4.1. Plant material

The bark of *S. racemosa* was procured from Ramnivas Darak & bros; Mansh; Dist: Neemuch (M.P.) Gujarat, India in dried form August 2012. It was identified and authenticated by Dr. Hariom N. Gupta; Head Of Department, Dravya Guna Department, Government Ayurved College, Vadodara by perceiving various pharmacognostical and physicochemical parameters. Voucher specimen (PHPL/HB/060) has been deposited at Pharmanza Herbal Pvt. Ltd., Dist. Anand, Gujarat, India. Dried bark powdered and passed through 60# sieve and packed in air tight container for the study.

4.2. Chemicals and equipment

4.2.1. Equipment

Instruments used in the study include microscope (M. Shah Trading Co., India), Hot air oven (D. K. Scientific, India), Muffle furnace (Lab line Stock Instruments, India) Digital automatic Karl-Fischer titrator (Lab India Analytical Inst. Pvt. Ltd. India), spray dryer (Lab Plant, UK) and UV Spectrophotometer (Shimadzu Corporation, Japan).

4.2.2. Chemicals

The Folin-Ciocalteau reagent was procured from Chemdyescorporation, India. Gallic acid was procured from Suvidhinath Laboratories, India. Karl-Fischer reagent (K/F) and methanol (K/F grade) were obtained from S. D. Fine Chem., India. Ash less filter papers (No. 41) were obtained from Sigma Aldrich, India. Other chemicals, solvents and reagents used were of analytical grade. The herbal extracts were prepared at Pharmanza Herbal Pvt. Ltd. Dist. Anand.

4.3. Pharmacognostical studies

4.3.1. Macroscopic studies

The bark species were subjected to macroscopic studies, which comprised of organoleptic characters of the drugs like color, odour, appearance, taste, smell, texture, fracture etc. according to the reported methods (Rajpal, 2005).

4.3.2. Microscopic studies

After collection, samples were immediately fixed in 70% alcohol for histochemical studies. Thin sections of bark (12 to 14 mm) were taken. Sections were stained with phloroglucinol and hydrochloric acid for two to three min. They were observed for the presence of lignified tissue (Khandelwal, 2006).

4.4. Preparation of extracts

4.4.1. Hot extraction method

The dried bark material of plant was pulverized and the powdered material (250 g) was extracted separately with methanol, ethanol and water using hot extraction method. After removing the biomass residues by filtration, pooled extracts were concentrated on rotary vacuum evaporator. The extracts were further dried using oven at 80°C except water extract. The water extract was dried using spray dryer (at inlet temperature: 168 ± 2°C, outlet temperature: 107 ± 3°C, blow Speed: 12 units and air Pressure: 0.6 kg/cm^2). Finally all dried extracts were weighed and yields were calculated which was shown in Table 4.1.

4.4.2. Successive solvent extraction method

The dried bark material (250 g) was extracted successively using soxhlet apparatus with hexane, chloroform, ethyl acetate, n-butanol at 40°C for 6 h and water using hot extraction method at 80°C for 6 h. After removing the biomass residues by filtration, pooled extracts were concentrated on rotary vacuum evaporator. The extracts were further dried using oven at 60°C except water extract. The water extract was dried using spray dryer (Table 4.1).

4.4.3. Cold maceration method

The dried bark material (250 g) was extracted using cold maceration method with extracting solvent (3×500 mL) at room temperature for 48 h by refreshing the solvent at the end of 48h. After removing the biomass residues by filtration, methanol extract was concentrated on rotary vacuum evaporator and water extract was dried using spray dryer (Table 4.1).

4.4.4. Fractionation of methanol extract

Methanol extract prepared by using hot extraction method was fractionated with n-hexane, chloroform, ethyl acetate, n-butanol and water. The fractions were dried and residue was used for the further study (Table 4.1).

4.5. Physicochemical evaluation

4.5.1. Foreign matter determination

WHO specifies that medicinal plant material should be free from any hazardous or toxic foreign matter and as far as possible free from innocuous foreign matter. Foreign matter was determined as per Pharmacopoeial requirement (Anonymous, 2001).

Dried bark powder of *S. racemosa* (Approximately 250g) was spreaded out ina thin layer over white sheet of paper and inspected with the unaided eye and separated the foreign matter by hand as complete as possible. The dried powder was weighed and percentage of foreign organic matter was determined from the weight of the powder taken (Table 4.2).

