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Pharmacognostical evaluation and comparative phytochemical screening of *Rumex vesicarius* L.

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Abstract

Pharmacognosy is a simple and reliable tool by which complete information of the crude drug can be obtained. Therefore in this context the detailed pharmacognostic study of various parts like leaf, stem, and root of *Rumex vesicarius* L. has been carried out with the aim to establish its pharmacognostical standards. The parameters selected were physicochemical, fluorescence analysis and preliminary phytochemical screening along with mineral analysis. In physico-chemical evaluation the ash values and extractive values were studied.. The powder of *Rumex vesicarius*.L was successively extracted with petroleum ether, chloroform, methanol and water by both cold maceration and hot soxhlet extraction for the identification of the best solvent and method. Preliminary phytochemical screening was carried out for all the extracts. Fluorescence analysis performed showed the wide range of fluorescence colours for the crude powder. The preliminary phytochemical screening shows maximum chemical constituents in the different extract obtained from cold maceration of different plant parts compared to extract obtained from hot soxhlet extraction. The principal constituents of *Rumex vesicarius* L. include phenols, tannins, flavonoids, saponins, triterpenoids, alkaloids, anthraquinones, quinines, reducing sugars, proteins, lipids and carbohydrates. The inorganic elementary analysis performed revealed the presence of sodium, chloride and iron. The present study indicates the pharmacognostical and physicochemical characteristics and preliminary properties of the different parts of *Rumex vesicarius*.L for the identification of the drug in the dry form. Thus plays a crucial role in standardization of crude drug.

Keywords: *Rumex vesicarius*.L,pharmacognostic evaluation,phytochemical screening, Hot extraction, cold maceration.

Introduction

Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers 'all plant parts' to be potential sources of medicinal substances [1]. Many medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) and proposed for their interesting multilevel activities. Amongst the medicinal plants used in Ayurvedic preparations for their therapeutic action, some have been thoroughly investigated and some of are still to be explored. One such plant is *Rumex sps.* The *Rumex* (Polygonaceae) genus comprises several species, of which leaves and roots have been used in traditional medicine for inflammation, blood purification, and constipation [2,4]. Because of their high oxalic acid content, they have been implicated in oxalic intoxication, mainly in children [3,4]. The growing interest in many *Rumex* species has led to the study of their biological activities, namely, the effect of *Rumex acetosa* in body weight and serum levels of amino acids and minerals [5], the psychopharmacological [6] and purgative effects of *Rumex nepalensis* [7], the antioxidant and cytotoxic agents from *Rumex patientia* [8], the antifertility action of *Rumex steudelii* [9], the antimicrobial and anti-inflammatory activities of *Rumex nervosus* and *Rumex abyssinicus* [10], the anti diarrheal effect of *Rumex maritimus* [11], and the antiviral activity of *Rumex bequaertii* [12]. The present investigation relay on *Rumex vesicarius* L.

Rumex vesicarius L. is a branched succulent herb which belongs to the family Polygonaceae and is distributed in India. Rumex vesicarius L. is one of green vegetable medicinally valuable plant and It is commonly called as "Bladder dock". The whole plant is medicinally important and cures several diseases. Rumex vesicarius L (Polygonaceae) traditionally used as aperients, astringent, diuretic, and cooling agent. The plant juice is useful in curing stomach heat, toothache, checks nausea and promotes appetite. Fruits are aperients and diuretic, eaten fresh against Jaundice, hepatic conditions, constipation and indigestion, roosted seeds are prescribed in dysentery [13].

The literature survey revealed that the systemic evaluation including pharmacognostical study of this plant is still lacking. No scientific parameters are available to identify the true plant material and to ensure its quality. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the material to be used as medicine. Thus, in the recent years there has been a rapid increase in the standardization of the selected medicinal plants of potential therapeutic significance. The process of standardization can be achieved by stepwise pharmacognostic studies. Despite of modern techniques, identification ofplant drugs of pharmacognostic studies is more reliable. The standards are utmost importance in not only finding out genuity but also in detection of adulterants in marketed drugs [14]. So, correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. The objective of the present study is to evaluate various pharmacognostic standards like microscopy of leaf, stem, and root; ash and extractive values, fluorescence analysis, preliminary phytochemical analysis and mineral analysis of *Rumex vesicarius* L.

