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PHARMACOGNOSTICAL STANDARDIZATION OF AERIAL PARTS OF *Rosmarinus officinalis* Var. Albus

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Key words: *Rosmarinus officinalis Albus*, Scalariform xylem vessels, Volatile oil, Flavonoids.

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ABSTRACT

***Plan**: Rosmarinus officinalis Albus is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white flowers grown in many parts of the world. It is widely used in the treatment of genitourinary conditions, liver diseases and scalp treatment.*

***Preface**: The present study was performed to highlight the micro-anatomical, physiochemical and phytochemical aspects of the plant.*

***Methodology:** The microscopical studies of leaf and stem were carried out separately. The chemo-microscopical studies were done using different staining reagents. Thin layer chromatographic analysis was carried out for the methanolic extract and compared with the standard rutin, quercetin and gallic acid.*

***Outcome**: The anatomy of the leaf, stem showed abundant number of branched covering trichomes, collapsed covering trichomes, Stellate, multicellular covering trichomes and fibres in leaf. Scalariform xylem vessels, pitted fibres were observed in stem. Fluorescence analysis showed wide range of fluorescence colours. Water soluble extractive values were found to be high which shows that most of the phyto-constituents may be polar soluble. The preliminary phytochemical chemical tests showed the presence of volatile oil, flavonoids, cardiac glycosides, phenolic compounds, steroids amino acids and proteins.*

1. INTRODUCTION

The aerial parts of *Rosmarinus officinalis* have good medicinal properties in traditional system of medicine. Phytochemical screening involves botanical identification, extraction with suitable solvents, purification and characterization of the active constituents. *Rosemarinus officinalis* Albus (garden rosemary, compass plant), belonging to the family Laminaceae.

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Rosemary is indigenous to south Europe and Asia. It is cultivated in Mediterranean basin and India. In folk medicine, it is used as an antispasmodic in renal colic and dysmenorrhea, in relieving respiratory disorders, and to stimulate growth of hair, rosemary extract also relaxes smooth muscles of the trachea, and intestine and it has anti-bacterial, hepato-protective and anti-tumorigenic activitie[1- 5]**.** Though there are lot of works conducted on rosemary in the western countries, no much emphasis has been given to the plant grown in Indian sub-continent. Hence with this intension we have performed the pharmacognostical standardization to evaluation the various parameters of aerial parts of Indian Rosemary.

Figure 1
Rosmarinus officinalis Albus

2. MATERIALS AND METHODS

2.1. Collection of plant materials

Rosmarinus officinalis Albus commonly known as Rosemary, it is indigenous to South Europe and Asia. It is cultivated in Mediterranean basin and India**.** Plant material was collected from the Central Institute of Medicinal and Aromatic plants, obtained from field station, Allalasandra, Bangalore 65, India, from the month of December to January. The plant material was identified and authenticated by Mr. Siddulu, Professor of botany department, Nagarjuna Government Degree College, Nalgonda, Andhra Pradesh. A herbarium was prepared and submitted for future references in the department of Pharmacognosy, under the voucher no: NCOP/Ph'cog/2011-2012/042.

2.1.1. Macroscopic and microscopic analysis [6, 8, 10]

The macroscopic characters such as colour, odour, taste, texture were studied for morphological investigation.

2.1.2. Micro anatomical study [6, 8, 10]

2.1.3. Transverse section (TS) of leaf and stem

Fresh leaf and stem of *Rosmarinus officinalis* Albus was used for this purpose. Free hand sectioning was performed to obtain a thin transverse section of leaf and stem, and microphotographs were taken for identification and arrangement of cells and tissues.

Longitudinal section (LS) of stem [6, 8, 10]

Fresh stem of *Rosmarinus officinalis* Albus was sectioned longitudinally and studied.

2.1.4. Powder microscopy [7]

Fresh aerial parts of *R.officinalis* were cleaned and then dried in the shade. It was then powdered separately with the help of electric grinder to a coarse powder. This was subjected to powder microscopy as per the standard procedures mentioned.

The powder was stained with different chemical reagents like phloroglucinol: HCl (1:1), iodine and safranin and finally observed under microscope to study the various cell contents.

2.1.5. Microscopical measurements [7, 10, 13]

The microscopic measurements like length of trichomes and length and width of fibres were performed as per standard reference books.

