Proximate, Mineral and HPTLC Fingerprints of Bael Fruits used by Tribes of Bhiwapur Tahsil Nagpur District, India

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Abstract:

Wild edible plants play an impotant role in human life and are the vital constituent of the traditional diet. People of the Bhiwapur tahsil are very close to the nature, wild fruits like *Aeglemarmelos* (Bael) which is one of the natural resources in the tahsil. They have the direct dependence on the wild plants for their sustenance. Due to the easy accessibility, the fruits are very commonly utilized by the tribal populations and travelers. The present investigation aimed to assess the proximate, mineral and phytochemical analysis of ripened fruits of a popularly known medicinal plant *Aeglemarmelos*. The study includes the estimation of Ash content, protein, carbohydrate (sugar), vitamins and mineral contents (Cu, Fe, Mn, Zn, Ca, Na, K) of Bael fruit. The water extract was screened for the qualitative phytochemical analysis and finally HPTLC fingerprints at 350 nm. The fresh fruits were found a rich source of protein (6.91±0.11 g/100g), carbohydrate (22.55±0.15 g/100g) fiber (7.26±0.23 g/100g) and energy (133.14 Kcal/100g). The fruits were found to contain high calcium (86.69±0.01 mg/100g) content and may be considered as the rich source to facilitate the rehabilitation of bone problems in human being. The Na/K and Zn/Cu ratio were found to be 0.012 and 12.26 may attribute the medicinal properties in cardiovascular disorders. The water extract showed the presence of carbohydrates, proteins, sterols, alkaloids, glycosides, saponins, polyphenols and flavonoids. The proximate, nutritional and phytochemical attributes reveals that the fruits are not only acting as supplementary foods but is the tonic requirements of the tribal's and deprived of poor Bhiwapur tahsil.

Keywords:
Bael, HPTLC, Proximate, Mineral, Tribe.

Introduction:

The evidence of man's dependency on plants for his survival can be demonstrated by palaeo-ethnobotanical findings from prehistoric archaeological sites [1]. In many tropical countries rural people traditionally harvest wide range of leaf of vegetables, roots, tubers and fruits because of its cultural uses, as a food supplement labeled as 'famine' or 'hunger' food. Plants not only provide the edible fruits but also have their importance in providing fodder, fuel and medicines. They play a significant role in the food and nutrient security of rural poor tribal's. Gathering of wild fruits is a common practice even today. These are often and the only fruits consumed, as tribal cannot afford cultivated commercial fruits as grapes, orange,

pomegranate, apple etc. The wild edible species are mostly gathered by the tribal, rural communities and forest dwellers for consumption value and taste during festivals. The general information, edibility and therapeutic properties of these wild fruits, their safety data and nutritional composition are in negligence [2].

Some wild fruits have been identified to have better nutritional value than the one that are cultivated [3]. As a result, in recent years, a growing interest has emerged to evaluate various wild edible plants for their nutritional features [4, 5]. By taking into consideration present study was designed to evaluate the nutritional and phytochemical traits of Bael fruits from Bhiwapur Tahsil.

Material and methods:

Study area and fruits collection

The study area is situated between the 20^0 35' and 21^0 44' N latitudes and between 75^0 53, and 80^0 East longitudes and is spread over the area of 61323.62 hectares of land. It includes 106 villages with a tribal population of 83,164 (2001). The tribal communities that fall in the villages are Banjara, Gond, Mana, Dhivar and Pardhi, of which Gond tribes are dominant. The vegetation is of deciduous type with a rainfall in average 45 inches [6] (Figure 1).

Field survey, collection of fruits and its related data, were carried out during the period of April 2011 to 2013, in different seasons. The specimens were identified by carefully matching them in the herbarium and authentically certified, by the Department of Botany Hislop College, Nagpur (Specimen no. 3254). Ethnic informers were consumed to locate and collect the plant along with the other informants. Useful information was gathered by interviewing the local people and elderly persons. Narurally growing wild ripe fruits were collected from the study area, outer cover, seeds were removed. The fruit pulp was shade dried, pulverized and coarse powder was utilized for their phytochemical and nutritional analysis (Figure 2).

