PHYSIOCHEMICAL INVESTIGATION OF *RAPHANUS SATIVUS* LEAVES WITH SPECIFIC REFERENCE TO THEIR PHARMACOGNOSTICAL EVALUATION

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***Abstract*: *Raphanus sativus* is made to develop pharmacognostical characters of leaf as macroscopic, microscopic and physical characters including histochemical analysis of leaf is carried-out. Pharmacognostical evaluation as color, odour, taste, size, shape, surface and powder microscopy of plant show the presence of epidermal cells, spiral vessels, anomocytic stomata, simple trichome, crystals of calcium oxalate and starch grain etc. Quantitative leaf microscopy to determine palisade ratio, stomata index and vein-islet number are carried out. The leaf epidermal studies are carried out on fresh specimens. Peels are removed mechanically through epidermal peeling off and stomatal index (SI) is calculated. The vein islet number and palisade ratio of lamina are determined according to the standard method. We prepared the extracts of plant with different solvents for determine the different extractive values by maceration, soxhlet exraction, successive extraction process and determination of ash values, pH value, moisture content , fluorescence analysis and phytochemical screening to developed the quality standards.**

***Keywords*: *Raphanus sativus*, Pharmacognostical study, Phytochemical identification, Leaves extraction, Fluorescence analysis.**

INTRODUCTION: *Raphanus sativus* Linn is popularly known as *Radish* in belongs to the family Brassicaceae. It is cultivated in plains throughout India up to 3000 m in the Himalayas and other hilly regions[1]**.** It is an edible root vegetable. Radishes have numerous varieties, varying in size, color and duration of required cultivation time & are grown and consumed throughout the world. Some radishes are annuals, and some are biennials[2]. Radish leaves usually are medium green, lobed and have a rough texture and characterized with either pinnate or entire leaf edge, and both types of leaves are simple[3]**.** Radish leaves are rich source of calcium, iron, and ascorbic acid. Leaves are characterized in terms of their physico-chemical, nutritional, antioxidant, antimicrobial, anticancer, hepatoprotective, anti-diabetic, gastrointestinal and uterine tone modulatory, anti-ulcer and cardio-modulatory activities properties[4]. Leaves presented higher percentage of protein, ash and crude fiber than roots. The most abundant free and bound phenolic compounds of roots and leaves were pyrogallol and vanillic acid; and epicatechin and coumaric acid, respectively. Aerial parts (leaves and stem) of Raphanus sativus, which are usually discarded were found to possess potent antioxidant and radical scavenging activity, as measured by standard antioxidant assays[5]**.**The extracts were subjected to antioxidant tests (Total reducing power and Total phenolic content), and preliminary phytochemical screening[6].

MATERIAL AND METHODS:

Collection and authentication of plant material: Samples of *Raphanus sativus* leaves was collected from Hamdard university campus,New Delhi, India(2018) and samples were identified by Taxonomist Prof. H.B.Singh, Department of Botany, NISCAIR. The specimen was studied in Pharmacognosy and Phytochemistry laboratory, Vivek College of Technical Education, Bijnor,U.P.

Macroscopical and Microscopic study: The fresh leaves were examined to macroscopical and microscopical study. The dried leaves were examined for powder microscopy using with different staining reagents for different types of microscopical characters.

Physicochemical Evaluation of Drug:

Determination of individual extractive values: The amount of soluble components extracted with different solvents from the powder plant material.

Maceration: The air-dried coarse drug powdered was macerated with different solvents like pet.ether, chloroform, water and methanol of in a closed flask and place for 24 hours, shaking frequently during 6 hours and allowing standing for 24 hours. After the filtration, evaporated to dryness in a dish and dried at 105ºC, to constant weight and get percentage yield.

Soxhlet extraction: The dried coarse powdered drug was packed in a Soxhlet apparatus separately for different solvents like pet.ether, chloroform, water and methanol. The each extract was evaporated till to dryness and extractive value was noted.

Successive Extraction: The dried coarse powdered drug was subjected for successive extraction in Soxhlet apparatus with different solvents as pet.ether, chloroform and methanol. The extract was evaporated till to dryness and extractive values were noted.

Determination of ash values:

Ash value is an essential parameter of a drug for the extent of adulteration and also establishes the quality and purity of the drug.

Determination of total ash values: After ignition of medicinal plant yield total ash constituting in which both physiological and non-physiological ash was present. The drug was incinerated in a silica crucible at temperature which not more than 450ºC. Then was cooled and weighed to get the total ash content.

Determination of Acid insoluble ash values: Sand and siliceous earth both forming acid insoluble ash represents. Ash is boiled with dil. HCl (6N) for 5 minutes. After that the insoluble matter collected on an ash less filter paper, rinsed with hot water and ignited at a temperature which not more than 450ºC to a constant weight.

