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Quality Aspects of Eucalyptus

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Abstract: *Eucalyptus is fastest growing species popularly known as gum tree, red iron tree, safeda and belonging to the family Myrtaceae. E. tereticornis and E. grandis are important commercial species with a clean straight bole and compact crown. Large scale plantations have been raised on forest and farm lands, community lands, field boundaries and road/rail/canal strips in India. It is most suitable species for degraded land, waterlogged areas, problematic soils etc. Although, it is a controversial tree because of high water consumption, nutrient depletion, allopathic effects etc., it is also source of pulp, paper, essential oil, timber, medicinal use, etc. Moreover it also provides ecological, socioeconomic and industrial services. Improvement in physical and chemical properties of on Sodic wastelands, heavy metal accumulation in different tissues of in mined soil; carbon sequestration potential, etc. were reported in studies. On unit basis of dry biomass produced, it consumes very little water compared to other trees. If bark of the tree is left on site, the balance of nutrients remaining is (80-88%) of inputs for N, P, K, Ca and Mg which lasts for several years without considering the original nutrients that are still present in soil. In the face of growing economy and increased demand for wood products, it remains to be the desired species that grows fast and produce wood to meet the demand of wood for fuel, construction and furniture materials. Relieving wood product scarcity, landscape re-greening, contribution to poverty reduction, biodiversity restoration and conservation are valuable contribution in forest sector.*

Keywords: Eucalyptus Oil, Quality Aspects, Physicochemical Properties, Formulation, Evaluation

I. INTRODUCTION

1.1 Morphology

Eucalyptus globulusLabill. Is an aromatic tree in the Myrtle Family (Myrtaceae) which commonly attains a height of 150-180 feet and a diameter of 4-7 feet. It has a straight trunk up to two-thirds of its total height and a well-developed crown. The central trunk and tap root are fringed with many lateral stems and roots. The tap root rarely exceeds a length of 10 feet. The light, yellow-brown bark is deciduous.

The leaves of the older branches are narrowly lanceolate, often curved, alternate and hung vertically. They are glossy, dark green, thick and leathery. They average in length from 1.5-2 dm. The leaves of the young shoots are ovate, opposite, sessile, and horizontal. They are covered with a grey, waxy bloom which is much thicker on the bottom surface of the leaf. Young stems are squared or winged. The white flowers are solitary in the axils on flattened stalks. They are approximately 4-5.5 cm wide. The sepals and petals are united to form a warty lid which is present on the bud and drops off at an thesis. The flower has many stamens. The ovary is four-loculed with many ovules. Flowers are most abundant from December to May.

The fruit is a hard, woody capsule, broadly top shaped or globose with a wide flat disc. It is loculicidally dehiscent at the top by four valves. The fruit is 2-2.5 cm across. The numerous seeds are approximately 2 x 1 mm (relatively small compared to other woody plant species).

1.2 Organoleptic Characters

Type: Simple
Colour: blue to green
Condition: Shaded area
Shape: narrow, sickle-shaped and hang down
Fruit: The fruit is a hard, woody globose capsule. The fruit is 0.8-1 in.
Margin: Smooth

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Base: Bark is either smooth , fibrous , hard , stringy , sepal and petals that fuses to from a cap
Venation: Bronchidrodromus venation
Size: Ht. 40-60 ft. Diameter 40-45 inchs
Shape: Round leaves
Odor: Ditinctcamphoraceous smell
Taste: Intensely Aromatic With a mic of Menthol , Citrus , And Pine

II. MACROSCOPIC EVALUATION

Prior to cutting the central part of the leaf blades in small pieces for cuticle isolation, leaf margins and midribs were removed with a scalpel. Leaf cuticles were subsequently isolated in a solution containing 5% cellulose and 5% pectinase (Novozymes, Bagsvared, Denmark), plus 1% polyvinylpyrrolidone (Sigma–Aldrich, Munich, Germany) and 2 mm sodium azide, which was set to ph 5.0 by adding sodium citrate (Guzmán et al., 2014a). Tissues were maintained in solution (changed after 2 weeks) at room temperature (23–25∘C) for 1 month, manually shaking the flasks at frequent time intervals. After the extraction period, clean intact adaxial cuticles were selected, thoroughly washed in deionized water, air-dried, and stored for further use.

