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RESEARCH ARTICLE

Pharmacognostic Standardization Parameters of *Roylea elegans* Wall (Aerial Parts)

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ABSTRACT

To evaluate the pharmacognostical study of *Roylea elegans* (aerial parts). The qualitative and quantitative microscopy, physicochemical evaluation, phytochemical screening and fluorescence analysis of the plant were done by the standard procedure recommended in the WHO guidelines. Macroscopic study shows that leaves were dark green with lemon like odor and bitter taste, 2-8 cm length and 1-8 cm wide, shape: ovate, hairy upper and lower surface, apex: acute and having reticulate veination, Stems: were light green Microscopic evaluation of the leaves powder shows the presence of trichomes (unicellular covering and glandular), upper epidermis, vessels, xylem fibres, wavy trichomes. The transverse section of the leaf shows the presence of epidermis layer followed by cuticle layer, lignified vascular bundles, trichomes, collenchyma, and palisade cells. Various pharmacognostical parameters help to evaluate the identification and standardization of *Roylea elegans* (aerial part).

KEYWORDS

Roylea elegans, Pharmacognostical Parameters

INTRODUCTION

Roylea elegans is a shrub of monotypic genus. The family lamiaceae contain 22 species. Plant *Roylea elegans* belonging to family lamiaceae. Leaves part of this plant (decoction) is traditionally used as a bitter tonic and also as a febrifuge. It is also used as a tonic in contusions. Leaves are used in skin disease and fever. The leaves of *Roylea elegans* contains various phytoconstituents like betulin, beta-sitosterol, beta-amyrin, stigmasterol, cetyl alcohol, glucose, fructose, arabinose and palmitic, stearic, oleic, gallic, oxalic and tartaric acids. The stems and leaves contain the diterpenes, calyenone, precalyone and calyone, and a triterpene, moronic acid.

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Phytoconstituents Precalyone shows antitumour activity against P-388 lymphocytic leukaemia. The Aerial parts of *Roylea elegans* exhibited spasmolytic and CNS-depressant activity[1].

Recent studies of *Roylea elegans* show the isolation of two furanoid diterpenes isomers royeleganin, royelegafuran. A further investigation of this plant has yielded a new triterpene, named here with moronic acid[4].

In India, People lives in Himalayan region traditionally use aerial parts of *R. elegans* (Titpati) for protect their liver and crushed leaves are given to infants against jaundice[2].

MATERIAL AND METHODS

Chemicals

All the chemicals which were used in study are of analytical grade and purchased from the Himedia Lab. Pvt. Ltd, Rankem.

Plant Selection and Identification

The plant material (*Roylea elegans)* investigated in the present study was collected from Nauni, near Solan, Himachal Pradesh. The plant material was identified and authenticated at the Herbarium of Council of Scientific and Industrial Research - National Institute of Science Communication and Information Resources (CSIR-NISCAIR), Delhi vide reference no. NISCAIR/RHMD/Consult/2014/2781/160.

Processing of Collected Plant Sample

The collected plant material was shade-dried for one week and then powdered using mortar and pestle or grinder and the powder was stored in air tight for use in phytochemical analysis and determination of pharmacopoeia standards.

Macroscopic Analysis

The macroscopical study includes the evaluation of organoleptic characters and external features of the leaf of selected plant material (*Roylea elegans*). The following macroscopic characters for the fresh leaves were noted size, shape, colour, surfaces, venation, margin, base, lamina, texture, colour and taste.

Microscopic Analysis

In microscopic evaluation was conducted on both grounds qualitatively and quantitative studies of *Roylea elegans* (aerial parts).

In this study transverse section and powder microscopy of aerial parts was carried out. Staining procedure was used as per standard procedures. The staining reagents used for staining procedure were phloroglucinol and conc. hydrochloric acid (1:1), Glycerol, safranin etc. The various characters were identified and studied.

