



ISSN 2278-4136
JPP 2014; 3 (4): 121-124
Received: 07-08-2014
Accepted: 10-09-2014

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Physico-chemical evaluation and biochemical quantification of crude drug powder (stem) of *Chassalia curviflora* (Wall. ex Kurz.) Thwaites; A folk medicinal plant

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Abstract

The medicinal plants are sources of important therapeutic aids for alleviating human health and improving the quality of life. The present study was undertaken with an objective to investigate the physico-chemical and biochemical analysis of the crude drug powder of the stem of *Chassalia curviflora* (Wall. ex Kurz.) Thwaites, an ethno-medicinally important plant belonging to coffee family (Rubiaceae). The physico-chemical studies showed foreign content 0.313%, moisture content 11.333 %, total ash content 11.416%, acid soluble ash 56.833%, water soluble ash 15.054 % and alcohol soluble ash 10.595. The result of biochemical contents showed that, the highest value of dry matter was $88.666 \pm 0.166\%$, followed by carbohydrate $63.027 \pm 0.023\%$, crude fibre $14.693 \pm 0.170 \%$, crude protein $13.125 \pm 0.004\%$, total ash $11.416 \pm 0.289\%$, moisture $11.333 \pm 0.166\%$ and crude fat $1.099 \pm 0.062\%$. The extractive values of the stem were determined by using different solvents. This information's are useful for the pharmacognostical evaluation of this plant material.

Keywords: *Chassalia curviflora*, Physico-chemical evaluation, Biochemical quantification.

1. Introduction

Ethno-botanical and ethno-pharmacological studies have been defined in many ways. It assumes great importance in enhancing our existing knowledge about the plants used by tribal folklore, the rich diversity assembled by them for their sustenance and the different means adopted by them for its preservation and conservation. The tribes have developed their own traditional ways of diagnosis and treatment of diseases. The medicinal plants constituted the main source of new pharmaceutical drugs. The evaluation of crude drug means confirmation of its identity and determination of its quality and purity.

The plant *Chassalia curviflora* (Wall. ex Kurz.) Thwaites are commonly known as 'neelakurinji' in Malayalam. It belongs to the family Rubiaceae which constitutes one of the largest flowering plant. It is an evergreen, erect shrub, up to 2 m tall, leaves elliptic ovate, flower pinkish-white with yellow throat, fruit drupe, purplish-black^[1]. It is used as a traditional medicinal plant in Western Ghats of Kerala, India. The Kani tribal folklore in Aarukani Hills, used the root and root bark of the *C. curviflora* to treat jaundice and wounds^[2]. In addition, the different parts of the plant are reportedly used for various medicinal properties such as headache, ulcers, sore throat, phlegm, rheumatism pneumonia eye and ear diseases^[3,4].

At present, studies on the physicochemical and biochemical quantification of this plant are scarce in literature. Therefore, in the present study an attempt has been made to assess the quality control parameters for further pharmacognostical standardization of this plant material.

2. Materials and Methods

2.1 Collection of plant material and identification

The taxonomically identified *Chassalia curviflora* (Wall. ex Kurz.) Thwaites plant stem was collected from "Paingottupuram" near Kuttikkattoor, Kozhikode, Kerala, India. A herbarium for morphological studies was prepared, identified and authenticated by a botanist.

A voucher specimen No: CALI- 6806 was deposited in the Department of Botany, Calicut University Herbarium, Kozhikode, Kerala.

2.2 Reagents and chemicals

All the reagents and chemicals used in present study were procured from M/s Merck India, Ltd. Bombay.

2.3 Preparation of crude drug powder

The collected plant stem of *C. curviflora* were cleaned and washed with running water and dried at room temperature for 2 to 3 weeks. The dried stem (Figure-1) was then powdered in a plant sample grinder at controlled temperature and stored in plastic container.



Fig 1: Dried stem

2.4 Physio-chemical analysis

2.4.1 Determination of foreign content

Approximately 100 gm of stem powder (Figure-2) of *C. curviflora* was taken and spread into a thin layer. The foreign matter was being detected by inspection with the unaided eye separated and weighed. The percent aged of foreign content was determined using the formula.

Percentage of foreign content =

$$\frac{\text{Weight of sample} - \text{Weight of foreign matter}}{\text{Weight of sample}} \times 100$$



Fig 2: Crude drug powder

2.4.2 Determination of moisture content

A small amount of powder was placed in a crucible with lid and put in a hot air oven for removal of moisture content at 100 ± 1 °C, overnight. The dried samples were weighed in crucible with lid after cooling to room temperature. The moisture content was calculated by the following formula.

