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Sweta Negi
GRD (PG) IMT, Department Of Pharmacognosy, 214-Rajpur Road, Dehradun, Uttarakhand, India

Dr. Arvind Negi
GRD (PG) IMT, Department Of Pharmacognosy, 214-Rajpur Road, Dehradun, Uttarakhand, India

Ankita Pokriyal
GRD (PG) IMT, Department Of Pharmacognosy, 214-Rajpur Road, Dehradun, Uttarakhand, India

Geetika Tripathi
GRD (PG) IMT, Department Of Pharmacognosy, 214-Rajpur Road, Dehradun, Uttarakhand, India

Corresponding Author:
Sweta Negi
GRD (PG) IMT, Department Of Pharmacognosy, 214-Rajpur Road, Dehradun, Uttarakhand, India

Pharmacognostical investigation of "*Adina cordifolia* (Roxb.) Brandis" Family-*Rubiaceae*, collected from areas of Haldwani District Nainital

Sweta Negi, Dr. Arvind Negi, Ankita Pokriyal and Geetika Tripathi

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Abstract
Ayurveda is a pelagic with lashing quantities of proclaimed and unrevealed medicinal gems. Due to the morphological structure and lack of knowledge certain medicinal gems of *Ayurveda* are debatable. Such as *Adina cordifolia* which is one of the controversial kind of drug. *Adina cordifolia* is the Saffron Teak, Yellow, Belonging to family *Rubiaceae*, subfamily *Cinchonoideae* is endowed from India, Southern Asia, Srilanka, Vietnam and Southern China. It is a buoyant, deciduous tree; hinges disintegrated all throughout the significant part of Indian deciduous forests and drift in the sub-himalayan zone up to an altitude of 900 m. Since immemorial times it has been utilized in folklore medicine. *Adina cordifolia* is a medicinal plant used for the treatment of chronic jaundice, skin disease, gastropathy, fever, cough, swelling in stomach, stomachache and several other diseases. In the present article *Adina cordifolia* was investigated for Pharmacognostical screening such as Morphological Identification, Organoleptic properties, Microscopic evaluation, Fluorescence Analysis, Determination of the moisture content, Determination of Ash values, Determination of percentage extractive, Loss on drying, and Determination of pH. Because *Adina cordifolia* is a controversial kind of drug having sparse research so this study will be benevolent to push scientist for co-ordinating their studies.

Keywords: *Adina cordifolia*, morphology, *Microscopical parameters*, fluorescence analysis

Introduction
Pioneer basins of natural or herbal medicines are procured by the Medicinal plants. The structural diversity of their phytoconstituents accomplishes them as an esteemed source of novel lead compounds for the quest of drugs to treat acute and chronic diseases. Because of rich biodiversity Indian subcontinent has richest plant based traditional medicinal system. Due to better recession and lesser side effects on human body these herbal medicines are primarily used for health care. The Indian Himalayan vicinity embraces about 1,748 different species of medicinal plants. The Garhwal Himalaya is acknowledging for its affluent bio-resources and ethano-culture diversity. The origin of drugs to the greatest extent depends on these natural products from plant, animal, microorganism and minerals, which is pre-owned in the therapy of human and animal disease [1, 2]. Respective plants encompass a diversity of phyto-pharmaceuticals with essential applications amidst the fields of agriculture and medical speciality. Plants have considerable capacity utilized as drugs and pharmacopoeia medication as an extended proportion of the world population hinge on traditional medicines of plant origin due to the deficient endow and overprice formulaic modern medicine. Numerous plants derived therapeutic agents play a very important role for the development of novel drug leads for the treatment and hindrance of diseases [2, 3].
Ayurvedic medicines are divided into three classes, namely Herbal, mineral and animal on the bases of material of origin. Among this, recently herbal formulation has procured extended significance and rising global awareness. Throughout the last few years in developed world, where market expansion occurred in USA and European countries the scenario is evident as substantial scale increase in the herbal formulation usage has been perceived [3]. It has been estimated according to the World Health Organization (WHO) that 80% of the world's inhabitants still anticipate mainly on traditional medicines for their health care. With about 45,000 plant species Indian subcontinent is well-known to be one of the humongous biodiversity centres [4]. Overall the history of mankind this richness of flora has denoted to its status as a reservoir of herbal. About 15,000 medicinal plants have been registered in India, from which the communities used 7,000-7,500 plants for curing different diseases. About 700 type of plants listed in *Ayurvedic* medicinal systems [5].