Materials and methods

Plant material collection and authentication- The different parts of plant *Rumex vesicarius*.L were collected from village khusnoor in Gulbarga district Karnataka. The specimens of plant were authenticated by Dept. of Botany, Gulbarga University, Gulbarga. A voucher specimen was submitted at Dept. of Botany Gulbarga University Gulbarga for future with the reference no. HGUG- 5012.
Drying and Pulverization- The different plant parts like stem, root , leaves and whole plant of *Rumex vesicarius*.L. were shade dried and pulverized and stored in an air tight container for future use.
Extraction of powdered plant material- Extraction of phytoconstituents was done using two techniques

- Hot Soxhlet extraction method, and
- Cold Maceration (at room temperature)

The air dried and coarsely powdered leaves stem, root and whole plant of *Rumex vesicarius* L. were successively extracted by cold maceration process and hot soxhlet extraction process using organic solvents Petroleum Ether, Chloroform, Methanol and water. All the extracts were evaporated to dryness and stored for future use.

Pharmacognostic studies

Physical evaluation- The ash values, extractive value and loss on drying were performed according to the officinal methods prescribed in Indian Pharmacopeia [15] and the WHO [16] guidelines on quality control methods for medicinal plants materials. Fluorescence analyses were carried out according to the method of Chase and Pratt [17] and Kokoski [18].

Phytochemical screening

Petroleum ether, chloroform, methanol and aqueous extract were subjected to comparative phytochemical analysis for the presence of various secondary phytoconstituents using standard procedure described by kokatte [19] and Horborne [20]. The extract residues of the plant were subjected to phytochemical screening to screen the presence of various active phytocompounds like phenols, tannins, flavonoids, saponins, alkaloids, primary metabolites like carbohydrates, proteins, lipids.

Mineral analysis

Calcium, Copper, Iron, Magnesium, Potassium, Sodium , and Zinc and Nitrogen in different parts of plant were determined using the standard methods given by NIN [21]. All the determinations were done in duplicates.

Result

Physico-chemical parameters

Physical appearance and color of different extracts of hot and cold extraction were recorded in (Table 1,2.). The physical evaluation of drugs is an important parameter in detecting adulteration or important handling of drug. The ash values, extractive values and moisture content of leaves were determined and the results are shown in (Table 3) .Mean of ash value were recorded. 20.81%, 21.19%, 22.68%, 10.57% of total ash value was present in whole plant, leaf, stem and root respectively. 10.25%, 1.9%, 7.75%and 4.75% of water soluble ash was present with 11.15%, 25.25%, 22.65% and 1% of acid insoluble ash in whole plant, leaf, stem, root respectively. The extractive value of different plant parts have been analyzed to find out the % of extractive values. The water soluble extractive value of whole plant, leaf, stem, root was obtained as 4.85%, 42.48%, 28.4%, 20.5% respectively. The alcohol soluble extractive value of whole plant, leaf, stem, root were 12%,1%,12.48%,7.2% respectively. The maximum % of moisture content was obtained as 20% in roots followed by leaf with 9.5%, whole plant with 9%, and stem with 6%.

Table 1. Nature and % yield of hot extract

EXTRACT		EXTRACTIVE VALUE	COLOUR	CONSISTENCE	PHYSICAL APPEARANCE
PE	WP	0.65%	Greenish black	oily	Semi solid mass
	LF	1.00%	Greenish black	oily	Semi solid mass
	ST	1.2%	Greenish black	oily	Semi solid mass
	RT	2.06%	Greenish Yellow	oily	Semi solid mass
CE	WP	1.8%	Greenish black	oily	Semi solid mass
	LF	3.00%	Greenish black	oily	Semi solid mass
	ST	1.3%	Greenish black	oily	Semi solid mass
	RT	3.01%	Greenish black	oily	Semi solid mass
ME	WP	2.9%	Greenish yellow	Gummy	Syrupy mass
	LF	3.6%	Greenish yellow	Gummy	Syrupy mass
	ST	2.7%	Greenish yellow	Gummy	Syrupy mass
	RT	4.21%	Greenish yellow	Gummy	Syrupy mass
AE	WP	11.21%	Brownish yellow	Waxy	Syrupy mass
	LF	9.80%	Brownish yellow	Waxy	Syrupy mass
	ST	5.5%	Brownish yellow	Waxy	Syrupy mass
	RT	6.21%	Brownish yellow	Waxy	Syrupy mass

PE-PETROLEUM EXTRACT, CE- CHOLOFORM EXTRACT, ME- METHANOL EXTRACT , AE-AQUEOUS EXTRACT.
WP-WHOLE PLANT, LF- LEAF , ST- STEM, RT- ROOT

