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EVALUATION OF *INVITRO* ANTIOXIDANT ACTIVITIES OF ACETONE EXTRACT OF *MUSSAENDA PHILIPPICA* FLOWER

Velayutham Mani Mala*, P. Esakki Raja, R. K. Arul Kishore, A. Aravindan, M. Mariselvi, M. Ramasamy, Arun Murugesan, B. Sathiyarajan

Department of Pharmaceutical Chemistry, Sri Ram Nallamani Yadava College of Pharmacy, Nallamani nagar, Kodikurichi, Tenkasi–627804, Tamil Nadu, India.

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***Corresponding Author: Velayutham Mani Mala**
Department of Pharmaceutical Chemistry, Sri Ram Nallamani Yadava College of Pharmacy, Nallamani nagar, Kodikurichi, Tenkasi–627804, Tamil Nadu, India. **DOI:** https://doi.org/10.5281/zenodo.18803167

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ABSTRACT

Aim: To evaluate the *In-vitro* antioxidant activity of *Mussaenda philippica* flowers in acetone extract. **Methodology:** The shade dried flower plant powder was extracted with acetone by continuous hot percolation method using Soxhlet apparatus. The antioxidant activity was determined by DPPH assay and FRAP assay. **Results:** At the DPPH assay concentration of 50 mg/ml, the DPPH assay revealed an inhibition percentage of **67%**, with an IC_{50} value of 8.72 mg/ml, in contrast to the standard ascorbic acid, which had an IC_{50} value of 33.7334 mg/ml. This indicates that MP ET antioxidant activity via the DPPH assay is **GOOD** when compared to Vitamin C. Further Investigation, MP ET included dose-dependent antioxidant properties within *in-vitro* FRAP assays. When placed against the standard ascorbic acid in comparable settings. The extract displayed **SIGNIFICANT** activity at a concentration of 100 mg/ml (1.62- OD) when in comparison to normal Vitamin C. **Conclusion:** An acetone extract of *Mussaenda philippica* flowers having more effective antioxidant activity. These *in- vitro* assays indicate that this plant extract are better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

KEYWORDS: Acetone, DPPH Assay, FRAP Assay, *In-Vitro* antioxidant activity and *Mussaenda philippica*.

INTRODUCTION

The worldwide use of medicinal plants has gained additional impact due to its natural origin and high therapeutic implications. *Mussaenda philippica* 'Aurorae' belonging to family Rubiaceae is a shrub, having small tube-like flowers, oblong-lanceolate, dark green, glossy leaves. It is well distributed throughout India, South East Asia and Philippines.[1]

Mussaenda genuses have various species having distinct novel Phyto-constituents which are preferred for the treatment as antioxidant, antimicrobial, anti-inflammatory agents. A rich resource for natural products is provided by ethno-botany which shows a new-step stone for research in drug and its development. In recent years, the use of traditional medicine of plant source has gained more interest [1]. It has been reported that more than 50% of all modern drugs in clinical usage are of natural products. The medicinal plants have been comprised about 8000 species and among them 50% accounts for higher flowering plant species of India which is yet to be explored.[2]

Antioxidants are a diverse group of compounds that play a crucial role in maintaining cellular homeostasis by neutralizing reactive oxygen species (ROS) and other free radicals generated during normal metabolic processes and environmental stress. Excessive accumulation of these reactive molecules leads to oxidative stress, which can damage lipids, proteins, and nucleic acids, thereby contributing to the development of numerous chronic diseases, including cardiovascular disorders, neurodegenerative conditions, cancer, and aging-related pathologies. Endogenous antioxidant defence systems-such as enzymatic antioxidants like superoxide dismutase, catalase, and glutathione peroxidase-work in tandem with exogenous antioxidants obtained from the diet, including vitamins C and E, carotenoids, and polyphenolic compounds. Understanding the mechanisms by which antioxidants mitigate oxidative damage is therefore essential for elucidating their role in disease prevention and therapeutic strategies.

MATERIALS AND METHODS

1. Collection and authentication of plant material

The flowers of *Mussaenda philippica* was collected from Tenkasi, Tirunelveli District of Tamil Nadu, India. Taxonomic distinguishing proof was produced using the Sri Parasakthi College for Women, Courtallam, Tenkasi, Tirunelveli District, Tamil Nadu, India. The Plant Powdered material was put away in a hermetically sealed holder. The flowers were shade dried and grind into a coarse powder. The powdered materials were stored in a air tight polythene bags until use.

