ISSN 2664-4142 (Print) & ISSN 2664-6749 (Online)
South Asian Research Journal of Pharmaceutical Sciences
Abbreviated Key Title: South Asian Res J Pharm Sci

| Volume-6 | Issue-1 | Jan-Feb 2024 | **DOI: 10.36346/sarjps.2024.v06i01.002**

Original Research Article

Evaluation of Phytochemical Screening, Antioxidant, and Thrombolytic Activity of Methanolic Extract of *Phlogacanthus thyrsiflorus*

Afrin Jahan Tamanna[1], Avijit Saha[2], Majedul Hoque[2*], Md. Aktaruzzaman[2], Md. Nahid Hasan[2]

[1]Department of Pharmacy, Southeast University, Dhaka-1213, Bangladesh
[2]Department of Pharmacy, Jahangirnagar University, Dhaka-1342, Bangladesh

***Corresponding Author:** Majedul Hoque
Department of Pharmacy, Jahangirnagar University, Dhaka-1342, Bangladesh

Article History
Received: 01.12.2023
Accepted: 05.01.2024
Published: 09.01.2024

Abstract: ***Objective*:** The main objectives of the study are preliminary phytochemical screening, antioxidant activity and in vitro thrombolytic activity evaluation of extract *Phlogacanthus thyrsiflorus*. ***Methods*:** Preliminary phytochemical analysis was done by using standard methods. Human blood clot lysis was used to evaluate the thrombolytic activities. The antioxidant activity is evaluated by free radical scavenging activity of DPPH (1,1-diphenyl-2-picrylhydrazyl) method and total phenolic content. ***Results*:** Preliminary phytochemical screening revealed the presence of various bioactive components like Tannin, Saponins**,** Phenol and Flavonoids. In thrombolytic assay, a significant clot lysis was observed plant extract compared to the negative control. The plant could bebeneficial in synthetic drug formulation by virtue of the presence of antioxidant activity. Thecrude extract of plant were found to have Antioxidant activity with the concentration of 20 µg/ml,40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml respectively compared to control and standard. In DPPH scavenging assay, IC50 values of extract and ascorbic acid were found to be 31.87 µg/ml and 52.04 µg/ml respectively. Total phenol content was 10.5 mg. ***Conclusion*:** In conclusion, with this study it could be concluded that the phytochemical, antioxidant assay and thrombolytic activity of methanol extract *Phlogacanthus thyrsiflorus*would give valuable information for further characterization and exploitation of this important medicinal plant.

Keywords: *Phlogacanthus thyrsiflorus*, extraction; phenol; DPPH; methanol; formulation; phenolic; phytochemical; gallic acid; antioxidant.

1. INTRODUCTION

Due to the unparalleled abundance of chemical diversity, natural products like plant extracts, whether as pure chemicals or as standardized extracts, present countless prospects for the development of new drugs [1]. In Asia, the usage of herbal remedies is a reflection of a long history of environmental interactions with humans. Many different compounds found in plants used in traditional medicine can be used to treat both viral and chronic illnesses [2]. Since ancient times, medicinal plants have been employed to treat illnesses. Medicinal plants are those that are used as the basic components of herbal therapy. The modern pharmacopeia has seven thousand medicinal chemicals that are derived from plants. Modern science and empirical knowledge from the past are combined in herbal medicine [3]. According to estimates from the U.S. Forest Service, plants constitute the source of 40% of medicinal medications in the Western world [4]. People in developing nations employ therapeutic plants in place of pharmaceuticals. The earth is home to many different species of plants. Among them are herbs, which come in a variety of forms, hues, and leaf types [5]. Among the various applications of medicinal plant taxonomy is the identification of the appropriate species of medicinal plants for the associated disease. Plant identification by hand takes a lot of effort and requires professional assistance [6]. The automatic identification and classification of medicinal plants is an issue that must be solved for the benefit of humankind as a whole. Therefore, automatic medicinal plant identification and categorization is useful for image processing research. One important stage in the identification of medicinal plants is feature extraction.

