IJP (2015), Vol. 2, Issue 2

(Research Article)



Received on 31 December 2014; received in revised form, 19 January 2015; accepted, 17 January 2015; published 01 February 2015

PHARMACOGNOSTIC AND PRELIMINARY PHYTOCHEMICAL SCREENING OF LEAVES OF *RAPHANUS SATIVUS*, LINN.

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Keywords:

Raphanus sativus, Ash value, Physicochemical, Phytochemical, Diuretic

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ABSTRACT: Raphanus sativus commonly known as radish is cultivated as a crop and having various medicinal uses. It is cultivated all over the world as an edible root vegetable. The present study deals with the pharmacognostic, preliminary phytochemical investigation of Raphanus sativus, Linn. belonging to family Cruciferae. In this, pharmacognostic studies are concerned for the determination of physicochemical parameters like ash values, extractive values, and loss on drying, fluorescence analysis also the macroscopic and microscopic evaluation carried out. The leaves were subjected to successive soxhlation using petroleum ether, ethyl acetate, alcohol, and water. The extracts thus obtained were studied for preliminary phytochemical investigation for detection of the presence of various chemical constituents like alkaloids, glycosides, steroids, tannins, saponins, fats and oils, flavonoids, etc.

INTRODUCTION: The term "Medicinal Plants" for worldwide trade specifies the plants having substances or substances of medicinal value, which have been biologically proved useful as drug or contain drug constituents that can be used as therapeutic agents or as models for new synthetic drugs and instruments in drug development and diagnostic purposes ¹. A Medicinal plant is defined as any substance with one or more of its organ containing properties that can be used for therapeutic purposes or which can be used as precursors for the synthesis of various drugs ².



DOI: 10.13040/IJPSR.0975-8232.IJP.2(2).93-97

Article can be accessed online on: www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.2(2).93-97

Raphanus sativus belonging to family Cruciferae is an annual or biennial bristly herb, with a white or brightly colored fleshy taproot. Stem simple or branched, erect, 20-100 cm. Leaves pinnatisect or pinnate, coarsely toothed. Flowers usually white or lilac with purple veins, borne in long terminal racemes. Fruits inflated, hardly or irregularly constricted, with a long tapering beak and filled inside with white pith between the seeds. Seed 2-8, globose, brown or yellow. Flowers and fruits during winter.

The various vernacular names of the plant are as Sanskrit- Moolaka. Hindi- Muli, Maharashtra-Mula. English- Radish. Cultivated throughout India and up to 3,000 m in the Himalayas as well as other hilly regions. It possesses various traditional uses like decoction of dry radish is given orally either alone or mixed with meat juice in piles; hot fomentation on piles with the paste made from

E- ISSN: 2348-3962, P-ISSN: 2394-5583

radish by admixture with water or vinegar stops bleeding. Extract of the dry root is a remedy for hiccough, influenza, dysentery, urinary troubles and colic (50 to 100g/day in divided doses); external application of the extract in the form of liniment is effective in erysipelas, edema from pulmonary diseases and an earache. Seeds are considered expectorant, diuretic, mild purgative. Green leaves of tender radish are considered diuretic and useful for constipation ^{3,4,5,6}.

MATERIALS AND METHODS:

Collection and Identification of Plant Material:

In the present study, the leaves of *Raphanus sativus*, Linn. (Family - Cruciferae), was collected from the local areas of Jalgaon district. The plants were authenticated from Agharkar Research Institute (ARI) Pune. (An autonomous, grant-in-aid research institute of the Department of Science and Technology (DST), Government of India). Soon after authentication, all the crude drugs were dried at room temperature until they free from moisture and subjected to physical evaluation with different parameters.

Macroscopic: The macroscopic characters such as size, shape, margin, nature, texture, apex, surface, color, odor, taste were studied for morphological investigation ⁷. The results are reported in **Table 1**.

TABLE 1: MACROSCOPIC CHARACTERS OF RAPHANUS SATIVUS, LINN. LEAF

S.	Parameters	Observation	
no.	Physical		
I	Shape	Lower leaves hairy, petiole lyrate, coarsely	
		toothed; uppermost leaves simple, sub-	
		linear but narrowed at the base	
II	Size	Thick $3.6-6.8 \times 21.3-4.6 \text{ cm } 20 \text{ cm}$	
III	Colour	Bright green	
IV	Odor	Characteristic	
V	Taste	Characteristic	

Microscopic: For microscopy, freehand section leaf was cut and stained according to the prescribed method ⁸.

