QUANTIFICATION OF SECONDARY METABOLITES FROM

ZIZIPHUS MAURITIANA LAM. BARK

SHASHANK BHATT & SURESH DHYANI

Department of Biotechnology, NIMS University, Jaipur, Rajasthan, India

ABSTRACT

The plants have specific role in the environment and human life. All parts of plants are useful but some specific compounds that are called primary and secondary metabolites are most useful. *Ziziphus mauritiana* has medicinal effect. In this reference dried bark of *Ziziphus mauritiana* was used and detected the secondary metabolites quantity. 2.2% alkaloids were found in the dried bark. 25.8% flavonoids were extracted from it. 92.65% saponins and 5µg/ml tannins were found in it. It controls all metabolic activities and is effective in anticancer, antimicrobial activity, liver damage treatment.

KEYWORDS: Alkaloids, Flavonoids, Saponins, Tannins, *Ziziphus Mauritiana* Bark

INTRODUCTION

Plants are the part of life for human, animals and also microorganisms. All of them depend on plants according to ecosystem. Plants are categorized in different classes in which some of them are for general and medicinal use. It provides oxygen in photosynthesis process. These points show the importance of plants in human and animal life. Most of the medicine production companies are using different parts of plants in preparing different types of medicines for different diseases. These parts have capacity to increase the immunity power. Medicinal plants contain various types of active compounds which are used in the treatment of different diseases and to relieve from pain [Okigbo et. al.; 2008]. The properties of medicinal plants base on the antioxidant, antimicrobial, antipyretic effects of the phytochemical in them [Cowman; 1999]. According to the world Health Organization, medicinal plants can be the best source to obtain a different variety of drugs. Medicinal plants have two types of metabolites; one is primary and the other secondary. Primary metabolites are directly involved in all processes while secondary are not involved directly and work as biocatalysts. Phytochemical are chemical compounds that are formed during the plant’s normal metabolic processes. The primary metabolites are carbohydrates, DNA, protein etc. while secondary are alkaloids, flavonoids, glycosides, lignins, saponins, sterols, phenols and tannins etc. [Harborne; 1973]. All secondary metabolites have specific functions as like saponins have antimicrobial activity. These are used in medicines preparation according to their role.

*Ziziphus* is a spiny small tree which belongs to the Rhamnaceae family. It grows in subtropical regions in warm temperature throughout the world. The leaves’ shape is alternate, ovate with depressed longitudinal veins at the base. It is about 2.5 to 3.2cm long and 1.8 to 3.8cm wide. It is dark green and glossy on upper phase and pale green on the lower side. The fruit is called ‘Bor’. It has different shapes of fruits according to the varieties.

The *Ziziphus mauritiana* Lam. fruit is oval or round shaped and can be 1-2.5 inches (2.5 to 6.25cm) long, depending on the kind of the fruit. The fruit pericarp bears more quantity of nutrients. The nutrient quantity per 100gm is 17 gm carbohydrate, 5.4 to 10.5 gm sugar, 0.07 gm fat, 0.8 gm protein, 81.6 to 83.0gm water etc. [Morton J.; 1987].

The extract of roots revealed the presence of alkaloids, flavonoids, glycosides, saponins and volatile oil. The methanolic extract showed the antidiarrhoel effect as exhibited a concentration dependent inhibition of the spontaneous
pendular movement of the isolated rabbit and inhibited acetylcholine induced contraction of rat ileum [D. Dahiru et. al.; 2006].

*Ziziphus mauritiana* has different types of medicinal characteristics. The ethanolic extract of *Ziziphus mauritiana* leaf was used and found that leaf extract protected rats against CCl4 liver injury by lowering AST, ALT, ALP, TB and lipid proxide levels. [D. Dahiru, William, E.T. and Nadro, M.S. 2005]. *Ziziphus jujube* Mill. was used traditionally as tonic and aphrodisiac. It is sometimes used as hypnotic sedative and anxiolytic, anticancer, anti-inflammatory, cognitive, antispastic, antifertility /contraception, hypotensive and antinephritic, cariotoxic, antioxidant, immunostimulent and wound leading properties [R.T. Mahajan, MZ Chopda; 2009].

**MATERIAL AND METHODS**

**Collection of Plant Material**

*Ziziphus mauritiana* Lam. is found in many parts of the world. I collected the ripened fruits at Mandsaur, district Mandsaur, Madhya Pradesh. District Mandsaur is situated at the northern projection of Madhya Pradesh between the parallels of latitude 23° 45' 50" North and 25° 2' 55" North, between the meridians of longitude 74° 42' 30" East and 75° 50' 20" East.

![Figure 1: Map of Madhya Pradesh](image1.jpg)

![Figure 2: Bark of Ziziphus Mauritian Tree](image2.jpg)
**Phytochemical Screening of Secondary Metabolites**

The barks were dried and then powdered using mixer grinder and kept to cold percolation process for 48 hours with petroleum ether, chloroform, 95% ethanol, methanol and distilled water. Then the extracts were filtered and used for preliminary phytochemical screening such as alkaloids, flavonoids, glycosides, lignin, phenols, saponins, sterols and tannins [Shashank Bhatt *et. al.*; 2011]. The quantitative estimations had occurred that were present in phytochemical screening.

**Quantitative Estimation of Alkaloids [Krishnaiah D. *et al.* 2009]**

5 gm bark sample was collected into 250 ml beaker and added 200 ml of 10% acetic acid in ethanol. The reaction mixture was kept for 4 hrs. and then filtered. Filtrate liquid was kept on water bath for ¼ to original volume. After this process, conc. NH$_4$OH was added into solution drop by drop and found precipitate. The whole solution was kept for some time for the settlement of precipitate. The precipitate was washed with dilute NH$_4$OH then filtered. The weight of precipitate was taken after drying it.