4.5.2. Determination of ash values

4.5.2.1. Total ash

Total ash method is designed to measure the total material remaining after ignition. This indicates both "physiological" ash which is derived from the plant tissue itself and "non-physiological" which the residue of extraneous matter (e.g. sand and soil) is adhering to the plant surface.

Two gm of accurately weighed bark powder was incinerated in a platinum crucible at a temperature not exceeding 450 ºC until free from carbon, cooled and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on an ashless filter paper, incinerated, along with filter paper, evaporated to dryness and ignite data temperature not exceeding 450ºC. The ash thus obtained was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug (Anonymous, 2001).

4.5.2.2. Acid insoluble ash

Acid insoluble ash measures the amount of silica present, especially as sand and siliceous earth. The ash obtained in the total ash method was taken and processed further for acid insoluble ash determination.

The ash obtained from above procedure was boiled for 5 min. with 25 mL of dilute hydrochloric acid and the insoluble matter was collected on an ashless filter paper. The insoluble matter thus obtained was washed with hot water and filter paper was ignited to a constant weight. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

4.5.2.3. Water soluble ash

The total ash was taken and processed further for determination of water soluble ash. Percentage of water soluble ash was calculated with reference to air dried drug.

The ash was boiled for 5 min with 25 mL of water, the insoluble matter collected on an ashless filter paper, washed with hot water and ignited for 15 min at a temperature not exceeding 450 ºC. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight was the water soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried drug.

4.5.3. Determination of extractive values

4.5.3.1. Water soluble extractive value

Five gm of the air-dried bark powder was macerated with 100 mL of chloroform water of the specified strength in a closed flask for 24 h, shaking at an interval of 6 h. It was then allowed to stand for 18 h. The macerate was filtered rapidly to prevent any loss of solvent. 25 mL of the

filtrate was evaporated to dryness in a tarred flat bottom shallow dish and dried at105 ºC to a constant weight and finally weighed. The percentage of water soluble extractive was calculated with reference to the air-dried drug (Anonymous, 2001).

4.5.3.2. Alcohol soluble extractive value

Five gm of the air-dried bark powder was macerated with 100 mL of alcohol of the specified strength in a closed flask for 24 h, shaking at an interval of 6 h. It was then allowed to stand for 18 h. The macerate was filtered rapidly taking precaution against any loss of solvent. 25 mL of the filtrate was evaporated to dryness in a tarred flat bottom shallow dish and dried at 105 ºC to a constant weight and finally weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug (Anonymous, 2001).

4.5.4. Moisture content

4.5.4.1. Moisture content determination by loss on drying

Loss on drying is the loss in weight in percent w/w resulting from loss of water and volatile matter of any kind that can be driven off under specific conditions. Moisture content was determined using oven method (Anonymous, 2001).

4.5.4.2. Moisture content determination by Karl-Fischer titrimetric method

Moisture content was determined using karl-fisher titrator (Lab India Analytical Inst. Pvt. Ltd. India) in the methanol and water extract of bark of *S. racemosa*.

4.6. Preliminary phytochemical investigation of methanol and water extract of *S. racemosa* (Chemical tests)

Methanol and water extract were scrutinized for proximate phytochemical analysis using reported qualitative tests for the alkaloids, flavonoids, carbohydrates, glycosides, tannins, proteins, resins and steroids and triterpenoids (Anonymous, 2002; Kokate, 2001; Khandelwal, 2006).

4.7. Quantitative analysis

4.7.1. Determination of total phenolics

Total phenolic content of extract was measured by Folin-phenol reagent method. In this method, phenols reacts with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline media and produced complex (molybdenum blue) which was measured at 760 nm using UV spectrophotometer (Chen et al., 2007).

The solutions of, Gallic acid (2mL of 100 µg/mL) reference standard, sample (2 mL of 100 µg/mL) and distilled water (as blank 2 mL) were transferred in respective clean and dry test

tubes. To these solutions, 2 mL of Folin reagent and 2 mL of 2 M NaOH were added in each test tube. Absorbance was noted at 760 nm on UV Spectrophotometer.

