

Studies Phytochemical Screening of Leaf Extract of *Clerodendrum Infortunatum* Linn

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ABSTRACT

ABSTRACT: The leaves of *Clerodendrum infortunatum* Linn, a member of the Verbinaceae family, contain compounds with biological activity. The current study set out to evaluate various extraction techniques in order to find the optimal ones for extracting flavonoid components from various solvents. Four different solvents were used to extract the leaves of *Clerodendrum infortunatum* Linn. To determine the percentage (%) yield of each extract, plant material was extracted using a variety of organic solvents in ascending order of polarity, including Petroleum ether (60–80°), Chloroform, Acetone, and Methanol. Each extract was subjected to a thin layer chromatography analysis to determine the amount of components it contained. Using various extraction techniques and (80%) ethanol, N.R. Fransworth extracted a flavonoid-rich fraction for comparative analysis.

KEYWORDS: Flavonoids, *Clerodendrum infortunatum* Linn leaves phytochemical screening, Different extraction methods, Different solvents TLC Rf value

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INTRODUCTION

Different species of *Clerodendrum* genus have been traditionally used over centuries and their antioxidant and hepatoprotective potential have Already been proved. Various parts of the plant are used by tribes in colic, scorpion sting and snake bite, tumors and certain skin diseases. The leaves are slightly bitter, cure inflammation, skin diseases and good in small pox. The plant parts are also used in Indian folk medicine in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy. The plant was found to contain triterpenes, steroids and flavonoids. The antioxidant, antimicrobial, anti-malaria, anthelmintic and analgesic activities of the plant have further created an upsurge in investigations on the plant. The objective of the present study was to extract the flavonoid from the leaves of *C. infortunatum* and investigate its analgesic activity effects in rat.

Clerodendrum infortunatum Linn Well known for their traditional uses in various regions around the globe. This is commonly known as Saraswati leaf other names are Bhant (Hindi), Bhagri (Sanskrit), Khanduchakka, Bhandari (Marathi) Bhat Ghetu (Bangali), Bhandika, Bhantaka,

Bhargi (Sanskrit).

Plant profile

- Kingdom: plantae.
- Order: lamiales.
- Family: lamilaceae.
- Genus: *Clerodendrum*.
- Species: *C. infortunatum*.
- Binomial name: *clerodendrum infortunatum* Linn.
- Vernacular name: Hill Glory Bowar, Khanduchakka, Bharanghi, Bhagri.



Fig. 1

MATERIALS AND METHODS

Plant material

Plant material the fresh leaves of *C.infortunatum* linn. Were collected locally (Nagpur district of Maharashtra) in autumn as it contains the maximum active constituents at that time. Which was then identified and authentication at Department of Botany, Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur.

Drying and size reduction

The leaves of *Clerodendrum infortunatum* linn. Were shade drying under normal environmental conditions and then subjected for size reduction to coarse powder in a mechanical grinder.

Preparation and extraction of plant material

About 1kg of coarse powder material was added subjected to successive extraction with 80% ethanol and 20% water (hydroalcoholic) extraction in sohxlet apparatus. The extraction was continued until solvent in the thimble becomes clear indicating the completion of the extraction. After extraction, the solvent was distilled off and extract was poured in petri plate and concentrated at room temperature.

Morphological characteristics of flavonoid rich fraction

Color, odor and taste were shown in table .8.1

Table 1: Morphological Characteristics of flavonoid rich fraction.

Sr. No.	Test	Characteristics of flavonoids rich fraction
1	Color	Greenish black
2	Odour	Characteristics
3	Taste	Sweet

Phytochemical screening

The Phytochemical screening tests are performed to

clerodendrum infortunatum linn leaves of hydroalcoholic extracts and the results are depicted in table 1.



