Physico-Chemical Screening of *Rosmarinus officinalis* L

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Abstract

This study evaluated the physico-chemical properties of *Rosmarinus officinalis* L. (rosemary) leaves. The leaves were collected, authenticated, and subjected to morphological, microscopical, and proximate analysis. The proximate values, including moisture content (10.12%), total ash (8.21%), water soluble extractive (9.1%), and alcohol soluble extractive (17.32%), were within pharmacopeial standards. Hydro-alcoholic extract of rosemary was prepared using 70% alcohol by soxhlation, yielding 7.12%. Phytochemical analysis revealed the presence of phenols, saponins, fixed oils, flavonoids, terpenes, and di and triterpenes. Quantification of phyto-constituents showed that rosemary contained 16.5% polyphenol, 26% flavonoids, and 0.96% sterol contents, indicating its potential as a rich source of flavonoid.

Keywords: *Rosmarinus officinalis* L, Soxhlation, glandular trichomes.

Introduction

Rosmarinus officinalis L also known as rosemary (Fam. Lamiaceae) is a herb native to the Mediterranean region, but it now cultivated all over the world[1]. This study focuses on evaluating the physico-chemical properties of rosemary leaves, including morphological, microscopical, and proximate analysis, as well as phytochemical investigation and quantification of phyto-constituents.

Rosmarinus officinalis L is mainly used as carminative, stimulant, diuretic, aperient, astringent, cholagogue, digestive, diuretic, emmenagogue and hypertensive [2,3] . The plant has been investigated for antimicrobial, antioxidant, insecticidal, hepatoprotective, anti-tumorogenic, Anti-inflammatory, Antinociceptive activities [4-20] . Extracts of R.officinalis L, contain flavonoids, phenols, volatile oil and terpenoids. Carnosol and carnosic acid are constituents of deodorized rosemary extract [21-23] .

In the present study we have evaluated Physico chemical properties of leaves of *Rosmarinus officinalis* L .

Materials and Methods

I.COLLECTION AND AUTHENTICATION OF SAMPLES

Dried leaves of *Rosmarinus officinalis* L. was used for the study. Rosemary was collected from Amruth Kesari.The sample drug has been identified and Authenticated by Dr. Shiddamalaya N, NADRI (National Ayurveda Dietetics Research Institute) Bangalore. The drug was air dried in shade and stored in polythene bags.

II PHARMACOGNOSTIC EVALUATION

The leaves of *Rosmarinus officinalis* L was subjected to morphological and microscopical examination. Proximate values like moisture content, total ash value, alcohol soluble extractive values and water soluble extractive values were determined.

Morphology

The leaves part was subjected to macroscopical identification based on colour, odour, taste and shape of the drug. Morphological observations are recorded in **Table no 1**

Microscopy:

The sample was subjected to powder microscopy.

i. Small quantity of powder of sample was taken in watch glass. To this a few drops of chloral hydrate was added and heated for 2-3 minutes. Small amount of clarified drug was mounted on a slide with a drop of glycerin, covered with cover slip and observed under microscope for presence of Calcium oxalate crystals.

ii. To visualize lignified tissues, 1:1 ratio of phloroglucinol: concentrated Hydrochloric acid was added to the clarified mixture. After 2-3 minutes, small amount of drug was mounted on a slide with a drop of glycerin, covered with cover slip and observed under microscope.

iii.To visualize starch grains, dilute Iodine solution was added to the clarified mixture. After 2-3 minutes, small amount of drug was mounted on a slide with a drop of glycerin, covered with cover slip and observed under microscope. The photographs of Rosemary shown in Photo no:

III Proximate analysis:

Proximate values such as moisture content, total ash value, alcohol soluble extractive values and water soluble extractive values were determined for the powdered drugs.

The results are represented in **Table no-2**

a: Moisture Content

Moisture content of the powder drug samples was determined by loss on drying method described in Ayurvedic pharmacopoeia of India.

Procedure: 5g of the drug powder were taken in previously weighed petri plate and kept in the oven at 105 ^{0}C for 5 hours. The Petri plate was then cooled in a desiccator and weighed. The difference in weight was taken. The drying and weighing was continued till, after 30 minute of heating and cooling a constant weight was obtained.

b: Determination of Ash values

The total ash was determined for air dried sample using procedure described in quality control methods for medicinal plant materials.

Total Ash Values Principle:

When vegetable drugs are incinerated, they leave an inorganic ash in some plants. The total ash is of importance and indicates to some extent the amount of care taken in the preparation of the drug. It is indicative of inorganic constituents and contaminants such as siliceous matter. While determining the total ash value carbon must removed at as low a temperature (450 0 C) as possible because alkali chlorides, which may be volatile at higher temperatures, would otherwise be lost. If carbon is still present after heating at a moderate temperature, the water-soluble ash may be separated and the residue ignited again, or the ash may

be broken up, with the addition of alcohol, and ignited. The total ash usually contains carbonates, phosphates, silicates and silica.

