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Proximate and phytochemical screening of the fruit and leaves extract of Bael (*Aegle marmelos*).

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***Abstract*:** *The study examined the fruits and leaves extract for the proximate, phytochemicals and nutrients composition of Aegle marmelos (Bael) using Standard methods. Proximate analysis revealed that moisture content, ash, protein, crude fiber, carbohydrate, pH and acid value occurred in appreciable amounts in both the fruits and leaves samples. The moisture content of the fruits was 51.7±0.8% and leave was 16.6±0.7%. The ash content of the fruit was 23.3±0.3% and leave was 17.8±1.1%. The protein content in fruit was 10.4±0.1% and leave was 13.40±0.02%. The fiber content was 30.7±0.7% and 12.9±0.4% in the leaves and fruits respectively. Carbohydrate content for leaves was 21.3±2.0% and in fruit was 1.82±0.85%. The pH value in the fruits and leaves was 2.50±0.06 and 1.93±0.53 respectively. Corresponding acidity value in fruits and leaves was 1.92±0.83 and 3.23±0.12 respectively. Phytochemical analysis revealed the presence of alkaloids, tannins, flavonoids, saponins, triterpernoids, steroids, anthraquinones and phenol in fruit and leaves samples analysed. However, cardiac glycosides and reducing sugar were absent in both the fruit and leaves. The concentration (mg/kg) in fruits was Ca (61.0), K (277.5), Na (10500), Cu (6.00), Zn (11.8), and Cd (0.05). Corresponding concentration (mg/kg) in leaves were Ca (61.3), K (6400), Na (6350), Cu (1.75), Zn (20.3), and Cd (0.07). The Aegle marmelos (Bael), fruit and leaves can play a significant nutritional role in human and livestock diet.*

Keywords: *Aegle marmelos, fruits, livestock diet, phyochemicals, proimate analysis,*

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I. Introduction

Fruits are a major component needed to form balanced diet needed by human body. They are also useful in medicine and treatment of diseases. There is increased awareness on the role of fruits in human diet in a good number of consumers. Fruits are one of the oldest forms of food and they play a leading role in human diet [1]. For a plant and its fruits to be acceptable to be of nutritional importance, it has to be assessed by the content of vitamins, fats and oils, carbohydrate, minerals, and proteins that are important for growth and development in human and animals [2]. Among other benefits of fruits in addition to their nutritional benefits include being used as anti-oxidant, anti-mutagenic, anti-carcinogenic, anti-viral and possession of anti-bacterial properties. These are active biological substances [3, 4]. The bioactive constituents present in plants such as alkaloids, flavonoids, and phenolic compounds have been reported to make them exhibit medicinal and physiological activities [5, 3, 6]. The use of plants for food and medicinal purpose by man started since primordial time. One of such plants is *Aegle marmelos* (Bael). It has good nutritional value and is rich in carbohydrates, fiber, minerals and vitamins [7] and for medicines.

Aegle marmelos commonly known as Bael, belong to the family Rutaceae. It is often referred to as a handsome multipurpose social tree called elephant apple. Bael's importance seems largely due to its panacea of medicinal qualities. All parts of this tree namely the root, leaf, fruit and the seed are used for curative purposes. The fruit has considerable medicinal value when it just begins to ripen [8]. The leaves of Bael (*Aegle marmelos*) are used extensively in the feeding of ruminants as well as for treating different ailments in herbal medicine. The fruit is eaten by humans when ripe, used as feed to animals and for ethnomedical treatment of diseases [9. 10]. All parts of *aegle marmelos* are medicinally useful. The leaves can be used as mild laxative or the inflammation of the mucous membrane having a free discharge and for asthma parts [11]. The fruit can be eaten during convalescence after diarrhoea and served as a potent remedy for dysentery. It has been reported in southern Chhattisgarh that traditional healers often use the fruit powder mixed with mustard oil for the treatment of burn cases. The fruits can also be used to treat medical issues in gastric troubles, constipation, laxative, tonic, digestive, stomach ache, brain and heart tonic, ulcer, antiviral, intestinal parasites, gonorrhea, epilepsy [12, 13]. Currently, focus is now on natural products derived from plant due to their diverse pharmacological properties, including antioxidant and antitumor activity [15] in their study reported that many fruits, shrubs, spices and herbs and leafy vegetables are used as food, food drinks and for medicinal purposes in Nigeria. With global increase in cost of production and living there is need to source for a cheap, renewable, easily available and

