

# Immunomodulatory, Cytotoxicity, and Antioxidant Activities of Roots of *Ziziphus mauritiana*

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Submitted: 10-09-2016

Revised: 08-11-2016

Published: 11-07-2017

## ABSTRACT

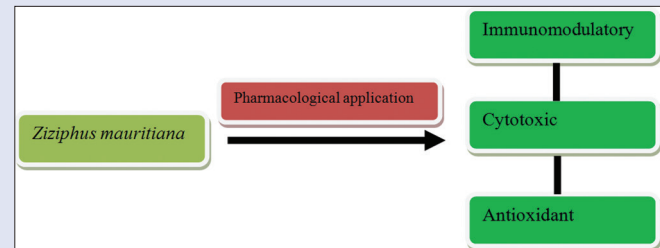
**Aims:** The study is conducted to evaluate the immunomodulatory, cytotoxicity, and antioxidant potential of *Ziziphus mauritiana* (Rhamnaceae). Phytochemical analysis of *Z. mauritiana* revealed the presence of alkaloids, anthraquinone glycoside, cardiac glycoside, saponin, tannin, and flavonoids. **Methodology:** The cytotoxicity of the plant *Z. mauritiana* was evaluated by brine shrimp lethality test. Antioxidant parameters such as superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) levels were calculated in the plasma of rats after chronic administration of 400 mg/kg of *Z. mauritiana* for 6 weeks. **Results:** The dichloromethane extract of the plant exhibited significant immunomodulatory activity, with inhibitory concentration 50% of 55.43 ± 7.9. The dichloromethane extracts of the plant showed 70% mortality at concentration 1000 µg/ml. SOD and T-AOC levels were increased while MDA level in the plasma was reduced in the plasma of rats treated with dichloromethane *Z. mauritiana*. **Conclusion:** This can be deduced that the root of *Z. mauritiana* has immunomodulatory, cytotoxic, and antioxidant potential.

**Key words:** Cytotoxicity and antioxidant potential, immunomodulatory, *Ziziphus mauritiana*

## SUMMARY

- Roots of *Z. mauritiana* was exhibited immunomodulator, cytotoxic and antioxidant activities
- *Z. mauritiana* showed potential antioxidant activity in rats

- This can be deduce that *Z. mauritiana* has cytotoxic, and antioxidant potential.



**Abbreviations used:** SOD: Superoxide dismutase; T-AOC: Total antioxidant capacity; MDA: Malondialdehyde; ZMRD: *Z. mauritiana* root extract of dichloromethane fraction; LD50: *Z. mauritiana* root extract of methanol fraction ZMRM, lethal dose 50.

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**DOI:** 10.4103/pm.pm\_398\_16

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## INTRODUCTION

There is a lot of archaeological evidence that humans used medicinal plants for better living, reducing disease, and improving quality of life. Several studies have described the use of plants in traditional medicine for many years and have recently gained much importance in the field of pharmacological industries. The knowledge and assessments of the biological properties of extracts from plants can serve as a source of future drug candidates in many areas of health.<sup>[1]</sup> The relevant literature shows that many plants or plant products have been used as an alternative source for the treatment of different diseases. Several of these herbs have been used to exert immunomodulatory effect in the treatment of various diseases. Medicinal plants with immunomodulatory activity are used for cases of organ transplant rejection or for the treatment of various autoimmune diseases.<sup>[2]</sup> Medicinal plants can inhibit or stimulate immune response which could be useful in the treatment of various human diseases. In particular, medicinal plants capable of inhibiting the cellular and humoral responses could have useful applications in the treatment of immunological disorders.<sup>[3]</sup>

The plant *Ziziphus mauritiana* belongs to the family Rhamnaceae. The family comprises 58 genera and almost 900 species.<sup>[4]</sup> Many species of the genus *Ziziphus* are found in Pakistan, Iran, Europe, America, India, and Afghanistan.<sup>[5]</sup> The plant is commonly known as Berry and Chotta Ber in Pakistan.<sup>[6]</sup> The plant is used to cure wide variety of diseases such as fever, ulcer, asthma, depression, anxiety, and inflammation.<sup>[7-9]</sup>

Plant is enriched with biological and phytochemical properties. The *Z. mauritiana* showed antioxidant, antimicrobial, antitumor, antidiabetic, and anticancer activities.<sup>[6,10,11]</sup>

The various species of genus *Ziziphus* showed antipyretic, antidiuretic, anti-inflammatory, and analgesic activities.<sup>[12,13]</sup> The genus *Sisypus* has found many photochemical constituents, cyclopeptide alkaloids such as Absenine B, Zazyphine D, amphibine C, and oxyphylline A.<sup>[14]</sup> The polysaccharides such as rhamnose and arabinose also reported from genus *Ziziphus*.<sup>[15]</sup>

## MATERIALS AND METHODS

### Plant collection

The roots of *Z. mauritiana* was collected from surroundings of Bahauddin Zakariya University in 2015 and identified by Dr. Zafrulla

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**Cite this article as:** Afzal S, Batool M, Ch BA, Ahmad A, Uzair M, Afzal K. Immunomodulatory, cytotoxicity, and antioxidant activities of roots of *Ziziphus mauritiana*. Phcog Mag 2017;13:S262-5.

