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# NUTRITIONAL COMPOSITION, ENZYME ACTIVITY AND ANTIMICROBIAL ACTIVITY OF WOOD APPLE (AEGLEMARMELOS) LEAVES

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## ABSTRACT
Wood Apple leaves, a medicinal and auyrvedic leaves is locally known as 'Bael leaves' we investigated nutritional composition, enzyme activities and biological activities (antimicrobial) of locally available Wood Apple (*Aeglemarmelos*) leaves. The nutritional composition of Wood Apple leaves was determined by standardmethod. On TLC, it was found that Ethyl-acetate (EA) and Methanol(M) extracts of Wood Apple leaves was a mixture of different type of compounds like steroid and terpenoid. Enzyme activity was also determined .The activities of amylase cellulase, invertas and protease were 0.251, 0.132, 0.075, 0.0512 mg/min/ml respectively. In vitro, antibacterial activity of crude methanol extract (ME) was determined against four gram positive and five gram negative bacteria. Leaves extract have antibacterial activity against *Shigella dysenteriae*, *Sarcinalutea*, *Escherichia coli*, *Bacillus megaterium*, *Salmonella typhi*. It was found that leaves extract showed no sensitivity againstfive tested fungi.

***KEYWORDS*:** Standard method, TLC, Enzymatic activity, Antibacterial activity, Antifungal activity.

## I.INTRODUCTION
The wood apple (*Aeglemarmelos*) or Bael tree (in Bengali) is a species native tree of Bangladesh, India, Pakistan, Nepal, Bhutan etc. It is also cultivated all over South Asia as well as in the Northern Malay Peninsula, Java, the Philippines and Fiji. It is the only member of the monotypic genus *Aegle*.[1] It is a mid-sized, slender, aromatic, armed, gum-bearing tree growing about18 meter in tall. Its fruit has a very hard rind which can be difficult to crack open, and contains sticky brown pulp and small white seeds. The leaves are pinnate with 5-7 leaflets, each leaflet is 25–35 mm long and 10–20 mm broad, with a citrus-scent when crushed.[2]

The fruit is eaten fresh or dried. If fresh, the juice is strained and sweetened to make a drink similar to lemonade. It can be made into sharbat (Hindi) or belpana (Bengali/Oriya language), a refreshing drink made of the pulp with water, sugar, and lime juice, mixed, left to stand a few hours, strained, and put on ice. For the fragrance, the leaves and small shoots are eaten as salad greens. Considering its Ayurvedic properties people usually soak the leaves nightlong in water and filter it to get strained liquid to drink.[3]

The tree has great medicinal importance. It comes under the 10 great trees of medicinal value. Its' leaves have a great medicinal value for Diabetics, Diarrhoea, Dyspepsia, Detoxification, Skin ailments, Asthma, Sinusitis, Cold fever etc. It is also a good source of Beta-Carotene and is good for lever and kidney problems. It also contains thiamin& riboflavin. It is widely used in the treatment of snake bites. It is also a good source of vitamin-C to prevent scurvy disease. It can be also used for blood purification as tea or drink. The Wood Apple leaves can relieve respiratory problems. It is responsible for curing chronic cough and sore throat which leads to respiratory ailments. It can help to destroy worms in the intestine and the tannin in Wood Apple leaves reduces the inflammation. Infusions of Wood Apple leaves are helpful to prevent pain or discomfort in stomach ulcers. The laxative properties of leaves and fruit help to prevent constipation. Juice of the tender leaves of Wood Apple mixed with milk and sugar gives excellent effect in treating bowel complaints of children. The powder of the leaves if rubbed on the body to kills the worms and keeps the body away from diseases.

## II. METHOD AND MATERIALS
### 2.1. Collection of Sample
During the summer seasons, Aeglemarmelos leaves were collected from Rajshahi University campus. After collection, it was cleaned and stored at room temperature.

**2.2. Preparation of crude extract**

At first 10 g of Wood Apple leaves were cut into small pieces and pasted in a mortar with pestle and then homogenized well with Pre-cold buffers of respective pH (for amylase: 0.1 M phosphate buffer pH 6.7and for invertase pH 7.0, for cellulase, citrate buffer, pH 5.0 and for protease 0.1M phosphate buffer, pH 7). The homogenate was filtered through a double layer of muslin cloth. After centrifugation at 6,000 rpm for 10 min the supernatant was used as crude enzyme extract and stored in the freeze.

**2.3 Assay of Enzyme activity**

Amylase activity was assayed following the method as described in laboratory Manual in Biochemistry.[4] The polyphenol oxidase activity as described in.[5] Cellulase activity was assayed by method described in.[6] Invertase activity was assayed following the modified method as described in methods in physiological Plant Pathology.[7] The protease activity was measured following the method of Kunitz. T.