Plant components have been used for many maladies, such as skin disorders, rheumatism, inflammation, syphilis, mental illness, epilepsy, hysteria, dehydration, and diarrhea, for a very long time in Southeast Asia [7, 8]. Many countries with limited resources, as well as many industrialized nations, use herbal medicine to cure specific diseases like coughs and to preserve overall health. Among these plants are ginseng, gingko, garlic, ginger, and echinacea, they are also blessing of nature [9, 12]. Over the ages, human groups have shared knowledge of their therapeutic qualities with one another. Plant species are utilized globally for a variety of purposes, including the treatment of infectious diseases. The biological characteristics of these plants are typically attributed to active chemicals generated during secondary metabolism. People are currently being cautioned by numerous studies about the dangers and risk posed by pathogenic germs that have developed resistance to newly developed medicines [10, 11].

Phlogacanthus thyrsiflorus is found usually in the sub- tropical Himalayas from Ravi to Bhutan, Upper gangetic plain, Bihar, India, China, Vietnam, Indonesia, Myanmar andBangladesh. North Bengal, plains and hills of Assam at an altitude of 1200 m andBangladesh. The plant occurs as an undergrowth in moist, shady places in parts of sub- Himalayan region and in Sal forests of Assam. It is gregarious and kills the vegetation beneath it. It is often cultivated as an ornamental plant for its handsome, laurel-like foliage and long spike of flowers. It can be propagated by cuttings in the rainy season. A gregarious evergreen shrub called *Phlogacanthus thyrsiflorus* is used to treat a variety of illnesses in traditional medicine. It is known that this plant contains hepatoprotective, antifungal, antibacterial, anti-inflammatory, anti-diabetic, anti-cancer, and hypolipidemic properties. Various plant parts have also reportedly been used as an antiseptic, pesticide, and antiallergic. *Phlogacanthus thyrsiflorus* has been shown to have a diverse array of chemical compounds, such as phlogan-thoside and diterpene glucoside. The plant was used to separate flavonoids, tannins, phytosterols, phenol, glycosides, fatty acids, galactoglycero lipid, and volatile oil as phytochemical components. The aim of this study was to screen methanolic extract of plant *Phlogacanthus thyrsiflorus* to display potent antioxidant activity in order to find possible sources for future novel anti- oxidant in pharmaceutical formulation.

2. METHOD AND MATERIALS

2.1 Collection of plants:

The fresh whole of the plant *Phlogacanthus thyrsiflorus* was chosen for pharmacological investigation. The leaf was collected in the month of October, 2021 from Chittagong District, Bangladesh. (Voucher specimen number: SEU-233B) and it was recognized by National Herbarium, Bangladesh.

2.2 Drying and grinding of the plant parts:

Leaves were separated from undesirable materials or plants or plant parts. Then the leaves were washed properly to remove dirt and shade dried for ten days. Shade dried was maintained to avoid degradation of the direct sunlight heat liable leaves component. These were then dried in an oven for 6 hours at considerably low temperature for better grinding. The dried plants were ground into coarse powder by a blender.

2.3 Storage and preservation of the plant parts:

The dried powder was stored in an airtight container against the re-absorption of moisture, oxidation, excessive heat or humidity, growth of molds and bacteria and infestation by insects and rodents and kept in a cool, dark and dry place until the next procedure. Proper storage is important to protect the drugs from all the above deteriorating factors and agents and maintain a high degree of quality in them.

2.4 Extraction of plant materials:

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. Crude plant drugs find their way in modern medicine system through continuous extraction followed by different isolation techniques and different pharmacological tests.

Chemical constituents from crude plant can be extracted by following two extraction procedures-

a) Cold extraction
b) Hot extraction

In our current study we used cold extraction method.

2.5 Procedure of Cold Extraction of the plant materials:

About 500gm of powder (leaves powder) was taken in a flat bottom glass container and soaked in 1.5L Methanol. The container with its content was sealed with aluminum foil and kept at room temperature for a period of 10 days accompanying occasional shaking and stirring. The extract was filtered through fresh cotton plug followed by Whatman No.1 filter paper. The filtrates were concentrated with a rotary evaporator under reduced pressure at 55°C temperature to

be ready for crude extract. Then the filtrate was kept in a water bath, turning the filtrate into semisolid. The gummy or semisolid filtrate dried at low temperature (39^0C).