Fig: 2. flavonoid Extract

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Phytochemical and preliminary screening of the extract:

Solubility: Solubility of the flavonoid extract in various solvents are shown in table 8.2

Table: 2. Solubility of the flavonoid Extract

Sr. No.	Solvent	Solubility
1.	Chloroform	Soluble
2.	Water	Soluble
3.	Petroleum ether	Sparingly soluble
4.	Methanol	Soluble
5.	Ethanol	Soluble
6.	Acetone	Soluble

The flavonoid extract of *Clerodendrum infortunatum* were soluble in chloroform, water, methanol, ethanol, and acetone and sparingly soluble in petroleum ether.

Chemical test for flavonoid extract (flavonoid rich fraction)

Table: 3. Chemical test for flavonoid extract

TEST	OBSERVATION	INFERENCE
1. Shinoda test	Magenta red color is observed	Flavonoids are present
2. Lead acetate test	Yellow precipitate are formed	Flavonoids are present
3. With sodium hydroxide	Yellow coloration (Thus decoloration after addition of HCL) (acid)	Indicating presence of flavonoids

TLC (Rf value) of flavonoid extraction

Table: 4. RF Value of the flavonoid extract

SR. NO.	Solvent System	No. of Spot	RF Value
1	Ethyl acetate : Benzene : Methanol : Water (75:5:10:10)	Two	0.96 and 0.49
2	Ethyl acetate : Formic acid : Methanol : Water (50:5:2:4)	Two	0.91 and 0.60
3	N- butanol : Acetic acid : Water (8:2:10)	Two	0.96 and 0.37
4	Ethyl acetate : Acetone : Formic acid : Water (50:30:10:10)	Two	0.86 and 0.36
5	Chloroform : Toluene : Ether : Acetic acid (60:60:15:5)	Two	0.42 and 0.45
6	Toluene : ethyl acetate : formic acid (50:40:10)	Two	0.32 ,0.75

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Fig:3 flavonoid extracts

A



Fig:4 flavonoid Extracts

B

- A) Toluene : ethyl acetate : formic acid (50:40:10)
- B) (60:60:15:5)Chloroform : Toluene : Ether : Acetic acid

- 1. Petroleum ether (60-80)
- 2. Chloroform
- 3. Acetone
- 4. Methanol

Extraction & chemical evaluation of plant material

Extraction of the plant material with various organic solvents in increasing order of polarity with the help of following solvents.

A) Table: 5. Percentage yield with various organic solvents

Solvents	% yield
Petroleum ether (60-80)	2.43 %W/W
Chloroform	2.2%W/W
Acetone	2.3%W/W
Methanol	2.37%W/W

Different solvent systems were tried for the complete separation of the phytochemical present in the flavonoid extract. The Extraction of the plant material with various

organic solvents in increasing order of polarity was identify the more percentage yield was found to be petroleum ether as compare to chloroform, acetone, and methanol.

Table: 6. TLC characterization of different extract.

SR. NO.	Organic Solvent	Solvent System	No. of Spot	RF Value
1.	Petroleum Ether	Benzene : Ethyl Acetate (8:2)	Two	0.84, 0.95
2.	Chloroform	Ethyl Acetate : MeHO (7:3)	Two	0.18, 0.84
3.	Acetone	Ethyl Acetate : MeHO (7:3)	Two	0.80,0.78
4.	Methanol	Ethyl acetate : formic acid : glacial acetic acid : water (100:11:11:26)	Two	0.90,0.86

Proximate analysis

The various ash values and extractive values determined quantitatively are shown in table 8.6

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a) Table:7. Ash value determination of extract

Sr. No.	Parameters	Result
1.	Total ash value	0.354% w/w
2.	Acid insoluble ash value	0.133% w/w

b) Table: 8. Extractive value

Solvents	Wt. of plant material	Colors of extract	Extractive value (%)
Aqueous (Water)	5g	Light yellowish	4.5% w/w
Alcohol (Ethanol) (90%)	5g	Dark greenish	3.6% w/w
Ether	4g	Greenish brown	3.5% w/w

