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Evaluation of phytochemical composition and *in-vitro* assessment of antioxidant and antimicrobial activities of various medicinal plant extracts

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Abstract
Investigating the qualitative and quantitative phytochemical analysis of medicinal plants is crucial for understanding their therapeutic potential and ensuring their safe usage. By identifying and quantifying the various phytochemical compounds present in these plants, researchers can better comprehend their mechanisms of action and potential health benefits. The phytochemical analysis of *Psidium guajava*, *Basella alba*, *Centella asiatica* and *Solanum torvum* reveals their significant therapeutic potential due to high levels of flavonoids, phenols, and tannins, as well as potent antioxidant and antimicrobial properties, supporting their traditional medicinal uses. The study identifies alkaloids, flavonoids, phenols, terpenes, saponins, and tannins in the medicinal plants, indicating their diverse chemical profiles. The extracts from these medicinal plants demonstrate powerful oxygen free radical scavenging abilities, comparable to the standard antioxidant L-Ascorbic acid. Further, studies show that the extracts exhibit significant antimicrobial activity, indicating their potential in inhibiting microbial growth. The findings support the traditional medicinal uses of the studied plants, validating their efficacy in ethnomedicine. Overall, research on the evaluation of phytochemicals and *in vitro* studies on antioxidant and antimicrobial activities provide valuable insights into the therapeutic potential of medicinal plants, facilitating the development of natural products for various health applications.

Keywords: Medicinal plants, phytochemicals, total phenols, flavonoids and tannins, anti-oxidant assay and anti-microbial activity

Introduction
Medicinal plants play a vital role in maintaining human health [1], with their historical use in disease management spanning centuries, contributing to the discovery of over half of modern pharmaceuticals [2]. Despite an estimated 500,000 plant species worldwide, only 1% have undergone phytochemical investigation, suggesting substantial potential for uncovering new bioactive compounds [3]. The World Health Organization reports that 65% to 80% of people in developing nations rely on medicinal plants for treatment [4], highlighting the ongoing expansion of traditional medicine worldwide [5]. These plants serve as valuable resources for developing novel therapeutic agents. Rich in potent bioactive secondary metabolites, they are extensively utilized in ethno medical and modern herbal practices to address diverse health conditions [6-7], offering safe and effective alternatives to synthetic chemicals [8-9]. Natural products extracted from medicinal plants such as neem, tulsi, amla, dhatura, and nimbu are abundant sources of biologically active compounds, forming the basis for developing new pharmaceuticals and medicines for combating microbial infections [10].
The current research is focused on identifying optimal phytomedicines for health maintenance from four plant species: *Psidium guajava* (Myrtaceae), *Basella alba* (Basellaceae), *Centella asiatica* (Apiaceae), and *Solanum torvum* (Solanaceae), renowned for their versatile medicinal properties [11]. These plants are utilized in traditional Indian systems of medicine, including Ayurveda and Siddha, with the belief that their whole plant material possesses rejuvenating properties. They are rich sources of various plant secondary metabolites such as alkaloids, phenols, flavonoids, tannins, glycosides, terpenoids, and pigmentation components like tannins and quinines. This study focuses on the leaf and fruit extracts of *Psidium guajava*, *Basella alba*, *Centella asiatica* and *Solanum torvum*, aiming to elucidate their phytoconstituents responsible for their pharmacological activities.

Materials and Methods

Chemicals and Reagents

Folin-Ciocalteu's Reagent, Sodium Carbonate, Ferric Chloride, Ferrous Sulphate and Acetic acid, Ferrous Sulphate and Acetic acid were from Fisher Scientific; Gallic Acid, Potassium Acetate, Potassium Persulfate were from Himedia; Quercetin, Trichloroacetic Acid, 2,2-diphenyl-1-picrylhydrazyl and Methanol were from CDH chemicals; Butylated Hydroxytoluene (BHT), Potassium ferrocyanide were from Rankem chemicals and Nutrient Agar media from Himedia. All chemicals were used were high pure and analytical grade.

