

ASSESSMENT OF MEDICINAL PLANTS THROUGH PROXIMATE AND MICRONUTRIENTS ANALYSIS

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Abstracts: The leaves, roots, bark and fruits of medicinal plants have various health-promoting effects on human and animals. These materials may be suitable singly or in combination as therapeutic agents and are important raw materials for manufacturing traditional and modern medicines. Indigenous medicinal plants have been playing a significant role in the economy of our country. Proximate compositions of seeds, aerial parts and roots of amla (*Embllica officinalis*), Bahera (*Terminalia belerica*) and Harad (*Terminalia chebula*) of indigenous origin were determined. The mineral contents [Iron (Fe), Copper (Cu), Zinc (Zn) and Manganese (Mn)] from the fruit pulp of these plants were determined. The moisture content (%), crude fat (%), ash (%), crude protein (%), crude fibre (%) and total carbohydrates (%) were evaluated in the proximate composition. It was found that the overall proximate composition in seeds was highest when compared to aerial parts and roots. Therefore, fruits of Amla, Bahera and Harad have good nutritional value and hold their potential for nutraceutical development.

Keywords: Medicinal plants, Micronutrients, Modern, Traditional

INTRODUCTION

Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practiced in India. Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.

Medicinal plants continue to play a vital role in the healthcare system of large proportions of the world's population [1]. Different herbs are obtained from different parts of the plant they may come from roots, leaves, barks, seeds or flowers of a plant [2].

Triphala is a widespread polyherbal drug, which has been used to treat a number of diseases in the traditional systems since the ancient times. Triphala is a composite mixture of three herbs Amalaki (*Embllica officinalis*), Haritaki (*Terminalia chebula*) and Vibhitaki (*Terminalia belerica*) also known as the 'three myrobalans'. *Embllica officinalis* Gaertn. belongs to Euphorbiaceae, and *Terminalia chebula*

Retz. *Terminalia belerica* belongs to Combretaceae family.

Different parts of *Terminalia chebula* Retz, *Terminalia belerica* Roxb, and *Embllica officinalis* Gaertn are widely used in the Indian traditional system of medicine [3]. The half ripe fruit of *T. belerica* and the pericarp of *T. chebula* fruit were reported to be purgative [3]. The fruit of *T. chebula* was traditionally used to cure asthma, urinary disorders, heart disease and it has cardiotonic activity [4,5]. In Ayurveda, the fruit of *E. officinalis* is used as a cardiotonic, cerebral and intestinal tonic [6], and it is also reported to have anticancer properties [7,6]. The fruit of *E. officinalis* is a rich source of vitamin C, a well-known antioxidant [8]. The crude extract of *E. officinalis* was reported to counteract the hepatotoxic and renotoxic effects of metals [7] due to antioxidant properties.

Embllica officinalis is commonly called the 'Indian gooseberry'. It belongs to the family Euphorbiaceae, and is known as Amla in Hindi, and Amalaki in Sanskrit. It is a small to medium-sized tree with a crooked trunk and spreading branches, and grayish-green bark that peels off in flakes. The branchlets are glabrous or finely pubescent, 10–20 cm long, usually deciduous, with the leaves simple, subsessile, and closely set along the branchlets. The leaves are light green, resembling pinnate leaves. The flowers are greenish-yellow, born in axillary fascicles, and give way to globose fruit. The fruits are depressed globose in shape, 1–2.5 cm in diameter, fleshy, and obscurely six-lobed, containing six trigonous seeds. They are green when unripe, and turn light yellow or brick red when mature.

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Terminalia chebula is a medium to large deciduous tree growing to 30 m (98 ft) tall, with a trunk up to 1 m (3 ft 3 in) in diameter. The leaves are alternate to sub opposite in arrangement, oval, 7–8 cm (2.8–3.1 in) long and 4.5–10 cm (1.8–3.9 in) broad with a 1–3 cm (0.39–1.18 in) petiole. They have an acute tip, cordate at the base, margins entire, glabrous above with a yellowish pubescence below. The fruit is drupe-like, 2–4.5 cm (0.79–1.77 in) long and 1.2–2.5 cm (0.47–0.98 in) broad, blackish, with five longitudinal ridges. The dull white to yellow flowers are monoecious, and have a strong, unpleasant odor. They are borne in terminal spikes or short panicles. The fruits are smooth ellipsoid to ovoid drupes, yellow to orange-brown in colour, with a single angled stone.