2.2. Chemo microscopy:

The fresh leaves and stem were taken and thin sections were cut with help of potato using a razor blade. Phloroglucinol and HCl, toluidine blue, Sudan III, safranin and rhodamine were used as staining reagents. The slides were neatly prepared and focused under a microscope to study the various cell contents and cell wall thickenings. [10, 11]

2.3. Fluorescence analysis [6 -12]

Powdered drug was treated with different chemical reagents and then observed under short wavelength, long wavelength and day light.

2.4. Proximate analysis [10]

The ash values, extractive values, loss on drying and fluorescence analysis were performed according to the officinal methods as described in Indian Pharmacopoeia and the WHO guidelines on quality control methods for medicinal plant materials.

2.5. Extraction

About 100g of the whole aerial parts of the plant was packed in thimble then defatted with petroleum ether and then extracted with methanol for 34hrs at 30˚C.The thick mass was evaporated with the help of rotary vacuum evaporator and percentage yield was calculated.

2.6. Phytochemical screening [8, 10]

The preliminary chemical tests for both extracts were carried out according to the standard procedure.

2.7. Thin layer chromatography (TLC) [14]

Methanolic extract of aerial parts of *Rosmarinus officinalis* Albus were subjected to TLC.

2.7.1. TLC for flavonoids

Adsorbent:	Pre-coated Silica Gel $60F_{254}$
Solvent system:	Toluene: Dioxane: Ethyl acetate (75.6: 21: 3.3)
	Toluene: acetic acid (60:20)
	Chloroform: acetone: formic acid (75: 16.5: 8.5)

Spraying reagent: 2% Aluminium chloride, Visualization: UV Chamber 360 nm.

3. RESULTS

3.1. Macroscopy

Leaves were leathery, opposite, with prominent midribs. The margins were entire and strongly revolute. Upper surface was green coloured, lower surface grey and woolly due to numerous trichomes. The stems were hard and woody in nature (Figure 1). The upper surface was brown colour and inner surface buff coloured**.** Flowers were white in colour with two stamens projecting far beyond the corolla. Stamens were with anther and style with stigma.

3.2. Microscopy

3.2.1. T.S of Rosmarinus officinalis Albus leaf

The transverse section of the leaf showed dorsiventral nature. The leaf was divided into lamina and midrib region (Figure 2).

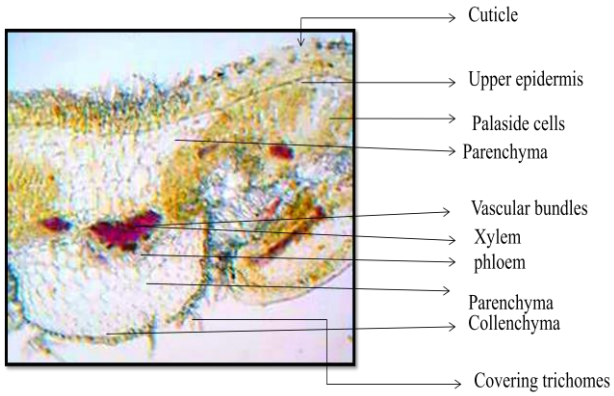

Figure 2: T.S of leaf stained with phloroglucinol & HCl acid (1:1)

3.2.2. Lamina region:

The T.S of the leaf was divided into two sections, the lamina and the midrib portion.

3.2.2.1. Lamina: It consists of a thin waxy cuticle present on the upper surface of the leaf. Below the cuticle a single layer of rectangular epidermal cells were observed. Abundant covering trichomes were observed which were identified to be stellate trichomes. The covering trichomes were branched uniseriate, multi-cellular (3- 4 celled) with sharp apex. Below the epidermis mesophyll parenchyma were observed which consists of palisade cells that were oblong cells near the upper epidermis. They were arranged perpendicular to the epidermal cells. These cells contain chlorophyll which help in photosynthesis. Next to this were the spongy mesophyll cells which contained the vascular elements. The spongy mesophyll cells were loosely packed, with few chloroplasts.

3.2.2.2. Midrib region

The cuticle and epidermal cells were observed in the midrib region too. But the palisade cells were found to discontinue in the midrib region. Below the epidermal cells 2-3 layers of collenchyma cells were observed. Below to this was found to be the loosely arranged parenchyma cells. Towards the central region arc shaped vascular bundles which consists of xylem in the central region phloem beneath to this was observed. Below the vascular bundle region 5-6 layers of loosely arranged parenchyma was seen. Strip of collenchyma cells of 1-2 layers above the lower epidermis was observed. Stellate covering trichomes were seen in the lower epidermis (Figure 2).