Sample Collection and Preparation

Fresh leaves of *Psidium guajava, Basella alba, Centella asiatica* and fruits of *Solanum torvum* were gathered from local areas in Mysuru, Karnataka. After thorough washing with tap water followed by distilled water, the samples were shade-dried at room temperature. Subsequently, they were finely ground into powder using a mechanical blender. The powdered leaves and fruits were then packed into a Soxhlet apparatus for sequential solvent extraction employing methanol, ethyl acetate, chloroform, and methanol-hexane mixtures at specific temperatures. The resulting extracts were filtered through Whatman filter paper No. 1, and the filtrate was concentrated using a rotary evaporator (Heidolph, Germany) through solvent evaporation techniques (Figure 1). Finally, the extracts were stored in storage vials at 4°C for further assays [12].

Phytochemicals analysis of the selected plant extracts

The preliminary qualitative screening of aqueous extracts from *Psidium guajava, Basella alba, Centella asiatica* and *Solanum torvum* fruits was conducted to assess the presence of phytochemicals including phenols, alkaloids, flavonoids, saponins, tannins, and terpenoids. Standard protocols for phytochemical analysis were followed to determine the presence of these compounds in the samples [13-14].

Quantitative analysis

Total Phenolic Content (TPC) Estimation

The total phenolic content of various extracts including ethyl acetate, chloroform, methanol, and methanol-hexane mixture from *Psidium guajava, Basella alba, Centella asiatica* and *Solanum torvum* fruits was determined using the Folin-Ciocalteu (FC) reagent method with slight modifications [15]. In brief, 200µl of 1:10 diluted samples were combined with 800µl of distilled water. Then, 0.5 mL of 1:1 diluted FC reagent was added and the mixture was incubated for five minutes at room temperature. Subsequently, 2mL of sodium carbonate solution (20%) was added and the mixture was further incubated for 60 minutes at room temperature. The absorbance was measured at 738nm. Gallic acid was used as a standard, and the concentration of phenolic compounds was expressed as gallic acid equivalents (GAE) in milligrams per gram of dry mass.

Total Flavonoid Content (TFC) Estimation

The total flavonoid content was determined using the aluminum chloride method with slight modifications [16]. In brief, 200µl of 1:10 diluted sample was mixed with 800µl of distilled water, followed by the addition of 1.5mL of methanol, 0.2mL of aluminum chloride solution (10%), and 0.2mL of potassium acetate solution (1M). The reaction mixture was then incubated for 30 minutes at room temperature, after which the absorbance was measured at 415nm. Quercetin was used as a standard, and the concentration of flavonoids was expressed as quercetin equivalents (QE) in micrograms per 100 grams of dry mass.

Total Tannin Content (TTC) Estimation

The quantitative tannin content in various leaf extracts was assessed using the method outlined by Lahare [17]. For this, 0.5 ml of the sample was mixed with 1.0 ml of 1% potassium ferric cyanide and 1.0 ml of 1% ferric chloride, and the final volume was adjusted to 10.0 ml with distilled water. The reaction mixture was allowed to stand at room temperature for 5 minutes, after which its absorbance was measured at 720 nm against a reagent blank. The tannin content was expressed as milligrams of tannic acid equivalents (TAE) per gram of extract.

Estimation of Antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of the various extracts was assessed using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, following the procedure outlined by Kalpoutzakis [18] with slight modifications. L-Ascorbic acid in methanol was used as the standard. Methanolic extracts of different concentrations (0.5, 1, 2, 4, 6, 8, 10 µg/ml) were prepared in 20 µL of methanol. DPPH was prepared by dissolving 2.95 mg in ethanol to achieve a concentration of 300 µM. The methanolic sample was dispensed into a 96-well microtiter plate, to which 180 µL of DPPH was added. The mixture was then incubated in the dark at 37°C for 30 minutes. Absorbance was measured at 515 nm. The half maximal inhibitory concentration (IC50) was calculated using the following equation.

$$\% \text{ Inhibition} = \frac{\text{Abs of blank} - \text{Abs of sample}}{\text{Abs of blank}} \text{ X } 100$$

Anti-Microbial activity

The recovered plant extracts were subjected to further analysis to evaluate their bactericidal activity against various bacterial species, including *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* (ATCC 6633). The bacterial cultures were obtained from the Nano biotechnology Laboratory, Department of Studies in Biotechnology, University of Mysore, Mysuru.

