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Original Article

Bioactive Compounds and Biological Activities of *Jacquemontia Pentanthos* (Jacq.) G. Don.: A Phytochemical and Pharmacological Study

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ABSTRACT

Jacquemontia pentanthos (Jacq.) G. Don., also referred to as Sky-blue Cluster vine, comprises a flowering vine that belongs to the Convolvulaceae family. This research focused on the investigation of phytochemical constituents, antioxidant and antibacterial efficacy of the plant parts of *J. pentanthos* (Jacq.) G. Don. different parts like leaves, stem, and flowers. This research mainly focuses on the investigation of qualitative & quantitative phytochemical screening, antioxidant and anti-bacterial activity of different plant parts. Plant extract was prepared in three solvent like methanol, hexane and distilled water by cold extraction method. From the qualitative phytochemical screening, different extract shows the presence kind of secondary metabolites like alkaloids, flavonoids, phenols, tannins, and phytosterols, and primary metabolites like protein and amino acids. The quantitative assays have been used to identify the total content of phenols, flavonoids, and tannins by using the spectrophotometric method. The antioxidant activity has been determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate assay (PMA), which revealed statistical data regarding the plant's capacity to scavenge free radicals and reduce oxidative stress. Antibacterial activity was determined against two different bacterial strains, including Gram +ve bacteria (*Staphylococcus aureus*) and Gram -ve bacteria (*Escherichia coli*), by performing the well diffusion method. The various phytochemical constituents show during the analysis of different plant parts and extraction solution. The highest flavonoid content was found in hexane extract of leaves, with value of 7.265 ± 0.0036. The highest phenolic content was found in methanol extract of flowers, with value of 6.302 ± 0. While the highest tannin content was found in methanol extract of leaves, with value of 2.348 ± 0.042. Hexane flower extract shows higher antioxidant activity with lowest IC50 value of 14.06 in DPPH assay, while hexane stem extract shows less antioxidant activity with highest IC50 value of 88.39 among all the extracts. In PMA antioxidant activity distilled water extracts of flower shows % inhibition of free radicals, with value of 86.06%. The leaf extracts demonstrate high antibacterial potential then the stem. The largest zone of inhibition was seen in fresh leaf methanolic extract 23mm. D.W. extracts not evaluate any zone of inhibition.

Keywords: *Jacquemontia pentanthos* (Jacq.) G. Don., DPPH, TPC, TFC, TTC, PMA, phytochemicals, antioxidant activity, antibacterial activity

INTRODUCTION

Jacquemontia pentanthos (Jacq.) G. Don is a plant of the Convolvulaceae family, which consists of 55 genera and 1650 species found in tropical and temperate locations worldwide. J. pentanthos (Jacq.) G. Don. is a popular decorative plant that grows in tropical and subtropical regions of India (Sultan and Rahman, 2016). J. pentanthos (Jacq.) G. Don. aerial parts were grown in Egypt for the first time, including

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

potential as a natural resource for therapeutic purposes (Eskander et al., 2019). The genus is known for its complexity at the species level, as some species intergrade and can be challenging to identify. There is limited knowledge on their biology and ecology, although it is assumed that they are pollinated by bees or similar insects. The majority of species are modest, twining or trailing, and have beautiful blue blooms. Although the genus has limited economic or ecological significance, some species, such as J. pentanthos (Jacq.) G. Don and J. tamnifolia (L.) griseb, are planted for ornamental purposes (Robertson, 1971).

Fig 1: Jacqueontia pentanthos (Jacq.) G. Don.

Phytochemicals are components of plants possessing protective or disease-preventing features. The study of natural phytocompounds is known as phytochemistry. Many of the phytochemicals are used to treat various human disorders (Gungor and Sengul, 2008; Roy and Datta, 2019). Phytochemicals accumulate in several plant components, including bark, roots, stems, leaves, flowers, fruits, and seeds. Phytochemical are classified into two categories based on their role in plant metabolism: 1.) Primary metabolites, 2.) Secondary metabolites (Yadav and Agarwala, 2011; Rana et al., 2023). Phytocompounds provides defense against an extensive variety of noncommunicable diseases, including diabetes, cardiovascular and neurological diseases, cancer, and many more. Inadequate nutritional intake of fruits, vegetables, spices, and other foods accounts for two-thirds of the fatalities globally (Lim et al., 2010). Antioxidants are substances that prevent and stabilize free radical damage by delivering antioxidants to cells (Hamid et al., 2010). Antioxidants eliminate harmful free radicals and byproducts from the body. Consumption of antioxidant-rich fruits and vegetables has been linked to a lower risk of some free radical-related disorders (Rahman et al., 2015). The antioxidant activity of medicinal plants is extensively understood and utilized for a variety of health advantages, including as lowering blood pressure, preventing cardiovascular disease, and lowering cancer risk. Medicinal plants consist of antimicrobial compounds that suppress the growth of bacteria, fungus, viruses, and protozoa through various processes. These compounds have therapeutic potential for treating resistant strains (Patel & Modi, 2024). The research presented here examines the amount of different secondary metabolites, their antioxidant and antibacterial activity. Qualitative and quantitative phytochemical analysis indicates the presence and total amount of phenol, flavonoids, tannins, and alkaloids in the vast majority of leaves.

MATERIALS AND METHODS

2.1 Plant collection & Sample preparation:

The plant Jacquemontia pentanthos (Jacq.) G. Don. was collected in January 2025 from the campus area of Gujarat University, Ahmedabad, Gujarat. Mature and healthy plant parts like leaves, stem, and flowers were collected for experiment work. The plant material was sun dried. Finely processed powder of plant parts with the help of blender.