Procedure:

2g of the ground air-dried sample powder was weighed into previously ignited, tarred silica crucible cooled in a desiccator. The material was spread evenly as a thin layer.It was ignited slowly to obtain a carbonized residue and placed in the muffle furnace and the temperature was adjusted to 450-500° C and allowed to ignite until it was white, indicating the absence of carbon. Crucibles were removed from muffle furnace, allowed to cool for 30 minutes in a desiccator and weighed without delay.

Total ash in mg per g of air dried material was calculated as shown below and results are expressed as % Ash.

Calculation:

Total Ash % = (B-C) x100/A Where, A-Sample weight in (g) B- Weight of dish + contents after drying (g) C- Weight of empty dish (g)

c: Extractive values

The % of alcohol soluble extractive value and % of water soluble extractive value were determined for the powdered drug using procedure described in Ayurvedic pharmacopoeia of India.

(i): Alcohol soluble extractive value

Procedure: 5g of the air-dried coarsely powdered drug was macerated with 100ml of alcohol in a glass stoppered flask for 24 hours, shaking the contents frequently during the first 6 hours and allowed to stand for 18 hours. Filtered rapidly taking precautions against loss of alcohol. 25ml of the filtrate was evaporated to dryness on a water bath in a tarred china dish; Dried at 105° C for 1 hour in a hot air oven, cooled in a desiccator and weighed. The process was repeated till the constant weight wasobtained. The percentage of alcohol-soluble extractive with reference to the air-drieddrug was calculated.

Calculation: Alcohol soluble extractive value % = (B – A) x 4 x 100 / W

Where, A- weight of the empty dish (g) B-Weight of dish + residue (g) W-Weight of plant material taken (g)

(ii): Water soluble extractive value

Procedure: 5gm of powdered drug was treated with 100ml of water at 80°C in a stoppered flask with frequent shaking during first 6 hrs and allowed to stand for 18 hrs. It was filtered rapidly after 24 hrs taking precaution against loss of solvent. 25ml of the filtrate was evaporated in a tared dish at 105°C, cooled in dessicator for 30 minutes and weighed. The process was repeated till a constant weight was obtained. The percentage of Water-soluble extractive with reference to the air-dried drug was calculated.

Calculation: Water soluble extractive value % = (B – A) x 4 x 100 / W Where, A-weight of the empty dish (g) B-Weight of dish + residue (g) W-Weight of plant material taken (g) Results of alcohol soluble and water soluble extractive values are given in **Table No 2**

Graphical representation of Moisture content, total ash, water soluble extractive and alcohol soluble extractive values of stem part of *Rosmarinus officinalis* L is shown in Graph No 1

IV. PREPARATION OF EXTRACTS BY SOXHLATION:

The leaves *of Rosmarinus officinalis* L. was ground using mixer grinder and powdered to mesh # 20 for Rosemary leaves. The powdered drugs were then extracted by soxhlation. The extract prepared were used for phyto-chemical study.

Soxhlation method:

Soxhlation, also called as hot percolation is a method of extraction where the plant material packed as a thimble in the soxhlet extractor is completely flushed with fresh solvent formed by boiling the solvent containing the extracted analytes. The extraction is continued until sufficient compound is extracted. The method is suitable for heat stable substances and for constituents which are less soluble in the menstrum in the absence of heat. Soxhlation is simple and cheap; Solvent use is limited and gives good and selective extraction. However Analytes must be sufficiently soluble without stirring and/ or slow heating.

Procedure:

Soxhlet extraction was performed using classical soxhlet extraction apparatus. 10g of the sample to be extracted was placed in a thimble and 40ml of 70% alcohol was taken in round bottom flask attached to thimble. Condenser was placed above the thimble. Extraction was performed for 8hrs with 70% alcohol. Finally extracts were evaporated to dryness using vacuum or rotary evaporator. The residues were weighed and the percentage yields were calculated and results are tabulated in **Table No:3**

V PHYTOCHEMICAL ANALYSIS

V.A: Qualitative Chemical tests

The hydro alcoholic extract of *R.officinalis* L was subjected to various chemical tests to detect the chemical constituents present in them. Test methods are described below.

Detection of Alkaloids

Extracts were dissolved individually in dilute HCl and filtered. The filtrates were used to test for the presence of alkaloids.

a) Mayer's Test

Filtrate was treated with Mayer's reagent (potassium mercuric iodide). Formation of a cream colored precipitate indicated the presence of alkaloids.

b) Wagner's Test

Filtrate was treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/ reddish brown precipitate indicated the presence of alkaloids.

c) Dragendroff's Test

Filtrate was treated with Dragendroff's reagent (solution of potassium bismuth iodide). Formation of red precipitate indicated the presence of alkaloids.

d) Hager's Test

Filtrate was treated with Hager's reagent (saturated picric acid solution). Formation of yellow colored precipitate indicated the presence of alkaloids.