Terminalia belerica (Gaertn.) Roxb. leaves are about 15 cm long and crowded toward the ends of the branches. It is considered a good fodder for cattle. *Terminalia belerica* seeds have an oil content of 40%, whose fatty-acid methyl ester meets all of the major biodiesel requirements in the USA, Germany and European Union. The seeds are called bedda nuts.

Triphala (in Sanskrit, tri = three and phala = fruits) is another important Ayurvedic medicinal preparation comprising three fruits: *Phyllanthus emblica* or *Embolica officinalis*, *Terminalia chebula*, and *Terminalia belerica*. Triphala is one of the most well-studied Ayurvedic formulations, and experiments have shown it to possess antibacterial, antifungal, free radical scavenging, antioxidant, anti-inflammatory, laxative, antiarthritic, anticataleptic, hypolipidemic, antihyperlipidemic, hepatoprotective, anti-stress, antidiabetic, antimutagenic, anticancer, chemopreventive, chemoprotective, radioprotective, and immunomodulatory properties.

Although, the medicinal properties and presence of antioxidants in these plant products are well recognized, data with regard to their chemical composition is scanty. It is necessary to evaluate the proximate and nutraceutical composition of those plants in addition to their components that promote health care. So, this present study is aimed to assess the proximate composition and nutritional parameters in different parts of Amla, Bahera and Harad viz. seeds, aerial parts and roots.

MATERIALS AND METHODS

- ❖ **Apparatus used:** In the present studies, the apparatus used are Spatula, Filter Paper, Water bath, Oven, Beaker, Test tubes, Sieve, Funnel, Measuring cylinder, Soxhlet Extraction unit, Hand grinder, Sample bottle, Detergent, Wash Bottle, Aluminium foil etc.
- ❖ **Sample collection:** The different parts of were Amla, Bahera and Harad viz. seeds, aerial parts and roots were procured from Chaudhary Charan Singh Haryana Agricultural University, Hisar.

- ❖ **Sterilization of glass wares:** All glass wares used during the experimental investigation were washed properly with detergent, rinsed with distilled water and air dried. They were also then sterilized in hot air oven by wrapping with Aluminium foil.

- ❖ **Sample preparation:** The procured plant samples were dried under shade and grinded into fine powder followed by transferring to airtight containers with proper labelling for future use.

- ❖ **Proximate analysis:** The proximate analysis (moisture, crude fibre, ash, crude fat, crude protein and Total carbohydrates) of all the samples were determined in triplicates as per the standard technique of AOAC.

Estimation of moisture content

The dried powdered samples (seed, aerial parts and roots) of Amla, Bahera and Harad (2g each) were taken in triplicates and dried initially at lower temperature (80–90°C) and finally at higher temperature (100–102°C) then weight of dried samples was noted until constant weights were obtained. The moisture content (%) was estimated using the formula as follows:

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

Estimation of crude fat content

The dried powdered samples (seed, aerial parts and roots) of Amla, Bahera and Harad (2g each) were taken in a thimble and placed in a Soxhlet extractor. Approximately 150–175 ml of petroleum ether was added up to one and a half siphons in a dried and pre-weighed round bottom flask connected to soxhlet assembly. The extraction was carried as long as for 8h. After extraction, weight of the round bottomed flask along with the extract was determined again when almost all the petroleum ether gets evaporated from RB flask upon heating. The crude fat contents (%) were calculated as follows:

$$\text{Crude Fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

Estimation of ash content

In a pre-weighed and ignited crucibles, the powdered samples (seed, aerial parts and roots) of Amla, Bahera and Harad (2g each) were transferred and placed in a muffle furnace (pre-heated 600°C). The crucibles having samples were placed directly from the furnace into a desiccator, and weight was taken after cooling. The formula for ash contents (%) is as follows:

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Initial weight of sample}} \times 100$$

Estimation of Crude protein content

The conventional microkjeldahl's method was adopted for the estimation of Crude protein content. Multiplication % of N with the factor of 6.25 is done for calculating crude protein.