3.2.3. Transverse and longitudinal section of Stem

The TS of young stem was divided into epidermis, cortex and pith, whereas in mature stem the epidermis was replaced by the cork region (Figure 3).

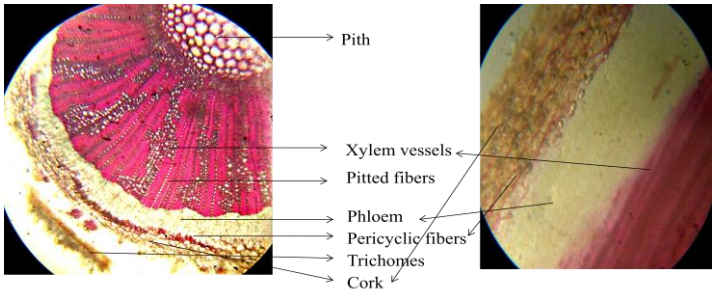

Figure 3: TS and LS of old stem

3.2.3.1. Epidermis:

It was outermost layer of the young stem and was formed of single layer of cells. The outer surface of the epidermis was covered by a thin layer of cuticle in younger stem and cuticle was absent in older branches of stem. Abundant number of covering trichomes were observed, the covering trichomes were uniseriate, multi-cellular branched (3- 4 celled) with pointed apex. The cork cells were observed in the older stems which were found to be polygonal in shape with brown colour. Trichomes were found to be absent in the older stems.

3.2.3.2. Cortex:

Cortex was made of loosely arranged parenchyma cells with patches of sclerenchyma cells, pericyclic fibres and vascular bundles. The pericyclic fibres were lignified and were found to be in 2 to 3 layers. Vascular bundles were present beneath the pericycle. Phloem was observed to be towards the outer region. Xylem region was found beneath the phloem. The xylem vessels and xylem fibres were seen. In the younger stems the vascular bundle region was observed to be narrow, whereas in the older stem the xylem fibres had taken up almost the major portion in the stem. Xylem vessels were found to be narrow and less in number (Figure 14). The fibres were observed to be lignified. A layer of endodermis was observed towards the central region below the vascular bundles.

The section of the stem before subjecting to staining techniques appeared pale green to yellow in colour (Figure 12, 13).

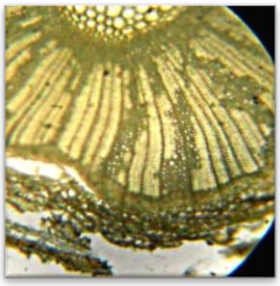

Figure 12:T.S of stem without staining

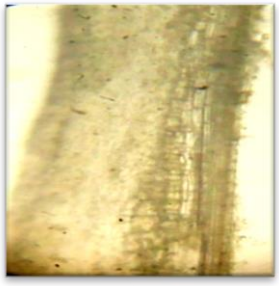

Figure 13: L.S of stem without staining

T.S and L.S of stem when treated with phloroglucinol and HCL stained xylem vessels pink colour due to the presence of lignin. TS and LS of stem stained with toluidine blue the lignified xylem vessels were stained bluish green colour and whereas the cutin and suberin containing cells were stained blue (Figure 14).

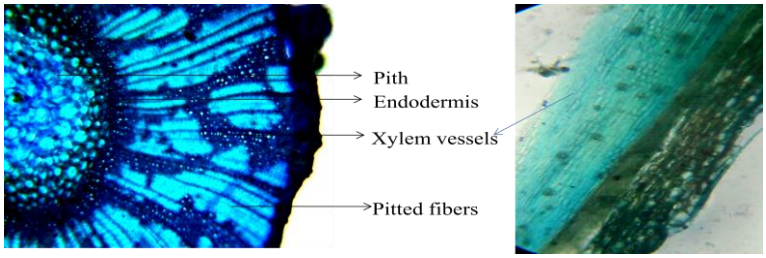

Figure 14: Transverse section and L.S of stem treated with toludine blue

TS and LS of stem treated with Sudan III imparts pink colour to the epidermal cells and trichomes indicating the presence of volatile oil (Figure 15). When the section was stained with rhodamine, increased fluorescence of scalariform xylem vessels were observed (Figure 16).