2.2 Preparation of Extracts:

Different plant extracts were prepared by the cold extraction method. 10g of plant powder was mixed with 100 mL of solvent and put in shaker for 24 hrs. Methanol, hexane, and distilled water selected as a solvent. After that filter the solution and air dried the filtrate in petri plates. Collect 30 mg of the dried filtrate from plate and dissolved in 30 mL of appropriate solvent.

2.3 Chemicals:

Plant extracts, Folin-Ciocalteau reagent, Folin-Ciocalteau Phenol reagent, sodium hydroxide, aluminum chloride, sodium carbonate, sodium nitrite, sodium phosphate, ammonium molybdate, DPPH powder, Agar powder, nutrient broth, distilled water.

2.4 Qualitative analysis: (Shaikh and Patil, 2020)

Table 1: Qualitative tests for phytochemical screening

No.	Name of test	Procedure	Result
Test for Alkaloids			
1.	Dragendroff's test	Few mL filtrate + 1-2 drops of Dragendroff's reagent	Reddish-brown ppt
2.	Mayer's test	Few mL filtrate + 1-2 drops of Mayer's reagent	Yellow ppt
Test for Flavonoids			
1.	Lead acetate test	1 mL extract + few drops 10% lead acetate solution	Yellow ppt
2.	Conc. H_2SO_4 test	1 mL extract + conc. H_2SO_4	An orange color
Test for Phenols			
1.	Iodine test	1 mL extract + few drops of dil. Iodine solution	Transient red color
2.	Lead acetate test	1 ml extract + 5 ml D.W. + 5 ml 10% lead acetate solution	White ppt
Test for Tannins			
1.	Braymer's test	1 mL extract + 3 mL D.W. + 3 drops of 10% ferric chloride solution	Blue green color
2.	10% NaOH test	0.4 mL extract + 4mL 10% NaOH + shake well	Formation of emulsion
Test for Phytosterol			
1.	Salkowski's test	Extract + few drops of H_2SO_4	Red color at base
2.	Hesse's response	5 mL aq. Extract + 2 mL chloroform + 2 mL conc. H_2SO_4	Red/ Pink color at base
Test for Proteins and Amino acids			
1.	Millon's test	2 ml filtrate + few drops of Millon's reagent	White ppt
2.	Xanthoproteic test	Extract + few drops of conc. Nitric acid	Yellow color sol.

2.5 Quantitative Analysis:

Secondary metabolites are measured using several spectrophotometric quantitative analyses.

2.5.1 Total Phenol Content (TPC):

According to Malick and Singh (1990), total phenolic content was measured by the Folin-Ciocalteau method. Take different fractions (0.2-1.0 mL) of standard/plant extract into test tubes (in triplets). Make the total volume 3 mL with water. Add 0.5 mL of Folin-ciocalteau reagent in all the test tubes. Give rest for 3 minutes and add 2 mL of 20% Na2CO3 solution in every test tube. Mix thoroughly; place the test tubes in boiling water for 1 minute only, cool the tubes at room temperature, and measure the absorbance at 650 nm in a UV spectrophotometer. Prepare the standard curve of gallic acid results and find the total % of phenol in a plant part with the help of the calibration curve.

2.5.2 Total Tannin Content (TTC):

As specified by Lahare et al. (2021), the Folin-Ciocalteau phenol method was employed for the quantification of total tannin content of the plant. Take different amounts of plant extract/standard (0.2-1.0 mL) in triplet series of test tubes. Add 7.5 mL distilled water in all the test tubes. After adding distilled water, 0.5 mL of Folin Ciocalteau phenol reagent was added to the mixture. Now, add 1 mL of 35% Na2Co3 and make the total volume 10 mL by adding distilled water. The mixture was mixed

thoroughly and left at room temperature for 30 minutes. After 30 minutes, measure the absorbance at 725 nm in a spectrophotometer. Tannic acid (1 mg/mL) was used as a standard solution. Calculate the total tannin content of the plant part using the equation of the standard curve of tannic acid.

2.5.3 Total Flavonoid Content (TFC):

The total flavonoid content was determined using the aluminum chloride colorimetric method with some modifications. Take different amounts of plant extracts/standard (0.2-1.0 mL) in triplet series of test tubes. Then, 0.3 mL of 5% sodium nitrite was added. After 5 minutes, 0.3 mL of 10% aluminum chloride was added. After rest of 6 minutes, add 2 mL of 1 M sodium hydroxide to the mixture. Then, add 3.3 mL of distilled water for dilute the mixture and mix thoroughly. The absorbance was measured at 510 nm in a spectrophotometer. The calibration curve of quercetin used as a standard used to determine total flavonoid content (Zhishen et al., 1999; Fattahi et al., 2014).

2.6 Antioxidant Assay:

Antioxidants are chemically stable substances that transfer electrons to neutralize free radicals, decreasing their harmful potential (Tsao et al., 2004). Antioxidants serve multiple functions, including hydrogen donation, radical scavenging, peroxide decomposition, singlet oxygen quenching, synergy, enzyme inhibition, and metal chelation (Pattni et al., 2023). This study investigated the antioxidant properties of Jacquemontia pentanthos (Jacq.) G. Don plant using various methodologies.

