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PRELIMINARY PHYTOCHEMICAL SCREENING, ESTIMATION OF TOTAL PHENOLIC, FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF *STYLOSANTHES FRUTICOSA* (RETZ.) ALSTON

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***Abstract*:** *Stylosanthes fruticosa* is a medicinally important plant traditionally used in folk medicine for various therapeutic purposes. The present study aimed to evaluate the phytochemical composition, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant potential of different solvent extracts of *S. fruticosa* leaves. Qualitative phytochemical screening revealed the presence of flavonoids, tannins, phenolics, alkaloids, coumarins, saponins, proteins, fixed oils, and reducing sugars in varying proportions among ethanol, methanol, acetone, and petroleum ether extracts. The acetone extract exhibited the highest total phenolic content (0.694 ± 0.032 mg GAE/g extract), while the ethanol extract showed maximum flavonoid content (0.700 ± 0.090 mg QE/g extract). Antioxidant activity assessed by DPPH free radical scavenging assay demonstrated that the methanolic extract possessed the highest antioxidant activity (50.052 ± 2.054%) with an IC_{50} value of 0.9509 mg/ml. The regression analysis indicated a strong linear relationship between concentration and scavenging activity (R^2 = 0.9766). The findings suggest that *S. fruticosa* is a promising source of natural antioxidants and bioactive phytochemicals with potential pharmaceutical applications.

Index Terms **-** Phytochemical analysis, Total phenolic content, Total flavonoid content, DPPH assay, Antioxidant activity, *Stylosanthes fruticosa*

I. INTRODUCTION

Medicinal plants are important reservoirs of biologically active compounds and have been widely explored for their therapeutic potential. In recent years, interest in natural antioxidants has increased considerably because oxidative stress is associated with several chronic disorders including cancer, diabetes, cardiovascular diseases, inflammation, and neurodegenerative disorders. Reactive oxygen species (ROS) generated during oxidative stress can damage cellular macromolecules such as proteins, lipids, and DNA (Halliwell & Gutteridge, 2015). Plant-derived antioxidants are considered safer alternatives to synthetic antioxidants due to their reduced side effects and broad pharmacological activities.

Phenolic compounds and flavonoids are among the major classes of secondary metabolites responsible for antioxidant activity. These compounds possess the ability to donate electrons or hydrogen atoms to

neutralize free radicals and thereby inhibit oxidative damage. They also exhibit antimicrobial, anti-inflammatory, anticancer, and cardioprotective properties (Kumar & Pandey, 2013). Therefore, estimation of total phenolic content (TPC) and total flavonoid content (TFC) has become an essential parameter in phytochemical investigations.

Stylosanthes fruticosa (Retz.) Alston belongs to the family Fabaceae and is widely distributed in tropical and subtropical regions. The plant has been traditionally used for several medicinal purposes. Previous phytochemical studies have reported the occurrence of alkaloids, flavonoids, tannins, glycosides, and phenolic compounds in the genus *Stylosanthes*. However, scientific information regarding the antioxidant potential and quantitative phytochemical composition of *S. fruticosa* remains limited. Therefore, the present study was undertaken to evaluate the phytochemical constituents, total phenolic and flavonoid contents, and antioxidant activity of different solvent extracts of *S. fruticosa* leaves.

II. RESEARCH METHODOLOGY

2.1 Collection and Identification of Plant Material

Fresh leaves of *S. fruticosa* (Fig.1) were collected from different localities of Amravati district, Maharashtra, India. The plant material was washed thoroughly with distilled water, shade dried at room temperature, and pulverized into fine powder using a mechanical grinder.

Fig. 1. *Stylosanthes fruticosa* (Retz.) Alston

2.2 Preparation of Plant Extracts

Fifty grams of dried powdered material was extracted separately with 250 ml each of ethanol, methanol, acetone, and petroleum ether. The mixtures were macerated for 30 minutes and kept for seven days with intermittent shaking. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator at 40–50 °C. The concentrated extracts were dried and stored at 4 °C until further analysis.

2.3 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of different solvent extracts was carried out using standard procedures described by Harborne, (1998) and Kokate, (1994). The extracts were screened for alkaloids, flavonoids, tannins, phenolics, coumarins, proteins, amino acids, reducing sugars, fixed oils, and saponins using specific chemical tests.

2.4 Estimation of Total Phenolic Content (TPC)

Total phenolic content was determined using the Folin–Ciocalteu method (Singleton *et al*., 1999). Briefly, 10 µl of extract was mixed with Folin reagent and sodium carbonate solution and incubated in the

dark for 30 min. Absorbance was measured at 750 nm using an ELISA reader. Gallic acid was used as standard and results were expressed as mg gallic acid equivalents (GAE)/g extract (Fig 2).

