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REGULAR ARTICLE

STANDARDIZATION OF HOMOEOPATHIC DRUG *RUTA GRAVEOLENS* L.

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SUMMARY

Ruta graveolens L. is a small, strong scented perennial herb belonging to family Rutaceae. It is having a broad sphere of action & hence a potential drug in homoeopathy. It has antihysteric, emmenagogic, ophthalmic, vermifuge, carminative, antiepileptic, revulsive, anthelmentic, abortive, spasmolytic properties. It has both curative and injurious on the fibrous and bony tissues, especially in the vicinity of joints. In the present paper, the pharmacognostic and physio-chemical investigations on the leaves and stem of the plant have been presented. Morphological and anatomical characters of leaf and stem are studied in detailed. Physio-chemical parameters of raw drug viz., moisture content, ash values, extractives values, as well as quantitative estimation of various phytochemicals have been studied.

Keywords: *Ruta graveolens*, Homoeopathic drug, pharmacognosy.

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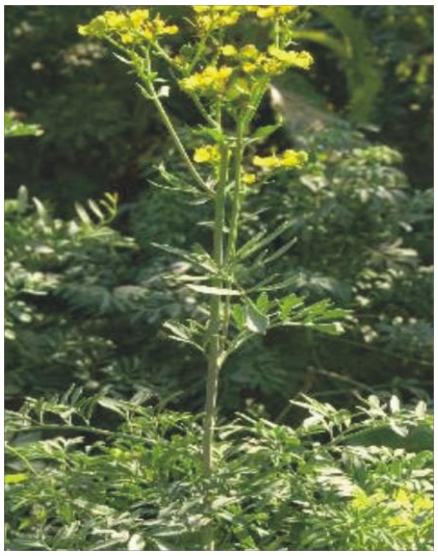
1. Introduction

Ruta graveolens L. [Rutaceae] is popularly known in Hindi as '*Sadab / Satap*', in English as '*rue/ bitter herb*' & in German "*Garten Raute*'. *Rue* has been valued for centuries as a bitter herb. The ancient Greeks & Romans held the plant in high esteem. Rue was alleged to be the antidote which Mercury gave it to Ulysses to counteract the drugged drink offered by Circe, the Enchantress. According to Mrs. Grieve, the effectiveness of *Ruta* in various diseases inspired the Greeks to name it *Ruta* after the word *reuo, i.e.* to set free. Folklore used finely chopped leaves as salads as a digestive aid. In the middle ages it was believed that it warded off plague & was credited with anti magical powers as well as allegedly cured countless ills [1]. It is a well known remedy for the treatment of various types of disorders in the Ayurvedic, Homoeopathic system of medicine in India [2]. Rue is the native of the South of Europe, cultivated in Northern gardens. Rue was first mentioned by Turner, 1562, in his Herbal and has since become one of the best known and most widely grown simples for medicinal and homely use.



Flowering twig of *Ruta graveolens*

It is mentioned by Hahnemann in Materia Medica Pura Vol. II pg 437. T.F. Allen in Encyclopedia of Pure Materia Medica, Vol. VIII, P.431, Hering in The

Guiding Symptoms of our Materia Medica, Clarke in Dictionary of practical Materia Medica.

It has antihysteric, emmenagogic, ophthalmic, vermifuge, carminative, antiepileptic, revulsive, Antihelminthic, abortive, spasmolytic properties. It appears to affects both injuriously and curatively the fibrous and bony tissues, especially in the vicinity of joints [3]. This is a medicinal plant which has been traditionally used as sedative, antihelminthic, menstrual and gastrointestinal disorders [4]. It is also used as hypotensive, antifertility & its anti-inflammatory effects have been claimed as further analgesic actions of this plant [5].

Ruta graveolens contains an essential oil, where the main components are 2-hendecanone (2-undecanone, methylnonylketone up to 60 %) and 2-nonanone (methylheptylketone) plus several more ketones and corresponding secondary alcohols. Methyl anthranilate and anethole glycol are also reported. The terpenoids represented are mainly by limonene, α-pinene, cuminaldehyde and l, 8-cineol. A component responsible for the bitter taste is rutin (7 to 8 % in the dried leaves), a polyphenolic flavonolone glycoside containing the disaccharid rutinose as sugar component. [6].

Furthermore, *R. graveolens* shows the presence of chemicals like kokusaginine, skimmianine, graveolinine, 2,3-dohydrokokusaginine, γ-fagarine, dictamnine, arborinine, rutamine, rutacridone, ribalinium, isopropyldihydroxy furoquinoline; xanthotoxin and an aliphatic ketone; rutin, isoimperatorin and psoralen; furocoumarin – β-D- glucopyranoside-rutarin and furocoumarin-rutaretin etc.[3].

