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Research Article

# PHYTOCHEMICAL SCREENING OF SECONDARY METABOLITIES OF *ZIZIPHUS MAURITIANA* LAM. BARK

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**ABSTRACT**

The present paper shows *Ziziphus mauritiana* Lam. has the medicinal properties. The plant bark's compounds were extracted with petroleum ether, chloroform, methanol, and distilled water through cold percolation method and found different types of secondary metabolic compounds. These are alkaloids, flavonoids, glycosides, phenol, lignins, saponins, sterols and tannins were presented. Mostly all main types of secondary metabolites were present in this research.

**Keywords:** Alkaloids, Flavonoids, Phenol, Saponins, *Ziziphus Mauritiana* Lam.

## INTRODUCTION

Medical field is the broad spectrum in which different areas are presented as like allopathic, ayurvedic and unani etc. But today, nearly about 80% population believes in ayurvedic medicines or traditional medicines that are prepared from plant materials. It does not show any side effect in human body [WHO; 1993]. Therefore, it is true that the plants have important role in human lifespan and human health. The primary and secondary metabolites show key role in plant metabolic activity by which it produces different types of energy and useful products.

Different types of plant products are used by animals and human as a feeder. Growing plants had been one of the cheapest sources of feeding for animals having crude protein of 14-25% [Abdu SB. *et.al.;* 2007, Simbaya J; 2002]. It had been estimated that vitamins and minerals are lacking in grassland pastures [Keay RWJ; 1989].

Different types of medicinal plants are found all over the world but their uses are not known to all the people. Plants have two types of metabolites, one of which is primary and secondary. Primary metabolites are directly involved in plant activities while secondary are not involved.

*Ziziphus* is one of which that is found in all over the world and have medicinal activity. *Ziziphus mauritiana* Lam. is one of which that is grown in dry places. It has different morphological changes by which it is divided into different species. It is generally used for feeding cattle, camel, goats getting resistance power against different types of pathogens [Morton J., 1987]. *Ziziphus mauritiana* Lam. belongs to the family of *Ziziphus* and to the kingdom; plantae, order; roasles, division; magnoliophyta, class; magnoliopsida, family; rhamnaceae, genus; Ziziphus, species; mauritiana. It is fast growing tree which is almost evergreen, but is deciduous during the dry season. Its height can reach upto 12 meter to 30 cm diameter at breast height [Singh J.P. 1973]. The leaves of *mauritiana* Lam. had been also used in the treatment of liver diseases, asthma and fever [Morton; 1987]. Different types of valuable contents as like carbohydrates, starch, proteins, sugar, mucilages and vitamins are abundantly found in *ziziphus* species [Clifford S.C. Paper-Characterization; 2002]. Fruits are useful in burning sensation, hyperdipsia, consumption, vomiting, constipation, flatulence, dyspepsia, nausea, leprosy, thirst, fatigue, pruritis, wounds and ulcers. Seeds are also useful in the treatment of encephalopathy, ophtalmopathy, cough and asthma, burning sensation, diarrhea, vomiting, general debility and insomnia. *Ziziphus mauritiana* Lam. bark is useful in dysentery, diarrhea, gingivitis, boils and ulcers [Kapoor LD.; 2005, Nadkarni KM.; 2002, Khare CP; 2004, Bhattacharjee SK; 2004, Sheth A.; 2005]. *Ziziphus mauritiana* Lam. bark has the compounds that can be used in the treatment of cancer. Cancer is the main problem all over the world.

## MATERIAL AND METHODS

### Collection of Plant Material

*Ziziphus mauritiana* Lam. is found all over the world. I had collected the plant barks material from Mandsaur district, Madhya Pradesh. Mandsaur District forms the northern projection of Madhya Pradesh. It lies between the parallels of latitude $23^0$ 45' 50" North and $25^0$ 2' 55" North, and between the meridians of longitude $74^0$ 42' 30" East and $75^0$ 50' 20" East.

### Preliminary Screening of Secondary Metabolites

The plant bark was dried and powdered using mixer grinder, and subjected to cold percolation process for 48 hours with petroleum ether, chloroform, methanol and distilled water. After this process, the extracts were filtered and used for preliminary phytochemical screening such as alkaloids (Iodine, Wagner, and Dragendorff's test), flavonoids (Pew's, Shinoda and NaOH tests), glycosides(Keller-Killani, Conc. $H_2SO_4$, and Molisch tests), lignin (Labat and Lignin tests), phenols (Ellagic acid and Phenol tests), sapnins (Foam and Haemolysis test), sterols (Libermann- Burchard, and Salkowski tests), tannins (Gelatin and Lead acetate tests) were carried out [Shashank Bhatt *et. al.,*2011].

