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Presence of antioxidants and nitric-oxide precursors in *Mimosa pudica* extract

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Abstract

Blood pressure lowering effect of *Mimosa pudica* induced by dieresis was reported earlier. As a diuretic it enhances urine outflow, decreases plasma volume, venous return; and thereby, reduce blood pressure. Besides the diuretic agent, some other blood pressure lowering substance may also be present in *Mimosa pudica.* Present study was undertaken to reveal the presence of antioxidants and nitrite in *Mimosa pudica* extract, which may help to reduce blood pressure. Methanolic extract of *Mimosa pudica* (using 80% methanol) was lyophilised to obtain dried *Mimosa pudica* Extract (MPE). For Total phenolic content estimation Folin's method and for estimation of flavonoids, Aluminium chloride method were followed. The radical scavenging and superoxide anion radical scavenging activity were measured following standardised methods. Nitrite content of MPE at different dilutions (10-100 µl in methanol) was measured following standardised procedure keeping sodium nitrite as the standard. Present study noted presence of favonoids and phenolic compounds and also noted antioxidant property in the aforesaid extract that exhibited DPPH+ and superoxide scavenging activities. Besides that, this study also revealed formation of nitrites in the extract of *Mimosa pudica* in a dose dependent manner. Nitrite is the precursor of nitric oxide (NO). NO is a potent vasodilator that decreases blood pressure. Present study indicated the presence of both antioxidants and nitrites in *Mimosa pudica* extract; both of which have blood pressure lowering properties indicating it as a blood pressure lowering agent; and helpful in the maintenance of vascular health.

Keywords: *Mimosa pudica* extract; Nitric oxide; Antioxidants; Blood pressure

1. Introduction

Mimosa pudica L, commonly known as Lajjabati or touch-me-not plant is a diffuse prickly under-shrub belonging to family Mimosaceae [1]. Among the variousmedicinal properties (e.g., wound healing, antidepressant, anticonvulsant, antioxidant, hypoglycaemic etc.) of *Mimosa pudica*,[2-7]anti-hypertensive and diuretic effects are some of the salient ones [8]. Diuretic enhances urine outflow, decrease plasma volume and venous return, decrease cardiac workload, oxygen demand and blood pressure [9]. Blood pressure can also be lowered by decreased peripheral resistance. Any substance that can cause vasodilatation, lowers peripheral resistance andthereby blood pressure. Besides that, reduction of oxidative stress by anti-oxidants helps to prevent hypertension, as indicated byprevious studies.[10,11]Consumption of dietary supplements and nutraceuticals containing antioxidants, showed blood pressure lowering effect[12].

The development and formation of nitric oxide (NO) is considered one of the most significant factors associated with vasodilation of the cardiovascular system. Furthermore, vasodilation is an integral part of the maintenance of vascular health, where the endothelium produces several vascular responses that are important in the regulation of vascular tone. The enhancement of NO anticipated from dietary supplements can have additional beneficial effects on

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hypertensive condition [13]. Present study was planned to explore whether extract of *Mimosa pudica* contains antioxidant properties and/ or something that has vaso-dilatory effect that helps to prevent hypertension or decrease blood pressure and maintain normal vascular health.

2. Material and methods

2.1. Drug preparation

The aerial parts of *Mimosa pudica* were shade dried in summer, 2019 and powdered. The coarse powder was extracted with hydro-methanol (20-80%) in soxhlet apparatus at 60^0C for 6 hours and solvent was removed under reduced pressure to obtain dried *Mimosa pudica* Extract (MPE)[14]. The extractive value was determined by gravimetric method and expressed as percentage yield.

2.2. Estimation of phenolic content

Total phenolic content in MPE was determined following Folin's method. In a test tube, 0.2 ml of MPE solution (1 mg/ml methanol) was mixed with 1.0 ml of Folin-Ciocalteu reagent and incubated in dark for 5 min. Then, 0.8 ml of 7.5% sodium carbonate solution was added and kept in dark for 30 min at room temperature. Finally, 3 ml of deionized water was mixed and absorbance was read at 765 nm using spectrophotometer. Total phenolic content was expressed as Gallic Acid Equivalent (GAE) in µg/mg of extract [15].

2.3. Estimation of flavonoids

Aluminium chloride method was followed to estimate flavonoids. In a test tube, 0.2 ml of MPE solution (1 mg/ml methanol), 0.8 ml methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of deionized water were mixed and absorbance was read after 30 min at 415 nm using spectrophotometer. The flavonoids were expressed as quercetin equivalent (QE) in µg/mg of extract [15].

