Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(6): 1305-1308
Received: 07-09-2019
Accepted: 09-10-2019

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Preliminary phytochemical and physico-chemical analysis of Saṁvartikā (Tender leaves) of kamala (*Nelumbo nucifera* Gaetrn.)

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Abstract
Objective: The purpose of the study is to evaluate preliminary phytochemical constituents of the Saṁvartikā (Tender leaves) of Kamala (*Nelumbo nucifera* Gaertn.).
Methods: The preliminary Phytoconstituents tested were Alkaloids, Carbohydrates, Reducing sugars, Protiens, Xantho proteins, Amino acids, Starch, Glycosides, Steriods, Tannins and p^H value. The Physico-chemical tests were Total moisture contents, Ash value, Soluable extracts and Foreign matter. These Parameters were evaluated with standard methods of Association of official Analytical chemists.
Results: From this study it is revealed that the dried tender leaves of *Nelumbo nucifera* Gaertn. showed the presence of Alkaloids, Carbohydrates, Reducing sugars, Tannins and a p^H value of 5.65 at 23.8˚C. The Physico-chemical study reveals the presence of Moisture content 6.55%W/W, Total Ash 10.97%W/W, Acid insoluable Ash 0.098%W/W, Water Soluable Extract 15.75%, Alcohol Soluable Extract 10.56% and Foreign matter -Nil.
Conclusion: The preliminary Phytochemical and Physicochemical screening is helpful do to further pharmacological activities.

Keywords: *Nelumbo nucifera*, phytochemicals, saṁvartikā

Introduction
KAMALA (*Nelumbo nucifera* Gaertn.) is a perennial aquatic herb bearing the famous Red or Rose pink coloured flower. It is found in ponds, lakes, marshes and flooded fields. Kamala have been known by the names as Sacred lotus, Indian lotus, Asian lotus, Lotus, East Indian Lotus etc. It is extensively described in almost all classical of Āyurveda that reflects its great medicinal value, it is edible used as food and medicine. It has miraculous cooling effect, Anti-haemorrhagic property, Anti- diabetic [1], Anti-platelet, Hepto-protective and Anti-estrogenic effect. Its leaves, seeds, flowers, root contains several Alkaloids [2] and Flavonoids which are beneficial in treating different ailments. The plant as a whole is also used to treat many pathological conditions [3].

Lotus Leaves

Fig 1: Tender leaves of Kamala (Saṁvartikā)

Phytochemical study
Aims and objectives: The Saṁvartikā (Tender leaves) of *Nelumbo nucifera* Gaertn. is subjected to Preliminary Phytochemical screening for the detection of various chemical constituents present.

Material and Methods

Sample collection and preparation: The sample drug Kamala Saṁvartikā Cūrṇa (*Nelumbo nucifera* Gaertn.) were collected from the ponds in Cuddalore Dist. Tamilnadu in Febuary 2018. Fresh Leaves were collected and rinsed with tap water for 2-3 times and shade dried and the dried parts were powdered using mechanical grinder and packed in Airtight container for further Analysis.

Preparation of aqueous extract and laboratory analysis

The aqueous extract of Lotus tender leaves was prepared by soaking 100 gm of dried Tender leaf powdered samples in 500ml of distilled water for 12 hrs. The filtrate of tender leaf powder is tested for the presence of various active principles namely Alkaloids, Carbohydrates etc.

Tests for Alkaloids

Mayer's Test: To 1 ml of the extract, 3 ml of Mayer's reagent was added, the formation of full white precipitate confirmed the presence of Alkaloids.

Test for Carbohydrates

Molisch Test: To 2 ml of the extract, 1 ml of α-naphthol solution and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet colour at the junction of the two liquids revealed the presence of carbohydrates.

Tests for reducing sugars

Benedict's test: Mix equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5 min. solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

Test for proteins

Biuret Test: To 1 ml of the extract, 1ml of 40% sodium hydroxide solution was added followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour showed the presence of proteins.

Xantho-protein Test

To 1 ml of the extract, 1ml of concentrated nitric acid was added. A white precipitate is formed, it is boiled and cooled. 20% of sodium hydroxide or ammonia is subsequently added; orange colour indicated the presence of aromatic amino acids.

Test for Amino-acids

Ninhydrin test: Heat 3ml test solution and 3 drops 5% Ninhydrin solution in boiling water bath 10 minutes purple or bluish colour appears.

Test for Starch

Iodine test: Mix 3ml of test solution and few drops of dilute iodine solution. Blue colour appears, it disappears on boiling and reappears on cooling.

Test for Tannins

To 1 ml of the extract, ferric chloride was added, formation of a dark blue or greenish black colour product showed the presence of tannin.

Tests for steroids

Salkowski Test: Dissolve the extract in chloroform and equal volume of concentrate sulphuric acid. Shake well. chloroform layer appears red and acid layer appears greenish yellow represented the steroid components in the tested extract.

Tests for Glycosides

Keller Killiani test: The extract is dissolved in a mixture of 1% Ferric sulphate solution in 5% glacial acetic acid. Add one or two drop of concentrated sulphuric acid. A blue colour develops due to the presence of deoxy sugars.