2.5 Estimation of Total Flavonoid Content (TFC)

Total flavonoid content was estimated using the aluminium chloride colorimetric method (Zhishen *et al*., 1999). Quercetin was used as the standard compound (Fig.4) and absorbance was recorded at 510 nm. The flavonoid content was expressed as mg quercetin equivalents (QE)/g extract.

2.6 Determination of Antioxidant Activity (DPPH Assay)

The antioxidant activity of different extracts was evaluated using the DPPH free radical scavenging assay described by Shimada *et al*., (1992). The reaction mixture consisted of 10 µl extract and 190 µl of 0.1 mM DPPH solution. The mixture was incubated at 37 °C for 5 min and absorbance was measured at 517 nm. Percentage inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \left(\frac{\text{Ac} - \text{As}}{\text{Ac}}\right) \times 100$$

where Ac is the absorbance of control and As is the absorbance of sample.

III. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Screening

Qualitative phytochemical analysis revealed the presence of several important secondary metabolites in different solvent extracts of *S. fruticose* (Table-1). Ethanol and methanol extracts showed the richest phytochemical profile with the presence of flavonoids, saponins, tannins, phenolics, coumarins, alkaloids, proteins, amino acids, and reducing sugars. Acetone extract contained flavonoids, phenolics, coumarins, alkaloids, fixed oils, and reducing sugars, whereas petroleum ether extract showed comparatively fewer phytoconstituents. Anthocyanins were absent in all extracts. The findings are in agreement with previous reports by Sandosh *et al*., (2013), Kumanan *et al*., (2014), and Peter, (2012), who reported the occurrence of diverse bioactive constituents in *S. fruticosa* through phytochemical and GC–MS analyses.

Table 1. Qualitative phytochemical screening of different solvent extracts of *Stylosanthes fruticosa*

S. No.	Sample Tested for	Name of Test	(Ethanol)	(Methanol)	(acetone)	(P.E)
1.	Flavonoids	H2So4 test	+	+	+	+
2.	Saponins	Foam Test	+	+	-	-
3.	Tannins	10% NaOH Test	+	+	-	+
4.	Phenolic comp	Lead acetate test	+	+	+	-
5.	Coumarin	NaOH Test	+	+	+	-
6.	Proteins and Amino Acids	Xanthoproteic test	+	+	-	-
7.	Alkaloids	Dragerdorff's test	+	+	+	-
8.	Fixed Oils &Fats	Spot test	+	-	+	+
9.	Reducing sugar	Fehling's test	+	+	+	+
10.	Anthocyanin	HCL test	-	-	-	-

+ Present; - Absent; PE: Petroleum ether

3.2 Total Phenolic Content

Among all solvent extracts, the acetone extract exhibited the highest total phenolic content (0.694 ± 0.032 mg GAE/g extract), whereas ethanol, methanol, and petroleum ether extracts showed negligible phenolic content (Fig.3). The results indicate that acetone is more efficient for extracting phenolic compounds from *S. fruticosa.*

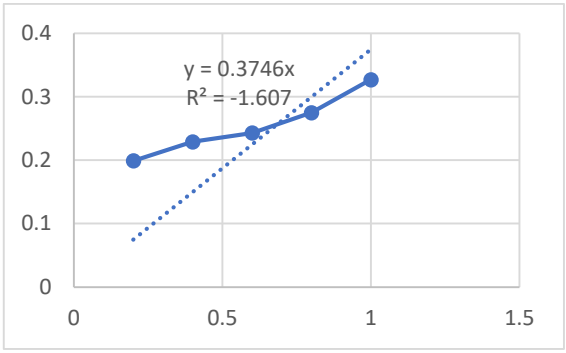

Fig.2. Calibration curve for Gallic acid

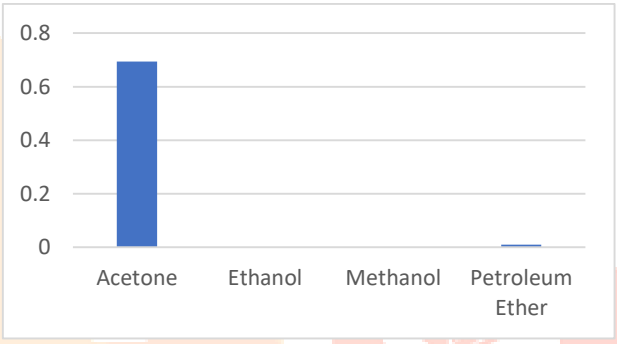

Fig. 3 Quantitative estimation of total phenolic content (mg GAE g^{-1} FW) in *Stylosanthes fruticosa*

3.3 Total Flavonoid Content

The ethanol extract showed the highest flavonoid content (0.700 ± 0.090 mg QE/g extract), followed by methanol extract (0.099 ± 0.016 mg QE/g extract) (Fig.5). No detectable flavonoid content was observed in acetone and petroleum ether extracts. The higher flavonoid content in ethanol extract indicates the suitability of polar solvents for flavonoid extraction.