India with its former magnificent stereotyped medicinal system and use of different plants pattern is one of eighth major centers of origin and diversification of domesticated taxa. India is one of the world's twelve mega diverse countries having rich biodiversity. Besides that, currently there is a global consensus on the benefits of phytopharmacy along with medicinal plant. In plant research and medicinal India inhabits a key position. Numerous Plants species which are extensively used as medicine need phytochemical investigation to search active medicaments [6]. Many Plants Species encompassing active ingredient of medicinal ethics are yet to be uncover. This aspect cues us to undertake phytochemical investigation of plants for the experiments and research. An extensive range of medicinal plant parts is utilized for extract as raw drugs and acquire variety of medicinal properties. The different parts used incorporate leaves, root, stem, flower, fruit, twigs exudates and modified plant organs. While for many herbal industries few of these raw drugs collected in larger quantities and traded in the market as the raw material [7].

"Adina cordifolia" is a buoyant, deciduous tree; underneath sound circumstances grows, over 30 m., but is conventionally about 14-20 m tall [8]. Hinges disintegrated all throughout the significant part of Indian deciduous forests and drift in the sub-himalayan zone up to an altitude of 900 m. Since immemorial times it has been utilized in folklore medicine. It is a medicinal plant used for the treatment of chronic jaundice, skin disease, gastropathy, fever, cough, swelling in stomach, stomach-ache and several other diseases. Leaves grow up to 25 cm or more over, predominantly circular or oval in shape, acute at the apex, heart-shaped at the base, slightly hairy especially when young, green or tinged with red or pink; from the base to the tip of the leaf a strong one nerves runs and 5-6 pairs of lateral ones, which unite near the margin of the leaf in a wavy line. Leaves come out in pairs, one on either side of a branch, their stalks connected by a pair of stipules. These are two leaf-like structures, up to 2.5 cm. long, enclosing and protecting the very young leaves and shoot apex; when the stipules fall away, they leave two clear lines, each encircling half of the branch. Leaf stalks are 5-10 cm, long [10]. Flowers are individually insignificant, being very small; but they come out in balls 2-3 cm. across; the tiny flowers are yellow to yellowish in color, often tinged with pink. When the little flowers open out, the most prominent parts are the styles, which form a sort of halo round the floral ball [11]. Fruits are minute, forming an almost solid ball, which when ripe is black or nearly black. Fruits are capsules like, splitting into two dehiscent cocci [12]. Seeds are numerous, narrow, small and tailed [13]. Leaves are shed arround February, and the tree remains leafless until about May-June; the stipules covering the buds are than very conspicuous. From June to August Flower balls are at their best. After the fruit has been shed properly about at the beginning of June of the following year, the fruit-heads appear black and are about 12 mm. across: the rains of the monsoon may bring them down and prepare the tree for the new flower balls [14].

In the present article *Adina cordifolia* was investigated for Pharmacognostical screening.

Table 1: Taxonomic classification of "*Adina cordifolia*" [15, 16]

Kingdom	**Plantae**
Class	Magnoliopsida
Sub-class	Asteridae
Super order	Gentiananae
Order	Gentianales
Family	Rubiaceae
Subfamily	Cinchonoideae
Genus	Adina
Specific epithet	Cordifolia
Botanical name	*Adina cordifolia* (Roxb.) Benth& Hook. F.
Synonym	Haldina Cordifolia (Roxb.)

