Symbiosis

www.symbiosisonline.org
www.symbiosisonlinepublishing.com

Research Article | International Journal of Plant studies | Open Access

Phytochemical Profiling and Antioxidant Activity of Lajwanti *Mimosa Pudica* Linn. In Vitro

Ganesh Chandra Jagetia[1*] and C. Lalhmangaihi

[1]*Maharana Pratap Colony, Scetor 13, Hiran Magri, Udaipur, India*

Received: July 11, 2018; Accepted: July 24, 2018; Published: July 26, 2018

***Corresponding author:** Ganesh Chandra Jagetia, 10 Maharana Pratap Colony, Sector-13, Hiran Magri, Udaipur-313002, India; E-mail: gc.jagetia@gmail.com*

Abstract

The plants have been a major source of phytomedicines for human healthcare and it is prudent to analyse various phytoconstituents present in them. The phytochemical analysis of *Mimosa pudica* Linn. (Lajwanti) showed presence of alkaloids, flavonoids, cardiac glycosides, phenols and saponins whereas tannins, and terpenoids were conspicuous by their absence. The total phenolic contents were estimated as gallic acid equivalent in petroleum ether, chloroform, ethanol and aqueous extracts of Lajwanti. Total phenolic contents increased in a concentration dependent manner in various extracts and a maximum amount of phenolic contents were estimated in chloroform extract at a concentration of 60µg followed by aqueous and petroleum ether extracts, whereas ethanol extract showed the least. The free radical scavenging assay of Lajwanti showed a concentration dependent rise in the scavenging of DPPH radical and a maximum scavenging activity was recorded at 800 µg/ml for all extracts except aqueous extract where this concentration was 900 µg/ml. Different extracts of Lajwanti scavenged superoxide radicals in a concentration dependent manner and a highest scavenging activity was observed for 800 µg/ml for chloroform and ethanol extracts, whereas 900 and 500 µg/ml for petroleum ether and aqueous extracts, respectively. The nitric oxide scavenging also showed a concentration dependent inhibition and the petroleum extract Lajwanti showed a maximum activity at 1000 µg/ml, chloroform extract at 600 µg/ml, whereas for ethanol and aqueous extracts this concentration was 500 µg/ml, respectively. The determination of reducing power showed that different extracts had a potent antioxidant action and a greatest reducing power was recorded for 80 µg/ml for all extracts of Lajwanti except petroleum ether where it was at 100µg/ml. Our study demonstrates the presence of phenols, alkaloids, flavonoids, cardiac glycosides and saponins in Lajwanti and its different extracts scavenged various free radicals in concentration dependent manner and possess a potent antioxidant activity.

KeyWords: *Mimosa Pudica*; Phenols, Flavonoids; Alkaloids; DPPH, Nitric Oxide; Reducing Power.

Introduction

Humans have used plants for various purposes including as a medicine since the advent of their history. The infliction of different diseases led to testing of several plants or natural products for their treatment. The medicinal use of plants has a long history of experimentation and their curing capacity of different diseases through several generations. Medicinal plants are found around the globe and they have utility locally as well as in other parts, where similar plants are present. The use of medicinal plants has led to evolution of traditional system of medicine in different cultures. The Indian system of medicine, the Ayurveda depends on natural and plant products for treatment of different diseases and it has been successfully used to treat various chronic ailments, where modern medicine has not been very successful [1]. There is no denying the fact that 200 years ago the pharmacopeia had a commanding position in the use of natural and plant products for healthcare. However, with the evolution of modern medicine the interest in the medicinal uses of plants and natural products waned [2]. Despite the widespread use of modern medicine for treatment World Health Organization estimates that 80% of the global population still relies on the plants and natural products for treatment of several ailments [3]. This may be due to the fact that plant and natural product-based systems are time tested and are older than the modern system of medicine and have no known adverse side effects despite their long-term use [1, 4]. The use of plants and natural products based medicinal systems are constantly gaining their acceptance even in the USA from a mere 2.5 to 12.1% between 1990 - 1997 up to 380% indicating the faith reposed in the oldest system of healthcare [5].

The use of oxygen by aerobic organism is indispensable for energy production and their survival. However, it comes with a price in the form of oxidative stress, which is produced due to use of oxygen for production of chemical energy during electron transport [6, 7]. The cells have inbuilt mechanisms to protect from the production of oxygen free radicals. However, overproduction of oxygen free radicals overwhelms the endogenous system causing inflammation and several diseases. Despite the fact that oxygen free radicals are injurious they are indispensable for several physiological processes required for survival [7, 8]. The excess oxidative stress can be combated by use of exogenous agents, which are able to neutralize the free radicals efficiently [9]. Since plants synthesize several metabolites and secondary metabolites in particular may be useful in combating acute and

chronic oxidative stress due to their ability to passivate oxygen free radicals.

Lajwanti or *Mimosa pudica* Linn. (Family: fabaceae) is also known as Chuimui in Hindi. It is also commonly known as sensitive plant, humble plant, shame plant and touch-me-not in English. The *Mimosa pudica* is a weed that grows as a creeping annual or perennial herb. The nature of Lajwanti is described in Ayurvedic scriptures as sheetha (cold) and its taste as tikta (bitter) and kashaya rasa (astringent). According to Ayurveda texts it possesses antiasthmatic, stimulant, pain-killing and antidepressant activities and balances kapha and pitta. The whole crushed plant is used to relieve itchiness and itch related diseases. The leaves of Lajwanti are used to treat cuts and bleeds, fistulous withers, hemorrhoids, hydrocele, pinkeye and scrofula. Lajwanti roots are utilized to cure angiopathy, bronchial asthma, dysentery, fevers, leucoderma, metropathy, swellings, jaundice, small pox, strangury and ulcers. The Ayurveda prescribes whole plant as treatment for dropsy, myodynia, rheumatoid arthritis and uterine tumors [10]. Lajwanti has been used clinically to treat menstrual disorders in women and long-term use did not induce any adverse side effects in this study [11]. Different parts of Lajwanti are considered to possess medicinal properties as they are used to treat dysentery, asthma, biliousness, burning sensation, fatigue, inflammations, leprosy, leukoderma, blood diseases vaginal and uterine complaints etc. [12]. It helps to cure Amoebic dysentery (raktaatisaara), diarrhea (athisaara), bleeding piles, and arrests bleeding [10]. Various extracts of Lajwanti have been reported to exert antiandrogenic, antibacterial, antinociceptive, anticonvulsant, antidiabetic, anti-inflammatory, antidepressant, antifungal, antimalarial, antioxidant, antitumor, antiulcerogenic, antihyperglycemic, antivenomic, immunomodulatory, hepatoprotective, antifertility, diuretic and wound healing activities [10,13, 14]. Lajwanti leaf extracts has been found to reduce pentylentetrazol and strychnine-induced seizures in mice and it also acts as antagonistic to the N-methyl-D-aspartate-induced turning behavior in mice [15]. The acute toxicity studies of chloroform and methanol extracts up to 5 g/kg orally did not cause any toxicity in mice [16]. Our recent study has shown that ethanol extract of Lajwanti accelerated wound healing, increased glutathione contents, activities of catalase, and superoxide dismutase and reduced lipid peroxidation. It also increased the DNA, collagen and hexosamine syntheses in the regenerating wounds of mice [1]. The presence of various medicinal properties in *Mimosa pudica* (Lajwanti) stimulated us to undertake its phytochemical analysis and its ability to scavenge different free radicals in vitro.

