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Research Article

PHYTOCHEMICAL STUDIES OF THE SECONDARY METABOLITES OF *ZIZIPHUS MAURITIANA* LAM. LEAVES

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ABSTRACT

The present paper shows the medical importance of *Ziziphus mauritiana* Lam. The plant leaves compounds were extracted with petroleum ether, chloroform, methanol, 95% ethanol and distilled water for 48 hours and found different types of secondary metabolites as glycosides, saponins, phenols, lignins and tannins were present.

Keywords: Glycosides, Phenol, Saponin, *Ziziphus Mauritiana* Lam.

INTRODUCTION

Plants have an important key role in the living and nonliving organisms. Today global warming is a major issue for environment safety. India has different types of regions like Himalayan regions, desert regions, highly rain regions. Madhya Pradesh is the state in which Malwa is one of its parts. This region has normal situations in which there is neither too much nor too low rains. These regions have different varieties of plants which are useful from medical point of view.

Medicinal plants have different types of useful contents that are primary and secondary metabolites. Primary metabolites are directly involved in metabolic process while secondary metabolites are not involved directly and work as catalyst.

Growing plants are one of the cheapest sources of feeding for animals, having crude protein of 14-25% [Abdu SB. *et.al* 2007, Simbaya J; 2002]. These plants provide vitamins and minerals which are lacking in grassland pastures [Keay RWJ; 1989]. *Ziziphus* is a very common plant that is easily available all over the world. Near about 40 species are available of *ziziphus* and one of which is, *ziziphus mauritana* Lam., very common. *Ziziphus* belongs to the kingdom; plantae, order; roasles, division; magnoliophyta, class; magnoliopsida, family; rhamnaceae, genus; *Ziziphus,* species *mauritiana*. This plant is generally grown in dry places. Its fruiting time is February to March. Morphological changes are also found in different climatic conditions.

The *ziziphus mauritiana* Lam. leaves are eaten by cattle, camels, goats etc. by which they found minerals, useful for their health [Morton J., 1987]. Carbohydrates, starch, proteins, sugar, muilages and vitamins are abundantly present in *ziziphus* species. Whole plant of *ziziphus mauritiana* Lam. is found to be used as a medicinal tonic.

Ziziphus mauritania's Lam. fruits have highly useful contents quantity that is useful for human heath. It is an evergreen shrub or tree up to 15 m tall and up to 40 cm in diameter. The leaves are alternate and elliptic, 2.5-3.2 cm long and with three distinct veins. In the leaf-corners are two spines, one long and straight the other small and curved. Although most of the trees bear spines, spineless individuals are not uncommon. Flowers are small and bisexual, yellow or greenish, borne on short stalks, 2-3 together in the leaf-corners [Liu, M. J.; Cheng, C. Y. 1995, Singh, J. P.; Singh, I.S. 1973].

MATERIAL AND METHODS

Collection of Plant Material

Ziziphus mauritiana Lam. is found all over the world. I had collected the plant leaves 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 leaves were dried in the shaded area and powdered using mixer grinder, and subjected to cold percolation process for 48 hours with petroleum ether, chloroform, methanol, 95% ethanol 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), saponins (Foam and Haemolysis test), sterols (Liberman-Burchard, and Salkowski tests), tannins (Gelatin and Lead acetate tests), Lignin (Labat and Lignin tests), Phenols (Ellagic acid and Phenol 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: Few 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 shade dried plant leaves were powdered and subjected to cold percolation with petroleum ether, chloroform, methanol, 95% ethanol and distilled water for 48 hours. The results of the phytochemical screening of leaves extract of *Ziziphus mauritiana* Lam. were presented in Table-1. Different types of secondary metabolites such as glycoside, phenol, lignin, saponins and tannins were presented while alkaloids, flavonoids and sterols were absented in *Ziziphus mauritiana* Lam. [Table-1].

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 show 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 agent of plants. [Mandal *et. al.;* 2005: Manjunatha, 2006].

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

Test	Petroleum Ether	Chloroform	Methanol	95% Ethanol	Distilled water
Alkaloids					
Iodine Test	-ve	-ve	-ve	-ve	-ve
Wagners Test	-ve	-ve	-ve	-ve	-ve
Dragendorff Test	-ve	-ve	-ve	-ve	-ve
Flavonoids					
Pews Test	-ve	-ve	-ve	-ve	-ve
Shinoda Test	-ve	-ve	-ve	-ve	-ve
NaOH Test	-ve	-ve	-ve	-ve	-ve
Glycosides					
Keller Killani Test	+ve	-ve	+ve	+ve	-ve
Glycosides Test	-ve	+ve	-ve	+ve	-ve
Conc. H2SO4	+ve	-ve	+ve	+ve	+ve
Molisch's Test	-ve	-ve	-ve	+ve	+ve
Phenol					
Ellagic Test	-ve	+ve	+ve	+ve	-ve
Phenol Test	-ve	-ve	+ve	+ve	-ve
Lignin					
Lignin Test	-ve	-ve	+ve	+ve	-ve
Labat Test	-ve	-ve	+ve	+ve	-ve
Saponins					
Foam Test	-ve	-ve	+ve	+ve	+ve
Haemolysis Test	-ve	-ve	+ve	+ve	-ve
Sterols					
Libermann- Burchard Test	-ve	-ve	-ve	-ve	-ve
Salkowski Test	-ve	-ve	-ve	-ve	-ve
Tannins					
Gelatin Test	-ve	-ve	+ve	+ve	+ve

Lead acetate Test	-ve	-ve	+ve	+ve	+ve

Fig. A: leaflet of *ziziphus mauritiana* lam.

CONCLUSION

According to our research works, I have concluded that different types of secondary metabolites are present that have different types of functions. It shows very effective function against pathogens. Hence, its leaves can be used in the treatment of liver diseases according to their function and also used in cancer treatment. The plant leaves should be used in the preparation of medicinal drug for the treatment of different types of cancer, antimicrobial and antifungal activity.

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