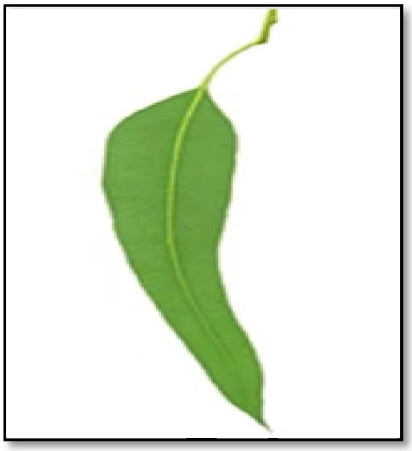

Figure 1: Eucalyptus leaf

III. MICROSCOPIC EVALUATION

Midrib and leaf blade T.S of Eucalyptus leaf. - Upper epidermis, Oil Gland, Lower epidermis, Palisade parenchyma, Sponge parenchyma, Sclerenchyma, Xylem, Phloem, Collenchyma, Calcium oxalate crysta.

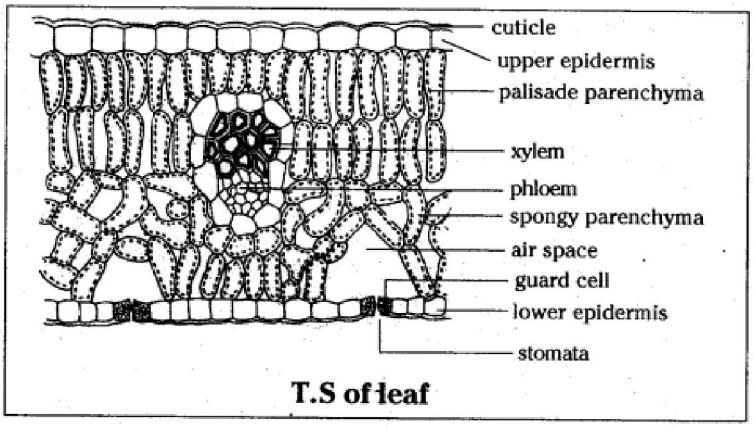

Figure 2: TS of Eucalyptus Leaf

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Nature of Lamina	Dorsi Ventral
Epidermal cell	Anticlinal walls
Trichome	Single Potent Insect Repllant
Stomata	Sunken
Nature of Lamina	Entire
Stomatal Number	300 Per sq m
Stomatal index	8%
Vein Islets number	1-6

2.1. Chemical Evaluation

Relative percentage chemical composition of essential extracted from seven Eucalyptus species.

Sr. no	Components	E.Citriodora	E.camaldulensis	E.crebra	E.teretiocornis	E.globules	E.melanophloia	E.microtheca
1.	1,8-Cineole		16.1	4.9	15.2	56.5	3.1	2.0
2.	A-Pinene	3.6	0.9	2.5	12.1	4.2	16	31.4
3.	B-Pinene			2.6		0.2	1.5	2.5
4.	Eugenol	3.9	0.6	0.7	1.8		1.7	11.8
5.	Eucalyptol	0.1			3.3	0.2	1.1	8.1
6.	Linalool	0.5	17	1.1	7.4	0.3	0.6	
7.	Terpinene-4-ol	0.3	5.3	10.2			1.2	
8	Geranial	0.1	6.6	3.6		0.1		1.4

Table 1: Chemical evaluation

Structures:

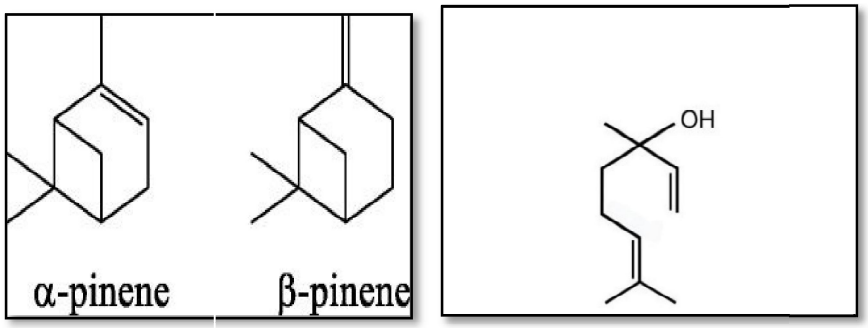

Figure 2: Pinene and Linalool

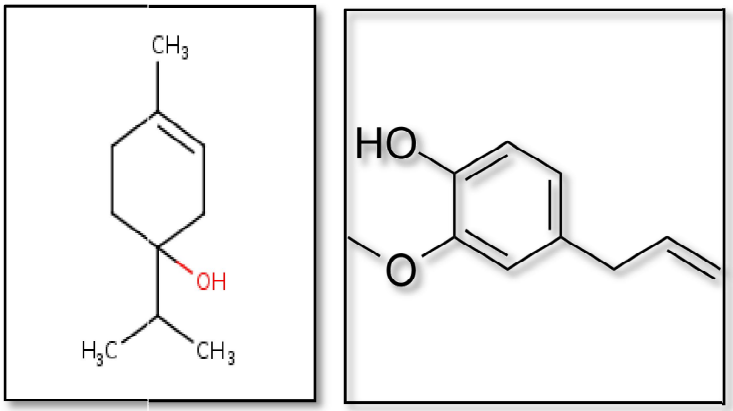

Figure 3: Terpinene-4-ol and eugenol

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III. BIOLOGICAL EVALUATION METHODS

In both fresh and dried form, leaves of eucalyptus are used as air fresheners and in medicinal teas. Oil – In this form, eucalyptus is added to cough and cold medicines, dental products, antiseptics and used directly to treat fevers. Oils are also used in industrial mining operations and as aromatherapy. Humans can't safely ingest eucalyptus. However, clinical studies have indicated that eucalyptus leaves and its oils have promising antifungal and antiseptic properties when applied topically.

3.1 In process quality evaluation and Quality assurance

WHO guidelines for good manufacturing practices, good laboratory practices: -

Unlike conventional pharmaceutical products, which are usually produced from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicines are prepared from materials of herbal origin, which are often obtained from varied geographical and/or commercial sources. As a result, it may not always be possible to ascertain the conditions to which they may have been subjected. In addition, they may vary in composition and properties. Furthermore, the procedures and techniques used in the manufacture and quality control of herbal medicines are often substantially different from those employed for conventional pharmaceutical products. Because of the inherent complexity of naturally grown medicinal plants and the often-variable nature of cultivated ones, the examples of contamination with toxic medicinal plants and/or plant parts and the number and small quantity of defined active ingredients, the production and primary processing has a direct influence on the quality of herbal medicines. For this reason, application of Gmps in the manufacture of herbal medicines is an essential tool to assure their quality.

3.2 Skilled human resources, requirements of well-equipped laboratories for quality Assurance

The establishment and maintenance of a satisfactory system of quality assurance and the correct manufacture and control of pharmaceutical products and active ingredients rely upon people. For this reason, there must be sufficient qualified personnel to carry out all the tasks for which the manufacturer is responsible. Individual responsibilities should be clearly defined and understood by the persons concerned and recorded as written descriptions. The manufacturer should have an adequate number of personnel with the necessary qualifications and practical experience. The responsibilities placed on any on individual should not be so extensive as to present any risk to quality. All responsible staff should have their specific duties recorded in written descriptions and adequate authority to carry out their responsibilities. Their duties may be delegated to designated deputies of a satisfactory qualification level. There should be no gaps or unexplained overlaps in the responsibilities of personnel concerned with the application of GMP. The manufacturer should have an organization chart. All personnel should be aware of the principles of GMP that affect them and receive initial and continuing training, including hygiene instructions, relevant to their needs. All personnel should be motivated to support the establishment and maintenance of high-quality standards. Steps should be taken to prevent unauthorized people from entering production, storage and quality control areas. Personnel who do not work in these areas should not use them as a passageway.

IV. QUALITY EVALUATION OF FINISHED PRODUCTS

Long term and short-term stability of herbal formulations using ICH guidelines:

Since the herbal substance or herbal preparation in its entirety is regarded as the active substance, a mere determination of the stability of the constituents with known therapeutic activity will not suffice. The stability of other substances present in the herbal substance or in the herbal preparation, should, as far as possible, also be demonstrated, e.g., by means of appropriate fingerprint chromatograms. Lt should also be demonstrated that their proportional content remains comparable to the initial fingerprint.