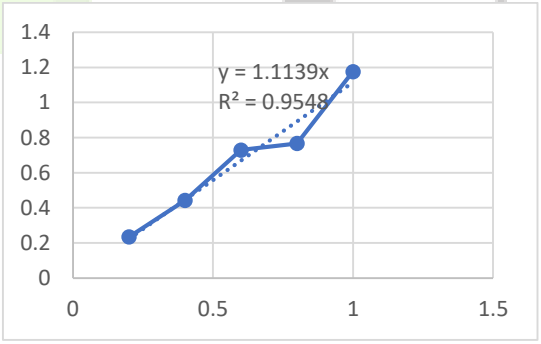

Fig.4. Calibration curve for Quercetin

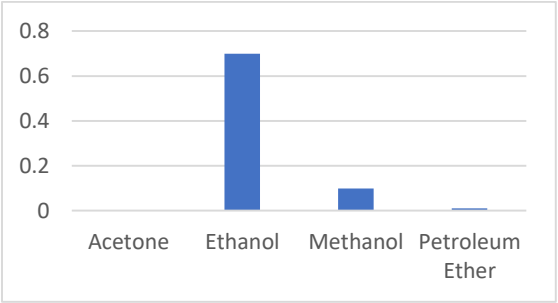

Fig.5. Quantitative estimation of total flavonoids content (mg QE g^{-1}FW) in *Stylosanthes fruticosa*

3.4 Antioxidant Activity

The antioxidant activity of different solvent extracts was evaluated using DPPH assay (Table. 2). Among the extracts tested, the methanolic extract exhibited the highest antioxidant activity (50.052 ± 2.054%), whereas ethanol extract showed moderate activity (18.814 ± 1.177%). Acetone and petroleum ether extracts showed no detectable antioxidant activity. The DPPH scavenging activity of methanolic extract increased with concentration, indicating concentration-dependent antioxidant activity. The regression equation obtained was y = 47.332x + 4.9896 with a regression coefficient of R^2 = 0.9766 and IC_{50} value of 0.9509 mg/ml (Table.3). The antioxidant activity of methanolic extract may be attributed to the synergistic effect of phytochemicals such as flavonoids, alkaloids, tannins, and coumarins. Similar antioxidant and pharmacological properties of *S. fruticosa* have been reported by Vadivel *et al*., (2025) and Bakrey *et al*., (2023). Compared to the methanolic extract, standard Ascorbic acid exhibited higher antioxidant activity with a lower IC_{50} value of 0.052 mg/ml, confirming its strong free radical scavenging ability.

Table. 2. DPPH radical scavenging activity (%) of selected extracts of *Stylosanthes fruticosa*

Sr.No.	Extraction Solvents	% Antioxidant Potential (Mean±SD)
1	Ethanol	18.814 ±1.177
2	Methanol	50.052±2.054
3	Acetone	0.000
4	P.E.	0.000

Table.3. Antioxidant activity of methanolic extract of *Stylosanthes fruticosa* at different concentrations with IC_{50} value and regression analysis.

S. No.	Conc (in mg)	% free radical scavenging activity	Y equation	R^2 value	IC_{50} (in mg)
1	0.2	12.086±1.004	y = 47.332x + 4.9896	R^2 = 0.9766	0.9509
2	0.4	26.332±1.845			
3	0.6	33.411±1.655			
4	0.8	45.064±1.253			
5	1.0	50.052±1.445			

4. Conclusion

The present study demonstrated that *S. fruticosa* possesses significant phytochemical and antioxidant properties. The plant extracts contained important secondary metabolites including flavonoids, tannins, phenolics, alkaloids, coumarins, and saponins. Among all extracts, the methanolic extract demonstrated the strongest antioxidant activity with an IC_{50} value of 0.9509 mg/ml. These findings indicate that *S. fruticosa* may serve as a valuable natural source of antioxidant and therapeutic compounds for future pharmaceutical applications.

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