Fig 1: Tip with leaf and flower bud

Fig 2: Leave with seeds

Fig 3: Seeds

Fig 4: Leave with flower

Pharmacognostic study [14-20]

The present Pharmacognostic study of "*Adina cordifolia"* includes the following:

1. Collection and Processing.
2. Morphological Identification.
3. Organoleptic properties.
4. Microscopic evaluation.
5. Fluorescence Analysis.
6. Physiochemical screening.

- Determination of the moisture content.
- Determination of Ash values:
  - Total ash
  - Acid soluble ash
  - Water soluble ash
- Determination of percentage extractive.
  - Alcohol soluble extractive
  - Water soluble extractive
- Loss on drying.

7. Determination of pH.

Material and Methods

1. Collection and Processing

- **Collection of plant sample:** The "*Adina cordifolia* (Roxb.) Brandis" plant leaves were collected from rural area of Haldwani in Nainital district of Uttarakhand.
- **Processing of plant sample:** The plant leaves were washed thoroughly and shade dried for two consecutive weeks and it was grinded to the uniform powde, this powder was used for evaluation of different parameters and preparation of different extracts.

2. Morphological identification

By the morphological characters the plant was identified properly and compared with the standard.

3. Organoleptic properties: colour, touch, odour and taste.

4. Microscopy evaluation [14-20]

Leaf and stem of "*Adina cordifolia"* were studied for Microscopy (T.S) and powder characters using compound microscope or inbuilt light microscope and photographs were taken. Using eye piece, stage micrometer and camera lucida Quantitative microscopical measurements were made. All the chemicals, reagents and solvents used are of A.R grade.

- **Microscopy of *"Adina codifolia"* leaf:** Microscopically the leaf sample was studied by taking transverse section (T.S) through different sections of leaf: Apex, Middle and Base of leaf. A section was cut from each part with small portion of lamina and thin section was stained with saffranin and examined under compound microscope.
- **Microscopy of *"Adina codifolia"* stem:** The thin transvers section of stem was cut, cleared with water, stained with saffranin and observed under compound microscope.
- **Powder microscopy:** On a clean glass slide fine powder sample of "*Adina cordifolia"* was mounted and clarified with clearing solution and examined for identification of diagnostic characters.

Quantitative microscopy

- **Stomatal Number and Stomatal index:** The upper epidermis of leaf between midrib and lamina was exfoliated and translucent area was cleared with clearing solution and mounted on a glass slide. On a blank sheet between 0.2mm square the stomata and epidermal cells were traced using prism type camera lucida under high power (45x). As per rule from each drawn square the number of epidermal cells and stomata were counted.

$$\text{Stomatal Index} = \frac{\text{Number of stomata}}{\text{Number of Stomata + Epidermal cells}} \times 100$$

- **Vein-Islet and Vein termination Number:** The leaf section between midrib and margin was macerated and decolorized with bleaching solution. The cleared lamina portion was mounted on a glass slide and vein islet and vein termination were traced on black sheet between 0.5mm square using low power (5x) and values were determined per sq.mm of leaf area between midrib and margin.
- **Palisade ratio:** The leaf sample of "*Adina cordifolia"* was disintegrated with con. HCl and caustic soda by boiling on water bath, further bleaching solution was used to bleach the leaf, and then clarified epidermal cells were traced. The average numbers of palisade cells were traced. The average numbers of palisade cells were calculated and palisade ratio was determined.

5. Fluorescence analysis

The leaves of "*Adina cordifolia"* were dried in shade to obstruct decomposition of active principle and grind to fine powder for the fluorescence study. A small quantity (1gm) of finely dried powder of leaves was treated with freshly prepared acid, alkaline solutions, and different solvents. They were subjected to study the fluorescence analysis in visible light, short UV light (254nm) and long UV light (365nm) [14-20].