Ullah Zafar, Plant Taxonomist, Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan. A voucher specimen number Stewart 469 (6) for *Z. mauritiana* was submitted to the department.

## Extraction

The freshly collected roots of *Z. mauritiana* (20 g) was taken and shade dried, ground, and extracted successively with solvents dichloromethane and methanol (3 × 6 L) at room temperature for 24 h. The dichloromethane and methanolic extract was concentrated under vacuum on Rotavapor model number Buchi-Rotavapor R.200. The dichloromethane extract was obtained brown crude extract (1.64 g) which was labeled as ZMRD. The other extract of methanol was obtained as dark brown crude extract (13.37 g) which was labeled as ZMRM. Results of the extraction of the plant are mentioned in Table 1.

## Phytochemical tests

Detection of alkaloids, anthraquinone glycosides, cardiac glycosides, tannin, catechin, flavonoids, and saponin was done using the gold standard methods as reported.<sup>[16]</sup>

## Brine shrimp lethality assay

10 mg of each plant extract was dissolved in 1 ml of dimethyl sulfoxide to prepare a stock solution. It was used to prepare further dilutions. 34 g of sea salt (Sigma) was mixed in a liter of distilled water. It was continuously stirred for 2 h. We used a rectangular tray having two compartments with sea salt saline for the hatching of brine shrimps. One compartment was covered for darkness. Eggs of brine shrimps were sprinkled in this dark compartment and placed for hatching for 24 h. Larvae were collected after 24 h with the help of pipette from lightened side. 100 µl (1000 µg), 10 µl (100 µg), and 1 µl (10 µg) of the plant extract stock were taken up and added in drum vials used in the assay. In each vial, we added 2 ml of saline and then with the help of pipette transferred 10 shrimps in each vial, raised the volume of saline in each vial up to 5 ml, and incubated at 25°C. After 24 and 48 h of incubation, number of survivors was counted using magnifying glass.

Then, using Abbot's formula calculations were made.

Percentage death = (control - sample)/control × 100 (Meyer *et al.* 1982).

## Immunomodulatory activity

The estimation of oxidative burst activity performed by chemiluminescence assay. Luminol-enhanced chemiluminescence assay was conducted following procedure.<sup>[17]</sup> The brief steps are given below:

1. Place 25 µl Hanks' balanced salt solution (HBSS)++ to a 96-well flat bottomed plate in a final volume of 0.1 ml
2. Add sample (drug or compound to be tested) and prepare serial dilutions to obtain very low level of activity
3. Add HBSS++ with no compound or drug to be used as control
4. Place 25 µl whole blood (1:50 dilution) or PMNCs (1 × 10<sup>6</sup>/ml) or MNCs cells (1 × 10<sup>6</sup>/ml) or macrophages (1 × 10<sup>6</sup>/ml) in suspension

of HBSS++ to each well, except B\* and blank

5. The mixture will be placed in incubator for 30 min in Luminoskan chamber about 37°C
6. After incubation, add 25 µl of luminol into each well
7. Place 25 µl serum opsonized zymosan, except A\* and blank
8. The luminometer used to monitor the phagocytosis studies for 50 min in the repeated scan mode. Peak and total integral chemiluminescence reading were expressed in the relatively light unit (RLU). The RLU was used to express the peak and total chemiluminescence.

## Antioxidant activity

Totally, 18 Wistar-Kyoto rats were recruited and acclimatized for 5 days before starting the chronic administration of *Z. mauritiana*. Animals were divided into 3 groups: control group receiving water, standard group receiving Vitamin C 1 g/kg, and third group receiving 400 mg/kg dose of *Z. mauritiana* for 6 weeks as reported.<sup>[18]</sup> These animals were given free access to water and food containing *ad libitum*.

## Measurement of oxidative stress parameters

Oxidative stress parameters such as superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (T-AOC) levels were measured after 6 weeks of chronic administration by drawing the blood by nipping the tail and collecting the plasma by centrifugation the blood at 10,000 rpm for 5 min from the plasma using kits (NJJC Bio Inc., Nanjing, China).

## RESULTS AND DISCUSSION

The preliminary phytochemical analysis of the plant showed the presence of alkaloids, flavonoids, tannins, saponin, and cardioglycosides [Table 2]. The dichloromethane and methanol extracts were obtained and subjected to investigate the cytotoxicity and immunomodulatory activity. The dichloromethane extract of the plant exhibited significant inhibitory of immunomodulatory activity [Table 3]. The dichloromethane extracts of the plant showed 70% mortality at concentration 1000 µg/ml [Tables 4 and 5]. The cytotoxicity of the plant showed due to the presence of flavonoids.

