ORIGINAL ARTICLE

Pharmacognostic standardization of Asian folk medicinal plant *Reinwardtia Indica* Dumort

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Abstract

Background: Recent scenario and global trends are moving toward the medicinal plant used as health-care product for the treatment of different disease or disorder, but the critical and essential issue to be considered in assuring the therapeutic efficacy and safety. Thus standardization of plant parts need to standardized according to the standard guideline for global acceptance of the product. *Reinwardtia indica* belongs to Linaceae family used as folk medicine in Asia in the treatment and management of boils, carbuncle, and as an antimicrobial agent in wound healing traditionally. **Objective:** The present study aimed at physicochemical standardization of *R. indica* leaves and stem part of the plant. **Materials and Methods:** In our investigation, leaves and stem part of *R. indica* were standardized based on microscopy, powder microscopy, physicochemical evaluations, extractive yields, and heavy metal analysis as per the International Regulatory Norms. **Results:** The results revealed that the pharmacognostic parameters have shown the leaves and stem part of the *R. indica* plant found within the standard limit as per regulatory norms. **Conclusion:** The data found after standardization can be adopted as a standard of the plant *R. indica,* and it can be used in the formulation of the health care product after pre-clinical and clinical investigations.

Key words: Heavy metal analysis, high-performance thin-layer chromatography, physicochemical, *Reinwardtia indica*

INTRODUCTION

Folk medicine which is used on the basis of traditional knowledge-based system has immense therapeutic effect in treatment and management of different diseases or disorders worldwide but it is need for the scientifically validation for global acceptance as a health-care product after regulatory approval as per the International guidelines.[1] These herbal medicines are having less side effect and immense therapeutic value, eco-friendly, and bio-friendly for common person. Nowadays, global trends also shift toward the herbal health-care product and replace the synthetic drugs because of the resistance, side effect, and less effective in chronic disease.[2]

The World Health Organization (WHO) also made the standard parameters to assess the quality, safety, and efficacy of herbal plant which is based on the scientific validation and also meets the regulatory norms for the approval of phytopharmaceutical product as per regulatory norms.[3]

The present study deals with *Reinwardtia indica* a folk medicine used in Asia for the treatment and management of boils, pimples, and carbuncle and as an anti-microbial agent in

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wound healing. The root paste of this plant also used with fruits of *piper nigrum* in the treatment of measles.[4] The investigation was carried out first time to develop standardization parameters of this plant. The objectives include performing both leaves and stem part of this plant microscopy, powder microscopic characterization, physicochemical analysis, heavy metal analysis, extractive values in different polarity-based solvents, and preliminary phytochemical screening.

MATERIALS AND METHODS

Collection of Plant Material and Identification

The stem and leaves of *R. Indica* Dumort. (family Linaceae) were collected in the month of September–February from Himalayan region of Uttarakhand. The botanical identity was confirmed by Voucher specimen number Lina.2015/1 by Dr. N K Dubey, Professor, Department of Botany, Banaras Hindu University, Varanasi.

Physicochemical Evaluations

Microscopy of leaves and stem part with powder microscopy of shade-dried powder was carried out using Nikon eclipse E200 microscope with Cat cam 300-3.0 MP Microscope camera. Physicochemical characteristics of leaves and stem samples were analyzed by quantitative analysis for total ash, water-soluble ash, acid-insoluble ash, water-soluble extractives, moisture content, and foreign content as per the standard techniques. Micromeritic characteristics such as bulk density, tap density, angle of repose, Hausner ratio and Carr's index were determined for leaves and stem samples. Hexane (H), chloroform (C), ethyl acetate (EA), ethanol (E), and hydro alcohol (HA) (70:30) extracts of leaves and stem samples were prepared by hot percolation method with Soxhlet apparatus for extraction and concentrated using rotary vacuum evaporator (Buchi R-210 Advanced, Switzerland) at 40–50°C under vacuum and were used for screening of constituents such as alkaloids, glycosides, coumarone, flavonoids, carbohydrates, anthraquinone, tannins, sterols, terpenes, phenol, protein, and amino acid. Fluorescence analysis was carried out as per the method of Chase and Pratt. Leaves and stem samples were analyzed for the presence of heavy metals such as lead (Pb), arsenic (As), cadmium (Cd), zinc (Zn), cobalt (Co), and mercury (Hg) by atomic absorption spectroscopy (AA 240, Varian, The Netherlands).[3]

RESULTS AND DISCUSSION

Microscopy of Leaves and Stem

The microscopy of transverse section of leaves and stem showed the anatomical identification [Figure 1]. The powder microscopy of leaf and stem is used for the identification of powder of plant. The leaf powder microscopy found anomocytic stomata, phloem fibers, trichomes, calcium crystal oxalate, cystolith, aleurone grains, fats, oil, starch grains, and mucilage at 40× magnification [Figure 2]. However, in stem powder, microscopy found epidermal cells, epidermis with starch grains, annular xylem tracheids, fibers, cork cells in surface view, and conducting strand (xylem) fats, oils, and starch grains at the same magnification [Figure 3].