nutritive source of material as active ingredients for food and medicine. *Aegle marmelos* fruit and leaf can conveniently provide the aforementioned. However, much attention has not been given to this plant in order to exploit its usefulness for food and medicine in Nigeria. The aim of this study is to determine the nutritional value as well as evaluate the proximate composition and phytochemical content of the fruit and leaves of *Aegle marmelos* in Bassa Kogi State, Nigeria. However, due to the possibility of contamination of the plant with heavy metal during growth, the fruits and leaves were also assessed for the presence of some metals.

II. Materials and Methods

2.1 Sample collection and crude extract extraction

Fresh fruits and leaves of *Aegle marmelos* were collected from Mozum, in Bassa Local Government Area of Kogi State, Nigeria (7° 51'0 "N 6°56 '0" E). The fresh fruit was cut open and pulp was scooped out, the pulp, epicarp and the leaves were washed with distilled water and rinsed severally with distilled water and air dried at ambient temperature in the laboratory. The dried materials were ground using a mortar and pestle.

2.2 Preparation of Plant Extracts

The crude extract in the ground samples was obtained by cold press method and filtered [16]. The extract was thereafter concentrated using a rotary evaporator with water bath and freeze dried. Aliquot portions of the crude plant extract residue were weighed and used for phytochemical screening and proximate analysis.

2.3 Phytochemical Screening

The screening of the samples to determine the presence of alkaloids, steroids, saponins, terpenoids, glycosides, flavonoids, tannins, proteins and carbohydrates is carried out using
standard procedures.

2.3.1 Test for Saponins

A 0.5g of extract was added to 5ml of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion, indicating the presence of sponins [3, 17].

2.3.2 Test for Terpenoids

A 0.5g of the extract was dissolved in 1ml of chloroform. 1ml of acetic anhydride was added, followed by the addition of 2ml of concentrated H_2SO_4. Formation of reddish violet colour indicates the presence of terpenoids [17].

2.3.3 Test for Tannins

A 0.5g of extract was treated with boiled distilled water and few drops of $FeCl_3$ were added, shaken to dissolve. Formation of brownish green colour indicates the presence of tannin [17].

2.3.4 Test for Reducing Sugar (Fehling's Test)

A 0.5g of the extract was dissolved in 5ml distilled water and filtered. The filtrate was hydrolysed with dilute HCl, neutralized with alkali (NaOH) and was added 2 mL of a 1:1 mixture of Fehling's A and Fehling's B. The mixture was heated to boiling in a water bath for 10 min and the presence of the presence of reducing sugars was confirm by the formation of red precipitate.

2.3.5 Test for Anthraquinones A 0.5g of the extract was boiled with 10ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform. The mixture was filtered and the filtrate shaken with equal volume of 10% ammonia solution. The formation of pink, red or violet colour in the aqueous layer indicates the presence of free anthraquinone [18].

2.3.6 Test for Steroids

A 0.5g of the extract was dissolved in 10ml of chloroform and equal volume of concentrated H_2SO_4 was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicate the presence of steroids.

2.3.7 Test for Cardiac Glycosides (Keller-Killiani Test)

To 0.5g of extract was added 5ml distilled water and 2ml of glacial acetic acid solution containing a trace amount of ferric chloride solution. To the mixture was added 0.5ml of concentrated H_2SO_4 on the wall of the test tube. A bluish-green colour appears in the acetic acid layer [19].

2.3.8 Test for Flavonoids

A 3 ml of test solution was treated with few drops of NaOH solution in a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids [20].

2.3.9 Test for Alkaloids

Mayer's test:

To a 1ml of test solution when treated with 1ml of Mayer's reagent drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids [18].

Dragendoff's test:
In another tube containing test solution was added 1ml of Dragendoff's reagent drop by drop. Observation of formed reddish-brown precipitate indicates the presence of alkaloids [18].