Mineral contents

To prepare the sample for mineral analysis, the ripe fruits were oven dried, pulverized to fine powder and used for dried ashing. In each case the powdered fruits were taken in a pre-cleaned and constantly weighed silica crucible and heated in a muffle furnace at 450°C till there was no evolution of smoke. The crucible was cooled at room temperature in a desiccator and carbon-free ash was moistened with concentrated sulphuric acid and heated on a heating mantle till fumes of sulphuric acid ceased to evolve. The crucible with suphated ash was then heated in a muffle furnace at 600°C till the weight of the content was constant (~2–3 h). One gram of sulphated ash obtained above was dissolved in 100 ml of 5% HCl to obtain the solution

ready for determination of mineral elements through atomic absorption spectroscopy (AAS) and flame photometry (FPM). Standard solution of each element was prepared and calibration curves were drawn for each element using AAS/FPM [7].

Nutritive value

Moisture content

The fully ripe fruit pulp was cut into small pieces and moisture content was examined by air oven method at 105°C and till to get the constant weight. The loss in weight was regarded as a measure of moisture content [8].

Ash content

For determination of ash content, 10 g of dried powdered sample was weighed in a quartz crucible. The crucible was heated first over a low flame till all the material was completely charred, followed by heating in a muffle furnace for about 3–5 h at 600 °C. It was cooled in a desiccator and weighed to ensure completion of ashing. To ensure completion of ashing, it was heated again in the furnace for half an hour, cooled and weighed. This was repeated consequently till the weight became constant (ash became white or grayish white). Weight of ash gave the ash content [8].

Fat content

Fat content was determined by extracting 2 g dried sample with petrol in a Soxhlet extractor, heating the flask on a heating mentle for about 6 h till a drop taken from the drippings left no greasy stain on the filter paper. After boiling with petrol, the residual petrol was filtered using Whatman no. 40 filter paper and the filtrate was evaporated in a pre-weighed beaker. Increase in weight of beaker gave crude fat [9].

Protein content

The protein was determined using micro Kjeldahl method. Two grams of oven-dried material was taken in a Kjeldahl flask and 30 ml conc. H_2SO_4 was added followed by the addition of 10 g potassium sulphate and 1 g copper sulphate. The mixture was heated first gently and then strongly once the frothing had ceased. When the solution became colourless or clear, it was heated for another hour, allowed to cool, diluted with distilled water and transferred to a 800 ml Kjeldahl flask, washing the digestion flask. Three or four pieces of granulated zinc, and 100 ml of 40 % caustic soda were added and the flask was connected with the splash heads of the distillation apparatus. Next 25 ml of 0.1 N sulphuricacid was taken in the receiving flask and distilled. When two-thirds of the liquid had been distilled, it was tested for completion of reaction. The flask was removed and titrated against 0.1 N caustic soda solution using methyl red indicator for determination of Kjeldahl nitrogen, which in turn gave the protein content [10].

Fiber content

The crude fiber content was determined to be reported along with the nutritive value. For determination of crude fiber, the estimation was based on treating the moisture and fat-free material with 1.25 % dilute acid, then with 1.25 % alkali, thus imitating the gastric and intestinal action in the process of digestion. Then 2 g of moisture and fat-free material was treated with 200 ml of 1.25 % H_2SO_4. After filtration and washing, the residue was treated with 1.25 % NaOH. It was the filtered, washed with hot water and then 1 % HNO_3 and again with hot water. The residue was ignited and the ash weighed. Loss in weight gave the weight of crude fiber [9].

Percentage carbohydrate was given by: 100 – (percentage of ash + percentage of moisture + percentage of fat + percentage of protein) [10].

Nutritive value was finally determined by: Nutritive value = 4 ´ percentage of protein + 9 ´ percentage of fat + 4 ´ percentage of carbohydrate [10].

Extraction and phytochemical screening

The decoction of dried coarse powder was prepared at 80 ^oC for about 2 h and lyophilized to get the dried water extract. The extract was screened for the presence of different primary and secondary metabolite using different phytochemical tests [11].