The secondary metabolites 2-Nonanone, 2-undecanone, chalepensin and geijerene are the main constituents found in the extracts from *Ruta graveolens* leaves, flowers, stems and roots respectively [7]. Three new glycosides, 3'-sinapoyl-6-feruloylsucrose, methylcnidioside A. and methylpicraquassioside A in addition to 4 known glycosides like 3',6-disinapoylsucrose, cnidioside A, rutin, were isolated from the *Ruta graveolens*. Their structures were elucidated by interpretation of IR, MS and 1D and 2D NMR spectra [18]. The furanocoumarins 5-methoxypsoralen, 8-methoxypsoralen, and the quinolone alkaloid graveoline were isolated as phytotoxic constituents from *Ruta graveolens* [8].

Toxicology

Ruta graveolens is toxic & its accidental ingestion causes stomach pain, vomiting, exhaustion, confusion & convulsions; large amounts may be fatal. The plant is irritant & vesicant, esp. in the heat of summer; handling of the flowers & fruit has produced erythema, with burning pain, itching & vesication [1].

2. Materials and Methods

The fresh leaves of *Ruta graveolens* were collected from Botanical garden of KSPM'S, Homoeopathic Medical College, M.I.D.C., Latur (M.S.) India. The leaves were washed and used for the present study. The macroscopic observations of the mature leaves were noted down. For microscopic studies, the epidermal peels and cross sections of the leaves were prepared and stained. Quantitative leaf microscopy was carried out for stomatal frequency, stomatal index, vein-islet and vein termination number. The histo-chemical colour reactions were performed by the standard methods [9, 10]. The line and cellular sketches of the figures were drawn using a Camera Lucida. Physio-chemical values such as the percentage of moisture (loss on drying at 105° C), total ash, acid insoluble ash, acid soluble ash, extractive values like petroleum ether-soluble extractives, alcohol-soluble extractives and water-soluble extractives were calculated according to the methods described in the Indian pharmacopoeia [11, 12]. Phytochemical studies such as quantitative analysis were done from the shade dried powdered material. Transections of leaf, stem and root were taken by free hand. Fresh and preserved materials were used. Sections were stained in safranin (1 %), light green (1 %). Microphotographs of leaves, stem and root sections were taken by using Jenaval and Mirax Laborec Cameras affixed to microscope.

The air dried leaf powder was used for the identification of phytochemcal constituents. The recommended procedures were followed for determining reducing sugar [13], Nitrogen content was estimated by micro-Kjeldahl method after digesting the sample with Conc. H_2SO_4 [17]. The percentage of crude protein was calculated by multiplying total nitrogen value with 6.25 [14]. Amino acid, protein and phenolics [15], like calcium [16], phosphorus [13] and potassium [17].

3. Results

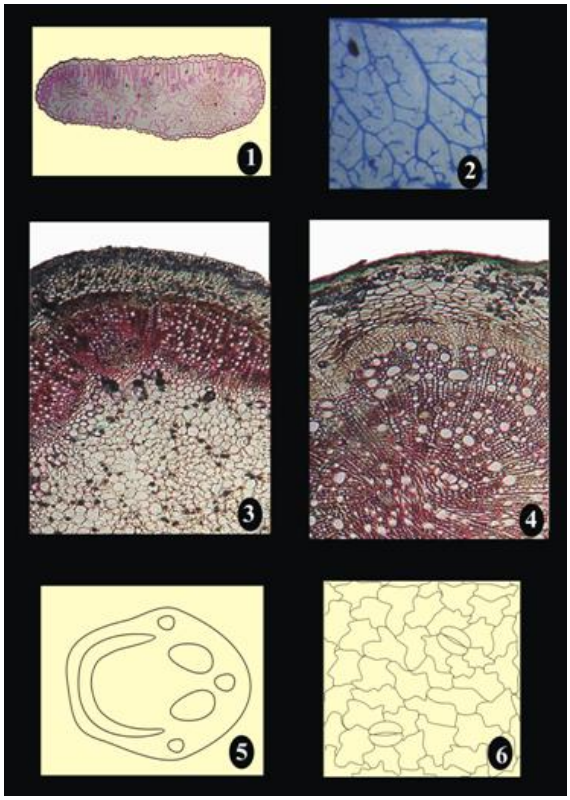


Fig. 1. Microscopic slides

a) Macroscopic characters

It is strong scented, erect, glabrous and glaucous, woody perennial herbs, 30-90 cm tall; stems terete. Leaves 2-3 pinnate, 5-10 cm long; spanthulate-oblong or linear-oblong, 2-10 by 1-4 mm. flowers in spreading corymbs; common peduncles 1-5 cm long, stout; partial peduncles 5-10 mm long, 2-bracteolate below the middle. Bracts ovate, 3-4 mm long, pubescent; bracteoles minute, long persistent. Pedicels 5- 10 mm long. Sepals distinct, ovate, 1.5-2 mm long, punclate. Petals yellow, oblong-ovate, slightly larger than sepals; linbs pactinate; claw short. Ovary 3-5 lobed, ovoid; style short; stigma capitate. Fruits ovoid, 6-10 mm long,separating into 3-5,apically dehiscent mericarps, gland-dotted. Seeds angled blackish.

