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DPPH FREE RADICAL SCAVENGING ASSAY OF MIMOSA PUDICA LINN. EXTRACTS AND L-ASPARAGINE AMINO ACID IN VITRO CONDITION

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ABSTRACT

Mimosa pudica is used in various disease like jaundice, leprosy, ulcer, smallpox and related to some blood disease as well in cancer treatment. In the present study chloroform, Ethanol, and Aqueous extracts of Mimosa pudica plant samples were obtained using Soxhlet apparatus. The study is a venture to analyze the antioxidant activity of plant extract and L-asparagine Chromatography (HPTLC). Free radical scavenging activity of M. pudica extract and L-Asparagine was also compared using 2, 2-diphenyl-1-picrylhydrazylradical scavenging assay (DPPH).

HPTLC inspection showed the presence of amino acids, amines and in ethanolic extract of M. pudica crude extract and aqueous crude extract showed antioxidant activity (IC_{50}= 0.229 mg/ml and IC_{50}=0.387mg/ml respectively) whereas chloroform extract and L-asparagine did not show any antioxidant activity.

The result indicate that ethanol and aqueous extract of Mimosa pudica exhibiting significant antioxidant activity.

Keywords: Antioxident, Mimosa pudica, L-asparagine, DPPH

INTRODUCION

Plants have been used since time immemorial to treat various disease including cancer and it is known that 75% of drug in the market are either derived from plants or plant metabolites have formed the main source of development of these drugs (Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod.*2016 and Rodrigues T, Reker *D Nat chem* 2016). *Mimosa pudica* known as 'chue mue' belongs to family Mimosaceae, is a stout straggling prostrate shrubby plant, with compound leaves that get sensitive on touching, pinous stipuzesles and globose pinkish flower heads, it grows as a weed in almost all parts of the country. Leaves and stem of the plant have been reported to contain mucilage and root contain tannins (Ghani A., 2003)

Nowadays there is an increase in interest worldwide in identification of pharmacologically potent antioxidant compounds and with no side effects. Such compounds plays an important role as health-protecting factors and it neutralizes the free radicals, which are unstable molecules and are linked with the development of a number of degenerative diseases and conditions including hepatic disease, immune dysfunction, cataracts and macular degeneration. Scientific reports suggest that antioxidants also reduce the risk for chronic disease and conditions. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl which are thereby involved in reducing the risk of diseases associated with oxidative stress.

The antioxidant activity of compounds can be determine by using the colorimetric DPPH assay, as described by Shimada et al., (1992) to determine the radical scavenging activity of the plant extracts.

The hydrogen donating capacity of test samples is quantified in terms of their ability to scavenge the relatively stable organic free radical DPPH and by consequent reduction. The absorption of the deep violet DPPH solution is measured at 517 nm, after which absorption decreases due to decolorization to a yellow-white color, in the event of reduction. This decrease in absorption is stoichiometric according to the degree of reduction (Arulpriya et al., 2010).

MATERIALS AND METHODS

Chemicals

L-Asparagine and all other chemicals were purchased from Hi-Media Laboratories (India)

Preparation of plant extract:

Mimosa pudica Linn. or Lajwanti plant belongs to family Fabaceae. The plants free from fungal infection were collected from the Amravati university campus in October to December. The whole collected plants were thoroughly washed with tap water so as to wash out dust and other extraneous materials and dried in shade before converting them into powdered form by grinder. Approximately 100gm powder of *Mimosa pudica* plant was extracted sequentially with chloroform, ethanol and water in a Soxhlet apparatus. (Jagetia GC, Lalhmangaihi 2017, Jagetia GC, Lyngdoh)

Preparation of Sample:

The free-radical scavenging activity was estimated by DPPH assay. The reaction mixture contained 10µl of test sample and positive control ascorbic acid 10µl and 180µl of methanolic solution of 0.1 mM DPPH radical. The mixture was then shaken vigorously and incubated at 37º C for 5 min. different concentration of the testing mixture were prepared. The absorbance was measured by 517 nm on ELISA plate reader indicating higher free radical scavenging activity, which was calculated

$$(\%)\text{Free radical scavenging effect} = \frac{[\text{Absorbance of control (Ac)} - \text{Absorbance of sample(As)}]}{\text{Absorbance of control (Ac)}} \times 100$$

Antioxidant Potential of Synthetic Compounds

The antioxidant activities was successfully performed of the both selected plant extracts and amino acid were analyzed *in vitro* for their antioxidant potential by DPPH (2, 2 diphenyl-1-picryl hydrazyl) free radical scavenging assay. Dose response curve was plotted between % inhibition and concentration. IC_{50} values were found out for plant extract as well as for compound.

