

Short Communication

PROXIMATE COMPOSITION ANALYSIS OF TWO MEDICINALLY IMPORTANT PLANTS  
*ACHYRANTHES ASPERA* AND *CISSUS QUADRANGULARIS*

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ABSTRACT

**Objective:** Nutritive status of components of two medicinally important plants *Achyranthes aspera* (seeds) and *Cissus quadrangularis* (stem) was determined. These plants have been evaluated for proximate principles to utilize them as a new dietary supplement.

**Methods:** Finely grounded samples of selected plants were subjected to chemical analysis by AOAC (1995). The total nitrogen content was determined following the standard Kjeldahl's method using Kel Plus Semi Automatic Nitrogen Analyzer (Pelican Equipments). Determination of ether extract (EE) was done with the help of Soxhlet's apparatus. For determination of dry matter (DM), crude fiber (CF), nitrogen detergent fibre (NDF), acid detergent fiber (ADF) and total ash (TA), standard conventional procedures were followed. The conventional Weende's method was followed for the calculation of nitrogen free extract (NFE). The method of Talapatra *et al.* (1940) was followed for the determination of calcium and phosphorus.

**Results:** The CP, CF, TA, NDF, Ca and P of *Achyranthes aspera* (seeds) and *Cissus quadrangularis* (stem) respectively were found to be 18.91 and 20.19, 21.03 and 19.19, 2.86 and 1.53, 27.00 and 21.00, 1.98 and 4.28 and 0.49 and 0.47.

**Conclusion:** The evaluation revealed that these plant parts may prove a good dietary supplement for their medicinal use as reported and can compensate the traditional sources which are in a shrinking state.

**Keywords:** Proximate analysis, *Achyranthes aspera*, *Cissus quadrangularis*.

India has a rich wealth of plants. India recognizes more than 2500 plant species which have medicinal values. Plants are like natural laboratories where a great number of chemicals are biosynthesized and in fact they may be found to be the most important source of chemical compounds. To use these medicinal plants as a supplement for improvement in health status in the determination of nutritive value is a basic requirement. Proximate analysis system is the conventional method to determine the elementary nutritional characteristics of plants.

*Achyranthes aspera* (Amaranthaceae) and *Cissus quadrangularis* (Vitaceae) popularly known as Apamarga and Hadjora (veld grape), respectively, are commonly available plants in India and are reported to possess various medicinal properties in Ayurveda an Indian system of medicine. These plants have been used traditional by many people in India, for the treatment of various ailments, but the report on its nutritive composition is scanty. The aim of this study was to determine the proximate composition (nutritive composition) of *Achyranthes aspera* and *Cissus quadrangularis* and thus amass data to support and encourage its usage in human nutrition and in disease treatment. The chemical composition of the plant gives its potential nutritive value hence in the assessment of quality of a plant the proximate principles are first determined by AOAC (1995) [1].

The Weende proximate analysis system includes the determination of moisture (DM=dry matter), ether extract (EE=crude fat), crude protein (CP), ash (TA), crude fiber (CF) and nitrogen free extract (NFE). Determination of calcium and phosphorus was done by the method of Talapatra *et al.* (1940) [3].

Healthy stem of *Cissus* was collected from various parks of Bikaner where it is cultivated as an ornamental plant whereas seed samples of *Achyranthes* were purchased from the shop of herbal medicine. The fresh sample of *Cissus* was immediately packed in tight polythene bags to avoid loss of moisture and purchased seeds of *Achyranthes* were dried separately at 100±5 °C to estimate the dry matter. The samples were taken in triplicate.

Determination of dry matter (DM)

The moisture of the sample is lost by volatilization caused by heat. The amount of material left after the removal of the moisture is the dry matter.

Procedure

Dishes were washed and dried overnight in an oven at 105 °C. These dishes were placed in desiccator, cooled, and weighed. Different 2.0 gm. of sample was weighed and placed into an oven at 105 °C for overnight. Dishes were removed, covered on the top and placed in desiccators and cooled. Removed from desiccator and weighed as quickly as possible.

Calculation

$$\text{Dry matter (\%)} = \frac{(\text{Wt.of dish} + \text{wt.of dried sample}) - \text{wt.of dish}}{\text{wt of sample before drying}} \times 100$$
$$= \frac{\text{Wt of dry sample} \times 100}{\text{wt of sample before drying}}$$

$$\text{Moisture content (\%)} = \frac{\text{Wt.of fresh sample} - \text{wt.of dry sample}}{\text{wt of fresh sample}} \times 100$$

Determination of Ash (TA)

The sample was ignited at 600 °C to burn off all organic material. The inorganic material which does not volatilize at that temperature was termed as ash.