Physicochemical Evaluation

The physicochemical parameters help in judging the purity and quality of the powder. The powder was evaluated for its physicochemical parameters and found that loss on drying was 7% which is not too high and hence could discourage bacterial and fungal growth and favor long storage.[5] Content of total ash of leaves was 11.02%; water-soluble value of ash was found to be 6.73% and acid-insoluble ash value was 4.08%. However, in comparison of leaves, stem has the less total ash value of 5.08%; water-soluble value of ash was found to be 4.32% and acid insoluble ash value was 1.59% [Table 1]. The extractive value of the leaf powder with H, C, EA, E, and HA was 6.85%, 6.57%, 3.714%, 10%, and 8%, respectively. The same with stem powder with H, C, EA, E, and HA was 4%, 3.42%, 3.14%, 7.42%, and 4.28% [Table 2]. The flow properties of a powder are essential in determining its suitability as a direct compression excipient. The flow properties are also confirmed by Hausner ratio and Carr's index.[6] Value <1.25 indicates good flow (20% Carr's index) and value >1.25 indicates poor flow (33% Carr's index). In the present investigation, both parameters

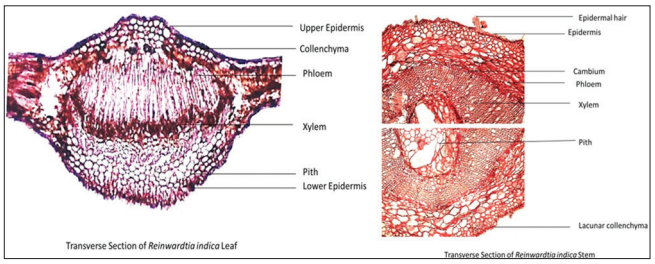

Figure 1: Microscopy of leaf and stem part of *Reinwardtia indica* plant

Table 1: Physiochemical characteristics of the plant *R. indica*

Physical parameter	Value (in w/w %)	
	Leaves	Stem
Foreign matter	2.13	0.89
Moisture content	12	6.23
Total ash content	11.02	5.08
Water-soluble ash	6.73	4.32
Acid-insoluble ash	4.08	1.59

R. indica: Reinwardtia indica

were found to be 1.43 and 23%, respectively, which indicate a poor flow [Table 3]. The fluorescence characteristic properties of the extract are enlisted in Table 4. These physiochemical characteristics are within the standard parameter according to international guideline which can be helpful in the preparation of the formulation of phytopharmaceutical product.[7].

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis of H, C, EA, E, and HA extracts was performed. Powdered drugs were subjected to successive solvent extraction with different solvents. The obtained extracts were screened according to the standard procedures mentioned and found maximum presence of phytochemicals in the EA, E, and HA extract of leaf and stem part of *R. indica* plant. The uses of these phytochemicals already reported in the several pharmacological activities such as cardiac protective, anti-inflammatory, antidiabetic, metabolic disorder, and neuroprotective. The presence of these phytochemicals in this extract also indicated the biological properties of this plant but it is need for scientific validation [Table 5].[8]

Heavy Metal Analysis

Nowadays, natural products are at risk of contamination by pollution, through air, water, and soil. Therefore, before use