The antibacterial activity was assessed using the disk diffusion method [19]. Initially, bacterial cultures were inoculated onto nutrient agar media plates using the spread plate method. Subsequently, disks were placed onto the nutrient agar plates. One disk contained Gentamicin (10 mg/ml), an antibiotic used as a positive control, while another disk was loaded with methanol as a negative control. Similarly, disks were loaded with extracts dissolved in methanol, ethyl acetate, methanol-hexane mixture, and chloroform (5 mg/ml). All plates were then incubated at 37°C for 24 hours. After incubation, the plates were observed for bacterial growth, and the antibacterial activity was determined by measuring the zone of inhibition (in millimeters) formed around the disks on each plate.

Results and Discussion

Preliminary Phytochemical Screening

Phytochemical screening plays a crucial role in identifying bioactive compounds with significant potential in medicinal sciences. The preliminary screening was conducted on leaf

extracts of *Psidium guajava*, *Basella alba*, *Centella asiatica* and fruits of *Solanum torvum* using various solvents such as chloroform, ethyl acetate, methanol-hexane mixture, and methanol (Figure 1). As indicated in Table 1, presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, phenols, and tannins. These compounds serve as valuable reservoirs of novel therapeutic agents with healing properties.

Table 1: Phytochemical analysis of different leafs and fruit extracts

Phyto Compounds	Test	Solvents															
		SET 1				SET 2				SET 3				SET 4			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Alkaloids	Wanger's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Mayer's test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phytosterols/ Terpenoids	Salkowski test	+	+	+	+	+	+	+	+	+	-	+	++	+	+	+	+
	Liebermann test	+	+	+	-	+	+	+	+	-	+	-		+	+	+	+
Saponins	Froth test	-	-	+	-	-	-	+	+	+	-	-	+	+	+	+	-
	Form test	-	-	+	-	-	-	+	+	-	+	+	+	-	-	+	+
Phenols	Ferric chloride test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Ellagic test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	Alk. reagent test	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
	Lead acetate test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrates	Benedicts test	-	-	+	-	+	+	+	+	-	+	-	+	-	-	-	+
	Fehlings test	-	-	+	-	+	+	+	+	-	-	+	+	-	+	+	+
Glycosides	Borntrager's test	+	+	+	+	+	-	-	+	-	+	+	-	+	+	+	+
Tannins	Ferric chloride test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin test		-	-	-	-	-	-	-	-	-	-	-	-	-	-		-

Set 1: *Pisidium guajava*, Set 2: *Basella alba*, Set 3: *Centella asiatica*, Set 4: *Solanum torvum* Extracts. A-Chloroform, B-Ethyl acetate, C-Methanol, D-Methanol & Hexane Mixture. (+: Present;-: Absent)

Fig 1: Sample Collection and Preparation different solvent extracts: Fresh leaves of A. *Basella alba* leafs, B. *Centella asiatica* leafs, C. fruit of *Solanum torvum and* D. *Psidium guajava* leafs

Total Phenol Content

The total phenol content in various extracts of *Psidium guajava*, *Basella alba*, *Centella asiatica* and fruits of *Solanum torvum* was presented in Table 2. Among the four extracts, the Methanol mixture extract exhibited the highest phenol content (102.4 mg of gallic acid equivalent (GAE) per gram of extract), followed by Ethyl acetate, Methanol extract, and Chloroform.

Table 2: Total phenol content of different plant extracts

Extracts	Total phenol content *psidium guajava* (µg GA/g ± SD)	Total phenol content *Basella alba* (µg GA/g ± SD)	Total phenol content *centella asiatica* (µg GA/g ± SD)	Total phenol content *Solanum torvum* (µg GA/g ± SD)
Methanol	120.85±0.115	73.111±0.038	58.740±0.045	50.481±0.027
Methanol-Hexane mixture	84.703± 0.044	68.703±0.020	59.111±0.060	52.888±0.059
Ethyl acetate	82.259 ± 0.024	72.814±0.080	60.730±0.017	42.074±0.014
Chloroform	65.407 ± 0.071	57.246±0.052	52.777±0.029	47.888±0.032

Total Flavonoid Content

The total flavonoid content in various extracts of *Psidium guajava*, *Basella alba*, *Centella asiatica* and fruits of *Solanum torvum* was determined. Among the extracts, Methanol and Ethyl acetate extracts exhibited the highest flavonoid content (as shown in Table 3).

