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Pharmacognostic properties of *Rivina humilis* L

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Abstract
The *Rivina humilis* belongs to the family Phytolaccaceae. The evaluation of physic-chemical properties of *R. humilis* extract was carried out by using standard methods. This analysis were done by using WHO recommended parameters such as pH, total alkalinity, total hardness, biological oxygen demand, chemical oxygen demand, and metal concentration etc. In this study the observed values are not exceeds the permissible limit of WHO. The study of heavy metal contamination in plants, serve to show the metal concentration status of the site, where plants have grown. This is a main tool for phytoremediation. The pharmacognostic properties analyses were done by using WHO recommended parameters such as loss on drying, total ash, water soluble ash, acid soluble ash, extractive values and successive extractive yield. The pharmacognostic characters enlisted in this study will help in finding of the crude drug; the standardization parameters laid down will ensure the efficacy of drug and also differentiate the drug from its adulterants.

Keywords: physico-chemical, total ash, pharmacognostic, extractive values, crude drug, standardization *Rivina humilis*

Introduction

Plant kingdom is an effective rich source of potential drug targets and more active molecules to be discovered. Plant based drugs are having a revived interest now-a-days because of the harmful effects of modern synthetic drugs. Natural products can play a pivotal role in the pharmaceutical industry as a drug or as drug carrier or bio enhancers [1]. Plants contain phytochemicals that can be used for therapeutic purpose or as precursors for pharmaceutical synthesis [2]. Herbal medicines are safe, inexpensive and have no adverse effects. They are effectively being used to treat numerous diseases but lack of documentation and severe quality control. They are also susceptible to adulteration and exchange which puts uncertainty on their efficacy. There are many methods but still most simple, dependable and easy method but still most simple, dependable and easy method is pharmacognostic study. Accurate identification and quality assurance of the staring material will help to continue the replicable quality of herbal drugs and provide to its safety and potency [3]. Herbal medicines as the major remedy in traditional medicine system have been made a great contribution to maintain human health [4, 5]. Pharmacognosy is the study of medicines derived from natural sources, mainly from plants. It basically deals with standardization, authentication and study of natural drugs. Most of the analysis in pharmacognosy has been done in identifying contentious species of plants, authentication of commonly used traditional medicinal plants through morphological, physicochemical and phytochemical analysis. The significance of pharmacognosy has been widely felt in recent times. Unlike taxonomic identification pharmacognostic study includes parameters which help in identifying contamination in dry powder form also. This is often request because once the plant is dried and made into powder form, it mislays its morphological identity and simply prone to adulteration [6]. Plants are the rich source of all the elements essential for human beings. Quantitative and qualitative determination of mineral elements present in plants is chief because the concentration and kind of minerals present essential often be specify on the label of a food. The quality of kind foods based on the concentration and kind of minerals what they have, also play a very important role against a type of degenerative diseases and processes, they may also prohibit and decrease wound from environmental pollutants and increase the ability to work and learn, some minerals are essential to a healthy diet (e.g. calcium, potassium, phosphorus and sodium) where as few can be toxic (e.g. Mercury, lead, aluminium and cadmium) [9]. Ash values are used to determine quality and purity of crude drug. It indicates presence of various adulterations like carbonate, oxalate and silicate. The water-soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and reveal contamination with earthy material. Moisture content of drugs should be at low level inhibiting the growth of bacteria, yeast or fungi during storage [6]. Estimation of extractive values determines the amount of the active components in a given amount of plant material when extracted with a specific solvent. The extractions of an crude drug with a specific solvent yield a solution containing various phytocomponents. The compositions of these phytocomponents depend on the nature of drug and the solvent used [7, 8]. It also indicates whether the crude drug is exhausted or not [7]. Total ash and insoluble ash are essential indices to illustrate the quality and purity of the herbal medicine. Total ash illustrates and acid insoluble ash is essential indices to illustrate the quality and purity of the herbal medicine. Total ash consists of physiological ash, which is derived from plant tissue itself and non-physiological ash that is usually from atmosphere contamination includes sand and soil. The amount and composition of ash remaining after combustion of plant materials differ highly according to the part of the plant,

age, treatment etc. The components of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant [9]. The matter of proper identification and appropriate quality that is lack of adulteration, sophistication, or substitution is an extremely important one in the field of herbal medicine [10]. The few variations in physicochemical and phytochemical properties might be due to the time harvesting age of the plant, environmental factor, griding process etc., [11]. Hence, the present study was undertaken to evaluate physic-chemical properties, ash values and extractive value using various solvents of *R. humilis* plant extract.