b) Microscopic characters

Leaf (1.1): - The leaf is dorsiventral and hypostomatic. The adaxial epidermis is of larger cells. The abaxial epidermal cells are comparatively smaller. Papillae absent, trichomes are lacking on both surfaces. Mesophyll is differentiated into palisade and spongy tissues. The palisade consists of one to two layers and a spongy tissue is of loosely arranged cells. Midrib vascular bundle is solitary and an arc shaped. In the midrib region the lower epidermis is follow by one to two layers of collenchymatous hypodermis followed by parenchymatous cortex. Vascular bundle i.e. midrib is lifted towards right corner of leaf.

Epidermis (Fig 1.2): - The epidermis cells are cubical to oval. The anticlinal epidermal cell walls are straight, arched or sinuous. The adaxial epidermal cells are large with thin walls as compared to the abaxial epidermal cells. The cells in the intercostals region are variously oriented. A well developed, cuticle is always present on both the surfaces.

Stomata : - Stomata are present on the lower surface of the leaf only. The shape of stomata is generally circular, oval or elliptical in outline. The stomata are anomocytic (Fig. 1.6).

Stem (Fig. 1.3): - The transverse section of the stem shows a circular outline. Epidermis has small compactly arranged; cell subsequent to the epidermis is a band of collenchyma of 4-5 layers, a zone of parenchyma 7-8 layers of cell. The stem possesses a dictiostele which encloses wide central pith. Phloem consists of sieve tubes, companion cells and phloem parenchyma, and xylem consists of vessels, tracheids and xylem parenchyma. Starch grains, calcium oxalate crystals of acicular form are found abundantly in stem.

T. S. of Node (Fig. 1.6): - From the central axial vascular tissue a median trace and then two lateral traces emerge out living behind three distinct gaps. The entire vasculature is surrounded by sclerenchymatous ring. The

three traces for axillary bud are given out. The node is trilacunar and three traced. Phyllotaxy is alternate.

Root (Fig. 1.4): - The transverse section of the root shows a circular outline. Epidermis has small compactly arranged cells. The cork consists of 5 to 7 rows of nearly cubical to rectangular cells of which, the cells of the peripheral rows are thick walled while the innermost one or two rows are with thin walled cells. Phellogen is single layerd. The cortex consists of tangentially elongated cells and small group of stone cell. Starch grains are present in the cortex. Secondary growth is prominent. Each phloem group is composed of narrow tangential strips of phloem fibers alternating with the thin-walled phloem-elements. Secondary xylem is well developed.

c) Quantitative microscopy

The leaf microscopic characters like stomatal frequency, stomatal index, vein islet number and vein termination were determined [Table 1].

Table 1: Quantitative microscopy.

Parameter	Range	Mean
Stomatal frequency	90-120	106
Stomatal index	9.09-12.19	10.64
Veinislet number	11.48-15.78	13.81
Vein termination	14.47-21.05	17.76
Palisade ratio	2.25-2.50	2.38

d) Histological colour reaction

The histological colour reactions were observed in the transverse section of the fresh leaf [Table 2]. The results indicated the presence of protein, starch, fats, alkaloids, saponins, tannins, and glyceride.

e) Physio-chemical characters

The physio-chemical characters like moisture content, total ash, acid insoluble ash, acid soluble ash and extractive values in chloroform, alcohol and water, of the dried leaf powder were calculated in terms of air dried sample [Table 3].

f) Phytochemical evaluation

The air dried leaf powder was used for the identification of phytochemcal constituents [Table 4]. The values obtained are presented in Graph 1.

Table 2: Histological colour reactions.

Test	Starch	Protein	Saponin	Fat	Glyceride	Alkaloid	Tannin
Drug	**+**	**+**	**+**	**+**	**+**	**+**	**+**

Table 3: Physical evaluation (% w/w).

Parameter	% (w/w)
Moisture content	7.55
Extractive values	
a) Petroleum ether	5.0
b) Alcohol	7.0
c) Water	11.5
Ash values:	
a) Total ash	11.8
b) A.I.A	1.0
c) A.S.A.	10.08

Table 4: Formulation of Mother Tincture.