Procedure

Clean crucibles were placed under a muffle furnace at 600 °C for one hour. Crucibles were moved from the furnace to a desiccator and cooled to room temperature. These crucibles were weighed quickly to prevent moisture absorption. 2.0 gm. of sample was added into dried silica crucibles. Placed in a muffle furnace and the temperature were held at 600 for 6 hours. The crucibles were taken to a desiccator and cooled to room temperature. After cooling crucibles, were weighed as quickly as possible to prevent moisture absorption. The ash sample was saved for mineral determinations.

**Calculation**

$$\text{Ash (\%)} \text{ on partial dry or as fed basis} = \frac{\text{Wt. of ash}}{\text{wt of sample}} \times 100$$

$$\text{Adjusting to dry basis} = \frac{\text{ash \% on as sample}}{\text{dry matter \% of as sample}} \times 100$$

**Acid Soluble and Insoluble Ash**

The residue left after boiling after total ash with 50% HCl is called acid insoluble ash and it is largely silica. The ash is digested with dilute HCl to dissolve inorganic salts.

**Procedure**

The residue obtained from the ash determination was used. Boiled with 20 ml 50% HCl being careful to avoid spattering filtered through ash less filter paper, and washed with hot water until acid-free. Filter paper and residue both was transferred into a dry porcelain dish and placed in a muffle furnace at 600 °C for 2 hours.

**Calculation**

$$\text{Ash Insoluble ash (\%)} = \frac{\text{Wt. of acid treated ash}}{\text{wt of sample}} \times 100$$

**Determination of Crude Fiber (CF)**

A moisture-free and ether extracted sample was digested first with a weak acid solution, then a weak base solution. The organic residue was gathered in a filter crucible. The loss of weight on ignition is equivalent to the crude fiber.

**Procedure**

About 2 gm. of the dried, fat-free sample was taken into a 600 ml beaker. 200 ml of hot sulphuric acid was added and the beaker was placed under the condenser and boiled gently for exactly 30 min. Distilled water was used in order to maintain volume and to wash down particles sticking to the sides. Filtered through Whatman No. 541 paper in a buchner funnel, using suction. Washing with boiling water has been provided. Residue was transferred back to the beaker and 200 ml hot sodium hydroxide solution was added. Replaced under the condenser and again brought to boiling within 1 min. After boiling for exactly 30 min, it was filtered through a porous crucible and washed with boiling water; 1% hydrochloric acid and then again with boiling water. Mashing was given twice with alcohol or acetone, dried overnight at 100°C, cooled and weighed. Ashed at 500°C for 3 hours, cooled and weighed. The weight of fiber was calculated by the difference in weight.

**Calculation**

Crude fiber (% of fat-free DM)

$$= \frac{(\text{Wt. of crucible+dried residue}) - (\text{Wt. of crucible+ash residue})}{\text{wt of sample}} \times 100$$

**Determination of crude fat (EE) (Soxhlet Method)**

Ether was continuously volatilized, then condensed and allowed to pass through the sample, extracting ether soluble materials. The extract was collected in a flask. When the process was completed, the ether was distilled and collected in another container and the remaining crude fat was dried and weighed.

**Procedure**

2 to 3 gm. of the dried sample was weighed and filled in the thimble. It was placed inside the soxhlet apparatus. Dry pre-weighed solvent flasks were connected beneath the apparatus and the required quantity of solvent was added and connected to the condenser. The heating rate was adjusted to give a condensation rate of 2 to 3 drops and extracted for 16 hours. On completion of this process thimble was removed and the apparatus was reclaimed ether using. Removal of ether was performed on a boiling water bath and flasks were dried at 105°C for 30 minutes. Cooled in desiccator and weighed.