Materials and Methods

Physico-chemical characters: The percentage of loss of weight on drying, total ash, acid insoluble ash, water soluble ash and residue on ignition were obtained from the species of *R. humilis* by employing standard methods of analysis as described in Pharmacopoeia of India [12].

Determination of loss of weight on drying: A known quantity (3 gm) of the whole plant dry powder of *R. humilis* was weighed separately and allowed to dry until a constant weight was obtained. From the initial and final weight, the loss of weight on drying was determined.

Determination of total ash: The whole plant dry powdered samples (3 gm) of *R. humilis* are taken in a previously weighted silica crucible and ignited carefully not exceeding dull red heat until the ash was free from carbon. Then, the crucible was cooled and weighed. The percentage of ash with reference to the sample was calculated.

Determination of water-soluble ash: A known amount of ash (0.15 gm) was boiled with 25 ml of distilled water. The insoluble matter was collected in a previously weighed sintered crucible, washed with hot water, dried to a constant weight and weighed. The insoluble matter was subtracted from the amount of ash taken and the amount of water-soluble ash of *R. humilis* was determined and recorded separately.

Determination of acid-insoluble ash: A known weight (0.15 gm) of *R. humilis* ash was boiled separately with 25 ml of diluted 2N-hydrochloric acid. The insoluble mater was collected in a previously weighed sintered crucible, washed with hot water, dried to a constant weight and weighed. The percentage of acid-insoluble ash with reference to the sample of *R. humilis* was calculated separately.

Determination of extractive value and successive extractive yield of plant samples: The extractive values of *R. humilis* are recorded separately to study the distribution of various constituents and all the raw ingredients of the formulation. Accurately weighed 4.0 gm of coarsely powdered air-dried whole plant material of *R. humilis* was placed in a glass stopper conical flask separately and macerated with 100 ml of the solvents such as chloroform, ethanol, ethyl acetate, hexane and water for 6hr shaking frequently, and then allowed to stand for 18 hr. The mixture was filtered rapidly taking care not to lose any solvent. Twenty-five milli litres of the filtrate was transferred to a flat-bottomed dish and evaporated to dryness on a water bath. The residue was dried at 105ºC for 6hrs cooled in desiccators for 30 min and weighed without delay [13]. The extraction yield is a measure of the solvents efficiency to extract specific components from the original material. The successive extractive yield of different solvents such as chloroform, ethanol, ethyl acetate, hexane and water was carried out in the whole plant dry powdered material of *R. humilis.* The extractive value and the successive extraction yield were calculated according to the method of [14].

The extractive value/the successive extraction yield (%) = (weight of the freeze – dried extract x 100)/(weight of the original sample).

Result

Physio-chemical properties of soil samples collected from *Rivina humilis* growing areas

The Physio-chemical properties of soil samples collected from *Rivina humilis* are presented in the table 1, Fig. 1. The colour of the soil was blackish brown. The alkaline pH was noted (7.80). The soil temperature was 16.21°C. Soil sample had high water holding capacity (1.45%) and bulk density (0.68 mg/kg) and Electric conductivity of soil was 1.63 dsm^{-1}. Total alkalinity and hardness was (1.55 mg/l and 62.02 mg/l) respectively. The dissolved oxygen of the collected soil was 2.41 mg/l. The BOD and COD of the soil were 0.80 mg/l and 0.14 mg/l. The concentration of ammonia was 1.20 mg/l available calcium 2.16%, available magnesium 0.11%, available potassium 120.60%, available phosphorous 105.26%, organic carbon 1.92%, total nitrogen 2.15%, chloride 23.85mg/l, calcium 5.60 mg/l, nitrite 0.60mg/l, magnesium 0.87 mg/l, potassium 0.85 mg/l, phosphate and sulphate was 0.22,22.93 mg/l respectively. The productivity of an ecosystem depends upon the quality of soil. The observed values are does not exceeds the permissible limit of WHO.

Metal concentration

The results of heavy metal analysis in the soil samples were collected and the values are depicted in the Table 1; Fig. 1. Arsenic, cadmium, chromium, silver were absent. Copper was present in 3.24 mg/kg. Amount of ferrous was 61.23 mg/kg. Metals such as lead, manganese, nickel, zinc are 9.22 mg/kg, 0.20 mg/kg, 2.50 mg/kg, and 12.03mg/kg respectively. While comparing the permissible limit of WHO, the observed values are not beyond the limit.