Section Cutting

Section cutting of petiole and stems were done. Sections were clear by using 10% KOH solution and stained by various reagents.

Powder Microscopy

In this study the dry leaves were powdered. The cleared powder mounted on slide with the help of glycerin. Then stain the cleared powder with the staining reagents such as phloroglucinol and conc. hydrochloric acid (1:1), Glycerol etc. Various identified characters were observed.

Physicochemical Analysis

Physicochemical analysis of powder of the selected plant material *Roylea elegans* (aerial parts) was determined according to the WHO guidelines and the official methods. In the physicochemical analysis various parameters such as ash values, extractive values, loss on drying.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the aerial parts extract mainly done for the evaluation of the various phytoconstituents such as steroids, tannin, Alkaloids, Flavonoids and glycosides were present in the aerial parts.

Thin Layer Chromatography (TLC)

The TLC plates will be washed and dried in oven. TLC plates will be prepared by the Pouring method. Silica gel G will be taken in a beaker and the slurry will be made with distilled water .The plates will be then tipped back and to spread the slurry uniformly over the surface .These plates will be dried at room temperature and then put in the oven at 100^{o}c for activation of TLC plates. Various solvents will be used for TLC for the investigation of different compounds which were present in extracts.[7]

Fluorescence Analysis

Fluorescence analysis is the one of the most important parameter for the evaluation of the quality, strength and purity of the selected plant material. The powdered material (aerial parts) was analyzed under the three region of light like visible, short U.V region and long U.V region after the treatment with various inorganic/organic reagents.

RESULTS AND DISCUSSION

Macroscopic Characteristics (Morphology)

The macroscopical characters such as colour, odour, taste, shape, margin, apex, base, surface

and size of *Roylea elegans* (leaf) were observed and shown in figure 1 and table 1.

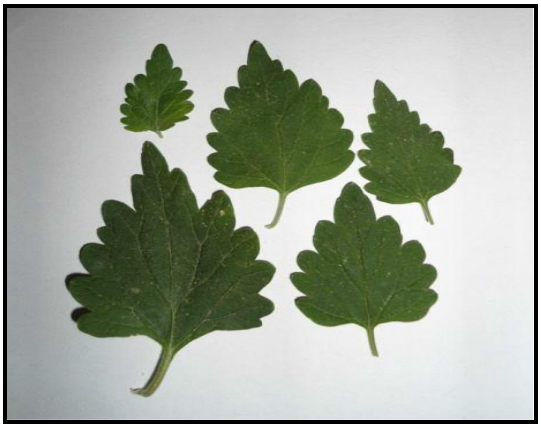


Figure 1: *Roylea elegans* leaf

Table 1: Macroscopical characters of *Roylea elegans* leaf

Macroscopical characters of *Roylea elegans* (leaf)

1	Condition	Fresh
2	Type	Alternate, broad, membranous
3	Size	Length 2-8 cm. Width 1-8cm
4	Shape	Ovate
5	Apex	Acute
6	Margin	Dentate margin
7	Venation	Reticulate venation
8	Base	Oblique
9	Petiole	Present
10	Surface	soft
11	Color	Upper surface dark green and lower surface light green
12	Odour	Lemon like

Microscopic Characteristics

T. S. of Leaf

Centralized epidermis is covering entire section of it; there are several layers of parenchyma bearing air in the cavity lying just above to multi layered chollenchymatous (thick walled cells) arrangement. There are abundant and radially developed vascular bundles.

