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AN ANALYSIS OF THE PHYTOCHEMICAL, MORPHO-ANATOMICAL, ANTIBACTERIAL AND ANTIOXIDANT STUDIES OF *Stachytarpheta cayennensis* (Rich.) Vahl

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ABSTRACT

Plants contain numerous secondary metabolites that can be used in the medicinal fields. Current study was undergone to find the phytochemicals and analyse the antibacterial, antioxidant activities in a weed *Stachytarpheta cayennensis*. Alkaloid, terpenoid, tannin, phenol, flavonoid, saponin, glycoside were quantitatively determined. In quantitative analysis saponin was found to be higher and terpenoid were found to be lower in 70% & 90% ethanolic extract. Antioxidant activity were determined by DPPH assay. Scavenging activity was found to be higher in high concentration for 70% & 90% ethanolic extract. Plant extracts were also used against bacterial strains by disc diffusion method. Maximum zone of inhibition were found to be in 100% extract against gram positive bacteria *Enterococcus faecalis* and gram neagative bacteria *Escherichia coli*. Anatomy of the plant was done which helps for the identification of plant.

Keywords: phytochemicals, antibacterial, anti-oxidant, *Stachytarpheta cayennensis*

1. INTRODUCTION

Weeds are plants that grow in undesirable locations. A weed is a plant that grows or reproduces aggressively or is invasive outside of its natural habitat [22]. Despite the fact that some of them are cultivated as useful plants in gardens. Antibiotics are quite important in our daily lives. It has numerous benefits, but it also has numerous drawbacks. Antibiotics can cause skin irritation, allergies, nausea and a variety of other unpleasant side effects.

The current study of *Stachytarpheta cayennensis*, a member of the verbenaceae family, is an attempt to determine its pharmacognostic significance and as a result, to develop alternative medications for various therapeutic treatments. This can also be used to prevent various side effects of antibiotics.The plant is an erect herb or shrub that is native to the United States. It is an invasive species in many other parts of the world .The study of epidermal cells, cuticle, trichome shape, stomatal arrangement and the number of vascular bundles in the root, stem and leaf of *S. cayennensis* aids as a useful tool for resolving taxonomic issues [2].

Several studies have recently been conducted on these plants all over the world to determine the active ingredients that can cure a variety of ailments. The antibacterial, anti-inflammatory, anti-diuretic, anti-viral, anti-cancer, and anti-fungal effects of these plants are mostly due to secondary metabolites. These compounds are known as phytochemicals. Antioxidants react to different types of free radicals and oxidants in different ways [3]. Plant-derived antioxidants that combat free radicals like reactive oxygen (ROS) and nitrogen species (RNS) have gathereda lot of attention in the last decade, which has led to increased efforts to develop plant-derived antioxidants [4,3.5]. Researchers will be able to learn more about *S. cayennensis* ability to resist pathogens by evaluating its antibacterial capabilities against the illnesses *Escherichia coli* and *Enterococcus fecalis.* Literature reports and ethnobotanical records imply that plants are the sleeping giants of the pharmaceutical industry, providing natural medications to cure diseases all over the world [6].

Fig. 1 : *Stachytarpheta cayennensis*

2. MATERIALS AND METHODS

Fresh Material of *Stachytarpheta cayennensis* was collected from Adat in Thrissur district, Kerala. Samples were collected in a polythene bag to retain freshness. Wash thoroughly under running tap water to remove debris.

2.1 ANATOMY OF PLANT

Leaf, stem and root of fresh plant were collected and free hand thin sections were made. Specimens then stained using safranine. Stained sections mounted in glycerine on a clean slide and observe under Labomed Microscope .

2.2STOMATAL INDEX

Epidermal layer of leaf were peel off to obtain the stomata and observe the number of stomata . Analyse three region of the field and calculate the stomatal index. Estimation can be done by using the formula:

Stomatal index (%) = No. of stomata / No. of stomata + No. of epidermal cells × 100

2.3EXTRACTION OF PLANT

Dried parts of whole plant were powdered and 5g powder soaked in 50 ml of 90% and70% ethanol. Filter the extract through whatman No. 1 filter paper and stored in refrigerator for further analysis.

2.4 QUALITATIVE ANALYSIS

Extracts were subjected to qualitative phytochemical analysis for identifying different bioactive constituents according to standard methods [7-12].

