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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *LINARIA RAMOSISSIMA* (WALL.) JANCH. (SCROPHULARIACEAE) STEM

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ABSTRACT
Linaria ramosissima (Wall.) Janch. (Scrophulariaceae) commonly known as 'Kanoti' & 'Bhintgilodi' is a reputed folklore drug. Literally 'Kanoti' means 'ear' representing ear shaped leaves of the plant. The drug is reputed for its anti diabetic properties and also having properties like Mutral, Rechak, Raktapittahar, Tika etc. The Review of the literature did not reveal much information regarding this plant, hence it was thought worth to investigate its pharmacognostical and phytochemical evaluation for its correct identity. Almost all the parts of plant possess medicinal properties but stem was first selected for its microscopical evaluation which includes the detail histological features involving transverse sections as well as powder microscopy both. priliminary phytochemical tests, florescence analysis & physicochemical parameters were investigated and besides this methanolic extract of the plant powder was also observed for Chromatographic evaluations. Important diagnostic characters like, striated cuticularised epidermis, simple & glandular trichomes, pericyclic fibres, pitted & spiral vessels etc. were observed while preliminary phytochemical & chromatographic study also reveals that it may be the constituents like saponin, flavonoid, alkaloid, tannin and other phenolic compounds are present. The finding of the study may be used as an useful key for identification and standardization of the drug.

KEYWORDS: *Linaria ramosissima* (Wall.) Janch., Scrophulariaceae, Kanoti, Pharmacognosy, Bhintgilodi.

INTRODUCATION

Linaria ramosissima (Wall.) Janch. (Scrophulariaceae), commonly known as Bhintgilodi in Gujarati, an important medicinal drug found in many parts of India[1-6], The herb grows on old abandoned walls of many ruined buildings during rainy season and found through out the year[1-6]. It is a perennial, slender, tomentose, prostrate herb. Leaves – ovate or sagitate – hastate measuring 1.2 – 4.5 cm x 0.6 – 2.5 cm,; flowers yellow, axillary, solitary or in terminal racemes; fruit, capsule about 0.4 – 0.6 x 0.2 – 0.4 cm, ovoid or sub globose seeds many minute[1-2]. The whole plant is considered to be clinically important and used as Rechak (purgative), Mutral (diuretic), Tikta (bitter), Raktapittahara (useful in bleeding disorders), etc[7] and pharmacologically it being claimed to having anti-diabetic property[1,3-7]. The pharmacognostical and phytochemical studies of stem, which is one of the important useful part of plant, has not been undertaken yet, therefore its detailed investigation in fresh as well as in powder form was carried out for identification point of view and to find out various medicinally important constituents from it.

MATERIAL AND METHODS

The plant was collected when it was in full bloom in rainy season, from the places located around the Jamnagar. After that, it was washed thoroughly with running water and then stem part were separated. and some of them were stored in FAA (Formalin90: Acetic acid 7: Alcohol 3) solution[8] for microscopic investigation and the remaining materials were dried under the shade. 40# powder was prepared and stored in well closed containers away from the light. Herbarium was also prepared and submitted to Pharmacognosy museum of I.P.G.T. & R.A., Jamnagar, vide Herbarium no. 6015, for future reference. Free hand sections were taken and observed as such to see their cell contents and then stained with phloroglucinol and hydrochloric acid to observe the lignifications of the cell wall.[9] Subsequently lignified elements were isolated by schultz's maceration process[9]. Sections and diagnostic characters of the powder were drawn by using camera lucida and their photographs were also taken separately. Powder drug treated with different reagents and were examined for fluorescence analysis under visible and UV light of long and short wavelengths for various color radiations.[10] preliminary qualitative tests were also performed to detect primary and secondary metabolites.[9] the powder was subjected to determine various physico-chemical constants by the standard procedures mentioned in API.[11] finally chromatographic method was also done for separation and confirmation of chemical compounds by using various solvents and spraying reagents by following standard procedures.[12]

RESULTS

MACROSCOPY: Stem of the plant is profusely branched, branches arising from the crown and up to 30 cm in length. Stem soft, cylindrical, measuring 0.75 – 1.2 cm in diameter, nodes somewhat swollen and prominent with distance of 3 – 4.5 cm; surface glabrous with few hairs on the upper side and tomentose on the lower side; fracture short; colour light greenish to dark green; odour not distinct; taste astringent and bitter.(fig no.1,2)