Table 3: Total flavonoid content of different extracts

Total Flavonoid Content *Psidium guajava* (µg Quercetin/g ± SD)	Total Flavonoid Content *Basella alba* (µg Quercetin/g ± SD)	Total Flavonoid Content *Centella asiatica* (µg Quercetin/g ± SD)	Total Flavonoid Content *Solanum torvum* (µg Quercetin/g ± SD)
66.75 ± 0.004	79.25 ±0.006	76.75 ±0.008	74.18 ±0.054
84.25 ± 0.064	86.75 ±0.028	69.25 ±0.036	79.35 ±0.005
99.25 ± 0.021	89.25 ±0.019	87.19 ±0.0011	75.94 ±0.013
96.75± 0.002	84.25 ±0.027	74.56 ±0.032	76.84 ±0.001

Total Tannin Content

The total tannin content in various extracts of *Psidium guajava*, *Basella alba*, *Centella asiatica* and fruits of *Solanum torvum* was estimated as Tannic Acid Equivalent. Among the four extracts, Methanol extract exhibited the highest tannin content. The total tannin content in different extracts is illustrated in Table 4.

Table 4: Total Tannin Content of different extracts

Extracts	Total Tannin Content *Psidium guajava* (µg TA/g ± SD)	Total Tannin Content *Basella alba* (µg TA/g ± SD)	Total Tannin Content *Centella asiatica* (µg TA/g ± SD)	Total Tannin Content *Solanum torvum* (µg TA/g ± SD)
Chloroform	7.827±0.184	8.107±0.106	7.827±0.184	7.017±0.258
Ethyl acetate	9.795±0.378	8.202±0.501	9.468±0.047	7.453±0.058
Methnaol	10.415±0.100	9.289±0.035	9.488±0.047	7.701±0.002
Methanol-Hexane mixture	8.817±0.089	8.851±0.112	8.576±0.177	7.487±0.056

Antioxidant activity

DPPH assay

The DPPH radical scavenging activity of extracts from *Psidium guajava*, *Basella alba*, *Centella asiatica* and fruits of *Solanum torvum* was depicted in Figures 2, 3, 4, and 5, respectively. Ascorbic acid was used as the standard. Among the four extracts, the methanol extract exhibited the highest activity, with the scavenging ability of the four extracts following the order of Methanol > Ethyl acetate > Methanol-hexane > Chloroform. The IC50 values of Standard Ascorbic acid and other solvents were depicted in Figures 2, 3, 4, and 5, respectively.

Anti-Microbial activity

The bactericidal activity of the plant extract was analyzed against various bacterial species, including *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* (ATCC 6633), as shown in Figure 6. *Psidium guajava* extracts, particularly Methanol and ethyl acetate, exhibited promising results, followed by *Basella alba*, *Centella asiatica*, and fruits of *Solanum torvum*. The analysis was conducted using the well diffusion assay, and the zone of inhibition was measured, as depicted in Tables 5, 6, 7, and 8.

Table 5: Anti-microbial activity of different leaf extracts of *Psidium guajava*

Bacterial strain	Gentamicin 10mg/ml [Standard]	Methanol [control]	Methanol extract	Methanol-Hexane extract	Ethyl acetate extract	Chloroform extract
			5mg/ml			
B. subtilis	20mm	-	11mm	7mm	10mm	7mm
E. coli	19mm	-	12mm	7.5mm	12mm	8mm
S. aureus	20mm	-	12mm	7mm	10mm	7mm
P. aeruginosa	20mm	-	10mm	6mm	10mm	8mm