Table 2. Nature and % yield of cold extract

EXTRACT		EXTRACTIVE VALUE	COLOUR	CONSISTENCE	PHYSICAL APPEARANCE
PE	WP	0.65%	Greenish black	oily	Semi solid mass
	LF	1.00%	Greenish black	oily	Semi solid mass
	ST	1.2%	Greenish black	oily	Semi solid mass
	RT	2.06%	Greenish Yellow	oily	Semi solid mass
CE	WP	1.8%	Greenish black	oily	Semi solid mass
	LF	3.00%	Greenish black	oily	Semi solid mass
	ST	1.3%	Greenish black	oily	Semi solid mass
	RT	3.01%	Greenish black	oily	Semi solid mass
ME	WP	2.9%	Greenish yellow	Gummy	Syrupy mass
	LF	3.6%	Greenish yellow	Gummy	Syrupy mass
	ST	2.7%	Greenish yellow	Gummy	Syrupy mass
	RT	4.21%	Greenish yellow	Gummy	Syrupy mass
AE	WP	11.21%	Brownish yellow	Waxy	Syrupy mass
	LF	9.80%	Brownish yellow	Waxy	Syrupy mass
	ST	5.5%	Brownish yellow	Waxy	Syrupy mass
	RT	6.21%	Brownish yellow	Waxy	Syrupy mass

PE-PETROLEUM EXTRACT, CE- CHOLOFORM EXTRACT, ME- METHANOL EXTRACT , AE-AQUEOUS EXTRACT.
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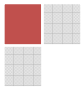

Table 3. Shows physicochemical parameters

SNO.	Parameters	Whole plant	leaves	stem	root
1	Total ash value	20.81	21.19	22.68	10.57
2	Acid insoluble ash	11.15	25.25	22.65	1
3	Water soluble ash	10.25	1.9	7.75	4.75
4	Water soluble extractives	40.84	42.48	28.4	20.5
5	Alcohol soluble extractives	12	1	12.48	7.2
6	Moisture content	09	9.5	6	20

Chemical and Fluorescence analysis

Chemical and fluorescence analysis are the tool to determine the kind of nature of the drug . The Chemical and Fluorescence analysis of the plant powder were done by treating the plant powder with various chemical reagent and separate observation were made under normal light and uv light, the colour changes of different parts of plant powder was recorded and presented in table (Table 4,5.) respectively.

Table 4. Chemical analysis of plant powder

S.NO	Treatment	Whole plant	Leaves	Stem	Roots
		Observation	Observation	Observation	Observation
1	Powder as such	Grey green	Light green	grey	Light green
2	Powder + water	Lemon green	Straw green	Grey green	Green
3	Powder+ acetic acid	Light green	Brownish green	Brownish green	Yellow
4	Powder + ferric chloride	Brown	Brown	Light brown	Brown
5	Powder+5%KOH	Green	Light green	Brown	Red
6	Powder +50%HCL	Light brown green	Dark green	Straw green	Lemon yellow
7	Powder+50 %H2SO4	Dark green	Brillent green	Dark brown	Dark brown
8	Powder + 50%HNO3	Reddish brown	Yellow	Yellow	Orange
9	Powder+ ethyl acetate	Grey green	Reddish brown	Orange	Pale yellow
10	Powder+ chloroform	Yellowish brown	Dark brown	Brown	Yellowish brown

Phytochemical screening

The comparative preliminary phytochemical screening of *Rumex vesicarius* L. was undertaken for the identification of different chemical constituents present in different parts, individual screening of both hot and cold extracts indicated the presence of all major phytoconstituents. The phytochemical screening of hot and cold exract were recorded in table (6,7) respectively. Petroleum ether, chloroform, methanol and aqueous extract by cold maceration showed the highest amount of phytoconstituents compared to extract by soxhlet extraction. In case of extract obtained by soxhlet extraction, phenols were detected in trace and alkaloids, tannins glycosides, flavanoids were completely absent in aqueous extract. Where as the aqueous extract by cold maceration indicated the presence of these phytoconstituents.

Mineral analysis

The AAS elemental analysis of the plant was presented in the table (8). The values of nitrogen, phosphorus, potassium, sulphur, calcium, magnesium were reported in percentage. While zinc, iron, manganese, copper were reported in parts per million (ppm).