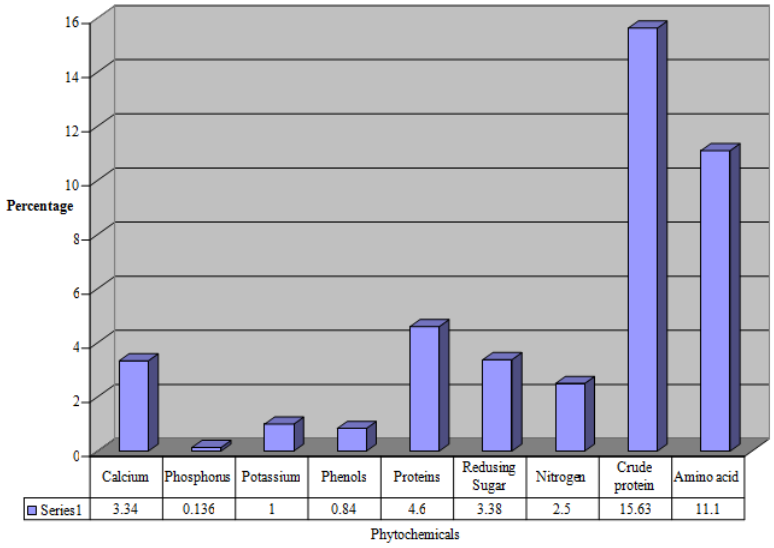
Drug Strength	1/10
R. graveolens in coarse powder	100 gm
Strong alcohol	730 ml
Purified water	300ml

To make one thousand ml of the mother tincture.

Table 5: Physico-chemical constants of Mother Tincture

Sr. No.	Parameter	Observations
1.	Organoleptic character	
	a) appearance	Clear, foam formed on shaking
	b) colour	Dark maroon
	c) odour	Fruity and aromatic
2.	Sediments	Absent
3.	Weight per ml	0.89 g
4.	Total solid	2.1 % w/v
5.	pH at room temperature	3.0-3.5
6.	Alcohol content	58-62% v/v

Chemical studies of *Ruta graveolens* (%)



4. Discussions

The present pharmacognostic study of *Ruta graveolens* provides useful information on its correct identity. Leaves are small, petiolated, decompound, segments cunates. It has glandulose appearance and a strong smell. The stem is rounded. The leaves and stem are medicinally important. The root as always admixed in the crude drug.

The epidermis cells are cubical to oval. The anticlinal epidermal cell walls are straight, arched or sinuous. The adaxial epidermal cells are large with thin walls as compared to the abaxial epidermal cells. The cells in the intercostals region are variously oriented. The palisade ratio is found as 2.25-2.50. Stomata are reported on the lower surface of the leaf only. The shape of stomata is generally circular, oval or elliptical in outline. The stomata are anomocytic (Fig. 1.6). The stomatal index on lower surface is reported as 10.46 (average) while stomatal

frequency is 106 (average). The veinislet number and vein termination number is noted as 13.81 and 17.76 in an average respectively (Fig. 1.2)

The leaf midrib vascular bundle is solitary an arc shaped. The lower epidermis is followed by one to two layers of collenchymatous hypodermis followed by parenchymatous cortex. The midrib Vascular bundle is lifted towards right corner of leaf.

The transverse section of the stem shows a circular outline. Epidermis has small compactly arranged cells; subsequent the epidermis is a band of collenchyma of 4-5 layers and a zone of parenchyma 7-8 layers of cell. The stem possesses a dictiostele which encloses wide central pith. Starch grains, calcium oxalate crystals of acicular are found abundantly in the region of the stem. (Fig. 1.3). From the central axial vascular tissue a median trace and then two lateral traces are emerge out living behind three distinct gaps. The entire vasculature is surrounded by sclerenchymatous ring. The three traces for axillary bud are given out. The node is trilacunar and three traced. Phyllotaxy is alternate (Fig. 1.5).

The transverse section of the root shows a circular outline. Epidermis has small cells compactly arranged. The cork consists of 5 to 7 rows of nearly cubical to rectangular cells of which, the cells of the peripheral rows are thick walled, but not lignified while the innermost one or two rows are thin walled. Phellogen is single layerd. The cortex consists of tangentially elongated cells and small group of stone cell. Starch grains are simple and are present in the cortex. Secondary growth is more. Each phloem group is composed of narrow tangential strips of phloem fibers alternating with the thin-walled phloem-elements. Xylem developed in large amount with linear manner (Fig. 1.4).

Physical standards like moisture content, ash values, extractive values etc. play an important role in the drug evaluation. In the present plant, moisture content is reported as 7.55. Acid insoluble ash content is low 1.0 % and total ash calculated is 11.8 %. The leaf shows higher extractive values in water than that of alcohol and petroleum ether (Table 3).

The preliminary phytochemicals studies carried out so far reveal the presence of various phytochemicals like alkaloids, stach, protein, fats, saponins, tannins and glyceroids (Table 2). the physico-chemical data for the raw drug has been summarized in Table 5 and that of mother tincture preparation in Table 4. The phytochemical quantitative percentage shows great variations. The values obtained are presented in Graph 1.

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