Reagents

- Digestion mixture: sulphuric acid and perchloric acid were mixed in the ratio of 9:1.
- N/100 sulphuric acid
- N/100 sodium hydroxide
- 40% sodium hydroxide
- Methyl red indicator

Method

100 mg of sample was taken in 100 mL micro-kjeldahl's digestion flask. (9:1) $K_2SO_4 : CuSO_4$ (\square 1g each) was added to it followed by 10 mL conc. sulphuric acid. Continuous heating was done to obtain clear solution. The flask was cooled and the sample contents were diluted and final volume was made upto 100 mL with double distilled water. 5 mL aliquots of the digest were transferred to conical flask. 10 mL of N/100 sulphuric acid was taken in the receiving flask. Then, 10 mL of the digested sample was transferred to the steam chamber of the apparatus followed by the addition of 10 mL of 40% NaOH. Ammonia was distilled till 30-40 mL of distillate was collected in the receiving flask. The contents in the receiving flask were titrated against N/100 NaOH and volume of NaOH used was noted. The end point was reached when colour changed from pink to yellow. The titration is performed with the blank under the identical conditions.

Calculations

Amount of nitrogen (%) = A-B

Where, A = Volume of NaOH used for blank

B = Volume of NaOH used for sample

Crude Protein content (%) in sample = Nitrogen content in sample \times 6.25

Crude fibre content

The modified method of Maynard (1970) was followed for the estimation of crude fibre content (%).

Reagents

- 1) 1.25% Sulphuric acid
- 2) 1.25% Sodium hydroxide

Method

The powdered sample (seed, aerial parts and roots) of Amla, Bahera and Harad (2g each) was weighed and transferred to a clean one litre beaker and added 200 mL of 1.25% (w/v) sulphuric acid. For heating, hot plate was used to place the beaker and the contents were allowed to reflux for 30 minutes' time was noted from onset of boiling and shaking is done after every 5 min. After 30 min beaker was removed from hot plate and filtration was performed using suction through a muslin cloth. Washing of the residue was done with hot water till it became free from acid, then the material was transferred to the same beaker and after the addition of 200 mL of 1.25% NaOH solution, the contents were again refluxed for 30 min. It was followed by the filtration again through muslin cloth with the help of suction pump and to free the residue from alkali, it was washed with hot water and then transferred to a crucible and placed in hot air oven, allowed to dry to constant weight at 80-110°C

and recorded its weight. The residue was ignited in muffle furnace at 550-660°C for 2-3 h, then cooled and weighed again. The loss of weight due to ignition is weight of crude fibre.

$$\text{Crude fibre content (\%)} = \frac{\text{Wt. of crude fibre}}{\text{Original wt. of sample}} \times 100$$

Total carbohydrates content

The difference is used for the calculation of Total carbohydrates content as follows:

Total carbohydrates content (%) =

$$100 - [\text{Moisture (\%)} + \text{Crude Fat (\%)} + \text{Ash (\%)} + \text{Crude Protein (\%)} + \text{Crude fibre (\%)}]$$

Minerals content**Reagents**

1) **Diacid mixture:** $HNO_3 : HClO_4$ (4:1) just before use.

2) **Hydrochloric mixture (1%):** Conc. HCl (1 mL) in 50 mL distilled water and Distilled water was used in making V_{final} as 100 mL.

Method

The powdered sample of fruit pulp of Amla, Bahera and Harad (\square 1g) was digested with 15 mL of diacid mixture ($4HNO_3 : 1HClO_4$) in a conical flask by heating on hot plate in open space till clear white precipitates settled down at bottom of conical flask. The precipitates were dissolved in 1% HCl prepared by dilution with double distilled water, filtered and V_{final} of filtrate was made 50 mL using double distilled water.