2.6.1 DPPH (2,2-diphenyl-1-picrylhydrazyl) Assay:

According to (germane et al., 2002; Pattni et al., 2023), the antioxidant capacity (estimated as free radical scavenging activity- %RSA) was determined using a stable free radical DPPH, with slight alterations to the method. The standard and plant extract series were produced in triplicate, with 1 mL of extract in each test tube at concentrations ranging from 20-100 µL/mL. Ascorbic acid is utilized as a standard. According to its light sensitivity, the DPPH solution was newly produced by dissolving 4 mg DPPH powder into 100 mL methanol (DPPH is water-insoluble) and maintained in a dark environment. A 3 mL DPPH solution was mixed with a series of extracts and incubated for 20-30 minutes. Post incubation, the purple mixture obtained by the addition of DPPH will be pale yellowish. That color transition clearly indicates the extract's capability to scavenge free radicals. The absorbance of incubated extracts was recorded at 517 nm with a spectrophotometer. The radical scavenging activity was calculated by recording absorbance and applying the equation below.

%RSA = (Control – Sample)/ (Control) * 100

Where, Control is the absorbance of the 3 mL DPPH solution and 1 mL methanol, without extract and Sample is the absorbance of the DPPH solution with plant extract.

2.6.2 Phosphomolybdate Assay (PMA):

The methodology described by (Prieto et al., 1999; Mankad et al., 2021), with a few changes. In the present study, 0.2 mL of plant extract (1mg/mL) and standard ascorbic acid solution (0.2-1 mL) were combined with phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4mM ammonium molybdate). The reaction solution was subsequently incubated in a water bath for 60 minutes at 90˚C. After cooling to room temperature, absorbance was determined at 695 nm. The antioxidant capacity was expressed as mg of ascorbic acid equivalents (AAE) /gram of extract.

2.7 Antibacterial activity:

Bacterial culture of Staphylococcus aureus and Escherichia coli was obtained from department of Microbiology and Biotechnology, Gujarat University. To evaluate the antibacterial activity of specific elements from plant J. pentanthos (Jacq.) G. Don. By using Agar well diffusion methods. To start with, dissolve 1.3g nutritional broth in 100mL of distilled water in a conical flask. The agar medium was prepared with 4.8g agar powder mixed with 260mL of distilled water. Following that, both the medium and petri plates were sterilized using an autoclave. After 30 minutes of sterilization, the flask was gently removed from the autoclave. 20mL of agar media was

poured to the sterilized petri dish and bacterial culture was inoculated in the nutrient broth. The whole procedure was completed in a laminar airflow cabinet near to the spirit lights. Then, it was left to solidify at room temperature for 24 hours. Staphylococcus aureus and Escherichia coli were introduced separately on to a nutrient agar plate using a cotton swap. Methanolic, acetone, and distilled water extracts of leaves and stems were used for this study. The cork borer was used to create wells on agar medium. Wells were filled with 50µL of the plant extract at specific concentration. Next, the plates were incubated at 37˚C for 24 hours. The inhibitory zone was evaluated using a zone scale. (Gameti et al., 2023).

RESULT AND DISCUSSION:

3.1 Qualitative analysis of phytochemicals:

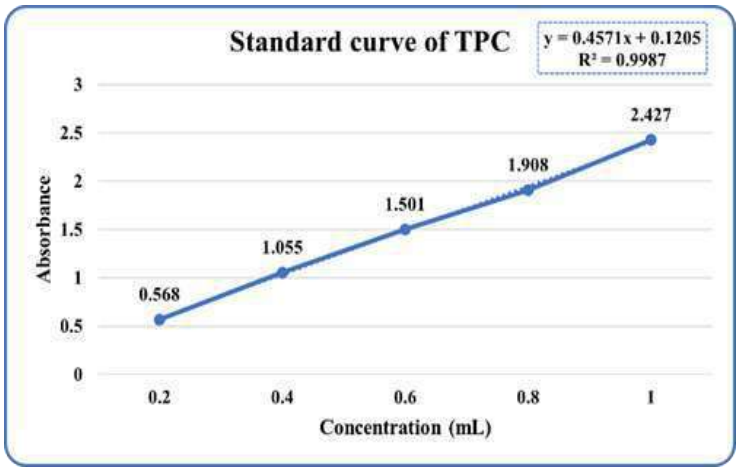
The preliminary tests for phytochemicals of J. pentanthos (Jacq.) G. Don. methanolic, hexane and distilled water extracts shows the presence of many secondary and primary metabolites in different parts leaves, stem, and flower. Methanolic and hexane extracts shows the presence of phenols, tannins, flavonoids, phytosterols, protein and amino acids in leaves, stems, and flowers. While alkaloids present in only methanol and hexane extracts of leaves. D.W. extract can not extract the phytocompounds.

Table 2: Result of qualitative analysis of plant J. pentanthos (Jacq.) G. Don.

Phytochemical	Biochemical test	Solvent and plant part								
		Methanol			Hexane			D.W.		
		L	S	F	L	S	F	L	S	F
Alkaloids	Dragendroff's test	+	-	-	+	-	-	-	-	-
	Mayer's test	+	-	-	+	-	-	-	-	-
Tannins	Braymer's test	+	+	+	+	-	+	+	-	+
	10% NaOH test	+	+	+	+	+	+	-	-	-
Phenols	Iodine test	+	+	+	+	+	+	-	-	+
	Lead acetate test	+	+	+	+	+	+	-	-	-
Flavonoids	Conc. H_2SO_4 test	+	+	+	+	+	+	-	-	-
	Lead acetate test	+	+	+	+	-	-	-	-	-
Phytosterols	Salkowski's test	+	+	-	+	-	-	-	-	-
	Hesse's response test	+	+	-	+	-	-	-	-	-
Proteins	Millon's test	+	-	+	+	-	-	-	-	+
	Xanthoproteic test	+	-	+	+	-	+	-	-	-