Detection of Carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

a) Molisch's Test

Filtrate was treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2 ml of conc sulphuric acid was added carefully along the side of the test tube. Formation of violet ring at the junction indicated the presence of carbohydrates.

b) Benedict's Test

Filtrate was treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicated the presence of reducing sugars.

c) Fehling's Test:

Filtrate was hydrolyzed with diluted HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicated the presence of reducing sugars.

d) Barfoed's Test

Extracts heated with Barfoed's reagent. (Copper acetate in water and glacial acetate). Formation of red colour indicated the presence of reducing sugar.

Detection of Glycosides

Extracts were hydrolysed with dil. HCl and then subjected to test for glycosides.

a) Modified Borntrager's Test

Hydrolysed extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and shaken with an equal volume of benzene. The benzene layer was separated and treated with ammonia solution.

Formation of rose-pink colour in the ammoniacal layer indicated the presence of anthranol glycosides.

b) Legal's Test :Hydrolysed extract was treated with sodium nitroprusside in

pyridine and methanolic alkali. Formation of pink to blood red colour indicated the presence of cardiac glycosides.

c) Liebermann Burchard' s Test

Hydrolysed extract was extracted with chloroform, to chloroform extract a few drops of acetic anhydride was added boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicated the presence of sterol aglycone.

Detection of Saponins

a) Foam Test

Extracts were diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min. Formation of 1 cm layer of foam indicated the presence of saponins.

Detection of Triterpenes and Phytosterols

a) Salkowski's Test

Extracts were dissolved in chloroform, chloroform solution was treated with a few drops of Concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicated the presence of triterpenes and steroids.

b) Libermann Burchard's Test

Extracts were dissolved in chloroform. To the chloroform solution few drops of acetic anhydride, was added boiled and cooled. Concentrated Sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicated the presence of phytosterols.

Detection of Fixed Oils and Fats

a) Stain Test

Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicated the presence of fixed oils and fats.

Detection of Resins

a) Acetone-water Test

Extracts were dissolved in acetone and filtered. Small amount of water was added to acetone solution and shaken. Appearance of turbidity indicated the presence of resins.

Detection of Phenols

a) Ferric chloride Test

Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols.

Detection of Flavonoids

a) Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids.

b) Lead acetate Test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicated the presence of flavonoids.

c) Shinoda Test To the alcoholic solution of extracts, a few fragments of magnesium ribbon and Conc.HCl were added. Appearance of magenta colour after few minutes indicated the presence of flavonoids.

Detection of proteins and amino acids

a) Xanthoproteic Test

The extracts were treated with few drops of Concentrated Nitric acid solution. Formation of yellow colour indicated the presence of proteins.

b) Ninhydrin Test

To the extract, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicated the presence of amino acid.

c) Biuret Test: The extracts were treated with 1 ml of 10% sodium hydroxide solution and heated. To this a drop of 0.7% copper sulphate solution was added. Formation of purplish violet colour indicated the presence of proteins.

Detection of Diterpenes and Triterpenoids

a) Copper acetate Test

Extracts were treated with few drops of copper acetate solution. Formation of emerald green color indicated the presence of diterpenes.

b) Tshugajen Test

Extracts were treated with chloroform and filtered. Excess of acetyl chloride and a pinch of zinc chloride was added, kept aside for some time till the reaction was complete and then warmed on water bath. Appearance of eosin red colour indicates the presence of triterpenes. Results of the chemical constituents of the extracts are tabulated in **Table no: 5**

V.B: Quantification of phytochemical constituents:

Qualitataive chemical test of extracts of R.officinalis L showed the presence of flavonoids, polyphenols and triterpenoids and sterols as the chief constituents. The extract obtained by soxhlation method were evaluated for content of different phyto-chemicals like flavonoids, polyphenols and sterols.

B.1. Estimation of total Flavonoid:

The principle of aluminum chloride colorimetric method for determination of flavonoids is based on the fact that aluminum chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the ortho- dihydroxyl groups in the A or B ring of flavonoids. Flavonols with C-3 and C-5 hydroxyl groups, such as quercetin form complexes with aluminum chloride, showing maximum absorbance at 415-440 nm. Generally the aluminum chloride complexes of compounds with more functional groups absorb more strongly at 415 nm and show the absorption maximum at longer wavelength.

Materials:

Quercetin hydrate: purity 95%, Acros organics, Belgium.

AlCl 3 : purity 98.0%, Finar chemicals limited Reagents, Ahmedabad.

Potassium acetate: purity 99.1%, Loba chemie, Mumbai.

Methanol: AR Grade, purity 99.8%, Himedia, Mumbai.

Acetic acid: purity 99.5%, SDFCL (s.d fine chemical limited), Mumbai.

Reagents

AlCl 3 10% → 10 g of AlCl 3 dissolved in 100ml distilled water.

Potassium acetate 1M → 9.82 g of potassium acetate in 90 ml distilled water adjust

PH 7.5 with 2M acetic acid.