2.4. Estimation of DPPH radical scavenging

The radical scavenging activity of MPE was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl). In a test tube, 0.1 ml of MPE in methanol at different known concentrations (10-100 µg/ml) was mixed with 3.9 ml of 0.135 mM DPPH solution and read after 30 min at 517 nm. Butylated hydroxyl toluene (BHT) was used as positive control. The sample concentration required to scavenge 50% of DPPH (IC50) was determined [16].

2.5. Estimation of superoxide anion radical scavenging

The incubation mixture contained 3 ml of Tris-HCl buffer (0.1 M, pH 7.4), 0.75 ml of nitroblue tetrazolium (300 µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 ml of MPE at different concentrations (10-100 µl in methanol). Finally, 0.75 ml of 120µM phenazine methosulphate was added to the mixture and exposed to light for 5 min at room temperature. The optical density of all samples was measured at 560 nm. L-Ascorbic acid was used as positive control. The percent inhibition of super oxide anion generation was expressed as IC50 [17].

2.6. Estimation of nitrite content

MPE at different dilutions (10-100 µl in methanol) was mixed to 0.5 ml of 0.025 M phosphate buffer saline (pH 7.4), 2 ml of 10 mM sodium nitroprusside and kept in room temperature for 150 min. Then, 1 ml of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% N-1-naphthylethylenediamine dihydrochloride) was added and incubated for another 30 min. Absorbance was estimated at 546 nm. Nitrite content was estimated with sodium nitrite as the standard [18].

2.7. Statistical analysis

Descriptive statistics were denoted and compared wherever applicable. The data were represented as Mean ± Standard Deviation (SD).

3. Results and discussion

Phenolic contents, flavonoids, DPPH+ scavenging activity, superoxide scavenging activity and nitrite contents in *Mimosa pudica* extract were shown in the following tables (Table 1 and 2) and diagram (Figure 1).

Table 1 Bioactive constituents

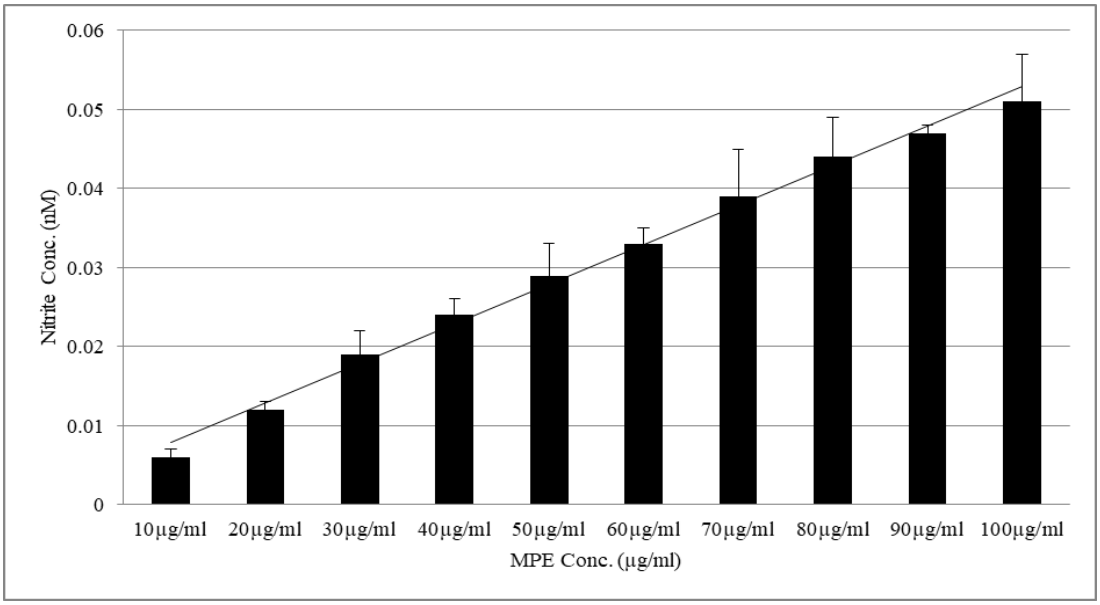
	Extractive value (%) Mean ± SD	**Phenolics (mg GAE/mg extract) Mean ± SD**	**Flavonoids (mg QE/mg extract)Mean ± SD**
MPE	14.25±0.78	106.55±0.72	69.38±0.446

n=6 in each test; 20-80 hydro-methanolic extractGAE= gallic acid equivalent; QE=quercetin equivalent

Table 2 Antioxidants

	IC_{50} (µg/ml MPE)Mean ± SD
DPPH radicalscavenging	39.86±0.54
Superoxide scavenging	72.05±0.68

n=6 in each test; IC_{50} means 50% inhibitory concentration



n=6 in each test; IC_{50} means 50% inhibitory concentration; y=0.005x+0.002; R^2=0.992;

Figure 1 *In vitro* nitrite formation in *Mimosa pudica* Extract [MPE]

4. Discussion

Results revealed that, extract of *Mimosa pudica* contains the phytochemicals that has antioxidant properties supported by DPPH+ scavenging and superoxide scavenging activities and nitrite as well.