2.5 QUANTITATIVE ANALYSIS

2.5.1 TEST FOR ALKALOID

Alkaloid content was estimated by Evans method [13].

2.5.2 TEST FOR TERPENOID

Determination of terpenoid was estimated by Ghorai method [14].

2.5.3TEST FOR TANNIN

Tannin was determined by Folin- Denis method [15].

2.5.4 TEST FOR FLAVONOID

Aluminium chloride method was used for the estimation of flavonoid content [16].

2.5.5 TEST FOR PHENOL

Phenol content of ethanol extract (70% & 90%) was determined using folin ciocalteu method [17].

2.5.6 TEST FOR SAPONIN

Estimation of saponin was made by vanillin sulphuric acid method [18].

2.5.7 TEST FOR GLYCOSIDE

Determination of Glycoside was enabled by the method of Evans , Shah and Seth [19, 20].

2.7 DPPH RADICAL SCAVENGING ASSAY

Antioxidant activity of *S. cayennensis* was determined by DPPH (2,2-Diphenyl-1-picrylhydrazyl) [21]. Add 3ml of extract and 1ml DPPH, incubate for 30 minutes in dark condition. Absorbance was measured by spectrophotometer at 517nm. Ascorbic acid was used as standard.

Percentage of inhibition of DPPH radical scavenging activity =

$$\frac{(\text{OD of control} - \text{OD of sample})}{\text{OD of control}} \times 100$$

2.8 ANTIBACTERIAL DISC DIFFUSION METHOD

Bacterial cultures of gram positive & gram negative bacteria were procured from Sudharma polyclinic. Petriplates were prepared by pouring 15-20ml of Muller - hinton agar & allowed to solidify the media. Plate was inoculated with streak plate method. 6mm diameter disc were prepared using sterile whatmann No.1 filter paper. Each disc was impregnated with 20µl of different solvent extract of plants. The differently concentrate impregnated disc were placed on plate in an even array using sterile forceps. The disc was gently pressed to ensure the contact with the agar surface. Amoxicillin disc was used as positive control and corresponding solvants were used as negative control [22].

2.9 STATISTICAL ANALYSIS

The mean and standard error for all analysis were calculated and reported. The data is the mean of three replicates. To evaluate significant relationship between experimental parameters by correlation and regression analysis, the F and T- tests were used [23].

3. RESULTS AND DISCUSSION

3.1 MACROSCOPIC STUDIES

In morphological studies, plant is a small, erect shrub that grows up to 2.5m tall. The stem is angular, branching, and woody at the base. Leaves were arranged in opposite directions, frequently hairy on both sides, serrated, wrinkled,and pointed at the apex. On the terminal shoot, a long, slender, hairy spike can be seen. The flowers are tiny, sessile, and purple in color and they are placed on a spike. The fruit consisted of two seeded kernels.

3.2 MICROSCOPIC STUDIES

3.2.1 T.S OF LEAF

In anatomical studies, transverse section of leaf shows the presence of a single layered top and bottom epidermis (Fig: 2). It consist of diacytic stomata and trichomes . Below the epidermis, 2-3 layers of cells followed by wide range of parenchymatous ground tissue can seen.On both sides, single-layered mesophyll cells were distributed and differentiated into palisade and spongy parenchyma. Vascular bundles made up of xylem and phloem can be seen in the midrib area. Xylem is located on the inside of the cell and is surrounded by phloem.

3.2.2 T.S OF STEM

Transverse section of stem shows a wavy outline at four angular positions (Fig: 2). On the epidermis, epidermal hairs can be detected. Upper 2-3 layer parenchyma cells and lower 3-4 layer collenchyma cells differentiate in the cortex. Above the vascular bundles, the sclerenchymatous bundle cap can be seen. Secondary xylem is surrounded by 2-3 layers of secondary phloem. Vascular bundles are concentric, with protoxylem pointing to the center and metaxylem pointing to the periphery. Parenchymatous pith can be found in the central region.

3.2.3 T.S OF ROOT

Transverse section of root reveals 2-3 layers of periderm, followed by cortex. The round cylinder of secondary xylem was surrounded by secondary phloem (Fig : 4).

3.3 STOMATAL INDEX

In the estimation of stomatal index numerous diacytic stomata can be found in the epidermis of plant. The stomatal index of the plant is relatively high. Stomatal index data were presented in table no: 1.