Standard solution (A): 10 mg of Quercetin was dissolved in100 ml methanol.

Sample solution (B): 200mg of each extract was dissolved in 10 ml methanol.

Procedure

0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml of standard solution (A) were taken separately into test tubes and diluted suitably with methanol to get 2.0 ml. To each of these tubes 0.1ml of 10% AlCl 3 solution; 0.1 ml of 1M potassium acetate solution was added, followed by 2.8 ml distilled water, the contents were mixed. All tubes were allowed to stand at room temperature for 30 min. Similarly 2ml of each of the sample solution (B) was taken in place of standard solution. To this 0.1 ml of 10% AlCl 3 solution; 0.1 ml of 1M potassium acetate solution and 2.8 ml of distilled water were added. The contents mixed and allowed to stand at room temperature for 30 min. A blank determination with 2 ml of methanol instead of sample/standard treated similarly was maintained. Absorbance of the reaction mixture was measured against reagent blank at 415 nm using UV (Schimadzu Double Beam) spectrophotometer.

The results, expressed as Flavonoid content of each of the extract, was calculated as mg % of quercetin using the standard calibration curve.

The standard calibration curve is shown in **Graph no 2**; The Flavonoid content is recorded in **Table no: 5**

B.2. Estimation of polyphenol content:

Polyphenols: Phenolic compounds are secondary constituents distributed widely in leaves, seeds, bark and flowers of the plants offering protection against UV rays and pathogens. These phenolics are also important constituents of human diet as they are found in fruits, vegetables as well as tea and wine. There are a variety of spectrophotometric and colorimetric methods which can be used for, quantification of various phenolic groups. The Folin-Ciocalteau colorimetric method described by Ghasemi et.al. was used for determination of total polyphenol content.

Principle for total polyphenol content determination by Folin-Ciocalteau method: This method is based on the reducing power of phenolic hydroxyl groups which detects all the phenols with varying sensitivities. The intensity of the blue complex can be estimatied with a spectrophotometer (λmax at 765 nm).

Materials:

Gallic acid: Sigma, Germany.

Methanol: AR Grade, purity 99.8%, Himedia, Mumbai.

Phenol reagent (Folin & Ciocalteau): SDFCL, Mumbai.

Sodium carbonate: purity 99.5%, Reachem laboratory chemical limited, Chennai.

Reagents: Sodium carbonate (1M): 28.615g of sodium carbonate in sufficient water to produce 100ml.

Folin-Ciocalteau reagent: 1 ml Folin reagent diluted in 10 ml distilled water.

Methanol : water (50:50v/v): 50 ml methanol in 50 ml water.

Standard solution (A): 500mg Gallic acid was dissolved 100ml Methanol: Water.

Sample solution (B): 500 mg of each extract was dissolved in 10 ml (Methanol:
water).

Procedure for determination of poly phenols by Folin-Ciocalteau method:

0.1, 0.2. 0.3, 0.5, 1 ml of standard solution (A) were taken separately into test tubes. To each of these tubes 5 ml of (1:10 diluted with distilled water) Folin-Ciocalteau reagent was added allowed to stand for 5 mins, 4 ml of 1M aqueous Na 2 C0 3 was added. The solution was made upto 10 ml with methanol: water (50:50v/v) the contents were mixed. All tubes were allowed to stand for 15 min. Similarly 0.5 ml of each of the sample solution (B) was taken in place of standard solution. To this 5 ml of (1:10 diluted with distilled water) Folin-Ciocalteau reagent was added and allowed to stand for 5 mins, 4 ml of 1M aqueous Na2C03 was added. The solution was made up to 10 ml with methanol: water (50:50v/v).The contents were mixed. All tubes were allowed to stand for 15 min. A blank determination with 1 ml methanol: water (50:50v/v) instead of sample/standards treated similarly was maintained. Absorbance of the reaction mixture was measured against reagent blank at 765 nm using UV Schimadzu Double Beam spectrophotometer. The polyphenol content of each of the extract was calculated as mg % of Gallic acid using the standard calibration curve.

The standard calibration curve is shown in Graph no: 3; the polyphenol content is
recorded in **Table no:5**

B.3. Estimation of total sterol content:

Sterols from drug powder was extracted by mixing with an alcohol-ether mixture and the solvent evaporated carefully using a water bath. The residue obtained thereby was dissolved in chloroform and subjected to the Liberman-Burchard reaction, which involves addition of sulphuric acid in the presence of acetic anhydride that gives a green chromophore. The Liberman-Burchard reaction with cholesterol gives much more color in chloroform than in glacial acetic acid. However, the reaction in chloroform is more sensitive to effect of such analytical variables as time, temperature etc, hence that more careful technical control is necessary. The spectrometric characteristics of the color in chloroform are similar to those of the color in acetic acid except the peak absorption in the red end of the spectrum is at 660nm instead of the 620-625nm range. The color intensity slowly increases and is also a function of

the extent of exposure to light. As the color is sensitive to bleaching by light, the color is allowed to develop for a known period of time in dark area and photometric measurements made as rapidly as possible (to avoid prolonged exposure to the light beam in photometer.)