**Calculation**

$$\text{Crude fat (\% of Dry matter)} = \frac{\text{Wt. of fat}}{\text{wt of sample}} \times 100$$

**Determination of crude protein (CP) (Kjeldahl Method)****Procedure**

Accurately 1 gm of sample was weighed and put in a digestion flask. 10 gm potassium sulphate, 0.7 gm mercuric oxide and 20 ml sulphuric acid were added. The flask was heated gently at an inclined angle until frothing subsides and then boiled until the solution clears. Boiling went on for an additional half hour. On cooling, about 90 ml. Distilled water was added, re cooling was done, 25 ml. Sulphide solution was added and mixed. Small pieces of boiling chip added at prevent bumping and 80 ml. of sodium hydroxide solution while tilting the flask so that two layers were formed. The condenser unit was connected rapidly, heated, and collected distilled ammonia in 50 ml. Boric acid/indicator solution. 50 ml of distillate was collected. On completion of distillation; the receiver was removed and titrated against standard acid solution.

**Calculation**

Nitrogen content of sample (%)

$$= \frac{\text{ml acid X Normality of standard acid}}{\text{wt of sample (gm)}} \times 0.014 \times 100$$

Crude protein content (%) = Nitrogen content X 6.25

Vishwakarma and Dubey (2011) [4] also determined the nutritive value of *Achyranthes aspera* and *Cissus quadrangularis* whole plant containing about 4.37 and 3.97 percent crude protein respectively which are much lesser than the findings obtained for stem of *Cissus quadrangularis* and seeds of *Achyranthes aspera* in this investigation. Tahira et al (2012) [2] reported nutritive value of *Achyranthes aspera* (Apamarga) containing about 25.99 percent crude protein which is higher than the CP values obtained in the seeds under study.

**Determination of nitrogen free extracts (NFE)****Procedure**

Nitrogen free extract of a sample was determined by difference after the analysis has been completed for ash, crude fiber, crude fat and crude protein.

**Calculation**

NFE on dry basis = 100% - (% ash on dry basis + % crude fiber on dry basis + % crude fat on dry basis + % protein on dry basis)

**Determination of neutral detergent fiber (NDF)****Procedure**

1 gm of air dried meshed sample was taken. 100 ml preheated neutral detergent solution, 2 ml of decalin and 0.5 gm sodium sulphite were added in order and refluxed for 60 min. regent was filtered off, washed thrice with hot distilled water under vacuum, vacuum removed, the mat was broken up and crucible was washed with hot water. Washing was given twice with acetone and suck dry. Crucible was dried at 100 for 8 hours and weighted. Yield of recovered N. D. F. was reported as the cell wall constituent.

**Calculation**

1. Cell wall constituents (%) (N. D. F.)

$$= \frac{(\text{Wt. of crucible+cell wall constituents}) - \text{Wt. of crucible}}{\text{wt of dry sample}} \times 100$$

2. Cell contents (%) = 100 - Cell wall constituents

3. Insoluble ash in neutral detergent (%)

$$= \frac{(\text{Wt. of crucible+ash}) - \text{Wt. of crucible}}{\text{wt of dry sample}} \times 100$$

**Determination of Acid Detergent fiber (ADF)****Procedure**

1 gm air dried meshed sample was taken. 100 ml acid detergent solution, 2 ml of decalin was added in order. Heated to boiling for 5-10 minutes. Heat was reduced as boiling begins and refluxed for 60 minutes from the onset of boiling. The regent was filtered off,

washed thrice with hot distilled water under vacuum. Again washed twice with acetone in the same manner and suck dried. Crucible was dried at 100°C for 8 hours and weighted.

#### Calculation

$$\text{Acid detergent fiber (\%)} = \frac{(\text{Wt. of crucible+fiber}) - \text{Wt. of crucible}}{\text{wt of dry sample}} \times 100$$

#### Determination of Calcium

##### Procedure

From the stock solution of acid soluble ash, 25 ml. of aliquot was taken in 250 ml. beaker. 50 ml. of distilled water, 10 ml. of freshly

prepared saturated Ammonium oxalate solution and 10 ml of concentrate hydrochloric acid was added. 2 drops of alcoholic methyl red indicator were added. Acidity was adjusted to pH 4.6 by adding drop by drop concentrated ammonium solution till an orange brown color precipitate begins to appear. Dilute ammonium solution (1:4) was added drop by drop till a white color precipitate appears. Content of beaker was kept overnight to allow the precipitate to settle down and the supernatant was filtered through Whatman filter paper No. 42. The precipitate was washed with hot distilled water several times to eliminate an excess of oxalates. The filter paper was then transferred to the same beaker and 100 ml of hot distilled water was added. It was dissolved in 10 ml of conc. H<sub>2</sub>SO<sub>4</sub> and was heated at 60-70°C then titrated against N/10 KMnO<sub>4</sub> until stable pink color appeared.