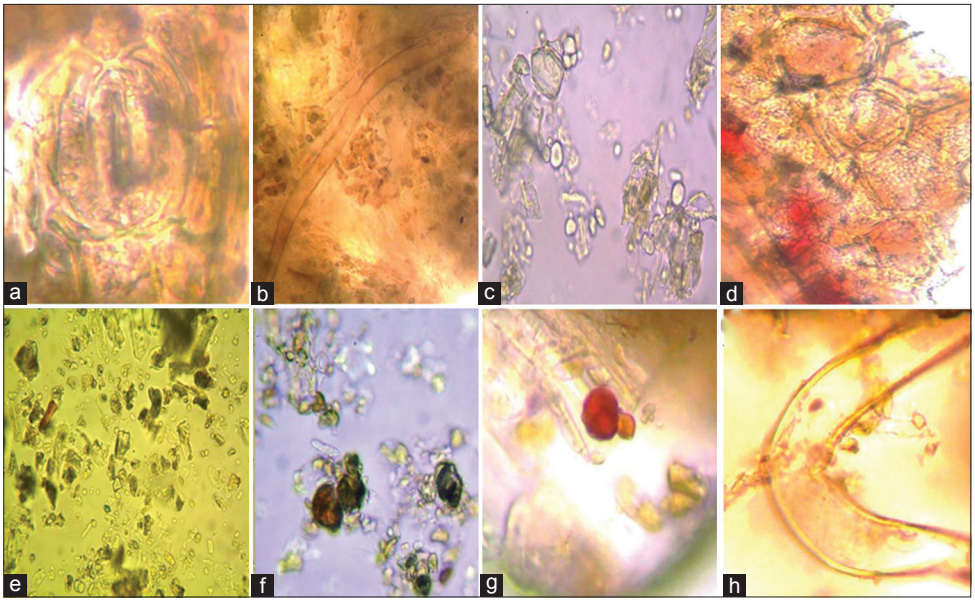

Figure 2: Leaf powder microscopy at 4× (a) anomocytic stomata, (b) phloem fibers and trichomes, (c) calcium crystal oxalate, (d) cystolith, (e) aleurone grains (f and g) fats, oil, and starch grains, (h) mucilage

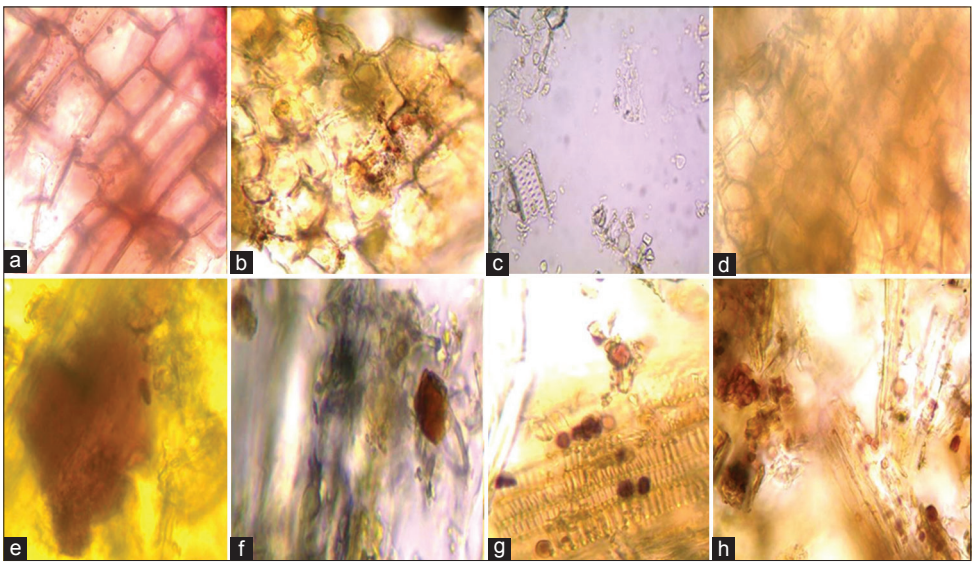

Figure 3: Stem powder microscopy at 4× (a) epidermal cells, (b) epidermis with starch grains, (c) annular xylem tracheids and fibers, (d) cork cells in surface view, (e) conducting strand (xylem) and brownish matter (f–h) fats, oils, and starch grains, respectively

Table 2: Percentage yield of leaves and stem part of plant ***R. indica*** in different polarity-based solvents

Parts of plant	Extract	Yield percentage
Leaves	H	6.85
	C	6.57
	EA	3.71
	E	10.0
	HA	8.1
Stem	H	4.2
	C	3.42
	EA	3.14
	E	7.42
	HA	4.28

R. indica: Reinwardtia indica, H: Hexane, C: Chloroform, EA: Ethyl acetate, E: Ethanol, HA: Hydro alcohol

Table 3: Powder characteristics of the plant ***R. indica***

Constants	Leaves	Stem
Hydration capacity g/g	4.25	7.15
Swelling Index	40	60
Moisture sorption capacity/g	0.89	1.2
Bulk Density g/ml	0.25	0.35
Tapped density g/ml	0.35	0.52
Hausner Ration	1.25	1.40
Carr index %	12	35

R. indica: Reinwardtia indica

the assessment of quality of the plant material should be standardized so that the concentration of active constituents in the product is consistent between batches and that the presence of unwanted impurities is minimized before use to the human. The parameters are set by the international guidelines which are related to safety and consistent therapeutic effects of the product.[9]