Short Term: In the case of an herbal medicinal product containing a herbal substance or herbal preparation with constituents of known therapeutic activity, the variation in content during the proposed shelf-life should not exceed ± 5% of the declared assay value, unless justified.

Long Term: In the case of a herbal medicinal product containing a herbal substance or herbal preparation where constituents with known therapeutic activity are unknown, a variation in marker content during the proposed shelf-life of ±10% of the initial assay value can be accepted if justified by the applicant.

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V. EVALUATION OF EUCALYPTUS OIL

5.1 The Specific Gravity

The ratio of the density of any substance to the density of some other substance taken as standard, water being the standard for liquids and solids, and hydrogen or air being the standard for gases. Based on the above table, the results showed the specific gravity of eucalyptus oil refining traditionally, 0.8491 and 0.8455 for the processing plant. Both the above results when compared with the value of Indonesian national standard the specific gravity values ranging between 0.900 and 0.930, showed a significant difference. This is due to traditional refining, treatment of raw materials eucalyptus leaves is not done. The increase in the value of the specific gravity is higher with the longer the storage time of eucalyptus leaves is expected because the eucalyptus oil obtained from the distillation of leaves that have been saved will have a constituent component weight fraction which is more and more so that the value of the the specific gravity of the oil will be higher. Treatment without stirring and stirring of leaves when storage give different results for the value of the specific gravity of eucalyptus oil produced. The value of the specific gravity of eucalyptus oil obtained from the leaves by stirring treatment is higher than the value of the specific gravity of eucalyptus oil obtained from the leaves without stirring. Analysis Of Eucalyptus Oil Distillates Traditionally

5.2 The Refractive Index

The refractive index can be determined on the basis of direct measurement of the angle of refraction oil by maintaining a constant temperature conditions. The value of the refractive index of eucalyptus oil or other essential oils can be determined by using a refractometer. The results showed the range of 1.464 (distillation traditional) and 1.467. This value is within the range of 1.450 to 1.470 in the national standard. According to Guenther, the value of the refractive index is also influenced either by the presence of water in the eucalyptus oil content. The more water content, hence the smaller the value of the refractive index. This is due to the nature of the water that is easy to refractin coming light.

5.3 Optical Rotation

No	Types of test	Requirement
1	State	
2	Color	Clear to Yellow-green
3	Odor	typical eucalyptus
4	The Specific gravity 20 C/20 C	0.900-0.930
5	The refractive index (nD20)	1450-1470
6	Solubility in Ethanol	1:1 to 1:10 clear
7	Optical Rotation	1.850 up to - 2.550

Optical activity, the ability of a substance to rotate the plane of polarization of a beam of light that is passed through it. (In plane-polarized light, the vibrations of the electric field are confined to a single plane.) The intensity of optical activity is expressed in terms of a quantity, called specific rotation, defined by an equation that relates the angle through which the plane is rotated, the length of the light path through the sample, and the density of the sample. Optical rotation eucalyptus oil can be determined by apparatus a polarimeter. Determination of the value of optical rotation eucalyptus oil is based on measuring the angle of the field in which the polarized light is rotated by the oil layer thickness and temperature. Optical rotation is a value obtained from the polarization of lightis rotated to the right (dextrorotary) or to the left (laevorotary) by essential oils which are placed in the light or light (Guenther, 1987). Values obtained optical rotation of eucalyptus oil in the study ranged from 1.850 up to - 2.550

Table 2 Quality Requirement of Eucalyptus Oil

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VI. APPLICATION OF CHROMATOGRAPHY FOE THE QUALITY EVALUATION OF EUCALYPTUS AND FORMULATION