Figure 1: Instruments for Cold extraction of the plant material

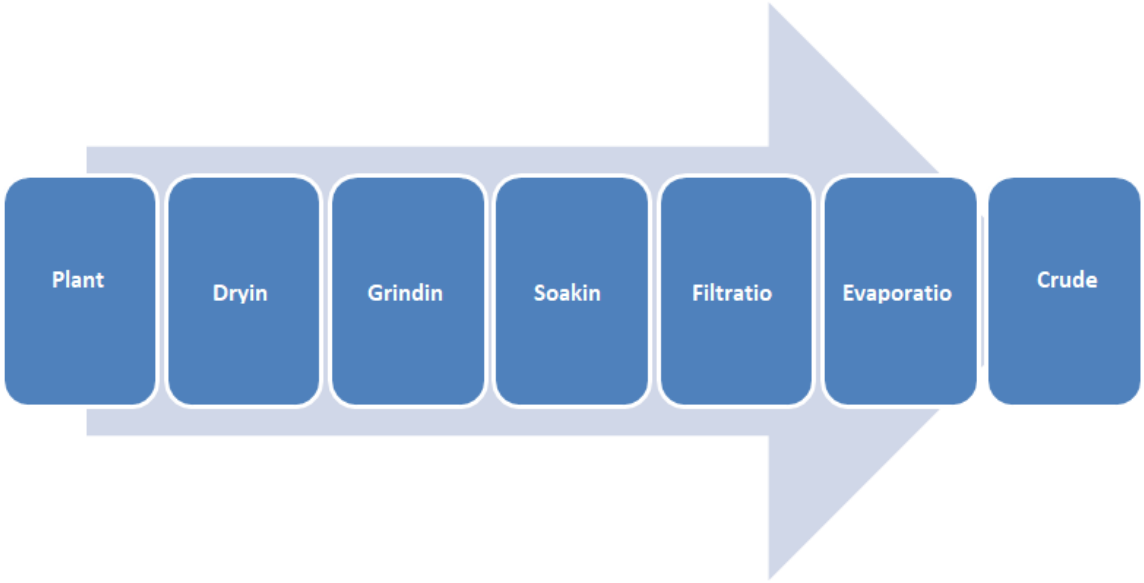

Figure 2: Schematic procedure of cold extraction

2.6 Pharmacological investigation of plant extract:

The following pharmacological investigations were done to determine the medicinal effect of the experimented extract:

a) Preliminary phytochemical analysis
b) In vitro thrombolytic activity
c) Antioxidant activity

3. RESULTS

Preliminary phytochemical screening revealed the presence of various bioactive components like Tannins, Saponins, Phenol and Flavonoids shown in Table 1.

Table 1: Phytochemical screening of ethanol extract of *Phlogacanthus thyrsiflorus*

Phytochemical test	Ethanol extract of Leaf
I. Alkaloids	
➢ Mayer‘stest	_ _
➢ Wagner‘stest	_ _
➢ Hager‘stest	_ _
➢ Dragendorff‘stest	_ _

Phytochemical test	Ethanol extract of Leaf
II. Carbohydrate	
➢ Molisch's test	_ _
➢ Benedict's test	_ _
➢ Fehling's Test	_ _
III. Saponins	
➢ Forthtest	++
➢ Foamtest	+-
IV. Phenols	
➢ Phenols	++
V. Tannins	
➢ Tannins	++
VI. Flavonoids	
➢ Alkaline reagent	++
➢ Lead acetate Test	+-

Key: Absent **--,** Low concentration **+-,** High concentration**++**

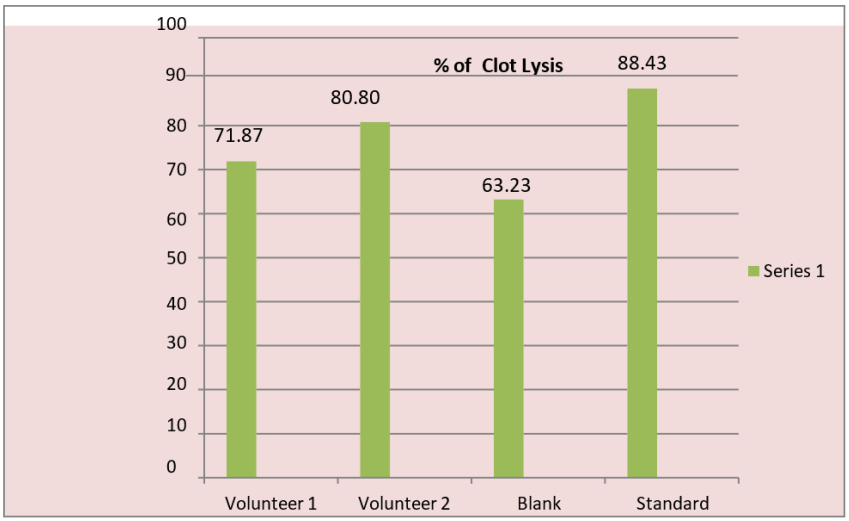

Figure 3: Thrombolytic activity of leaf extraction *Phlogacanthus thyrsiflorus*

The percentage % of lysis found from Volunteer 1, 2, Blank and Standard are 71.87, 80.80, 63.23 and 88.4 respectively shown in (Figure – 3).