RESULTS AND DISCUSSION**Proximate composition****Moisture content**

The statistics of moisture content in different parts of Amla, Bahera and Harad is given in Table 4.1. Moisture content in seeds, aerial parts and roots of Amla was 34.12, 7.85 and 5.36%, respectively. Moisture content in seeds, aerial parts and roots of Bahera was 32.35, 7.64 and 4.48%, respectively. The corresponding values of moisture content in Harad were 17.27, 7.58 and 3.28%, respectively.

Crude Fat content

The data of crude fat content in different parts of Amla, Bahera and Harad is given in Table 1. Fat content in seeds, aerial parts and roots of Amla was 2.95, 1.10 and 0.19%, respectively. Fat content in seeds, aerial parts and roots of Bahera was 3.44, 0.55 and 4.23%, respectively. The corresponding values of fat content in Harad were 2.97, 1.40 and 0.58%, respectively.

Ash content

The data of ash content in different parts of Amla, Bahera and Harad is given in Table 1. Ash content in seeds, aerial parts and roots of Amla was 11.28, 3.75 and 4.78%, respectively. Ash content in seeds, aerial parts and roots of Bahera was 11.75, 2.75 and 0.84%, respectively. The corresponding values of ash

content in Harad were 8.56, 2.61 and 3.48%, respectively.

Crude Protein content

The data of crude protein content in different parts of Amla, Bahera and Harad is given in Table 1. Crude Protein content in seeds, aerial parts and roots of Amla was 10.39, 13.93 and 7.63%, respectively. Protein content in seeds, aerial parts and roots of Bahera was 18.29, 3.17 and 1.71%, respectively. The corresponding values of crude protein content in Harad were 18.45, 3.70 and 2.24%, respectively.

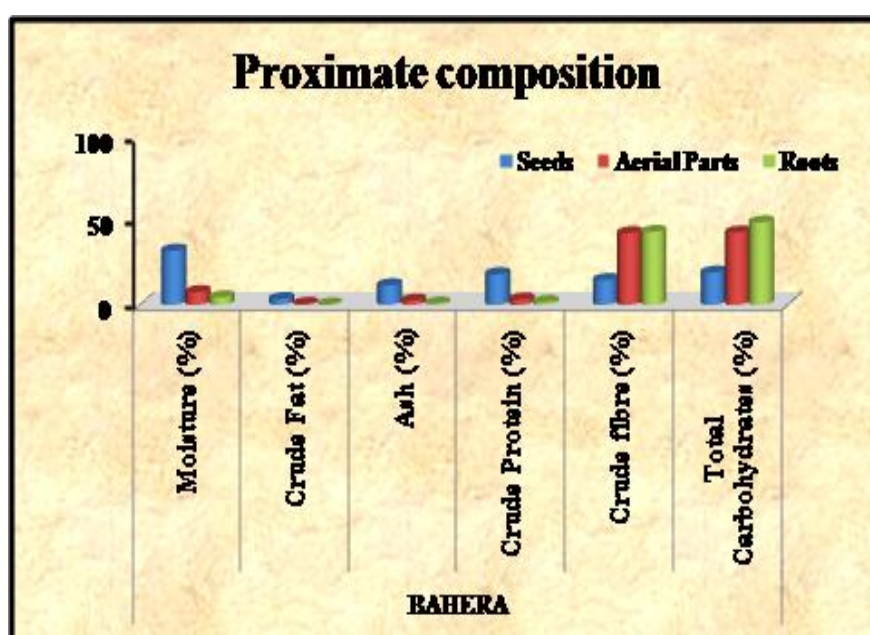
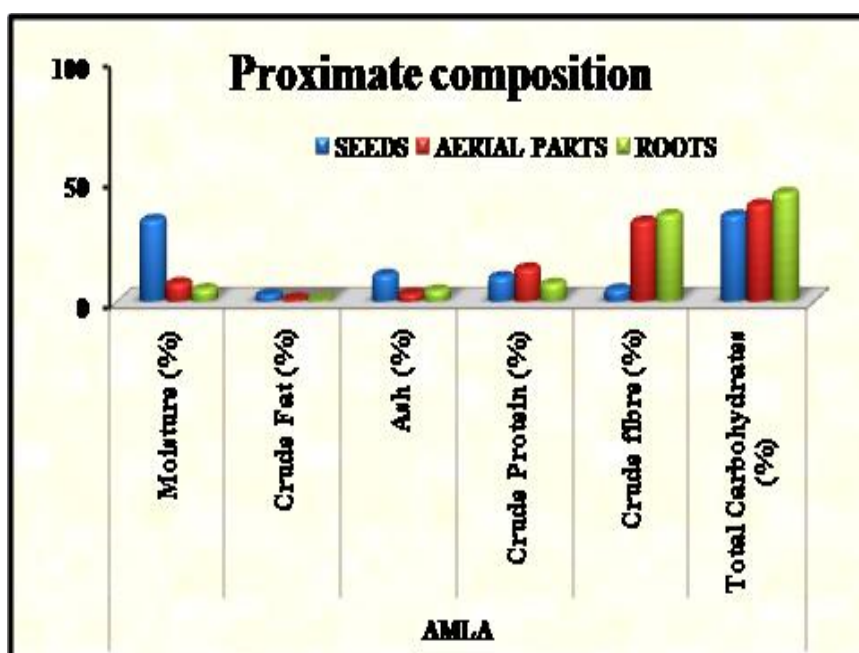
Crude fibre content