Percentage of moisture content =

$$\frac{\text{Weight of sample} - \text{Weight of dried sample}}{\text{Weight of sample}} \times 100$$

2.4.3 Determination of total ash contents

Ash represents the inorganic matter content of the sample which was determined by the method of AOAC [5]. Approximately one gram dried sample in a crucible was charred over a low flame and kept in a muffle furnace set at 550–600 °C for 2-3 hours. It was then cooled in a desiccator and weighed to ensure completion of ashing. It was once again heated in the furnace for half an hour, cooled and weighed. The procedure was repeated consequently till the weight became constant. Total ash content was calculated by the following formula.

$$\text{Percentage of ash} = \frac{\text{Weight of ashed sample}}{\text{Weight of sample taken}} \times 100$$

2.4.4 Determination of acid/alcohol/water soluble ash

The soluble ash was determined by using different solvents such as 5N HCl, alcohol and distilled water. The ash obtained was digested with 25 ml of solvent for 20- 30 minutes in a boiling water bath. The content in the crucible was filtered by using ash less filter paper (Whatman filter paper No: 42). The filter paper with residue was removed carefully without any loss, folded, put in the same crucible, dried in hot air oven and ignited in muffle furnace at 600 °C for 1 hour. Then it was cooled in a desiccator and weighed. The soluble ash value was calculated by the following formula.

$$\text{Percentage of soluble ash} = \frac{\text{Weight of soluble ash}}{\text{Total weight of ash}} \times 100$$

2.4.5 Determination of extracting values

Coarsely chopped (5 gm) stem powder of *C. curviflora* was subjected to macerate for 24 hours in a closed flask using 100 ml of different solvent viz. alcohol, chloroform, ethyl acetate, hexane, petroleum ether and distilled water. The flask was frequently shaken during the first 6 hours and then allowed to stand for 18 hrs. After 24 hrs, the contents in the flask were filtered using Whatman No: 42 filter paper. In a flat bottomed shallow dish, 25 ml of filtrate was evaporated to dryness, dried at 105 °C and weighed. Percentage of soluble extractive was calculated with reference to the air dried powder.

Percentage of extracting value =

$$\frac{\text{Weight of flask with extract} - \text{Weight of empty flask}}{\text{Weight of sample}} \times 100$$

2.5 Biochemical quantification

The biochemical quantification of ash, crude fibre, crude protein, carbohydrate, crude fat, dry matter and moisture content of the stem powder of *C. curviflora* were done using standard proximate analysis techniques. [6]

2.5.1 Determination of dry matter

The dry matter determined using the weight difference method was estimated by deducting percent moisture from hundred as described by James [7].

$$\text{Dry matter (\%)} = 100 - \% \text{ of moisture}$$

2.5.2 Determination of crude fat

One gram of crushed dried sample was taken in the paper thimble kept in a pre-weighed flask of soxhlet extractor. 80 ml of petroleum ether was poured on the flask and refluxed for 8 hours. The flask was cooled in a desiccator and the weight of crude fat extracted was taken. The percent crude fat was determined by using a formula.

$$\text{Crude fat (\%)} = \frac{\text{Weight of flask with fat} - \text{weight of empty flask}}{\text{Weight of original sample}} \times 100$$

2.5.3 Determination of crude fibre

One gram of the defatted plant material was taken in a spout free beaker and boiled in 200 mL of 1.25% sulphuric acid for 30 minutes. The content was then filtered and washed with hot distilled water to neutralize and transferred again to the beaker and boiled in 200 mL of 1.25% sodium hydroxide for 30 minutes. It was again filtered and washed with hot distilled water for neutralization. The crucible was dried in an oven at 100 ± 5 °C in a hot air oven overnight (10-12 hours), cooled in a desiccator and weighed to a constant weight. Latter, the crucible with its content was put in a muffle furnace at 550-600 °C for 2-3 hour for complete burning of organic matter. It was then cooled in a desiccator and weighed to a constant weight. The percent fibre was determined from the formula.

$$\text{Crude fibre (\%)} = \frac{W_1 - W_2}{\text{Weight of sample}} \times 100$$

Where,

W1- The crucible with crude fibre was cooled and weighed.

W2- The content of the crucible was ignited over a low flame until charred and then kept in a muffle furnace and weighed.

2.5.4 Determination of crude protein

Total nitrogen (N) content was determined with the help of Kjeldahl method described by Pearson [8]. The protein determination was divided into three steps.

2.5.4.1 Digestion

0.5 g of dried plant material was taken in the digestion flask. To this 1 g of digestion mixture (copper sulphate and sodium sulphate) and 15 ml of concentrated sulphuric acid was added. The solution was heated until it became clear and frothing ceased. It was then boiled gently for another 2 hours, cooled and digested with 30 ml of water with constant mixing. The digest was transferred to 250 ml standard flask and necessary amount of distilled water up to the mark of the flask was added.

2.5.4.2 Distillation

The distillation step was carried out in a Kjeldahl apparatus, 20 ml of 4% boric acid was taken and one drop of methyl red indicator was added. 10 ml of aliquot of digested material was transferred to the distillation assembly and 20 ml of sodium hydroxide (40%) solution was added to it. The completion of distillation was indicated by a change of colour of boric acid from pink to blue.

2.5.4.3 Titration

The boric acid with trapped ammonia was titrated with 0.1N hydrochloric acid. The colour of boric acid changed again to pink.