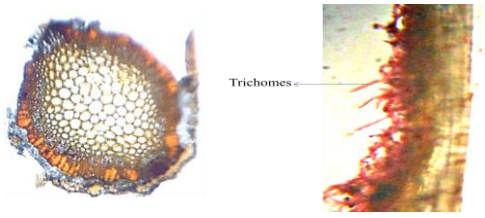

Figure 15: T.S and L.S of young stem treated with SudanIII

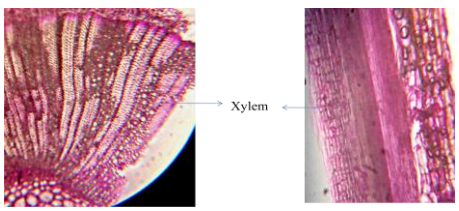

Figure 16: T.S and L.S of stem treated with Rhodamine

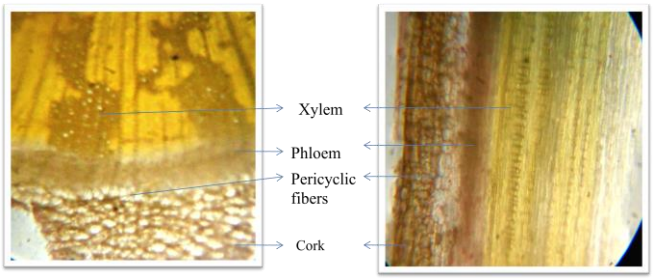

Figure 17: T.S of stem treated Schulze's reagent

TS and LS of stem treated with Schulze's solution (Chlor-zinc-iodide) stained lignin yellow, cutin and suberin yellow to brown, whereas no bluish coloured stained was observed which confirmed that starch was totally absent (Figure 17). The TS of stem when stained with safranin the lignin present in the phloem region showed faint pink colour and the cellulose were observed as blue colour (Figure 18).

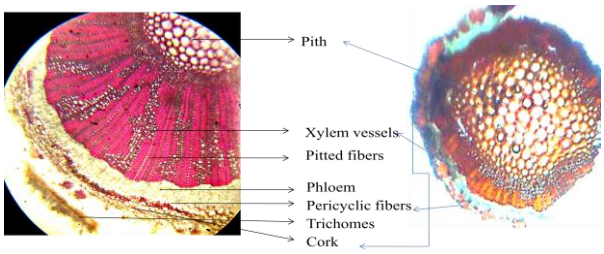

Figure 18: T.S of old and young stem treated with safranin

Pith was large in the young stems and had taken up the major portion in the section. It was made of thin walled, big polygonal cells with intercellular spaces. In the case of older stem the pith was found to be small since major portion of the stem was occupied by the xylem fibres (Figure 15).

3.2.4. Powder microscopic analysis of leaf

Powder microscopy of leaf revealed the presence of trichomes and fibres (Figure 4). Trichomes were found long slender with pointed apex. They were uniseriate, unicellular covering trichome. This was identified as the main tissue of diagnostic importance. It also consists of collapsed covering trichomes, branched trichomes, multicellular trichomes (2-3 celled) and stellate trichomes. Fibres present in leaf powder were long and less in number.

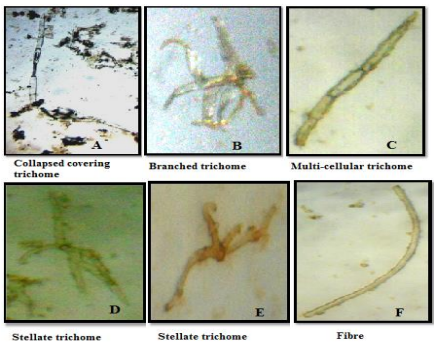

Figure 4: Powder microscopy of leaf

3.2.4.1. Powder microscopic analysis of stem:

The powder of stem revealed the presence of brownish coloured polygonal shaped cork cells (Figure 5). They were widely scattered in the slide. Abundant lignified xylem vessels with pitted walls were observed. Long lignified fibres in groups as well as isolated were observed in the view.

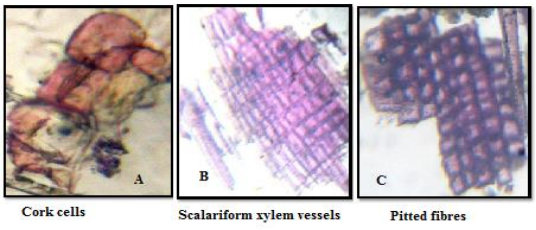

Figure 5: Powder microscopy of stem

3.3. Chemomicroscopy

Transverse section of the leaf was stained with various chemicals and reagents to study the cell wall thickenings and cell contents present in the leaf of *Rosmarinus officinalis*. When the leaf section was treated with Phloroglucinol and HCL, the lignified cells were stained pink in colour (Figure 6).