Materials:

Standard cholesterol solution:

Alcohol-ether mixer

Acetic acid: purity 99.5%, SDFCL (s.d fine chemical limited), Mumbai.

Acetic anhydride

Sulphuric acid: LR Grade, purity 98% SDFCL (s.d fine chemical limited), Mumbai

Reagents:

Liberman- Burchard reagent: 0.5 ml of sulfuric acid dissolved in 10 ml of acetic anhydride. Covered and kept in ice bath.

Alcohol-ether mixer: 1:3 ratios (1ml of alcohol and 3ml of ether)

Standard cholesterol: 100mg of cholesterol dissolved in 100ml of chloroform.

Sample preparation: 10 mg extract dissolved in 10ml of alcohol-ether mixture.

Procedure:

0.1, 0.2, 0.3, 0.4 and 0.5ml of standard solution (A) were taken separately into test tubes and each test tube volume made up to 5 ml of acetic acid. Finally the volume was made up to 10ml with acetic anhydride-sulphuric acid reagent (Liberman- Burchard reagent). Similarly 5ml of the sample solution (B) was taken in place of standard solution and volume is made up to 10ml with acetic anhydride- sulphuric acid reagent. Then all the test tubes were kept in dark for 30min. Absorbance of the reaction mixture was measured against reagent blank at 660 nm using UV (Schimadzu Double Beam) spectrophotometer. The results, expressed as sterol content of each of the extract, was calculated as mg % of cholesterol using the standard calibration curve.

The standard calibration curve is shown in **Graph no 4**; The cholesterol content is recorded in **Table no: 5**

RESULTS

COLLECTION AND AUTHENTICATION OF SAMPLES

Dried leaves of *Rosmarinus officinalis* L used for the study was collected from Amruth Kesari The sample drug was identified and Authenticated by Dr. Shiddamalaya N, Ref no: Drug Authentication/SMPU/ NADRI/ BNG/ 2011-2012/ 681 Bangalore, Karnataka.

***Rosmarinus Officinalis* L**

PHARMACOGNOSTIC EVALUATION

Morphology

The aerial parts of samples were subjected to macroscopic identification based on colour, shape, size, odour, taste and features of the drug. Morphological Details of the drugs are shown in **Table no: 1**

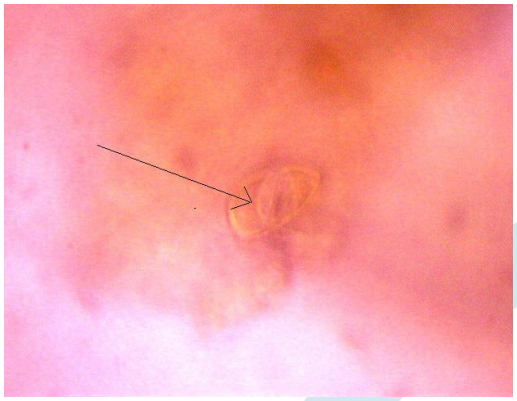
Characteristics	***Rosmarinus Officinalis* L.Leaves**
Colour	Green on dorsal surface and white on vertical surface.
Shape	leaves similar to pine needles
Size	2-4 cm (0.8-1.6 in) long and 2-5 mm broad
Extra Features	The leaves are sessile, tough, linear to linear-lanceolate and have wide recovered edges.
Taste	Bitter and astringent

Table 1: Morphological features of leaves of *R.Officinalis* L

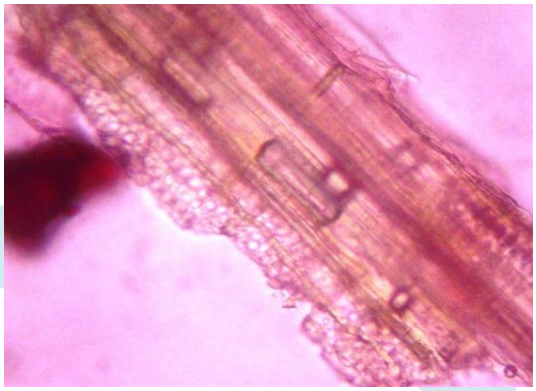
Microscopic analysis:

The sample was subjected to powder microscopy. Microscopic features were observed and compared with standard description in Indian Herbal Pharmacopeia. The microscopic characters for rosemary are; Diacytic stomata, hypodermal cells, Group of fibres, covering trichomes and glandular trichomes