The different metals present in the soil, air, and water (HMs, atomic weights 63.5–200.6 g/mol, and a specific gravity <5 g/cm^3) are accumulated as part of industrial pollutants, heavy-duty electric power generator emissions, automobile exhaust, refuse burning, municipal wastes, and pesticides used in agriculture. Plants are uptake the metals through both roots and foliage (deposition and adsorption). In the human body, HMs act as micronutrients and are important for the functioning of vital processes in the permissible limit. For example, iron is an essential component of hemoglobin. However, the excess amount of metals is hazardous for human health, for example, Hg and As caused nervous system and cardiac damage.[10]

On the basis of permissible data defined by the WHO and Ayush (India), we found the amount in relative irradiance leaves and stem [Table 6] accordingly Hg (0.93 ± 0.04 ppm and 0.82 ± 0.04 ppm), As (0.93 ± 0.04 ppm and 0.94 ± 0.03 ppm), Co (0.060 ± 0.003 ppm and 0.002 ± 0.00 ppm), Cd (0.044 ± 0.005 ppm and 0.001 ± 0.00 ppm), Pb (0.526 ± 0.01 ppm and 0.633 ± 0.01 ppm), and Zn (1.93 ± 0.03 ppm and 0.377 ± 0.02 ppm). The result also revealed that leaf and stem part of plant *R. indica* can be used as for human health-care product or in the manufacturing of the herbal formulation.

Table 4: Fluorescence characteristics of ***R. indica*** extract

Plant parts	Extractives	Visible light	White light	UV light
Leaves	H	Light green	Yellowish green	Pale yellow
	C	Green	Green	Dark green
	EA	Pale green	Yellowish green	Dark green
	E	Dark green	Dark green	Pale green
	HA	Pale green	Yellowish green	Brown
Stem	H	Light green	Green	Yellowish green
	C	Green	Green	Dark green
	EA	Pale green	Yellowish green	Pale green
	E	Yellowish green	Yellowish green	Pale green
	HA	Pale green	Yellowish green	Brown

UV: Ultraviolet, H: Hexane, C: Chloroform, EA: Ethyl acetate, E: Ethanol, HA: Hydro alcohol *R. indica: Reinwardtia indica*

Table 5: Preliminary phytochemical screening of ***R. indica*** extract

Constituents	Stem part extract					Leaves part extract				
	H	C	EA	E	HA	H	C	EA	E	HA
Alkaloids	–	–	+	++	+	–	–	+	+	+
Glycosides	–	+	–	+	+	+	–	–	++	+
Coumarin	–	+	+	–	+	++	++	+	+	+
Flavonoids	+	+	++	+	+	–	+	+	++	+
Carbohydrates	–	–	+	+	+	–	–	–	++	+
Phlobatannin	–	–	–	+	+	–	–	+	+	+
Anthraquinone	–	+	–	–	–	–	–	+	–	–
Saponin	–	–	–	+	+	–	–	+	+	+
Triterpenes	–	–	–	–	+	–	+	+	+	+
Starch	+	–	–	–	+	–	–	–	–	+
Tannin	+	–	–	–	–	+	–	–	–	+
Phenol	+	+	++	++	++	+	++	+	++	++
Protein	+	–	–	–	+	–	–	–	+	+
Amino acids	–	–	–	+	+	–	–	–	+	+

H: Hexane, C: Chloroform, EA: Ethyl acetate, E: Ethanol, HA: Hydro alcohol (70:30), *R. indica: Reinwardtia indica*

Table 6: Heavy metal analysis of ***R. indica*** leaves and stems powder

Part of the plant	Hg	As	Co	Zn	Cd	Pb
Ayush India and the WHO limit	1 ppm	3 ppm	Not mentioned	27.4 ppm WHO edible plant	0.3 ppm	10 ppm
Leaves	0.93±0.04	0.93±0.04	0.060±0.003	1.93±0.03	0.044±0.005	0.526±0.01
Stem	0.82±0.04	0.94±0.03	0.002±0.00	0.377±0.02	0.001±0.00	0.633±0.01

Hg: Mercury, As: Arsenic, Co: Cobalt, Zn: Zinc, Cd: Cadmium, Pb: Lead, WHO: World Health Organization, *R. indica: Reinwardtia indica*

CONCLUSION

The present work was carried out for the standardization of *R. indica* plant part leaves and stem which is found suitable for herbal product formulation as per standard parameters. Heavy metal quantity was also found within the standard limit as per given by the regulatory authorities.[3] Hence, the pharmacognostic parameter of the plant *R. indica* will be helpful in the further preclinical and clinical study. The presence of phytochemicals also indicated that it can be used to develop the new lead phytomolecule in treatment and management of lifestyle disease or disorder.

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