Determination of Water-soluble ash values: The ash was in dissolved distilled water after that the insoluble part of ash collected on an ash less filter paper which ignited at 450ºC to a constant weight. The weight of soluble part of ash is noted by subtracting the weight of insoluble part from the ash.

Florescence analysis: Florescence analysis of the powder drug was exposed to in daylight and UV light (254 and 366 nm) and treated with different reagents like sodium hydroxide, picric acid, iodine, hydrochloric acid, nitric acid, pet.ether, ferric chloride, chloroform etc.

Phytochemical screening: The different extracts of the selected drugs like Petroleum ether extract, Chloroform extract, Methanolic extract, aqueous extract were reported to preliminary phytochemical investigation for the detection of secondary metabolites. The plant extracts may provide the information regarding various types of phytoconstituents present such as Alkaloids, Carbohydrates, Flavonoids, Protein, Saponins, mucilage, resins, fat and lipids etc[7].

Determination of PH:

pH 1% solution: Drug was dissolved in distilled water, filtered this and noted pH of the filtrate with a standardized glass electrode.

pH 10% solution: Drug was dissolved in distilled water, filtered this and noted pH of the filtrate with a standardized glass electrode.

Determination of moisture content: Excess of water in medicinal plant will encourage the microbial growth and also presence of fungi and insect resulting in deterioration and hydrolysis. Weighed drug and dried in oven at 105^0C temp. For 1 hour, then cool in desicator and weight.

Loss on Drying = Wt. before drying – wt. after drying X 100 / wt. sample taken.

Heavy metals residue: Heavy metals were determined such as lead, arsenic, mercury and cadmium in the leaf extract of the plant by using Atomic Absorption Spectrophotometer[8].

Pesticide residue: According to American Organisation of Analytical Chemist (AOAC) by using GC-MS method pesticides residue were determined such as pyrethroids, organochlorines, organophosphates in the leaf extract of the plant[8].

Aflatoxin analysis: According to American Organisation of Analytical Chemist (AOAC) by using HPLC method Aflatoxin were analysed in leaf extract of the plant[8].

RESULT AND DISCUSSION:

Macroscopical study: The leaves of *R. sativus* were green in colour, pinnate shape, smooth surface, 18-30cm in size, slightly bitter in taste and characteristic odour as shown in **Table 1.**

TABLE 1: MACROSCOPIC CHARACTER OF LEAVES OF *RAPHANUS SATIVIS*

S.no.	Parameters	Observation
1.	Colour	Green
2.	Odour	Characteristic
3.	Taste	Slightly bitter
4.	Size	18-30 cm in length
5.	Shape	Pinnate
6.	Surface	Smooth

Microscopic study:

Transverse section of leaves: Transverse section of leaves through the mid rib showed upper epidermis and lower epidermis, single layer epidermal cells with thin cuticle. Mesophyll made up of upper 2 to 3 layer compactly arranged palisade parenchyma enriched by chlorophyll pigment. Lower layer arranged in to 6 to 8 layers of spongy parenchyma between the palisade spongy parenchyma

the vascular strands are passed. Through mid rib shows below the upper epidermis and lower epidermis 1 or 2 layers of collenchymatous layer are present.

Quantitative Microscopy: The slides of surface preparation of leaf are prepared and subjected to quantitative microscopic examination. The parameters such as vein termination, vein islet and stomatal numbers, stomatal index and palisade ratio of the leaf as shown in **Table 2.**

TABLE 2: QUANTIVATIVE MICROSCOPY OF LEAVES OF *RAPHANUS SATIVUS*

S.no.	Parameters	Observation
1.	Stomatal no.	
	Upper surface	7 ± 11
	Lower surface	8 ± 14
2.	Stomatal index	
	Upper surface	20 ± 24
	Lower surface	22 ± 26
3.	Vein islet no.	7 ± 9
4.	Vein termination	12 ± 14
5.	Palisade ratio	4 ± 5

Physicochemical evaluation: The various physicochemical parameters were determined by using air dried powder plant material as shown in **Table 3.**

TABLE 3: PHYSICOCHEMICAL EVALUATION OF POWDER DRUG OF LEAVES OF *RAPHANUS SATIVUS*

S.no.	Parameters	Result %w/w
1.	**Maceration extraction**	
	Petroleum ether	0.87
	Chloroform	0.42
	Methanol	0.62
	Hydroalchohal	0.75
2.	**Soxhlet extraction**	
	Petroleum ether	2.22
	Chloroform	3.90
	Methanol	5.03
	Hydroalchohal	8.03
	Aqueous	0.87
3.	**Successive extraction**	
	Petroleum ether	3.05
	Chloroform	5.68
	Methanol	9.09
4.	**Ash values**	
	Total ash	5.58
	Acid insoluble ash	1.74
	Water soluble ash	3.83
5.	pH of 1% solution	6.8
	pHof 10% solution	7.3
6.	Moisture content	5.6

Determination of Heavy metal residue: As per WHO, the determination of heavy metals was carried out in the extract of *R. Sativus* leaves by using Atomic absorption spectrophotometer as shown in **Table 4.**