**2.4. Brine Shirmp Lethality Bioassay**

This bioassay can be used as a convenient monitor for screening and fractionation in the discovery and monitoring of bioactive natural products.[8, 9]

**2.4.1 Preparation of simulated seawater**

38 g of sea-salt (non ionized NaCl) was weighed accurately, dissolved in one liter of sterilized distilled water and then filtered off to get clear solution. The pH of the seawater was maintained between 8 and 9 by using NaHCO3 solution.

**2.4.2 Hatching of brine shrimp**

*Artemia salina* leach (brine shrimp eggs) collected from the pet shop was used as the test organism. Simulated sea water was taken in the small tank and the shrimp eggs (1.5 g/l) were added to one side of the tank and this side was covered. The shrimps were allowed for one days to hatch and immature as nauplii (larvae.).Constant oxygen supply was carried out and constant temperature (around 37˚C) was maintained during the hatching time. The hatched shrimps were attracted to the lamp on the other side of the divided tank through dam. These nauplii were taken for this bioassay.

**2.4.3 Preparation of test sample**

The final concentration of the samples in these vials becomes 10, 20, 40, 60 and 80 µg/ml respectively. For each concentration, control experiment was done. The experiment was repeated three times.

**2.4.4 Application of brine shrimp nauplii**

15 living nauplii were transferred to each of the vials. A magnifying glass was used for convenient counting of the nauplii. If the counting of 10 nauplii was not being possible accurately, then a variation in counting from14-16 might be allowed.

**2.4.5. Counting of nauplii**

After 24-hours of incubation, the vials were observed using a magnifying glass and the numbers of survivors in each vial were counted. The percentage of mortality of the nauplii was calculated for each concentration and the $LD_{50}$ values were determined using probit analysis.[10]

**2.5. Antibacterial Activity Study**

**2.5.1 Principle of agar discs diffusion method**

In the discs diffusion assay, the surface of a nutrient agar medium contained in a petri dish was uniformly inoculated with the test bacterial culture. Test sample solution was applied on filter paper disc with the help of a micropipette and dried in room temperature. The filter paper discs were then placed on each of the Petri dishes previously inoculated.

**2.5.2 Procedure to determine antibacterial activity**

Antibacterial activity was determined keeping the Petri-dishes in room temperature 6-12 h. This method was developed by Bondi and standardized by Bauer et al in 1966 for susceptibility test.

**2.6 Antifungal Activity Study**

Antifungal activities of different extracts were tested against seven fungi by using disc diffusion technique, because it is essentially a quantitative or semi-quantitative test indicating the sensitivity or resistance of micro-organism to the test material. In vitro antifungal screening is a useful technique for the detection of new lead compounds for the development as potential new antibiotics.

**III. RESULT AND DISCUSSION**

**3.1 Enzyme Activity of Wood Apple leaves (*Aegle sp.*)**

Amylase, an enzyme having physiological, commercial, and historical significance, also called diastase. Two types of amylase are recognized. β- Amylase (in plants and bacteria) which can only remove the terminal two glucose molecules each time it reacts, and α- amylase in animal. α-amylase and β-amylase catalyze the reaction in Starch degradation. The activity of amylase in Wood Apple leaves was found to be (0.251± 0.05 mg /min/ml). Polyphenol oxidase is also known as phenoloxidase, tyrosinase, dopaoxidase, Catecholoxidase and potatooxidase. The enzyme catalyzes the oxidation of monophenols and orthodiphenpls particularly tyrosine and p-cresol, orthodiphenols such as adrenaline, pyrogallol and substituted catechols are important substrates of the enzyme. The equation, which catalyzed by polyphenol oxidase is. Invertase, which hydrolyzes sucrose into glucose and fructose, occurs in many plants and microorganisms. The expression and distribution of plant invertases has been especially well documented, because these are considered to play an important role in sugar metabolism.[11] Proteases are proteolytic enzymes catalyze the hydrolysis of protein. During germination the rapid mobilization of storage protein in the cotyledons of seedling require the action of protease. Cellulase are used to perform various functions

including removing cell walls or crude fiber to release valuable components (flavors, enzymes, polysaccharides and other proteins) from plant cells to improve nutritional value of animal feeds or to prepare plant protoplast for genetic research (Mandels, M 1985). The activity of cellulase was examined in (0.132± 0.002mg /min/ml).

In brine shrimp lethality bioassay, the crude Methanol (M) extract and Ethyl-acetate (EA) extract showed positive results indicating that the extracts were biologically active. The $LD_{50}$ values of Methanol and Ethyl-acetate were 41.o8 and 44.39 ppm respectively, whereas positive control ampicillin trihydrate showed $LD_{50}$ value of 21.37 ppm as shown in the table 3.2. The mortality was not observed in the negative control experiment.