Table 6: Anti-microbial activity of different leaf extracts of *Basella alba*

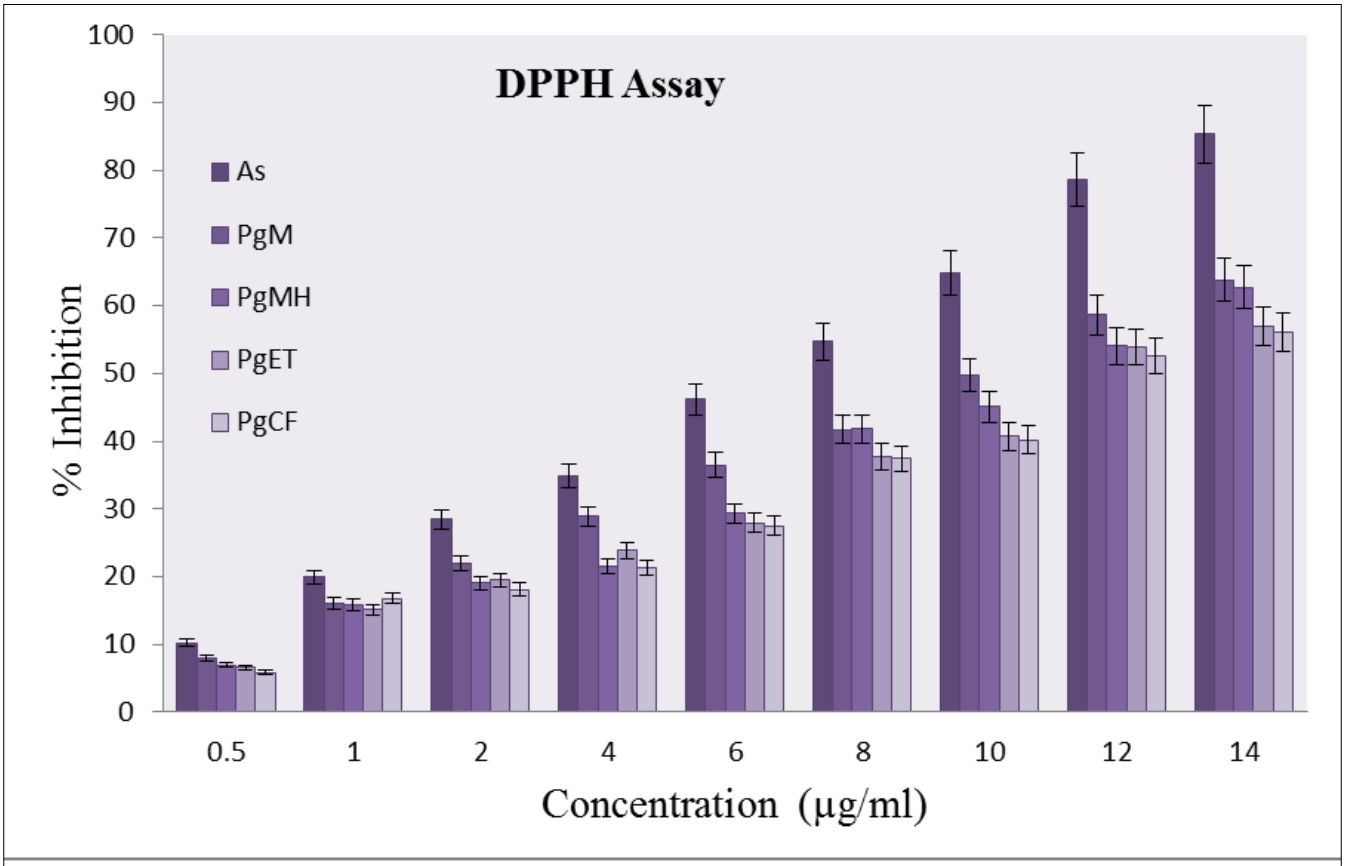
Bacterial strain	Gentamicin 10mg/ml	Methanol [control]	Methanol extract	Methanol-Hexane extract	Ethyl acetate extract	Chloroform extract
			5mg/ml			
B. subtilis	21mm	-	10mm	7mm	8mm	5mm
E. coli	20mm	-	11mm	6mm	9mm	3mm
S. aureus	20mm	-	10mm	7mm	8mm	4mm
P. aeruginosa	19mm	-	10mm	6mm	7mm	5mm