The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the change in absorbance and percentage of scavenging activity is calculated. The activity was increased by increasing the concentration of the sample extract. The antioxidant activity of methanol extract of *Phlogacanthus thyrsiflorus* leaf was evaluated by DPPH radical scavenging assay shown in (Table – 2), (Figure 4, 5).

Table 2: DPPH radical scavenging activity of methanol extract of *Phlogacanthus thyrsiflorus* Leaf

Name of thesample	Abs. Control	Conc	Abs Standard	% Scavenging	IC50 µg/ml
Ascorbic acid(Standard)	0.936	20	0.899	3.952991	31.87
		40	0.652	30.34188	
		60	0.339	63.78205	
		80	0.145	84.50855	
		100	0.086	90.81196	
Crude methanol extract of *Phlogacanthus thyrsiflorus*	0.936	20	0.756	19.23	52.04
		40	0.643	24.9	
		60	0.496	40.6	
		80	0.359	61.64	
		100	0.120	87.17	

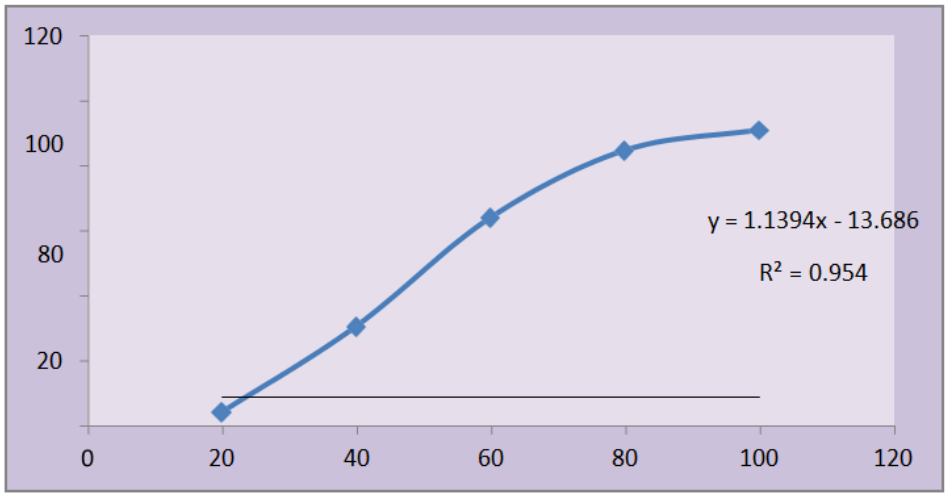

Figure 4: DPPH radical scavenging activity of standard Ascorbic acid

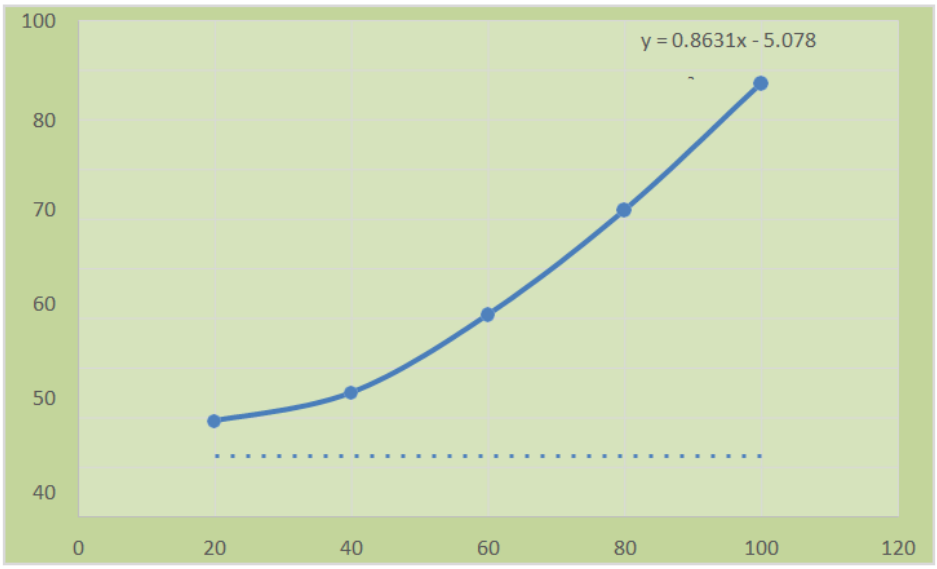

Figure 5: DPPH radical scavenging activity of methanol extract of *Phlogacanthus thyrsiflorus*

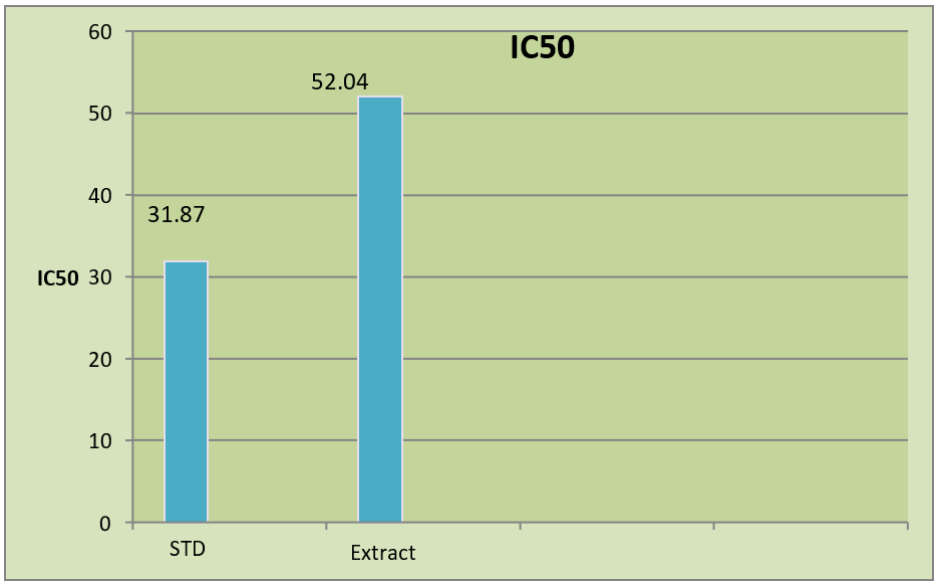

Figure 6: IC50 (µg/ml) values of methanol extract and Ascorbic acid (STD) for DPPH radical scavenging activity