2. Preparation of plant extract

The flowers powder samples of *Mussaenda philippica* were extracted with Acetone Solvent at temperature between 50-60^0 C by using Soxhlet Extractor. The solvent was evaporated by rotavapor to obtained viscous semi solid masses.

3. *In-vitro* Antioxidant studies

I. DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl- Hydrate) Assay

A frequently used method for assessing how well antioxidants work to eliminate free radicals involves the DPPH test. During this procedure, a steady free radical known as DPPH is combined with a plant extract, and the resulting colour change is monitored. A more significant change in colour indicates stronger antioxidant capabilities. (*Baliyan S. et al., 2022*).

a) Principle

The capability of the extracts to counteract free radicals was gauged utilizing the DPPH radical scavenging test, adhering to the procedure detailed by Blois and Desmarchelier (Blois MS (1958) and (Desmarchelier et al., 1997). The aptitude of plant extracts to contribute hydrogen atoms was assessed by monitoring the fading of colour in a methanol mixture incorporating 2,2-diphenyl-1- picrylhydrazyl (DPPH). If antioxidants exist, the DPPH solution, which starts off with a violet or purple shade, changes to yellow tones. (*Rahman MM et al., 2015*).

b) Chemicals and reagents

DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl- Hydrate), Ethanol, Distilled water, Ascorbic acid, Test Sample.

c) Procedure

An arrangement of DPPH at a concentration of 6×10-5M in methanol was made by dissolving 7.89mg in 100ml, which implies for 250ml, you'd utilize (7.89/100) ×250. At that point, 500µl from each test arrangement was set into an Eppendorf tube. Each concentration was tried three times. Following, 500µl of the DPPH solution was included to the test arrangement and blended well. This blend was shaken and cleared out at room temperature for half an hour. The absorbance was at that point measured at 520nm. Ascorbic corrosive served as the positive control, whereas refined water acted as the negative control. The rate restraint compared to the standard was calculated utilizing the condition underneath, and the IC_{50} values were too decided. Graph Pad Prism 9 software (*Blois MS. 1958 and Karan SK et al., 2012*).

II. FRAP (Ferric Reducing Antioxidant Power) Assay

The ability of antioxidants to convert ferric ions into ferrous ions is evaluated through the FRAP assay. This method measures the reduction potential, allowing for an estimation of a sample's antioxidant capacity. An enhancement in reduction potential indicates a rise in antioxidant effectiveness (Munteanu IG and Apetrei C 2021).

a) Principle

The FRAP measure depends on the quick transformation of ferric-tripyridyltriazine (Fe3-TPTZ) in tests by cancer prevention agents, driving to the arrangement of ferrous-tripyridyltriazine (Fe2-TPTZ), which is characterized by its blue tone. By presenting the FRAP reagent to different known concentrations of Fe2+ arrangements, a standard bend is made, empowering the assurance of Fe2+ levels within the tests, which reflects their "antioxidant capacity." This strategy draws motivation from the inquire about conducted by Benzie and Strain. This test measures the antioxidant potential over a extend of tests. The FRAP measure accomplishes this by changing over ferric particles to ferrous particles through cancer prevention agents found within the tests. When ferric particle diminishment happens, a blue coloration is created, which is calculated colorimetrically. The assessment of the antioxidant potential in tests utilizes a standard bend based on ferrous press. The comes about are detailed as fe2+ counterparts spoken to in µm or as a FRAP esteem (Benzie IFF and Strain JJ 1996; Kurniawati P, et al 2017).

b) Chemicals and reagents

0.2 M, PH 6.6 phosphate buffer, Potassium ferricyanide (K3Fe (CN) 6), 10% Trichloroacetic acid, Distilled water, 0.1% Fecl3, Ascorbic acid, Test Sample.

c) Procedure

For ideal and steady comes about, as it were crisply gathered tests ought to be utilized. The extraction from plant materials can be performed with a assortment of solvents such as water, methanol, ethanol, or acetone. The fitting extraction procedure is decided by the nature of the particular test being prepared. A weakening arrangement was made with the plant substances. To each of the tubes, 2.5 ml of phosphate buffer (0.2 M, pH 6.6) was presented. Each tube's substance was blended altogether. In this way, 2.5 ml of a 1% potassium ferricyanide K3F(CN)6 arrangement was included to each test. Taking after this, each response blend was unsettled energetically employing a vortex shaker. The tests were set to hatch at a temperature of 50°C for roughly 20 minutes. Once the hatching was completed, 2.5 ml of

