

MIMOSINE - A NOVEL SUBSTANCE AND EVALUATE THE LIPID PEROXIDATION

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ABSTRACT

Objective: The mimosine is a novel substance extracted and purified from *Mimosa pudica*. The present study is to evaluate lipid peroxidation (LPO).

Methods: The sox halation is used to differentiate extracts. Mimosine is purified by gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS). The present study was conducted to evaluate the hepatoprotective activity of ethanol extract of *M. pudica* against alcohol-induced albino rats. This was evident from significant of *M. pudica* on LPO and antioxidants liver of rats.

Results: By GC–MS and LC–MS, the mimosine is purified and it shows the highest peak. The phytochemical analysis of *M. pudica* and the activity of the enzyme antioxidants such as superoxide dismutase is 176.83 g and peroxidase is 0.989 g, were found to be present in the plant extract of *M. pudica*. In rat treated with toxic dose of alcohol, a significant increase in serum and liver were observed. Simultaneous oral administration of the aqueous extract of *M. pudica* reduced the lactate dehydrogenase (LDH) activity to a significant extent compared to control. LDH, a cytosolic liver marker enzyme, is a regulator of many biochemical reactions in the body tissues and the fluid.

Conclusion: The result obtained in our work, existence of bioactive substance proves that it is mainly used as a traditional medicine. The phytochemical analysis indicated the presence of alkaloids, steroids, terpenoids, tannin, phenolic, saponins, proteins, and amino acids. The liver showed that the induction of alcohol causes hepatic necrosis and injury, but it is cured by *M. pudica*.

Keywords: *Mimosa pudica*, Gas chromatography–mass spectrometry, Liquid chromatography–mass spectrometry, Mimosine, Liver homogenate.

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INTRODUCTION

Mimosa pudica indicated the presence of alkaloids, carbohydrates, steroids, flavonoids, glycosides, turpinoids, phenols, tannins, saponins, coumarin, C-glycosylflavones, gums, and mucilages. It possesses a high microbial and antifungal activity which also reduce headache [1-3]. *M. pudica* has been reported to contain mimosine (an alkaloid), free amino acid, sitosterol, linoleic acid, and oleic acid. For medicinal benefits, several plants have been identified as a foundation of various effective and prevalent medicines. From the period of the ancient times until today, these herbs used as traditional medications, have been widespread, and have proved to be efficient in curing diseases across the world. It has been demonstrated that *in vitro* screening techniques may possibly give the required prime perceptions that have been important in selecting crude plant extracts with conceivably valuable characteristics for further chemical, microbiological, and pharmacological examinations. The number of medicinal preparations has been advocated in tradition system of medicine, especially in Ayurveda for treating liver disorders [4-8].

Hepatic cell injury caused by various toxicants such as chemotherapeutic agents, antituberculosis drugs, carbon tetrachloride, paracetamol, chronic alcohol consumption, and pathogenic microbes is well reported [9-11]. People are finding and exploring the advantages associated with herbal contraceptives due to their lower side effects. Herbal contraceptives have lower cost of development as compared to modern medicine. This review explores the recent advances in development that offers many benefits for shifting the paradigm for herbal contraceptives [12-15]. From times, immemorial, references have been made for the plants that possess antifertility properties also [16-19].

MATERIALS AND METHODS

Materials

M. pudica were taken from Agricultural University, Thrissur, and the mimosine is purified from Sapala Organics, Hyderabad, and lipid peroxidation (LPO) studies were done in Amala Medical College, Thrissur, Kerala.

Preparation of extract

The coarsely powdered leaves (300 g) of *M. pudica* were extracted to exhaustion in a Soxhlet apparatus at 50°C with 500 ml of ethanol and methanol separately. The extract was filtered through a cotton plug, followed by Whatman filter paper and then concentrated using a rotary evaporator at a low temperature (40–60°C) and reduced pressure to provide ethanolic and methanol extractive of 8.20 g separately.

Preliminary phytochemical analysis

The ethanolic extract and methanol extract of *M. pudica* was subjected to identify the presence of various phytoconstituents, namely alkaloids (Mayer's test), steroids and terpenoids (Liebermann–Burchard test), glycosides (modified Borntrager's test), tannin and phenolic compounds (ferric chloride test), flavonoids (lead acetate test), saponins (foam test), and proteins and amino acids (Xanthoproteic test).