2.3.10 Test for phenols

To a 10 ml of sample was added distilled water, heated and filtered. To the filtrate was added 1% $FeCl_3$. A blue black coloration indicated that phenol was present

2.4 Procedures for Proximate Analysis

The Association of Official Analytical Chemists (AOAC) method [21] was used for the determination of moisture, ash, crude fiber and crude protein. Crude protein was estimated by multiplying the sample percentage nitrogen content by a factor 6.25. Carbohydrate was determined by the difference between 100% (accepted total value of nutritional status) and the sum of the values of protein, moisture, fiber and ash [22].

2.4.1 pH determination

The pH was read using a pH meter following the procedure describes by Onwuka [23].

2.4.2 Ash content determination

Ash was obtained by the incineration of 2.0g sample in a muffle furnance (LMF4 from Carbolite, Bamford, Sheffield England) at 600^o for 4 hours until it turned white. The sample was then removed from the furnace, cooled in a desiccator and re-weighed. The weight of the ash was then calculated.

2.4.3 Determination of moisture content

Moisture content was determined by heating 2.0g portion of each of the sample in an oven (Plus 11 Sanyo Gallenkamp PLC, England) at 105^oC until constant weight was obtained. The loss in weight was calculated as the moisture content.

2.4.4 Determination of Acid Value

About 5g of oil sample was weighed into conical flask. 25ml of 95% v/v alcohol was added and 1ml of phenolphthalein indicator. The solution was titrated with 0.1M potassium hydroxide until the colour changed to pink [22].

2.4.5 Determination of Crude Protein

Protein value was estimated by determining the nitrogen value according to improved Kjeldahl method as described by AOAC [23] and multiplying by a factor of 6.25.

2.4 .6 Determination of Fibre Content

Crude fibre content was determined as outlined in AOAC [24]using Gallenkhamp muffle furnace at 550°C and the result was expressed in percentage.

2.4.7 Carbohydrate Content Determination

The nitrogen free method of AOAC[21].
was used. The carbohydrate is calculated as weight by difference between 100 and the summation of other proximate parameters as Nitrogen free Extract (NFE) percentage carbohydrate:
Carbohydrate content = 100 – (protein (%) + moisture (%) + Crude fibre (%) + ash (%)).

2.5 Mineral Determination

Mineral contents of *Aegle marmelos* (Bael) were determined by atomic absorption spectrometry, flame photometry and spect according to the methods of AOAC [21].
A 1.0g of the powdered sample was added 12ml of HNO_3 and the mixture was left overnight at room temperature. Then 4.0 ml $HClO_4$ was added to the mixture and the mixture was evaporated on a hot plate in a fume cupboard until the appearance of white fumes. The digest was then filtered and kept for Flame Photometry and Flame Atomic Absorption Spectrophotometer (FAAS) analysis using PerkinElmer Atomic Absorption Spectrometer, model (AA200).

III. Results and Discussion

3.1 Phytochemical screening

The result of the phytochemical screening is given in Table 1. The screening of the fruits and leaves extract of *Aegle marmelos* revealed the presence of active constituents like saponins, tannins, steroids, anthranquinons, flavanoids and alkaloids. However, cardiac glycosides and reducing sugar were not found in both extracts. The presence of these constituents may have accounted for the various therapeutic uses of the fruits and leaves of *Aegle marmelos*. It has been well documented of the therapeutic uses of plants in traditional medicine and their antimicrobial activity against various pathogenic microorganisms based on the phytochemicals present in them [17, 25, 26]. It has been reported that all parts of *aegle marmelos* are medicinally useful. The fruit has considerable medicinal value and has been well documented [12, 13]. Oil extracted from bael leaves gives relief from recurrent colds, cough and respiratory infections [8]. Reports have it that decoction of the root and sometimes the stem bark is useful in intermittent fever and palpitation of the heart. Similarly, the decoction can be given with sugar and fried rice to check diarrhea and gastric irritability in

children [27]. The presence of phytochemical constituents is essential to the usage of this plant for medicinal purpose. This is important in herbal medicine. Low record of adverse effects of usage of herbal remedy compared to synthetic drugs has been reported [28]. The increase in usage of plants with medicinal value is as a result of perceived resistance of current available synthetic drugs. Resistance to current available synthetic drugs is becoming a concern to public health. It has been reported that bioactive phytochemical contents give rise to physiological and pharmacological effects which is the key factor of a plants medicinal value [29, 30].