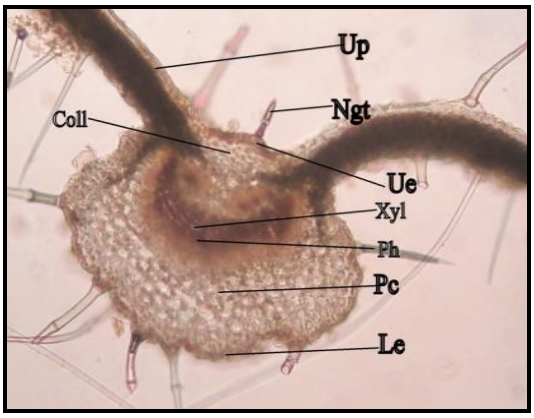


Figure 2: T. S. of *Roylea elegans* leaf

Up- upper palisade**, Ngt-** non glandular trichome**, Ue-** upper epidermis**, Xyl-** xylem**, Ph-** phloem**, Pc-** parenchyma, **Coll-** collenchyma**, Le-** lower epidermis

T. S. of Stem

Figure 3 showed a single layered epidermis as outermost covering with numerous glandular trichomes and unicellular covering trichomes. Cortex consists of 2-4 layers of chlorenchyma cells followed by 2-4 layers of parenchyma cells. Groups of lignified pericyclic fibres were scattered in form of ring throughout the cortex. Phloem region was crushed and vascular bundles were separated by 2-3 cells wide medullay rays which extend upto cortex. Xylem tissue was composed of xylem fibres, xylem vessels and xylem parenchyma.

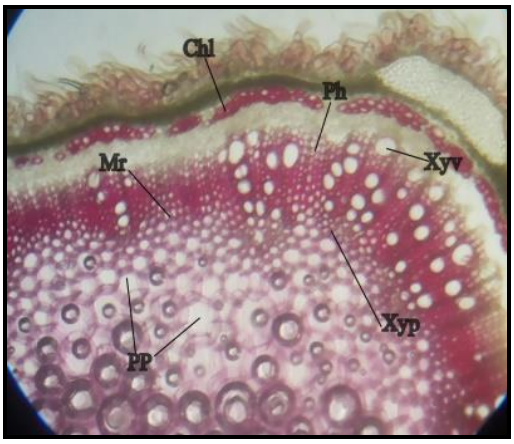


Figure 3: T. S. of *Roylea elegans* (Stem)

Chl- chlorenchyma, **Ph-** phloem, **Xyv-** xylem vessel, **Xyp-** xylem parenchyma, **Pp-** pith parenchyma, **Mr-** medullary ray

Powder Microscopy

The powder microscopy of the Aerial parts of *Roylea elegans* showed the presence of the unicellular covering trichomes and trichomes shown in figures 4 & 5. The trichomes were found to be unicellular shown in figure 6. The structures were found to be lignified when stained with phloroglucinol and conc. Hydrochloric acid (1:1) shown in figures.