HPTLC Fingerprint of Extract

A CAMAG HPTLC system equipped with a sample applicator Linomat IV using 100 ml syringe and connected to a nitrogen tank; twin trough plate development chamber; CAMAG TLC scanner-3 with winCATS software. Each HPTLC plate precoated, silica gel G 60 F254 size 10 X 10 cm accommodated ten tracks of samples, applied according to following settings: bandwidth 4 mm; distance between bands 5 mm; The plates were developed to 8 cm in a twin trough glass chamber, saturation time 30 min, scanning mode Absorbance/Reflectance; temperature 20±5 ^oC and separation technique ascending, using different mobile phase [12].

Results and discussion:

Nutritional analysis

The importance and awareness of nutrition is public health issues, has resulted in the increase demand of knowledge of the biochemical nutrients of foods. Carbohydrates and sugars highly contribute for energy (133.14 kcal/100g) signify the role of bael fruit as good source of nutrition. About 14 elements are essential to human health, deficiency of which create health problem. Human bodies daily need more than 100 mg of major minerals (N, P, K, Ca, Mg, Na) and less than 100 mg of minor minerals (Cu, Fe, Zn, Mn, Co, Br, Si) [13].The processing methods produce Na and K ratio less than 1 which is in accordance with the recommended ratio [14]. As per

the results of mineral analysis, the Na/K found 0.012 which is within the recommended ratio for good human health. The fruit contain high content of potassium act as an electrolyte for maintaining the homeostasis and act as power generator inside the cells of human body. Calcium is the second highest mineral (86.69±0.01) present in Bael fruit which is reported, very essential in muscle contraction, oocyte activation, building strong bones and teeth, blood clotting, nerve impulse, transmission, regulating heart beat and fluid balance within cells [15]. The other important minerals (Fe, Cu, Zn and Mn) were found in the range of 1.29±0.01 to 15.82±0.02 (Table 1). Excessive ratio of zinc to copper (>16) from dietary sources causes imbalance in their bioavailability and has been linked to increased risk of cardiovascular disorders [16]. The less Zn/Cu ratio (12.26) in Bael fruit may contribute its several therapeutic applications. The nutritional analysis revealed that the fruit is not only acting as supplementary food but is the tonic requirement of the tribal's, and deprived of poor Bhiwapur.

Phytochemical analysis

The phytochemical screening of water extract from the dried ripe fruits of *Aeglemarmelos* revealed the presence of major bioactive compounds including phyto-sterols, carbohydrate, protein, alkaloids, glycosides, polyphenols, flavonoids and saponins which may retain a wide range of pharmacological actions (Table 2).

HPTLC Fingerprint

By HPTLC extracts shown five different peaks in the chromatogram of water extract.

Table 1: Nutritional analysis of Bael fruits pulp

Nutritional Characteristics (DW)	Result
Total ash (g/100g)	2.63±0.07
Moisture (g/100g)	58.95±0.12
Protein (g/100g)	6.91±0.11
Fat (g/100g)	1.70±0.17
Fiber (g/100g)	7.26±0.23
Sugar (g/100g)	6.38±0.09
Carbohydrate including sugar (g/100g)	22.55±0.15
Copper (mg/100g)	1.29±0.01
Iron (mg/100g)	3.32±0.03
Manganese(mg/100g)	1.53±0.03
Zinc (mg/100g)	15.82±0.02
Calcium (mg/100g)	86.69±0.01
Sodium (g/100g)	0.02±0.07
Potassium (mg/100g)	1.66±0.09
Calorific value (Kcal/100g)	133.14

Each value represents the mean ± SD of three determinations (n = 3) on dried weight of fruit pulp (DW) basis.

Table 2-Preliminary phytochemical analysis of Bael fruits water extract

Phytoconstituents	Test	Observations	Aqueous extract
Alkaloids	Dragendroff's	Orange colourppt produced	+
Alkaloids	Mayer's Test	Cream colouredppt produced	+
Alkaloids	Wagner's test	Reddish brown colourppt produced	+
Flavonoids		Magenta (brick) red colour produced	+
Proteins	Biuret test	Violet or purple colour produced	+
Proteins	Millon's test	Red colour produced	+
Carbohydrates	Molisch's test	Red or dull violet colour produced	+
Carbohydrates	Fehling's test	Yellow or red colourppt produced	+
Phytosterols	Liebermann-Burchard test	Dark red or pink colour produced	+
Glycosides	Baljet test	Yellow to orange colour produced	+
Glycosides	Keller-Killiani test	Two layer reddish brown colour produced, in upper layer turns bluish green colour produced	+
Phenols	Ferric chloride test	Deep blue or black colour produced	+
Saponins	Foam test	Persistent form produced	+

Where (+) and (-) indicate the presence and absence of phyto-constituents respectively.