6.1 HPTLC of Ellagic Acid HPTLC Chromatographic Condition

Chromatography was performed on a 20 x 10 cm with 0.2 mm thickness pre-coated HPTLC Polyamide F 234 glass plate. Sample and standard spots were applied to the plate as 6 mm wide band with an automatic TLC Applicator Linomat V with nitrogen flow in the dosage speed of 90 nl/s, 10 mm from the bottom. Densitometry scanner was performed on CAMAG Scanner III at 394 nm. The plates were prewashed by methanol and activated at 60 ºc for 10 min prior to chromatography. The slit dimension was kept at 5x0.45 and 40 mm sec-1 scanning speed was employed. The mobile phase consisted of ethyl acetate: formic acid: water in the ratio of 17:2:3 and 10 ml of mobile phase per chromatography was used. Linear ascending development was carried out in 10 x 20 cm twin glass chamber saturated with the mobile phase and then it was dried completely. Further it was derivatised with freshly prepared natural product reagent + PEG (poly ethylene glycol) solution. The chromatographic condition was given in the Table 1 and Chromatogram was shown in Figure

Instrumentation:

The HPTLC system (CAMAG, Muttenz, Switzerland) consists of a CAMAG TLC scanner III integrated with Win CATS software (version 1.4.2), a Limomat 5 (fitted with 100 mL syringe), a TLC applicator connected to a nitrogen cylinder, a twin-trough glass chamber (20 X10 cm), a plate heater and a derivatization chamber. Sartorius analytical micro balance, ultra sonicator (DC1500H MRC), micropipettes and micro-pore filtration set etc were also used.

Sample Preparation

The sample solution was prepared by dissolving 25 mg of crude ethanol extract in 25 mL of ethanol. Standard solution of ellagic acid was prepared by dissolving 5 mg in 1mL of DMSO and make up to 25 mL with ethanol.

HPTLC Analysis of Allagic Acid

Sample : Allagic Acid

Solvent system : Toulene : Choloroform : Ethly Acetate : Formic Acid.

2 : 6 : 6 : 2

Sample preparation: Powdered sample extracted with methanol. The extract concentrated and the residue dissolved in methanol.

Detection: By using System elution A, External standard Method and Calculating Peak Area.

Rf Value : 0.47

Chromatograph of Eucalyptus Leaf.

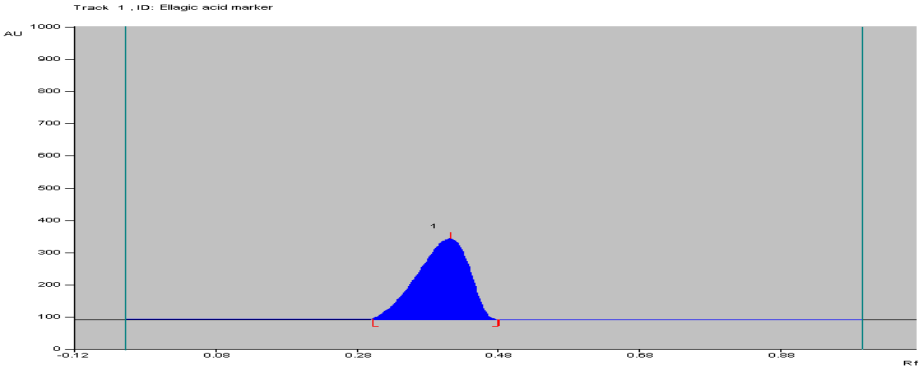

Figure 4: Chromatograph of eucalyptus leaf.

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VII. QUALITY EVALUATION OMETHODS OF EUCALYPTUS AND FORMULATIONS

7.1 Determination of moisture content of Eucalyptus

Procedure:

Weigh about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100° C or 105 C, until two consecutive weighing do not differ by more than 0.5 mg. Cool in a desiccators and weigh. The loss in weight is usually recorded as moisture.

Note:

1. A very useful form of dish for the determination of moisture as a thin flat porcelain dish. If a platinum dish is available, it may be used.
2. The burning of the powder should proceed slowly and the material must not be allowed to catch fire or to give off smoke as dense fumes.
3. The desiccation method is useful for materials that melt to a sticky mass at elevated temperatures.
4. The most common method for the determination of moisture is heat the drug till one gets constant weight at 00, as is done for digitalis. However, many substances loose other volatile constituents, or some of their constituents undergo change with consequent loss of weight at a temperature of 100%, other methods are used for materials of these types.
5. If the solvent is anhydrous, water may remain absorbed in it leading to false results. Hence, it is advisable to saturate the solvent with water before use.