4.7.2. Determination of alkaloid content

Sample (Approximate 1g) was weighed and transferred in to a 150 mL conical flask. To it 100 mL ether: methanol (80:20) and 5 mL dilute ammonia solution were added and kept aside for 1 h, was shaken frequently and filtered and washed the residue with100 mL of ether: methanol (80:20) and transferred to separating funnel. Gum tragacanth was added to stimulate the stratification. To the total ether methanol solutions 1 N sulphuric acid was added. Shaken well and allow it to separate. The lower layerwas discarded. The extraction was continued with 25 mL of 0.5 N sulphuric acid: methanol (75:25), procedure was repeated for 2 times. The mixed acid solution was washed with 10 mL of chloroform twice. The chloroform layer was discarded and acidic solution was transferred to separator. Make it alkaline with 5 mL dilute ammonia solution and 25 mL of chloroform was added until the complete extraction of alkaloids achieved. Washed the each chloroform extract with 10 mL of water. The chloroform layer was transferred in pre weighed beaker. Evaporate it on the water bath. 2 mL of methanol was added and evaporate to dryness till constant weight was achieved (Rajpal, 2005).

4.7.3. Determination of saponins

Accurately 5g of sample was weighed in a petridish. Entire sample was transferred in to a clean and dry ground glass stoppered round bottom flask. To this 50 mL of 90 % v/v methanol was added. The content was mixed well and then refluxed for half an hour. Then cooled and filtered. The residue was washed with 90 % v/v methanol till washings were almost colourless. The methanol extracts were combined and evaporated on water bath to obtain the thick paste. This residue was treated with 25 mL petroleum ether by refluxing for half an hour. The petroleum ether layer was separated and discarded. Now this residue was treated with 25 ˚C of chloroform by refluxing for half an hour. The chloroform layer was separated and discarded. Then residue was washed with 25 mL of ethyl acetate by refluxing for half an hour. The ethyl acetate layer was separated and discarded. To ethyl acetate washed residue, 5 mL of 90 % v/v methanol was added. Shake the content well to dissolve the residue. Now poured this content drop wise with constant stirring in to a beaker containing 25 mL of acetone to obtain the precipitates, flask was rinsed with 90 % v/v methanol and transferred to the above beaker. The organic layer was decanted and the precipitates were dried to constant weight at temperature of 100 ºC in an oven. Percentage of total saponins was calculated (Rajpal, 2005).

4.7.4. Determination of crude fiber

Crude fiber was estimated by acid-base digestion with 1.25 % w/v H_2SO_4 and 1.25 % w/v NaOH solutions. The residue after crude lipid extraction was put into a 600 mL beaker and 200 mL of boiling 1.25 % w/v H_2SO_4 solution was added. The content was boiled for 30 min, cooled, filtered through a filter paper and residue washed with three 50 mL of boiling water. The drained residue was returned to the original beaker and 200 mL of boiling 1.25 % w/v NaOH solution was added. The content was boiled for 30 min, filtered, washed as above, residue drained and washed with 25 mL of ethanol. The filter paper containing the residue was dried in an oven at 130 °C to constant weight and cooled in a desiccator. The residue was scrapped into pre-weighed porcelain crucible, weighed, ashed at 550 °C for 2 h, cooled in a desiccator and reweighed. Crude fiber content was expressed as percentage loss in weigh on ignition (Rajpal, 2005).

4.7.5. Determination of total tannin content

Total tannin was determined as per titrimetric method. About 0.5 g of substance was weighed in a 500 mL conical flask and dissolved using 50 mL of water. 250 mL of water and 25 mL indigo sulphonic acid were added and Titrated with 0.02 M $KMnO_4$ solution until color change appeared green to golden yellow. The burette reading (X mL) was noted and blank titration was performed using the same procedure omitting the sample. The blank reading (Y mL) was noted. Percentage was calculated by using the following formula with reference to dry weight of substance taken (Anonymous, 2001).

% of tannins = (X-Y) mL x 0.004157 x Molarity of $KMnO_4$ x 100/Weight (g) of substance taken x 0.02

4.7.6. Determination of total flavonoid content

Drug powder (3 g) was refluxed with 50 mL of alcohol on water bath for half an hour. The content was filtered and repeated the procedure twice. Alcohol was evaporated under vacuum. The residue was taken up repeatedly with 25, 15 and 15 mL of hot water. Shake above aqueous extract with 15 mL of ethyl acetate in separating funnel; the ethyl acetate layer was collected. Washed with water and evaporated to dryness the ethyl acetate layer. Methanol (15-20mL) was added for purification and evaporated it in pre weighed beaker and the weight of flavonoid content was noted. The percentage of flavonoid content in the extract was calculated (Rajpal, 2005).