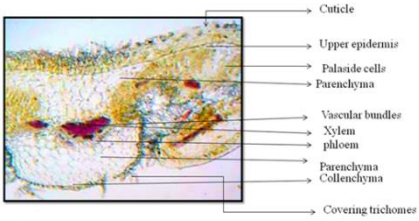

Figure 6: T.S of leaf treated with phloroglucinol and HCl

When leaf section was stained with Sudan III the epidermal cells and trichomes stained pink in colour, due to the presence of volatile oil (Figure 7). TS of the leaf treated with Rhodamine, the cells with volatile principles were clearly observed with pink fluorescence whereas the collenchyma cells were seen as blue fluorescence (Figure 8). Section stained with Schulze's solution exhibited lignin in yellow colour, cutin and suberin as yellow to brown (Figure 9). When the TS of the leaf were stained with safranin the lignified cells were stained maroon- pink, whereas the volatile oil containing cells were stained orange-pink (Figure 10). The TS of the leaf when stained with toluidine blue which is a polychromatic stain imparted lignin containing cells blue to bluish-green (Figure 11).

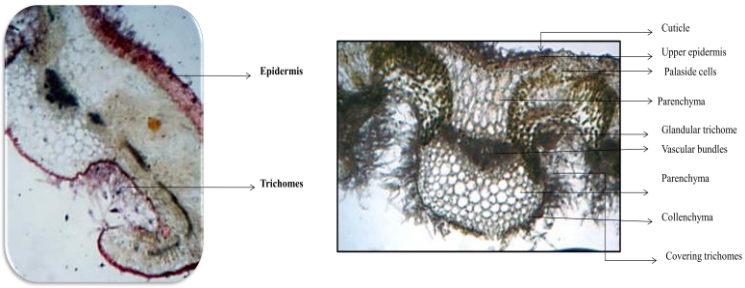

Figure 7: T.S of Leaf stained with Sudan III & without staining.

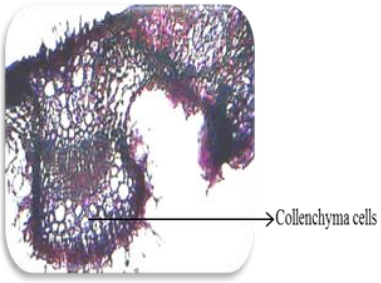

Figure 8: T.S stained with rhodamine

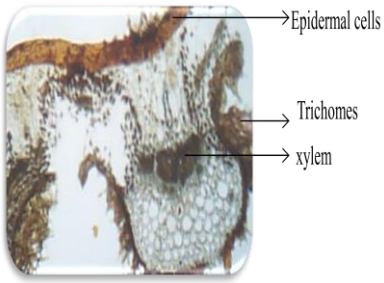

Figure 9: T.S stained with Schulz's solution

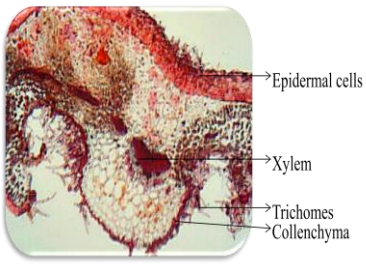

Figure10: T.S stained with safranin

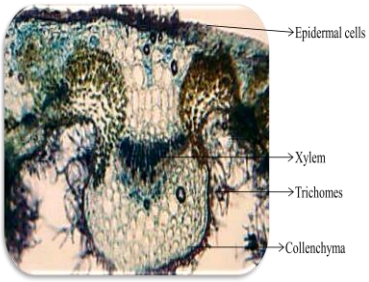

Figure 11: T.S stained with toluidine

In fibres it was observed that toluidine blue O showed the lignin first developing in the primary wall by colouring it a clear blue-green, then, as lignification proceeded, the blue-green staining progressed into the middle lamella and finally the secondary wall. When leaf and stem stained with toludine blue shows presence of suberin, starch, cutin, lignin and cellulose in the cell walls.

3.4. Organoleptic evaluation of Rosmarinus officinalis Albus leaf powder

- Color -- Green
- Odour -- Aromatic and camphoracious
- Taste -- Slightly spicy
- Fracture -- Uneven

3.4.1. Organoleptic evaluation of Rosmarinus officinalis Albus stem powder

- Color -- Buff
- Odour -- Aromatic
- Taste -- Slightly Bitter
- Fracture -- Uneven, Flaky

3.4.2. Organoleptic evaluation of Rosmarinus officinalis Albus flower

- Color -- White
- Odour -- Aromatic

3.5. Microscopic measurements

Measurements of length and width of fibres and trichomes were performed. Minimum, average and maximum length of trichomes were found to be 17.5µ-431.625µ-733.56 µ, respectively. The minimum, average and maximum width of trichomes were found to 1µ-29.7µ-55,23 µ, respectively. Minimum, average and maximum length of fibre was determined to be 16µ-426µ-987.22 µ, respectively. Minimum, average and maximum width of fibre was observed to be 1µ- 25.5µ- 73.36 µ, respectively.

