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AN EVALUATION OF THE MORPHO-ANATOMICAL, PHYTOCHEMICAL, ANTIOXIDANT ACTIVITY AND ANTIBACTERIAL EFFICACY OF TRIUMFETTA RHOMBOIDEA JACQ.

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ABSTRACT

The purpose of the study was to investigate the phytochemical, antioxidant and antibacterial properties in 70% and 90% ethanolic extract of *Triumfetta rhomboidea* (EETR). The preliminary screening revealed the presence of alkaloid, phenol, terpenoid, tannin, saponin, steroid, coumarin, flavonoid and glycoside in both the extracts. The total phytochemical content was compared individually to corresponding standard phytochemicals. The highest phytochemical content was shown in 90% ethanolic extract. The EETR showed moderate activity against DPPH (2, 2'-diphenyl-1-picrylhydrazyl). The antibacterial assay were also performed.

Key words: Antioxidant activity, antibacterial assay, *Psuedomonas aeruginosa*, *Enterococcus fecalis*

1.INTRODUCTION

Angiosperms are the most diverse group of land plants, with over 300,000 species of seed-bearing flowering plants [20]. There are roughly 100 species in the genus *Triumfetta* (Tiliaceae family). Members of the genus can be found in open wastelands and along road sides throughout the world's tropical and subtropical areas [21, 12]. It is represented by 5 species in the Flora of the Madras Presidency. The genus is known for its bur-like fruits and belongs to the tiliaceae family's Grewieae tribe [19]. Antioxidants are chemical compounds that help the physiological system lessen or prevent oxidative stress. Plants are a good source of antioxidants that are produced naturally [22]. *Triumfetta rhomboidea* Jacq is an antioxidant-rich medicinal plant. The herb has been utilised in traditional medicine since ancient times [28]. Plants include a huge number of chemical elements that can be used to make medications. Plant biochemical agents include alkaloids, tannins, flavonoids and phenolic chemicals. Plant phytochemical screening is required in order to discover and develop novel medicinal agents with improved efficacy [30].

Plants have been used for therapeutic purposes since prehistoric times. Secondary metabolites are responsible for the plant's medicinal effect. Secondary metabolites are significant plant ingredients that can be employed for a variety of therapeutic purposes [16]. Alkaloids are secondary metabolites that contain basic nitrogen atoms and are found in plants. By scavenging free radicals, alkaloids can prevent numerous degenerative diseases before they start. Alkaloids derived from plant extracts have been utilised for a variety of therapeutic applications, including snake bite treatment, fever treatment, insanity treatment and liquid medication. Alkaloids are known for their medicinal properties, yet they are also exceedingly poisonous. They have allelopathically active compounds that work against microorganisms, herbivores, and insects, as well as other plants [24]. Tannins are phenolic molecules, which are another significant secondary metabolite. These high-molecular-weight chemicals are present in plant leaves, bark, stems, fruits, wood, roots and other places and are mostly involved in plant defence mechanisms. These are water soluble and can create tannin-protein complexes by binding with proteins [14]. Glycosides are plant-derived chemicals that contain one or more sugars as well as alcohol, phenol, or a complex structure [15]. Other secondary metabolites

include flavonoid, coumarin and others. The current study investigated the morpho anatomical, phytochemical, antioxidant and antibacterial efficacy of *Triumfetta rhomboidea.*

2.MATERIALS AND METHODS

The plant material was collected from Kannadi in Palakkad district. A mature plant with leaves and root were collected. The plant material was identified with the help of flora of presidency of Madras by [10]. The whole plant of *Triumfetta rhomboidea* was washed thoroughly in running tap water. Then it was allowed for shade drying. The dried material was powdered using a blender. The powdered material was stored in a clean and air tight container.

2.1.STOMATAL INDEX [23]

Triumfetta rhomboidea mature leaves were peeled off, stained with saffranin and examined under a microscope. The formula was used to calculate the number of stomata and epidermal cells.