6. Physiochemical screening

Determination of the moisture content

Air dried coarse powder of "*Adina cordifolia"* leaves was weighed around 2 gms in a previously weighed Petri plates. Petri plates with the samples were kept in the oven and maintained at 110ºc for drying. After 3 hrs Petri plates were taken out weight was noted down. This procedure is repeated three times until the constant weight is reached [14-20].

$$\text{Moisture content of the drug (\%)} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

Determination of Ash values [14-20]

Total ash

Air dried coarse powder of "*Adina cordifolia"* leaves was weighed around 2 gms in 3 heated silica crucibles avoiding any moisture content. The coarse drug powder ignited to 100º -150 ºC in an electric ignition till the charring of the drug material and kept in an incinerator at 50 ºC, temperature allowed rolling back to Zero; then it was removed from furnace and cooled in desiccators to room temperature and weighed [14-20].

$$\text{Total ash} = \frac{\text{Weight of residue}}{\text{Weight of the sample}} \times 100$$

Acid insoluble ash

The total ash, which was procure, was boiled for 5 minutes with 25ml of diluted hydrochloric acid, the insoluble matter was collected on an ash less filter paper which was washed with hot water and ignited to constant heat [14-20].

$$\text{\% of acid insoluble ash} = \frac{\text{Difference in weight}}{\text{Weight of sample}} \times 100$$

Water soluble ash

The total ash obtained above was boiled with 25ml of distilled water for 5 minutes. The insoluble matter was collected on an ash less filter-paper, washed with hot water and ignited to constant weight at low temperature. The weight of the insoluble matter was subtracted from the weight of total ash, represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug. The result was calculated with reference to the air dried drug [14-20].

$$\% \text{ of water insoluble ash} = \frac{\text{Difference in weight}}{\text{Weight of sample}} \times 100$$

Determination of percentage extractive

Alcohol soluble extractive

Air dried coarse powder of "*Adina cordifolia"* leaves was weighed around 5 gm and macerated with 100 ml of 95% ethanol in a closed flask for 24 hours agitated frequently. Next day the solution was filtered and 25 ml of this filtrate was added to the first 6 hours and allowed standing for 18 hours. Subsequently, it was filtered speedily with precautions against loss of the solvent. Evaporated 25ml of the filtrate to dryness in a tared flat bottom shallow dish dried at 105 ˚C and weighed. The percentage of ethanol soluble extractive value with reference to the air dried drug was calculated [14-20].

$$\% \text{ alcohol soluble extractive} = \frac{\text{Difference in weight}}{\text{Weight of the sample}} \times 100$$

Water soluble extractive

Air dried coarse powder of "*Adina cordifolia"* leaves was weighed around 5 gm and macerated with 100 ml of chloroform water in a closed flask for 24 hours agitated frequently. Next day the solution was filtered and 25 ml of this filtrate was added to the first 6 hours and allowed standing for 18 hours. Subsequently, it was filtered speedily with precautions against loss of the solvent. Evaporated 25ml of the filtrate to dryness in a tared flat bottom shallow dish dried at 105 ˚C and weighed. The percentage of water soluble extractive value with reference to the air dried drug was calculated [14-20].

$$\% \text{ water soluble extractive} = \frac{\text{Difference in weight}}{\text{Weight of the sample}} \times 100$$

Loss on drying

The powdered leaves of "*Adina cordifolia"* was dried to constant weight in the oven at 85-90 ˚C. The test was performed in triplicate and percentage of loss on drying was calculated [14-20].

$$\% \text{ Loss on drying} = \frac{\text{Difference in weight}}{\text{Weight of the sample}} \times 100$$

7. Determination of pH

pH was evaluated at room 25 ºC temperature. Apparatus was Calibrated using different buffer solution up to pH 7, then water soluble and alcohol soluble solutions was kept ready, and the electrodes were immersed in both the solutions and readings were recorded [14-20].

Result and Discussion

Pharmacognostic studies

Morphological identification

Adina cordifolia is a substantial deciduous tree with dark grey bark with exfoliating in asymmetrical woody scales, orbicular briefly acuminate leaves, Yellow flowers in globose pedunculate heads and dehiscent capsule.