Materials and Methods

Chemicals

Petroleum-ether, chloroform, ethanol, ferric chloride, hydrochloric acid, olive oil, ethyl acetate, liquid ammonia., sulphuric acid, glacial acetic acid, butanol, Folin Ciocalteau reagent, naphthalene diamine dihydrochloride (NDD), Greiss reagent, sulfanilamide, H3PO4, EDTA (ethylenediamminetetraacetic acid), ferrous ammonium sulphate, ferric chloride, Nash reagent, DMSO (dimethylsulfoxide), NaOH, methanol, 1,1-diphenyl-1-picrylhydrazyl (DPPH), sodium nitroprusside, and trichloroacetic acid (TCA) were procured from Merck India, Mumbai. All chemicals were of analytical grade.

Preparation of extract

The identification of *Mimosa pudica* or Lajwanti (family: Fabaceae) was carried out by the Department of Horticulture Aromatic and Medicinal Plants, Mizoram University, Aizawl, India. The whole non-infected plants were collected from the Mizoram University campus in the months of September to December, they were cleaned, and shade dried. The whole dried plants were chopped into small pieces and powdered in an electrical grinder. The dried powder of Lajwanti was sequentially extracted in petroleum ether, chloroform, ethanol and water using Soxhlet apparatus. The extracts of *Mimosa pudica* from petroleum ether (MPPE), chloroform (MPCE), ethanol (MPEE) and distilled water (MPAE) were collected and evaporated to dryness for further use.

Phytochemical analysis

The phytochemical analysis of Lajwanti was carried out for the presence of different phytochemicals as follows: -

Alkaloids

The alkaloid assay was carried out by mixing 0.1g of the Lajwanti powder with 0.5 ml of Mayer's reagent or Dragendorff's reagent. The formation of a creamy (Mayer's reagent) or reddish-brown precipitate (Dragendorff's reagent) indicated the presence of alkaloids [18, 19].

Flavonoids

Three different methods were used to test the presence of flavonoids in all the extracts [17-19]. Five ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each sample followed by the addition of a concentrated H_2SO_4. Appearance of a yellow colour (disappeared on standing) in each extract indicated the presence of flavonoids.

A few drops of 1% aluminum solution were mixed with an aliquot of each filtrate. The development of yellow colour indicated the presence of flavonoids.

A small volume of each sample was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Appearance of yellow colour indicated the presence of flavonoids.

Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath for 10 minutes, filtered while hot and cooled before conducting the following tests:

Frothing: 3 ml of filtrate was diluted with distilled water to make up the volume up to 10 ml and shaken vigorously for 2 minutes. The formation of a fairly stable froth indicated the presence of Saponins in the filtrate.

Emulsification: 3 drops of olive oil were added to the solution

obtained by diluting 3 ml filtrate to 10 ml distilled water and shaken vigorously for a few minutes. The formation of a fairly stable emulsion indicated the presence of Saponins [18, 19].

Cardiac glycosides (Keller-Killani test)

The cardiac glycosides assay was carried out by adding 5 ml of the sample in 2 ml of glacial acetic acid containing one drop of ferric chloride solution and underlaid with 1 ml of concentrated sulphuric acid. The appearance of brown ring at the interface indicated the presence of deoxysugar, which is characteristic of cardenolides [18, 19].

Phenols

Generally, 0.2 g of different Lajwanti samples was treated with 5% ferric chloride solution. The formation of deep blue color indicated the presence of phenols [18, 19].

Tannins

About 0.5 g of dried powdered samples was boiled in 20 ml of distilled water in a test tube and filtered. A few drops of 0.1% ferric chloride was added to the filtrate. The formation of brownish green or a blue-black colour indicated the presence of tannins [18, 19].

Terpenoids

The presence of terpenoids was detected as follows:

Salkowski test: Five ml of the sample was mixed with 2 ml of chloroform and carefully overlaid with 3 ml concentrated sulphuric acid. The formation of a reddish-brown precipitate at the interface indicated the presence of terpenoids [17, 18].

Quantitative determination of total phenols

Total phenolic contents were estimated by Folin–Ciocalteu colorimetric method with minor modifications [20-22]. Different extracts of Lajwanti (0.5 ml) were mixed with10% Folin–Ciocalteu phenol reagent (5ml) and kept for 5 min. Thereafter, 4ml of 6% sodium carbonate was added and the mixture was left for 90 min at room temperature. The absorbance of the mixture was measured at 750 nm using a UV-Visible spectrophotometer (SW 3.5.1.0. Bio spectrometer, Eppendorf India Ltd., Chennai). Standard calibration curve for gallic acid in the range of 0–200µg/ml was prepared in the same manner and results are expressed as mg gallic acid equivalent per100 gram of dried extract.

Assay of free radicals and antioxidant activity

The free radical scavenging and antioxidant activities of different extracts of Lajwanti were determined by grouping them as follows: -

MPPE: - The free radical scavenging activity was determined by using 100, 200, 400, 600, 800 and 1000 µg/ml of petroleum extract.

MPCE: - The ability of 100, 200, 400, 600, 800 and 1000 µg/ml chloroform extract to scavenge various free radicals was estimated in cell free system.

MPEE: - The different doses of ethanol extract Viz. 100, 200, 400, 600, 800 and 1000 µg/ml were utilized to assay the scavenging activity of different free radicals.

MPAE:- The free radical scavenging ability of 100, 200, 400, 600, 800 and 1000 µg/ml aqueous extract was studied in cell free system.

The ability of petroleum, chloroform, ethanol and aqueous extracts of Lajwanti to scavenge different free radicals was determined using the following protocols: -

DPPH free radical scavenging assay

The DPPH free radical scavenging activity of *Lajwanti* was estimated as described earlier [23]. Various concentrations of extracts of Lajwanti (0.5 ml each) were mixed thoroughly with 1 ml methanol solution of 0.1 mm 2, 2-diphenyl-1-picrylhydrazine (DPPH). The mixture was allowed to stand for 30 min in the dark. The absorbance was recorded at 523 nm using a UV/VIS Spectrophotometer. An equal amount of DPPH and methanol were used as standard and blank, respectively. The scavenging activity was calculated using the following formula: -

$$\text{Scavenging (\%)} = (A_{control} - A_{sample}) / A_{control} \times 100$$

Where Asample is the absorbance of the test sample and Acontrol is the absorbance of the control.

Superoxide anion scavenging assay

Superoxide ($O_2^{\bullet-}$) anion radical scavenging activity of different extracts of Lajwanti was estimated as described earlier with minor modifications [24]. The reaction mixture contained 0.2 ml of NBT (1mg/ml of solution in DMSO), 0.6 ml different extracts, 2 ml of alkaline DMSO (1 ml DMSO containing 5 mm NaOH in 0.1 ml H_2O) in a final volume of 2.8 ml. The absorbance was recorded at 560 nm using a UV-VIS spectrophotometer. The blank consisted of pure DMSO instead of alkaline DMSO.

Nitric oxide scavenging assay

Nitric oxide scavenging activity of various extracts of Lajwanti was assayed using a standard protocol [25]. Sodium nitroprusside (5 mm) in phosphate buffered saline was mixed with different concentrations of the petroleum ether, chloroform, ethanol or aqueous extract of Lajwanti and incubated for 150 minutes at 25°C. The samples were mixed with Greiss reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with napththylenediamine was read at 546 nm using a UV-VIS spectrophotometer. The inhibition of nitric oxide formation was determined with respect to standard potassium nitrite in the same way with Griess reagent. The results have been expressed as potassium nitrite equivalent which has been used as a standard.