3.2 Result of Quantitative analysis:

3.2.1 Total phenolic content (TPC):



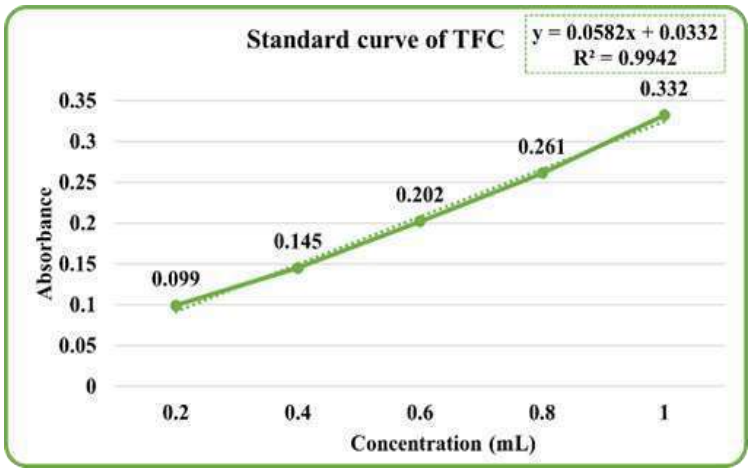
Graph 1: Standard Curve for TPC

Methanolic extract of leaves shows the highest TPC content with value of (6.295 ± 0.009). So, across all plant parts extracts, indicating that methanol and hexane are the most effective solvent for extracting phenols. Distilled water showed negative phenol content in leaves (-0.117 ± 0.0004) and stems (-0.117 ± 0.0012), but lower in flowers (0.235 ± 0.0020).

Table 3: Result of Total phenolic content in plant J. pentanthos (Jacq.) G. Don.

Sr. no.	Plant part	Volume	Total phenolic content extract		
			Methanol	Hexane	Distilled water
1.	Leaves	1 mL	6.295 ± 0.009	6.295 ± 0	-0.117 ± 0.0004
2.	Stem	1 mL	6.265 ± 0	6.295 ± 0	-0.117 ± 0.0012
3.	Flowers	1 mL	6.302 ± 0	6.203 ± 0.004	0.235 ± 0.0020

3.2.2 Total flavonoid content (TFC):



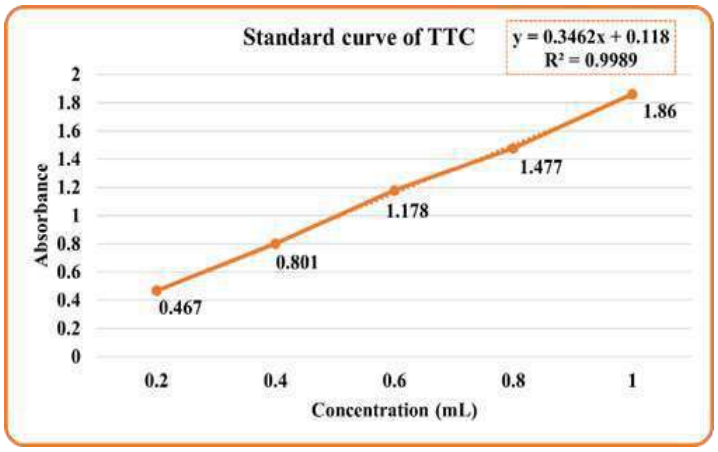
Graph 2: Standard curve for TFC:

Hexane extract of leaves shoes the highest flavonoid concentration with value of (7.265 ± 0.003), on other hand for stem, methanolic extract of stem shows the highest flavonoid content with value of (5.048 ± 0.001). In flower flavonoid is extracted in higher amount in methanolic extract (4.447 ± 0.001). Distilled water has least ability to solubilize flavonoids.

Table 4: Result of total flavonoid content in plant J. pentanthos (Jacq.) G. Don.

Sr. no.	Plant part	Volume	Total flavonoid content extract		
			Methanol	Hexane	Distilled water
1.	Leaves	1 mL	5.907 ± 0.041	7.265 ± 0.003	2.024 ± 0.014
2.	Stem	1 mL	5.048 ± 0.001	4.447 ± 0.005	1.045 ± 0.0009
3.	Flowers	1 mL	4.447 ± 0.001	3.124 ± 0.003	1.010 ± 0.002