3.2 Proximate Composition Analysis

The proximate analysis of the fruits and leaves extract result is shown in Table 2. The mean moisture content for the fruits and leaves were 51.7±0.8% and 16.6±0% respectively. The high moisture content of the fruit could attributed to the thick back which helps to prevent loss of water through evaporation and may a supplement to table water in human diet when consumed. Report has it that high moisture content in fruits or foods reduces the shelf life of such fruit or food [31]. However, moisture in food determines the keeping quality of the food, the speed of digestion, absorption and finally the rate of assimilation of such food within the body system. Similarly, it also plays a significant role for the easy elimination of digestive waste from the body system [32]. The ash content in the fruits ranged from 23.0% to 23.50% with a mean value of 23.3±0.3%. Corresponding values in leaves ranged from 17.09% to 19.00% with mean value of 17.8±1.1%. These values were significantly higher than 0.87% and 0.47% respectively for *S. aethiopicum* and *S. macrocarpon* [33]. Similarly, this study result was higher than result of

Table 1: Phytochemical analysis of *Aegle marmelos* (Bael)

Phytochemical	Observation	
	Fruits	Leaves
Saponins	+	+
Tannins	+	+
Terpernoids	+	+
Cardiac glycosides	–	–
Reducing sugar	–	–
Steroids	+	+
Anthranquinons	+	+
Flavanoids	+	+
Alkaloids	+	+

Table 2: Percentage Proximate Composition of *Aegle marmelos* (Bael)

Characteristics	Fruits				Leaves			
				Mean				Mean
pH	2.40	2.50	2.60	2.50±0.06	1.93	1.90	1.96	1.93±0.03
Moisture %	51.8	51.73	51.67	51.7±0.8	17.27	15.86	16.67	16.6±0.7
Ash %	23.0	23.40	23.50	23.3±0.3	19.00	17.23	17.09	17.8±1.1
Protein %	10.0	10.4	10.5	10.4±0.1	13.39	13.43	13.40	13.40±0.02
Crude fiber %	12.41	13.07	13.07	12.9±0.4	31.37	30.07	30.72	30.7±0.7
Acidity value%	2.80	1.80	1.16	1.92±0.83	3.3	3.1	3.3	3.23±0.12
Carbohydrate%	2.79	1.4	1.26	1.82±0.85	18.97	22.76	22.12	21.3±2.0

S. gilo (9.75%) and *S. anguivi* (7.60%) reported by Adeyeye and Fagbohun [34]. Ash content is a reflection that the fruits are rich in mineral elements. The recorded pH mean for the leaves (1.93±0.03) was lower than that of the fruit with pH mean of 2.50±0.06. Similar low pH values of 3.8, 4.2, 3.5, 2.9, 3.83 and 3.82 have been reported for fruits like apple, tomato, orange, *Carisa carindas*, *Punica granatum* and *Capparis decidua*, respectively [35, 36]. It was reported that lower pH of sample is favourable for higher shelf life [35]. However, Timberlake has reported that low pH value can give an astringent taste to the fruit [36]. The acidity values were similar to the pH values.

The mean protein content of *Aegle marmelos* (Bael) was 10.4±0.1% and 13.40±0.02% for the fruits and leaves respectively. This is an indication of that the plant is a potential rich source of protein supplement for animals. The leaves and fruits therefore may serve as an alternate source of feed. However, the protein content is lower than 27.74% reported for *Vitex doniana* leaves, 20.72% for *Moringa oleifera* and 19.1% for *leptadenia haetate* leaves [37]. Crude fibres are also constituents of many fruits and vegetables. The mean percentage fibre of 12.9±0.4% and 30.7±0.7% was recorded for the fruits and leaves respectively. The observed range of 12.41% to 13.07% for the fruits and 30.07% to 31.37% for the leaves are significantly higher than a range of values of 0.1% and 6.8% reported for selected fruits [38] but compared favourably with those reported on dry weight basis 10 – 41% for some fruits [39]. High fibre content present in the leaves of the plant is not too good in human nutrition as it may lead to intestinal irritation due to human inability to digest them easily [40, 41, 42,