Reducing Power

The antioxidant potential of different extracts of Lajwanti was determined by estimating its ability to reduce ferric ion

to ferrous ion. The reducing power of MPPE, MPCE, MPEE and MPAE was determined according to the earlier method [26]. Briefly, different concentrations (1-100 µg) of various extracts in distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH6.6) and potassium ferricyanide (2.5 ml, 1%); the mixture was incubated at 50°C for 20 min followed by the addition of 2.5 ml of trichloroacetic acid (10%). The mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 1%). The absorbance was measured at 700 nm with a UV-VIS spectrophotometer. Increased absorbance of the reaction mixture indicated increased reducing power.

Results

The results of phytochemical analyses and free radical scavenging activities of different extracts of Lajwanti are shown in (Tables 1-2) and figures 1-5.

Table 1: The phytochemical analysis of *Mimosa pudica*.

Name of the phytochemical	Present / Absent
Alkaloids	+
Flavonoids	+
Phenols	+
Cardiac glycosides	+
Saponins	+
Tannins	-
Terpenoids	-

(+ indicates presence whereas – indicates absence)

Table 2: Total phenolic contents in different extracts of *Mimosa pudica.*

Concentration (µg/ml)	Phenolic contents as gallic acid equivalent mg/100 g of dry weight			
	Petroleum ether extract	Chloroform extract	Ethanol extract	Aqueous extract
10	4.32	9.28	6.57	7.32
20	5.62	14.04	11.77	13.86
30	8.53	17.13	12.83	18.24
40	9.5	21.21	12.49	23.34
50	12.31	28.77	13.74	25.98
60	20.09	33.73	14.14	28.98
70	20.74	26.85	17.36	32.34
80	21.92	26.66	19.71	34.92
90	25.06	25.13	22.82	37.44
100	28.76	25.67	23.25	49.26

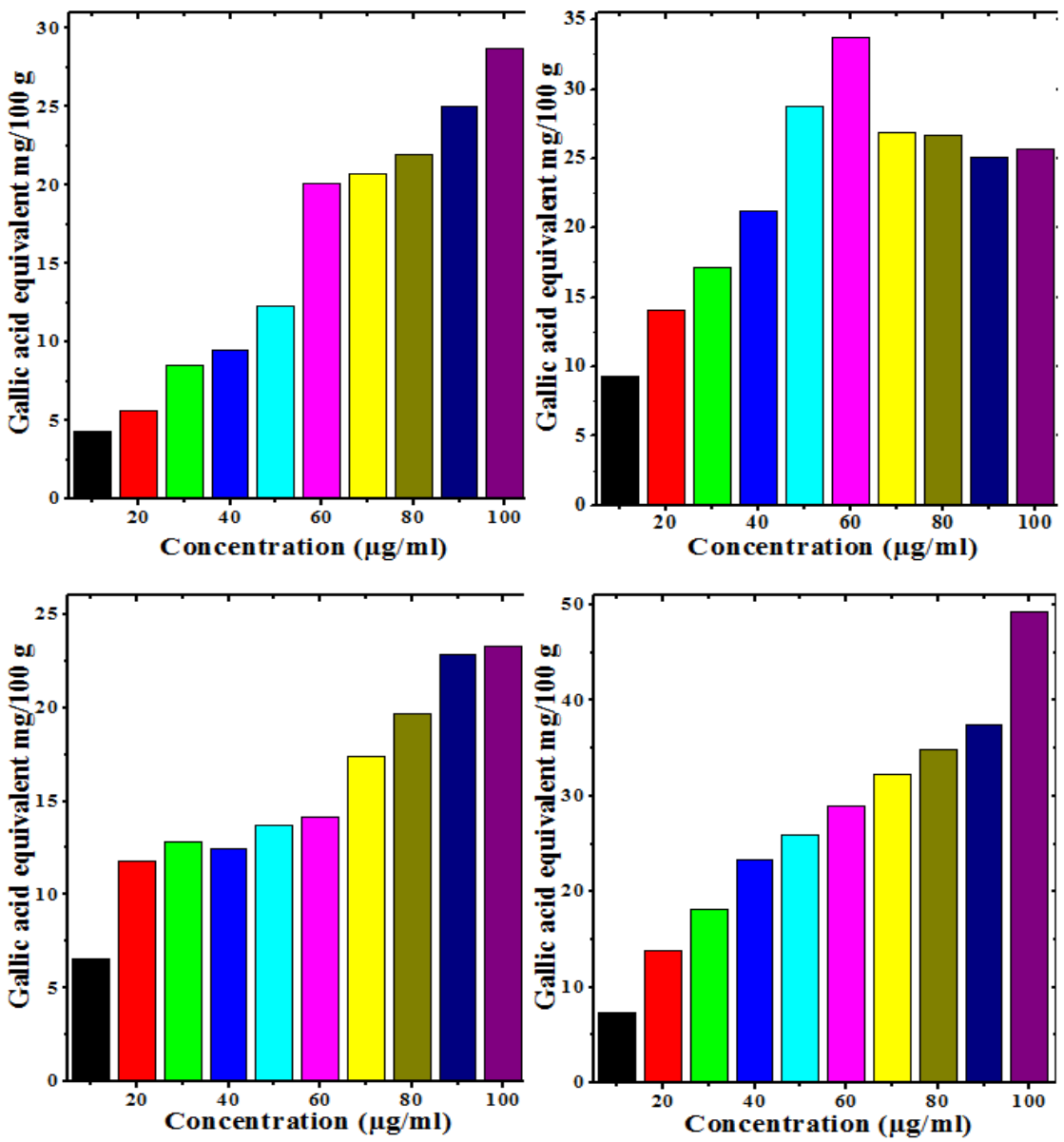

Figure 1: Total phenolic contents in the different extracts of *Mimosa pudica.* Upper left: Petroleum ether extract; Upper right: Chloroform extract; Lower left: Ethanol extract and Lower right: Aqueous extract.