Table 1: Physico-chemical properties of soil samples collected from *Rivina humilis* L. growing areas.

Sl. No.	Physico-Chemical Properties	Concentrations		Permissible limit of WHO
1	Colour	Blackish brown		---
2	pH	7.80		6.5 – 8.5
3	Temperature	16.21	°C	---
4	Water holding capacity	1.45	%	---
5	Bulk density	0.68	mg/kg	---
6	Electric conductivity	1.63	dsm^{-1}	---

7	Total alkalinity	1.55	mg/L	200.00
8	Total hardness	62.02	mg/L	300.00
9	Dissolved oxygen	2.41	mg/L	4.00
10	BOD	0.80	mg/L	3.00
11	COD	0.14	mg/L	6.00
12	Ammonia	1.20	mg/L	5.00
13	Available calcium	2.16	%	---
14	Available magnesium	0.11	%	---
15	Available potassium	120.60	%	---
16	Available phosphorous	105.26	%	---
17	Organic carbon	1.92	%	---
18	Total nitrogen	2.15	%	---
19	Chloride	23.85	mg/L	250.00
20	Calcium	5.60	mg/L	75.00
21	Nitrite	0.60	mg/L	40.00
22	Nitrate	1.74	mg/L	45.00
23	Magnesium	0.87	mg/L	30.00
24	Potassium	0.85	mg/L	30.00
25	Phosphate	0.22	mg/L	500.00
26	Sulphate	22.93	mg/L	200.00
	Metal concentration			
27	Arsenic (As)	NP	mg/kg	100.00
28	Cadmium (Cd)	NP	mg/kg	0.20
29	Chromium	NP	mg/kg	100.00
30	Copper (Cu)	3.24	mg/kg	5.00
31	Ferrous (Fe)	61.23	mg/kg	200.00
32	Lead (Pb)	9.22	mg/kg	25.00
33	Manganese (Mn)	0.20	mg/kg	5.00
34	Nickel (Ni)	2.50	mg/kg	25.00
35	Silver (Ag)	NP	mg/kg	0.20
36	Zink (Zn)	12.03	mg/kg	30.00

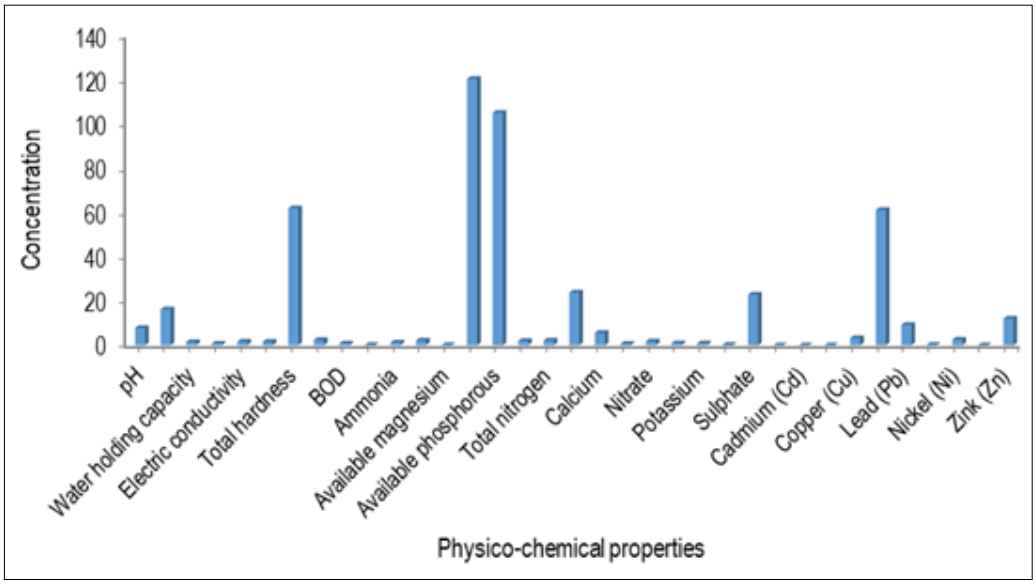

Fig 1: Physico-chemical properties of soil samples collected from *Rivina humilis* growing areas