Many natural products have played a significant role in maintaining and improving the quality of human life. The modulation of immune response to alleviate many diseases has been one mechanism of interest for pharmacology studies. A great number of medicinal plants have been shown to stimulate or inhibit immune response. In the present study, dichloromethane and methanolic extract obtained from roots of this plant was investigated for its cytotoxic effects and immunomodulatory activity. For this purpose, at first, the present study evaluated the cytotoxic activity of the extract of the plant by brine shrimp lethality activity. It was observed that ZMRD extract showed highest activity at 1 g dose having LD<sub>50</sub> values 47 µg/ml when compared to standard drug etoposide 7.46 µg/ml. In contrast, methanolic extract was 50% less effective at same dose, indicating that the dichloromethane extract is better in cytotoxicity.

**Table 1:** Result of extraction of roots of *Ziziphus maritiana*

Plant name	Part used	Solvent	Weight of extract (mg)	Abbreviation for the extracts
<i>Ziziphus maritiana</i>	Roots parts (20gm)	Dichloromethane	1.64gm	ZMRD
		Methanol	13.37gm	ZMRM

**Table 2:** Results of phytochemical screening of *Ziziphus mauritiana*

Name of plants	Alkaloid	Anthraquinone Glycoside	Cardiac Glycosides	Saponins	Tannin	Flavanoid
<i>Ziziphus maritiana</i> (Root part)	+	+	+	+	+	+

+: Present, -: Absent

**Table 3:** Results of immunomodulatory studies dichloromethane and methanolic extracts of roots of *Ziziphus mauritiana*

Sample code	Concentration (mg/ml)	%Inhibition	IC <sub>50</sub> ± SD
ZMRD	25 µg/ml	12.34	55.43±7.9
ZMRM	25 µg/ml	NIL	NIL
Ibuprofen	3 g/ml	4.6	23.2±1.8

**Table 4:** Result of Brine Shrimp (*Artemiasalina*) lethality bioassay of dichloromethane extracts of roots of *Ziziphus mauritiana*

Dose (µg/ml)	No. of shrimps	No. of survivors	LD50 (µg/ml)	Std. drug	LD50 (µg/ml)
10	30	23	47.0284	Etoposide	7.4625
100	30	12			
1000	30	1			

**Table 5:** Result of brine shrimp (*Artemiasalina*) lethality bioassay of methanolic extracts of roots of *Ziziphus mauritiana*

Dose (µg/ml)	No. of shrimps	No. of survivors	LD50 (µg/ml)	Std. drug	LD50 (µg/ml)
10	30	21	6298.7330	Etoposide	7.4625
100	30	22			
1000	30	16			

**Table 6:** Oxidative stress parameters investigated after 6 weeks. \*Denotes significant difference  $P < 0.05$  in comparison vs. Control while #represents comparison with Control + Vit.C

Groups	SOD (U/ml)	T-AOC (Umol/L)	MDA (nmol/ml)
Control	10±0.22	21±1	8±0.4
Control+Vit.C	20±0.30*	30±1*	3±0.3*
Control+Zm	14±0.30*#	25±2*#	5±0.4*#

U/ml: Micron per millilitre; (Umol/mL): Micromol per litre; (nmol/mL): Nanomoles per litre

Environmental pollutants and dietary habits cause disturbances in immune activities, and diet containing micronutrients and antioxidants is known to prevent these alterations.<sup>[19]</sup> The use of herbs as an immunomodulator can modulate the body's defense system. With this point, both extracts were also employed to explore immunomodulatory activity. It was observed that methanolic extract ZMRM did not show any activity when compared to standard drug ibuprofen while dichloromethane extract ZMRD showed 12.35% inhibition with inhibitory concentration 50% 55.43 ± 7.9 when compared to standard drug brufen having 4.6% inhibition. This immunomodulatory activity of ZMRD opens a gateway to explore the different activities such as anti-inflammatory, antiangiogenic, antileukotriene, and immunosuppressant of extract.

Oxidative stress plays an imperative role in cardiac and vascular abnormalities in different types of cardiovascular diseases that are why any antioxidant therapy may be beneficial for combating these diseases.<sup>[18]</sup> Natural plants have been used as antioxidant extensively to combat with different diseases. In the human body, mostly SOD and MDA are in the balance, while most of the time, SOD is predominated in normal conditions. Increased MDA indicates the presence of free radicals and onset of diseases left ventricular hypertrophy.<sup>[20]</sup> These free radicals are culprit for the onset of many diseases where MDA is elevated locally and systemically. Antioxidants are responsible to counteract these free radicals and increase the antioxidant mechanisms of the body such as SOD and T-AOC. It has been observed in present

study that exogenous administration of *Z. mauritiana* roots after 6 weeks of oral treatment significantly reduced ( $P < 0.05$ ) the plasma levels of MDA and significantly increased (all  $P < 0.05$ ) the levels of SOD and T-AOC in the plasma when compared to control group as shown in Table 6. These findings are in line with the findings of previously reported data,<sup>[21]</sup> which explored antioxidant role of *Z. mauritiana* in liver protection. These findings indicate the emerging role of this medicinal plant as an antioxidant in different pathological ailments.

## CONCLUSION

The present study has explored that *Z. mauritiana* roots extract, particularly dichloromethane, exhibited cytotoxicity, immunomodulatory, and antioxidant activities.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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