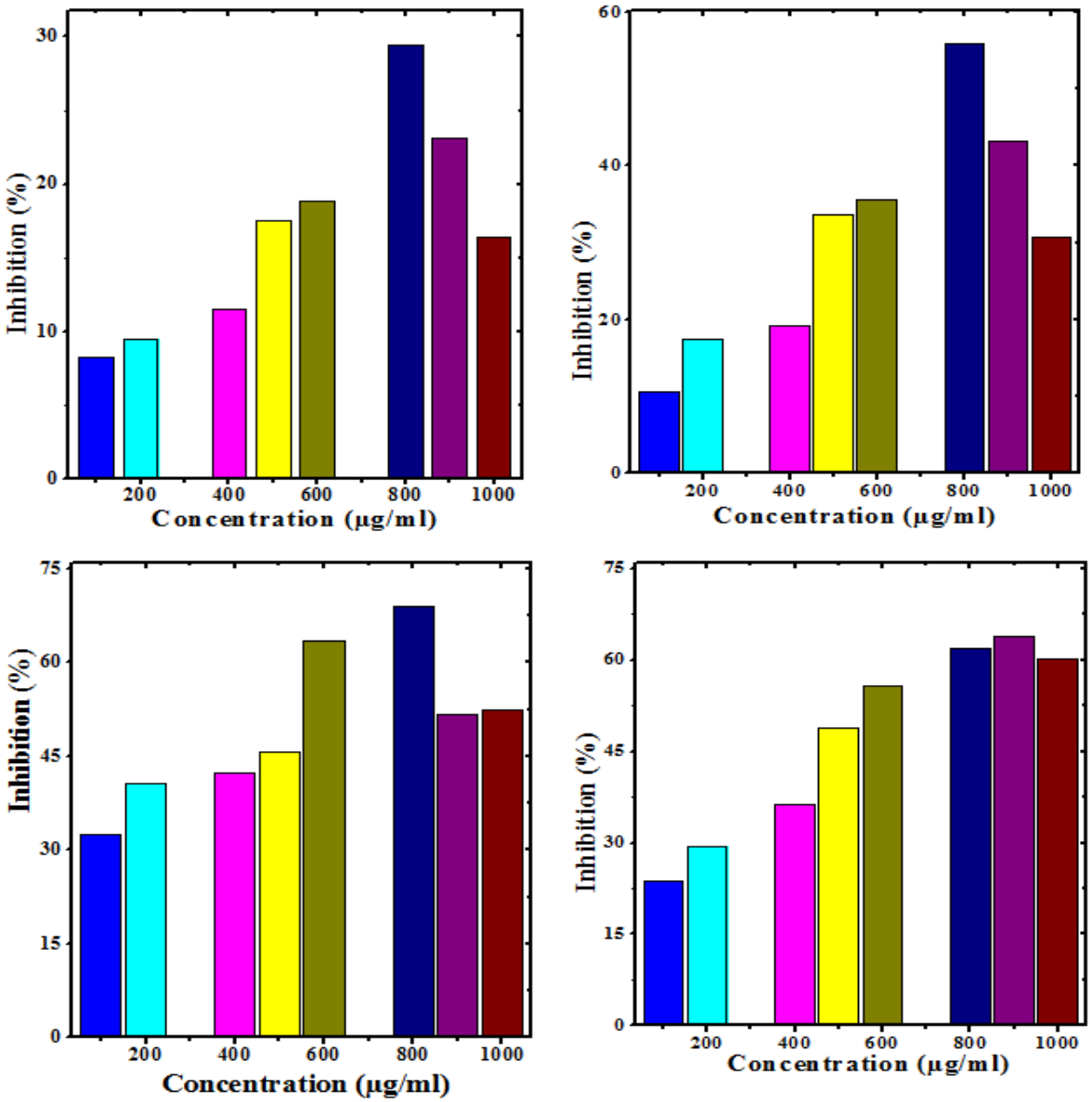

Figure 2: Effect of different extracts of *Mimosa pudica* on the scavenging of DPPH free radicals in cell free system in vitro. Upper left: Petroleum ether extract; Upper right: Chloroform extract; Lower left: Ethanol extract and Lower right: Aqueous extract.

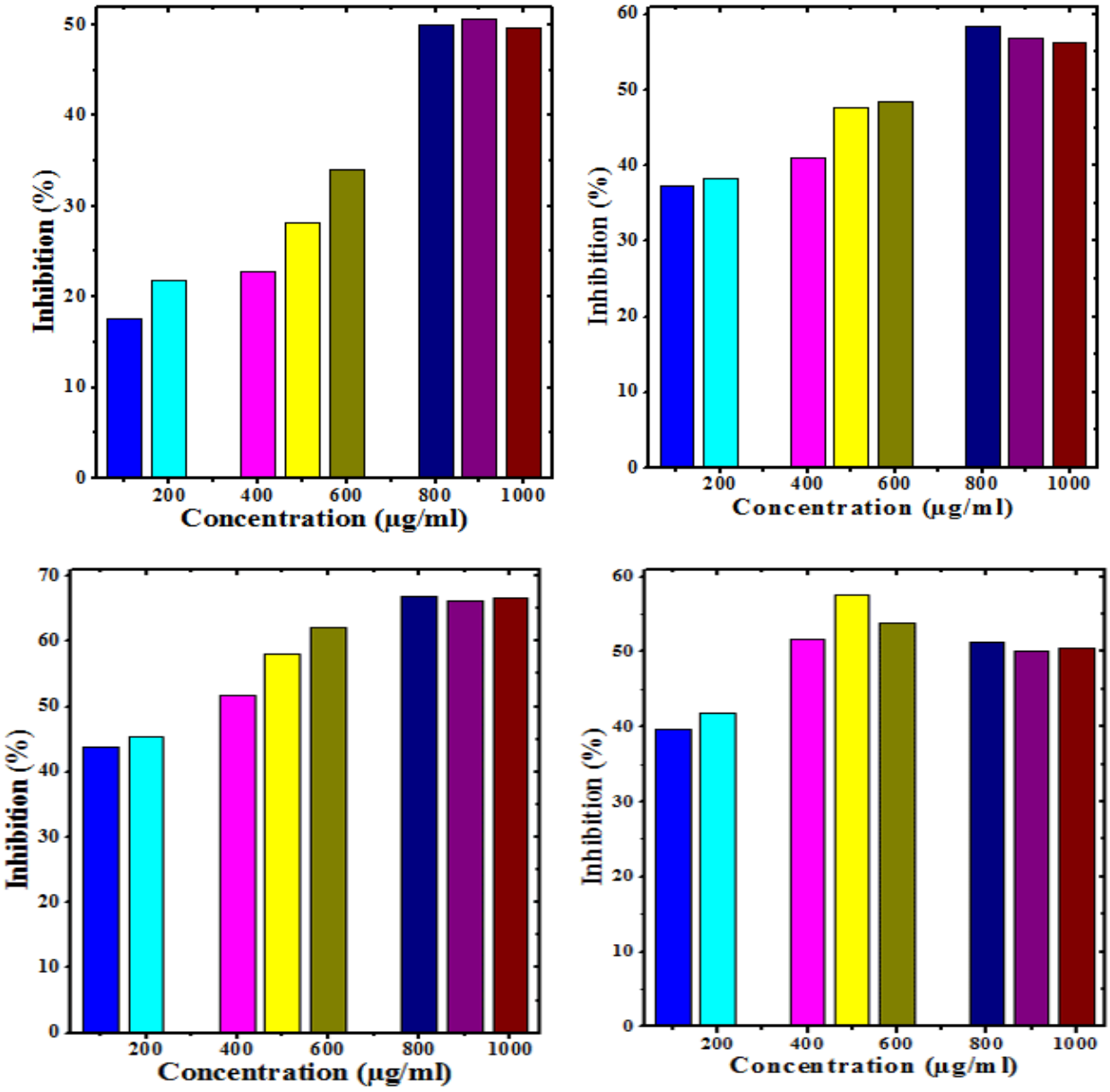

Figure 3: Effect of different extracts of *Mimosa pudica* on the scavenging of superoxide anion free radicals in cell free system in vitro. Upper left: Petroleum ether extract; Upper right: Chloroform extract; Lower left: Ethanol extract and Lower right: Aqueous extract.