Physicochemical Evaluation: The extractive values, ash values, and loss on drying ⁹, fluorescence analysis ¹⁰ were performed according to the official methods. The results are reported in **Table 2** and **3**.

Preparation of Extracts: In the present study, the crude drugs were carefully selected and shade dried. The dried material was reduced to coarse

powder in a mechanical grinder and passed through a sieve no. 40 to obtain a powder of desired particle size. About 200 gm of powdered material was subjected to exhaustive extraction successively with petroleum ether, ethyl acetate, and ethanol at a temperature of 45-50 °C to about 40 cycles per batch for 8 batches. The extraction was continued until the solvent in the thimble became clear then few drops of solvent were collected in a test tube during the completion of the cycle (during siphoning) and chemical test of that solvent was performed. Extraction was completed only when a chemical test shows negative results. Finally, the drug was be macerated with chloroform water. After each extraction, the solvent was distilled off rotary evaporator, and the extract was concentrated at low temperature. These extracts were used for phytochemical investigation.

TABLE 2: PHYSICOCHEMICAL ANALYSIS OF RAPHANUS SATIVUS, LINN, LEAVES

S. no.	Parameters	Observation
I	Physical tests	
	Nature	Coarse powder
	Colour	Green
	Odor	Characteristic
	Taste	Characteristic
II	Extractive value	
	Pet. ether	2.40% w/w
	Ethyl acetate	5.50% w/w
	Alcohol	4.75% w/w
	Aqueous	5.80% w/w
III	Loss on drying	7.00% w/w
IV	Ash values	
	Total ash	11.50% w/w
	Acid-insoluble ash	1.10% w/w
	Water soluble ash	1.8% w/w

TABLE 3: POWDER ANALYSIS OF RAPHANUS SATIVUS, LINN. WITH DIFFERENT CHEMICAL REAGENTS

11111	GENTE		
S.	Reagent	UV (254 nm)	UV (366 nm)
no.		Fluorescence	Fluorescence
1	Powder as such	Green	Light brown
2	Powder + 1N NaOH	Brown green	Blackish
3	Powder + 50% HCl	Green	Blackish
4	Powder $+50\%$	Green	Blackish
	H_2SO_4		
5	Powder + 50% HNO ₃	Light green	Blackish
6	Powder + Iodine	Dark green	Dark brown
	solution		
7	Powder + 5% FeCl ₃	Green	Dark brown

Phytochemical Screening: The preliminary phytochemical screenings of extracts were carried out as per standard procedures ¹¹⁻¹⁷. The results are reported in **Table 4**.

TABLE 4: PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF RAPHANUS SATIVUS, LINN. LEAVES