The physical properties of various parts (root, stem, leaf, mixture and fruit) of *Rivina humilis* were determined and the data are presented in Table 2 and in Fig. 2. The pharmacognostic properties like loss on drying, total ash, water soluble ash, acid soluble ash extractive values and successive extractive values were analyzed. The average values are expressed as percentage of air-dried materials. Regarding the loss on drying property, ethanol extract of mixture sample exhibited maximum value (8.12%) and minimum loss on drying property was reported in petroleum ether extract of fruit sample (6.15 %). Maximum total ash value (6.98%) was reported in root sample ethanol extract and minimum total ash (6.01%) was reported in leaf sample chloroform extract. Maximum water-soluble ash was exhibited in mixture sample of ethanol solvent (7.12%) and minimum water-soluble ash was reported in aqueous extract of fruit sample (6%). Regarding the acid soluble ash values, ethanol extract of mixture sample has maximum value (7.71%), minimum value was exhibited in fruit sample of aqueous extract (6.21%).

Successive extractive yield was maximum in ethanol extract of mixture sample (7.65%) and ethyl acetate extract of fruits had minimum values (6.15%). Maximum extractive value was reported in ethanol extract of mixture sample (7.48%) and minimum extractive value was in fruit sample of aqueous extract (6.02%).

Table 2: Pharmacognostic properties of *Rivina humilis* solvent extracts

Sl. No.	Solvent extracts	Plant samples	Pharmacognostic properties					
			LOD (%)	TA (%)	WSA (%)	ASA (%)	EV (%)	SEY (%)
1	Aqueous	Root	6.90	6.10	6.30	6.91	6.18	7.01
		Stem	6.72	6.80	6.18	6.80	6.50	7.00
		Leaf	7.00	6.12	6.02	6.60	6.15	6.89
		Mixed	7.02	6.19	6.40	6.51	7.01	6.80
		Fruit	6.20	6.06	6.00	6.21	6.02	6.62
2	Chloroform	Root	7.80	6.10	6.78	7.01	6.81	6.60
		Stem	7.20	6.04	6.90	7.08	6.85	6.58
		Leaf	7.14	6.01	7.00	7.15	6.15	7.01
		Mixed	7.30	6.15	7.15	7.19	6.65	7.12
		Fruit	7.03	6.00	6.52	6.89	6.10	6.42
3	Ethyl acetate	Root	7.02	6.90	6.80	6.90	6.89	6.18
		Stem	7.10	6.10	6.52	6.87	6.70	6.36
		Leaf	7.08	6.08	6.60	6.70	6.60	6.28
		Mixed	6.80	6.45	6.98	6.81	6.70	7.01
		Fruits	6.72	6.02	6.61	6.82	6.69	6.15
4	Methanol	Root	7.15	6.10	6.80	7.20	7.02	7.14
		Stem	7.10	6.76	6.95	7.58	6.90	7.04
		Leaf	7.42	6.90	6.50	7.40	7.01	7.15
		Mixed	8.10	6.91	7.01	7.67	7.45	7.60
		Fruits	7.08	6.10	6.52	7.05	6.85	7.00
5	Petroleum ether	Root	6.90	6.78	6.20	6.90	7.01	8.20
		Stem	6.18	6.68	6.12	6.87	7.04	7.80
		Leaf	6.40	6.85	6.18	6.50	6.90	6.50
		Mixed	6.52	6.90	7.00	6.82	7.11	6.90
		Fruits	6.15	6.58	6.69	6.50	6.95	6.58
6	Ethanol	Root	7.10	6.98	6.83	7.10	7.08	8.29
		Stem	7.09	6.68	6.80	7.60	6.87	7.07
		Leaf	7.01	6.71	6.49	7.48	6.52	7.23
		Mixed	8.12	6.78	7.12	7.71	7.48	7.65
		Fruits	8.01	6.02	6.25	6.91	6.50	6.90

LOD –Loss on drying; TA –Total ash; WSA –Water soluble ash; ASA –Acid soluble ash;
EV –Extractive values; SEY –Successive extractive yield;