Result: Moisture content of eucalyptus as per IP 9-22%

7.2 Determination of Foreign Matter of Eucalyptus

Foreign organic matter means the material consisting of any one or all of the following substances:

1. Material not collected from the original plant source.
2. Insects, moulds or other animal contamination.
3. Parts of the organ or organs from which the drug is derived other than the parts named in the definition and description.
4. Any other organ than those named in the definition and description.

Procedure:

Take weighed quantity of crude drug (500 G root\stem\dark, 250 G leaves \ flowers \ seed fruit, 50 G cut plant material). Spread it in a thin layer and sort the foreign material by using magnifying lens (10 x) or suitable sieve or by visual inspection. Pass the remaining sample through a sieve no. 250 to remove dust (mineral admixture). Weigh the portion of sorted foreign matter and determine % w/w of it.

If foreign matter resembles plant material, take pooled sample and apply physical /chemical test or microscopy. Determine the proportion of foreign matter from the sum of the proportion fail to respond to the test.

7.3 Determination of Extractive Values of Eucalyptus

It is useful for the evaluation of a crude drug. It gives idea about the nature of the chemical constituents present in a crude drug. Useful for the estimation of constituents extracted with the solvent used for extraction. Employed for material for which as yet no suitable chemical or biological assay exists.

Methods: There are two methods named as Cold Maceration and Hot Maceration

7.3.1 Determination of Alcohol-Soluble Extractives

Procedure:

Weigh about 4 g of the coarsely powdered drug in a weighing bottle and transfer it to a Dry 250 ml conical flask. Fill a 100 ml graduated flask to the delivery mark with the solvent (90% alcohol). Wash out the weighing bottle and pour the washings, together with the remainder of the solvent into the conical flask. Cork the flask and set aside for 24 hours, shaking frequently. Filter into a 50 ml cylinder. When sufficient filtrate has collected, transfer 25 ml. Of the filtrate to a weighed, thin porcelain dish, as used for the ash values determinations. Evaporate to dryness on a water-bath and

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complete the drying in an oven at 105° C for 6 hrs. Cool in a desiccator for 30 minutes and weigh immediately. Calculate the percentage w/w of extractive with reference to the air-dried drug.

Calculation:
25 ml of alcoholic extract gives = x g of residue
100ml of alcoholic extract gives = 4x of residue
Since 5g of air-dried drug gives 4x g of alcohol (90 %) soluble residue
100 g of air-dried drug gives 80x g of the alcohol (90%) solute residue. Since Alcohol (90 %) soluble extractive value of the sample=80x%.
Result: Alcohol soluble extractive value of Eucalyptus as per IP 12.35%

7.3.2 Determination of Water-Soluble Extractives
Procedure: Steps are similar to those mentioned in the previous experiment. Use chloroform water instead of alcohol (Chloroform acts as a preservative).
Result: Water soluble extractive value of eucalyptus as per IP 17.2%

7.4 Determination of Ash Values of Eucalyptus :
Ash values:
It is used to determine quality and purity of a crude drug and to establish the identity of it. Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. These are present in definite amount in a particular crude drug hence, quantitative determination in terms of various ash values helps in their standardization. Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects Total ash value. Such variables are removed by treating with acid (as they are soluble in hydrochloric acid) and acid insoluble ash value is determined e.g., Rhubarb, Liquorice etc. Used to determine foreign inorganic matter present as an impurity. Acid soluble ash value of crude drug is always less than total ash value.

7.4.1 Determination of Total Ash Value
Procedure:
Weigh and ignite flat, thin, porcelain dish or a tared silica crucible. Weigh about 2 g of the powdered drug into the dish/crucible. Support the dish on a pipe-clay triangle placed on a ring of retort stand. Heat with a burner, using a flame about 2 cm high and supporting the dish about 7 cm above the flame, heat till vapours almost cease to be evolved; then lower the dish and heat more strongly until all the carbon is burnt off. Cool in a desiccator. Weigh the ash and calculate the percentage of total ash with reference to the air-dried sample of the crude drug.If a carbon free ash cannot be obtained in this way, then any one of the following methods can be used.
Exhaust the charred mass with hot water, collect the residue on an ashless filter paper incinerate the residue and filter paper, add the filtrate, evaporate to dryness and ignite at a temperature not exceeding 450°C. Cool the crucible; add 15 ml of alcohol, break up the ash with glass-rod burn off the alcohol and again heat the whole to a dull red heat. Cool, weigh the ash.
Calculation:
Weight of the empty dish = x
Weight of the drug taken = y
Weight of the dish + Ash (after complete incineration) = z
Y g of the crude drug gives (z-x) g of the ash
Wt. Of the ash = (z-x) g
100 g of the crude drug gives $\frac{100}{y}$X (z-x) g of the ash
Total ash value of the sample = $\frac{100(z-x)}{y}$ %
Result: Ash value of eucalyptus as per IP – 6.29%

