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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ASSAY OF PLANT STROBILANTHES DYERIANA MAST

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ABSTRACT

Strobilanthes dyeriana Mast. is ever green, tropical shrub belonging to family Acanthaceae. It is ornamental plants grown for its metallic – purple stripes radiating from the middle of the vein and for its foliage that is dark green colours. In present study, the effects of three different solvents such as methanol, acetone and aqueous were investigated to determine the presence of phytochemicals , total phenolic content, total flavonoid content, antioxidant assay and FT-IR assay in leaves of Strobilanthes dyeriana Mast. The result concluded that flavonoids, tannins, phenols, proteins, terpenoids, cardiac glycosides are present in leaves extracts. In TPC, the highest content was observed in methanol extract of leaves and lowest content was observed in aqueous extract of leaves while in TFC, the highest content was observed in acetone extract of leaves and lowest content was observed in methanol (636.163 μ g/ml) and lowest in aqueous extract (198.716 μ g/ml).

Keywords: Strobilanthes dyeriana Mast, Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH Assay, FT-IR Assay

INTRODUCTION

The Strobilanthes dyeriana Mast. commonly known as Persian Shield belong to genus Strobilanthes of family Acanthaceae (Raghavendra et al., 2017). Acanthaceae family member Strobilanthes genus comprises over 350 species worldwide, with the majority found in Kashmir, Bhutan, Bangladesh, and the Khasi Mountains of northeast India (at least 46 species in India). The native of the plant is Myanmar (Burma) (Armitage., 2001). It is a soft-stemmed, tropical, evergreen shrub or subshrub that was chosen for planting primarily for its magnificent iridescent purple foliage. Persian Shield is cultivated for its eye-catching foliage. (EE Gamrod,2003) The leaf may seem metallic due to its silvery iridescent lustre that might cover it. The favored climate zones are subtropical/monsoonal and tropical, and the native habitat is terrestrial. The Latin words "Strobilos" (cone) and "Anthos" (flower) combine to form the name "Strobilanthes" (flower or stalk). (Xiao-Ling et al., 2022)

Ornamental Plants in ethnobotany

Ethnobotany is the study of interactions between humans and plants in diverse cultural settings and within the framework of biocultural ecology, where both the natural and cultural sphere are taken into consideration. (Albuquerque and Hurrell, 2010; Hurrell and Albuquerque, 2012).

Phytochemicals

The term "phytochemical" refers to a broad range of substances that naturally occur in plants and is loosely defined as "plant (Phyto) chemical." (Huang et.al., 2016) (Khant, et al., 2021) Phytochemicals are biologically active, naturally occurring chemical substances found in plants (from the Greek term Phyto, which means "plant") (Prajapati, et al. 2018) Typically, they play a part in plant growth or defense against adversaries, pathogens, or predators and have biological activity in the plant host (Molyneux et.al ,2007). And which are more beneficial to human health than the macro- and micronutrients they are linked to (Velavan., 2015) (Patel and Modi, 2018).

Fruits, vegetables, legumes, whole grains, nuts, seeds, fungus, herbs, and spices all contain a variety of dietary phytochemicals (Mathai.,2000). Common sources include whole tomatoes, wheat bread, grapes, strawberries, beans, cherries, legumes, raspberries, cabbage, garlic and onions, garlic (Moorachian., 2000).

Phytochemicals build up in so many plant parts, including that of the bark, roots, stems, leaves, flowers, fruits, or seeds (Yadav and Agarwala.,2011) (Rutuba, et al., 2021) According to their function in plant metabolism, phytochemicals are divided into two categories: 1. Primary metabolites 2. Secondary metabolites



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MATERIAL AND METHOD

• Plant Material:

The Strobilanthes dyeriana Mast. plant was collected in January from Krishna Nursery at Vijay Char Rasta and Jay Bajrangbali Nursery at Memnagar Road in Ahmedabad, Gujarat. For the experiment, fresh leaves of Strobilanthes dyeriana Mast. plant was taken.

• Preparation of Sample:

The entire plant material was dried in the air until all water molecules get vanished. Following drying, the plant material was finely ground using a mechanical blender into a powder before being put into airtight containers for later usage.