Extraction of mimosine

The fresh mimosa leaves of *M. pudica* were washed then heated at 100°C for 3 min. After that, it was grinded and extracted; then, filtered extract juice was separated and purified extract was freeze dried and purified by gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS) procedures.

GC-MS

Analysis was carried out on an HP 5890 GC system, coupled to an HP MD 5973 quadruple mass spectrometer. The compounds were separated on an HP-5MS capillary column (30 m, 0.25 mm i.d., 0.25 mm film); split injection was used for both samples: The split a ratio was 20:1. The column oven temperature was maintained at 40°C for 1 min after injection.

LC-MS

The LC is used in LC-MS and it is combination of high-performance LC (HPLC) with MS. The principle of separation in HPLC or LC-MS is normal phase mode or reverse phase mode of adsorption. Normal phase constricts with polar stationary phase and with non-polar solvent or mobile phase and reverse phase constricts with non-polar stationary phase and with polar solvent/mobile phase LC-MS were conducted using ultra-performance LC system. Chromatographic separation was achieved using HPLC column (100 mm×4.6 mm, 3 μm with a gradient system consisting of 10 mm ammonium acetate with 0.001% formic acid. The initial mobile phase which contains 15% of methanol which was increased linearly to 76% after 11 min, then decreased back to the initial mobile phase. After 11.6 min and held for 3.4 min to reequilibrate the LC column, total chromatographic run

time was 15 min. Retention time for each analyte was calculated in the software.

Characterization of mimosine

Effect of pH

The fresh purified mimosine was dissolved in buffers of different pH value ranging from 1.5 to 9.0 so that the final concentration of mimosine is equal to 8 μM/L, and this mimosine solutions at room temperature for 12 h after that see the readings of pH [20].

Effect of temperature

The fresh purified mimosine was dissolved buffers so that the concentration of mimosine in the buffers is equal to 8 μM/L, and this mimosine solution was placed in thermoregulator at 30, 40, 50, 60, 70, 80, 90, and 100°C for 2 h.

Assessment of liver function

Blood was collected from all the groups by puncturing the retro-orbital plexus and could clot at room temperature, and serum was separated by centrifugation at 2500 rpm for 10 min. The serum was used for the estimation of biochemical parameters to determine the functional state of the liver.

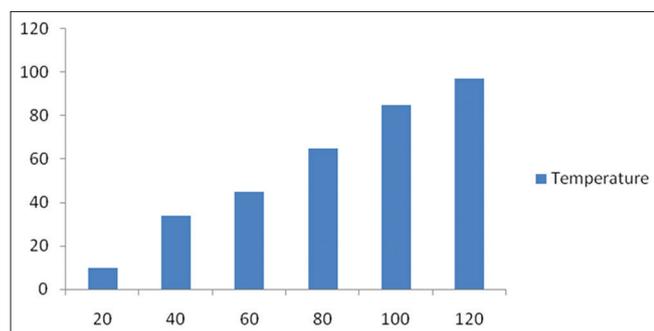


Fig. 1: Effect of temperature on mimosine

Table 1: Phytochemical analysis of *M. pudica*

S. No	Phytochemicals	Ethanol	Methanol
1	Alkaloids	+ve	+ve
2	Steroids	+ve	-ve
3	Terpenoids	+ve	+ve
4	Glycosides	-ve	-ve
5	Tannins	+ve	+ve
6	Phenolic	+ve	+ve
7	Flavonoids	+ve	+ve
8	Saponins	-ve	-ve
9	Proteins and amino acids	+ve	+ve