Microscopic Characters: Diagramatic TS of the stem is circular to oval in outline shows, a layer of epidermis bearing trichomes, which being plenty in the parts near the crown, but few on young branches, and centrally located pith encircled by a ring of stellar tissue, capped with well developed ring of pericyclic fibers along with narrow cortex.(fig no.3)

Detailed transverse section of the stem shows, a layer of epidermis bearing trichomes which gets easily detached and traversed with stomata at places mostly, covered with thick striated cuticle, mature parts consisting mostly glandular, multi cellular stalk and uni – bicellular cup shaped head and rarely simple, uni to multi cellular trichomes, underneath this, lies 3 – 5 rows of horizontally running, oval shaped chlorenchymatous cortex oftenly becomes parenchymatous too. Pericycle is characterized by single layered, discontinuous, thick walled fibres occasionally associated with isolated, reticulated parenchymatous cells at places, outside shielded with a distinct endodermis and inside with a narrow band of parenchymatous phloem and uni to bi seriate medullary rays in continuation with that of xylem which is composed of vessels, tracheids, parenchyma and fibres; pith is wide, parenchymatous but at places found pitted with beaded wall and lignified. (fig no.4)

POWDER

Organoleptic characters – Course, gritty, Pale greenish in colour with astringent, bitter taste and devoid of any odour.

The diagnostic characters of the powder shows various fragments of trichomes like glandular with multicellular stalk and uni – bi cellular, cup shaped head, simple, uni to multicellular; fragments of vessels with pitted and spiral thickening; isolated and in groups of thick walled, sclerenchymatous fibres; thin walled striated cuticularised epidermal cells in surface view with stomata and isolated parenchymatous cells with pitted thickening and beaded wall from the pith region.(fig no.5)

Isolation: Dimentions of the lignified elements of the vessels and fibres has been mentioned in table no.1 (Fig no.6)

PHYSICO-CHEMICAL PARAMETERS

Various physico chemical tests were performed as per the standard procedures mentioned in Ayurvedic Pharmacopoeia and their results were noted down. the moisture content was 6.20% w/w, total Ash 3.36% w/w, acid insoluble Ash 0.42% w/w, Alcohol soluble extractive 16.17% w/w while the water soluble extractive was found to be 17.67% w/w and pH value was 5.73.

FLUORESCENCE TEST

Powder drug dried in shade and treated with different reagents and were examined for fluorescence anlysis under visible and Ultra Violet light of long and short wavelengths. Various color radiations observed and results are shown in Table – 2.

PRELIMINARY QUALITATIVE ANALYSIS

Preliminary qualitative analysis for the presence of various functional groups was carried out on the methanol soluble extractive and the result is shown in Table – 3.

HPTLC STUDY

The methanolic extract was examined for HPTLC using the solvent system of Toluene: Ethyl Acetate: Glacial Acetic Acid in ratio of 5.5:4.5:0.2 & stationary phase – silica gel (Procoated Silica Gel TLC plates of merck). The developed plate was observed under 254 and 366 nm.Vanillin H_2SO_4 was used for derivatization and visualization, and then observed under day light with the conditions like, Camag Linomat V Application mode, Camag Twin trough Development Chamber, 30 min Chamber Saturation time, 30 min Development Time, Camag Scanner II,. Deuterium lamp & Tungstun lamp for Detection, and Win cats software Data System..The observations are tabulated in table- 4.The Chromatographic study revealed that the plant may contains saponins, flavanoids, alkaloid, steroids, tannin and phenols. (Fig no.7)

DISCUSSION

Pharmacognostical and Phytochemical evaluation of Stem of *Linaria ramosissima* (Wall.) Janch., provides scientific parameters that will be useful in assessment of identification and authentification of the drug. Simple and glandular trichomes, spiral and pitted vessels and lignified pitted parenchymatous cells serves as an important microscopic diagnostic characters and results of phytochemical & chromatographic study also reveals that it may be the constituents like like saponin, flavonoid, alkaloid, tannin and other phenolic compounds are present.