4.8. Analysis of probable contaminants

4.8.1. Heavy metal content (Lead)

In present days mostly drugs are commercially cultivated using modern methods. In such cases the study of heavy metals is to be checked as per WHO requirement. Heavy metal was determined as per the Pharmacopoeial requirement (Anonymous, 1996).

Reagents

Lead nitrate stock solution: Lead nitrate (159.8 mg) was accurately weighed in a 1000 mL of volumetric flask, 100 mL of water was added to dissolve, to which 1 mL of nitric acid was added, and then diluted with water to 1000 mL.

Standard lead solution: Lead nitrate stock solution (10 mL) was diluted with water to 100 mL. Each mL of standard lead solution contains the equivalent of 10 µg of Lead.

PH 3.5 Acetate buffer: PH 3.5 Acetate buffer of ammonium acetate and dissolved in 25 mL of water, and 38 mL of 6 N HCl was added, pH 3.5 was adjusted, with 6 N ammonium hydroxide or 6 N HCl if necessary, and diluted with water to 100 mL, and content was mixed.

Thioacetamide TS: Accurately weighed 4 g of thioacetamide and dissolved in 100 mL of water.

Glycerin Base TS: To 200 g of glycerin, water was added to bring the total weight to 235 g. 140 mL of 1 N sodium hydroxide and 50 mL of water were added.

Thioacetamide-Glycerin Base TS: Thioacetamide TS (0.2 mL) and glycerin base TS (1 mL) was mixed, and heated on water bath for 20 seconds. Use the mixture immediately.

Preparation of reference standard: standard lead solution 2 mL (20 µg/mL) was taken in a color comparison tube and diluted with water to make 25 mL. The pH was adjusted between 3.0 and 4.0 with 1 N acetic acid or 6 N ammonium hydroxide and further diluted with water to 40 mL and content was mixed.

Sample Preparation: Accurately weighed 4 g of raw material powder or extract powder in a suitable crucible. Sufficient sulfuric acid was added to wet the substance and carefully ignite at the low temperature until thoroughly charred. To this 2 mL of nitric acid and 5 drops of sulphuric acid were added, and heat cautiously until white fumes no longer evolved. It was ignited in a muffle furnace at 500-600 °C until the carbon is completely burnt (no longer than 2 h). If carbon remains, few drops of sulphuric acid was added, evaporated and again ignited. Cooled, 5 mL of 6 N HCl was added and digested on steam bath for 10 min. Cooled and transferred to test tube, rinsed the crucible with 5 mL of 6 N HCl. This was used for further procedure.

Procedure

The pH was adjusted 9.0 cautiously with ammonium hydroxide in each of the tubes containing standard preparation, sample preparation. Allowed to cool and adjusted the pH 8.0 using glacial

acetic acid then 0.5 mL was added in excess. The pH was checked; if necessary adjusted to a pH between 3.0 and 4.0 with 1 N acetic acid or 6 N ammonium hydroxide. Filtered if necessary, washed the filter with few mL of water in to a 50 mL color comparison tube, and then diluted with water to 40 mL. To these test tubes 2 mL of pH 1.5 acetate buffer, 1.2 mL of thioacetamide-glycerin base were added, diluted with water to 50.0 mL, mix and, allowed it to stand for 2 min. The downward surface over white surface was viewed.

4.8.2. Determination of microbial load

Crude drugs are bound to contaminate with the pathogenic or non-pathogenic microorganisms. For the crude drugs ready for internal use the upper limits of certain microorganisms like *E. coli*, *Salmonella*, total aerobics (10^5) has been given by WHO. Microbial load was determined as per the Pharmacopoeial requirement (Anonymous, 1996). Dried bark powder, methanol extract and water extract were evaluated for the microbial contamination.

For total plate count (TPC)

Sample (10 g) was accurately weighed and was transferred to 90 mL of sterile phosphate buffered working solution flask. Content was mixed well. 1 mL of above suspension was transferred in to two set of sterile petridish. About 20 mL sterile soyabean casein digest agar (previously cooled at about 40 ºC) was aseptically transferred. Mixed well, allow it to solidify the agar. The plates were transferred to an incubator at temperature of 37$\pm$2 ºC for a period of 24$\pm$2 h. On second day the plates were examined for growth of microbes and the colony forming units (CFU) was counted by using electronic colony counter. Same procedure was followed on third and fourth day. The growth of microbes was noted.

For yeast and moulds

Same procedure was followed as above using sabouraud dextrose agar in place of soyabean casein digest agar. The growth of yeast and moulds was noted.