RESULT

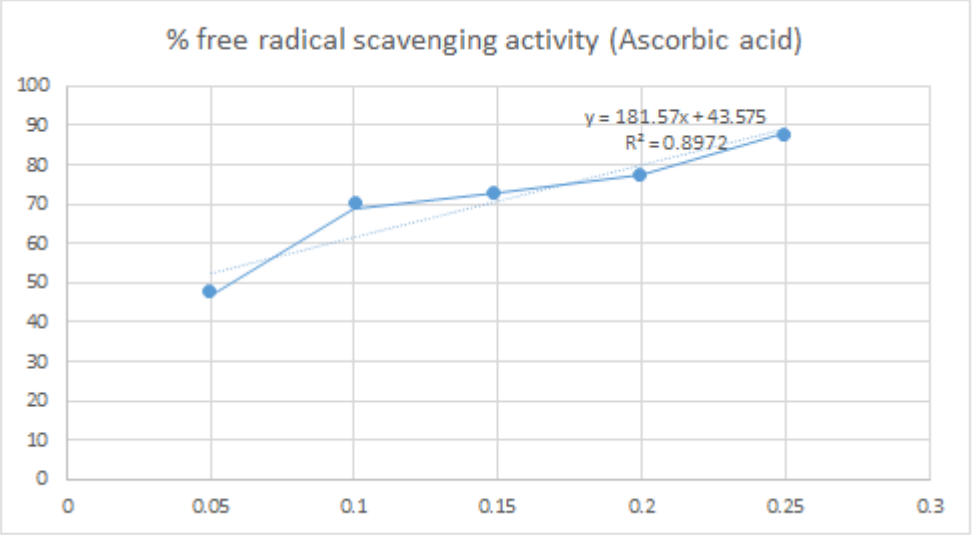
Determination of antioxidant IC50 of the Samples using DPPH assay

Table : DPPH free radical scavenging percent inhibition (Ascorbic Acid)

S. No.	Conc. (in mg)	% free radical scavenging activity	Y equation	R^2 value	IC_{50} (in mg)
1	**0.05**	48.945±1.256	181.57x + 43.575	0.8972	
2	**0.10**	58.925±2.321			
3	**0.15**	72.649±2.587			
4	**0.20**	77.458±1.698			
5	**0.25**	88.070±3.644			0.035

Table 1: DPPH free radical scavenging percent inhibition (Ascorbic Acid)

*All the data statistically analyzed with mean±SD (n=3)

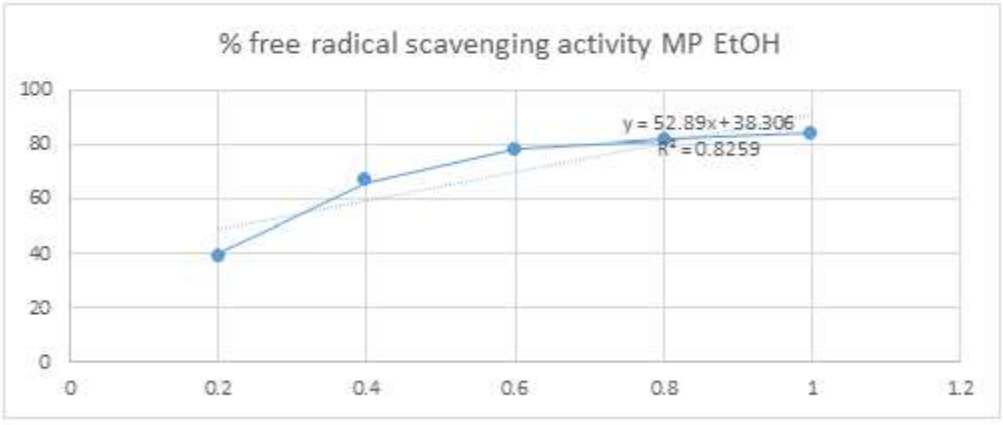


Graph 1: % free radical scavenging activity (Ascorbic Acid)

Table : DPPH free radical scavenging percent inhibition MP EtOH					
S. No.	**Conc. (in mg)**	**% free radical scavenging activity**	**Y equation**	**R^2 value**	**IC_{50} (in mg)**
1	**0.2**	39.778±2.124	52.89x + 38.306	0.8259	
2	**0.4**	65.643±2.122			
3	**0.6**	78.423±2.096			
4	**0.8**	81.734±2.406			
5	**1.0**	82.622±1.968			0.229

Table 2: DPPH free radical scavenging percent inhibition MP EtOH

*All the data statistically analyzed with mean±SD (n=3)