The scavenging activity of methanol extract of *Phlogacanthus thyrsiflorus* Leaf was more than that of Ascorbic acid (Standard). IC50 of ascorbic acid and methanol extract of *Phlogacanthus thyrsiflorus* Leaf were 31.87(µg/ml) and 52.04µg/ml respectively. The result are shown in (Table–2) and (Figure–6) methanol extract of *Phlogacanthus thyrsiflorus* Leaf showed activity of DPPH.

Phenolic content of crude methanolic extract of *Phlogacanthus thyrsiflorus* was determined using Folin-Ciocalteu reagent. Phenolic content of the samples were calculated on the basis of the standard curve for gallic acid as shown below in the (Table–3). The results were expressed as mg of gallic acid equivalent of dried extracts. The values were mean of triplicate experiments and represented.

Table 3: Absorbance of gallic acid at different concentrations after treatment with Folin-Ciocaltue reagent

Conc. (µg/ml)	Abs Control	Abs Sample (Gallic acid)
6.25	0.047	0.219
12.5	0.047	0.323
25	0.047	0.669
50	0.047	1.272
100	0.047	2.693
200	0.047	4.000

Table 4: Determination of total phenolic content of *Phlogacanthus thyrsiflorus*

Concentration	Abs of Sample	m=Wt. of PlantExtract	c	C	V	c*V	A=(c*V)/m	Mean	Standard Deviation
(µg/ml)		(gm)	µg/ml	mg/ml	ml	mg	mg/gm		
200	0.251	0.0002	2.1	0.0021	1	0.0021	10.5*	7.875	3.712
200	0.230	0.0002	1.05	0.00105	1	0.00105	5.25		

Phenolic content of methanol extract of *Phlogacanthus thyrsiflorus* was found 10.5 mg represented in Table 4. The absorbance values of the extract of *Phlogacanthus thyrsiflorus* was compared with the standard solution of gallic acid equivalents.