M. pudica: *Mimosa pudica*, +ve: Positive, -ve: Negative

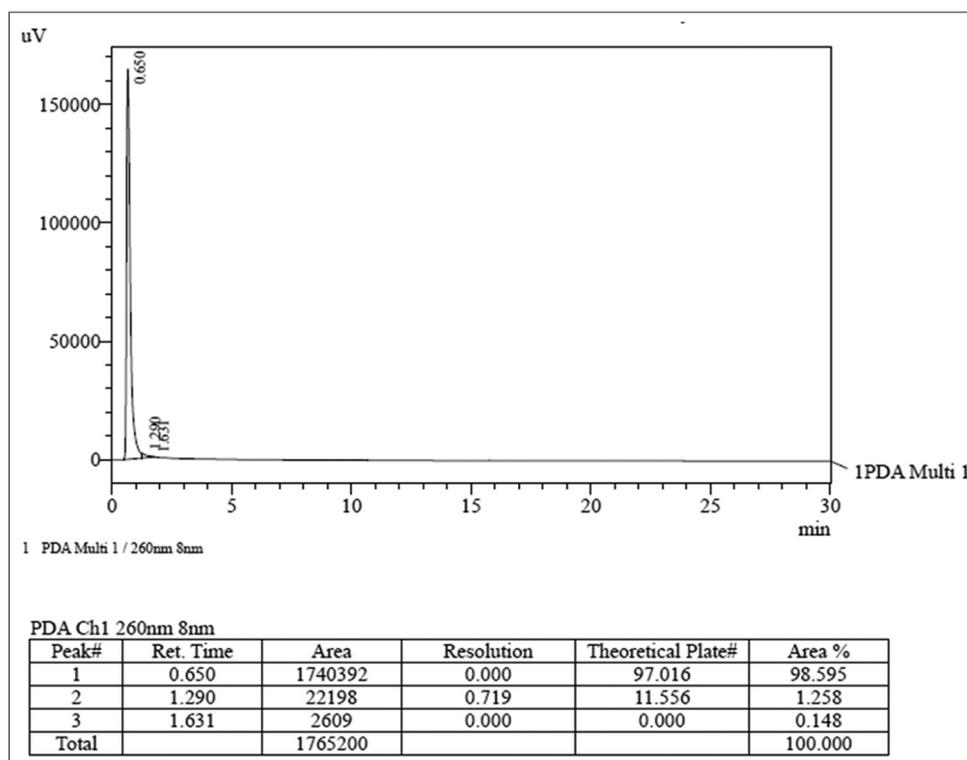


Fig. 2: Liquid chromatography-mass spectrometry analysis graph of *Mimosa pudica* leaves

Table 2: Effect of *M. pudica* on LPO and antioxidants in liver of rats

S. No	Parameter	Group I	Group II	Group III	Group IV
1	SOD units	6.324±0.05	4.432±0.04	6.246±0.04	6.437±0.02
2	CAT units	92.12±0.46	62.32±0.55	72.32±0.51	86.32±0.71
3	GPx units	4.32±0.05	3.31±0.09	3.316±0.09	4.132±0.06
4	GST units	1.632±0.04	2.732±0.05	2.32±0.15	1.832±0.09
5	Reduced GSH (nmol/g tissue)	62.32±0.55	43.32±0.59	47.72±0.59	57.72±0.59
6	Vitamin-C (mg/g tissue)	0.832±0.55	0.32±0.64	0.52±0.64	0.652±0.04
7	LPO (nmol of MDA/g tissues)	1.32±0.55	1.92±0.56	1.42±0.16	1.22±0.16

M. pudica: *Mimosa pudica*, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GST: Glutathione-S peroxidase, GSH: Glutathione, LPO: Lipid peroxidation, MDA: Malondialdehyde

Toxicity studies

Healthy Wistar albino rats of either sex weighing 150–200 g maintained under standard laboratory conditions were used for acute oral toxicity test per Organization for Economic Cooperation and Development guidelines 423 (OECD, 1996) [21]. Male albino Wistar rats of body weight 150 g were obtained from Amala Medical College, Thrissur. The rat was housed in large spacious cages and was fed on standard pellet diet and water *ad libitum*. The animals were well-ventilated area. The experimental procedures were carried out as per strictly animal ethics committee's rules and regulations of this institute.

RESULTS

By GC–MS and LC–MS, the mimosine is purified and it shows highest peak (Fig. 1). The mimosine temperature and PH is shown in Figs. 2 and 3. The phytochemical analysis of *M. pudica* (Table 1) and the activity of the enzyme antioxidants such as superoxide dismutase (SOD) is 176.83 g and peroxidase is 0.989 g, were found to be present in the plant extract of *M. pudica*. In rat treated with toxic dose of alcohol, a significant increase in serum and liver were observed. Simultaneous oral administration of the aqueous extract of *M. pudica* reduced the lactate dehydrogenase (LDH) activity to a significant extent compared to controlled; a cytosolic liver marker enzyme is a regulator of many biochemical reaction in the body tissues and the fluid.