The percent of protein was calculated by the formula.

$$\text{Protein (\%)} = \frac{V \times 1.4 \times 6.25 \times 0.1N \text{ HCl} \times \text{Vol (used)}}{W \times A \times 1000} \times 100$$

Where,

V - Titer value (Volume of HCl used).

1.4 - Weight of nitrogen expressed in gram in the formula.

6.25 - Protein factor.

W - Weight of powdered sample.

A - Aliquot digested sample used for distillation.

2.5.5 Determination of Carbohydrate

The percentage of carbohydrate in the sample was calculated by using this formula.

$$\text{Carbohydrate (\%)} = 100 - (\text{ash} + \text{moisture} + \text{crude protein} + \text{crude fat}) \%$$

3. Result and Discussion

The physicochemical parameters are mainly used in judging the purity and quality of the drug. The parameters of crude stem powder of *C. curviflora* were estimated based on the standard procedures. The results showed that the stem powder contained foreign content of 0.313% and an ash value of 11.416%. The percentages of acid soluble ash, water soluble ash and alcohol soluble ashes are depicted Fig-3. The highest plant extractive yield was obtained from water. The extractive values of the powdered stem using water, ethyl acetate, ethanol, chloroform, hexane, and petroleum ether are presented in Table-1. Thenmozhi *et al* (2013) had reported an extractive yield 0.4, 0.3 and 0.6 (w/w) from the fruits of *C. curviflora* using acetone, ethanol and water respectively as solvents. The ash value and foreign contents gave an idea of the inorganic content or other impurities present along with the crude drug. Besides the extractive values provided the additional information which might be useful in the determination of adulterated drug powder.

Table 1: Data showing the extractive values of *C. curviflora* stem powder.

SL. No.	Extract	Colour of extract	Yield* (%)
1	Ethanol	Dark green	04.294 ± 0.079
2	Chloroform	Green	03.373 ± 0.109
3	Ethyl acetate	Pale green	04.410 ± 0.036
4	Hexane	Yellowish green	01.700 ± 0.238
5	Petroleum ether	Yellowish green	01.099 ± 0.062
6	Water	Brown	06.863 ± 0.332

Note:* Mean of 3 readings ± SEM

The percentages of dry matter, moisture, crude fibre, crude protein, crude fat, ash and carbohydrate contents of the leaves of *C. curviflora* are shown in table-2. The plant stem has a crude fibre content of $14.693 \pm 0.004\%$, ash $11.416 \pm 0.289\%$, crude protein $13.125 \pm 0.004\%$ and crude fat $0.1099 \pm 0.062\%$. Further, biochemical quantification (proximate content) helps to set up certain standards for dried drugs and the determination of nutritive values. There are no further reports to support the findings.

During last few decades, there has been an increasing urge in

the study of medicinal plants and their traditional uses in different parts of the world. Herbal remedies are considered as the oldest form of health care known to mankind on this earth. The traditional system of medicine that have evolved over the centuries within various communities, are still maintained as a great traditional knowledge base in herbal medicine. Physico-chemical study taken up with *C. curviflora* suggested that the drug powder has high water soluble extractive values which might be due to its high content of water soluble bioactive components.

Table 2: Data showing the biochemical content in *C. curviflora* stem powder.

S. No.	Extract	Yield* (%)
1	Crude fat	1.099 ± 0.062
2	Crude protein	13.125 ± 0.004
3	Total ash	11.416 ± 0.289
4	Moisture contents	11.333 ± 0.166
5	Dry matter	88.666 ± 0.166
6	Crude fiber	14.693 ± 0.004
7	Carbohydrate	63.027 ± 0.023

Note:* Mean of 3 readings ± SEM

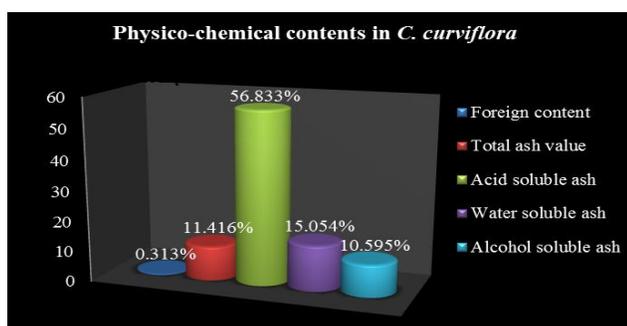


Fig 3: Showing the physicochemical values of *C. curviflora* stem powder.

4. Conclusion

Chassalia curviflora (Wall. ex Kurz.) Thwaites is an ethno-medicinal plant belongs to coffee family. The physicochemical and biochemical analysis of the crude drug powder of stem reported in this research work could be further useful to evaluate pharmacognostical properties of this plant.

5. Acknowledgement

Financial support from Indian Council of Agricultural Research through the World Bank funded National Agricultural Innovation Project (NAIP/Com-4/C2066/2007-2008) and National fund project (NFBSFARA/BSA-4004/2013-14) is thankfully acknowledged.

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