3.6. Fluorescence analysis

The fluorescence analysis revealed wide range of fluorescence colours. In comparison it was observed that leaf shows mostly green colour when it was treated with the different reagents and stem showed buff colour. The fluorescence colours observed for leaf and stem powder are tabulated in table 1and 2**.**

3.7. Proximate analysis

Ash Values such as (Total ash, Acid insoluble ash, Water soluble ash, sulphated ash) water soluble extractive, alcohol soluble extractives, Loss on Drying (Moisture content) and crude fibre content were determined by standard method and the results were tabulated (Table 3).

Successive solvent extracts

Colour, Consistency and yield of successive extracts of aerial parts of *Rosmarinus officinalis* Albus (Table 4).

3.8. Phytochemical screening

The powdered crude drug after defatting with petroleum ether was extracted with methanol and water. These extracts were subjected to phytochemical screening for determining the presence of phytoconstituents. The methanolic extract exhibited the presence of proteins, amino acids, fats and oils, steroids, glycosides, phenolic compounds, flavonoids and volatile oil (Table 5).

3.8.1. Thin layer chromatographic analysis (TLC)

TLC of extract was compared with standard drugs quercetin, rutin and gallic acid. The results obtained are tabulated in table 6. After derivatization with aluminium chloride solution the fluorescent quenching of the spots were observed (Figure 19, 20, 21).

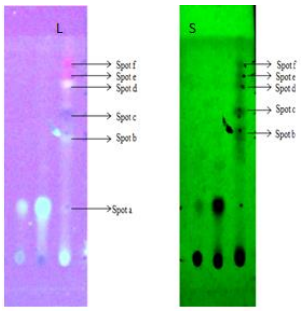

Figure 19: TLC of flavonoids solvent system- Toluene: Dioxane: Ethyl acetate (75.6:21:3.3)

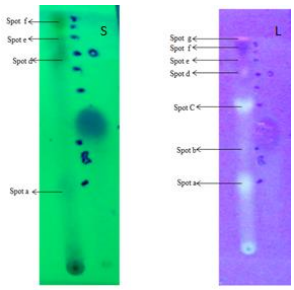

Figure 20: TLC of flavonoids solvent system-Toluene: acetic acid (40:20)

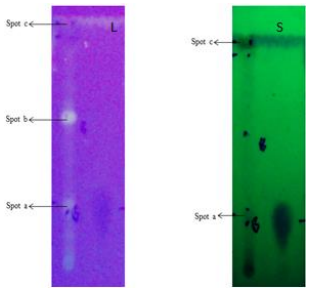

Figure 21: TLC of flavonoids in the solvent system- Chloroform: acetone: formic acid (75: 16.5:8.5)

3.9. Fluorescence analysis of leaf and stem powder

The fluorescence analysis of leaf and stem of the plant showed different fluorescent colours. The leaf powder showed different shades of green colour whereas the stem powder showed buff to black colour (Table 7 and 8).

4. DISCUSSION

The quantitative determination of pharmacognostical parameters is useful for setting standards for crude drugs. Identification of plant drugs by pharmacognostic studies is more reliable. According to the World Health Organization (WHO, 1998), the macroscopic and microscopic description of a medicinal plant is 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. Macroscopic studies play an important role for primary identification of drugs[15]. The leaves of *Rosmarinus officinalis* were needle shaped with abundant trichomes, green in colour and aromatic. The young stems were pale green in colour and aromatic, whereas the older stems were buff coloured, hard and fibrous in nature.

Microscopic studies or a structural detail helps the secondary identification of drugs. The microscopical studies of the leaf showed the presence of non-glandular trichomes and glandular capitate trichomes, stellate trichomes, unicellular trichomes, branched trichomes, multicellular trichomes and covering trichome with bulbous base and pointed apex which can be considered as the tissue of diagnostic importance in the leaf.

Capitate trichome consists of a basal cell, a short mono-or bicellular stalk and unicellular secretory head which secrete volatile oil. These trichomes can be correlated with morphological characteristics since they were in abundance.