3.4 PHYTOCHEMICAL ANALYSIS

Phytochemical study of *S. cayennensis* was carried out in ethanolic extracts with concentrations of 70% and 90%. (Table No: 2). The extract included alkaloids, phenol, tannin, flavonoid, saponin, terpenoid, glycoside, and cardiac glycoside, among other phytochemicals. The study did not include phlobatannin, anthroquinone, quinine, coumarin, or steroids.

The quantitative analysis validated the phytochemicals detected in the preliminary tests. The ethanolic extract of *S. cayennensis* was used to evaluate alkaloid, phenol, terpenoid, saponin, flavonoid, tannin, and glycoside (Table No: 3). In 90 % ethanol extract, the plant has a higher saponin content (376.64 $\pm$ 1.33 mg/ml) and a lower terpenoid content (17.97$\pm$ 0.10 mg/ml). Saponin was abundant in the 70 % ethanolic extract, with 141.58 $\pm$ 0.67mg/ml, but total terpenoid concentration was lower (17.45 $\pm$ 0.06mg/ml). The plant extract contained moderate amounts of alkaloid, tannin, flavonoid, and phenol.

3.5 ANTIOXIDANT ACTIVITY

The 70 % and 90 % ethanolic extracts of *S.cayennensis* were tested for DPPH free radical scavenging activities (Table No:4). Scavenging activity was greater at 100µl plant extract concentration , 50.81 $\pm$ 0.23 mg/ml in 70% ethanolic extract and 66.16 $\pm$ 0.26 mg/ml reported in 90% ethanolic extract In a 20 µl concentration, the free radical scavenging activity was determined to be low in both ethanolic extracts.

3.6 ANTIBACTERIAL ACTIVITY

Table No. 5 shows the antibacterial activity of *S. cayennensis* in the 70% and 90% ethanolic extract. The data showed low antibacterial activity in 65 % plant extract against *Enterococcus faecalis &Escherichia coli* (2.33 $\pm$ 0.33 mm) in 70 % ethanolic extract and (5.33 $\pm$ 0.33) in 90% plant extract. The antibacterial activity and zone of inhibition against the identified pathogen were highest in 100 % plant extract (6.33 $\pm$ 0.33 mm& 5.33$\pm$ 0.33 mm) in 70% plant extract and (7.33 $\pm$ 0.33) in 90% extract concentration.

3.6 STATISTICAL ANALYSIS

A multiple linear regression using concentration as independent variable and DPPH as dependent variable. Multiple R is the correlation coefficient. Multiple R in 70% ethyl alcohol and 90% ethyl alcohol was 0.974 and 0.990 respectively, which indicates fairly strong linear relationship between DPPH and concentration (Table No: 6). The P- value were less than significant level ($P<0.05$), reject null hypothesis. Sample provides strong enough evidence to conclude that two variables means are not equal.