The antifungal activities of methanol extract against seven pathogenic fungi were investigated by using the doses of 100µg/ disc, 200µg/ disc and 300 µg/ disc. The standard antibiotic disc of nystatin (100µg/disc) was used for comparison. The results of antifungal activity (zone of inhibition) of test materials against respective fungi were given in the Table 3.4. It was found that Methanol (M) extracts showed no zone of inhibition against all the tested fungi.

**Table-3.1: Summarize of Enzyme activity Assay**

| Name of the enzymes | Enzyme activity (mg/min/ml) |
|---|---|
| Amylase | 0.251 ± 0.05 |
| Cellulase | 0.132 ± 0.002 |
| Invertase | 0.075 ± 0.001 |
| Polyphenol oxidase | 0.420 ± 0.001 |
| Protease | 0.0512± 0.001 |

**Table-3.2: Toxicity of different extracts of Wood Apple leaves (*Aegle sp.*) from against brine shrimp nauplii.**

| Sample | $LD_{50}$ (ppm) | 95% Confidence Limit | Regression equation | Chi-squared $\chi^2$ |
|---|---|---|---|---|
| Ampicillin Trihydrate | 21.38 | 10.81-42-27 | Y=1.4223X + 3.1169X | 0.174 |
| Methanol Extract | 41.08 | 21.45576-50.38271 | Y=3.319492+1.107848X | 0.3480177 |
| Ethyl-acetate extract | 44.39 | 18.4931-45.34508 | Y=3.567452+1.114717X | 0.3043496 |

**Table 3.3: *In vitro* antibacterial activity of Methanol extract and Kanamycin.**

| Test bacteria | Methanol extract of leaves (µg/disc) | | | Kanamycin (µg/disc) |
|---|---|---|---|---|
| | 200 | 400 | 600 | 30 |
| | Zone of inhibition (diameter in mm.) | | | |
| *Staphylococcus aureus* | R | R | R | 22 |
| *Bacillus subtilis* | 8 | 9 | 11 | 20 |
| *Sarcina lutea* | 6 | 8 | 9 | 19 |
| *Bacillus Cereus* | 6 | 7 | 9 | 19 |
| *Shigella sonnei* | R | R | R | 20 |
| *Salmonella typhi* | 6 | 8 | 10 | 19 |

"R'' Resistance.

**Table-3.4: *In vitro* antifungal activities of Methanol extract (M) and Nystatin.**

| Test fungi | M | | | Nystatin (100µg/disc) |
|---|---|---|---|---|
| | (100µg/ disc) | (200µg/ disc) | (300µg/ disc) | |
| *Penicilium sp* | - | - | - | 25 |
| *Aspergillus flavus* | - | - | - | 28 |
| *Aspergillus niger* | - | - | - | 24 |
| *Fusarium species* | - | - | - | 26 |
| *Mucor* | - | - | - | 25 |
| *Aspergillus fumigatius* | - | - | - | 24 |
| *Candida albicans* | - | - | - | 23 |

***Symbol:* '-'** no sensitivity.

## IV. CONCLUSION

Wood Apple leaves have a great medicinal importance. This leaves also used as salad ingredients in many Asian countries. Database analysis of vegetables available in the region would be of value to educators and public health officials positioned to provide dietary advice to the food stressed populations. From nutritional analysis it was found that tender leaves contained some important

nutrients like sugar, protein, vitamin-C, crude fiber, phenolic compounds, steroidal compounds and some essential minerals, which are important for public health. Very small amounts lipid, total phenol, water soluble protein, vitamin-c and crude fiber, sugar and starch content were higher in tender leaves than aged leaves. From the study on nutritional composition, it can be concluded that younger leaves is more nutritious. From enzyme activity assay it was found that Wood Apple leaves might be used as a source of some enzymes such as tannin, amylase, cellulase, invertase, polyphenol oxidase, protease etc. From the antibacterial activity studies it was found that crude methanol extracts of Wood Apple leaves showed activity against many pathogenic bacteria, used in this study. The methanol extracts have lower cytotoxicity; therefore, the extract of vegetable may be use in the treatment of diseases caused by pathogenic bacteria. From the antifungal activity studies it was found that both Methanol extract and Ethyl-acetate extract has no activity.

On the basis of above findings, it can be concluded that we can use Wood Apple leaves as an important source of nutrients and ayurvedic medicine for the people of Bangladesh. We can produce enzyme from wood apple for commercial purposes. As methanol extract of wood apple showed antibacterial activity from this finding we can design drug for the treatment of human being.

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