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PROXIMATE ANALYSIS AND STANDARDIZATION OF LEAVES: *LEPTADENIA RETICULATA* (RETZ) WIGHT AND ARN. (JEEVANTI)

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ABSTRACT

Leptadenia reticulata (Retz) wight & Arn belongs to family Asclepiadaceae is an important medicinal plant comes under "Jeevaniya Dravya Ghana. This herb is used as Rasayana drug and having different potentials against human wellbeing. The aim of the present study was to investigate (a) nutritional values, (b) physico- chemical parameters, (c) phytochemical classes, (d) development of Thin Layer Chromatography and (e) anti-oxidant activity of the leaves and tender stalk of *L. reticulata.*

According to the results, 16 61 of total ash, 2.8% of acid insoluble ash, 5.9% of water-soluble ash, 35.8% of protein, 2.8% of crude fat, 23.4% of carbohydrates, 14.23 of dietary fiber, 1.5% of magnesium, 0.03% of iron and 0.97% of calcium were present in the leaves and tender stalk of *L. reticulata.* Antioxidant potential of leaves and tender stalk of *L. reticulata* (IC_{50}: 18.56 ± 0. 29 µg/mL) was lower than that of L -ascorbic acid (IC_{50}: 6.10 ±0.21 µg/mL). Presence of phenolic compounds and flavonoids may be contributed for the observed antioxidant activity. In conclusion, this study reveals that *L. reticulata* is a good source for essential nutrients with medicinal properties.

KEYWORDS: *Leptadenia reticulata*, proximate analysis, phytochemical classes, physico-chemical parameters, anti-oxidant activity, fingerprint profiles.

INTRODUCTION

Herb is a concentrated food that provides nutritional value like vitamins, minerals along with health benefits to the human body. It is estimated that there are 2, 50,000 species of higher plants on earth of which more than 80,000 are medicinal [1]. Herbs are more compatible with body because of their effects; therefore they are more suitable, especially in case of long consumption.[2] *Leptadenia reticulata* (Retz) Wight and Arn. belonging to family: Asclapadeciae, commonly named as Jeevanti, comes under "Jeevaniya Dravya ghana" according to Ayurvedic texts. *L. reticulata* is found in most of the parts in India: in Gujarat, Maharashtra, sub-Himalayan tracts from Punjab to Sikkim and Khasi hills with ascending up to an altitude of 900 m. [3]

L. reticulata is a twining shrub; stems with corky deeply cracked bark; with an ash coloured or buff white exterior, bears vertically elongated lenticels, whitish smooth interior and possesses camphor like smell. Branches numerous, leaves thinly coriaceous or lanceolate with hairy surface and leathery texture. 3.8-7.5 by 2- 4.5 cm, ovate, acute, glabrous above, more or less finely pubescent (specially on the nerves) beneath, base rounded or subcordate (rarely sub-acute); petioles 6-20 mm. long, puberulous. Flowers are greenish – yellow. [4] Mainly, the roots and the whole plant are used for medicinal purposes. [5] Its flowers and tender leaves are used as vegetable. [6] Most of the herbal preparations contain *L. reticulata* with other medicinal herbs and they have property of improving the health of the body. *L. reticulata* is considered to be a tonic (Rasayana) drug and is used to vitalize, nourish and rejuvenate the body. [7] It is mainly advisable to those who suffer from weak, debility or a lack of energy. It has great value in general debility, involuntary seminal discharge, as a stimulant and snake bite. [8] tonic, restorative, wound healer and in mouth ulcer[9]. In addition, *L. reticulata* exhibited antiepileptic. [10] hepatoprotective. [11] anti-anaphylactic. [12] activities in animal models and antibacterial activity[13]. The aim of the present study was to investigate (a) nutritional values, (b) physico- chemical parameters, (c) phytochemical classes, (d) development of Thin Layer Chromatography and (e) anti-oxidant activity of the leaves and tender stalk of *L. reticulata.*

MATERIALS AND METHODS

Plant material

L. reticulata plants leaves and the tender stalks of *L. reticulata* (Jeevanti) were collected from

Jamnagar, India and dried plant materials were authenticated by Botanist, Vidyaratnam Research and Development center, Kerala, India, according to the standards of Ayurveda Pharmacopeia in India.

Determination of protein, crude fat, carbohydrates, dietary fiber, magnesium, calcium and iron contents of *Leptadenia reticulata* leaves and the tender stalks

AOAC methods were used to investigate the protein, crude fat, carbohydrates, dietary fiber, magnesium, calcium (921.01:2000) and iron (AOAC, 999.11:2000) [14] contents.

Determination of physico-chemical parameters of *Leptadenia reticulata* leaves and tender stalks

Physico-chemical parameters were determined according to methods described in guide lines of WHO (2000) [15].