For *Escherichia coli*

Approximate 1 g of sample was weighed in conical flask of 100 mL; 20 mL of sterilized lactose broth was added and was incubated at 37± 2 ºC for a period of 24±2 h. On second day the content was mixed well. From this suspension aseptically streak loopful culture and was transferred to a sterile Mac conkey agar plate by using sterile loop and was allowed to incubate at same condition. The plate was observed on third day for colony characters. The suspect colony was transferred individually by inoculating loop to surface of eosin methylene blue (EMB) agar.

Development of brick red colour indicates presence of *E. coli* (gram negative) followed by gram staining where *E. coli* appeared as red coloured straight rods (gram negative).

For *Salmonella*

Approximate 1 g of sample was weighed in a conical flask of 100 mL. Sterilized lactose broth (20 mL) was added and incubated at 37±2 ºC for a period of 24±2 h. On second day the content was mixed well. From this suspension aseptically transferred 1 mL in to flask containing 10 mL selenite broth and 10 mL tetrathionate medium. Content was incubated at same condition. On third day the content was mixed well and aseptically streak loopful culture to brilliant green agar plate. Content was incubated at same condition. The plate was observed on fourth day. On brilliant green agar plate colony would appear pink or white and opaque indicating the presence of bacilli (gram negative) and followed by it the gram staining was performed where *Salmonella* appeard as red flagellated (gram negative) bacilli.

For *Staphylococcus aureus*

Approximate 1 g of sample was weighed in a conical flask of 100 mL and sterilized fluid soyabean casein digest broth (20 mL) was added and incubated at 37±2 ºC for a period of 24±2 h. On second day the content was mixed well. From this suspension aseptically streak the plate of mannitol salt agar. Content was incubated at same condition. The plate was observed for colony characters on third day. Colony appears circular grey to black with light coloured margins surrounded by the opaque zone which indicates presence of gram positive cocci and the gram staining was performed. *S. aureus* appears as violet, spherical gram positive coccus which is non-motile and non-sporing.

4.9. Results and discussion

4.9.1. Pharmacognostical study of bark of *S. racemosa*

4.9.1.1. Macroscopic study of bark of *S. racemosa*

The bark surface was rough. Blaze was about 7.5-13 mm in size. It was shortly fibrous, pale yellow, finely mottled with pale orange brown. Mature stem bark occurred in channeled or curved pieces, few flat pieces also occurred in thickness up to 1 cm. Outer surface is uneven and rough due to fissures and cracks. The colour was grayish brown to grey externally and pale to whitish-brown internally, taste was astringent and feebly bitter. Fracture was short and granular in cortical region and somewhat fibrous in inner region.

4.9.1.2. Microscopy of bark of *S. racemosa*

4.9.1.2.1. Transverse section of bark

Transverse section of mature bark showed a wide cork of thin-walled, rectangular cells arranged in radial rows, cork cambium 1-3 layered. Secondary cortex showed zone of thin-walled, oval and tangentially elongated parenchymatous cells towards outer side and rounded cells towards inner side, a number of stone cells, in singles or in groups present, scattered throughout the region having highly thickened walls with distinct pits. Secondary phloem has been composed of sieve elements, phloem parenchyma, phloem fibres and stone cells. Phloem parenchyma was found to be thin walled, oval to rectangular, containing prismatic crystals of calcium oxalate. Phloem fibres were found to be lignified and present in singles or in groups. Isolated fibres were found to be spindle shaped with pointed ends. Group of stone cells as rounded patches have been distributed throughout phloem region. The medullary rays were found to be uni to multiseriate consisting of rectangular cells having brown coloring matter; broader medullary rays dilating towards outer phloem region (Figure 4.1).

4.9.1.2.1. Powdered drug analysis of bark of *S. racemosa*

Colour of powder was brownish, with characteristic smell and slightly bitter taste. Microscopically examination of the stem bark powder showed lignified phloem fibers and sclereids, calcium oxalate crystal, cork cells with reddish brown matter and medullary rays (Figure 4.2).