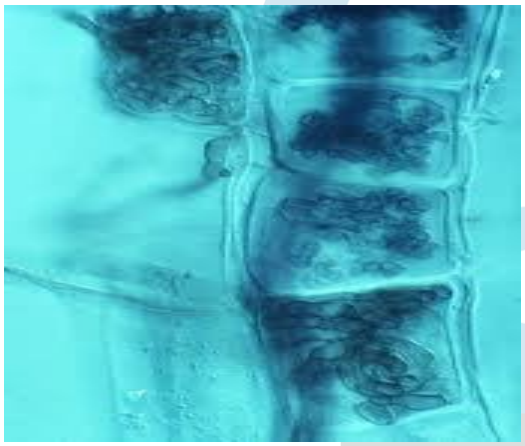
Photo no : 2 Photographs of the Microscopic characters for Rosemary powder



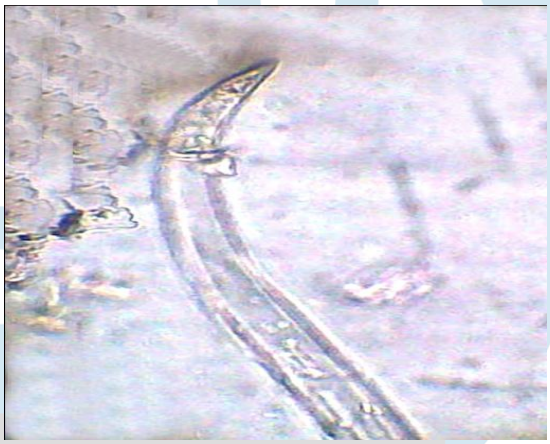
Stomata



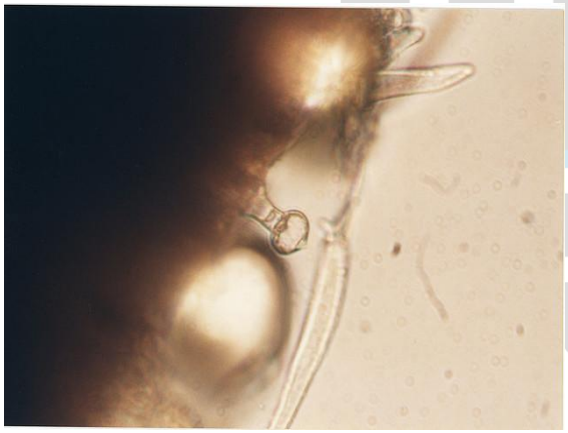
Group of fibres



Hypodermal cells



Covering trichomes



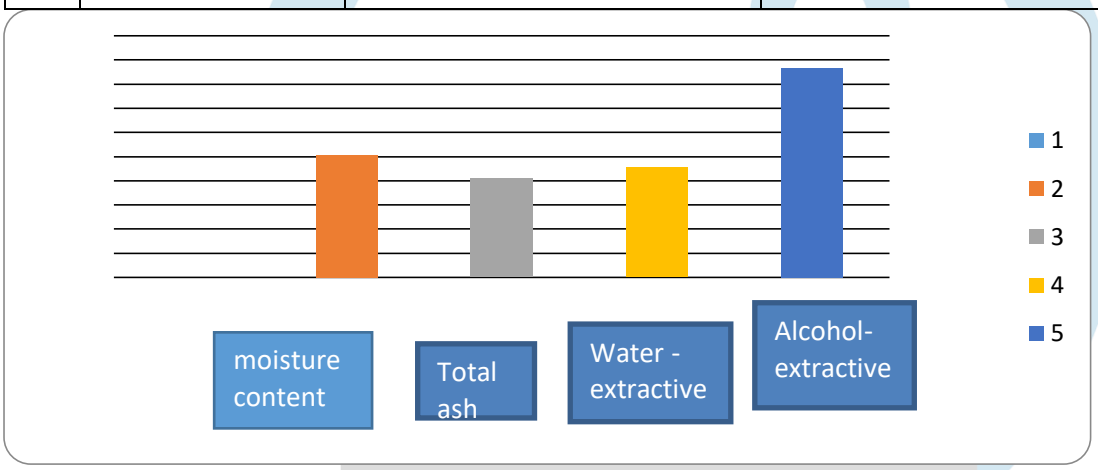
Glandular trichomes

Proximate analysis

The moisture content, total ash, water soluble extractive and ethanol soluble extractive values of the drugs are tabulated in **table no 2**

Table no: 2 Proximate values of *R.officinalis* L

SI No	Parameters	Values obtained w/w on dry Wt basis *of R.officinalis*	standards as per API[68] and BP[69] *R.officinalis*
1	Moisture content	10.12%	NMT-12%
2	Total ash	8.21%	NMT-9%
3	Water soluble extractive	9.1%	NMT-10%
4	Alcohol soluble extractive	17.32%	NLT-15%



Graph 1: Graphical representation of proximate values of leaves of *R. officinalis* L

PREPARATION OF THE EXTRACTS BY SOXHLETION METHOD

The extracts of *Rosmarinus officinalis* L soxhletion and evoparated using rotary evaporator. The % yield of the extract was calculated and results are shown in **Table 3.**

Table 4: Data showing percentage extraction yield of *Rosmarinus Officinalis* L from Soxhlet extraction

SI No.	SOXHLETION METHOD	% YIELD OF EXTRACTS
1	*Rosmarinus Officinalis* L	7.12%

PHYTOCHEMICAL ANALYSIS

Qualitative chemical test:

Prepared extract was subjected to qualitative chemical tests in order to find out chemical constituents present. Results are tabulated in **Table: 4**

The aqueous alcoholic extract of *R. Officinalis* prepared by soxhlet extraction method showed the presence of Phenols, saponins, fixed oils, flavonoids, terpenes and di and triterpenes.