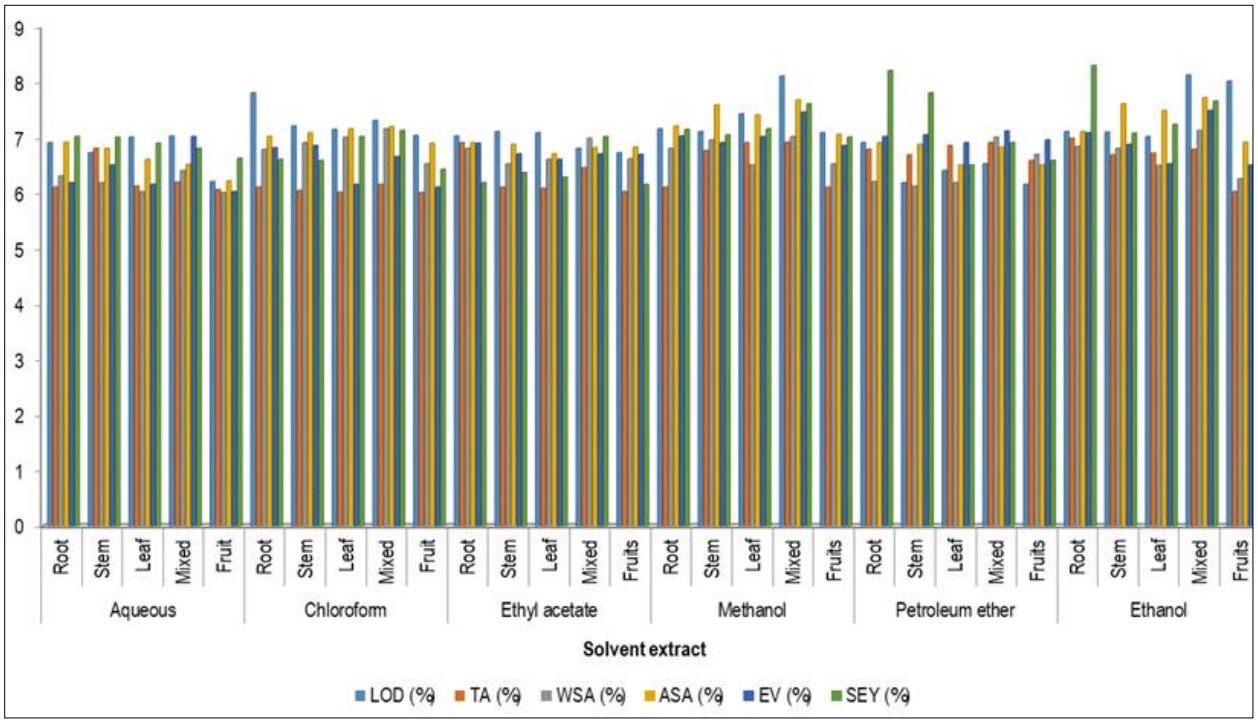

Fig 2: Pharmacognostic properties of *Rivina humilis* solvent extracts

Extractive values of *Rivina humilis* powder sample

The extractive values of different parts of *R. humilis* (root, stem, leaf, mixture and fruit) were calculated in various solvents and the data are presented in Table 3; Fig. 3. The extractive values were varies in different plant parts tested. In root sample the extractive values range from (5.24 ± 0.01 % to 8.21 ± 0.05%), (5.46 ± 0.02 to 8.84 ± 0.06%) in stem sample, (6.18 ± 0.03 % to 9.24 ± 0.07%) in leaf sample,

(6.29 ± 0.24% to 9.56 ± 0.70%) in mixture sample and (5.14 ± 0.08% to 8.20 ± 0.06) in fruit sample respectively. The extractive value of *R. humilis* samples recorded in different solvent extracts is arranged in the following descending order: Ethanol > water > methanol > chloroform > ethyl acetate > petroleum ether in root sample; Ethanol > water > methanol > ethyl acetate > chloroform > petroleum ether in stem sample; Ethanol > water > methanol > petroleum ether > ethyl acetate >chloroform in leaf sample; Ethanol > water > methanol > petroleum ether > ethyl acetate > chloroform in mixture sample and ethanol > water > methanol > ethyl acetate > chloroform > petroleum ether in fruit sample of *Rivina humilis* while comparing the solvents, ethanol was exhibited a maximum extractive values.