Stomatal index (%) = (S/S+E) X 100

S – Number of stomata E – Number of epidermal cell

2.2.EXTRACTION OF PLANT

The powdered sample was used for the preparation of plant extract. 5 g powdered sample was extracted with 70% and 90% ethanol. The sample was kept in a conical flask and covered with aluminium foil for one week. The extract was filtered by using Whatman No.1 filter paper and stored it in the refrigerator.

2.3.QUANLITATIVE ANALYSIS

The extract was subjected to phytochemical screening for the detection of various phytochemical constituents using standard methods. [6, 7, 13, 17, 18, 26]

2.4.QUANTITATIVE ANALYSIS

2.4.1.TEST FOR ALKALOID

The total alkaloid content was determined by Evans method [8].

2.4.2.TEST FOR PHENOL

The total phenolic content of 70% and 90% of ethanolic extract of *Triumfetta rhomboidea* Jacq was analysed by Folin-Ciocalteu method [27].

2.4.3.TEST FOR SAPONIN

Saponin content of plant extracts was determined by vanillin-sulphuric acid method [2].

2.4.4TEST FOR FLAVONOID

The total flavonoid content was estimated by aluminium chloride method [5].

2.4.5. TEST FOR TERPENOID

Total terpenoid content present in the extract was analyzed by Ghorai's method [11].

2.4.6. TEST FOR TANNIN

The total tannin content present in the extract was determined by Folin-Denis method [9].

2.4.7. TEST FOR GLYCOSIDES

Total glycoside was determined using Baljet reagent. [8, 4]

2.5.ANTIBACTERIAL ASSAY

2.5.1.COLLECTION OF TEST BACTERIAL CULTURES

Two different bacterial cultures of gram positive and gram negative bacteria were procured from, Sudharma polycylinic, Thrissur.

Gram negative bacteria – *Psuedomonas aeruginosa*

Gram positive bacteria - *Enterococcus fecalis*

2.5.2.ANTIBACTERIAL DISC DIFFUSION METHOD

6mm diameter disc was prepared using sterile Whatmann No.1 filterpaper. Plant extract (2g/20ml) prepared using solvents (70% ethanol and 90% ethanol). Each disc impregnated with 20µl of different solvent extracts of plants. Amoxicillin disc was used as positive control and corresponding solvents used as negative control. Antibacterial activity of plant extract was determined by disc diffusion method. Petri dishes was prepared by pouring 15 – 20 ml of Mueller-Hinton agar and allowed to solidify the media. Plate was inoculated with streak plate method. After that we place our impregnated disc, on plate in an even array using sterile forceps. Then we gently pressed the disc to ensure contact with the agar surface. Amoxicillin disc used as positive control and solvent impregnated disc considered as negative control and both are placed equally spaced on upper side of petriplate. Plates are incubated at 35ºC for 18-24 hours. After incubation plates we examined for measuring zone of inhibition. Zone of inhibition measured in millimetres. [25]

2.6.DPPH RADICAL SCAVENGING ASSAY

The antioxidant study of *Triumfetta rhomboidea*, estimated by using DPPH (2,2-Diphenyl-1-picrylhydrazyl) by spectrophotometer [3].

2.7.STATISTICAL ANALYSIS

The mean and standard error for each analysis were calculated and reported. The average of three replicates was used to arrive at the results. Using correlation and regression analysis, the F and T tests were used to see if there was a significant association between experimental parameters. The statistical and graphical analyses were carried out using Microsoft Excel 2019 and the free R program version 2.15.1. (http://www.r-project.org/)

3. RESULTS AND DISCUSSION

3.1.MACROSCOPIC STUDIES

Triumfetta rhomboidea is a 150-cm-tall annual woody shrub. Hairy, erect stem. Simple, alternating, rhomboid to ovate, pubescent leaves of various diameters, palmately 3-lobed basal leaves. The flowers are small, yellow, grouped and opposite the leaves. Pentamerous flowers are those that have five petals. Sepals five, ten stamens, ovary five celled,cells two ovuled, style filiform, stigma 2-5 lobed, Fruits have hooked spines and are indehiscent, so they attach to animals' fur, clothing and hair for dissemination.