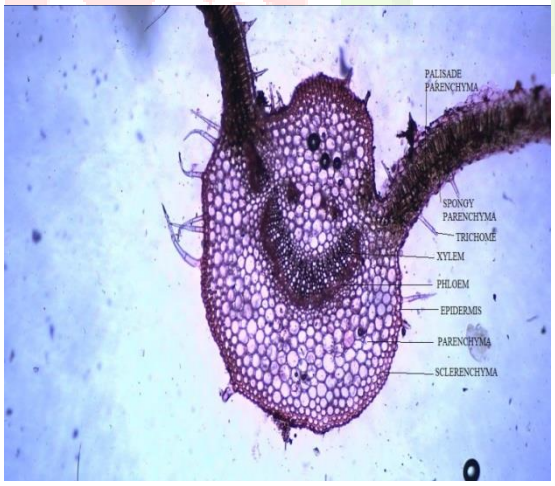

Figure 2: Transverse section of leaf

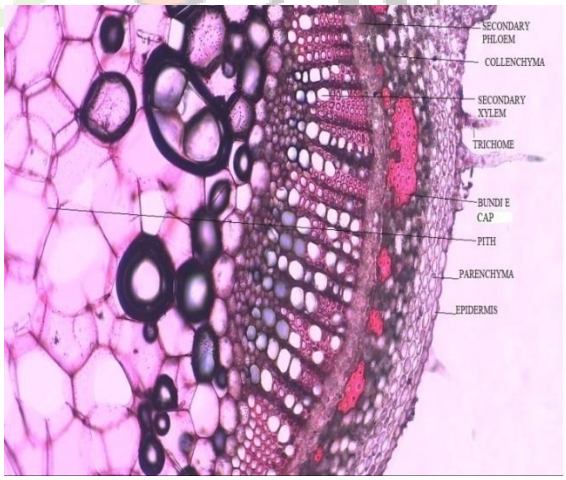

Figure 3: Transverse section of stem

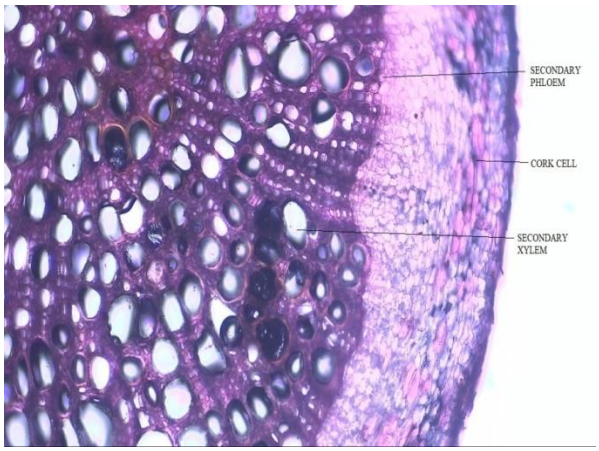

Figure 3: Transverse section of root

Table 1: Estimation of stomatal index of *S.cayennensis*

S. cayennensis	No. of stomata	No. of Epidermal cells	Stomatal Index (%)
Region 1	18	54	25
Region 2	15	50	23.08
Region 3	17	51	25

Table 2: Phytochemical constituents of ethanolic extract of *Stachytarpheta cayennensis*

Sl.No	Constituents	Chemical test	Extracts	
			70% Ethanol	90% Ethanol
1	Alkaloid	Mayer's test	-	-
		Dragendroff's test	++	+
		Hager's test	-	-
		Wagner's test	++	+
		Tannic acid test	-	-
2	Phenol	Ferric chloride test	-	-
		Lead acetate test	++	+
		Gelatin test	+	-
		Potassium dichromate test	-	-
		Alkaline reagent test	-	-
3	Flavonoid	Alkaline reagent test	-	-
		Shinoda test	-	-
		Ethyl acetate – ammonia test	-	-
		Pew's test	+	+
4	Tannin	Ferric chloride test	+	++
		Lead acetate test	-	-
		Potassium dichromate test	-	-
5	Saponin	Frothing test	+++	+
		Sodium bicarbonate test	-	-
6	Terpenoid	Salkowski's test	++	+
		Hesse's test	+	+
7	Glycosides	Keller kiliani test	-	-
		Borntrager's test	-	-

		Modified Borntrager's test	-	-
		Legal test	++	++
8	Steroids	Salkowski's test	-	-
		Libermann – sterol test	-	-
9	Coumarin	Test of Coumarin	-	-
10	Quinine	Test of Quinine	-	-
11	Anthraquinone	Test of Anthraquinone	-	-
12	Cardiac glycoside	Test of Cardiac glycoside	-	+
13	Phloba tannin	Test of Tannin	-	-

Table 3: Quantitative analysis of ethanolic extract of *Stachytarpheta cayennensis*

PHYTOHEMICAL	PLANT EXTRACT	
	70% Ethanol (mg/ml)	90% Ethanol (mg/ml)
Alkaloid	30.20±1.11	55.75±1.11
Tannin	21.74±0.48	24.66±0.73
Phenol	50.45±0.17	88.00±0.17
Flavonoid	22.25±0.17	370.08±0.29
Saponin	141.58±0.67	376.64±1.33
Terpenoid	17.45±0.06	17.97±0.10
Glycoside	40.56±0.42	41.39±0.42

Values are Mean ± Standard error

Table 4: DPPH Free radical scavenging activity of *Stachytarpheta cayennensis*

Sl.No.	Concentration(µl)	DPPH Activity(%)		
		Ascorbic acid (mg/ml)	70% Ethanol (mg/ml)	90% Ethanol (mg/ml)
1	20	13.31±0.23	35.08±0.23	38.70±0.10
2	40	31.85±0.23	41.26±0.13	46.40±0.10
3	60	58.06±0.23	43.01±0.13	56.13±0.10
4	80	74.73±0.13	50.00±0.23	61.20±0.10
5	100	81.32±0.13	50.81±0.23	66.16±0.26
IC50 VALUES		57.93	89.72	49.34