• Preparation of Extraction:

The extract of Strobilanthes dyeriana Mast. leaves by hot extraction method by using a Soxhlet extractor. And the solvents used for this method are methanol, acetone, aqueous (Distilled water). 10mg leaves powder extracted with 100ml solvent (10mg/100ml).

• Qualitative analysis: (Harborne 1998, Kokate & Ramman 2000).

1. Test for Alkaloids

A small amount of the crude extract was individually dissolved in dilute hydrochloric acid and then filtered.

• **Mayer's Test:** 1 ml of filtrate was taken and add 1 ml Mayer's reagent. Yellow colour precipitates indicate the presence of alkaloids.

• **Wagner's Test:** 1 ml of filtrate was taken and add 1 ml Wagner's reagent. Brown/reddish colour precipitates indicate the presence of alkaloids.

• **Hager's Test:** 1 ml of filtrate was taken and treated with 1 ml Hager's reagent. Yellow colour precipitates indicate the presence of alkaloids.

• **Dregendroff Test:** 1 ml of filtrate was taken and add 1 ml Dregendroff reagent. Presence of orange colour precipitates indicate the presence of alkaloids.

2. Test for Carbohydrates

Crude extracts were dissolved in 5 ml distilled water and then filter it. The filtrate is used as test solution.

• **Molish Test:** Take 1 ml of extract and add 1 ml Molish reagent into it and shake it well. Then add 1 ml of concentrated sulphuric acid side by side. Red violate ring indicate the presence of carbohydrate.

• **Fehling Test:** 2 ml filtrate were hydrolyzed with 1 ml diluted hydrochloric acid (1 N) and neutralize with 1ml alkaline solution (10% NaOH). Then heat in water bath and then add Fehling A & B solution. The formation of red precipitation indicate the presence of carbohydrates.

• **Barford's Test:** Take 1 ml of filtrate and add 1 ml Barford's reagent. Boil the mixture for 2 min. The formation of red ppts indicate the presence of carbohydrates.

• **Bendict's Test:** Take 1 ml of filtrate and add 1 ml Bendict's reagent. The mixture is heated in a boiling water bath for 2 mins. The formation of orange red ppts indicate the presence of reducing sugar.

3. Test for Glycosides

• **Borntrager's Test:** Take 2 ml of extract and add 3 ml of chloroform and shake it well. Now add a few drops of ammonium solution. Pink color indicate the presence of glycosides.

• **Legal's Test:** To 1 ml of extract add 1 ml of sodium nitroprussides and 1 ml of pyridine. Formation of pink or red colour indicate the presence of cardiac glycoside.

• **Keller-Killiani Test:** To 2 ml of test solution add 2-3 drops of glacial acetic acid and add 1% ferric chloride mixed with concentrated sulphuric acid. Formation of two-layer lower reddish brown and upper bluish green.

4. Test for Proteins

• Millon's Test (Mercuric Nitrate Solution): Take 2 ml of extract and add 1-2 ml of Million's reagent. White ppts indicates the presence of proteins.

• **Biuret Test:** Take 2 ml of extract and add 0.5 ml of CuSO₄, 1 ml of ethanol, 1 KOH pellet. Pink colour of ethanolic layer indicate the presence of proteins.

5. Test for Phenols

• **Ferric chloride Test:** To the extract treated with 2 ml of ferric chloride solution. Formation of blue/black colour ppts indicate the presence of phenols.

• Lead acetate Test: 1 ml of extract add 0.5 ml of 10% lead acetate solution. Formation of white precipitates indicate the presence of phenols in extract

• **Folin-Cioculten Test:** 0.5 ml of extract add 1 ml of Folin-Cioculten solution. Formation of bluish green precipitates indicate the presence of phenols in extract.

6. Test for Flavonoids



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• Alkaline Test: Take 1 ml of extract and add 1 ml of 10% sodium hydroxide solution. Formation of intense yellow colour, which was disappeared after addition of 2 ml concentrated sulphuric acid, indicate the presence of flavonoids.

• Lead acetate Test: To 1 ml of extract add 1 ml of 10% lead acetate solution. Formation of yellow precipitates indicate the presence of flavonoids in extract.