3.2. MICROSCOPIC STUDIES

3.2.1. T.S OF STEM

The epidermis, cortex and stele of a typical *Triumfetta rhomboidea* Jacq . The epidermis is composed of a single layer that is tightly packed. There are a lot of uniseriate epidermal hairs. After the epidermis, there are 2-3 layers of chlorenchyma and collenchyma. Patches of pericycle can be found around the vascular bundles. 6-20 sclerenchymatous cells are seen in each patch. Cambium is present and secondary growth is possible. Xylem parenchyma surrounds the xylem. There is phloem present. At the center, there is a conspicuous pith. Starch deposition can be found in cells.

3.2.2. T.S OF ROOT

The cork, cortex, and stele of a typical root of *Triumfetta rhomboidea* Jacq). Cork is made up of 2-3 layers, with 3-4 layers of parenchymatous cells between them. Vascular bundles follow the cortex. Starch deposition can be found in all cells. There are medullary rays present. Pith is missing.

3.2.3. MACERATION

Maceration showed the presence of xylem vessels, different kinds of thickening and xylem fibers.

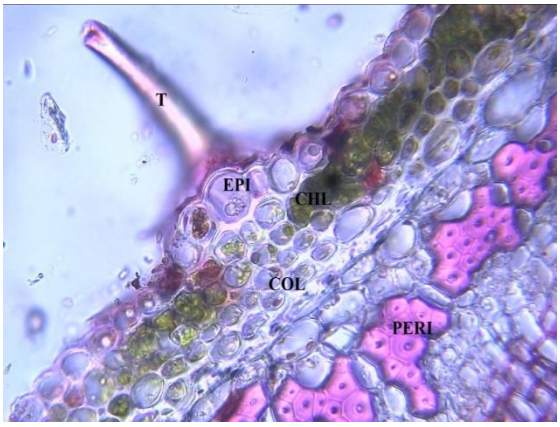

Fig.1(a)

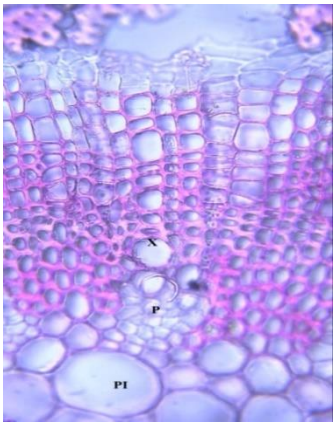

Fig.1(b)

Figure 1(a, b): Transverse section of stem at a magnitude of 40X

Abbreviations: T – Trichome, EPI – Epidermis, CHL – Chlorenchyma, COL – Collenchyma, PERI – Pericycle, X – Xylem, P – Phloem, PI – Pith

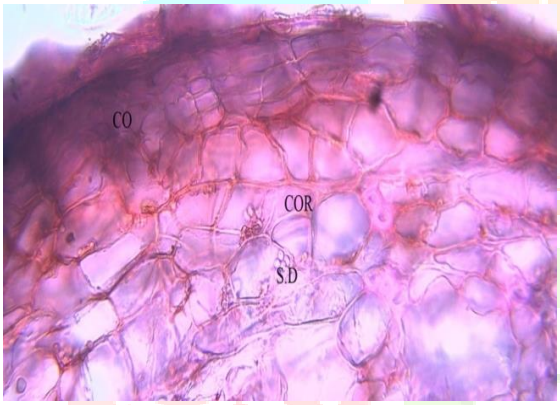

Fig.2(a)

