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Preliminary Pharmacognostical Standardisation of *Ruta graveolens* L. Aerial Parts

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Abstract: *Ruta graveolens* L. belonging to family Rutaceae is commonly known as Common rue and locally as Sudab in India. It is an important medicinal plant used in capillary fragility, for eye diseases, as stimulant and emmenagogue. As the herb is used widely in the Indian traditional system, it was thought worthwhile to undertake the standardization of its aerial parts. Aerial parts consist mainly of leaves that are 3-5 inch long, flowers are tetramerous and fruits are 4-5 lobed. In the powdered form it had pungent odor and exceedingly bitter taste. Microscopical examination of powder of aerial parts showed fragments of epidermis, glandular trichomes, stone cells, lignified xylem elements and abundant calcium oxalate crystals. Successive extractive value was highest in aqueous extract (16.08% on dry weight basis). Mean ash values (%) were 8.13 (total), 2.01 (acid insoluble ash) and 1.02 (water soluble ash). Loss on drying was found to be 4.03% and pH values of aqueous extract was 6.74. Bitterness value of aerial parts was 1.28; foaming index was less than 100. Screening of all extracts indicated the presence of all phytoconstituents except saponins. TLC fingerprints of extracts of aerial parts were also developed.

Key words: Common rue, Sudab, Rutaceae

INTRODUCTION

Ruta graveolens L. (Rutaceae) is an important medicinal plant which is used all over the world. The genus name Ruta comes from Greek word reuo meaning to set free which indicates herbs reputation in treatment of diseases (Duke, 2002). It is locally known as Sudab or Sadab (Kiritikar and Basu, 1999). Herb is used in capillary fragility (Tyler *et al*., 1988), for eye diseases, as stimulant and emmenagogue (Trivedi, 2006; Bhattacharjee, 2005). Report on standardisation of *R. graveolens* is not available. Moreover, locally *R. graveolens* and *Euphorbia dracunculoides* L. (Euphorbiaceae) share the same local name i.e., Sudab. They are often misidentified and used as substitutes. But these two drugs differ in their chemical constituents, they are bound to differ in their actions. Thus it was thought worthwhile to undertake the preliminary pharmacognostical standardization of *R. graveolens* aerial parts. Moisture content, extractive, ash value, swelling index, bitterness and R_f values are relatively simple parameters for development of preliminary standards (Evans, 1997). Standardisation of rue aerial parts was carried out for widely accepted parameters.

MATERIALS AND METHODS

Materials

Aerial parts were collected from *R. graveolens* herbs growing in natural habitat in Madhya Pradesh, India, in January, 2008 and identified by H.B. Singh, Taxonomist, NISCAIR, Pusa Institute, Delhi. Voucher specimen of the plant (NISCAIR 876/60) has been retained in the institute for reference purpose.

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Methods

Aerial parts of *R. graveolens* were examined systematically to observe morphological characters followed by microscopy of aerial parts. Extractive values were determined for cold, hot and successive extraction methods. Standard methods were followed to determine the total, acid insoluble and water-soluble ash. The foreign matter percentage in the crude drug was determined. Calibrated digital pH meter was used to measure the pH of 1 and 10% aqueous extracts and also loss on drying was noted. Standard procedures were followed for recording the swelling and foaming indices. Determination of bitterness value of the drug sample with reference to quinine hydrochloride was carried out as described by WHO (Anonymous, 1998).

The powder of aerial parts were subjected to the fluorescence analysis after being separately treated with water, NaOH, H_2SO_4, HCl, picric acid, ammonia solution, methanol, 5% iodine solution chloroform and examined under UV light as well as in daylight. Different colors were observed after treating the powder with NaOH, H_2SO_4, HCl, HNO_3, glacial acetic acid, chloroform, picric acid, ammonia solution, methanol and 5% iodine solution. The petroleum ether, dichloromethane, chloroform, ethyl acetate, acetone, methanol and aqueous extract residues of aerial parts were subjected to phytochemical screening for detection of plant constituents viz., sterols, alkaloids, tannins, flavonoids, proteins, amino acids, carbohydrates (including sugars), resins and lipids. TLC profiling was done as per the method described by Stahl (1969). Petroleum ether, dichloromethane, chloroform, ethyl acetate, acetone and methanolic extracts were subjected to TLC to find out the nature and approximate number of the compounds present.

RESULTS AND DISCUSSION

The morphological examination revealed that leaves were 2-3 pinnatisect, covered with ashy bloom when fresh, alternate, 3-5 cm long; fruits were tetramerous, yellow in terminal corymbs; petals were five in number with dentate or wavy margin, ovary large. Fruits were 4-5 lobed many seeded capsule, dehiscing at the apex, lobes indehiscent. Morphological studies are in agreement with the previous findings (Anonymous, 2003; Singh and Dey, 2005). On the basis of these morphological details *R. graveolens* and *E. dracunculoides* can be differentiated when intact. But the identification becomes difficult in bailed or powdered samples. So, it is tried to establish the pharmacognostical and phytoanalytical parameters for its standardization and quality control, especially in the powder form. The powder has an exceedingly bitter and acrid taste while as odor is pungent. Powder microscopy of *R. graveolens* showed abundant fragments of thin walled, polygonal epidermis with thick striated waxy cuticle, glandular trichomes and sunken anomocytic stomata. Lignified fibres with simple pits, aseptate, 40-50 μ diameter were also present. Vessels were lignified with simple pits, 50-120 μ in diameter. Calcium oxalate crystals were irregular shaped and abundant.