3.2.3 Total tannin content (TTC):

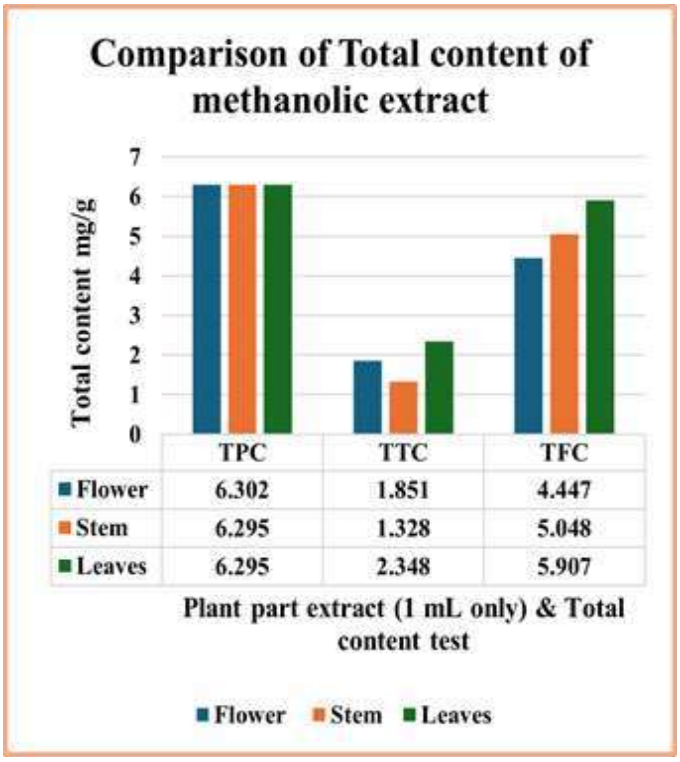


Graph 3: Standard Curve for TTC

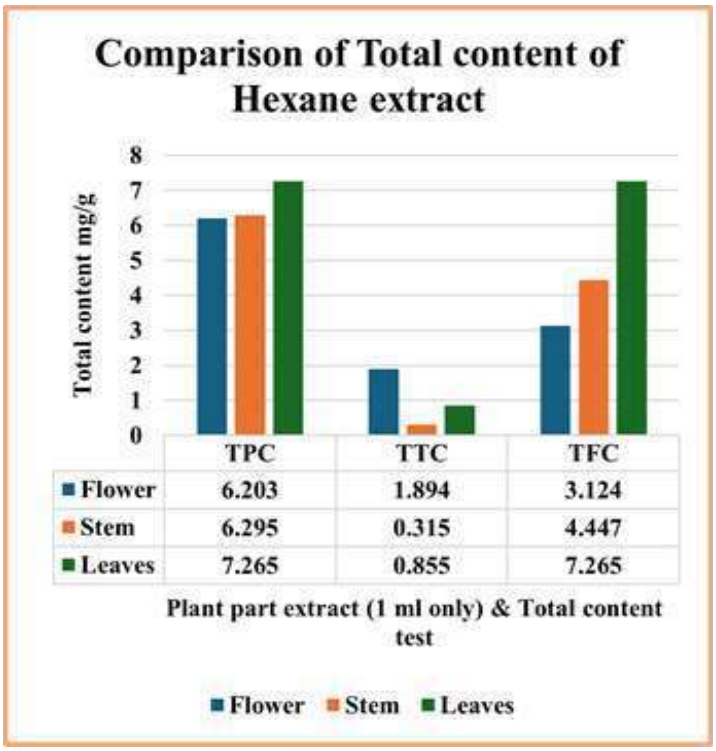
Methanol extract of leaves exclude more tannin content with value of 2.348 ± 0.042, from all plant parts compared to hexane and distilled water, indicating that methanol has a greater ability to solubilize tannins. Hexane exhibits moderate extraction, while D.W. extracts lowest number of tannins.

Table 5: Result of total tannin content in plant J. pentanthos (Jacq.) G. Don.

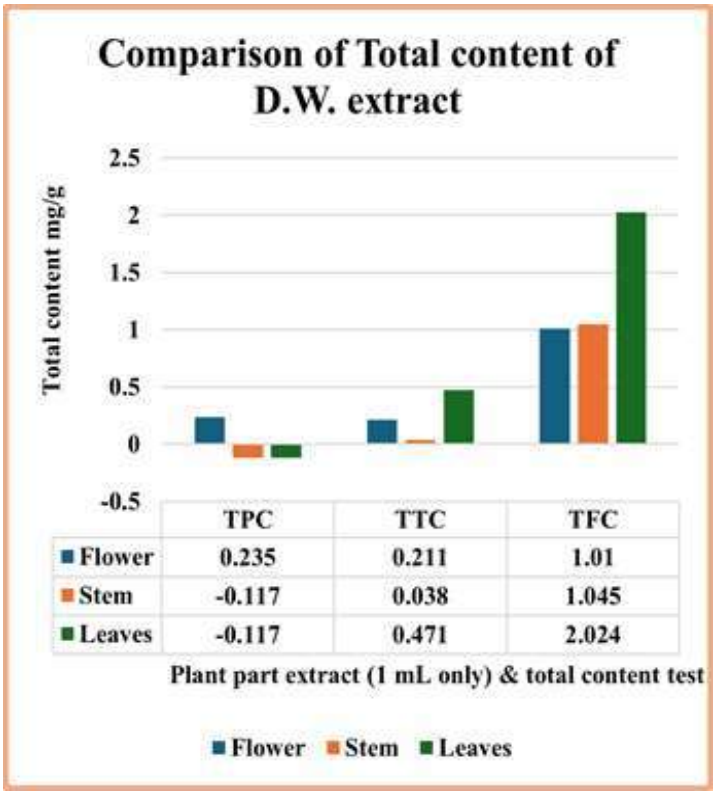
Sr. no.	Plant part	Volume	Total tannin content extract		
			Methanol	Hexane	Distilled water
1.	Leaves	1 mL	2.348 ± 0.042	0.855 ± 0.005	0.471 ± 0.0093
2.	Stem	1 mL	1.328 ± 0.018	0.315 ± 0.002	0.038 ± 0.01
3.	Flowers	1 mL	1.851 ± 0.17	1.894 ± 0.004	0.210 ± 0.0009