The data of crude fibre content in different parts of Amla, Bahera and Harad is given in Table 1. Crude fibre content in seeds, aerial parts and roots of Amla

was 5.23, 33.45 and 36.22%, respectively. Crude fibre content in seeds, aerial parts and roots of Bahera was 14.84, 42.85 and 43.57%, respectively. The corresponding values of crude fibre content in Harad were 16.45, 48.62 and 40.67%, respectively.

Total carbohydrates content

The data of total carbohydrates content in different parts of Amla, Bahera and Harad is given in Table 1. Total carbohydrates content in seeds, aerial parts and roots of Amla was 35.98, 40.42 and 45.30%, respectively. Total carbohydrates content in seeds, aerial parts and roots of Bahera was 19.30, 43.23 and 49.22%, respectively. The corresponding values of total carbohydrates content in Harad were 36.31, 43.49 and 49.76%, respectively.



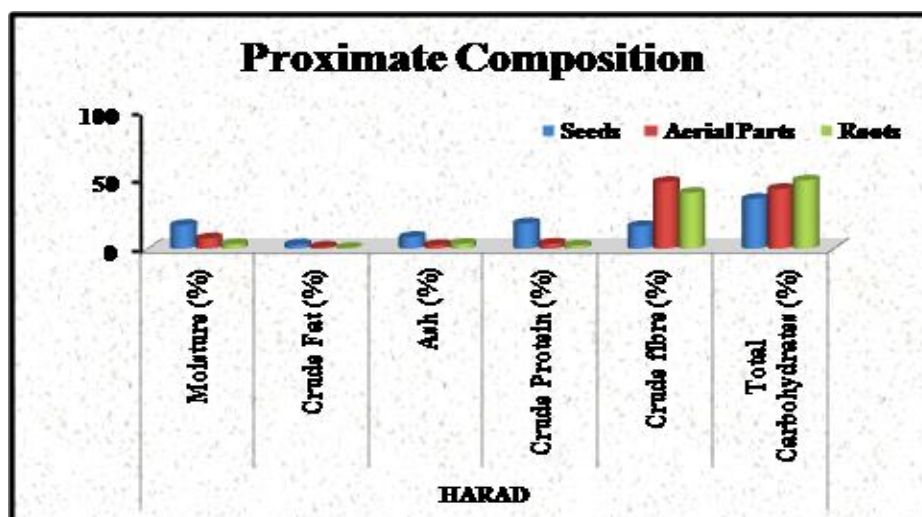


Fig. 1. Proximate composition in different parts of Amla, Bahera and Harad

Table 1. Proximate composition in different parts of Amla, Bahera and Harad

| Plant | Parameter | Seeds | Aerial Parts | Roots |
|--------|-------------------------|--------------|--------------|------------|
| AMLA | Moisture (%) | 34.12 ± 0.1 | 7.85±0.03 | 5.36±0.01 |
| | Crude Fat (%) | 2.95 ± 0.03 | 1.10±0.003 | 0.70±0.06 |
| | Ash (%) | 11.28 ±0.01 | 3.25±0.01 | 4.78±0.01 |
| | Crude Protein (%) | 10.39±0.02 | 13.93±0.02 | 7.63±0.01 |
| | Crude fibre (%) | 5.23±0.02 | 33.45±0.01 | 36.22±0.02 |
| | Total Carbohydrates (%) | 35.98±0.10 | 40.42±0.04 | 45.30±0.01 |
| BAHERA | Moisture (%) | 32.35 ± 0.03 | 7.64±0.02 | 4.48±0.01 |
| | Crude Fat (%) | 3.44 ± 0.01 | 0.55±0.01 | 0.19±0.01 |
| | Ash (%) | 11.75±0.01 | 2.56±0.02 | 0.84±0.01 |
| | Crude Protein (%) | 18.29±0.02 | 3.17±0.02 | 1.71±0.01 |