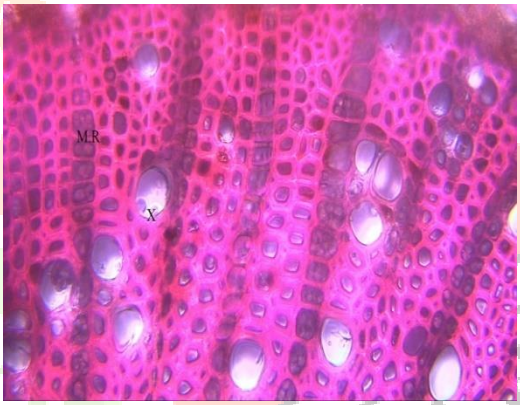

Fig.2(b)

Figure 2(a, b): Transverse section of root at a magnification of 40X

Abbreviations: CO – Cork, COR – Cortex, X – Xylem, M.R – Medullary ray, S.D – Starch deposition

Fig.3(a)

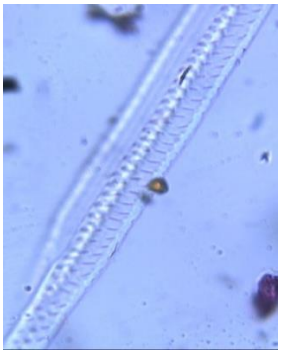

Fig.3(b)

Fig.3(c)

Figure 3: Maceration of stem; (a): Vessel element, (b): Xylem vessel, (c): Xylem tracheids

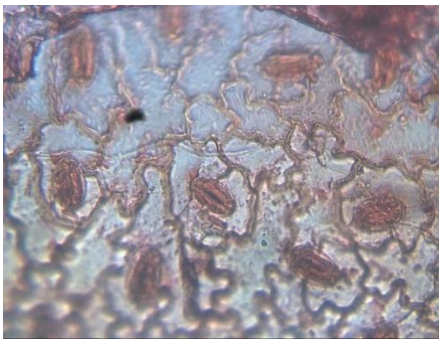

Fig.4(a)

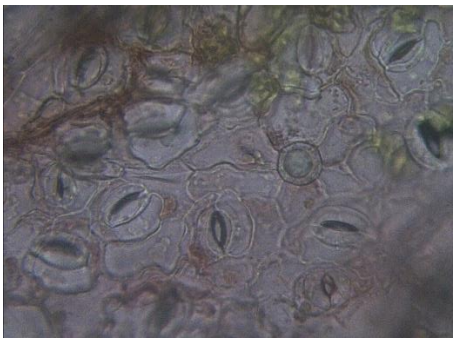

Fig.4(b)

Figure 4: Stomata of young leaves of *Triumfetta rhomboidea*; (a): Diacytic stomata, (b): Paracytic stomata

3.3. STOMATAL INDEX

Both diacytic and parasitic stomata can be seen on a juvenile leaf of *Triumfetta rhomboidea*

The stomatal index of *Triumfetta rhomboidea* juvenile leaves was 27.60 %.

3.4. PHYTOCHEMICAL ANALYSIS

The extract of *Triumfetta rhomboidea* Jacq was subjected to a qualitative phytochemical study, and the extract included numerous phytochemical elements, as shown in Table 2. Standardization of pharmaceuticals and refashioning the traditional medical system are critical in the expanding globe and the outbreak of new diseases. The identification and differentiation of plants from other species is aided by taxonomic, anatomical, phytochemical, and genetic investigations. The qualitative phytochemical examination of plants in various extracts reveals the presence of several phytochemicals [1]. In the 70 % and 90 % ethanolic extracts, phytochemical screening revealed the presence of alkaloid, flavonoid, phenol, tannin, terpenoid, saponin, glycoside, steroid and coumarin. Also lacking from both extracts are chemicals such as quinine, anthraquinone, cardiac glycosides, and phlobatannin.