Hot water extractable matter

Accurately weighed 4.0 g leaves and tender stalks of *L. reticulata* was placed in a glass stoppered conical flask. Water (100 mL) was added to the flask and it was weighed to obtain the total weight, including the flask. Then, the flask was shaken well and allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h. Then it was cooled and weighed. The weight was readjusted to the original total weight by adding required amount of water. The flask was shaken well and filtered rapidly through a dry filter paper (90mm Diameter Whatman ®). After that, 25 mL of the filtrate was transferred to a tared flat bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h, cooled in a dessicator and weighed. Finally, extractable matter was calculated.

Determination of moisture content

The powdered material (1g) was placed in a moisture dish and dried to a constant weight in an oven at 105 °C. The weight loss of the dried sample was calculated as:

$$\% \text{ Moisture Content} = \frac{\text{Weight loss}}{\text{Weight of Sample}} \times 100$$

Total ash content

The powdered material (2 g) was accurately weighed, in a previously ignited and tared crucible. The material was spread in an even layer and ignites it by gradually increasing the heat to 500-600 °C using muffle furners until it turned into white ash, indicating the absence

of carbon. The crucible was cooled in desiccators and weighed. The content of total ash in the dried material was calculated as:

$$\% \text{ Total Ash} = \frac{\text{Total Ash Weight x 100}}{\text{Weight of Sample}}$$

Acid-insoluble ash content

HCl (2M, 25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min using a hot plate. The watch glass was rinsed with 5 mL of hot water and the rinsed contents added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the acid insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight.

$$\% \text{ Acid Insoluble Ash} = \frac{\text{Acid Insoluble Ash Weight x 100}}{\text{Weight of Sample}}$$

Water soluble ash content

Water (25 mL) was added to the crucible containing the total ash and boiled for 5 min. The water insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The weight of this residue was subtracted from the weight of total ash and the content of water soluble ash calculated.

$$\% \text{ Water Soluble Ash} = \frac{\text{Total Ash Weight- Water Insoluble residue x 100}}{\text{Weight of Sample}}$$

Screening of preliminary phytochemical compounds in *Leptadenia reticulata* leaves and tender stalks

The qualitative phytochemical tests were performed for phenolic compounds, saponins, glycosides and flavanoids using water extract and ethanolic extract (both hot and cold) according to the method described by Fansworth (1996) [16] with some modifications.

Development of Thin Layer Chromotograply (TLC) fingerprints of *Leptadenia reticulata* leaves and tender stalks

Methanol (50 ml) was added to 4.0 g of the sample and stirred well for 30 min. The extract was thenfiltered through a funnel and the filtrate evaporated using a rotovapour (Buchi, B-480) and the residue was redissolved in 20 mL methanol. Each extract (2 and 4 µL) was spotted on TLC plates.

Adsorbent : Silica gel-GF_{254}

Solvent system : ethyl acetate: dichloromethane: cyclohexane (0.5:3.5:1 v/v/v).

Detection

Direct visualization : Anisaldehyde was sprayed to the TLC plate and heated at 105 ^{o}C for 5 min.

Scanning : Densitometer (CS – 9301PC, Shimadzu, Japan at 254 nm (Before spraying)

Determination of antioxidant activity of *Leptadenia reticulata* leaves and tender stalks by 2, 2 – diphenyl - 1 – picrylhydrazyl (DPPH$^{\cdot}$) scavenging assay

The antioxidant activity was determined by measuring the remaining concentration of DPPH$^{\cdot}$ as described by Navarro et al (1993) [17] with some modifications[18]. In this assay, known concentrations of (0 - 100 µg/mL) *L. reticulata* methanolic extract, butylated hydroxyl toluene (BHT) and L – ascorbic acid were prepared in different test tubes by adding MeOH up to 1.5 mL. Three milliliters of methanolic solution of DPPH (2 mg/100 mL in MeOH) were added to these tubes and shaken vigorously. The tubes were allowed to stand at room temperature for 5 min. and the absorbance was measured at λ 517 nm. Control was prepared as above by adding MeOH instead of test solution. Both BHT and L – ascorbic acid served as positive controls. This experiment was done in triplicates. The percentage of radical scavenging activity (RSA) was calculated using the following equation;

Percentage of RSA = $[(A_0 - A_s)/ A_0] \times 100$

Where A_0 is the absorbance of the control and A_s is the absorbance of the sample at λ 517 nm. IC_{50} values denote the concentration of sample required to scavenge 50% DPPH$^{\cdot}$ free radicals.

Quantitative determination of total polyphenolic content of *Leptadenia reticulata* leaves and tender stalks

The total polyphenolic content was estimated according to the Folin – Ciocalteu method[19]. Known concentrations of *L. reticulata* methanolic extract (0.1 mL) was diluted with distilled water (0.9 mL) and mixed with 5 mL of 10 fold diluted solution of Folin – Ciocalteu reagent. Four milliliters of saturated sodium carbonate solution was added to the above mixture and shaken. The absorbance of the reaction mixture was measured at λ 765 nm after 2 h. Total phenolic content was expressed as Gallic acid equivalents (mg gallic acid/g extract).