TABLE 4: HEAVY METALS RESIDUE ANALYSIS OF LEAVES OF *RAPHANUS SATIVUS*

S.no.	Heavy metals	Concentration
1.	Cadmimum(Cd)	0.22 ± 0.02
2.	Arsenic (As)	0.34 ± 0.12
3.	Mercury (Hg)	0.38 ± 0.05
4.	Lead (Pb)	0.35 ± 0.09

Determination of Aflatoxin residue: The detection of aflatoxin such as B1, B2, G1, G2 was carried out in the extract of *R. Sativus* leaves as shown in **Table 5.**

TABLE 5: AFLATOXIN RESIDUE ANALYSIS OF LEAVES OF *RAPHANUS SATIVUS*

S.no.	Parameters	Method	Results	MDL
1.	Aflatoxin B1	AOAC 990.332	Not detected	1.0µg/kg
2.	Aflatoxin B2	AOAC 990.332	Not detected	1.0µg/kg
3.	Aflatoxin G1	AOAC 990.332	Not detected	1.0µg/kg
4.	Aflatoxin G2	AOAC 990.332	Not detected	1.0µg/kg

Determination of Pesticide residue: According to AOAC guidelines pesticide residue was carried out in the extract of *R. Sativus* leaves as shown in **Table 6.**

TABLE 6: PESTICIDE RESIDUE ANALYSIS LEAVES OF *RAPHANUS SATIVUS*

Sno.	Pesticide	Test method	Results	MDL
1	α-BHC	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
2	β-BHC	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
3	γ-BHC	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
4	δ-BHC	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
5	α-Chlordane	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
6	β-Chlordane	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
7	Heptachlor	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
8	Heptachlor_Epoxide	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
9	α-Endoulfan	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
10	β-Endoulfan	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
11	Endrin	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
12	Endrin_Aldehyde	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
13	Total DDE	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
14	Total DDD	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
15	Total DDT	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
16	Alachlor	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
17	Butachlor	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
18	Monochlor	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
19	Malathoin	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
20	Methyl – parathion	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
21	Chlorpyrifos	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
22	Ethion	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
23	Diazinone	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
24	Phosphamidon	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
25	Simazine	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
26	Atrazine	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
27	Fenitrothion	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
28	Mevinphos	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
29	Dimethoate	AOAC790.52/EPA525.5	Not detected	0.01mg/kg
30	Phorate	AOAC790.52/EPA525.5	Not detected	0.01mg/kg

PHYTOCHEMICAL ANALYSIS: The presence and absence of various phytoconstituents to the preliminary chemical test of extracts is subjected as shown in **Table 7.**

TABLE 7: PHYTOCHEMICAL EVALUATION OF THE LEAVES EXTRACT OF *RAPHANUS SATIVUS*

S.no.	Constituents	Extracts Petrolium ether	Chloroform	Alcoholic	Aqueous
1.	Carbohydrate	-	-	-	+
2.	Phenolic compound	-	-	+	-
3.	Alkaloids	+	-	+	+
4.	Flavonoids	-	+	+	-
5.	Lipids	-	-	-	-
6.	Saponins	+	+	+	-
7.	Steroidas	-	-	+	+
8.	Amino acids	-	-	-	-
9.	Proteins	-	+	+	+
10.	Terpenoids	-	-	+	+

(-: Absent, +: Present)

Fluorescence Analysis: The air dried powder of the leaves was subjected in lights and UV light with different chemical reagents to be observed as shown in **Table 8.**

TABLE 8: FLUORESCENCE ANALYSIS OF POWDER OF LEAVES OF *RAPHANUS SATIVUS*

S.no	Reagent	Day light	UV light 254nm	UV light 366nm
1.	Powder such as	Green	Light green	Blue
2.	Powder treated with dist. water	Green	Dark green	Dark brown
3.	Powder treated with 1N NaOH	Dark green	Light green	Green
4.	Powder treated with HNO_3	Green	Dark brown	Blue
5.	Powder treated with H_2SO_4	Light brown	Dark green	Dark brown
6.	Powder treated with Iodine	Green	Dark brown	Blue
7.	Powder treated with conc. HCL	Dark green	Brown	Blue
8.	Powder treated with ammonia	Light green	Dark green	Brown
9.	Powder treated with ferric chloride	Dark green	Brown	Dark green
10.	Powder treated with picric acid	Light yellow	Yellow	Dark yellow
11.	Powder treated with pet. Ether	Dark green	Green	Dark brown
12.	Powder treated with chloroforrn	Dark green	Brown	Dark green

CONCLUSION: The present study is an attempt to develop the phamacognostic, physicochmemical and phtytochemical standards parameters to be used for identification, purity, quality and extracts of the leaves of *Raphanus sativus.* Clinical evaluation of these plants in human beings may be carried out for the above promising pharmacological activities.

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