Results and Discussion

Table 1: Results of phytochemical analysis of kamala Saṁvartikā Cūrṇa

S. No.	Phytochemical	Test name	Result
I	Alkaloids	Mayer's Test	Present
II	Carbohydrates	Molisch Test	Present
III	Reducing Sugars	Benedicts Test	Present
IV	Proteins	Biuret Test	Absent
V	Xantho proteins	Xantho protein test	Absent
VI	Amino acids	Ninhydrin Test	Absent
VII	Starch	Iodine test	Absent
VIII	Tannins	Ferric chloride test	Present
IX	Steroids	Salkowski reaction	Absent
X	Glycosides	Keller – killiani test	Absent
XI	P^H	5.65	5.65 at 23.8 ℃

Along with these *Flavonoids* are also present in Lotus leaves [4].
pH of Kamala Saṁvartika Cūrṇa: 5.65 at 23.8 ℃

Physicochemical study identity, purity and strength

AIM

To study the Identity, Purity and Strength of dried Tender leaves of *Nelumbo nucifera* Gaertn.

Objectives

Physicochemical studies such as Moisture content, Total ash, Foreign matter, Acid insoluable ash, Water soluables extract, Alcohol soluable extract were determined according to WHO guidelines on quality control methods for medicinal plants [5].

Materials and Methods

Methodology

1. **Loss on drying / Moisture content:** 10 gm of trail drug samples are placed after accurately weighing it in a tarred evaporating dish. After placing the above said amount of sample in a tarred evaporating dish is dried at 105° C for 5 hours and it is weighed. After drying tarred evaporating dish was allowed to cool in desiccators for 30 minutes and then weighed the remnant material.

$$\text{The \% of Loss on drying} = \frac{\text{Difference in weight after heating}}{\text{Weight of sample taken}} \times 100$$

2. Determination of ash values of a crude drug

Ash values

- Used to determine quality and purity of a crude drug
- Ash contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc.

A. Determination of total Ash value

- Weigh and ignite flat, thin, porcelain dish or a tarred silica crucible
- Weigh about 2g 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 2cm high and supporting the dish about 7cm. above the flame; heat till vapors 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.

Total ash value of the sample = 100 (z-x)/ y%

B. Determination of acid – insoluble ash value

Proceed as per the steps mentioned in the procedure for determination of total ash value of a crude drug. Further –

- Using 25ml of dilute hydrochloric acid, wash the ash from the dish used for total ash into a 100ml beaker
- Place a wire gauze over a Bunsen burner and boil for 5 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 vapors cease 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

$$\text{Acid insoluble Ash value of the sample} = \frac{a}{y}\%$$

3. Determination of extractive values

Extractive Values

1. Useful for the evaluation of a crude drug
2. Give idea about the nature of the chemical constituents present in a crude drug
3. Useful for estimation of specific constituents, soluble in that particular solvent used for extraction.

A. Determination of Alcohol soluble Extractives

- Weigh about 5g of the powdered drug in a weighing bottle and transfer it to a dry 250ml. conical flask
- Fill a 100ml 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 24hrs, shaking frequently
- Filter into 50ml cylinder. When sufficient filtrate has collected, transfer 25ml. of the filtrate to a weighed, thin porcelain dish, as used for the ash values determinations
- Evaporate to dryness on a water bath and complete the drying in an oven at 100 ºC
- Cool in a desiccator and weigh
- Calculate the percentage w/w of a extractive with reference to the air dried drug

B. Determination of water soluble extractives

- Weigh about 5g of the powdered drug in a weighing bottle and transfer it to a dry 250ml. conical flask
- Fill a 100ml graduated flask to the delivery mark with the solvent (90% Chloroform water). 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 24hrs, shaking frequently
- Filter into 50ml cylinder. When sufficient filtrate has collected, transfer 25ml. of the filtrate to a weighed, thin porcelain dish, as used for the ash values determinations
- Evaporate to dryness on a water bath and complete the drying in an oven at 100 ºC
- Cool in a desiccator and weigh
- Calculate the percentage w/w of a extractive with reference to the air dried drug

Results

Table 1: Results of Identity, Purity and Strength of Tender leaves of *Nelumbo nucifera* Gaetn.

Parameter	Results
	Kamala Saṁvartikā Patra
Moisture Content	6.55%w/w
Total ash	10.97% w/w
Acid insoluble Ash	0.098 % w/w
Water soluble Extract	15.75 % w/w
Alcohol soluble Extract	10.56% w/w
Foreign Matter	Nil

Conclusion

The Saṁvartikā (Tender leaves) was subjected to preliminary Phytochemical and Physicochemical Analysis. The extractive values are being useful for the further extraction of phytoconstituents from the plant. The Alcoholic soluable extractive indicates the presence of polar constituents like Phenols, Flavonoids etc. The Total ash is particularly important in the evaluation of purity of drugs i.e. the presence or absences of foreign matter such as metallic salts or silica. The total Ash of Saṁvartikā tender Kamala leaf is 10.97%W/W this may be due to presence of Calcium oxalate crystals and the metallic salts like Sodium, Potassium, Calcium, Magnesium, Chloride, Copper and Silver in PPM units. It was found that the leaf extract of *Nelumbo nucifera* Gaertn. Shows the presence of Alkaloids, Carbohydrates, Reducing sugars and Tannins which are beneficial in treating many ailments [6].

Acknowledgement

Authors are thankful to Department of Ayush, State drug testing laboratory, dr. B.R.K.R. Govt. Ayurvedic college, Hyderabad-500038. For providing facilities for the successful completion of the study.

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