4. DISCUSSION

In this present study the extract of *Phlogacanthus thyrsiflorus* was subjected to the preliminary phytochemical screening, antioxidant assay, in vitro thrombolytic activity to justify their traditional use. Phenolic compounds have redox properties, which allow them to act as antioxidants. As mentioned methanolic extract were tested for the determination of total phenol. The plant extract showed standard phenolic compound. The plant could bebeneficial in synthetic drug formulation by virtue of the presence of antioxidant activity. The crude extract of plant were found to have Antioxidant activity with the concentration of 20 µg/ml,40 µg/ml, 60 µg/ml, 80 µg/ml, 100 µg/ml respectively compared to control and standard. In DPPH scavenging assay, IC50 values of extract and ascorbic acid were found to be 31.87 µg/ml and 52.04 µg/ml respectively, and total phenol content was 10.5 mg.

5. CONCLUSION

From the ancient time medicinal plants are one of the most important and reliable sources for safe medication. The medicinal plants are used for discovering and screening of phytochemical constituents which are very helpful for manufacturing of new drugs. From this study it can be concluded that the phytochemical, antioxidant assay, in vitro thrombolytic activity of P. thyrsiflorus might give valuable information for further characterization and exploitation of this important medicinal plant. Medicinal plant might be potential for drug development that can possess excellent therapeutic effect with less side effect. Further study need to make more evidence and information profile about medicinal plant.

Disclosure of Conflict of Interest: None declared.

Author Contribution: All author contributed significantly to design and development of this work.

REFERENCES

1. Cosa, P., Vlietinck, A. J., Berghe, D. V., & Maes, L. (2006). Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *J Ethnopharmacology, 106*(3), 290–302. doi: 10.1016/j.jep.2006.04.003
2. Duraipandiyan, V., Ayyanar, M., & Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC complementary and alternative medicine*, *6*, 1-7. doi: 10.1186/1472-6882-6-35.
3. Mohammed, A. H. (2019). Importance of medicinal plants, *Res Pharmacy Health Sci*, *5*(2), 124-125.

4. https://www.fs.usda.gov/wildflowers/ethnobotany/medicinal/index.shtml
5. Wu, S. G., Bao, F. S., Xu, E. Y., Wang, Y. X., Chang, Y. F., & Xiang, Q. L. (2007, December). A leaf recognition algorithm for plant classification using probabilistic neural network. In *2007 IEEE international symposium on signal processing and information technology* (pp. 11-16). IEEE.
6. Putri, Y. A., Djamal, E. C., & Ilyas, R. (2021, March). Identification of medicinal plant leaves using convolutional neural network. In *Journal of Physics: Conference Series* (Vol. 1845, No. 1, p. 012026). IOP Publishing. doi: 10.1088/1742-6596/1845/1/012026
7. Shanghai, P. R. (1977). Jiangsu new medical college. Dictionary of Chinese Materia Medica. China: Shanghai Scientific and Technical Publishing House, p.1874.
8. Hoque, M. (2023). Centella asiatica: A mini review of its medicinal properties and different uses. *World Journal of Advanced Research and Reviews, 19*(02), 1185–1191. https://doi.org/10.30574/wjarr.2023.19.2.1699
9. Rakotoarivelo, N. H., Rakotoarivony, F., Ramarosandratana, A. V., Jeannoda, V. H., Kuhlman, A. R., Randrianasolo, A., & Bussmann, R. W. (2015). Medicinal plants used to treat the most frequent diseases encountered in Ambalabe rural community, Eastern Madagascar. *Journal of ethnobiology and ethnomedicine*, *11*(1), 1-16.
10. Singh, R. (2015). Medicinal plants: A review. *J Plant Sci*, *3*(1), 50-55. Doi: 10.11648/j.jps.s.2015030101.18
11. Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Virulence mechanisms of bacterial pathogens*, 4(2), 481-511. doi: 10.1128/microbiolspec.VMBF-0016-2015.
12. Hoque, M. (2023). Importance of Green Chemistry and its Implementation for Healthy Environment. *International Journal of Research Publication and Reviews*, *4*(8), 838-845.