Table 3: Extractive values of *Rivina humilis* powder samples

Sl. No.	Solvent extracts	Extractive values* of *Rivina humilis* (%)				
		Root	Stem	Leaf	Mixture	Fruits
1	Ethyl acetate	6.16 ±0.01	6.26 ±0.03	6.32 ±0.05	6.52 ± 0.12	6.12 ± 0.01
2	Chloroform	6.24 ±0.03	6.04 ±0.01	6.18 ±0.03	6.29 ± 0.24	6.01 ± 0.04
3	Ethanol	8.21 ±0.05	8.84 ±0.06	9.24 ±0.07	9.56 ± 0.70	8.20 ±0.06
4	Methanol	7.36 ±0.02	7.86 ±0.05	8.08 ±0.04	8.14 ± 0.56	7.12 ± 0.05
5	Petroleum ether	5.24 ±0.01	5.46 ±0.02	6.66 ±0.03	6.93 ± 0.06	5.14 ± 0. 08
6	Water	8.01 ±0.06	8.52 ±0.09	9.14 ±0.06	9.34 ± 0.04	8.00 ± 0.02

* -All values are mean of triplicate determinations; ± -Standard error

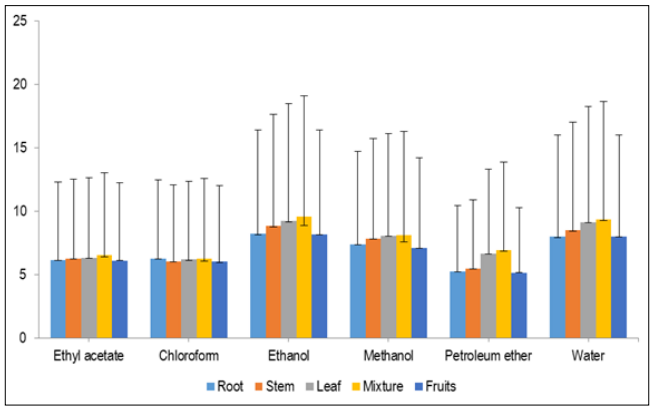

Fig 3: Extractive values of *Rivina humilis* powder samples

Discussion

Indian system of medicine utilize majority of the crude drugs which are of plant origin. It is significant that standards need to be set down to control and check the identity of the plant and confirms its quality before use [15]. Pharmacognosy is a study of drugs having their origin in plant and animal kingdoms. The subject pharmacognosy can also be indicate as an applied science that deals with biological, biochemical therapeutic and economic features of natural drugs and their components [16]. Standardization of crude drug is integral part of establishing its correct identity. The quantitative confirmation of some pharmacognostic parameters is useful for setting quality for crude drugs [17]. The physicochemical analysis of plant drugs is an important for identifying adulteration or incorrect handling of drugs [18]. *Anogeissus latifolia* root showed the absence of heavy metal contamination (Arsenic, Lead, Cadmium, Zinc) and so considered safe for use if the drug is used in making of any formulation [19]. Ash values are important quantitative standards for the evaluation of the quality, identity and purity of crude drugs especially in their powdered form [20, 21]. Total ash may include both physiological and non physiological ash. Physiological ash consists of sand and siliceous earth. Physiological ash may comprise mineral nutrients also. Non-physiological ash is resembled by acid insoluble ash [22]. The total ash method is applied to measure the total amount of material remaining after ignition [18]. Acid insoluble ash is a part of total ash and measures the amount of silica present as sand and siliceous earth. The value of the total ash and acid insoluble ash represent the difference between mineral adulterants and divergence from the natural ash [23]. Water soluble ash is the water-soluble portion of the total ash [24]. The water-soluble extractive value was indicating the presence of sugar acids and inorganic compounds present in the *Eclipta alba* plant sample [25]. Low moisture content is always desirable for higher stability of drugs [19]. Ash value represents the level of cleanness and high values may be due to unsuitable handling procedures during the sample collection process [26]. The extractive values give an idea about the chemical composition of the drug [27]. To evaluate the consistency of nature and the amount of chemical constituents present in drugs, the extractive values are used [28]. The Pharmacognostic studies are essential and has to be performed for all the medicinal plants which are being studied for any particular activity.

They act as reference standard and are diagnostic features of that particular plant. Similar studies are reported for many other plants by other researchers [29, 30, 31, 27]. Pharmacognostical studies has been carried out on large of plants *viz*. *Astercantha longifolia* [32], *Andrographis paniculata* [33], *Cathesranthus roseus* [34], *Salvia* [35, 36], Vasakhanda kushmandaka [37].

Conclusion

In the present study of *R. humilis*, the pharmacognostic characters like quality, safety and standardization were examined completely. The information from the present study will provide data which is helpful for the correct identification and authentication of this *R. humilis* plant and also it helps to prevent adulteration.

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