7. Test for Saponin

• **Froth Test:** Few mg extract diluted with 5ml in distilled water and shake it well for 15 mins. Formation of 1 cm of foam indicate that saponin is present.

8. Test for Terpenoids

• **Salkowski Test:** Take 1 ml of extract and then add 2 ml chloroform & 3 ml of concentrated sulphuric acid. Appearance of reddish brown colour indicate the presence of terpenoids.

• **Copper acetate Test:** To 1 ml of extract add 1 ml of 5% copper acetate solution. Green colour precipitates indicate the presence of terpenoids.

9. Test for Fixed oils and fats

• **Filtered paper Test:** Take small amount of extract and press it between the filtered paper. Leave oil strain on the filtered paper. The oil strain indicate the presence of fixed oils and fats.

10. Test for Cardiac Glycosides

• **Legal Test:** Take 1 ml of extract and add 1 ml of pyridine and 1 ml of 20% sodium nitroprusside. Pink or red colour indicate the presence of cardiac glycosides.

11. Test for Phytosterols

• **Liebermann Burchard's Test:** Take 1 ml of extract and add 2-3 ml of acetic anhydride solution and 2 ml of conc. sulphuric acid. Violet or green colour indicates the presence of phytosterols.

• **Salkowski Test:** Take 1 ml of extract and then add 2 ml chloroform & 3 ml of concentrated sulphuric acid. Appearance of reddish brown colour indicates the presence of phytosterols.

Estimation of Total Flavonoid Content in leaves of Plant

500 μ l of the extract of different parts of plants were mixed with 1500 μ l of 95% methanol and then 100 μ l of Aluminum chloride (10%) and Potassium acetate (1M) was added respectively and make volume up to 10ml with distilled water and agitated. Incubation was done for 20-30 minutes at room temperature. The absorbance was assessed at 415 nm against a blank having all the reagent without the sample using spectrophotometer. Measurement was done in triplicates and the total flavonoid quantified by the standard curve of quant

• Estimation of Total Phenol Content in leaves of Plant

One ml of the extracts of different parts of plants were thoroughly mixed with 10 ml of distilled water, added 1.5 ml of Folin-Ciocalteu reagent. After 5 minutes, 4 ml of 20% sodium carbonate (Na2CO3) was added and adjusted with distilled water up to 25 ml and agitated. Then incubated for 30 minutes at room temperature. The absorbance was measured at 765 nm against a blank having all the reagents excluding the sample using spectrophotometer. This procedure was repeated 3 times for each extract. The total phenols were quantified by the standard curve of gallic acid solution which was prepared using the similar procedure. ($R^2 = 0.998$) (Patel and Modi, 2018)

ANTIOXIDANT ACTIVITY

• DPPH assay (2, 2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay according to (Chang et al. 2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

Different volumes $(2 - 20\mu)$ of plant extracts were made up to 40μ l with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. (Shah, et al. 2021; Patel, et al. 2021)

FT-IR Assay

FTIR spectrophotometric analysis, the extracts were centrifuged at 3000 rpm for 10 min, and then using a highpressure vacuum pump, filtered using Whatman No. 1 filter paper. Using the same solvent, the sample has been diluted to a ratio of 1:10. Using a Perkin Elmer Spectrophotometer system, FTIR analysis was carried out to identify the characteristic peaks in the 400–4000 cm-1 range and their functional groups. The FTIR's peak values were noted. Every analysis was performed twice for the validation of the spectrum (Karpagasundari and Kulothungan., 2014 ; Patel and Modi, 2022)



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RESULTS AND DISCUSSION

• Yield Value

Comparing all of the values of extracts, the yield extractive value was highest in methanolic extract and lowest in acetone.