Organoleptic properties

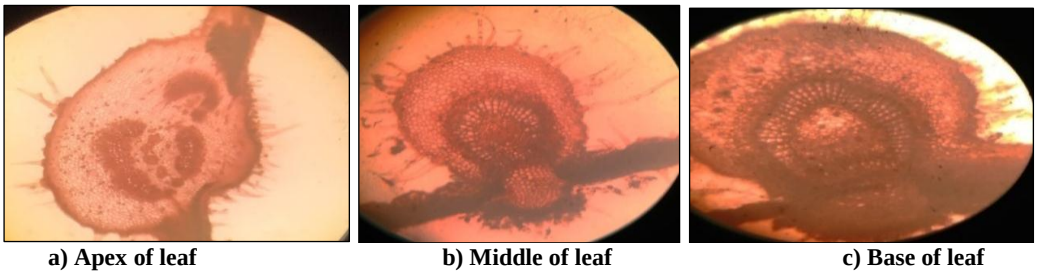
Table 2: Organoleptic properties

Parameters	Result obtained
Color	Green
Odour	Slightly pungent, causes chocking
Touch	Slightly coarse
Taste	Bitter

Microscopic evaluation of "*Adina cordifolia"*

Leaf microscopy

a. **Apex of leaf:** T.S shows underdeveloped pith, calciform, convolute and magnified pitted elements with inclined fibres and parenchyma, some remains of polygonal to rounded parenchyma cells with intercellular spaces, few semi-transparent resinous droplets.
b. **Middle of leaf:** T.S Shows Bifacial structure in midrib and lamila region: single layer upper epidermal cells which are rectangular with cuticulsrized outer walls and presence of trichomes, epidermal layer of lamina continuous over midrib region also with numerous trichomes. Mesophyll differentiates to palisade and parenchyma. Palisade cells are single layer compact and radially elongated. Numerous sheets of Parenchyma roughly arranged with intercellular spaces, few translucent resinous droplets. Lower epidermis is indistinguishable to upper epidermis with stomata and numerous trichomes.
c. **Base of leaf:** T.S shows convolute elements with inclined fibres and parenchyma, some remains of parenchyma with polygonal to rounded cells with intercellular spaces, few semi-transparent resinous droplets. Single layer upper epidermal cells which are rectangular with cuticulsrized outer walls and presence of trichomes, Lower epidermis is indistinguishable to upper epidermis with stomata and numerous trichomes.



a) Apex of leaf **b) Middle of leaf** **c) Base of leaf**

Fig 5: Leaf Microscopy

Stem Microscopy: T.S of *Adina cordifolia* stem shows cortex with hypodermis (collenchymas) and endodermis (starch cells) present above pericycle and vascular bundles, the outer epidermis with stomata and trichomes, two pipe like tissues xylem and phloem separated by cambium, Pith in the center.

