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Quantitative Estimation of Secondary Metabolites from *Mimusops elengi* L.

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Abstract: ***The present attempt was done to analyze the secondary metabolites from the medicinally important plant Mimusops elengi L. (Spanish cherry tree). The quantitative estimation of bioactive molecules from the leaves and bark showed the presences of flavanoids, terpenoids, saponins, flavonols and tannins. However, the amount of these metabolites varies in leaves and bark. The results obtained clearly indicates that bark contains highest amount of Tannins (8.3 ±0.051 mg/gm TAE), Flavanoids (6.8 ± 0.02 mg/gm CE), Flavonols (1.1 ± 0.13 mg/gm QE), Saponins (35.8 ± 2.04), Terpenoids (2.8 ± 0.36) compare to leaves which contain Tannins (3.6 ±0.09 mg/gm TAE), Flavanoids (3.5 ± 0.02 mg/gm CE), Flavonols (0.1 ± 0.05 mg/gm QE), Saponin (7.60±2.03), Terpenoids (2.0 ± 0.18). Hence bark served to have the potential source of drug for various ailments.***

Keywords: Secondary metabolites, Flavanoids, Flavonols, Terpenoids, Tannins

1. Introduction

The plants that possess therapeutic properties and exerting beneficial pharmacological effects on the animal body are generally designated as medicinal plants [1]. Plants are endowed with various phytochemical. These phytochemical are divided into primary and secondary metabolites. Secondary metabolites consist of alkaloids, saponins, quinones, coumarins, steroids, phenols, flavonoids, tannins and various other metabolites. Secondary metabolites have crucial role in plant development as well as in the interaction of a plant with its biotic and abiotic environment. It has been accepted that these secondary metabolites perform various biological activities such as antioxidant, anti-inflammatory, anti-cancer, anti-tumor, antidiabetics and many more [2, 3]. Thus the aim of the present work is to determine the secondary metabolites quantitatively from the medicinal plant *Mimusops elengi* L.

Mimusops elengi L. (Sapotaceae) are used as a remedy in the indigenous system of medicine for centuries. Internally bark skin is magnanimous in leucorrhoea, menorrhagia and is also notorious to have antiulcer activity. It is also used as a tonic, febrifuge, as a gargle for odontopathy, inflammation and bleeding of gums. Leaves are used in snake bite and applied as a paste on wounds [4, 5].

2. Materials and Methods

Collection and Authentication of Plant

Fresh leaves and Bark of *Mimusops elengi* L. were collected from the campus of DDU Gorakhpur University and authenticated by Herbarium Department of Botany, DDU Gorakhpur University Gorakhpur. The leaves and bark were washed and dried under shade. The dried bark and leaves were then pulverized with the help of mechanical grinder and kept in sealed jar at 4^0C temperature in refrigerator.

Preparation of Plant extract

The dried leaves and bark powder was weighed in a selected quantity and is subjected to soxhlet apparatus using methanol. After effective extraction solvent was concentrated using rotatory evaporator. The crude extracts thus obtained were used for quantitative estimation.

Determination of Tannin

Tannin content was determined by the procedure of Van-Burden and Robinson (1981) with slight modification. 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min. Tanin content was calculated from a calibration curve using tannic acid as standard and the result were expressed as tannic acid equivalent [6].

Determination of Saponin

Saponin content was determined by the procedure of Obadoni and Ochuko (2001). The samples were ground and 20 g of each were put into a conical flask and 100 Cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the

samples were dried in the oven to a constant weight. The saponin content was calculated as percentage [7].

$$\text{Percentage of Saponin} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Determination of Flavanoids

Flavanoid content was determined by the procedure of Kim et al., 2003. 100 mg of plant material was extracted with 100 ml of methanol using soxhelet apparatus and filtered through whatman filter paper. To 0.1 ml of diluted aliquots of extract, 4 ml of distilled water were added followed by 0.3 ml of 5% Sodium nitrite. The mixture was allowed to react for 5 min followed by 0.3 ml of 10% Aluminum chloride solution. Tubes were incubated for 5 minutes at ambient temperature. Finally the reaction mixture was treated with 2 ml of 0.1 M Sodium hydroxide. Immediately the volume of reaction mixture was made to 10 ml with distilled water. The absorbance of pink color developed was determined at 510 nm. Flavanoid content was calculated from a calibration curve using catechin as standard and the result were expressed as catechin equivalent [8].

Determination of total Flavonols

Total flavonols content was determined using the method of Kumaran and Karunakaran. 2 ml of the plant extract (1 mg/mL) was mixed with 2 ml of $AlCl_3$ prepared in ethanol and 3 ml of 50 g/L sodium acetate solution. The mixture was incubated at 20 ^{0}Cfor 2.5 h after which the absorption was measured at 440 nm. Total flavonoids content was calculated as quercetin (mg/g) using the calibration curve [9].

Determination of Terpenoids

Terpenoids content was determined by the procedure of Ferguson, 1956. About 10 gm of leaves and bark powdered was taken and soaked in alcohol for 24 hours. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids [10].

Statistical Analysis

All the experiments were performed in triplicates and the results were expressed as Mean ± S.D.