### Preliminary Screening of Phytochemical Test

### Phytochemical Screening

The filtrate obtained was subjected to preliminary phytochemical screening.

### Test for Alkaloids

**Iodine Test:** A few drops of dilute iodine solution were added into 3 ml test solution added. Blue colour appeared; and disappeared on boiling and reappeared on cooling [Khandewal K.R., 2008].

**Wagner's Test**: Few drops of Wagner's reagent were added into 2 to 3 ml extract. Formation of reddish brown precipitate indicates the presence of alkaloids [Kokate C. K. *et. al;* 2001].

**Dragendorff's Tests**: Few drops Dragendorff's reagent were added into 2 to 3 ml extract. Formation of orange brown precipitate indicates the presence of alkaloids [Kokate C. K. *et .al;* 2001].

### Test for Flavonoids

**Pew's Tests**: zinc powder was added into 2-3 ml. extract, followed by drop wise addition of con. HCl. Formation of purple red or cherry colour indicates the presence of flavonoids [Peach K., Tracey MV. 1956].

**Shinoda Tests**:- 2-3 ml. extract and few fragments of magnesium metal were added into a test tube, followed by dropwise addition of

concentrated HCl. Formation of magenta colour indicates the presence of flavonoids [Kokate C. K. *et. al;* 2001].

**NaOH Tests**: 2-3 ml. of extract and few drops of sodium hydroxide solution were added into a test tube. Formation of intense yellow colour that became colourless on addition of few drops of dilute HCl indicates the presence of flavonoids [Khandewal K.R., 2008].

**Test for Glycosides:**

**Keller-Killani Test**: Glacial acetic acid was added into 2 ml. extract and one drop 5% $FeCl_3$ and conc. $H_2SO_4$. Reddish brown color appears at the junction of the two liquid layers and the upper layer of bluish green indicates the presence of glycosides [Kokate C. K. *et. al;* 2001].

**Glycosides test**: 1 ml. water was added into the small amount of extract and shaked well. Then aqueous solution of NaOH was added. The appearance of yellow colour indicates the presence of glycosides [Treare GE, Evans WC. 1985].

**Concentrate $H_2SO_4$ Test**: 2ml. glacial acetic acid, one drop of 5% $FeCl_3$ and conc. $H_2SO_4$ were added into 5ml extract, the appearance of brown ring indicates the presence of glycosides [Khandewal K.R., 2008].

**Molisch's Test**: 2 drops of Molisch's regent was added into 1 ml of extract, and 2 ml of concentrate $H_2SO_4$ was added carefully into above solution. Formation of violet ring at the junction indicates the presence of glycosides [Kokate C. K. *et. al;* 2001].

**Test for Phenols**

**Ellagic Acid Test**: The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turned muddy or niger brown precipitate occurred in the extract. It indicates the presence of phenols solution [Gibbs R.D., 1974].

**Phenol Tests**: 0.5 ml of $FeCl_3$ (w/v) solution was added into 2 ml of test solution, formation of an intense colour indicates the presence of phenols [Gibbs R.D., 1974].

**Test for Lignins**

**Lignin test**: 2 ml of 2% (w/v) furfuraldehyde was added into the test solution. Formation of red colour indicates the presence of lignin [Gibbs R.D., 1974].

**Labat test**: The test solution was mixed with gallic acid; it developed olive green colour indicating the positive reaction for lignins [Gibbs R.D., 1974].

**Test for saponins**

**Foam Test**: The extract was diluted with 20 ml of distilled water and was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam, indicates the presence of saponins [Kokate C. K. *et. al;* 2001].

**Haemolysis Tests**: - one drop of extract and one drop of blood was placed on the glass slide. Hemolytic zone appeared [Kokate C.K., 1994].

**Test for Sterols**

**Liebermann-Burchard Test**: chloroform was mixed into 2ml. extract. 1-2 ml. acetic anhydride and 2 drops of concentrated $H_2SO_4$ were dropped into the test tube. First red, then blue and finally green colour indicates the presence of sterols [Kokate C. K. *et. al;* 2001].

**Salkowski's Test**: 2ml chloroform and 2 ml concentrated $H_2SO_4$were added to the 2 ml extract and shook well. The layer of red chloroform and acid shows greenish yellow fluorescence. It indicates the presence of sterols [Kokate C. K. *et. al;* 2001].