Table -1	Showing	yield	values	of	different	extract

Sr. No.	Selected Plant	Yield Value					
	Part	Methanolic	Extract	Acetone	Extract	Aqueous	Extract
		(gm.)		(gm.)		(gm.)	
1.	Leaves	1.774		0.441		0.783	

Phytochemical Analysis of different plant extract

The Phytochemical Analysis of Strobilanthes dyeriana Mast. in methanol, acetone, aqueous extracts of leaves show the presence of many secondary metabolites like Flavonoids, Tannins, Phenols, Proteins, Terpenoids, Cardiac Glycosides in methanol extract. Carbohydrates, Glycosides, Fixed oil & Fats, Steroids Flavonoids, Tannins, Phenols, Proteins, Terpenoids, Cardiac Glycosides in acetone extract. Flavonoids, Tannins, Phenols, Proteins, Terpenoids, Cardiac Glycosides, Saponins, Carbohydrates in aqueous extract. Many secondary metabolites are absence in methanol extract are alkaloids, Carbohydrates, Fixed oil & Saponins, Glycosides, Steroids. In acetone extract alkaloids, Saponins are absent. And in aqueous extract alkaloids, Glycosides, Fixed oil & Fats, Steroids are absent.

Table -2 Showing the result of Phytochemical Analysis of Strobilanthes dyeriana leaves in three different
extracts like methanol, acetone, aqueous.

Sr. No	Phytochemical Test	Solvent		
		Methanol	Acetone	Aqueous
Alkaloid	5			
1	Mayer's Test			
2	Wagner's Test			
3	Hager's Test			
4	Dragendroff Test			
Carbohy				
1	Molish Test			
2	Fehling Test			+
3	Barford's Test			
4	Bendict's Test		+	
Glycosid				
1	Borntrager's Test			
2	Legal's Test		+	
3	Keller-Killiani Test			
Proteins				
1	Millon's Test (Mercuric Nitrate Solution)	+	+	+
2	Biuret Test			
Phenol/ 7	Tannins			
1	Ferric chloride Test	+	+	+
2	Lead acetate Test	+	+	+
3	Folin-Cioculten Test	+	+	+
Flavonoi	ds			
1	Alkaline Test	+	+	+
2	Lead acetate Test	+	+	+
Saponins				
1	Froth Test			+
Fixed Oi	ls & Fats			
1	Filtered paper Test		+	
Terpenoi	ds			
1	Salkowski Test			
2	Copper acetate Test	+	+	+
Cardiac	Glycosides			
1	Legal Test	+	+	+
Steroids				
1	Liebermann Burchard's Test		+	



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2 Salkowski Test				
	2	Sarowski lest	 	

• Total Phenolic Content (TPC)

The maximum value of absorbance of Total Phenolic Content obtain in methanol extract is 0.443 ± 0.034 and the lowest value of absorbance obtain in aqueous extract is 0.281 ± 0.003 at the 1000 µg/ml concentration measured at the 765nm in leaves of Strobilanthes dyeriana Mast.

Table- 3 Shows the total phenolic content of the different extract of leaves of Strobilanthes dyeriana Mast.

Sr.	Concentration	TPC ABSORBANCE (EXTRACT)				
No.	(µg/ml)	Methanol	Acetone	Aqueous		
1.	1000	0.443±0.034	0.398±0.060	0.281±0.003		

• Total Flavonoid Content (TFC):

The maximum value of absorbance of Total Flavonoid Content obtain in acetone extract is 0.965 ± 0.059 and the lowest value of absorbance obtain in methanol extract is 0.503 ± 0.020 at 1000 µg/ml concentration measured at the 415nm in leaves of Strobilanthes dyeriana Mast.

Table- 4 Shows the total Flavonoid content of the different extract of leaves of Strobilanthes dyeriana Mast.

Sr.	Concentration (µg/ml)	TFC ABSORBANCE (EXTRACT)	
No.		Methanol	Acetone
1.	1000	0.503±0.020	0.965±0.059

Antioxidant Activity

DPPH Scavenging Activity of Standard

The maximum value of the DPPH Scavenging Activity is 38.771 ± 0.003 at 1000 µg/ml and minimum is 18.277 ± 0.003 at 200 µg/ml concentration in methanol solvent. The maximum value of the standard DPPH Scavenging Activity is 71.951 ± 0.003 at 1000 µg/ml concentration and minimum is 55.042 ± 0.008 at 200 µg/ml concentration in acetone solvent. The maximum value is 70.470 ± 1.851 at 1000 µg/ml concentration and minimum is 40.733 ± 2.150 at 200 µg/ml concentration in aqueous solvent measured at 517nm.