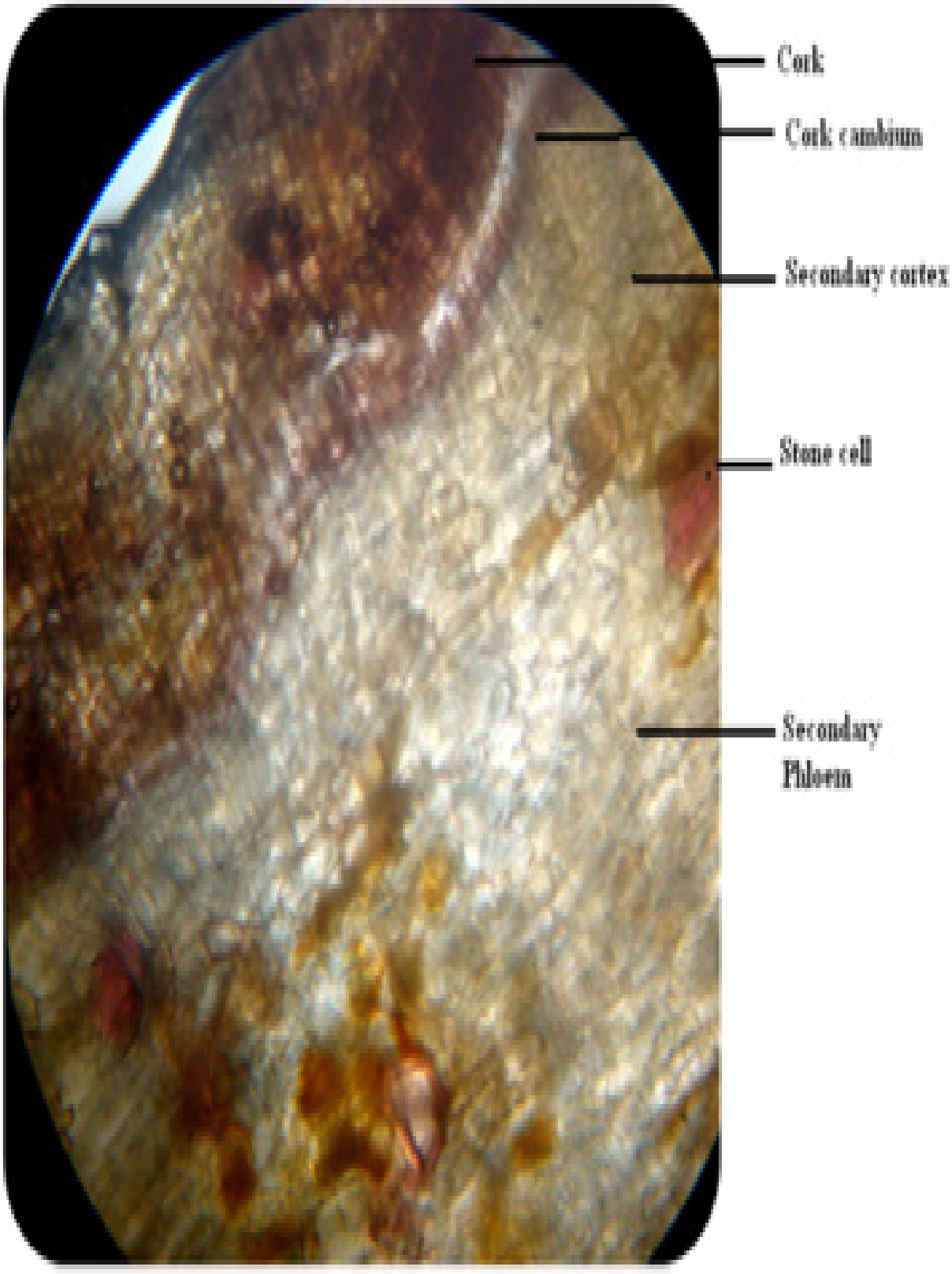

Figure 4.1: Microscopical diagram of bark of *S. racemosa* (450X)