Table 3: Rf of the different components in extract

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned
1	-0.01	36.5	0.00	137.3	14.96	0.03	0.0	1262.8	3.95	unknown *
2	0.39	2.3	0.44	15.9	1.73	0.47	7.7	498.4	1.56	unknown *
3	0.49	7.1	0.57	225.6	24.58	0.60	59.3	7183.8	22.48	unknown *
4	0.60	59.4	0.62	79.8	8.69	0.64	71.6	1807.6	5.66	unknown *
5	0.64	71.8	0.69	459.5	50.05	0.81	28.1	21203.9	66.35	unknown *

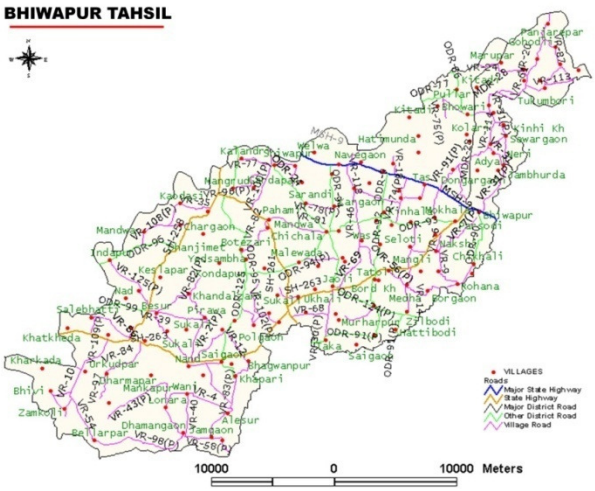

Figure 1: Map of BhiwapurTahsil, District Nagpur, Maharashta, India

Figure 2: Aeglemarmelos tree and fruits.

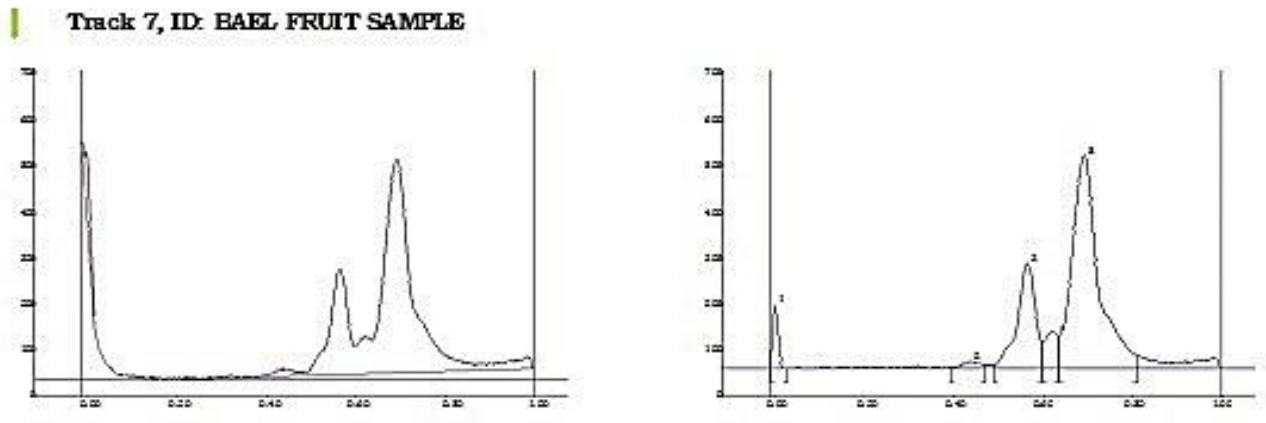

Figure 3: HPTLC Chromatogram of Bael water extract

Conclusion:

The result highlighted significance of wild fruit as a cheap source of nutrient for the rural and tribal people. It brings into focus the rich nutritional composition of the fruit and the scope for their use as an alternative source of bio-nutrition. The mineral analysis indicated the scope of using wild edible fruit for dietary supplement. It has valuable ingredients as micro-minerals (Fe, Cu, Zn and Mn) and macro-minerals (Ca, NA and K). The HPTLC fingerprints and phytochemical analysis of water extract helps in setting the standards for its quantitative standardization. Many other fruits of the forest therefore need to be analysed which could help in selecting promissory species for inclusion in agro and farm-forestry and re-forestratio nprogrammes. Plantation of wild fruit helps to sustain the wild animals. There is a need to explore more wild fruits that will add new dimensions towards traditional management and conservation of plant wealth.

Acknowledgement:

The author is thankful to the tribes and rural people of Bhiwapur tahsil for providing and sharing their knowledge regarding the above fruit and its uses in day to day healthcare practices.

References:

[1] **Sawian J. T., Jeeva S, Lyndem FG, Mishra BP, Laloo RC.** Wild Edible Plants of Meghalaya, North-East India. Nat Prod Rad 2007; 6: 410-426.

[2] **Aloskar L. V., Kakkar K .K., Chakra O. J.** Second Supplement to Glossary of Indian Medicinal Plants with Active Principles, Part-I (A-K), 1965-1981, NISC, CSIR, New Delhi, 1992; 265-266.

[3] **Jayanti, Agarwal K., Saini P**. Nutritional Assessment of Leaves of Wild Edible Plant *Urticaardence*. Ind J Pharm Biol Res 2013; 1: 53-59.

[4] **Nazarudeen A.** Nutritional Composition of Some Lesser-Known Fruits Used by Ethnic Communities and Local Folks of Kerala. Ind J Traditional Knowl 2010; 9: 398-402.

[5] **Aberoumand A, Deokule S. S.** Studies on Nutritional Values of Some Wild Edible Plants from Iran and India. Pak J Nutr 2009; 8: 26-31. http://www.nagpur.nic.in/zpnagpur/English/BhivapurTehsil.htm 2014.

[6] **Janani G. K., Sondhi S. M**. Determination of Mineral Elements in Some AyurvedicBhasmas Used for the Cure of Various Ailments. Ind Drugs 1995; 32: 187–190.

[7] **Anonymous.** The Ayurvedic Pharmacopoeia of India, Part-I, NISCOM, CSIR, New Delhi, 1999, vol. II, p. 191.

[8] **Chopra SL, Kanwar J. S.** Analytical Agricultural Chemistry, Kalyani Publications, New Delhi, 1991, vol. IV, p. 297.

[9] **Indrayan A. K., Sharma S, Durgapal D, Kumar N, Kumar M.** Determination of Nutritive Value and Analysis of Mineral Elements for Some Medicinally Valued Plants from Uttaranchal. CurrSci 2005; 89: 1252-1255.

[10] **Khandelwal K. R.** Practical Pharmacognosy, Techniques and Experiments. 08th ed. Pune: Nirali Prakshan; 2001, 15-21.

[11] **Vyas J., Itankar P., Tauqeer M., Kelkar A., Agrawal M.** Development of HPTLC method for estimation of piperine, guggulsterone E and Z in polyherbal formulation. Pharmacog J 2013; 5: 259-64.

[12] **Mahadkar S., Valvi S., Rathod V.** Nutritional Assessment of Some Selected Wild Edible Plants as a Good Source of Mineral. Asian J Plant Sci Res 2012; 2: 468-472.

[13] **Caunii A., Cuciureanu R., Zakar A. M., Tonea E., Giuchici C.** Chemical Composition of Common Leafy Vegetables. StudiaUnivVG SSV 2010; 20: 45-48.

[14] **Piste P., Didwagh S., Mokashi A.** Calcium and its Role in Human Body. Int J Res Pharm Biomed Sci 2013; 4: 659-668.

[15] **Ma J., Netts N. M.** Zinc and Copper Intakes and Their Major Food Sources for Older Adults in the 1994-96 Continuing Survey of Food Intakes by Individuals (CSFII). J Nutr 2000; 130: 2838–2843.