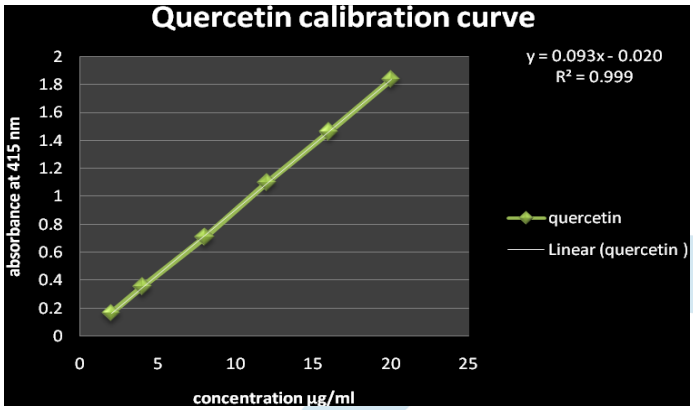
Chemical constituents	Tests	ROSEMARY
Alkaloids	Meyers test	-ve
	Dragendroff's test	-ve
	Wagner's test	-ve
	Hager's test	-ve
Carbohydrates	Molisch's test	-ve
	Benedict's test	-ve
	Fehling's test	-ve
	Barfoed's test	-ve
Glycosides	Modified Borntragers test	-ve
	Legal's test	-ve
	Liberman buchard's test	+ve
Saponin	Foam test	+ve
Fixed Oils And Fats	Stain test	+ve
Resins	Acetone-water test	-ve
Phenols	Ferric chloride test	+ve
Flavonoids	Alkaline reagents	+ve
	Lead acetate	+ve
	Shinado test	+ve
Protein And Amino Acid	Xanthoproteic test	-ve
	Ninhydrin test	-ve
	Biuret test	-ve
Diterpenes And Triterpenoids	Copper acetate	+ve
	Tshugajen test	-ve
Triterpenes And Phytosterols	Salkowski's test	+ve
	Liberman buchard's test	+ve

Table:4 Qualitative Chemical Tests Of The Hydro alcoholic extract of *Rosemarinus officinalis* L

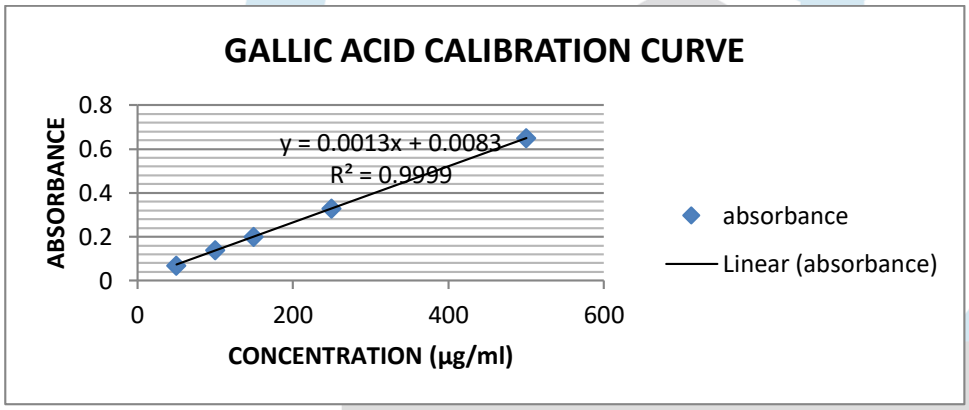
Note: + ve Indicates presence of phytoconstituents; whereas – ve Indicates absence of phytoconstituents.

Determination of Phyto-constituents in the extracts

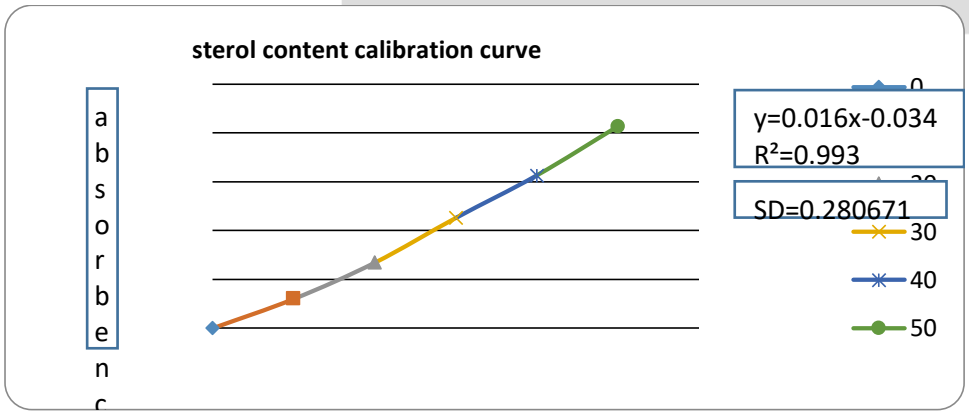
The total phenol content, total flavonoid content and Total sterol content in hydroalcoholic extracts of *R. Officinalis* L was determined by colorimetric method previously reported. Results are tabulated in **Table: 5**