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7.4.2 Determination of Acid-Insoluble Ash Value

Procedure: Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Further using 25 ml of dilute hydrochloric acid, wash the ash from the dish used for total ash into a 100 ml beaker. Place a mere gauze over a Bunsen burner and boil for five minutes. Filter through an 'ashless filter paper, wash the residue twice with hot water. Ignite a crucible in the flame, cool and weigh. Put the filter-paper and residue together into the crucible; heat gently until vapour ceases to be evolved and then more strongly until all carbon has been removed. Cool in a desiccator. Weigh the residue and calculate acid-insoluble ash of the crude drug with reference to the air-dried sample of the crude drug.

Calculation: Similar to previous experiment.

Weight of the residue = a gy g of the air-dried drug gives 'a' g of acid -insoluble ash

100 g of the air-dried drug gives $\frac{100\times a}{y}$ G of acid-insoluble ash.

Acid-insoluble ash value of the sample = $\frac{100\times a}{y}$ %

Result: Determination of acid-insoluble ash value – 5.33% W/W

7.5 Chemical Tests for Eucalyptus Oil

Phytochemical screening of leaves extract was done by using both quantitative and qualitative methods. The procedures used to detect the presence of various phytochemical compounds are described below:

7.5.1 Quantitative Methods

Phenolic Contents:

The determination of total phenolic content was done usingThe procedure given by Singleton. Different concentrations of sample were pipetted out From 0.2 to one ml in test tubes. The final volume was made in each test tube to three ml with the help of distilled water. 0.5 ml of folinciocalteau reagent was added to test tubes and incubated at room temperature for 3 min then two ml of sodium carbonate was added to it and mixed it thoroughly. Test tubes were kept in boiling water bath for one min. Cooled

The test tubes under tap water. The absorbance was measured at 720 nm. TheReference standard was taken as gallic acid.

Determination of Flavonoids

Determination of flavonoids was done by procedure given by Zhuang et al, 199. The extract and standard was taken. The different aliquots pipetted out with different concentrations. Then five ml distilled water and three mlalcl3 (1:10, w/v) were added. After six min, two ml CH3-COOK (1M) was added and the total volume was made up to10 ml and absorbance was measured against a blank at 415 nm. Quercetin served as the standard compound for the preparation of calibration curve.

Qualitative Methods:

The phytochemical tests were performed by the method given by Harborne, 1973.

Foam Test:

Two ml of extract was dissolved in 3 ml distilled water and shaken vigorously. A stable top layer of foam was formed, indicating the presence of saponins in the sample.

Hansch Test:

Two ml of extract was taken in a test tube. One ml of concentrated H2SO4 was added from the side walls of the test tube and the formation of a brown ring suggested the presence of carbohydrates.

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Tannin Test:
To 0.5 ml of extract solution one ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.

F
lavonoid Test:
Two ml of filtrate was taken. Five to six drops of concentrated hcl and a few magnesium filings were added to it. Appearance of red color indicates the presence of flavonoids.

Phenol Test:
In two ml of extract, a pinch of ferric chloride was added. Appearance of green colour indicates the presence of phenols.

Protein Test:
Two ml of extract was taken, and one to two drops of nitric acid was added. Development of yellow color indicates the presence of proteins.

Quinone Test:
Two ml of extract was taken, few drops of concentrated H2SO4 were added and appearance of red color indicates the presence of quinones.

Fat Test:
The extract was tapped on the filter paper. Appearance of oil on the filter paper showed the presence of fat in the extract of eucalyptus

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