The mean values of different extractive have been indicated in the Table 1. Cold extractives have lesser value than the individual hot extractives, which indicated the superiority of hot extraction process. Successive extractive have higher values in case of water (16.08% on dry weight basis) that indicated the presence of polar compounds in aerial parts.

Table 1: Extractive values of *R. graveolens* L. aerial parts with different solvents

Extraction method	Petroleum ether	Dichloro methane	Chloroform	Ethyl acetate	Acetone	Methanol	Water
	(Mean±SD)						
Cold	1.58±0.14	0.33±0.14	0.91±0.14	1.33±0.14	2.25±0.25	6.08±0.14	6.16±0.14
Hot	1.90±0.14	0.66±0.14	1.58±0.14	1.41±0.14	2.83±0.14	6.33±0.14	6.50±0.25
Successive	2.08±0.14	1.41±0.14	5.91±0.38	2.66±0.14	1.91±0.29	10.33±0.14	16.08±0.29

Results are mean of three observations, SD = Standard Deviation

Mean ash values (%) were found to be 8.13 (total), 2.01 (acid insoluble ash) and 1.02 (water soluble ash). Total ash value was relatively higher due to the high content of carbonates, phosphates, silicates and silica. Foreign matter was 0.35%. Low value of foreign matter indicated first hand collection of plant from Madhya Pradesh, India. Loss on drying was found to be 4.03% on account of loss of water and volatile chemicals. The average pH of aqueous extract was 6.74 and bitterness value was found to be 1.28, but no swelling was observed indicating the absence of mucilage in aerial parts. Foaming index was less than 100. The color reactions of fluorescence analysis and powder drug reaction with different reagents have been shown in Table 2 and 3, respectively.

Phytochemical screening was undertaken for the identification of different type of chemical constituents present in the aerial parts. Screening of all extracts indicate the presence of all major phytoconstituents i.e., alkaloids, amines, flavonoids, carbohydrates, phenolic, resins, but saponins were absent (Table 4).

Table 2: Fluorescence analysis of *R. graveolens* L. aerial parts

Solvent used	UV light (254 nm)	UV light (366 nm)
Powder as such	Brown	Dark green
Cold water	Brown	Blackish green
Hot water	Brown	Dark green
Picric acid	Brown	Yellowish green
Ammonia solution	Yellow	Brownish green
1 N NaOH	Greenish yellow	Blackish green
Methanol	Brownish white	Light green
Acetic acid	Green	Yellow
Conc. H_2SO_4	Brown	Brown
Conc. HNO_3	Bright Sky blue	Brown
Conc. HCl acid	Dark green	Light green
Dil. H_2SO_4	Brown	Greenish black
Dil. HCl	Brown	Greenish black
Dil. HNO_3	Brown	Greenish black
5% iodine solution	Black	Brownish

Table 3: Powdered drug reaction of *R. graveolens* L. aerial parts with different chemicals

Chemical treatments	Observation
Powder as such	Green
Picric acid	Green
Ammonia solution	Yellowish green
1 N NaOH	Green
Methanol	Light green
Acetic acid	Green
Conc. H_2SO_4	Black
Conc. HNO_3	Green
Conc. HCl acid	Green
5% iodine solution	Greenish black

Table 4: Phytochemical screening of *R. graveolens* L. aerial parts extracts

	Extract						
Class of compounds	Pet. ether	Dichloromethane	Chloroform	Acetone	Ethylacetate	Methanol	Water
Alkaloids	+	+	+	+	+	+	+
Amino acids	+	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+	+
Phenolics	+	+	-	-	+	+	+
Steroids	+	+	+	+	+	+	+
Resins	+	+	-	-	-	-	-
Proteins	-	-	+	-	+	+	+
Tannins	+	+	-	-	-	-	-
Saponins	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	+	+
Mucilage	-	+	+	+	+	+	+

+: Present, -: Absent

Table 5: TLC fingerprint profiles of *R. graveolens* L. aerial part extracts

Extracts	No. of spots	R_f values
Pet. ether	6	0.08, 0.41, 0.54, 0.72, 0.88, 0.94
Acetone	7	0.01, 0.32, 0.41, 0.55, 0.64, 0.73, 0.91
Dichloromethane	8	0.01, 0.09, 0.31, 0.42, 0.53, 0.73, 0.89, 0.95
Ethyl acetate	8	0.01, 0.26, 0.34, 0.39, 0.53, 0.62, 0.73, 0.87
Chloroform	8	0.01, 0.09, 0.27, 0.39, 0.51, 0.72, 0.89, 0.96
Methanol	8	0.01, 0.09, 0.27, 0.39, 0.51, 0.72, 0.89, 0.96

Solvent system: Toluene: Ethyl acetate: Diethyl amine: Formic acid (1.3: 0.6: 0.2: 0.5)

TLC fingerprints of aerial parts were developed and represented in Table 5. The solvent system i.e., Toluene: Ethyl acetate: Diethyl amine: Formic acid (1.3: 0.6: 0.2: 0.5) was worked out on hit and trial basis and gave best resolution of spots without overlapping and was applicable to all extracts.

CONCLUSION

The aerial parts of *R. graveolens* have been subjected to pharmacognostic standardization including phytochemical screening. The existing knowledge regarding rue may be increased by present investigation and may be quite useful for the quality control of various formulations containing rue. The main highlight of the present work is that it will be helpful to ascertain the correct identity of Sudab for which there is *E. dracunculoides* available in the drug market. The preliminary standardization studies of aerial parts of *E. dracunculoides* are underway in our laboratory. The current report will also help researchers and scientists design strategies for resolving cases of misidentification of plant material.

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