Graph no 2: The calibration curve with standard Quercetin for the determination of flavonoids by $AlCl_3$ colourimetry method



Graph no 3: The calibration curve of gallic acid for determination of polyphenol by Folin Ciocalteau method.



Graph no 4: The calibration curve of Cholesterol for determination of sterol content using Liberman-Burchard reagent.

Table 5: Content of total phenols, total flavonoid and total sterol content in hydro alcoholic extract of *R. Officinalis* L

Extract	Total flavonoids % as quercetin	Total phenols % as gallic acid	Total sterols %
Rosmarinus Officinalis L	26	16.5	0.96

DISCUSSION

Rosemary was procured from Amrut kesari depot a dealer in medicinal herb. The drug was air dried in shade powdered to # size 20. Pharmacognostic studies of the drug showed that both drugs confirmed to microscopic and morphological description from standard texts and monograph [24] . Proximate analysis of the drug shows moderate amounts of water (9.1%) and alcoholic soluble extractive (17.3%). The proximate values were within limits from standard monographs [25,26]

As drug yielded high extractive with water as well as alcohol. The hydro-alcoholic extract was used for evaluation of phytoconstituents. Besides alcohol is a good solvent for extraction of most of the phyto constituents: hydro-alcoholic extract of rosemary was prepared using 70% alcohol by soxhlation for 8 hrs. The yield of extractive was however less by soxhlation as compared to the extractive values obtained by overnight maceration as done for proximate values determination. Lesser duration of extraction may have affected the yield of extract. The values reported in published reports however are contractory to each other [17,27.] The season of collection, drying conditions, drug solvent ratio, time of extraction, temperature and method of extraction effect the yield of the extractive to any solvent. Phytochemical studies of the two drugs showed that Rosemary contains phenols, saponins, fixed oils, flavanoids, terpenes and sterols

Quantification of phytoconstituents viz: polyphenols, flavonoids and sterols contents in the hydro-alcoholic extract was done by reported colorimetric methods. Rosemary extract showed high flavanoid content (26%w/w) and a polyphenol content (16.5%w/w). Flavonoid content of amla was 8%. Both drugs showed low quantity of sterols.The polyphenol content of rosemary was determined here is higher than earlier reports [28,29] . Flavonoid and sterol content however are not reported for the drug. We have found rosemary extract was rich in flavonoids.

CONCLUSION

Rosemary powder was prepared and subjected for morphology, microscopy, proximate analysis and phytochemical investigation. Extraction of R.officinalis was done by soxhlation. Rosemary yielded 7.12% . Phytochemical studies showed presence of phenols, fixed oils, flavonoids and terpenes in R.officinalis. Quantification of phyto-constiuents showed that Rosemary contained 16.5% polyphenol, 26% flavanoids and 0.96% sterol contents.

SUMMARY

The present study aimed at evaluation of leaves of *Rosmarinus officinalis* L for Physical and phytochemical investigation.

Rosmarinus officinalis L leaves was obtained from commercial supplier – AmrutKesari depot, Bangalore. The identity of the drug was ascertained by its morphological and Pharmacognostic evaluation; and authentication was done by Dr. Siddamalaya N, NADRI, Bangalore.

Rosmarinus officinalis leaves powder # 20 was prepared and subjected for Morphology, Microscopy(stomata, fibres, hypodermal cells and covering trichomes) and proximate analysis like Moisture content, total ash, water soluble extractive value and alcohol soluble extractive value. All the parameters are within the pharmacopeial standards.

Alcoholic extracts of *Rosmarinus officinalis* leaves powder was prepared by soxhletion. The yield of the both extracts is determined. The *R.Officinalis* Yield was 7.12%.The optimized extract of *R.Officinalis* was subjected to phytochemical analysis. The Rosmary extract showed the presence of Phenols, saponins, fixed oils, flavonoids, terpenes and di and triterpenes. To correlate the biological activity of the extract and to check for quantitative differences of different extracts with respect to different constituents, the total flavonoid, total phenol and total sterol content was determined. An aluminium chloride colorimetric method was used for determining total flavonoid content, colorimetric method for determination of total phenol content and the Liberman-Burchard reaction method was used for determination of total sterol content. Rosemary extract showed a polyphenol content of 16.5%; flavonoid content of 26% and sterol content of 0.96%.

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