Table- 5 Shows the DPPH Scavenging Activity of standard of the different extract of leaves of Strobilanthes dyeriana Mast.

Sr. No.	Concentration(µg/ml)	DPPH SCAVENGING ACTIVITY (%) (STANDARD)			
		Methanol	Acetone	Aqueous	
1.	200	18.277 ± 0.003	55.042 ± 0.008	40.733 ± 2.150	
2.	400	22.608 ± 0.004	57.816 ± 0.004	49.900 ± 1.971	
3.	600	27.693 ± 0.005	62.180 ± 0.004	56.300 ± 1.042	
4.	800	34.592 ± 0.003	67.955 ± 0.005	63.613 ± 2.042	
5.	1000	38.771 ± 0.003	71.951 ± 0.003	70.470 ± 1.851	

The value of DPPH Scavenging Activity of extract in methanol solvent: the maximum value of the DPPH Scavenging Activity is at 65.609 ± 0.009 at $1000 \ \mu g/ml$ concentration and minimum is 22.104 ± 0.004 at $200 \ \mu g/ml$ concentration that the value of DPPH Scavenging Activity of extract in acetone solvent: the maximum value of the DPPH Scavenging Activity is at 44.135 ± 0.015 at $1000 \ \mu g/ml$ concentration and minimum is 15.416 ± 0.023 at $200 \ \mu g/ml$ concentration. The value of DPPH Scavenging Activity of extract in aqueous solvent was the maximum value of the DPPH Scavenging Activity is at 82.421 ± 0.014 at $1000 \ \mu g/ml$ concentration and minimum is 49.786 ± 0.014 at $200 \ \mu g/ml$ concentration measured at 517nm.

Table- 6 Shows the DPPH Scavenging Activity of extract of the different extract of leaves of Strobilanthes
dyeriana Mast.

Sr.	Concentration	DPPH SCAVENGIN	DPPH SCAVENGING ACTIVITY (%) (EXTRACT)			
No.	(µg/ml)	Methanol	Acetone	Aqueous		
1.	200	22.104 ± 0.004	15.416 ± 0.023	49.786 ± 0.014		
2.	400	37.613 ± 0.017	23.717 ± 0.021	57.547 ± 0.019		
3.	600	51.309 ± 0.017	32.327 ± 0.027	68.257 ± 0.017		
4.	800	63.242 ± 0.022	37.483 ± 0.023	74.932 ± 0.013		
5.	1000	65.609 ± 0.009	44.135 ± 0.015	82.421 ± 0.014		

The IC₅₀ Values of Antioxidant activity (DPPH) of standard and extract: the maximum IC₅₀ Value shown in standard of various solvent is methanol 719.729 and lowest in aqueous 430.409. In extract maximum IC₅₀ Value shown in various solvent is methanol 636.163 and lowest in aqueous 198.716.



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Table- 7 Shows the IC_{50} values of antioxidant activity (DPPH) extract of the different standard and extract of leaves of Strobilanthes dyeriana Mast.

Sr.	IC ₅₀ VALUES						
No.	Methanol		Acetone		Aqueous		
	Standard	Extract	Standard	Extract	Standard	Extract	
1.	719.729	636.163	439.166	572.261	430.409	198.716	

• FTIR Analysis

Methanol solvent shows the leaves absorption spectra's peaks and shows different bands present at: 3324.8 cm⁻¹ Carboxylic acid (O-H Stretching), 2944.6 to 2832.8, 1449.9 cm⁻¹ Alkane (C-H Stretching), 2594.2 to 2527.1 cm⁻¹ Thiol (S-H Stretching), 1707.1 cm⁻¹ Aliphatic Ketone (C=O Stretching), 1412.7, 1021.3 cm⁻¹ Sulfate and Sulfoxide (S=O Stretching). 1114.5 cm⁻¹ Secondary alcohol (C-O Stretching).