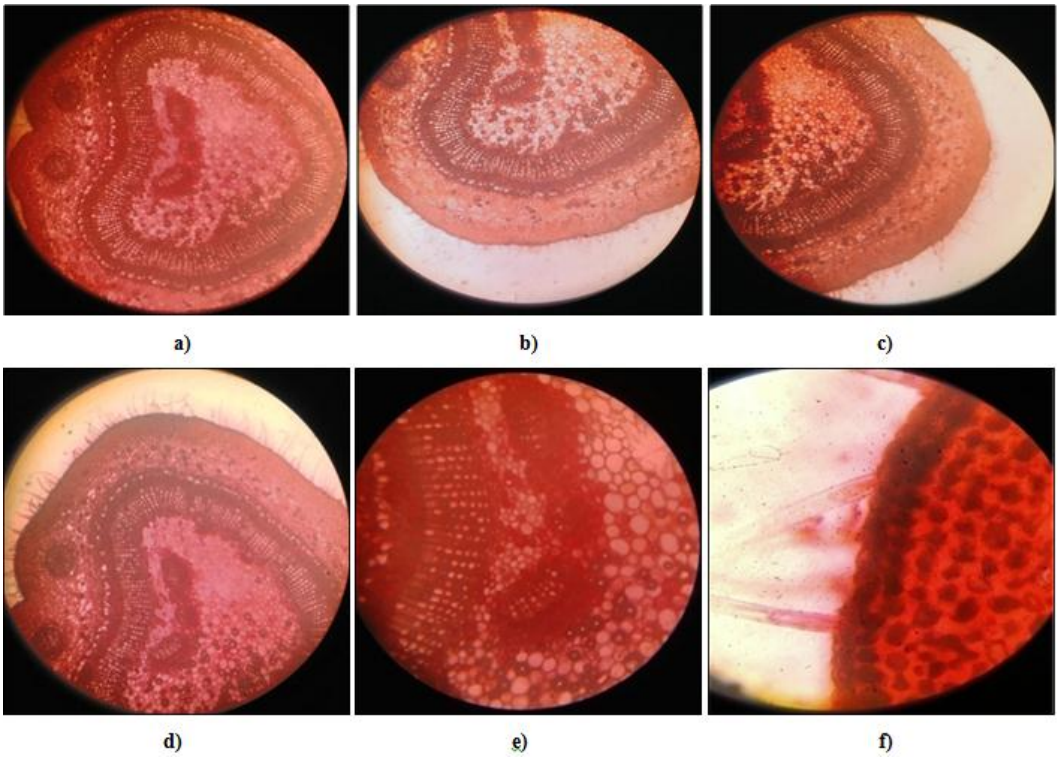


Fig 6: Stem Microscopy

Powder microscopy

Remains of busted tracheary tissue with calciform, convolute and magnified pitted elements with inclined fibres and parenchyma, some remains of polygonal to rounded parenchyma cells with intercellular spaces, fragments of xylem, fibres and part of small group of fibres, trichomes, few semi-transparent resinous droplets are found in the powder microscopic evaluation of *Adina cordifolia*.

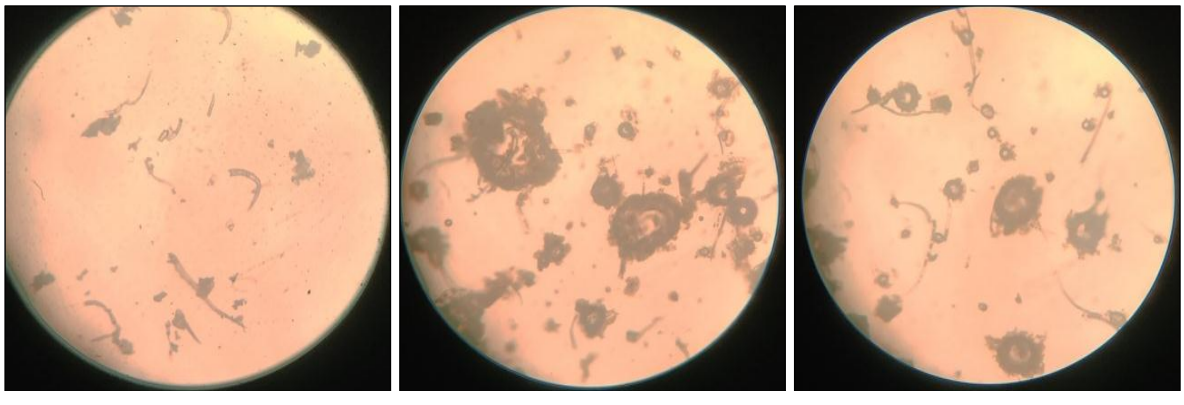


Fig 7: Powder Microscopy

Table 2: Quantitative microscopy

Sr. No.	Numbers		Apex	Middle	Base
1.	Stomatal Number	Upper surface	10-14	45-50	15-23
		Lower surface	42-50	75-90	22-30
2.	Stomatal index		42.7-61.8	68.22-70.42	55.15-62.7
3.	Vein-Islet		15-20	25-30	15-20
4.	Vein Termination Number		10-13	15-22	11-16
5.	Palisade ratio	Palisade No.	33	41	23
		Epidermal cell No.	4	4	4
		Palisade Ratio	1:8	1:10	1:5