S. no.	RELIMINARY PHYTOCHEMICAL INVESTIGATION Chemical Tests	PEE	ETA	ALCE	AQE
1	Tests for Carbohydrates				
	Molish's test (General test)	_	_	_	+
	Tests for reducing sugars				
	Fehling's test	_	_	_	+
	Benedicts test	_	_	_	_
	Test for Monosaccharides				
	Barfoeds test	_	_	_	_
	Test for Pentose sugars	_	_	_	_
	Tests for Hexose sugars				
	Tollen's phloroglucinol test for galactose	_	_	_	_
	Cobalt chloride test	_	_	_	_
	Test for Non-reducing sugars	_	_	_	_
	Tests for Non-reducing polysaccharides (starch)				
	Iodine test	_	_	_	_
	Tannic acid test for starch	-	_	-	-
	Test for Gums	-	-	-	-
2		-	-	-	
2	Tests for Proteins				
	Biuret test (General test)	-	+	-	-
	Millions test for proteins	-	+	-	-
	Xanthoprotein test	-	+	-	-
	Test for proteins containing sulfur	-	+	-	-
	Precipitation test	-	+	-	
3	Tests for Amino acids				
	Ninhydrin test (General test)	-	-	-	-
	Test for tyrosine	-	-	-	-
	Test of tryptophan	-	-	-	-
	Test for cysteine	-	-	-	-
4	Tests for Fats and Oils				
	Solubility test	=	_	-	-
	Saponification test	_	_	-	_
	Paper staining test	_	_	_	_
5	Tests for Steroids				
	Salkowski reaction		_	+	+
	Liebermann – Burchard reaction	_	_	+	+
	Liebermann reaction	_	_	-	+
6		-	-		Т
0	Tests for Triterpenoids				
	Salkowski reaction	-	-	+	+
	Liebermann – Burchard test	-	-	+	+
7	Tests for Glycosides				
	Tests for Cardiac Glycosides:				
	Baljet test	-	-	-	-
	Legals test (Test for cardenolides)	-	-	-	-
	Test for deoxy sugars (Killer Killani test)	-	-	-	-
	Liebermann's test (Test for Bufadenoloids)	-	-	-	-
	Tests for anthraquinone glycosides:				
	Borntraggers test	-	+	+	+
	Modified Borntragger's test	-	-	-	-
7	Tests for Saponin glycosides				
	Foam test	-	+	+	-
	Hemolysis test	-	+	+	-
	Test for Cyanogenetic glycosides, Guinard-reaction or				
	Sodium picrate test	-	-	-	_
	Tests for Coumarin glycosides				
	Alkaline reagent test	_	_	_	_
	NaOH Soaked paper test	_	_	_	_
8	Tests for Flavanoids				
U	Ferric Chloride test				
		-	+	+	-
	Shinoda test	-	+	+	-

	Alkaline reagent test	-	+	+	-
	Lead acetate test	-	+	+	-
9	Tests for Alkaloids				
	Dragendroff's test	+	-	+	+
	Mayers test	+	-	+	+
	Hagers test	+	-	+	+
	Wagners test	+	-	+	+
	Murexide test for purine alkaloids	-	-	-	-
10	Tests for Tannins and Phenolic compounds				
	5% FeCl ₃ solution	-	-	-	-
	Lead acetate solution	-	-	+	-
	Gelatin solution	-	-	+	-
	Bromine water	-	-	+	-
	Acetic acid solution	-	-	-	-
	Dilute iodine solution	-	-	+	-
	Dilute HNO ₃	-	-	+	-
	Dilute potassium permanganate solution	-	-	+	-
11	Tests for Saponins				
	Foam test	+	+	+	-
	Raymonds test	+	+	-	-
	Hemolysis test	+	+	+	-
	Bromine water test	+	+	+	-
	Legal's test	-	-	-	-
12	Test for Lipids	-	-	-	-

RESULTS AND DISCUSSION: The standardization of raw material is very important to the first step towards ensuring the quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The standardization of crude drugs is important before work carried out. The morphology, any microscopy, physicochemical tests are the first step towards establishing the identity and the degree of purity of such materials and should be carried out before any tests are undertaken. The result of this study as follows:

Microscopic Characters of Leaf: Petiole appears nearly circular in outline with two lateral wings; epidermis single layered, covered with thick cuticle; hairs unicellular, present only on the upper side; cortex 6-12 layers of the oval to polygonal, thin-walled, parenchymatous cells; collateral vascular bundles arranged in a ring. Midrib appears biconvex in outline; epidermis on both side covered with thin cuticle; epidermis followed by 6-12 layers of parenchymatous cortex on both sides; vascular bundle three in number, one central and two lateral. Lamina dorsiventral; epidermis on either surface

with thin-cuticle; palisade 2-3 layers; spongy parenchyma 4-5 layers; anisocytic stomata present on both surfaces.

E- ISSN: 2348-3962, P-ISSN: 2394-5583

CONCLUSION: The morphological microscopy, physicochemical parameters are the tools for the standardization of the crude drug. The results of this work support the importance of Raphanus sativus in various aspects. The present work confirms that various extracts of leaves of Raphanus sativus, shows the presence of various chemical constituents such as carbohydrates, steroids, triterpenoids, alkaloids, and glycosides. These phytochemical and physiochemical values is useful for determining the quality and purity of the drug.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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E- ISSN: 2348-3962, P-ISSN: 2394-5583

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How to cite this article:

Warke PD and Kale MK: Pharmacognostic and preliminary phytochemical screening of leaves of *Raphanus sativus*, Linn. Int J Pharmacognosy 2015; 2(2): 93-97. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.2(2).93-97.

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