Total ash value, water soluble ash value were found to be in range. The water soluble extractive value was found to be more in percentage yield of hydroalcoholic extract as compared to alcohol soluble extractive value. It indicates that

the polar constituents are present more in the hydroalcoholic extract of *Clerodendrum infortunatum* Linn

A) Table 9. Chemical Test of different extracts

Test	Observation	Inferences
1) Petroleum ether (60-80)	Green color appeared in chloroform layer	Sterol present
a) Salkowski test sample (1-2 mg) was dissolved in 1ml of chloroform (CHCl ₃) and 1ml of concentrated H ₂ SO ₄ was added		
2) Chloroform	Green color appeared in chloroform layer	Sterol present
a) Salkowski test Test sample (1-2 mg) was dissolved in 1ml of chloroform (CHCl ₃) and 1ml of concentrated H ₂ SO ₄ was added		
b) Shinoda test :- Small quantity of test residue was dissolved 5ml of ethanol (95% v/v) and treated with few drops of conc. HCl and 0.5g of magnesium metal.	Brownish black color are form	Flavonoids are absent
c) Molisch test : Sugar test molisch reagent (1ml) was mixed with 2ml of test solution and then 1 ml of conc. H ₂ SO ₄ was added.	Green ring at the junction of two liquid	Carbohydrate are absent
3) Acetone	Blue-black color are observed	Tannins are present
a) Test for Tannins		
1) Ferric chloride :- Test sample (2ml) was mixed with FeCl ₃ solution (5%)		

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2)Lead acetate:- Test solution was mixed with lead acetate solution and observed for the formation of white ppt	White ppt are form	Tannins are present
b) Shinoda test: Test for flavonoids Small quantity of test residue was dissolved 5ml of ethanol (95%v/v) and treated with few drops of conc. HCl and 0.5g og magnesium metal	Brown color are observed	Flavonoids are absent
4) METHANOL		
a) Test for Tannins	Blue-black color obsered	Tannins are present
1) Ferric chloride :- Test sample (2ml) was mixed with FeCl ₃ solution (5%)		
2)Lead acetate:- Test solution was mixed with lead acetate solution and observed for the formation of white ppt	White ppt appeared	Tannins are present
b) Test for flavonoids (Shinoda test) Small quantity of test residue was dissolved 5ml of ethanol (95%v/v) and treated with few drops of conc. HCl and 0.5g og magnesium metal	Magenta- red color are observed	Flavonoids are present

The different solvent extract of *Clerodendrum infortunatum* Linn. Shows the presence of pertolium ether and chloroform, steroids present and acetone, methanol have flavonoids, and tannin present and chloroform extract Carbohydrate, flavonoids are absent.

SUMMARY & CONCLUSION

- The preliminary phytochemical screening showed that the leave extract showed the positive test in Molisch test, Shinoda test, Alkali test, Lead acetate test and salkowski test.
- Rf value of leave extract was calculated using various mobile phase like Ethyl acetate : formic acid : glacial acetic acid : water (100:11:11:26), Toluene : ethyl acetate : formic acid (50:40:10), Chloroform : Toluene : Ether : Acetic acid (60:60:15:5)^j
- Extraction of the plant with various organic solvents in increasing order of polarity with the help of various solvents were performed and the percentage yield was calculated. The preliminary phytochemical study of the extract of different solvent was done and also the Rf value was calculated by using thin layer chromatography. The comparative study of the leave

extract with the standard of Quercetin was performed and the Rf value was calculated.

- The leaves of *Clerodendrum infortunatum* Linn exhibited the abundant presence of flavonoids in 80% hydroalcoholic extract.
- The alcohol soluble extractive value was found to be 3.6% w/w.
- Among the various organic solvents used, the highest percentage yield was obtained with petroleum ether.
- Among the various extraction methods, the soxhlet extraction method yielded highest percentage yield as compared other extraction methods

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