**Test for Tannins**

**Gelatin Test**: Gelatin (gelatin dissolves in warm water immediately) solution was added into the extract. Formation of white precipitate indicates the presence of tannins [Treare GE, Evans WC. 1985].

**Lead acetate test: F**ew drops of 10% lead acetate solution were added into 5 ml of extract. Formation of yellow or red precipitate indicates the presence of tannins [Treare GE, Evans WC. 1985].

**RESULT AND DISCUSSION**

The plant bark was powdered and subjected to cold percolation with petroleum ether, chloroform, methanol, and distilled water for 48 hours. The results of the phytochemical screening of bark extract of *Ziziphus mauritiana* Lam. were present in Table-1. Different types of secondary metabolites such as alkaloids, flavonoids, glycosides, phenol, lignins, saponins, sterols and tannins were presented. *Ziziphus mauritiana* Lam. bark is very effective compared to other part because most parts of secondary metabolites are present in it [Table-1].

Flavonoids have inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show ant-allergic, antimicrobial and anticancer activity by which it can be used for different diseases that is generally found in bark.

Tannins have general antimicrobial and antioxidant activities [Rievere *et. al*., 2009].

Current reports show that tannins may have potential value such as cytotoxic and antineoplastic agents [Aguinaldo *et. al*., 2005]. Saponins have antifungal properties [Aboada and Efuwape, 2001; Mohanta *et. al*., 2007]. These contents are shown different types of activity against different pathogens. Therefore, it can be used in the treatment of diseases.

Saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss etc. according to medical field. It is a bioactive antibacterial agents of plants [Mandal *et. al*. 2005: Manjunatha, 2006].

Plant steroids have cardiotonic activity, possess insecticidal and antimicrobial properties. It is generally used in herbal medicines and cosmetic products (Callow; 1936).

Phenolic compounds have anti-oxidative, antidiabetic, anticarcinogenic, antimutagenic and anti-inflammatory (Arts and Hollman; 2005, Scalbert *et. al.;* 2005).

**Fig. A: The bark of *ziziphus mauritiana* lam.**

**Table 1: Phytochemical study of *ziziphus mauritiana* lam. bark**

| Test | Petrolium Ether | Chloroform | Methanol | Distilled water |
|---|---|---|---|---|
| **Alkaloids** | | | | |
| Iodine Test | -ve | -ve | -ve | -ve |
| Wagners Test | -ve | -ve | +ve | -ve |
| Dragendorff's Test | -ve | -ve | +ve | -ve |
| **Flavonoids** | | | | |
| Pews Test | -ve | -ve | -ve | -ve |
| Shinoda Test | -ve | -ve | +ve | -ve |
| NaOH Test | -ve | -ve | +ve | -ve |
| **Glycosides** | | | | |
| Keller- Killani Test | +ve | +ve | +ve | +ve |
| Glycosides Test | +ve | +ve | +ve | +ve |
| Conc. H2SO4 | +ve | +ve | +ve | +ve |
| Molishs Test | +ve | +ve | +ve | +ve |
| **Phenol** | | | | |
| Ellagic Test | -ve | -ve | +ve | -ve |
| Phenol Test | -ve | -ve | +ve | -ve |
| **Lignin** | | | | |
| Lignin Test | -ve | -ve | +ve | +ve |
| Labat Test | -ve | -ve | +ve | +ve |
| **Saponins** | | | | |
| Foam Test | -ve | -ve | +ve | -ve |
| Haemolysis Test | -ve | -ve | +ve | -ve |
| **Sterols** | | | | |
| Libermann- Burchard Test | +ve | +ve | +ve | -ve |
| Salkowski Test | +ve | +ve | +ve | -ve |
| **Tannins** | | | | |
| Gelatin Test | -ve | -ve | +ve | +ve |
| Lead Acetate Test | -ve | -ve | +ve | +ve |

## CONCLUSION

According to this research work I have concluded that different types of secondary metabolites are present that have different specific functions against pathogens, bacteria, viruses etc. mostly all secondary metabolites are present in the mauritiana bark that can be used in the treatment of cancer, allergy most common diabetic disease. Hence, its bark can be used in the treatment of cancer, mutation and other bacterial and virus disease. The plant bark should be used in the preparation of medicinal drug for the treatment of cancer, antimicrobial and antifungal activity.

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