| | Crude fibre (%) | 14.84±0.02 | 42.85±0.01 | 43.57±0.01 |
| | Total Carbohydrates (%) | 19.3±0.04 | 43.23±0.02 | 49.22±0.03 |
| HARAD | Moisture (%) | 17.27± 0.01 | 7.58±0.02 | 3.28±0.01 |
| | Crude Fat (%) | 2.97 ± 0.01 | 1.40±0.11 | 0.577±0.01 |
| | Ash (%) | 8.56±0.003 | 2.61±0.01 | 3.48±0.01 |
| | Crude Protein (%) | 18.45±0.03 | 3.70±0.01 | 2.24±0.01 |
| | Crude fibre (%) | 16.45±0.02 | 48.62±0.01 | 40.67±0.01 |
| | Total Carbohydrates (%) | 36.31±0.02 | 43.49±0.03 | 49.76±0.02 |

The above mentioned plants were selected to compare the proximate parameters and micronutrients composition. Since many of these herbal products are used orally, therefore, to know proximate and nutrient analysis of these products and raw material used therein plays a crucial role in assessing nutritional significance and health effects [9-11]. The result of proximate analysis shows variant concentration/proportions of bio-chemicals and other contents. The difference found in the proportion of proximate parameter of these medicinal plants might be attributed to the conditions on which the plant species are harvested along with environmental parameters [12,13].

Mineral composition

Iron (Fe) content

The data of Fe content in fruit pulp of Amla, Bahera and Harad is given in Table 2. Fe content in the fruit

pulp of Amla, Bahera and Harad was 62.13, 220.53 and 30.05 ppm, respectively.

Copper (Cu) content

The data of Cu content in fruit pulp of Amla, Bahera and Harad is given in Table 2. Cu content in the fruit pulp of Amla, Bahera and Harad was 5.94, 6.37 and 7.33 ppm, respectively.

Zinc (Zn) content

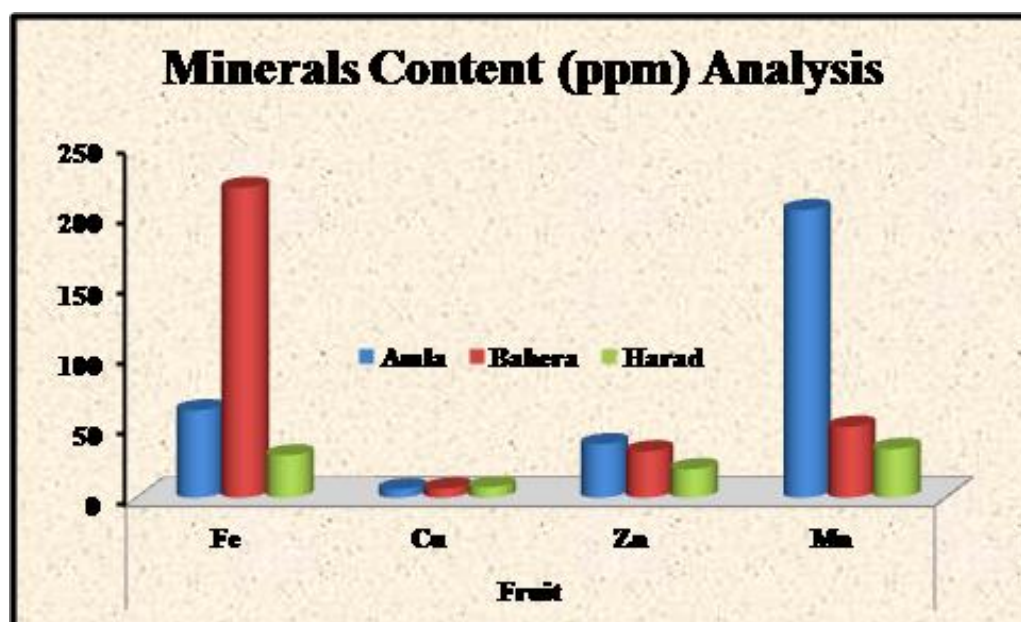
The data of Zn content in the fruit pulp of Amla, Bahera and Harad is given in Table 2. Zn content in the fruit pulp of Amla, Bahera and Harad was 38.04, 32.87 and 20.18 ppm, respectively.

