated and the solvent was completely removed. They were freeze dried and stored in the vacuum desiccator until further use. Preliminary phytochemical screening was carried out to identify the chemical constituents

Preliminary Phytochemical Screening for ethanolic Extract:[7,11-15]

Test for Carbohydrates

a) Molisch test (General test)

Test for Proteins[16]

a) Biuret test

b) Millon's test

Test for Amino acids [17]

a)Ninhydrin test

Test for Steroids [18-20]

a) Salkowski test

b) Liebermann-burchard test

c) Liebermann's test

Test for Terpenoids [22-24]

Test for Saponins [25-27]

Foam formation test [28-30]

Column Chromatography of Active Extracts

TLC- Characterization of Bioactive Fractions:[31,32]

After pharmacological evaluation of ethanol and aqueous fractions the only active fraction was evaluated by thin layer chromatography for determination of phytocomponents by following ways.

In the twin trough chamber with different solvent systems:

Solvent system I-chloroform: ethylacetate: formic acid (5:4:1)

In solvent system II- toluene: acetone: formic acid (4.5:4.5:1).

In solvent system III- ethyl acetate: ethanol: water (100:13.5:10),

In solvent system IV-ethyl acetate: formicacid: aceticacid: water (100:11:11:26) used.

After pre-saturation with mobile phase, 20 min for development was used. After the run, plates were dried and allowed for visualization [34-36]. After development, initially three spots were visualized in UV chamber (254, 365 nm). In the present study, different visualizing reagents were used such as NH3 vapour, vanillin HCl, 10 % alcoholic KOH, Folin-Ciocalteu. Always freshly prepared reagents were used to detect the better bands on the TLC plates. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples. After visualized in UV chamber and spray reagents, the spots were shown in different in colour. The Rf values were measured correctly and carefully.

a**) Detection of Steroids**

Solvent system used

Toluene: Ethyl acetate (9: 1)

Ethyl acetate: Methanol: Acetic acid (70:20:10)

Spray reagents

(i) Vanillin-Sulphuric acid reagent:

0.5 g vanillin is dissolved in 100 ml sulphuric acid- ethanol (40+10). Heated at 120^0c until maximum spot color intensity is reached.

Color observed - blue, blue-violet or pink colored spots.

(ii) Anisaldehyde-Sulphuric acid reagent:

0.5 ml of anisaldehyde was mixed with 10 ml glacial acetic acid, followed by 85 ml of methanol and 5 ml of concentrated sulphuric acid, in that order. The developed TLC plate was sprayed with reagent, heated at 100^0c for 5-10 minutes.

Color observed: blue, blue-violet or pink colored spots.

b) Detection of Alkaloids

Solvent system used

Toluene: Ethyl acetate: Formic acid (50:40:10)

Spray reagents

Sulphuric acid reagent

1% solution of concentrated sulphuric acid in ethanol. The developed TLC plate was sprayed with reagent, heated at 100^0c for 3-5 minutes.

Color observed: red-violet or brown colored spots.

c) Detection of Flavonoids

Solvent system used

Toluene: Ethyl acetate: Glacial acetic acid: Water (100:11:11:26)

N-Butanol: Acetic acid: Water (4:1:5)

Toluene: Ethyl acetate (9: 1)

Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)

Spray reagents

Anisaldehyde-Sulphuric acid reagent:

0.5 ml of anisaldehyde was mixed with 10 ml glacial acetic acid, followed by 85 ml of methanol and 5 ml of concentrated sulphuric acid, in that order. The developed TLC plate was sprayed with reagent, heated at 100^0c for 5-10 minutes.

Color observed: yellow-green spots.

d) Detection of Saponin

Solvent system used

Toluene: Ethyl acetate (9:1)

Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)

Spray reagents

Anisaldehyde-Sulphuric acid reagent

0.5 ml of anisaldehyde was mixed with 10 ml glacial acetic acid, followed by 85ml of methanol and 5 ml of concentrated sulphuric acid, in that order. The developed TLC plate was sprayed with reagent, heated at 100^0c for 5-10 minutes.

Color observed: green spots.

e) Detection of Tannins

Solvent system used

Toluene: Acetone: Ethyl acetate (3:1:2)

Ethyl acetate: Formic acid: Acetic acid: Water (100:11:11:26)

f) Detection of Glycoside

Solvent system used

Ethyl acetate: Ethanol: Water (100: 16.5: 13.5)

Methanol: water: chloroform (35:10:65)

Visualization: Under UV -365, Violet –blue color observed

Spray reagents

Sodium nitropruside reagent

1.5gm of Sodium nitroprusside is dissolved in 5 ml of 2N HCl, 95 ml of methanol and 10 ml of 25% ammonium hydroxide solution are added and solution is filtered.

Color observed: Orange-red.

Acute Toxicity Studies: [33, 34]

Organization for Economic co-operation and Development (OECD) regulates guideline for oral acute toxicity study.