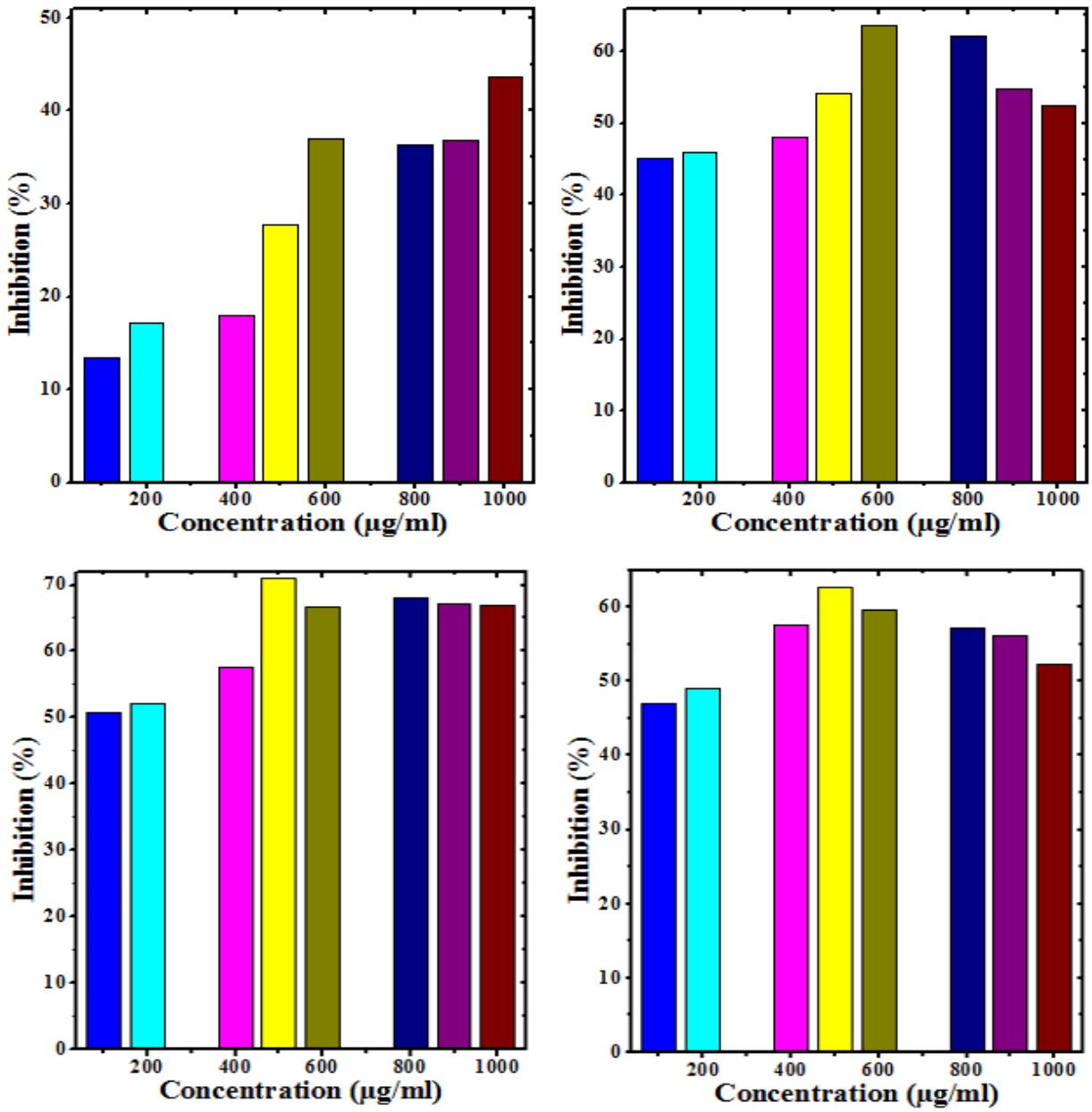

Figure 4: Effect of different extracts of *Mimosa pudica* on the scavenging of nitric oxide anion free radicals in cell free system in vitro. Upper left: Petroleum ether extract; Upper right: Chloroform extract; Lower left: Ethanol extract and Lower right: Aqueous extract.

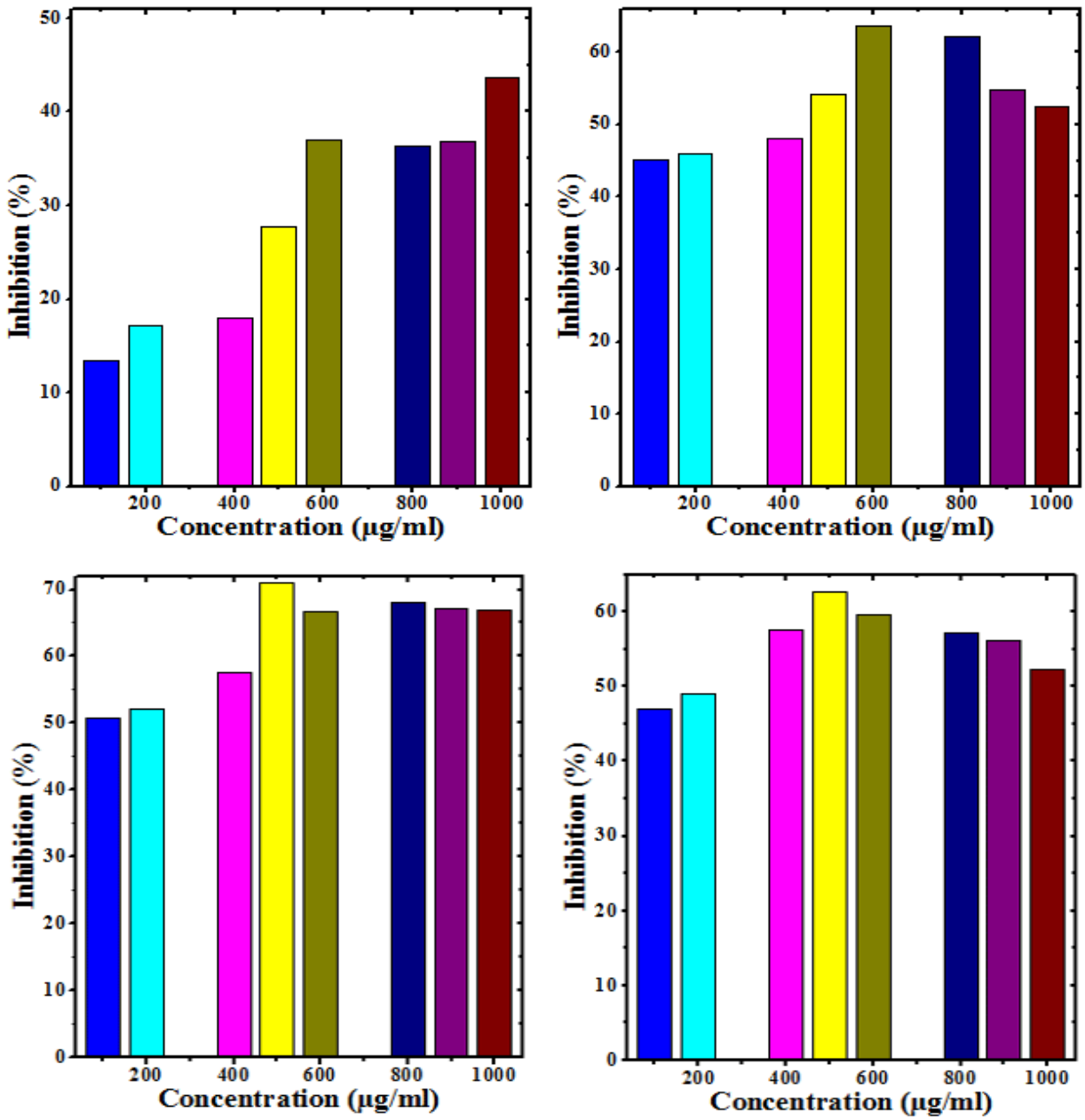

Figure 5: Effect of different extracts of *Mimosa pudica* on the reducing power in cell free system in vitro. Upper left: Petroleum ether extract; Upper right: Chloroform extract; Lower left: Ethanol extract and Lower right: Aqueous extract.

Phytochemical Analysis

The phytochemical analysis of *M. pudica* showed the presence of various phytochemicals including alkaloids, cardiac glycoside, flavonoids, phenols and saponins. The tannins, and terpenoids were conspicuous by their absence (Table 1).