3. Result and Discussion

The present study showed that the extract of leaves and bark of *Mimusops elengi* L. contains various bioactive biomolecule in different quantities. Plant tannins, one of the major groups of antioxidant polyphenols found in food and beverages, have attracted a lot of attention in recent years because of their multifunctional properties beneficial to human health [11]. Tannin content of *Mimusops elengi* L. was determined using standard curve equation derived from standard curve of tannic acid (y = 0.006x+0.162 R^2 = 0.990) (fig. 1). The result revealed that the bark contains highest amount of Tannins (8.3 ±0.051 mg/gm TAE) compare to leaves (3.6 ±0.09 mg/gm TAE).

Flavonoids and flavanol not only quench radicals in a similar way as those phenolic acids (chemically most of the flavonoids have an active phenolic group), but they can also chelate metal ions and therefore decrease the metal-catalyzed radical reactions such as lipid oxidation. Among the biological activities, flavonoids are active against free radicals; free radical mediated cellular signaling, inflammation, allergies, platelet aggregation, microbes, ulcers, viruses, tumors and hepatotoxins [12, 13]. Flavanoid content was determine using standard curve of catechin (y=0.003x+0.031 R^2 = 0.944) (fig.2). Flavanoids content of bark was 6.8 ± 0.02 mg/gm CE and flavonols was 1.1 ± 0.13 mg/gm QE. While flavanoid content of leaves was low 3.5 ± 0.02 mg/gm CE and flavanols was 0.1 ± 0.05 mg/gm QE.

Saponin has received numerous attention due to their various biological activities that including hepatoprotective, antitumor, antimicrobial and anti-inflammatory activities [14]. Saponin content of bark was higher (35.8 ± 2.04) compare to leaves (7.60±2.03). Terpenoids are mainly involved in the defense against insects and environmental stress, and are related to the repair of damage and wounding [15]. Higher content of terpenoids was reported from bark (2.8 ± 0.36) compare to leaves (2.0 ± 0.18).

4. Conclusion

Plant secondary metabolites are found to play a crucial role in pharmaceuticals. The biological properties of the medicinal plants are due to the presences of these secondary metabolites. Intake of these secondary metabolites in the form of drug may promote health benefits, protecting against chronic disorders, such as cancer, cardiovascular and neurodegenerative diseases. Therefore the present attempt was to investigate the secondary metabolites which can form the base for future research work.

Table 1: Quantitative determination of secondary metabolites from *Mimusops elengi* L

Secondary Metabolites	Leaves	Bark
Tannin	3.6 ±0.09 mg/gm TAE	8.3 ±0.051 mg/gm TAE
Terpenoids	2.0 ± 0.18	2.8 ± 0.36
Flavanoids	3.5 ± 0.02 mg/gm CE	6.8 ± 0.02 mg/gm CE
Flavonols	0.1 ± 0.05 mg/gm QE	1.1 ± 0.13 mg/gm QE
Saponins	7.60 ±2.03	35.8 ± 2.04

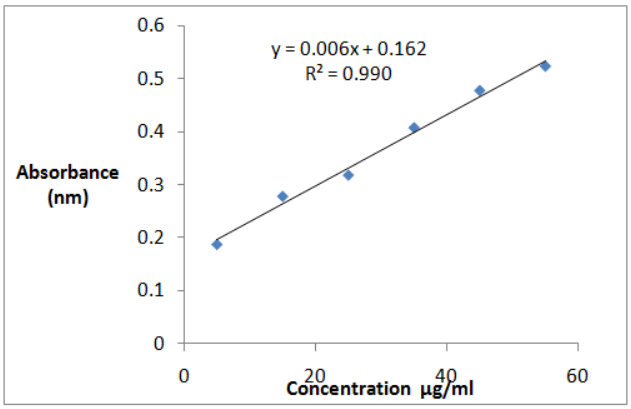

Figure 1: Standard curve of Tannic acid

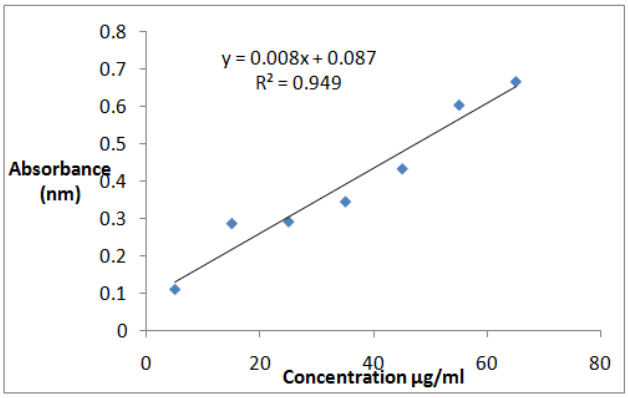

Figure 2: Standard curve of Quercetin

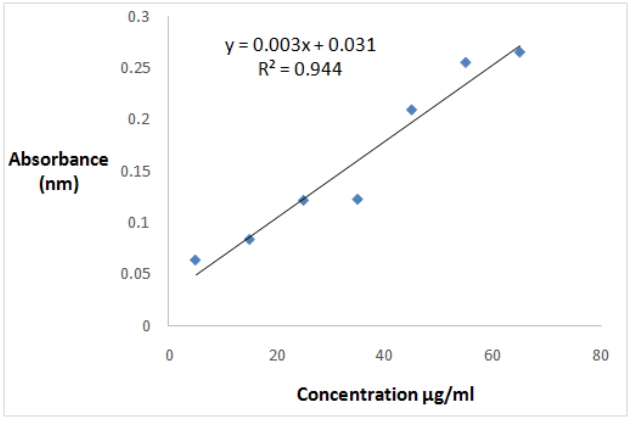

Figure 3: Standard curve of Catechin

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