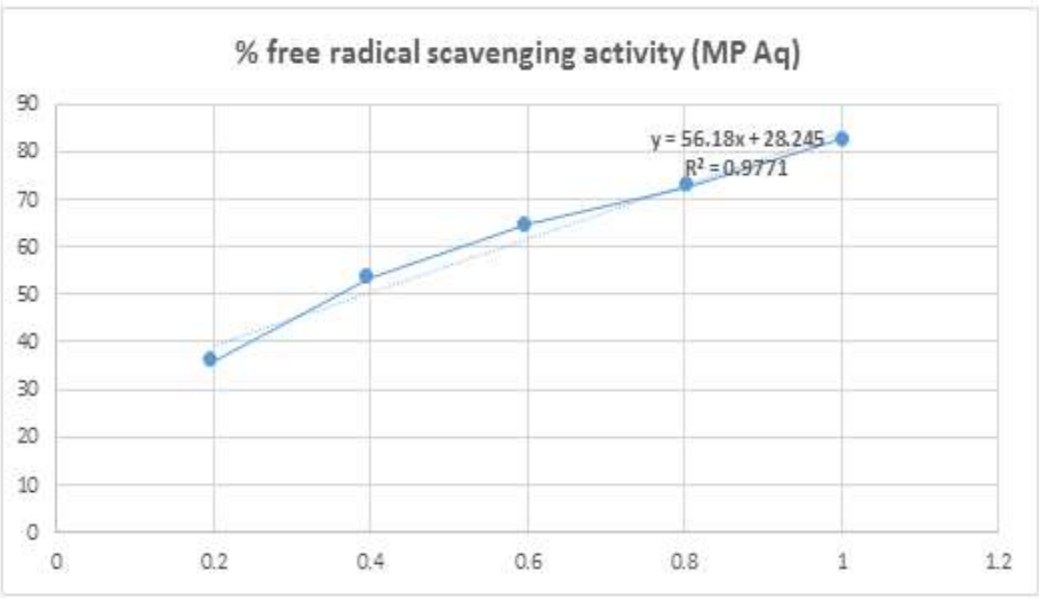
Graph 2: % free radical scavenging activity MP EtOH

Table : DPPH free radical scavenging percent inhibition MP-Aq					
S. No.	**Conc (in mg)**	**% free radical scavenging activity**	**Y equation**	**R^2 value**	**IC_{50} (in mg)**
1	**0.2**	36.190±2.078	56.18x + 28.245	0.9771	
2	**0.4**	53.397±1.785			0.387

3	**0.6**	64.869±2.085			
4	**0.8**	72.482±3.056			
5	**1.0**	82.827±2.458			

Table 3: DPPH free radical scavenging percent inhibition MP-Aq

*All the data statistically analyzed with mean±SD (n=3)



Graph 3: % free radical scavenging activity MP EtOH

Antioxidant Effect of *M. Pudica* and L-Asparagine

The DPPH radical scavenging percent inhibition ascorbic acid is shown in fig. 1. Moreover antioxidant activity was observed in ethanol and aqueous extract of *Mimosa pudica* plant at the concentration of 1mg/ml but in case of L-asparagine and chloroform extract did not show any scavenging activity at the same concentration (1mg/ml). Our result indicated that *M. pudica* aqueous and ethanol extract exhibit high antioxidative activity compared to L-asparagine.

DISCUSSION

The enormous challenge for phytochemical and pharmacological inspections involves the identification of the specific compound that are accountable for the beneficial effects and their modes of action, hence their utility considered as therapeutic drugs. *Mimosa pudica* linn. is a widely known herbal medicine throughout the world. Numerous studies have delineate the pharmacological organization and benefits of *M. pudica*. Jagetia GC (2017) has shown the most important of these biologically active constituents of plants are alkaloids,flavonoids, tannins, steroids and phenolic compounds. According to Joseph B et., al (2013) and Jagetia GC et.,al (2017). Lajwanti is analgesic, antidepressant, alexipharmic, antiasthematic, antitumor, antiulcer, stimulant and vulnerary moreover, it is a remedy for asthma, dysentery, leprosy, inflammation, leukoderma, fatigue, blood disease, vaginal and uterine complaints. The crushed whole plant is applied in itchiness and itch related diseases.

Moreover in plants L-asparagine is the major nitrogen storage and transport compound, and it may also accumulate under stress conditions (Sieciechowicz et al. 1988) and according to Avramis V.I Asparaginase : Biochemical pharmacology and mode of drug resistance in (2012) recent advancement in cancer cell metabolism suggest that asparagine plays critical role in solid tumor progression, and it is therapeutically explorable.

DPPH has been used to estimate the free radical scavenging activity of the natural antioxidant. DPPH which is a radical itself with a yellow colour, changes into a stable compound antioxidant and the extent of the reaction

depends on the hydrogen donating ability of the antioxidant. Cragg GM, (2005) and Ghani A. (2003). In this study out of three extract and L-asparagine only two extract which was ethanol and aqueous showed potential free radical scavenging activity with IC_{50} value of 0.229 mg/ml and 0.387 mg/ml respectively this extracts of *Mimosa pudica* has ability to scavenge DPPH radicals suggests that it can react with free radicals to convert them to more stable products and terminate radical chain reaction. Moreover asparagine and chloroform extract did not show any antioxidant activity.

CONCLUSION

The present study indicate that L-asparagine exhibited less or no antioxidant activity. Whereas ethanol and aqueous extract of Mimosa pudica exhibiting significant antioxidant activity than chloroform extract.

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