**Table 1: Chemical composition of *Cissus quadrangularis* and *Achyranthes aspera***

Parameter	<i>Cissus stem</i> (in %)	<i>Achyranthes seeds</i> (in %)
Dry Matter (DM)	97.54±0.94	96.2±0.88
Crude Protein (CP)	20.19±0.09	18.91±0.03
Crude Fiber (CF)	19.19±0.05	21.03±0.06
Ether Extract (EE)	02.55±0.02	02.57±0.04
Total Ash (TA)	01.53±0.01	02.86±0.01
Nitrogen Free Extract (NFE)	55.02±0.11	51.75±0.14
Neutral Detergent fiber (NDF)	21.00±0.52	27.00±0.47
Acid Detergent fiber (ADF)	12.33±0.23	13.00±0.28
Calcium (Ca)	04.28±0.01	1.98±0.03
Phosphorus (P)	0.47±0.20	0.49±0.35
Organic Matter (OM)	98.48±0.29	97.14±0.24
Total Carbohydrate (TC)	74.21±0.16	72.98±0.20

# Results are mean of triplicate determinations±SD

#### Calculation

$$\text{Calcium (\%)} = \frac{\text{ml of } \frac{N}{10} \text{ KMnO}_4 \text{ used} \times 0.02 \times 10}{\text{Gm of sample taken for ashing}} \times 100$$

#### Determination of phosphorus (P)

##### Procedure

From the stock solution of acid soluble ash, 25 ml. of aliquot was taken in 250 ml. beaker. 10 ml. of concentrated Nitric acid and 10 ml. of freshly prepared saturated Ammonium molybdate solution was added. At this stage, yellow colored precipitate of phosphorus-ammoniummolybdate began to appear. The beaker was kept overnight to allow the precipitate to settle down and the supernatant was filtered through Whatman filter paper No. 42. The precipitate was washed with two three times with 2% Nitric acid solution and several times with 3% Potassium nitrate solution, until the precipitate was free of acid. The filter paper was subsequently transferred to the same beaker and was dissolved in 25 ml of N/10 Sodium hydroxide solution. 2 drops of Phenolphthalein were added and titrated against N/10 standard Nitric acid solution. The phosphorus content was calculated as follows.

#### Calculation

$$\text{Calcium (\%)} = \frac{\text{ml of } \frac{N}{10} \text{ NaOH used} \times 0.001347 \times 10}{\text{Gm of sample taken for ashing}} \times 100$$

#### Determination of total carbohydrate (TC) and organic matter (OM)

The total carbohydrate was calculated by formula **TC = CF+NFE**

Organic matter **OM = CP+CF+EE+TA+NFE**

The average chemical composition of the samples of both plants (*Cissus quadrangularis* and *Achyranthes aspera*) (table 1), reveals the variation in the chemical composition of both plants. The crude protein content on the dry matter basis was found to be higher in *Cissus quadrangularis* (20.19), in comparison to *Achyranthes aspera* (18.91). The ether extract in both plants was found to be almost similar.

The CF (Crude fiber) content of these plants was on higher side having 19.19 in *Cissus* and 21.03 in *Achyranthes*. Total ash values were found to be higher in *Achyranthes aspera* (2.86) in comparison to *Cissus quadrangularis* (1.53). Nitrogen free extract (NFE) value was also found higher in *Cissus quadrangularis* (55.02) in comparison to *Achyranthes aspera* (51.75) this could be attributed to higher concentration of crude fibre present in *Achyranthes aspera*. Neutral detergent fibre (NDF) and Acid detergent fibre (ADF) were considerably lower in *Achyranthes aspera* (27) in comparison to *Cissus quadrangularis* (21). The total carbohydrate and organic matter were comparable in *Cissus quadrangularis* and *Achyranthes aspera*. Calcium is found considerably higher in *Cissus quadrangularis* (4.28) whereas phosphorus content was comparable in both plants.

On the basis of results of nutritive value it could be opined that stem of *Cissus quadrangularis* and seeds of *Achyranthes aspera*, may prove to be good dietary supplement due to their rich CP and CF content and can compensate the traditional sources which are in shrinking state. The result also shows that these plants can provide essential nutrients to human beings and animals though feeding trials are required to verify. Authors are thankful to University grant Commission (UGC) for providing funds to pursue this research work.

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