Quantitative determination of total flavonoid content of *Leptadenia reticulata* leaves and tender stalks

The total flavonoid content was determined using the Dowd method as described by Meda et al (2005). [20] In this experiment, 5 mL of 2 % $AlCl_3$ in methanol was mixed with the same volume of *L. reticulata* methanolic extract in known concentrations. After 10 min. the absorbance of the reaction mixture was measured at λ 415 nm. Total flavonoid content was expressed as quercetin equivalents (mg quercetin/g extract).

Statistical analysis

Data were analyzed by using Mann Whitney test and findings of $P < 0.05$ was considered to indicate statistical significance. All data were presented as Mean ± SEM. All the values were express as dry weight of the sample and they were performed in triplicates.

RESULTS AND DISCUSSION

Epidemiological evidences have shown that consumption of reasonable amount of dietary fiber (20 – 35g/day) lower risk of a number of chronic diet related diseases such as diverticular disease, coronary heart disease, obesity, type 2 diabetes mellitus, irritable bowel syndrome, etc (Abidemi, 2013). Present study revealed that 14.2% of dietary fiber contain in *L. reticulate.* Accordingly, above diseases can be easily delayed by consuming tender leaves of this plant. Further, *L. reticulata* is rich in carbohydrate (23.4%) and it will be stated as good energy source. According to results, the plant is rich in protein and crude fat (35.8% and 2.8% respectively).This makes them good for health especially in debilitated patient who has proper digestive capacity. Physico-chemical parameters and phytochemical screening is helped to define the amount of soluble constituents in medicinal plant material and they helpful in determining the quality, purity and detecting adulteration or improper handling of a crude drug. Total ash value of the plant was 16.6%. On incineration, crude drugs normally

leave an ash consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium and it involves the oxidation of the component of the product. A high ash value is an indicative of contamination, substitution and adulteration. The water soluble ash is the good indicator either previous extraction of water soluble salts. The value of water soluble ash was found to be 5.90. The acid insoluble ash value is not much high and it indicates contamination with earth and sand (Prabhu et al, 2012).

Leaves and tender stalks of *L. reticulata* contain variety of phytoconstituents. Both water and ethanolic extracts (hot and cold) revealed the presence of phenolic compounds, steroid glycocydes, tannins and coumarins. Saponins were present in hot ethanolic, hot, and cold-water extracts. Alkaloids were present only in hot ethanol extract. Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action (Frankel, 1995). The radical scavenging activity of methanolic leaf extract of *L. reticulata* was (IC_{50}: 18.56 ± 0. 29 µg/mL) lower than that of L – ascorbic acid (IC_{50}: 6.40 ±0.21 µg/mL) and BHT (IC_{50}: 10.00 ±0.29 µg/mL). The mean total polyphenolic content and mean total flavonoid contents of *L. reticulata* were 55.6 ± 0.50 mg gallic acid equivalents/g extract and 22.9 ± 0.80 mg quercetin acid equivalents/g extract respectively. Free radicals such as oxygen, superoxide and hydroxyl are biologically important substances which naturally release from human tissues. The highly reactive radicals can cause oxidative damage to DNA, lipids and proteins (Boveris et al, 2007; Fritz et al, 2003). Therefore, free radicals result in many disorders like cancer, cardiovascular diseases and diabetes mellitus (Velioglu et al, 1998; Vaya and Aviram, 2001). Main compounds carried out free radical scavenging are substances having antioxidant activity such as flavonoid and phenolic compounds or phenolic-rich plant extracts. Some possible mechanism of action by which Rasayana can be correlated in terms of modern scenario are as antioxidant action and nutritive function (Singh et al, 2014). At present, people use to buy many market products for keep health and beauty. Though, several modern and synthetic drugs are available in the market against to the oxidative damages of the tissues, and they may have some adverse effects than the natural. Consuming natural antioxidants is an alternative solution to this problem as they contain large amounts of phenolic compounds, high antioxidant properties and free radical scavenging activities.

Table 1: Nutritional values and mineral contents in leaves and tender stalks of *Leptadenia reticulate.*

	Percentage (%)
Protein	35.76±0.49
Crude Fat	2.83±0.14
Carbohydrate	23.4±0.45
Dietary fiber	14.23±0.14
Magnesium	1.46±0.02
Iron	0.03±0.00
Calcium	0.97±0.6

Values are expressed as mean ± SEM., n = 3

Table 2: Physico-chemical parameters in leaves and tender stalks of *Leptadenia reticulata*

Physico-chemical parameters	% w/w(Dry wt basis) *Leptadenia reticulata*
Total ash	16.61 ± 0.3
Acid insoluble ash	2.83 ± 0.2
Water soluble ash	5.90 ± 0.7
Hot ethanol extractable matter	13.11 ± 0.4
Cold ethanol extractable matter	6.54 ± 0.1
Hot water extractable matter	31.52 ± 0.4
Cold water extractable matter	5.94 ± 0.1

DWB – Dry Weight Basis

Values are expressed as mean ± SEM., n=3

CONCLUSION

The present study could be used as a diagnostic tool for the standardization and identification of *L. reticulata* for their safe use and is helpful in preventing its adulteration. Further, this reveals the important role of medicinal plant for the maintenance of healthy life and normal body function.

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