**Quantitative Estimation of Flavonoids [Krishnaiah D. *et al.* 2009]**

10gm of fruits pericarp powder was repeatedly shaken with 100 ml of 80% aqueous methanol at room temperature. The whole solution was then filtered through filter paper. The filtrate was kept on water bath and waited until solution evaporated into dryness. The sample was constantly weighed until it got a fixed weight.

\[
\% \text{ Flavonoids} = \frac{W_2 - W_1}{W_{\text{Sample}}} \times 100
\]

Where:

- $W_1$ = Weight of empty filter paper
- $W_2$ = Weight of filter paper + Flavonoids extract.

**Quantitative Estimation of Saponins [Obadoni BO, Ochuko PO; 2001]**

20gm of bark powder was dispersed in 200ml of 20% aqueous ethanol. The suspension was heated over a hot water bath for 4 hrs. with constant stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into 250 ml separating funnel and 20ml of diethyl ether was added and shaken vigorously.

The aqueous layer was recovered while the ether layer discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation, the samples were dried in oven to constant weight and saponins content were calculated in percentage.

\[
\% \text{ Saponins} = \frac{W_2 - W_1}{W_{\text{Sample}}} \times 100
\]

Where

- $W_1$ = Weight of evaporating dish
- $W_2$ = Weight of dish + Sample.
Quantitative Estimation of Tannins

Estimation of tannins was carried out by using Folin-Denis reagent [Anonymous, 1980]. Tannin-like compounds were reduced into phosphotungstomolybdic acid in alkaline solution to produce a highly coloured blue solution, the intensity of which was proportional to the amount of tannins. 500 mg sample was transferred into 250 ml conical flask. After adding 75 ml water, it was boiled for 30 min. Centrifuged at 2000 rpm for 20 min. collected the supernatant in 100ml volumetric flask and made up the volume. Transferred 1 ml of the sample from 100ml volumetric flask and added 75 ml water. Added 5 ml Folin-Denis reagent. Mixed 10 ml sodium carbonate solution and diluted it with distilled water and volume made upto 100 ml., shaked well and read the absorbance at 700 nm after 30 min.

RESULTS AND DISCUSSIONS

The quantities of primary and secondary metabolites are vary in different plant species. The primary metabolites are found in all plant’s parts but secondary are not compulsory. Alkaloids have dramatic physiological activities; therefore they are widely used in the development of medicines (Harborne, 1973; Okwu, 2005). 2.2% alkaloids were present in *Ziziphus mauritiana* Lam. bark. Some alkaloids may be useful against HIV infection (McMahon *et al.*, 1995; Sethi, 1979) as well as intestinal infections associated with AIDS (McDevitt *et al.*, 1996). Nitrogenous compounds are comprised in alkaloids and that are widely used as therapeutic agents in the management of cancer (Noble, 1990).

Flavonoids are one of the important compounds that have different activities. They can prevent oxidative cell damage and have strong anti-cancer activity and protect against all stages of carcinogens. They have the capacity to reduce the risk of heart diseases (Urquiaga and Leighton, 2000). 1.4% flavonoids were present in dried bark sample of *Ziziphus mauritiana* Lam. plant. The flavonoids have antioxidant property and their protective effect against cardiovascular diseases. Saponins are a special class of glycosides. These have soapy characteristics (Fluck, 1973). Saponins have active antifungal activity (Sodipo *et al.*, 1991). 87.05% saponins were present in bark sample. It shows the good activity against fungi. Tannins are the highly antimicrobial agents that are water soluble polyphenolic compounds. Tannins inhibit the growth of fungi, yeasts, bacteria, and viruses (Chung *et al.*, 1998). The sample tannins were calculated in comparison to standard tannic acid. 33 µg/ml tannins were found in bark sample. Tannins have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects.

CONCLUSIONS

Different types of secondary metabolites have different activities. The *Ziziphus mauritiana* Lam. bark have most of the secondary metabolites that are highly effective against different diseases. *Ziziphus mauritiana* Lam. bark have alkaloids, flavonoids, saponins and tannins. 87.05% saponins quantity showed high effects against different fungi species. The presence of these metabolite compounds showed the medicinal effect of this plant. Hence it can be kept into medicinal plant category. The research shows, *Ziziphus mauritiana* Lam. bark has some specific compounds that can be used in the treatment of diseases like cancer and HIV.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Secondary Metabolites</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Alkaloids</td>
<td>2.2%</td>
</tr>
<tr>
<td>02</td>
<td>Flavonoids</td>
<td>25.8%</td>
</tr>
<tr>
<td>03</td>
<td>Saponins</td>
<td>92.65%</td>
</tr>
<tr>
<td>04</td>
<td>Tannins</td>
<td>5 µg/ml</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

Praying and dedicating my research article to Maa Saraswati, the goddess of knowledge and wisdom, I am unable to find words to express my deepest gratitude to my parents, Mr. Krishna Kumar Bhatt and Mrs. Subhadra Bhatt, whose encouragement helped me to go ahead on this bright path. I share the credit of my work to respectable elder brother, Mr. Mayank Bhatt who very often guided me in the work.

The research work would have been a dream, had it not been enlightened my way, by my well wishers and the above respectables. Last but not least, the Almighty God is unforgettable without whose kindness nothing could happen.

REFERENCES