Table 7: Anti-microbial activity conducted by well diffusion assay using different leaf extracts of *Centella asiatica*

Bacterial strain	Gentamicin 10mg/ml	Methanol [control]	Methanol extract	Methanol-Hexane extract	Ethyl acetate extract	Chloroform extract
			5mg/ml			
B. subtilis	20mm	-	7.5mm	7mm	4.5mm	3.5mm
E. coli	19mm	-	7mm	7.5mm	5mm	3mm
S. aureus	20mm	-	7mm	7mm	5.5mm	4mm
P. aeruginosa	20mm	-	8mm	6mm	4mm	5mm

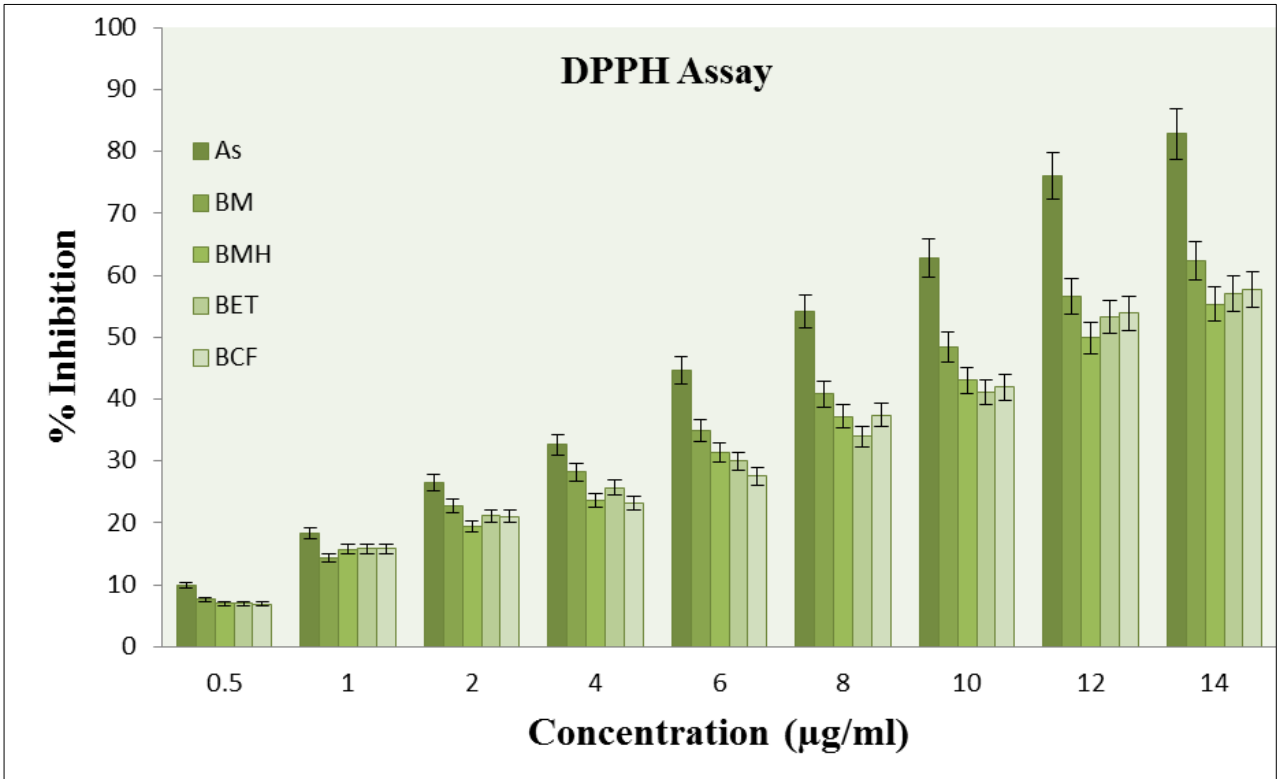
Table 8: Anti-microbial activity of different leaf extracts of *Solanum torvum*