It was observed that peltate trichomes were absent in this variety which shows the difference among plants of different varieties. The collenchymatic cells were observed towards the lower epidermal cells. The main tissue of diagnostic importance of the stem was found to be scalariform xylem vessels. The pitted fibres were found in the stem. The microscopy of stem also showed presence of covering trichomes which consists of volatile oil. The chemo-microscopical study is application of chemical tests to small quantities of its histological section which helps for the study of different constituents of drugs. This study gives chemical composition of the cell walls of the tissue in the plant. When leaf and stem stained with phloroglucinol and HCl, it shows the presence of lignin in xylem, when it is stained with Sudan III the entire trichomes were stained pink which shows the presence of volatile oils in the trichomes. When stained with safranin all lignified and cutinized tissues turned red and chloroplasts pink. Schulze's solution (Chlor-zinc-iodide) stained lignin yellow, cutin and suberin yellow or brown. Absence of starch grains were observed as no blue-violet stain was observed. Polychromatic stain toluidine blue O stained lignin containing cells blue to blue-green. Rhodamine fluorescence quenching of cells were observed in UV long wavelength mainly in the epidermal region. The microscopical measurements set a limit and range for identification of authenticity that can be present in the plant specimen. Measurements of the length of the trichomes in the leaf and stem were performed. Test for ash values was performed to determine quality and purity of a crude drug.

Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes inorganic variables like calcium oxalate, silica, carbonate content of crude drug affects 'Total ash value'. Such variables were removed by treating with acid and then acid insoluble ash value was determined.

Extractive values useful for the evaluation of a crude drug which gives an idea about the nature of the chemical constituents present in a crude drug and also useful for the estimation of specific constituents, soluble in that particular solvent used for extraction. In this plant water extractive values were more when compared with the methanolic extractive values.

Fluorescence studies of the leaf and stem gave a wide range of colour changes at day light, UV-chamber (256nm and 365nm). These colour changes reflect the nature of the chemical components present in the pant parts when exposed to the respective chemical reagent[16]. Hence, this parameter is very important technique for the proper identification of the plant species. The powder of the different parts of the plant was subjected to the preliminary chemical tests showed the presence of various constituents like alkaloids, flavonoids, cardiac glycosides, tannins, phenolic compounds, resin and volatile oils. Hence the methanol was selected as the suitable solvent for the major extraction. The information obtained from preliminary phytochemical screening will be useful in finding out the authenticity of the drug.

Chromatography is strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and therefore be used for authentication and identification of the herbal products. In general, the methods for quality control of herbal medicines involve macroscopic and microscopic examinations and analytical inspection using instrumental techniques such as thin layer chromatography. Aluminium chloride was the reagent for unsaturated 1, 2- and 1, 3-dihydroxyketones.

Aluminium chloride forms fluorescent complexes with flavonoids[17]. The colours of the fluorescing zones can depend on the concentration of the aluminium chloride solution. The TLC analysis of flavonoids showed fluorescent quenching after spraying with the aluminium chloride.

TLC analysis of aerial parts showed spots for flavonoids and the Rf values were compared with that of standard quercetin and rutin which confirms that the plant contain these compounds or their derivatives.

Table 1: Fluorescence Analysis Leaf Powder of *Rosmarinus officinalis* Albus

Leaf powder treated with different chemical reagents	*Day light*	*Short wavelength*	*Long wavelength*
Conc.HNO_3	Yellow	Green	Dark green
Conc.H_2SO_4	Light green	Light green	Green
Conc.HCl	Yellow	Green	Dark green
Methanol	Green	Dark green	Yellow
Chloroform	Light green	Green	Brown
Petroleum ether	Brown	Light green	Black
Glacial acetic acid	Yellowish orange	Green	Yellow
Glacial acetic acid + Conc.HNO_3	Yellow	Green	Greenish black
5%$CuSO_4$	Light green	Light green	Green

Leaf powder treated with different chemical reagents	*Day light*	*Short wavelength*	*Long wavelength*
5%$CuSO_4$	Light green	Light green	Green
Dragendorffs reagent	Yellowish green	Green	Blackish brown
Wagner's reagent	Light green	Green	Greenish black
Mayer's reagent	Light green	Green	Greenish brown
Benedicts reagent	Light green	Light green	Green
Picric acid	Yellow	Greenish yellow	Blackish brown
Chloroform	Light green	Green	Yellowish brown
$FeCl_3$	Light green	Green	Black
Dil.HCL	Light brown	Light green	Black
Dil.HNO_3	Brown	Light green	Black
Dil.H_2SO_4	Brown	Light green	Black
Cold water	Brown	Light green	Black
10%NaOH	Light brown	Light green	Black
Ninhydrin	Light brown	Light green	Black
10%NaOH+Conc.HNO_3	Brown	Light green	Black