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TABLE –1 MEASUREMENT OF THE ISOLATED ELEMENTS FROM THE STEM

Stem	Length	Width
Fibres	374.76 µ - 1665.6 µ	13.88 µ - 55.52 µ
Xylem vessels		
a) Spiral vessels	208.2 µ - 485.8 µ	13.88 µ - 27.66 µ
b) pitted vessels	208.2 µ - 555.2 µ	13.88 µ - 55.52 µ

TABLE – 2 FLUOROSCENCE ANALYSIS

Material	Visible Light	UV Light	
		Short (254 nm)	Long (366 nm)
Aqeons extract	Light brown	Greenish brown	Green
Methanol extract	Light olive green	Dark olive green	Red
Chloroform extract	Olive green	Light yellowish olive green	Light red
Powder + Hcl	Dark brown	Light green	Greenish blue
Powder + H2SO4	Dark reddish brown	Light grey	Florescent blue
Powder + NaOH	Light brown	Greenish brawn	Dark brown

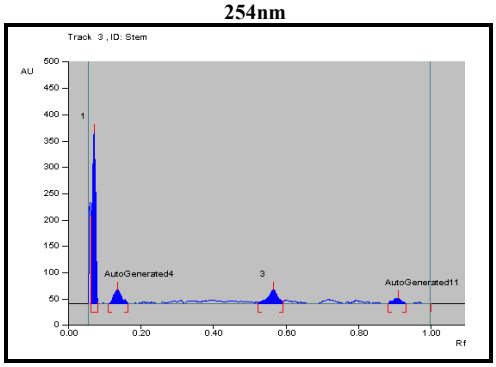
TABLE – 3 PRELIMINARY QUALITATIVE ANALYSIS

Material	Test/ Reagent	Functional gp	Observation	Result
Alcoholic extract of dried stem powder	Dragendorff's reagent	Alkaloids	Orange Brown ppt	+ve
	Wagner's reagent	Alkaloids	Reddish brown ppt	+ve
	5% fecl3	Tannin & Phenolic compd.	Deep blue black color	+ve
	Gelatin solution	Tannin & Phenolic compd.	White ppt	+ve
	Borntrager's test	Antnraquinone glycosides	No color change	-ve
	Biuret reagent	Protein	No color change	-ve
	Molisch's test	Carbohydrate	Violet ring was observed at the junction	+ve
	Fehling's test	Carbohydrate	First yellow, then brick red ppt observed	+ve
	Salkowoki	Steroids	Greenish yellow fluorescence	+ve
	Liebermann-buchard	Steroids	First red, then blue and finally green color appears	+ve
	Lead Acetate	Flavonoids	Yellow ppt	+ve
	Shaking in test-tube	Saponins	Frothing with honeycomb appearance	+ve

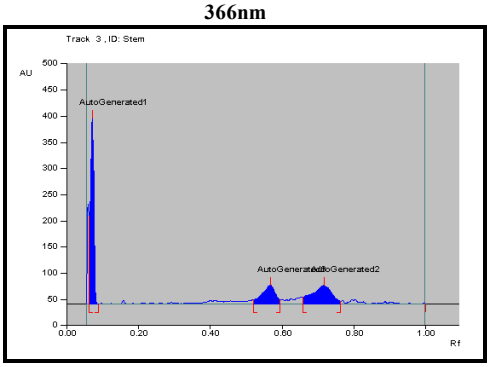
TABLE – 4 HPTLC ANALYSIS OF METHANOLIC EXTRACT

Condition	No. of Spots	Rf value
Short Uv (254 nm)	04	0.07
		0.14
		0.56
		0.91
Long UV (366 nm)	03	0.07
		0.57
		0.72
After spraying Vanillin H2SO4	05	0.05
		0.24
		0.31
		0.55
		0.83

254nm

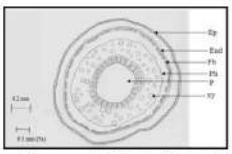


366nm



Natural habitat of Plant Fig no.01

Stem of the plant Fig no.02

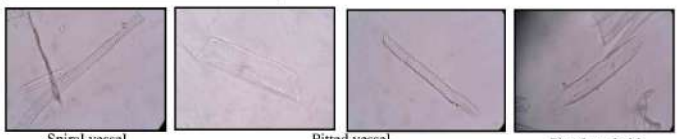


Diagrammatic Section Fig no. 03

Section photograph of Stem (Fig no.4)

Powder character of Stem (Fig no.05)

Isolated elements of the Stem (Fig no.06)

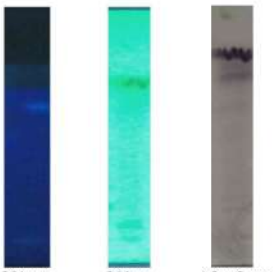


Fibres

Abbreviation:

Cu=Cuticle, Ep = Epidermis, End = Endodermis, Fb = Fibres, Ph = Phloem, Xy = Xylem, P=Pith

HPTLC PHOTOS



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