Oxidative stress is referred to imbalance between reactive oxygen species and antioxidant system to detoxify the reactive intermediate or to repair the resulting damage. Insufficient levels of anti-oxidants or inhibition of antioxidant enzymes can cause oxidative stress [19]. Antioxidants are reducing agents like thiols, ascorbic acid or polyphenols that inhibit oxidation of other molecules by being oxidised by themselves [20,21].

Previous studies revealed that antioxidants present in green tea reduced sympatho-excitation and lowered blood pressure.[10] Use of potassium magnesium, L-arginine, vitamin C, cocoa flavonoids, beet-root juice, co-enzyme Q10, controlled-release melatonin and aged garlic extract, different dietary supplements and nutraceuticals most of which are antioxidant agents with a high tolerability and safety profile have blood pressure lowering effect [12]. Furthermore, phenolic compound of a plant-leaf was found to reduce blood pressure and oxidative stress in spontaneously hypertensive experimental animal model [11]. Present study noted presence of favonoids and phenolic compounds and also noted antioxidant property in the aforesaid extract that exhibitedDPPH+ and superoxide scavenging activities.

As mentioned earlier, among the various medicinal properties of *Mimosa pudica*, [2-7] diuretic effect is one of the salient ones, which might be due to its interference with the Na^+-K^+-Cl^- co-transport carrier in the luminal membrane of the thick ascending limb of Loop of Henle, similar to the mechanism of action of furosemide. The active principle of the extract is mainly the alkaloid L- mimosine that caused increased urine volume and urinary excretion ofNa^+-K^+-Cl^- like that of furesemide.[8] Diuretic enhance urine outflow, decrease plasma volume and venous return, decrease cardiac workload, oxygen demand and blood pressure [9].

Blood pressure can be lowered by decreased peripheral resistance also. Nitric oxide (NO) causes vasodilatation, thereby decrement of peripheral resistance andblood pressure. Present study indicates formation of nitrites in the extract of *Mimosa Pudica* in a dose dependent manner, as indicated in Figure1. Nitrite is the precursor of nitric oxide (NO). As NO is a gas and its presence is transient, instead of NO its precursor nitrite has been estimated in the extract.

In vivo, nitric oxide is synthesized from arginine by NO synthase. This enzyme in nervous tissue and endothelium is activated by agents that increase intracellular Ca^{++} concentration including vasodilators acetylcholine and bradykinin.NO is inactivated by haemoglobin.

Nitrate (NO_3^-) supplementation (either as beetroot juice or sodium nitrate ($NaNO_3^-$) has shown reduction in blood pressure, enhancement of blood flow, elevation of driving pressure of oxygen in microcirculation to areas of hypoxia or exercising tissue. Inorganic nitrate enhance plasma NO_2^- levels. NO_2^--related NO signalling increases blood flow and targets hypoxic areas of the body [22].

In vascular smooth muscle, influx of Ca^{++} via voltage gated Ca^{++} channel produce a diffuse moderate increase in cytosolic Ca^{++} that initiates contraction. At the same time,Ca^{++}influxalso initiates Ca^{++} release from sarcoplasmic reticulum via ryanodine receptorand high local accumulation of Ca^{++} produced by this Ca^{++} sparks attaches β-subunit of Ca^{++} activated BK-channels (Big K^+-channels), increases their activities, through which K^+ efflux occurs in a high rate.Increased K^+ efflux alters membrane potentials, shuts off voltage gated Ca^{++} channels and produces relaxation [23].

NO formed in endotheliumfrom arginineby NO synthase diffuses to vascular smooth muscle cells and activates soluble guanylyl cyclase to produce Cyclic Guanosine Monophosphate (cGMP). cGMP can activate cGMP-specific protein kinase that affect ion channels andCa^{++} homeostasis or may increase the activity of phophatases or all of these and in turn mediates the relaxation of vascular smooth muscle [22].

Result of the present study revealed the presence of both antioxidants and nitrites in *Mimosa pudica* extract; both of which possess the blood pressure lowering properties. It seems that *Mimosa pudica* extract holds a high promise as a blood pressure lowering agent and may also help in the maintenance of vascular health.

5. Conclusion

Therefore, it may conclude that safe antihypertensive medicine may be obtained from *Mimosa pudica.* Further studies are required to confirm its antihypertensive principles.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare there is no conflict of interest.

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