Methods for Acute Toxicity Study [35]

Nine adult albino rats were divided into three groups, each with three individuals. All of the animals were fasting for the night. The fruit extract of *Randia Dometorum*dissolved separately in 1% CMC and given orally at dosages of 300, 1000, and 2000 mg/kg body weight, respectively. The animals were monitored for 2 hours and then for another 4 hours for any signs of death. Gross behaviour, pupil size, general motor activity, convulsion, water intake, faecal output, writhing, response to tail pinching, sedation, and any other toxic signs were monitored for 72 hours, after which the animals were maintained under observation for another 14 days.**6.**

RESULTS AND DISCUSSION

A pharmacognostic research was conducted and the results were presented below.

Macroscopical Examination

The colour, odour, taste, texture, size, and form of plant were evaluated morphologically. They were determined to be morphologically similar to reference material.

Morphology:

Figure 1:Randia Dometorum Roots Morphology

Figure 2:Randia Dometorum leaves Morphology

Microscopy:

Crude Powdered Drug Microscopy of **Figure 10- Randia Dometorum Roots & leaves Morphology** examines numerous microscopic techniques employed in the investigation of structural and cellular characteristics in order to establish their botanical origin. These techniques are effective for distinguishing between species that have comparable physical characteristics. To authenticate herbal medications nowadays, a range of procedures are available, ranging from simple morphological inspection to physical and chemical analysis, as well as DNA molecular biology. Powder microscopy is the most cost-effective approach for initial authentication due to its low cost. Botanical microscopy is a one-of-a-kind, useful, quick, and cost-effective evaluation method that is used to authenticate and analyse medicinal plants.

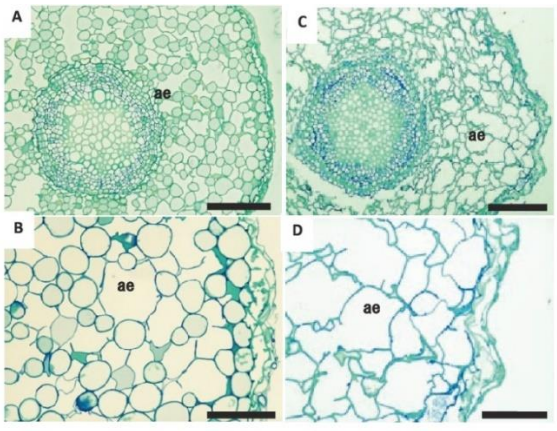

Figure 3: Transverse section of Randia Dometorum Roots & leaves Morphology

Determination of foreign organic matter
Foreign organic matter of extract of **Randia Dometorum Roots & leaves** was found to be 0.7% w/w when observed under 6X lens.

Determination of moisture content
Moisture content of ethanolic extract of **Randia Dometorum Roots & leaves** was found to be within limit.

Table 1:Moisture contentobservation

Time (hrs.)	Randia Dometorum Roots (%w/w) Ethanolic Extract	Randia Dometorum leaves (%w/w) Ethanolic Extract
0	0.000	0.000
01	0.204	0.203
02	0.214	0.211
03	0.222	0.217
04	0.215	0.213

Determination of Ash value
Ash value of was done. The total ash, acid insoluble ash, and water-soluble ash were determined and mentioned below,

Table 2: Ash Value details

Sr. No.	Evaluation Parameters	Randia Dometorum Roots Value (%w/w)- Extract	Randia Dometorum leaves (%w/w) Ethanolic Extract
1.	Total ash value	4	2.8
2	Acid insoluble ash value	0.7	1.2
3	Water soluble ash value	0.2	0.9

Determination of Extractive values
Extractive value of fruit parts was done. The water soluble and Alcohol soluble extractive values were determined and mentioned below,

Table 3: Extractive values

Sr. No	Extractive values	Randia Dometorum Roots Extractive value (%w/w)- Extract 1	Randia Dometorum leaves (%w/w) Ethanolic Extract
1	85% Ethanol soluble extractive values	13	11
2	Water soluble extractive values	5.8	4.7

In the case, the ethanol-soluble extractive value was found to be larger than the water soluble extractive value, indicating that the chemicals present in the ethanolic extract are highly soluble in alcohol. This might help us isolate the most active components from the plant.

Extract Characteristics
%Yield of various extracts of **Randia Dometorum Roots**were tabulated in below table

Table 4:%Yield of various extracts of Various Extract-1 of Randia Dometorum Roots

Sr. No.	Evaluation Parameters	Color	Nature	Randia Dometorum RootsPercentage Yield (% W/W)
1.	Petroleum ether	Green	Semisolid and sticky	12.69 %
2.	85% Ethanol	Dark Green	Semisolid	48.12 %
3.	Chloroform	Dark Green	Jelly like	7.40%
4.	Ethyl acetate	Green	Semisolid	8.20 %
5.	Aqueous	Dark brown	Sticky powder	8.91%

In the case of extracts 1, the percentage yield was found to be 48.12% in ethanol, which is more as compared to the yield with other solvents.

Preliminary Phytochemical Screening

The presence of steroids, terpenoids, flavones, and saponins has been confirmed in preliminary phytochemical screening of **Randia Dometorum Roots**.