Acetone solvent shows the leaves absorption spectra's peaks and shows different bands present at: 3570.8 to 3414.2 ,1420.1 cm⁻¹ alcohol (O-H Stretching), 1710.8 cm⁻¹ Aliphatic Ketone (C=O Stretching), 1356.8, 1032.5cm⁻¹ Aliphatic Sulfate and Sulfoxide (S=O Stretching), 1218.8, 1092.1cm⁻¹ Alkyl aryl ether and Secondary alcohol (C-O Stretching), 786.5 cm⁻¹ Halo compound (C-Cl Stretching).

Aqueous solvent shows the leaves absorption spectra's peaks and shows different bands present at: 3324.8 cm⁻¹ Aliphabetic primary amine (N-H Stretching), 2355.7 to 2310.9 cm⁻¹ Carbone dioxide (O=C=O Stretching), 2199.1 cm⁻¹ Alkyne (CEC Stretching), 2165.6 cm⁻¹ Thiocyanate (S-CEN Stretching), 2124.6 cm⁻¹ Azide (N=N=N Stretching), 2020.2 cm⁻¹ Isothiocyanate (N=C=S Stretching), 1949.4 cm⁻¹ Allene (C=C=C Stretching), 1848.8 cm⁻¹ Aromatic compound (C-H Stretching), 1722.0 cm⁻¹ Aliphatic ketone (C=O Stretching), 1636.3 cm⁻¹ Conjugated alkene (C=C Stretching), 1285.9 cm⁻¹ Aromatic amine (C-N Stretching).

Sr. No	Extract	Wave number(cm ⁻	Band Interaction	Band Assignment	Possible Compound
1	Methanolic	3324.8	Strong broad	O-H Stretching	Carboxylic acid
		2944.6	Medium	C-H Stretching	Alkane
		2832.8	Medium	C-H Stretching	Alkane
		2594.2	Weak	S-H Stretching	Thiol
		1707.1	Strong	C=O Stretching	Aliphatic Ketone
		1449.9	Medium	C-H Bending	Alkane
		1412.7	Strong	S=O Stretching	Sulfate
		1114.5	Strong	C-O Stretching	Secondary alcohol
		1021.3	Strong	S=O Stretching	Sulfoxide
2	Acetone	3570.8	Medium	O-H Stretching	Alcohol
		3414.2	Strong broad	O-H Stretching	Alcohol
		3004.2	Medium	C-H Stretching	Alkene
		1710.8	Strong	C=O Stretching	Aliphatic Ketone
		1420.1	Medium	O-H Bending	Alcohol
		1356.8	Strong	S=O Stretching	Sulfonate
		1218.8	Strong	C-O Stretching	Alkyl aryl ether
		1092.1	Strong	C-O Stretching	Secondary alcohol
		1032.5	Strong	S=O Stretching	Sulfoxide
		786.5	Strong	C-Cl Stretching	Halo compound
3	Aqueous	3302.4	Medium	N-H Stretching	Aliphatic primary amine
		2355.7	Strong	O=C=O Stretching	Carbon dioxide
		2199.1	Weak	CEC Stretching	Alkyne

 Table- 8 Shows the FTIR Analysis of the different extract of leaves of Strobilanthes dyeriana Mast.



	2165.6	Strong	S-CEN stretching	Thiocyanate
	2124.6	Strong	N=N=N	Azide
			Stretching	
	2020.2	Strong	N=C=S Stretching	Isothiocyanate
	1949.4	Medium	C=C=C	Allene
			Stretching	
	1848.8	Weak	C-H Bending	Aromatic compound
	1722.0	Strong	C=O Stretching	Aliphatic ketone
	1636.3	Medium	C=C Stretching	Conjugated alkene
	1285.9	Strong	C-N Stretching	Aromatic amine

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CONCLUSION

The study suggest that, the leaves of Strobilanthes dyeriana Mast. plant contain significant and active phytochemical components in three different solvents like phenols, tannins, flavonoids, cardiac glycosides, terpenoids, proteins. In total phenolic content maximum values was seen in methanol and in total flavonoids content in acetone. The high IC_{50} Values of antioxidant activity (DPPH assay) obtains in methanol. That shows the methanolic extract shows high power to resistance against environmental stress, pest etc. which help to develop therapeutic drugs. Many compounds found in leaves of Strobilanthes dyeriana Mast. plant by using FTIR method which are useful for medicinal studies.

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