The quantitative estimation of secondary metabolites in ethanolic extract was also a focus of this work. The study uses seven secondary metabolites found in plants, including alkaloid, phenol, tannin, terpenoid, flavonoid, saponin and glycosides. Phytochemicals such as alkaloid, tannin, phenol, flavonoid, saponin, terpenoid and glycoside were quantified using a spectrophotometric method. The total phytochemical content was higher in 90% ethanolic extract (298.67±1.67, 163.64±0.28, 599.82±0.24, 715.06±0.29, 3665.93±1.33, 57.20±0.16, 23.78±0.14 mg/ml) and lower in 70% ethanolic extract (15.33±1.67, 95.86±0.28, 524.86±0.16, 144.33±0.29, 1669.27±1.76, 44.82±0.10, 20.53±0.08 mg/ml).

3.5. ANTIOXIDANT ACTIVITY

The DPPH free radical scavenging activity of an ethanolic extract of *Triumfetta rhomboidea* was assessed in this work. Minerals, ions, nutrients, primary metabolites, and other substances are abundant in plants. Plants also contain a variety of secondary metabolites with antioxidant and medicinal activities. Plants need reactive oxygen species (ROS) because they participate in the metabolic process and provide tolerance to diverse stressors. When reactive oxygen species (ROS) oxidise, they damage cells and potentially induce mutations. The oxidation of ROS can cause a variety of disorders, including cancer, cardiovascular disease, liver damage, and so on. Antioxidants, by their free radical scavenging activity, were able to resist and defend against the damage produced by ROS [29].

The antioxidant activity was determined by DPPH method. The maximum DPPH activity in *Triumetta rhomboidea* was found in 90 % ethanolic extract at a concentration of 100 µg /ml (50.35 $\pm$0.37) and less in a 70 % ethanolic extract at a concentration of 20 µg /ml (37.23$\pm$ 0.13).

3.6. ANTIBACTERIAL ASSAY

In this work, the antibacterial activity of an ethanolic extract of *Triumfetta rhomboidea* was investigated against *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The minimum zone of inhibition was found in 65% extract *Enterococcus faecalis* (4.33 $\pm$ 0.33 µg/ml) and *Pseudomonas aeruginosa* in 85% extract (2.33 $\pm$ 0.33 µg/ml) in both 70 and 90% EETR.

3.7. STATISTICAL ANALYSIS

In *Triumfetta rhomboidea*, a linear relationship between solvent concentration and DPPH inhibition was discovered, with correlation coefficient values of 0.982 and 0.928 for 90% and 70 % ethyl alcohol, respectively. The beta coefficients for 90% and 70% ethyl alcohol were 0.043 and 0.11, respectively, which were statistically significant at the 0.05 level. As a result, the null hypothesis that each of the solvent concentrations contributes significantly to the DPPH predictor is accepted. As a result, two solvents emerged as the most powerful predictors of DPPH.

Table 1. Stomatal index of young leaf of *Triumfetta rhomboidea* Jacq

Sl.No	No. of stomata	No.of Epidermal cells	S/(S+E)	Stomatal Index (%)
1	15	40	0.2727	27.27
2	16	38	0.2962	29.62
3	14	40	0.2592	25.92
	Mean	27.60		

S - No. of stomata

N – No. of epidermal cells

Table 2. Qualitative phytochemical analysis of *Triumfetta rhomboidea* Jacq

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 for coumarin	++	+++
10	Quinine	Test for quinine	-	-
11	Anthraquinone	Test for anthraquinone	-	-
12	Cardiac glycosides	Test for cardiac glycosides	-	-
13	Phloba tannin	Test for phloba tannin	-	-

(-) Absent, (+) Present, (++) Moderate, (+++) Abundant

Table 3. Quantitative phytochemical analysis of 70% and 90% ethanolic extracts of *Triumfetta rhomboidea* Jacq