Graph 4: Comparison of total content from methanolic extract



Graph 5: Comparison of total content from hexane extract



Graph 6: Comparison of total content from D.W. extract

3.3 Result of antioxidant activity:

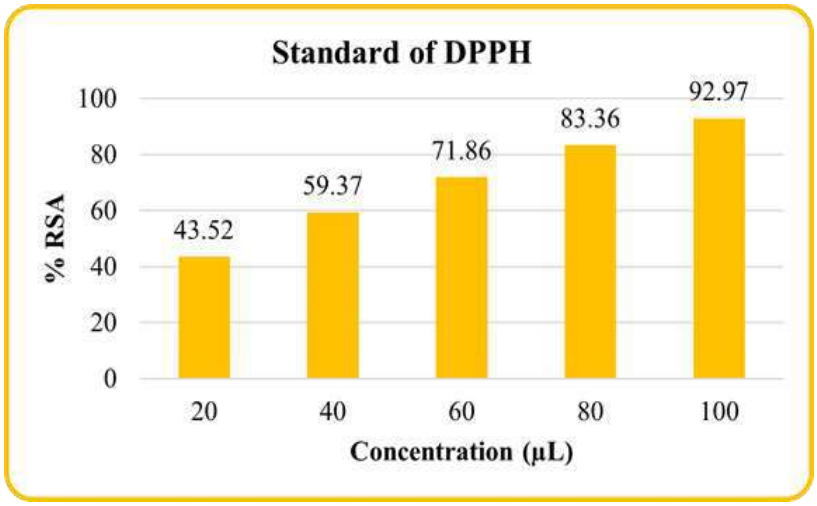
3.3.1 DPPH Assay:

The antioxidant activity measures as a percentage of its radical scavenging activity. The IC50 value was calculated using the %RSA calibration curve, which indicates the concentration of extracts required to inhibit 50% of free radicals. A lower IC50 value implies more antioxidant activity. The least IC50 value is found in hexane leaf extract with value of 14.06, indicate maximum antioxidant activity among

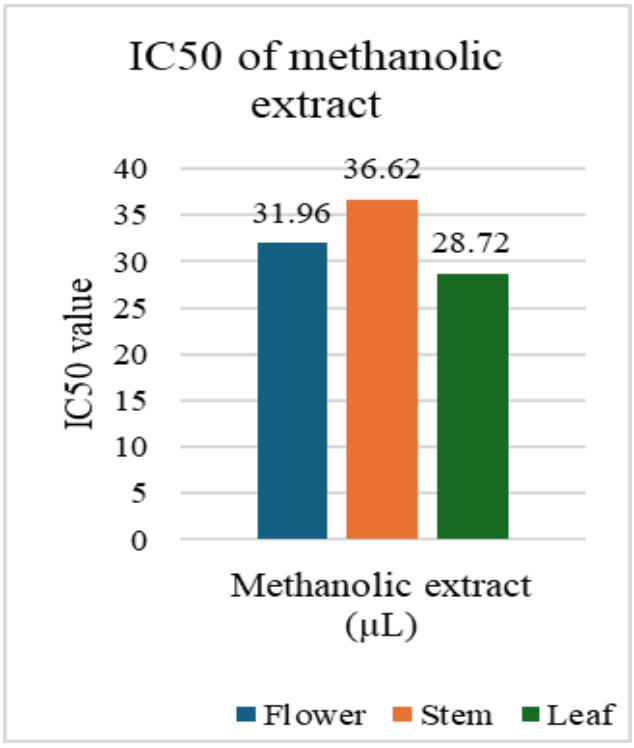
all the extracts. While, hexane extract of stem shows highest IC50 value 88.39, indicating the least antioxidant ability.

Table 6: Result of IC50 values of different part of plant J. pentanthos (Jacq.) G. Don.

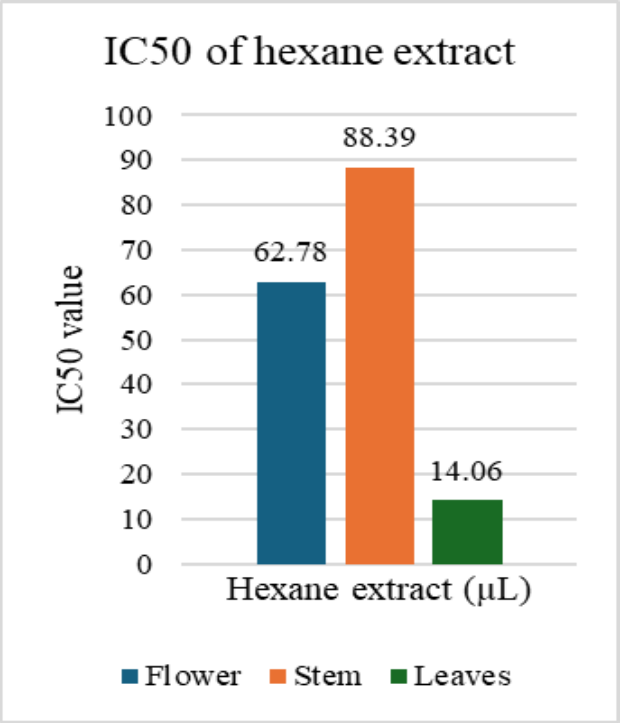
Plant part	IC50 value (methanolic extract)	IC50 value (Hexane extract)	IC50 value (distilled water extract)
Leaves	31.96	62.78	25.96
Stem	36.62	88.39	49.34
Flower	28.72	14.06	63.45