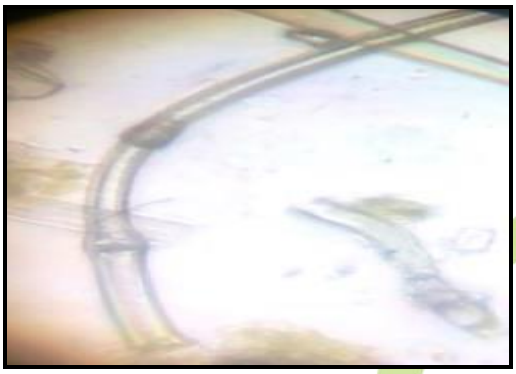


Figure 4: Multicellular covering

Figure 5: Unicellular Covering Trichomes Trichomes

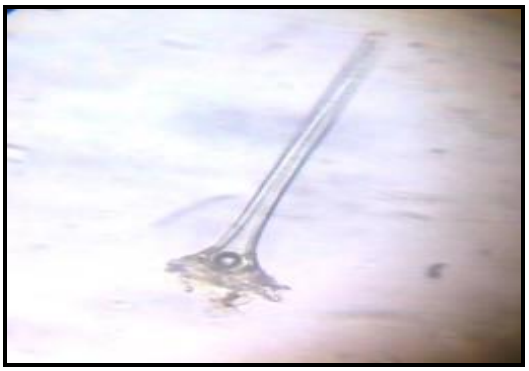


Figure 6: Glandular Trichomes

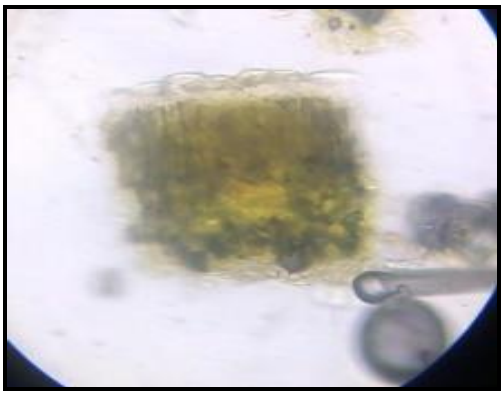


Figure 7: Mesophyll

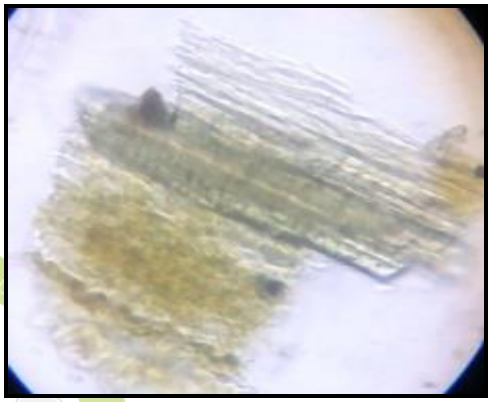


Figure 8: Xylem Vessels

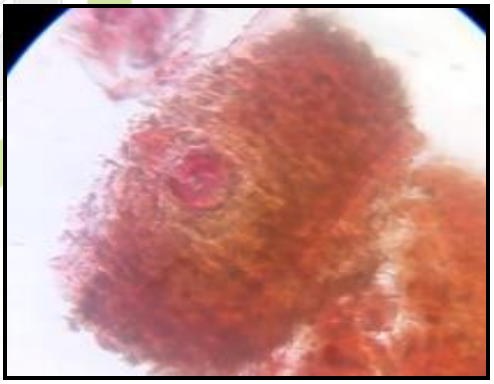


Figure 9: Oil Ceels

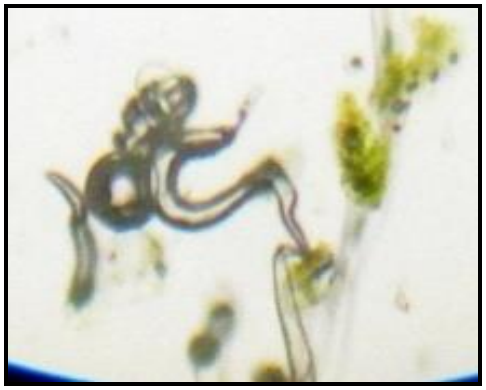


Figure 10: Wavy Trichomes

Figure 11: Scarliform Vessels

Physicochemical Analysis

The results of the analysis discussed in the table 2, 3 and 4.

Table 2: Physicochemical analysis of *Roylea elegans* Aerial parts

Sr. No.	Ash value	% w/w
1.	Total ash	6.66 %
2.	Acid insoluble ash	0.66 %
3.	Water soluble ash	3.33 %
4.	Sulphated ash	14 %