PHYTOCHEMICAL	PLANT EXTRACT	
	70% Ethanol (mg/ml)	90% Ethanol (mg/ml)
Alkaloid	15.33±1.67	298.67±1.67
Tannin	95.86±0.28	163.64±0.28
Phenol	524.86±0.16	599.82±0.24
Flavonoid	144.33±0.29	715.06±0.29
Saponin	1669.27±1.76	3665.93±1.33
Terpenoid	44.82±0.10	57.20±0.16
Glycoside	20.53±0.08	23.78±0.14

Table 4. DPPH free radical scavenging activity of 70% and 90% ethanolic extracts of *Triumfetta rhomboidea* Jacq

Sl.No.	Concentration(µg/ml)	DPPH Activity (%)		
		Ascorbic acid (mg/ml)	70% Ethanol (mg/ml)	90% Ethanol (mg/ml)
1	20	15.19±0.13	37.23±0.13	46.71±0.20
2	40	34.01±0.36	38.98±0.13	47.62±0.10
3	60	58.87±0.23	42.88±0.36	48.02±0.18
4	80	76.48±0.27	44.49±0.36	48.94±0.18
5	100	81.99±0.13	45.56±0.23	50.35±0.37
IC50 VALUES		56.24	133.67	98.72

Table 5. Antibacterial assay of 70% and 90% ethanolic extracts of *Triumfetta rhomboidea* Jacq

Antibacterial activity (Zone of inhibition) (mm)		
Concentration(µg/ml)	Organism	
	Enterococcus faecalis	*Pseudomonas aeruginosa*
Amoxicillin	3.33 ± 0.33	3.33 ± 0.33
70% Ethanol	2.33 ± 0.33	2.33 ± 0.33
65% extract	4.33 ± 0.33	3.33 ± 0.33
85% extract	5.33 ± 0.33	2.33 ± 0.33
100 %extract	6.33 ± 0.33	4.33 ± 0.33
Amoxicillin	3.33 ± 0.33	3.33 ± 0.33
90% Ethanol	2.33 ± 0.33	2.33 ± 0.33
65% extract	4.33 ± 0.33	3.33 ± 0.33
85% extract	5.33 ± 0.33	2.33 ± 0.33
100% extract	6.33 ± 0.33	4.33 ± 0.33

Table. 6 Student T-test and Anova for DPPH scavenging and concentration of the extracts of *Triumfetta rhomboidea*

Parameters	Multiple R	R-square	df	Slope	Y-intercept	t-value	P-value	95% confidence level	
								lower	Upper
90% ethylalcohol & DPPH	0.982	0.963	1	0.043	45.75	8.88	0.003	0.028	0.058
70% ethylalcohol & DPPH	0.978	0.957	1	0.111	35.18	8.15	0.004	0.068	0.154

One way anova (concentration of extracts and DPPH variations significant) ($P < 0.05$)

4.CONCLUSION

Triumfetta rhomboidea Jacq, a member of the tiliaceae family, was examined in the current study for its morphology, anatomy, qualitative and quantitative analysis, antioxidant capacity and antibacterial activity. The plant was extracted by using ethanol as solvent. As solvents, ethanol at 70 % and 90 % concentrations were both employed. The phytochemical screening of the plant suggest that the plant contain phytochemicals like alkaloids, phenols, flavonoids, tannins, terpenoids, saponins, steroids, glycosides and coumarins which are responsible for antioxidant and antibacterial activity. These phytochemicals were present in the 70% and 90% ethanolic extracts of *Triumfetta rhomboidea* Jacq. The quantity of phytochemicals present in both the extract were estimated using standard methods.

In the current investigation, it was discovered that the plant *Triumfetta rhomboidea* Jacq pocess good antibacterial efficiency against *Enterococcus faecalis* and *Pseudomonas aeruginosa*. The plant has antioxidant activity. Numerous minerals, ions, phytochemicals, and other components are abundant in plants. Due to environmental effect and evolution, variations among the presence and quantity of phytochemicals will occur in all plants. Evolution of new species or variants may also happen. Therefore, there is always space in plant science for investigations of phytochemicals.

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