Total phenolic contents

The total phenolic contents of the different extracts were measured by Folin Ciocalteau reagent in terms of gallic acid equivalent. The total phenolic content increased in a concentration dependent manner in MPPE and the maximum phenolic contents were observed for 100 µg/ml the highest concentration screened (Figure 1). Analysis of MPCE exhibited a concentration dependent increase in the total phenolic content, where greatest total phenolic contents were observed at 60 µg/ml, which declined thereafter (Figure 1). The total phenolic content in MPEE increased gradually but in a concentration dependent manner and the maximum phenolic contents were observed at 80 µg/ml, the highest concentration evaluated (Figure 1). The total phenolic content increased in a concentration dependent manner in aqueous extract up to 100 µg/ml, the highest concentration evaluated (Figure 1).

Free radical scavenging and antioxidant activities

DPPH

The MPPE showed a concentration dependent elevation in the free radical scavenging activity and a maximum effect was observed for a concentration of 800 µg/ml (29.46%) that declined thereafter (Figure 2). MPCE inhibited the generation of DPPH radicals in a concentration dependent manner with a highest radical scavenging activity at a concentration of 800 µg/ml (55.81%) that gradually declined thereafter (Figure 2). The scavenging potential of MPCE was better than MPPE (Figure 2). The evaluation of DPPH scavenging activity in the MPEE increased in concentration dependent manner and a maximum scavenging effect was observed at a concentration of 800 µg/ml (69.02%). With increase in MPEE concentration the scavenging activity declined suddenly at 900 µg/ml and gradually thereafter (Figure 2). The DPPH scavenging effect of MPAE elevated in a dose dependent fashion and the greatest effect was observed at 900 µg/ml (63.94%) and a decline thereafter (Figure 2). The scavenging activity of all extracts was highest at 800 µg/ml, except MPPE, which was least effective (Figure 2).

Superoxide

The superoxide radicals showed a concentration dependent elevation in the free radical scavenging activity of MPPE and a maximum effect was seen at 900 µg/ml (50.65%) and a decline thereafter (Figure 3). The pattern of superoxide scavenging for MPCE was similar to that of MPPE however, the maximum activity of 58.44% was found at 800 µg/ml (Figure 3). The ability of MPEE to inhibit the generation of superoxide increased in a concentration dependent manner up to 800µg/ml (66.88%) and remained almost similar thereafter (Figure 3). Determination of superoxide radical scavenging activity of MPAE showed a concentration dependent elevation in the free radical scavenging activity up to 500µg/ml (57.47%), which declined gradually thereafter (Figure 3).

Nitric oxide

MPPE inhibited the generation of NO radical in a concentration dependent manner up to 1000 µg/ml (43.59 %) the maximum concentration studied (Figure 4). The MPCE also showed a concentration dependent rise in the scavenging of NO radicals with a greatest effect for 600µg/ml (63.53%) and a decline thereafter (Figure 4). The scavenging activity was greater than petroleum ether extract (Figure 4). MPEE inhibited nitric oxide radical generation in a concentration dependent fashion and a maximum effect was observed at a concentration of 500 µg/ml, where approximately 71% scavenging was observed (Figure 4). The scavenging activity declined thereafter. MPAE exhibited a concentration dependent elevation in the scavenging of NO radicals and a maximum effect was observed at a concentration of 500µg/ml (62.68%) and gradual decline thereafter (Figure 4). The ethanol extract was most efficient inhibitor of NO generator in vitro (Figure 4).

Reducing Power

Ferric reducing power showed a concentration dependent rise as indicated by a continuous inhibition of ferric radicals after treatment with MPPE and a maximum effect was observed at 100 µg/ml in the MPPE (Figure 5). The pattern of rise in the ferric reducing power for MPCE was similar to the petroleum extract except that the greatest reducing power was recorded at 80 µg/ml MPCE (Figure 5). MPEE also reduced ferric irons to ferrous ions in a concentration dependent fashion and a maximum reducing power was seen at 80µg/ml that declined marginally thereafter (Figure 5). MPAE also exhibited a concentration dependent rise in the ferric reducing power and the maximum reducing power was observed at 100µg/ml MPAE (Figure 5).

Discussion

Nature has been a major source of medicinal agents for humans since the advent of their history [4, 27, and 28]. Various medicinal plants have been used for thousands of years in daily life to treat various human diseases all over the world [29, 30]. Several cultures of the world have a long history of use of folk medicines as their ancestors painstakingly created primitive medicines from plants and natural products when faced with natural calamity and infliction of different diseases. The oldest system of medicine, the Ayurveda originated in Vedic times and is more than 5000 years old. It uses 2000 medicinal plants for the treatment of different ailments and now it has been recognized by Western world as a therapeutic paradigm [31]. Sequel scientific evaluation of herbal and natural products could provide new inputs for the development of new therapies for human healthcare. Lajwanti is used in Ayurveda in the conditions of leukoderma, angiopathy, metropathy, ulcers, dysentery, swellings, jaundice, bronchial asthma, small pox, strangury, fevers, hydrocele, hemorrhoids, fistulous withers, scrofula, pinkeye, cuts and bleeds, vesical calculi, dropsy, rheumatoid

arthritis, myodynia, and uterine tumors, itching and other skin infections [10, 12]. Therefore, we were interested to evaluate phytochemical constituents and the ability of *Mimosa pudica* i.e. Lajwanti to scavenge free radicals in vitro.

Plants synthesize several phytochemicals including secondary metabolites that are used as a weapon of defence against infection, predation and environmental stress. These phytochemicals also help in pollination, growth and are responsible for myriads of colours of flowers and fruits [32]. The secondary plant metabolites are of great medicinal value to humans and help to treat various ailments [32, 33]. The phytochemical analysis of whole Lajwanti plant showed the presence of alkaloids, cardiac glycoside, flavonoids, phenols and saponins. Alkaloids, saponins, flavonoids and phenoilcs have been reported in the whole Lajwanti plant earlier [34]. However, in addition to that these authors also reported the presence of tannins, steroids, fixed oils and mucilage for which no attempt was made in the present study. However, we were unable to detect tannins in the present study. A study on methanol extract of Lajwanti leaves has been found to contain terpenoids, diterpenes, glycosides, alkaloids, phenols, quinines, tannins, saponins and coumarins [35-37]. The ethanol extracts of leaves and roots of Lajwanti were found to possess tannins and steroids, whereas flavonoids, saponins, alkaloids and anthraquinones were absent [38]. The roots of Lajwanti have been reported to synthesize flavonoids, alkaloids, phytosterol, tannins, glycosides and fatty acids [39]. Our studies on *Croton caudatus, Oroxylum indicum, Milletia pachycarpa* and *Helicia nilagirica* have shown the presence of different phytochemicals in these plants [33, 40, and 41].

The use of oxygen is an indispensable part of aerobic life however, due to its highly oxidizing (loosing electron) nature it causes deleterious effects. During respiration the utilization of oxygen in electron transport chain generates free oxygen radicals, which if not neutralized produce oxidative stress [6, 7]. The free radicals are indicated in several human diseases including aging, arthritis, autoimmune disorders, diabetes, rheumatoid arthritis, cataract, cardiovascular disorders, neurodegenerative diseases and cancer [42].

DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a dark-coloured crystalline powder made up of stable free-radical molecules. It is a most commonly used radical assay to ascertain the antioxidant potential of any substance. DPPH radical imparts a deep violet colour in solution, which becomes colourless or pale yellow when neutralized and converted into DPPH-H [39]. Many plants extracts have been reported to scavenge DPPH radical in vitro [43-46]. The MPPE, MPCE MPEE and MPAE inhibited DPPH free radicals in a concentration dependent manner. The ethanol extract of *Mimosa pudica* has been reported to scavenge the DPPH radical in a dose dependent manner earlier [47]. A moderate DPPH scavenging has also been observed by Lajwanti in an earlier study [48]. The hexane leaf extract of Lajwanti has been also reported to scavenge DPPH radicals [49]. However, there is no comprehensive study of DPPH scavenging as carried out in the present study. The different extracts of other plants have been reported to scavenge DPPH free radical in a concentration dependent manner earlier from this laboratory [45, 46, 50, and 51].