Discussion

The improvement in the quality control and standardization of herbal drugs has led to the development of effective quality medicines from plants. However herbal formulations involve use of fresh (or) dried plant parts. Correct knowledge of such crude drug is very important aspect in preparation, safety and efficacy of the herbal products [22]. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs [23]. In the present study, different parts were evaluated qualitatively by studying various physicochemical parameters and phytochemical screening. Detailed pharmacognostical studies for different parts of *Rumex vesicarius* L. been studied. Further, aerial part of *Rumex vesicarius*.L forms the major ingredient in salad and other preparations, so the present study is useful in establishing a monograph detail for the different parts of *Rumex vesicarius.L.*

Table 5. Fluorescence analysis of plant powder

S.NO	Treatment	Whole plant		Leaves		Stem		Root	
		Observation under normal light	Observation under UV light	Observation under normal light	Observation under UV light	Observation under normal light	Observation under UV light	Observation under normal light	Observation under UV light
1	Powder as such	Grey green	Grey	Light green	Light green	Green	Green	Green	Green
2	Powder + 1N NaOH (aqueous)	Yellowish green	Brown	Dark green	Brown	Reddish yellow	Brown	Brick red	Brown
3	Powder+ 1N NaOH (alcoholic)	Pale yellow	Yellow	Light green	Light green	Light green	Yellow	Red	Red
4	Powder+50% HNO3	Yellowish brown	Reddish brown	Brownish green	Dark green	Yellow	Dark brown	Orange	Brown
5	Powder+50% H2SO4	Green	Dark green	Light green	Pale green	Brown	Black	Brown	Black
6	Powder +1 N HCL	Pale yellow	Green	Yellow	Light brown	Pale yellow	Black	Pale yellow	Lemon green
7	Powder+5%K OH	Yellowish green	Brown	Yellow	Orange	Yellow	Fluorescent green	Brown	Dark green
8	Powder + ethanol	Green	Blackish green	Green	Dark Green	Green	Yellowish brown	Green	Lemon green
9	Powder+ Hexane	Yellowish green	Light orange	Light green	Dark green	Light green	Light green	Pale green	Light green
10	Powder+ methanol	Light yellow	Light blue	Brown	Dark brown	Light green	Light green	Light green	Yellowish green

Table 6. Qualitative analysis of primary and secondary metabolites of hot extract

S.NO.	TESTS	PEE				CE				ME				AE			
		WP	LF	ST	RT	WP	LF	ST	RT	WP	LF	ST	RT	WP	LF	ST	RT
1	CARBOHYDRATES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	PROTIENS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	STEROIDS	_	_	_	+	+	+	+	+	_	_	_	_	-	-	-	-
4	GLYCOSIDES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	ALKALOIDS	_	_	_	_	_	_	_	+	+	+	+	+	+	+	+	+
6	TANNINS	_	_	_	_	_	_	_	_	+	_	_	+	+	+	+	+
7	SAPONINS	+	+	+	+	+	+	+	+	_	_	_	_	-	-	-	-
8	FLAVANOIDS	_	_	_	_	_	_	_	_	+	+	+	+	+	+	+	+
9	PHENOLS	_	_	_	+	_	_	_	_	+	+	+	+	+	+	+	+
10	OILS AND FATS	+	+	+	+	+	+	+	+	_	_	_	_	-	-	-	-

PEE-PETROLEUM EXTRACT, CE- CHOLOFORM EXTRACT, ME- METHANOL EXTRACT , AE-AQUEOUS EXTRACT.
WP-WHOLE PLANT, LF- LEAF , ST- STEM, RT- ROOT

In the present investigation different parts of *Rumex vesicarius*.L powder was evaluated for its ash values, extractive values and loss on drying. The total ash is particularly important in the evaluation of purity of the drugs, i.e the presence or absence of foreign organic matter such as metallic salts and/or silica [24]. The ash value of whole plant, leaves, stem, root of *Rumex vesicarius* L. is 20.81%, 21.19%, 22.68%, 10.57% respectively. These ash values of *Rumex vesicarius* L. are indicative of the impurities present in the drug. In

Table 7. Qualitative analysis of primary and secondary metabolites of cold extract

S.NO.	TESTS	PEE				CE				ME				AE			
		WP	LF	ST	RT	WP	LF	ST	RT	WP	LF	ST	RT	WP	LF	ST	RT
1	CARBOHYDRATES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	PROTIENS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	STEROIDS	_	_	_	+	+	+	+	+	_	_	_	_	-	-	-	-
4	GLYCOSIDES	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
5	ALKALOIDS	_	_	_	_	_	_	_	+	+	+	+	+	+	+	-	-
6	TANNINS	_	_	_	_	_	_	_	_	+	+	+	+	+	+	+	+
7	SAPONINS	+	+	+	+	+	+	+	+	_	_	_	_	-	-	-	-
8	FLAVANOIDS	_	_	_	+	_	_	_	+	+	+	+	+	-	-	-	-
9	PHENOLS	_	_	_	+	_	_	_	+	_	_	_	_	-	-	-	+
10	OILS AND FATS	+	+	+	+	+	+	+	+	_	_	_	_	-	-	-	-