Table 3: Fluorescence Analysis

Sr. No.	Interaction of powder drug with different reagent	Colour produced under visible light	Colour produced under UV-radiation.	
			Short (254nm) wave length	Long (365nm) wavelength
1.	Drug (P) as such	Light Green	Light Green	Dark Green
2.	P + 5% $FeCl_3$	Dark Green	Black	Black
3.	P + Con.H_2SO_4	Brown	Green	Brown
4.	P + HNO_3	Redish Brown	Light Green	Black
5.	P + HCl	Greenish Brown	Light Green	Black
6.	P + Acetic acid	Green	Light Green	Dark Green
7.	P + Iodine	Greenish Brown	Green	Black
8.	P + Picric acid	Yellowish Green	Light Green	Black
9.	P + 50% ethanol	Dark Green	Green	Light Green
10.	P + 50% Methanol	Greenish Brown	Brown	Green
11.	P + $CHCl_3$	Green	Yellowish Green	Brown
12.	P + 10% Na_2CO_3	Dark green	Brown	Light Green
13.	P + 1N NaOH	Yellowish Green	Greenish Brown	Black
14.	P +Benzene	Green	Green	Black

Table 4: Physiochemical screening of *Adina cordifolia*

Parameters			Values (%)w/w
1.	Determination of the moisture content		7.00
2.	Determination of Ash values	Total ash	5.45
		Acid soluble ash	0.65
		Water soluble ash	2.18
3.	Determination of percentage extractive	Alcohol soluble extractive	12.00
		Water soluble extractive	10.00
4.	Loss on drying		8.40

Table 4: Determination of pH

Sample	pH Alcohol	pH Water
Adina cordifolia	5.62	4.54

Conclusion

The plant was collected from Haldwani district, Nainital Uttarakhand. The plant is accounted for its different exercises. "Traditionally the *Adina cordifolia* plant has a large demand due to its treatment of many chronic and acute diseases with great benefits. *Adina cordifolia* is an India, Thailand, Ceylon, and Burma; scattered in mixed deciduous forests used by traditional healers for the treatment of chronic cough and uses in jaundice, stomachache, fodder and swelling in stomach. Therefore, extracts of dry leaf powder of *Adina cordifolia* plant could be seen as a good source for useful drugs".

The conventional medicine implementation firmly put forward for this plant along with it is propound that furthermore work ought to be achieved such as to isolate, purify, and distinguishe the active constituents culpable for the activity of the plant. The Pharmacognostic inquisition of *Adina cordifolia* assists in the Identification and verification of taxon. Microscopical review centres on different attributes, for example, a cross over area *Adina cordifolia* leaf through midrib extends bifacial structure in midrib and lamila region: single layer upper epidermal cells which are rectangular with cuticulsrized outer walls and presence of trichomes, epidermal layer of lamina continuous over midrib region also with numerous trichomes. Mesophyll differentiates to palisade and parenchyma. Palisade cells are single layer compact and radially elongated. Numerous sheets of Parenchyma roughly arranged with intercellular spaces, few translucent resinous droplets. Lower epidermis is indistinguishable to upper epidermis with stomata and numerous trichomes and helps in additional investigation of the species. Present work is an endeavour to accumulate large pharmacognostic, Macroscopical assessment, Microscopical assessment, Standardization boundaries, Quantitative Analytical boundaries, Powder Microscopy, Fluorescence examination of powder and concentrates, cell reinforcement activity, *Adina cordifolia* contained significant organically dynamic mixtures.

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