10% trichloroacetic corrosive (TCA) was presented to each test. The test tubes experienced centrifugation at 3,000 rpm for a term of 10 minutes. From the coming about centrifuged tests, 2.5 ml of supernatant was gotten in unmistakable test tubes. In those recently procured isolated test tubes, 0.5 ml of ferric chloride (FeCl3) was joined. This brought about within the arrangement of a somewhat blue tint. Hence, absorbance was evaluated at a wavelength of 700 nm. A test with the next concentration displayed more prominent absorbance, while the speak connected to tests with lower concentrations. Ascorbic corrosive served as the positive control, whereas refined water acted as the negative control. The test was rehashed three times. At long last, the rate of antioxidant action and IC_{50} values were computed utilizing GraphPad Crystal 9 computer program (Benzie IFF and Strain JJ 1996; Kurniawati P, et al 2017).

RESULT AND DISCUSSION

In-vitro Antioxidant

In the current study, *Mussaenda philippica* (MP ET) exhibited *in-vitro* antioxidant activity as assessed by the DPPH assays. At a concentration of 50 mg/ml, the DPPH assay revealed an inhibition percentage of **67%**, with an IC_{50} value of 8.72 mg/ml, in contrast to the standard ascorbic acid, which had an IC_{50} value of 33.7334 mg/ml. This indicates that MP ET antioxidant activity via the DPPH assay is **GOOD** when compared to Vitamin C. Further Investigation, MP ET included dose-dependent antioxidant properties within *in-vitro* FRAP assays. When placed against the standard ascorbic acid in comparable settings. The extract displayed **SIGNIFICANT** activity at a concentration of 100 mg/ml (1.62- OD) when in comparison to normal Vitamin C.

1. DPPH (2,2-Diphenyl-1-Picryl-Hydrazyl- Hydrate) Assay

Analysis

To analyse data statistically, you need to determine the actual absorbance of the plant material. This involves subtracting the blank plant absorbance from the assay absorbance of the plant. Essentially, after preparing your dilution series, you'll measure the colour intensity of the plant material using a spectrophotometer, which will give you the absorbance reading for the blank plant. For instance, after diluting the plant, it will have a certain colour, and when you measure its colour intensity, you might find the absorbance to be A for the blank plant. If the DPPH assay shows an absorbance of B, then the actual absorbance for the assay would be calculated as B minus A. Therefore, to determine the true absorbance, subtract the absorbance of the blank from the absorbance of the assay. Subsequently, you must determine the percent inhibition using this formula:

% Inhibition = (Absorbance of DPPH blank – True absorbance) x 100% / Absorbance of DPPH blank.

To determine the DPPH solution's absorbance when clear, take a spectrophotometric measurement after the DPPH arrangement is prepared. By duplicating this process for positive and negative controls, construct a concentration-log time graph. Afterwards, the IC_{50} should be determined through a nonlinear regression model with GraphPad Prism software. At that point by comparing the test comes about with controls, you'll be able decipher the information discoveries.

Table 1: *Mussaenda philippica* (MP ET) *In vitro* antioxidants activity by DPPH assay

S. NO	Concentration(mg)	COD	SOD	%inhibition	Average	IC50 (mg/ml)
1	50 mg	0.27	0.08	70%	67± 4.61	
2		0.27	0.10	62%		
3		0.27	0.08	70%		
1	100 mg	0.27	0.13	51%	47 ± 3.51	

2		0.27	0.14	48%		**8.72 mg/ml**
3		0.27	0.15	44%		
1	150 mg	0.27	0.11	59%	60 ± 5.56	
2		0.27	0.12	55%		
3		0.27	0.09	66%		
***In vitro* Standard vitamin C**						
1	100 mg	0.27	0.02	92%	90 ± 2.30	
2		0.27	0.02	92%		
3		0.27	0.03	88%		

Table 2: *Mussaenda philippica* (MP ET) Antioxidant IC_{50} Value (mg/ml) Compared to Standard VIT C IC_{50} Value (mg/ml)