PEE-PETROLEUM EXTRACT, CE- CHOLOFORM EXTRACT, ME- METHANOL EXTRACT , AE-AQUEOUS EXTRACT.
WP-WHOLE PLANT, LF- LEAF , ST- STEM, RT- ROOT

Table 8. Shows mineral analysis

S.NO.	N %	P %	K %	S %	Ca %	Mg %	Zn ppm	Fe ppm	Mn ppm	Cu ppm
WHOLE PLANT	4.33	0.40	4.13	0.13	2.44	0.26	34.800	148.100	50.100	29.100
LEAF	4.74	0.39	2.24	0.13	3.35	0.38	30.100	397.800	62.200	12.000
STEM	3.24	0.38	2.96	0.10	1.96	0.26	30.600	555.000	24.100	8.600
ROOT	2.17	0.24	4.43	0.06	1.86	0.13	22.700	153.100	62.800	15.200

N- nitrogen, P-phosphorus, K- potassium, S- sulphur, Ca- calcium, Mg- magnesium, Zn- zinc,Fe- iron, Mn- manganese, Cu- copper

the present study whole plant, leaves, stem has more acid insoluble ash than the water soluble ash. The ash value is generally the index of purity as well as identity of the drug. The pharmacognostic investigation on physicochemical characteristics and fluorescence analysis shows that authentic botanical of this crude drug will prevent adulteration substitution and has a crucial role in standardization of crude drug.

Many phytocompounds fluoresce when suitably illuminated. The fluorescence colour is specific for each compound. The nonfluorescent compound may fluorescent if mixed with impurities that are fluorescent. The fluorescent method is adequately sensitive and enables the precise and accurate determination of the analyze over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples [25]. In the present study, the powdered whole plant, leaves, stem, root of *Rumex vesicarius*.L emitted wide range of color under daylight and under uv light. Florescence analysis of powders of different parts of *Rumex vesicarius* L. (whole plant, leaf, stem, root) gives a clue if powder are in adulteration, thus can be used as a diagnostic tool for testing the adulteration. Presence or absence of certain important compounds in an extract is determined by color reactions of the compounds with specific chemicals which act as dyes. This procedure is pre-requisite before going for detailed photo chemical investigation.

Non standardized procedures of extraction may lead to the degradation of phytochemicals present in the plants and may lead to the variations thus leading to the lack of reproducibility. Thus in the present study the comparison of the two extraction methods indicated that the yield of extract by cold maceration extraction is less compared to the hot soxhlet extraction. However the comparative preliminary phytochemical screening indicated that cold maceration extraction yielded significantly more number of phytoconstituents than hot soxhlet extraction method. The possible reason may be in case of hot soxhlet extraction the most volatile parts of the plant may be damaged or lost with exposure to heat [26]. Thus the overall data suggest that the use of the cold maceration method will yield a more accurate assessment of the number of phytoconstituents in plant while either method can be used for the isolation of specific phytoconstituents. And also successful prediction of botanical compounds from plant material is largely dependent on the type of the solvent used and the method of extraction followed.

Minerals are required for normal growth, activities of muscles and sketal development (such as calcium), cellular activity and oxygen transport (copper and iron) , chemical reaction in the body and intestinal absorption (magnesium), fluid balance and nerve transmission (sodium and potassium) ; as well as the regulation of acid-base balance (phosphorus). Iron is useful in prevention of anemia and other related diseases [27]. Manganese plays a role in energy production and in supporting the immune system. It also works with vitamin k to support blood clotting and with b complex vitamins to control the effects of stress. Zinc is useful for protein

synthesis, normal body development and recovery from illness [28]. Deficiency of these nutrients and minerals are known to affect the performance and health.

Conclusion

Rumex vesicarius L. *is* widely cultivated in India for its culinary purposes. The pharmacognostic investigation on physicochemical characteristics and fluorescence analysis shows that authentic botanical of this crude drug will prevent adulteration substitution and has a crucial role in standardization of crude drug. These parameters will also guide in the proper identification of the plant species from other species of *Rumex* as well as help in authentication of the purity of the plant. The chemical composition of the *Rumex vesicarius* L. indicates that it is a good source of minerals. By above all these parameters we can build up a suitable plant profile which paves way for further studies on the plant for the presence of active compounds and their biological activity.

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