43]. However, reports have linked decreased in incidence of heart disease, various types of cancer and diverticulosis due to high fibre consumption [45]. High levels of fibre in food also help in digestion and prevention of colon cancer [46]. Previous studies have opined that high fibre content present in vegetables may be employed in the management of diabetes, obesity, colon cancer and gastrointestinal disorder [46, 45]. The mean carbohydrate value of 1.82±0.85% of the fruit is significantly lower than the 21.3±2.0% recorded for the leaves. The leave can be considered as a good source of crude carbohydrate. Carbohydrate is said to contribute to fat metabolism and spare proteins as an energy source. It also acts as a mild natural laxative for humans and forms a substantial percentage of the human diet [47]. Pogozelski *et al*. (2005) has reported that a diet that does not contain carbohydrate can result to muscle break down, ketosis and dehydration [48].

3.3 Elemental Analysis

The result of metals obtained for the fruits and leaves of *Aegle marmelos* in this study compared with standard Mineral Recommended Dietary Allowance [49] as in Table 3 shows that the level (mg/kg) of Ca, K, Na, Zn, Cu and Cd in fruits was 61.0, 277.5, 10500.0, 11.8, 6.00 and 0.05 respectively. Corresponding levels (mg/kg) in the leaves were 61.3, 6400.0, 6350.0, 20.3, 1.75 and 0.07 respectively. The level of the metals in the fruit was in the following order Na> K> Ca>Zn>Cu> Cd. The order in the leaves was the same except that K was the highest. The fruit and the leaves can serve as good source minerals to the body. Significant amount of Cu and Ca were observed in both the fruit and the leaves. The fruits and the leaves when taken according to recommended dietary allowance can solve the problem of anemia as Cu deficiency in the body can lead to the manifestation of anemia and bone abnormalities [50]. Similarly, the recorded level of Ca will equally solve problem of bone condition. The level of K (277.5 mg/kg) in the fruit was within recommended dietary allowance. However the level (6400.0 mg/kg) in the leaves can be injurious to the body as concentration was significantly higher than 500 mg/kg recommended. Similarly, the level of Na in both the fruits and leaves were quite high with values of 10500.0 mg/kg and 6350.0 mg/kg respectively. Sodium concentration is significantly higher compared to 45mg/100g reported for *Senna obtusfoliat* and 5.00±06 mg/100g reported for *Tribus terrestris* leaves [51]. Although Zn is a cofactor of about 200 enzymes or more that play significant role in metabolic pathways however, if present in high levels in human body can be toxic due to its interference with copper metabolism. In this study the level of Zn in the leaves was above the recommended dietary allowance. The study values compared to 0.02 mg/100g reported for *Diospyrus mespilliformis* [54], 0.10±0.00 mg/100g reported for *Terrestris* leaves and 6.85±1.00 mg/100g reported for *Talium triangulare* by Fasuyi [55]. The concentration of Cd 0.05 mg/kg in the fruit and 0.07 mg/kg in the leaves are within FAO/WHO regulatory limit of 0.2 mg/kg for vegetables. The determination of Cd though at low level is of concern due to the toxic nature of the metal in human system. Cadmium accumulates primarily in the kidneys and has a long biological half-life in humans of 10-35 years [56].

Table 3: Level of some metals (mg/kg) in *Aegle marmelos* (Bael) fruits and leaves compared with Standad and Recommended Dietary Allowance

Metals	Fruits	Leaves	FAO/WHO Standard[a]	Recommended dietary allowance[b]
Ca	61.0	61.3	200	1200
K	277.5	6400.0	-	500
Na	10500.0	6350.0	-	2000
Zn	11.8	20.3	0.60	15
Cu	6.00	1.75	40	2.3
Cd	0.05	0.07	0.2	175[c]

a: FAO/WHO, 1993[52]
b: Eze and Obinna, 2004[53]
c: µg/g

IV. Conclusion

From the results of the proximate and phytochemical analysis, we can conclude that the leaves and fruits of *Aegle marmelos* (Bael) are important sources of proximate, phytochemicals and nutrients. The fruits and leaves of the plant are of significant nutritive value and can play a great role as good sources of minerals needed for the maintenance of good health. The fruit is healthy for human consumption and the leaves can be fed to ruminants.

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