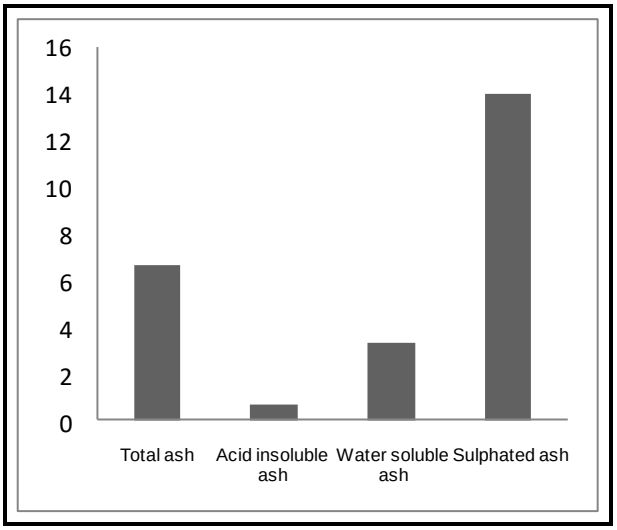
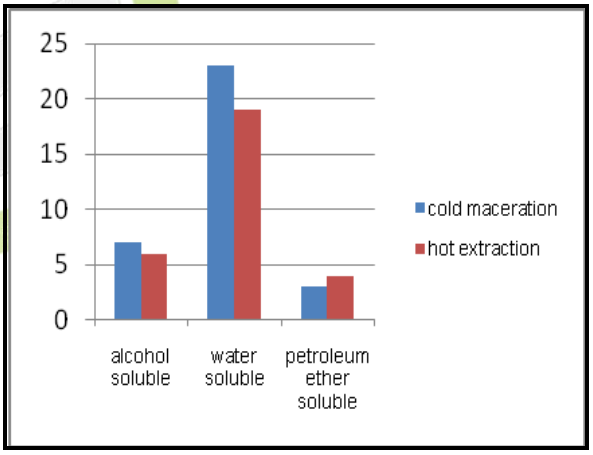


Table 3: Extractive values of *Roylea elegans* Aerial parts

Sr. No.	Extractive value	Methods	% w/w
1.	Alcohol soluble	Cold maceration	7 %
2.	Alcohol soluble	Hot extraction	6 %
3.	Water soluble	Cold maceration	23 %
4.	Water soluble	Hot extraction	19 %
5.	Petroleum ether soluble	Cold maceration	3 %
6.	Petroleum ether soluble	Hot extraction	4 %



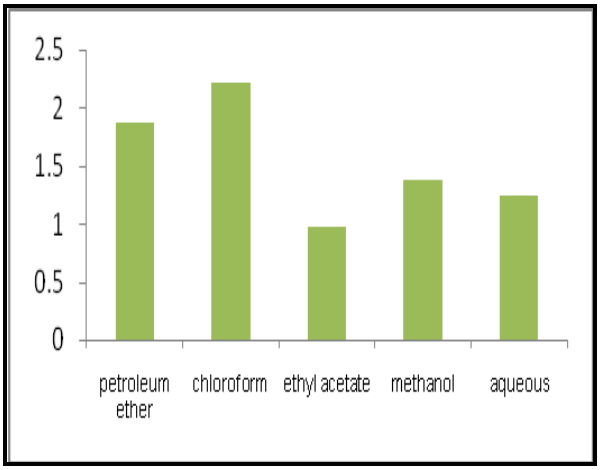


Table 4: Physical parameters include % yield, consistency and colour

Extracts	Fluorescence Analysis Under UV Lamp			Consistency	yield (%w/w)
	Long UV (colour)	Short UV (colour)	Visible		
Petroleum ether extract	Blackish	Blackish green	Brownish green	Sticky semi solid	2.8
Chloroform extract	Dark violet	Black	Blackish green	Slightly solid	3.32
Ethyl acetate extract	Dark blue	Brown	Dark brown	Sticky semi solid	1.89
Methanol extract	Parrot green	Dark green	Blackish green	Sticky paste	5.6
Water extract	Dark brown	Brown	Blackish	Solid	5.95

Phytochemical Screening

Table 5: Phytochemical screening of Roylea elegans (Aerial part)

S. No.	Phytochemical Tests	Pet. Ether extract	Chloroform extract	Ethyl acetate extract	Methanol extract	Water extract
1.	Carbohydrate	-	-	-	-	-
2.	Protein	-	-	-	-	-
3.	Amino acid	-	-	-	+	+
4.	Fats	+	-	-	-	-
5.	Steroid	-	-	+	-	-
6.	Triterpenoides	+	-	-	-	-
7.	Glycosides	-	-	-	-	-
8.	Flavonoids	-	-	+	+	-
9.	Alkaloids	-	+	-	-	-
10.	Tannins	-	-	-	-	-