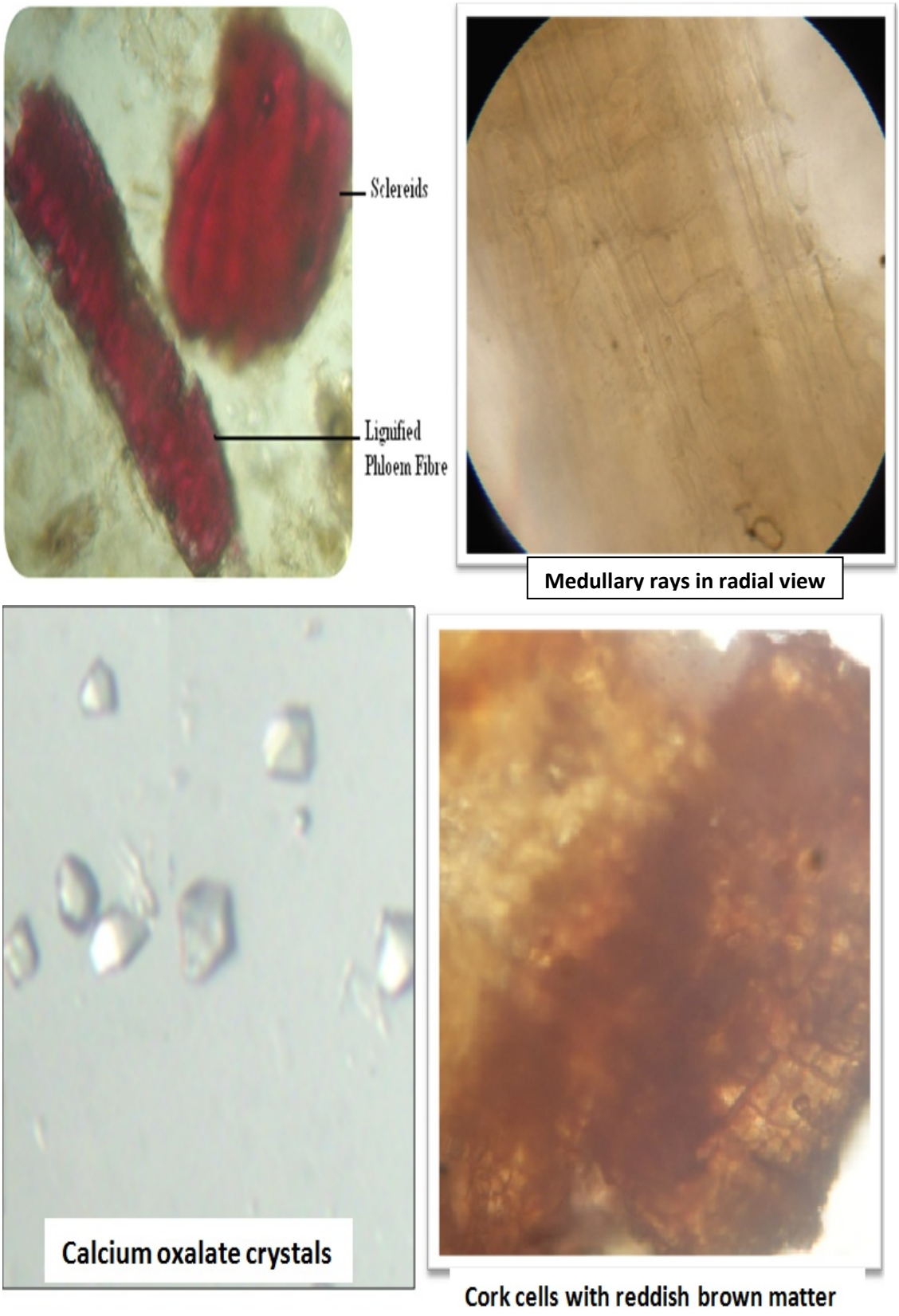

Figure 4.2: Powder characteristics of dried bark (450X)

Table 4.1: Different extracts and fractions prepared from bark of *S. racemosa*

Sr. no.	Name	Appearance
1	Ethanol extract (Hot)	Dark brown colour (4.20%w/w)
2	Methanol extract (Cold)	Light brown colour (4.30%w/w)
3	Methanol extract (Hot)	Dark brown colour (6.79%w/w)
4	Water extract (Hot)	Light brown colour (10.4%w/w)
5	Hexane extract	Dark greenish sticky residue (1 %w/w)
6	Ethyl acetate fraction of hexane extract	Reddish brown sticky residue (0.8 %w/w)
7	Chloroform fraction of hexane extract	Light brown color residue (0.8 %w/w)
8	n-butanol fraction of hexane extract	Dark brown color residue (2 %w/w)
9	Water fraction of hexane extract	Light brown color residue (6% w/w)
10	Hexane fraction of Methanol extract	Reddish brown sticky residue (0.9%w/w)
11	Ethyl acetate fraction of methanol extract	Dark brown sticky residue (4.5%w/w)
12	Chloroform fraction of methanol extract	Light brown sticky residue (3.9w/w)
13	n-butanol fraction of methanol extract	Reddish brown sticky residue (2.6%w/w)
14	Water fraction of methanol extract	Light brown powder (6.4%w/w)

4.9.2. Physicochemical evaluation

In drug powder foreign matter was found to be 1.14 % w/w. Foreign matter was removed by sieving and picking method from the dried drug powder and used for further study (Table 4.2). Determination of physico-chemical parameters indicated that parameters were found below the limits as per Pharmacopoeial requirement (Table 4.3).