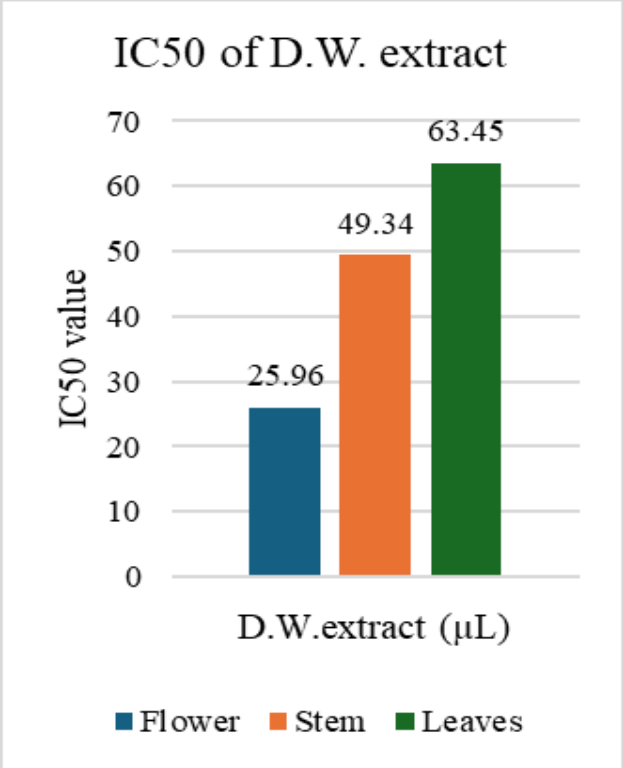
Graph 7: Standard curve of DPPH



Graph 8: Comparison of highest IC50 value of methanolic extract

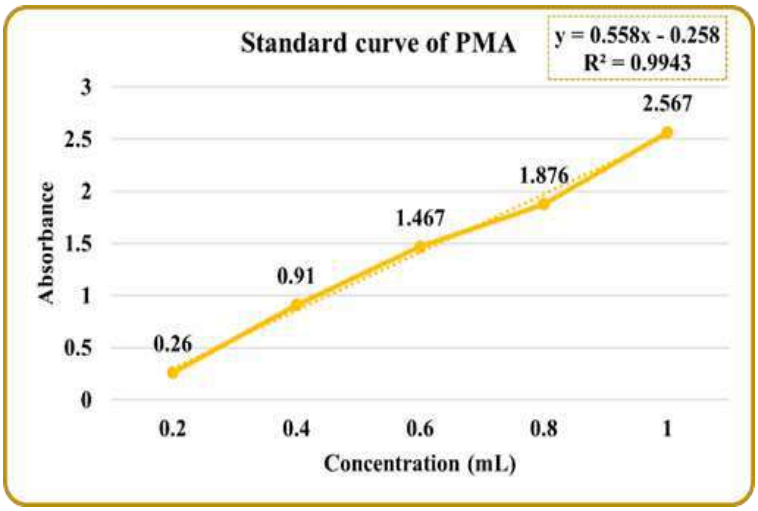


Graph 9: Comparison of highest IC50 value of hexane



Graph 10: Comparison of highest IC50 value of D.W. extract

3.3.2 Phosphomolybdate assay:



Graph 11: Standard curve of PMA assay

The PMA method is quantitative, as the total antioxidant capacity (TAC) is measures in ascorbic acid equivalents. The total antioxidant capacity of J. pentanthos (Jacq.) G. Don. was determined at a concentration of 0.2 mL. The D.W. flower extract shows highest %inhibition with value 86.06%. The lowest %inhibition found in methanolic leaves extract with value of 64.13.

Table 7: Result of phosphomolybdate assay of plant J. pentanthos (Jacq.) G. Don.

Plant part	Concentration	Extract	Total antioxidant capacity in mg AAE/g	% Inhibition
Leaves	0.2 mL	Methanol	0.961 ± 0.014	64.13
	0.2 mL	Hexane	1.045 ± 0.006	58.06
	0.2 mL	D.W.	1.045 ± 0.006	84.39
Stem	0.2 mL	Methanol	0.729 ± 0.002	80.77
	0.2 mL	Hexane	0.858 ± 0.003	71.48
	0.2 mL	D.W.	0.670 ± 0.004	85.03
Flower	0.2 mL	Methanol	0.837 ± 0.002	73.03
	0.2 mL	Hexane	0.837 ± 0.002	73.03
	0.2 mL	D.W.	0.656 ± 0.0012	86.06

The total antioxidants capacity of both methanolic with value of (0.961 ± 0.014) and hexane with value of (1.045 ± 0.006) extracts of leaves is higher compared to stems and flower. Methanolic and hexane extracts show strong antioxidant activity, however the D.W. extract has a lower TAC value, indicating a higher inhibitory percentage.

3.4 Antibacterial activity:

The antibacterial activity of three extracts methanolic, hexane, and D.W. of J. pentanthos (Jacq.) G. Don. was investigated by agar diffusion method against selected human pathogens such as Staphylococcus aureus and Escherichia coli. The maximum zone of inhibition 23 mm seen in the methanolic extract of fresh leaf against S. aureus. The leaves and stem show major efficacy to restrict the growth of S. aureus; while only leaf methanolic and hexane extracts can inhibit the E. coli with 19 mm and 17 mm zone of inhibition respectively.

Table 8: Result of antibacterial activity of plant J. pentanthos (Jacq.) G. Don.