The superoxide free radicals are generated during coupling of oxygen in the electron transport in mitochondria. As such superoxide radical is less toxic however, it is converted in the presence of iron into a highly reactive toxic hydroxy radical [7]. Furthermore, superoxide radicals generated as a result of incomplete metabolism of oxygen damage important biomolecules in the cells directly or indirectly after formation of H_2O_2, hydroxyl radicals, peroxynitrite or singlet oxygen [7, 52]. Therefore, removal of superoxide is essential in reducing oxidative stress-induced injuries. The MPPE, MPCE MPEE and MPAE suppressed the generation of superoxide radicals in a concentration dependent manner indicating its usefulness as an antioxidant agent. The studies regarding the scavenging of superoxide radicals by different extracts of Lajwanti are scanty. However, ethyl acetate root extract of Lajwanti has been reported to scavenge superoxide radicals with an IC50 value of 85.85 ± 0.84 µg/ml [53]. Various extracts of other plants have been reported to scavenge superoxide free radicals in a concentration dependent manner earlier [45, 46, and 51].

The nitric oxide is an important free radical that is essential during wound healing and acts as a signalling molecule for several physiological processes of the nervous, renal, gastrointestinal, and cardiovascular systems [54]. However, its excess production leads to oxidative stress and pathological implications [7, 55]. The hypertension, meningitis, excessive bleeding, rheumatoid arthritis, systemic sclerosis, hepatopulmonary syndrome, severe asthma and septic shock are associated with excess NO production, which could be even life threatening in certain circumstances [56, 57]. This indicates that excess production of NO needs to be suppressed and the agents which can inhibit the generation of NO production could act as a useful paradigm to treat diseased related to excess NO formation. The MPPE, MPCE MPEE and MPAE inhibited NO free radical generation in a concentration dependent manner. An earlier study has reported scavenging of NO radicals where the ethyl acetate root extract of Lajwanti was most effective followed by methanol extract whereas the petroleum ether extract was least effective [53]. An observation similar to ours in the present study. Similarly, chloroform leaf extract of Lajwanti has been reported to scavenge NO free radicals in an earlier study [58]. The hexane leaf extract of Lajwanti has been also reported to scavenge NO radicals [49]. However, systematic reports on the scavenging of NO radical by Lajwanti are lacking. Similarly, different plant extracts have been shown a concentration dependent nitric oxide scavenging ability earlier [45, 46, 51, and 59].

The reducing power assay is a convenient and simple method to estimate antioxidant potential of any chemical or plant extracts. The different extracts of Lajwanti including petroleum, ether, chloroform, ethanol and water have been found to increase the ferric reducing ability indicating its antioxidant nature. The reports regarding the evaluation of reducing power of different extracts of Lajwanti are scanty. However, the chloroform root extract of Lajwanti has been reported to show reducing power [58]. Similarly, it has been found to increase the reducing power

of ferric ions in a concentration dependent manner in an earlier study [47]. The estimation of reducing power of different extracts of Lajwanti showed that the alcohol and water extracts were most potent [60].

The exact mechanism of radical scavenging ability is not known. However, it may be due to the presence of total phenolic contents in all extracts which showed a concentration dependent elevation akin to the free radical scavenging. The synthesis of flavonoids, phenols, saponins and cardiac glycosides by Lajwanti may have also been responsible for the free radical scavenging ability of Lajwanti in the present study.

Conclusions

The phytochemical analysis of Lajwanti shows that it consists of various phytochemicals such as flavonoids, alkaloids, phenols, saponins and cardiac glycosides which may be the reason why it is used for the treatment of a variety of diseases in Ayurveda. The antioxidant activity of petroleum ether, chloroform, ethanol and aqueous extracts seems to be due to the scavenging of DPPH, superoxide and nitric oxide radicals and their ability to reduce ferric ions in a concentration dependent manner. The present study suggests that Lajwanti could be a potential rich source of natural antioxidants and it may ward off against aging, cancer, immune dysfunction, and other oxidative stress related diseases, and accrue health benefits to humans.

References

1. Jagetia GC, Lyngdoh R, Lalramchuana, Borah BK. *Mimosa pudica* (Lajwanti) accelerates repair and regeneration of deep dermal excision wound in swiss albino mice. Int J Complement Alt Med. 2017;9(2):00293.
2. Ernst E. The efficacy of herbal medicine –an overview. Fundam Clin Pharmacol.2005;19(4):405–409.
3. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177.
4. Jagetia GC. Phytochemical composition and pleotropic pharmacological properties of jamun, *syzygium cumini* skeels. J Explor Res Pharmacol 2017;2(2):54-66.
5. Eisenberg D, David RB, Ettner SL, Appel S, Wilkey S and Van Rompay M. Trends in alternative medicine use in the United States; 1990–1997. JAMA 1998;280(18):1569–1575.
6. Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol. 2003;552(2):335-344.
7. Lushchak VI. Free radicals, reactive oxygen species, oxidative stresses and their classifications. Ukrainian Biochem J. 2015; 87(6):11-18.
8. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180-183.
9. Carocho M, Ferreira IC. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food Chem Toxicol. 2013;51:15-25.
10. Sanaye MM, Joglekar CS, Pagare NP. Mimosa- A brief overview. J Pharmacog Phytochem. 2015;4(2):182-187.
11. Vaidya GH, Sheth UK. *Mimosa pudica* (Linn.) its medicinal value and pilot clinical use in patients with menorrhagia. Ancient Sci Life. 1986;5(3):156-160.
12. Chauhan BS. Johnson, DE. Germination, emergence and dormancy of *Mimosa pudica*. Weed Biol Managem. 2009;9(1):38-45.
13. Zaware BB, Chaudhari SR, and Shinde MT. An Overview of *Mimosa pudica* linn: Chemistry and Pharmacological Profile. Res J Pharmaceut Biol Chem Sci. 2014;5(6):754-761.
14. Muhammad G, Hussain MA, Jantan I, Bukhari SNA. *Mimosa pudica* L., a high-value medicinal plant as a source of bioactives for pharmaceuticals. Compreh Rev Food Sci Food Safety. 2016;15:303-315.
15. Bum EN, Dawack DL, Schmutz M, Rakotonirina A, Rakotonirina SV, Portet C, Jeker A, Olpe HR, Herrling P. Anticonvulsant activity of *Mimosa pudica* decoction. Fitoterapia. 2004;75(3-4):309-314.
16. Paul J, Khan S, Asdaq SMB. Wound healing evaluation of chloroform and methanolic extracts of *Mimosa Pudica* roots in rats. Int J Biol Med Res. 2010;1(4):223-227.
17. Trease GE, Evans WC. Pharmacognosy. 11th Ed. 1989; Brailliar Tiridel Can. McMillian publishers.
18. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis (3rd Edn), London: Chapman and Hall. 1988.
19. Doughari JH. Phytochemicals: extraction methods, basic structures and mode of action as potential chemotherapeutic agents, phytochemicals - A global perspective of their role in nutrition and health. Dr. Venkateshwara Rao (Ed.), 2012; InTech, Rijeka, Croatia.
20. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total Phenolics in selected fruits, vegetables, and grain products. J Agri Food Chem. 1998;46(10):4113-4117.
21. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and Phenolics compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74(17):2157-2184.
22. Chlopicka J, Pasko P, Gorinstein S, Jedryas A and Zagrodzki P. Total Phenolics and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT-Food Sci Technol. 2012;46(2):548-555.
23. Leong LP, Shui G. An investigation of antioxidant capacity of fruits in Singapore markets. Food Chem. 2002;76(1):69-75.
24. Hyland K, VoisinBanoun H, Auclair C. Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system. Anal Biochem. 1983;135(2):280-287.
25. Marcocci L, Packer L, Droy-Lefaix MT, Sekaki A and Gardes-Albert M. Antioxidant action of *Ginkgo biloba* extracts EGB 761. Method Enzymol. 1994;234:462–475.
26. Oyaizu M. Studies on products of browning reaction. Jpn J Nutri Diet. 1986;44(6):307-315.
27. Kinghorn AD, Pan L, Fletcher JN, Chai H. The relevance of higher plants in lead compound discovery programs. J Nat Prod. 2011;74(6):1539-5155.
28. Patridge E, Gareiss P, Kinch MS, Hoyer D. An analysis of FDA-approved drugs: natural products and their derivatives. Drug Dis Today. 2016;21(2):204-207.
29. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016; 21(5):559.