Table 4.2: Determination of foreign matter

Weight of sample	Type of foreign matter	Foreign matters (g)	Foreign matters (%w/w)
250g	Animal matter	0.00 g	Nil
	Mineral matter	2.85 g	1.14

Table 4.3: Determination of physico-chemical parameters

Parameters	**Value obtained on dry weight basis (% w/w)***	**Value described in API (% w/w)**
Total ash value	11.25 ± 0.06	NMT 12 per cent
Acid insoluble ash value	0.49 ± 0.09	NMT 1 per cent
Water soluble ash value	6.27 ± 0.02	NMT 10 per cent
Alcohol soluble extractive value	9.93 ± 0.21	NLT 9 per cent
Water soluble extractive value	18.73 ± 0.38	NLT 15 per cent
Moisture content (LOD)	5.87 ± 0.03	----
Karl fisher method	4.89±0.07	----

*n=3 (Results are expressed as mean ±SEM)

4.9.3. Preliminary phytochemical analysis

Phytochemical screening of the methanol extract and water extract of bark of *S. racemosa* showed the presence of various phytoconstituents (Table 4.4).

Table 4.4: Preliminary Phytochemical analysis of *S. racemosa*

Chemical tests	Methanol extract	Water extract
Carbohydrates	+	++
Proteins	---	---
Saponin	+	+
Tannins	++	+
Alkaloids	+	+
Glycoside	+	+
Flavonoids	+	---
Steroids/Triterpenes	+	---
Resins	+	+

+:Present, ++:Abundantly present --- : Absent

4.9.4. Quantitative determination of secondary metabolites

Various secondary metabolites were determined according to the reported methods, which are shown in Table 4.5. It was found from the quantitative determination that content of phenolics markedly higher in the methanolic extract. The content of phenolics, flavonoids, alkaloids, saponins as well as tannins was found to be higher in methanolic extract as compared to the aqueous extract.

Table 4.5: Quantitative determination of secondary metabolites

Estimation of content	Methanol extract	Water extract
Total Phenolic	15.72±0.28%	11.70±0.08%
Total flavonoid	0.17±0.04%	0.12±0.01%
Total alkaloid	0.23±0.02%w/w	0.18±0.01%w/w
Total saponin	0.26±0.01%w/w	0.13±0.007%w/w
Total tannin	1.28±0.02%	1.06±0.06%
Crude fiber	0.246%w/w	

4.9.5. Probable contaminants

Bark powder was analysed for the probable contaminants such as heavy metals and microbial load. Heavy metal content (lead) was found below100 ppm. Bark powderwas free from the microbial contaminants which should below the limit of detection as per the WHO guideline (Table 4.6). Yeast,moulds and *E. coli* were found to be present in small amounts but *Salmonella* and *S. aureus*were found absent and the results were found to be complying with WHO standards.

Table 4.6: Determination of microbial load

Sr. no.	Drug	Microbial Load				
		TPC	*Yeast and molds*	*E. coli*	*Salmonella*	*S. aureus*
1.	Drug powder	High to count	Present	Present	Absent	Absent
2.	Methanol extract	Less than 10^5	Absent	Absent	Absent	Absent
3.	Water extract	Less than 10^5	Absent	Absent	Absent	Absent

4.10. Conclusion

Pharmacognostical, physicochemical and phytochemical parameters are set to ascertain identity, purity and quality of bark of *S. racemosa*. The physicochemical evaluation of drug powder of bark of *S. racemosa* revealed the standard quality and purity of the drug and also indicated the authenticity of the drug. Phytochemical screening of the methanol extract and water extract of bark of *S. racemosa* showed the presence of various phytoconstituents such as triterpenoids, phenolics, steroids, alkaloids, glycosides and tannins. Heavy metal content was found below detection limit. In addition from the result of microbial load it was found that raw material showed the presence of TPC, yeast and molds, *E.coli* but were found to be absent in both methanolic and aqueous extracts. *S.aureus* and *Salmonella* were found to be absent in raw material as well as both the extracts. Above findings assure the identity and quality of the drug and used for further investigations.

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