Hepatoprotective efficacy of *M. pudica*

The present study on the hepatoprotective efficacy of *M. pudica* on ethanol-induced liver damage in rats was carried out, and results obtained are discussed (Fig. 4). The activity of the enzyme antioxidants such as SOD is 176.83 g and peroxidase is 0.989 g, was found to be present in the plant extract of *M. pudica*. Non-enzymatic antioxidants, such as phenols are 27.63 g, flavonoids are 0.436 g, reduced glutathione (GSH) is 216.19 g, Vitamin-E is 179.81 g, and tannins are 87.678 g, were analyzed in the aqueous extract of *M. pudica*.

LPO

The activity of the enzyme antioxidants such as SOD, peroxidase, catalase (CAT), and polyphenol oxidase was found to be present in the plant extract of *M. pudica*. Non-enzymatic antioxidants such as phenols, flavonoids, carotenoids, reduced GSH, ascorbic acid, α -tocopherol, and total proteins were analyzed in the aqueous extract of *M. pudica*. In rat treated with toxic dose of alcohol, a significant increase in serum and liver were observed. Simultaneous oral administration of the aqueous extract of *M. pudica* reduced the LDH activity to a significant extent compared to control. LDH, a cytosolic liver marker enzyme, is a regulator of many biochemical reactions in the body tissues and the fluid. Disturbances in cell membrane were estimated by measuring the leakage of LDH. A significant reduction in the activity of antioxidant enzyme such as SOD and CAT were observed in rats intoxicated with alcohol (Group II), but coadministration with *M. pudica* activity according to below Table 2.

DISCUSSION

Phytochemical exhibits wide range of biological effects as constituents at their antioxidant properties [22-24]. The phytochemical analysis of the crude extract indicated the presence of alkaloids, glycosides,

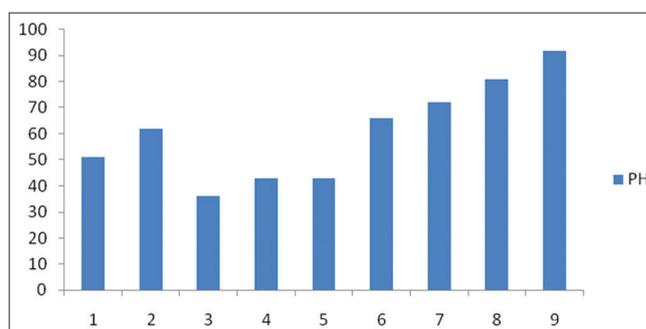


Fig. 3: Effect of pH on mimosine

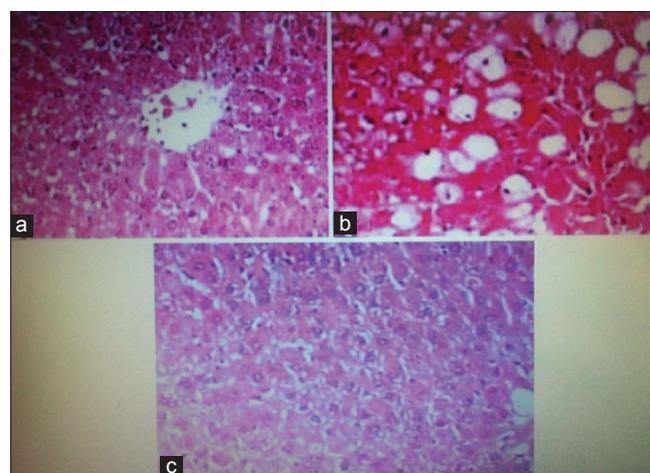


Fig. 4: Hepatoprotective activity: (a) Normal hepatic architecture, (b) Alcohol control - hepatic necrosis, (c) Cell regeneration

tannins, proteins, amino acids, and steroids. These compounds are known to be biological active and, therefore, aid the antimicrobial activity. The liver showed that the induction of alcohol causes hepatic necrosis and injury.

CONCLUSION

It can be aided that the ethanol extract of *M. pudica* exhibited a hepatoprotective effect induced hepatotoxicity [25-27]. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant. Histopathological observation also supported the above findings. The liver showed that the induction of alcohol causes hepatic necrosis and injury. The recovery of clinical symptoms is possible attributable to the regenerating effect of *M. pudica* extract.

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AUTHORS' CONTRIBUTIONS

Both the authors contributed equally in protocol preparation, ethical approval, content of the experiment, data collection, analysis, and manuscript preparation.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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