S.NO	Concentration (mg)	Average	IC50 value
1	50	67 %	**8.72 mg/ml**
2	100	47 %	
3	150	60 %	
Standard Ascorbic acid vitamin C			
1	50 mg	91%	**IC_{50} value**
2	100 mg	87%	**33.7334 mg/ml**
3	150 mg	86%	
4	200 mg	92%	
5	250 mg	84%	

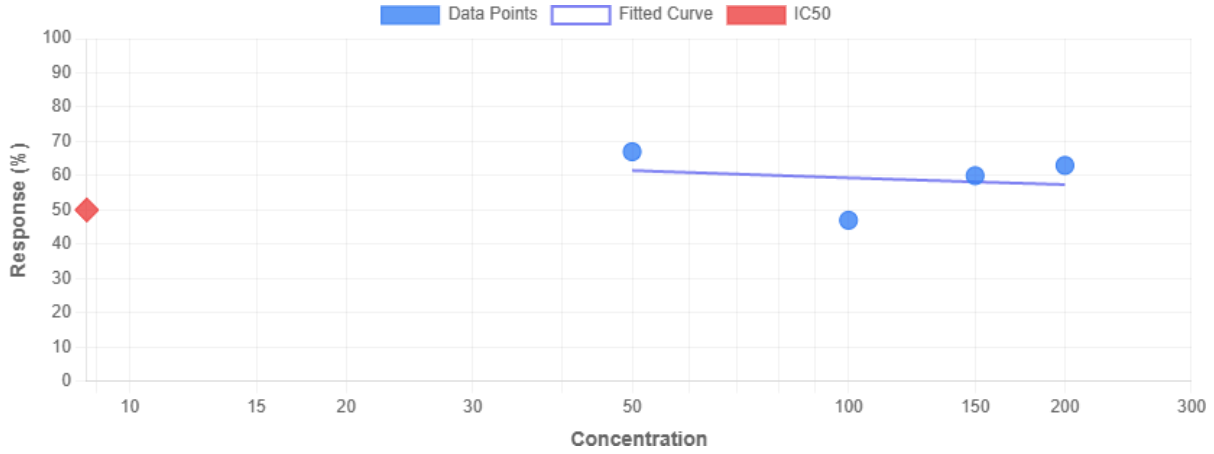

Fig. 1: *Mussaenda philippica* (MP ET) Anti-Oxidant Activity IC_{50} Value (mg/ml).

Fig. 2: Antioxidant activity By DPPH assay.

Fig. 3: Antioxidant activity By DPPH assay – Standard drug.

2. FRAP (Ferric Reducing Antioxidant Power) Assay Analysis

Based on the absorbance readings from your test, it is basic to compute the normalized rate of antioxidant movement for both the tests and controls. Furthermore, plotting a log-concentration versus time chart is required, and recognizing the IC_{50} ought to be performed through a nonlinear relapse show utilizing Chart Cushion Crystal program. A while later, by assessing the test results in connection to the controls, you'll observe the suggestions of the information discoveries.

Table 3*: Mussaenda philippica* (MP ET) *In vitro* antioxidants activity by FRAP assay.

S.NO	Concentration(mg)	OD	Average
1	50 mg	1.59	1.60
2		1.61	
3		1.61	
1	100 mg	1.63	1.62
2		1.61	
3		1.61	
1	150 mg	1.59	1.61
2		1.63	
3		1.63	
Standard			
S.NO	**Concentration(mg)**	**OD**	**Average**
1	50 mg	1.65	1.64
2		1.63	
3		1.65	
1	100 mg	1.64	1.64
2		1.65	
3		1.65	
1	150 mg	1.67	1.65
2		1.63	
3		1.66	

Fig. 4: Antioxidant activity By FRAP assay.

Fig. 5: Antioxidant activity By FRAP assay – Standard drug.

CONCLUSION

Antioxidant defences are created when multiple proteins and chemicals work together to eliminate and counteract reactive oxygen species (ROS). While the connection between ROS and the progression of tumours is complex and varies depending on the situation, new studies point to the possibility that ROS neutralization could encourage tumour formation and spread in a number of cancer types through a range of processes (Hawk, M. A., et al 2018). Prior research cantered on an Acetone Extract of *Mussaenda philippica* Flowers has reinforced its capacity to neutralize harmful free radicals, thereby validating its remarkable antioxidant strength.

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