Manganese (Mn) content

The data of Mn content in the fruit pulp of Amla, Bahera and Harad is given in Table 2. Mn content in the fruit pulp of Amla, Bahera and Harad was 204.74, 50.38 and 34.57 ppm, respectively.

Table 2. Minerals content (ppm) in the fruit pulp of Amla, Bahera and Harad

| Plant part | Minerals ↓ Plants → | Mineral content (ppm) | | |
|------------|------------------------|-----------------------|----------------|-------------|
| | | Amla | Bahera | Harad |
| Fruit | Fe | 62.13 ±0.04 | 220.53 ± 0.238 | 30.05±0.019 |
| | Cu | 5.94 ± 0.052 | 6.37 ± 0.120 | 7.33±0.145 |
| | Zn | 38.04±0.012 | 32.87±0.291 | 20.18±0.007 |
| | Mn | 204.74 ± 0.15 | 50.38± 0.01 | 34.57±0.088 |
| SE(m) | | 0.09 | 0.225 | 0.085 |
| CD at 5% | | 0.316 | 0.795 | 0.301 |
| CV% | | 0.20 | 0.503 | 0.642 |
| SE(d) | | 0.13 | 0.319 | 0.121 |

**Fig. 2.** Minerals content (ppm) in the fruit pulp of Amla, Bahera and Harad

The mineral content analysis of the medicinal plant species showed considerable variation among different micronutrients. However, for some species difference or higher concentration was recorded,

which may be due to prevailing environmental and soil conditions and the season when the plants were collected for analysis [13-15].

CONCLUDING RESULTS

Trends in Proximate Composition

| Parameter | Order |
|-------------------------|---|
| Moisture (%) | Amla>Bahera>Harad (Seeds>Aerial parts> Roots) |
| Crude Fat (%) | Bahera>Harad>Amla (Seeds>Aerial parts> Roots) |
| Ash (%) | Bahera>Amla> Harad (Seeds>Roots>Aerial parts) |
| Crude Protein (%) | Harad>Bahera>Amla (Seeds>Aerial parts> Roots) |
| Crude Fibre (%) | Harad>Bahera>Amla (Roots>Aerial parts> Seeds) |
| Total Carbohydrates (%) | Harad>Amla>Bahera (Seeds>Aerial parts> Roots) |

Trends in Minerals Composition

| Minerals composition | Order |
|----------------------|-------------------|
| Fe content (ppm) | Bahera>Amla>Harad |
| Cu Content(ppm) | Harad>Bahera>Amla |
| Zn Content (ppm) | Amla>Bahera>Harad |
| Mn Content (ppm) | Amla>Bahera>Harad |

CONCLUSION

Different parts of these medicinal plants namely Amla, Bahera and Harad were analyzed in order to get some useful information to be used in the preparation of therapeutic and nutraceutical foods. As there are no chief reports in literature on detailed proximate composition and nutritional parameters of medicinal plants' parts, this paper should be considered as a contribution to the course, being, a far from the knowledge for the active constituents formation from these medicinal parts of herbal plants.

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