Values are Mean ± Standard error

Table 5: Antimicrobial activity of ethanolic extract of *Stachytarpheta cayennensis*

Zone of inhibition (mm)		
Concentration (µl)	Isolated organism	
	Enterococcus faecalis	*Escherichia coli*
Amoxicillin	2.33 ± 0.33	2.33 ± 0.33
70% Ethanol	2.67 ± 0.33	1.33 ± 0.33
65% Extract	2.33 ± 0.33	2.33 ± 0.33
85% Extract	4.33 ± 0.33	4.33 ± 0.33
100% Extract	6.33 ± 0.33	5.33 ± 0.33
Amoxicillin	4.33± 0.33	4.33± 0.33
90% Ethanol	3.33± 0.33	3.33±0.33
65% Extract	5.33± 0.33	5.33 ±0.33
85% Extract	6.33± 0.33	6.33 ± 0.33
100% Extract	7.33 ± 0.33	7.33 ± 0.33

Values are Mean ± Standard error

Table 6: Studied T-Test and ANOVA for DPPH scavenging and concentration of the extracts of *Stachytarpheta cayennensis*

Parameters	Multiple R	R^2	df	Slope	Y-intercept	t-value	P-value	95% confidence level	
								lower	upper
70% Ethyl alcohol extract & DPPH	0.974	0.948	1	0.201	31.97	7.41	0.005	0.115	0.287
90% Ethyl alcohol extract & DPPH	0.990	0.981	1	0.349	32.80	12.32	0.001	0.259	0.439

Plant is erect, with a four angled green stem, oppositely arranged serrated leaves and little purple coloured sessile flowers grouped on the spike [24]. The findings can also be used to compare species from the same family. Chandler identified plant traits that set them apart [25]. Anatomical characteristics matched those of idu [26]. Trichomes, diacytic stomata, a variety of cell types, and vascular tissues are all common characteristics. These traits help identify plants and act as a taxonomic key.

Phytochemical study reveals a number of beneficial chemicals. Alkaloids, coumarin, terpenoid, phenol, tannin, glycoside and flavonoid are among the compounds. All of these chemicals were discovered at various concentrations. The presence of alkaloid, tannin, saponin, flavonoid, cardiac glycoside and the absence of steroid in the study by Edeoga backed confirmed our findings [27]. However, the absence of terpenoid and the presence of phlobatannin appear to contradict the current study. *S.cayennensis* has been found to contain terpenoid by a number of other researchers. This would allow the plant to be used to defend against a number of illnesses. They were utilized to make medications in response to a variety of ailments such as malaria, cancer, and heart problems. The extraction procedure and solvent utilized determined the different quantities of phytochemicals in plants.

The antioxidant activity of the plant extract contributes to the antiplasmodial activity [28]. The presence of numerous polyphenolics was responsible for the plant's antioxoidant properties. Phenolic and flavanoids were found to be better hydroxyl scavengers in these studies. Polar extracts, such as ethanol are high in phenolic content. Anti-oxidant properties were mostly due to the presence of phenol and flavonoid [29].

The antimicrobial property of the extract is due to the activities of the plant, which are poisonous to bacteria due to enzyme inhibition. Due to the existence of poisonous compounds that can be employed to synthesis natural control products rather than artificial pesticides, these plants have the ability to control the pest [30].

4. CONCLUSION

Weeds are vegetation that thrives in undesirable locations. It expanded quickly and is now present in most locations. The abiotics we take daily could have a number of negative impacts.These issues might be resolved by compounds that have been identified from the plants. Current research showed higher phytochemical constituents in 70% ethanolic extract. Variation in the presence of biochemicals occurs were due to the solubility and polarity of the solvent. Quantitative analysis showed higher phytochemical content in 90% ethanolic solvent. Maximum Zone of inhibition was exhibited in the 90% ethanolic extract for both bacteria. DPPH free radical scavenging activity was also found to be more in 90% extract.

Work shows the antibacterial, antioxidant properties that enable the plant to treat several diseases. Plants have antioxidant properties. Bioactive compounds in the plant can be used to produce several drugs in the Pharmaceutical industries. This might become a solution to prevent the side effects of antibiotics. Plant contain several polyphenols like flavonoids, phenols which responsible for the antioxidant properties . Antioxidant activities of the plant contribute to the antiplasmodial activities.

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