Plant part	Extract	Zone of inhibition with bacterial strain in millimeter	
		Staphylococcus aureus	Escherichia coli
Leaves	Methanolic (10/50)	16 mm	19 mm
	Acetonic (10/50)	14 mm	17 mm

	D.W. (10/50)	-	-
	Fresh methanolic	23 mm	-
	Fresh acetonic	19 mm	-
	Fresh D.W.	-	-
Stem	Methanolic (10/50)	15 mm	-
	Acetonic (10/50)	18 mm	-
	D.W. (10/50)	-	-
	Fresh methanolic	20 mm	-
	Fresh acetonic	21 mm	-
	Fresh D.W.	-	-

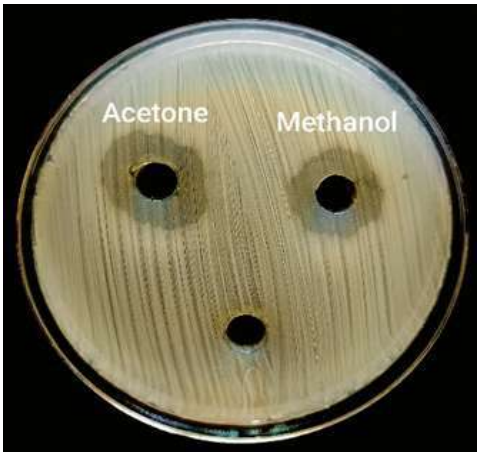


Fig 1: Antibacterial activity of J. pentanthos (Jacq.) G. Don. fresh stem extract against S. aureus with zone of inhibition of 21 mm (acetonic extract) and 20 mm (methanolic extract).

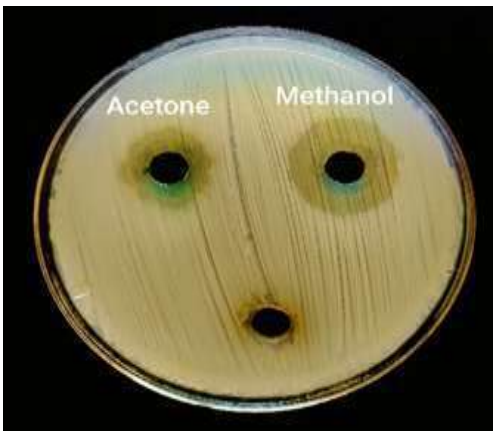


Fig 2: Antibacterial activity of J. pentanthos (Jacq.) G. Don. fresh leaves extract against S. aureus with zone of inhibition of 19 mm (acetonic extract) and 23 mm (methanolic extract).

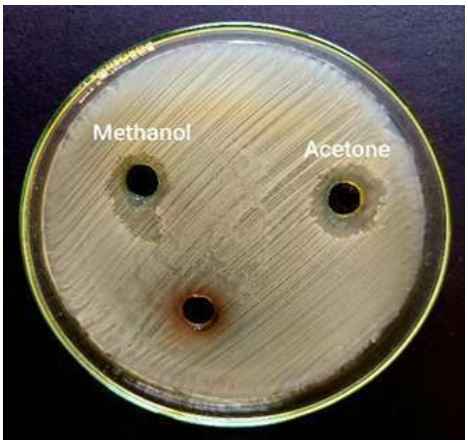


Fig 3: Antibacterial activity of J. pentanthos (Jacq.) G. Don. stem extract against S. aureus with zone of inhibition of 15 mm (methanolic extract) and 18 mm (acetonic extract).

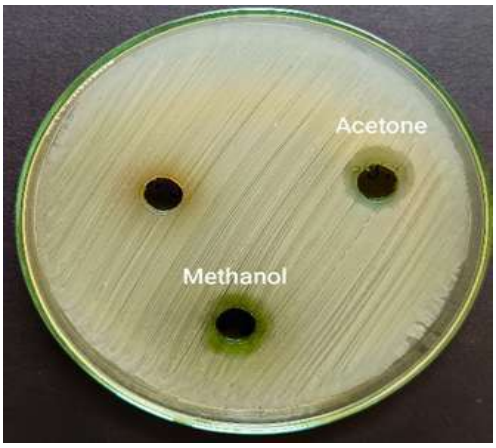


Fig 4: Antimicrobial activity of *J. pentanthos* (Jacq.) G. Don. Leaf extracts against S. aureus with zone of inhibition of 16 mm (methanolic) and 14 mm (acetonic).

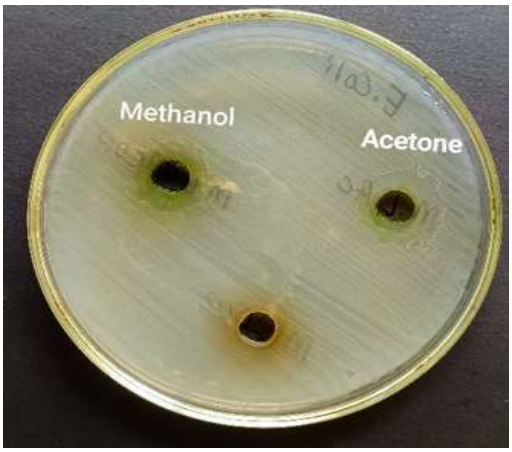


Fig 5: Antibacterial activity of *J. pentanthos* (Jacq.) G. Don. Leaf extracts against E. coli with zone of inhibition of 19 mm (methanolic) and 17 mm (acetonic).

CONCLUSION:

The research indicates that the plant J. pentanthos (Jacq.) G. Don. have a valuable amount of phenol, flavonoid, and tannin compounds, which are known for their antioxidant and antibacterial activities. The DPPH and PMA assays, reveals that the plant extracts can effectively scavenge the free radicals and have potential to decrease oxidative stress. While, the antibacterial assay shows that the J. pentanthos (Jacq.) G. Don. plant have capability to restrict the growth of bacteria S. aureus and E. coli., which shows the plant as a natural source of antibacterial compounds. The plant shows potential to restrict the S. aureus more

than the E. coli, so the extracts may have capability to treat skin diseases which cause by the bacteria S. aureus.

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