The role of various constituents was screened in all extracts. The results of this preliminary phytochemical study are summarized in the table below.

Table 5:Preliminary Phytochemical Screening of Various Extracts of Randia Dometorum Roots

Extracts	Randia Dometorum*extract*
Tests for Carbohydrates	
Molish Test	+
Fehling Test	-
Benedict Test	-
Test for Monosaccharide	
Barfoed's Test	-
Test for Non-reducing polysaccharides	
Iodine Test	-
Test for Proteins	
Biuret test	+
Millions test	-
Tests for Steroids	
Salkowaski reaction	-
Libermann Burchard reaction	-
Libermann reaction	-
Tests for Terpenoids	+

Test for Saponin	
Foam test	+
Tests for Flavonoids	
Shinoda test	+
Lead acetate Test	-
Sod-hydroxide Test	-
Test for Tannins & Phenolic compounds	
$FeCl_3$	+
Lead acetate	+

+ Indicates presence of phytoconstituents, - Indicates absence of phytoconstituents

TLC- Characterization of extract *of Randia Dometorum* Roots

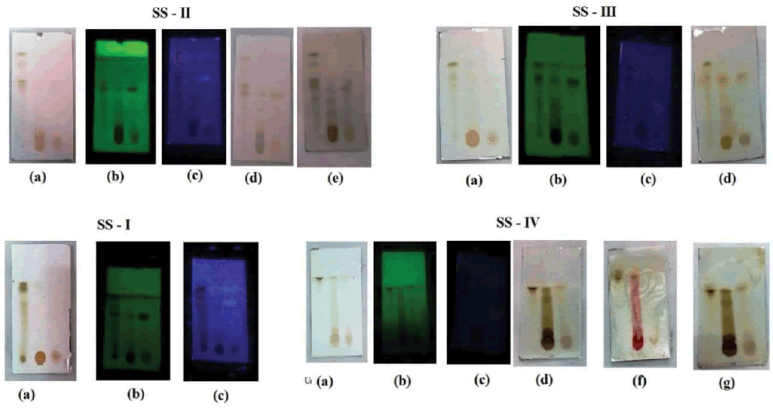

Figure 4: TLC Analysis of all plant extracts in various solvent system

SS- Solvent System; a-under visible light; b- under UV short wave length; c- under UV long wave length; d- under NH3 spray; e- under Folin-Ciocalteu spray, f-under vanillin HCl spray; g-under potassium hydroxide in ethanol spray.

Table 6: Thin Layer Chromatography of extract

Sr. no.	Chemical constituent	Rf- value
1.	Alkaloids	Petroleum ether :0.73 Ethanol : 0.89 Chloroform : 0.84
2.	Glycoside	Ethanol : 0.70 Chloroform : 0.62
3.	Flavonoid	Petroleum ether: 0.74 Ethanol : 0.82 Chloroform : 0.89
4.	Tannin	Ethanol : 0.60
5.	Steroids	Petroleum ether: 0.81 Ethanol : 0.74 Chloroform : 0.45 Ethyl acetate : 0.83
6.	Saponin	Petroleum ether: 0.87

Pharmacological Screening of isolated extracts-:
The extracts of **Randia Dometorum Roots**were then tested for acute toxicity study.

Acute Toxicity-:
The acute oral toxicity in mice indicated that extract of *Randia Dometorum Roots* (Ethanolic Extract) were nontoxic at 200-400mg /kg body weight.

Table 7:Acute toxicity profile

Groups	No. of animals in group	Dose (mg/kg)	Results
Ethanolic Extract	3	200	No toxic sign
	3	300	No toxic sign
	3	400	No toxic sign

Similarly, the extract of *Radian Dumetorum*leaveswere then tested for acute toxicity study.
The acute oral toxicity in mice indicated that extract of *Randia Dometorum leaves*(Ethanolic Extract)were nontoxic at 200-400mg /kg body weight.

Table 8: Acute toxicity profile

Groups	No. of animals in group	Dose (mg/kg)	Results
Ethanolic Extract	3	200	No toxic sign
	3	300	No toxic sign
	3	400	No toxic sign

CONCLUSION
Herbal remedies are widely available and come in huge quantities. Therefore, the purpose of this study was to attempt to standardize and assess the efficacy of herbal therapy in complex diseases such as diabetes. Examining Randia Dometorum root and leaves via macro and microscopical lenses was part of the pharmacognostic study. The yield and phytochemical content of the various extracts were among the many attributes studied. The hydroalcoholic combination of Randia Dometorum has a higher extractive yield value than the aqueous or other solvents. An initial phytochemical evaluation of the extract was conducted by employing thin-layer chromatography (TLC) and various chemical tests to identify alkaloids, flavonoids, terpenoids, and tannins, among others. Different chemical constituents were identified in various solvent systems through TLC analysis.Acute toxicity studies indicated the non-toxic nature of the ethanolic extract at tested doses.

CONFLICT OF INTEREST
Nil

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