Table 6: TLC results of various extracts in different solvent systems

Sr. No	Extract	Solvent system	Spots	R_f value
1.	Petroleum Ether	Toluene: ethyl acetate (4.5: 0.5)	7	0.1, 0.2, 0.4, 0.5, 0.6, 0.64, 0.7
2.	Chloroform	Toluene: methanol (4.5: 0.5)	6	0.18, 0.21, 0.4, 0.6, 0.72, 0.90
3.	Ethyl Acetate	Toluene: ethyl acetate (4.5 : 0.5)	4	0.3, 0.5, 0.71, 0.75
4.	Methanolic	Toluene: ethyl acetate: GAA (4: 0.5: 0.5)	6	0.28, 0.5, 0.53, 0.58, 0.66, 0.75
5.	Aqueous	n- butanol: GAA: methanol: DW (3: 1: 0.5: 0.5)	2	0.35, 0.4

Fluorescence Analysis

S. no.	Reagents	Visible	Short U-V	Long U-V
1.	Powder as such	Light green	green	brown
2.	50% H_2SO_4	Yellowish green	Henna green	Dark green
3.	Methanol	Light green	Emerald green	brown
4.	Ethanol	green	green	orange
5.	Conc. H_2SO_4	Dark brown	Dark green	Brownish violet
6.	Conc. HCl	Pale green	Emerald green	Blackish green
7.	NH_3	Pale green	Parrot green	Black green
8.	5 % KOH	Pale green	Emerald green	Dark brown
9.	5 % $FeCl_3$	Pale green	Henna green	Blackish violet
10.	50 % HNO_3	Yellow	Cascade green	Dark green
11.	5 % NaOH	Yellow	Sea green	Walnut brown
12.	1N HCl	Creamy	Yellowish green	Green
13.	1N Methanolic NaOH	Henna green	Emerald green	Yellowish brown
14.	1N Ethanolic NaOH	Henna green	Grass green	Light brown

Thin Layer Chromatography- TLC

TLC of various extracts of *Ficus palmata* leaves shows different Rf values.

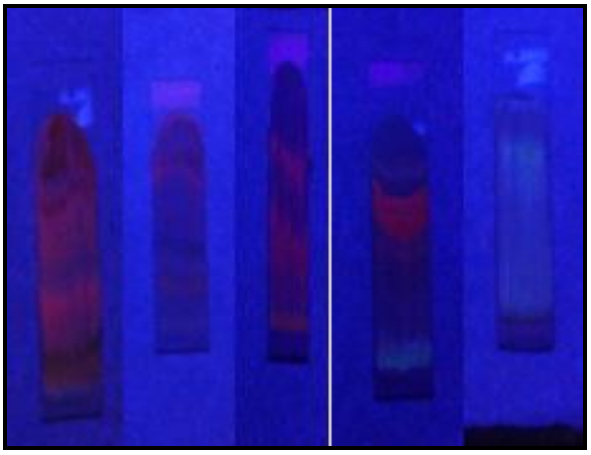


Figure 16: Long UV

CONCLUSION

To ensure quality of herbal medicines is necessary for its uses. For ensuring quality of starting material is authentification followed by creating numerical values of standards for comparison. Various Pharmacognostical parameters for easy identification like microscopy& physicochemical analyses are few of the basic protocol for standardization of herbals. The information obtained from the preliminary Phytochemical screening will useful for finding about the chemical nature of the drug. The total ash value, extractive value and florescence analysis will be helpful in identification and authentification of the plant material. The Pharmacognostical and phytochemical evaluation of *Roylea elegans*

leaves can provide useful information for the identification and authentification of the plant.

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