30. Thomford NE, Dzobo K, Adu F, Chirikure S, Wonkam A, Dandara C. Bush mint (Hyptis suaveolens) and spreading hogweed (*Boerhavia diffusa*) medicinal plant extracts differentially affect activities of CYP1A2, CYP2D6 and CYP3A4 enzymes. J Ethnopharmacol. 2018;211:58-69.
31. Pandey MM, Rastogi S, Rawat AK. Indian traditional ayurvedic system of medicine and nutritional supplementation. Evid Complem Alt Med. 2013.
32. Falcone Ferreyra ML, Rius S, Casati P. Flavonoids: biosynthesis, biological functions, and biotechnological applications. Front Plant Sci. 2012;3:222.
33. Zoremsiami J, Jagetia GC. The Phytochemical and thin layer chromatography profile of ethnomedcinal plant *Helicia Nilagirica* (Bedd). Int J Pharmacog Chinese Med. 2018;2(2):000131.
34. Pal P, Datta S, Basnett H, Shrestha B, Mohanty JP. Phytochemical analysis of the whole plant of *Mimosa pudica* (Linn.). UJPSR. 2015;1(1):1-9.
35. Gandhiraja N, Sriram S, Meenaa V, Srilakshmi JK, Sasikumar C and Rajeswari R. Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. Ethnobot Leaf. 2009;13:618-624.
36. Kaur P, Kumar N, Shivan TN. Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. J Med Plants Res. 2011;5(22):5356-5359.
37. Mohan SM, Pandey B, Rao SG. Phytochemical Analysis and Uses of *Mimosa pudica* Linn. in Chhattisgarh. IOSR J Environ Sci Toxicol Food Technol. 2015;1(3):1-4.
38. Ranjan RK, Kumar MS, Seethalakshmi I, Rao MR. Phytochemical analysis of leaves and roots of *Mimosa pudica* collected from Kalingavaram, Tamil Nadu. JCHPS. 2013;5(5):53-55.
39. Pande M, Pathak A. Preliminary pharmacognostic evaluations and phytochemical studies on roots of *Mimosa pudica* (Lajwanti). Int J Pharm Sci Rev Res. 2010;1(1):50-52.
40. Shantabi L, and Jagetia G.C. Phytochemical profiling of kam-sabut, *Croton caudatus* Geiseler. RRJ. Bot. Sci. 2015;4(1).
41. Lalrinzuali K, Vabeiryureilai M, and Jagetia GC. Phytochemical and TLC profiling of *Oroxylum indicum and Milletia pachycarpa*. J Plant Biochem Physiol. 2015;3:3.
42. Valko M, Leibfritz D, Moncol J. Free radicals and antioxidants in normal physiological functions and human disease", *Int. J. Biochem. Cell Biol.* 2007;39(1):44-84.
43. Goldschmidt S, Renn K. Amine oxidation IV. Diphenyl-trinitrophenylhydrazyl, *Chem. Ber*. 1922; 55:628-643.
44. Jagetia GC, Venkatesh P, Baliga MS. Evaluation of the radioprotective effect of *Aegle marmelos* (L.) Correa in cultured human peripheral blood lymphocytes exposed to different doses of γ-radiation: a micronucleus study. *Mutagenesis.* 2003;18(4):387-393.
45. Shantabi L, Jagetia GC, Ali MA, Singh TT and Devi SV. Antioxidant potential of *Croton caudatus* leaf extract *in vitro*. *Transl. Med. Biotechnol*. 2014;2(6):1-15.
46. Lalrinzuali K, Vabeiryureilai M, Jagetia GC, Lalawmpuii PC. Free radical scavenging and antioxidant potential of different extracts of *Oroxylum indicum in vitro. Adv. Biomed. Pharm*. 2015;2(3):120-130.
47. Zhang J, Yuan K, Zhou WL, Zhou J, Yang P. Studies on the active components and antioxidant activities of the extracts of *Mimosa pudica* Linn. from southern China. Pharmacogn Mag. 2011;7(25):35–39.
48. Arokiyaraj S, Sripriya N, Bhagya R, Radhika B, Prameela L and Udayaprakash NK. Phytochemical screening, antibacterial and free radical scavenging effects of Artemisia nilagirica, *Mimosa pudica* and *Clerodendrum siphonanthus*–An in–vitro study. Asian Pacific J Trop Med. 2012;2(2):601-604.
49. Almalki MA. In-Vitro Antioxidant Properties of the Leaf Extract of *Mimosa pudica* Linn. Ind J Sci Technol. 2016;9(13):1-6.
50. Lalrinzuali K, Vabeiryureilai M, Jagetia GC. The analysis of antioxidant activity and phenolic contents of selected medicinal plants of Mizoram. Genomics Appl. Biol. 2015;6(11):1-12.
51. Lalhminghlui K. Jagetia GC. Evaluation of the Free radical scavenging and antioxidant activities of Chilauni, *Schima wallichii* Korth in vitro. Future Sci OA. 2018;4(2);272.
52. Kirkinezosa IG, Morae CT. Reactive oxygen species and mitochondrial diseases. Cell Dev. Biol. 2001;12(6):449–457.
53. Kamboj PR, Kalia AN. Evaluation of in-vitro (non and site specific) antioxidant potential of *Mimosa pudica* roots. Int J Pharmaceut Sci. 2011;3:497-501.
54. Ciervo C, Zipp C. Nitric oxide in health and disease–its role in the practice of medicine. Osteopath Family Physic. 2011;3(2):66-73.
55. Hou YC, Janczuk A, Wang PG. Current trends in the development of nitric oxide donors. Curr Pharmaceut Desig. 1999;5(6): 417–441.
56. Malinski T. The vital role of nitric oxide. Oakland J. 2000;1:47-56.
57. Högman M. Extended NO analysis in health and disease. J Breath Res. 2012;6(4):047103.
58. Rekha R, Hemachander R, Ezhilarasan T, Keerthana C, Saroja DL and Saichand KV, et al. Phytochemical analysis and in-vitro antioxidant activity of *Mimosa pudica* Lin., leaves. Res J Pharm Technol. 2010;3(2):551-555.
59. Baliga MS, Jagetia GC, Rao SK, Kiran Babu S. Evaluation of nitric oxide scavenging activity of certain spices *in vitro*: A preliminary study, *Nahrung/Food*. 2003;47(4):261–264.
60. Lalithambigai G, Sakthiganapthi M, Yoganandam GP, Gopal V. In-vitro anti-oxidant and free radical scavenging activity of "thottal surungi churnam". Inter J Phytother. 2017;7(2):7-11.