Table 2: Fluorescence analysis of stem of *Rosmarinus officinalis* Albus

Stem powder treated with different chemical reagents	*Day light*	*Short wavelength*	*Long wavelength*
Conc.HNO_3	Brown	Green	Black
Conc.H_2SO_4	Black	Black	Black
Conc.HCl	Yellow	Green	Black
Methanol	Buff	Light green	Yellowish brown
Chloroform	Buff	Light green	Yellowish brown
Petroleum ether	Buff	Light green	Yellow
Glacial acetic acid	Buff	Light Green	Black
Glacial acetic acid + Conc.HNO_3	Brown	Light Green	Black
5%$CuSO_4$	Buff	Light green	Black
Dragendorffs reagent	Brown	Green	Black
Wagners reagent	Buff	Light Green	Black
Mayers reagent	Buff	Light Green	Black
Benedicts reagent	Buff	Light green	Black
Picric acid	Buff	Light green	Black
Chloroform	Buff	Light Green	Yellowish brown
$FeCl_3$	Yellow	Light Green	Black
Dil.HCl	Buff	Light green	Black
Dil.HNO_3	Buff	Light green	Black
Dil.H_2SO_4	Buff	Light green	Black
Cold water	Buff	Light green	Black
10%NaOH	Buff	Light green	Black
Ninhydrin	Buff	Light green	Black
10%NaOH+Conc.HNO_3	Buff	Light green	Black

Table 3: Proximate analysis of *Rosmarinus officinalis* Albus

S.No.	*Parameters*	*Values % (w/w)*
	Ash values	
1	Total ash	8.35%w/w
2	Acid insoluble ash	3.45%w/w
3	Water soluble ash	16.5%w/w
4	Sulphated ash	21%w/w
5	***Loss on drying***	98.66%w/w
6	***Crude fiber content***	30.93%w/w
7	***Extractive values***	
8	Water soluble extractives	17.6%w/w
9	Alcohol soluble extractives	0.12%w/w

Table 4: Successive solvent extracts of *Rosmarinus officinalis* Albus

Extracts	*Aerial parts of rosemary extract*				
	Colour			*Consistency*	*Yield %w/w*
	Long wave length	Short wave length	Day light		
Petroleum ether	Black	Dark brown	Dark green	Sticky	3.6
Methanol	Dark green	Green	Green	Powdery	3.57
Water	Dark green	Green	Green	Powdery	4.93

Table 5: Phytochemical screening of *Rosmarinus officinalis* Albus

Chemical constituents	*Methanol extract*	*Aqueous extract*
Carbohydrates	–	–
Proteins	+	+
Amino acids	+	–
Fats and oils	+	+
Steroids	+	+
Glycosides	+	–
Alkaloids	–	–
Flavanoids	+	+
Volatile oil	+	+

Table 6: TLC profile of *Rosmarinus officinalis* Albus.

Solvent system	*Long wavelength*		*Short wavelength*	
	Spot colour	*Rf*	*Spot color*	*Rf*
Toluene: Dioxane: Ethyl acetate (7.56: 2.1: 0.33)	Blue fluorescence	0.258	Brown fluorescence	0.689
	Brown fluorescence	0.689	Brown	0.724
	Brown	0.724	Yellow	0.810
	Yellow	0.810	Light pink	0.931
	Light pink	0.931	Orange	0.982
	Orange	0.982		
Standard (Rutin and quercetin)	Blue fluorescence	0.258	Brown	0.258
Toluene : acetic acid (4:2)	Blue fluorescence	0.285	Blue fluorescence	0.285
	Light blue fluorescence	0.532	Yellow	0.753
	Blue fluorescence	0.688	Light brown	0.935
	Yellow	0.753	Brown	0.974
	Light pink	0.935	Orange	1
	Brown	0.974	Purple (gallic acid)	0.580
Standard (Gallic acid)	Orange	1		
	Blue- purple	0.580		
Chloroform: acetone: formic acid (7.5: 1.65: 0.85)	Blue fluorescence	0.272	Brown	0.272
	Blue fluorescence	0.581	Pink	0.981
Standard (gallic acid)	Light pink	0.981	Brown	0.272
	Purple	0.272		

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