Bacterial strain	Gentamicin 10mg/ml	Methanol [control]	Methanol extract	Methanol-Hexane extract	Ethyl acetate extract	Chloroform extract
			5mg/ml			
B. subtilis	21mm	-	6mm	7mm	4.5mm	3.5mm
E. coli	20mm	-	7mm	7.5mm	5mm	3mm
S. aureus	20mm	-	7mm	7mm	5.5mm	4mm
P. aeruginosa	19mm	-	6mm	6mm	4mm	5mm



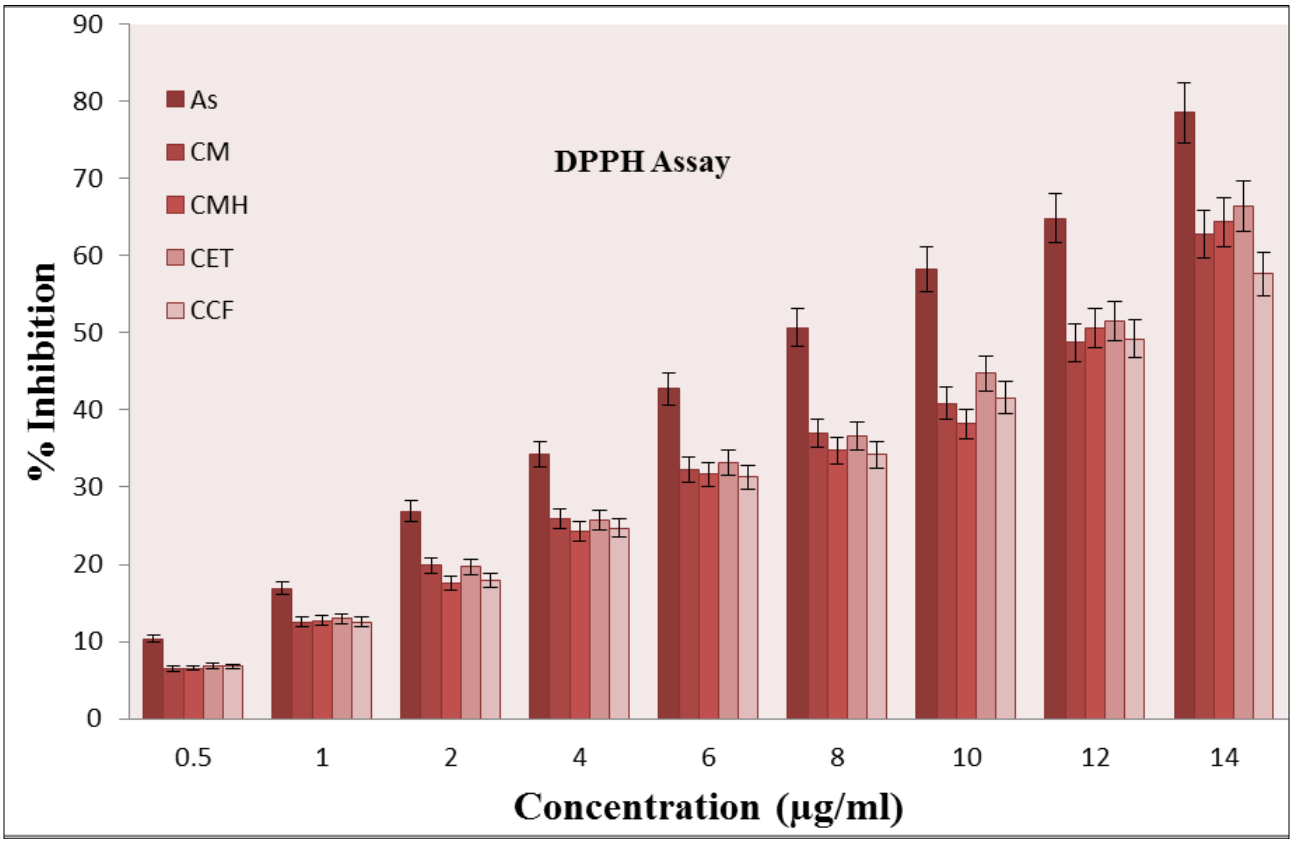
Sample	IC_{50} Value
Standard Ascorbic acid[As]	12.068 µg/ml
Methanol extract of Psidium guajava[PgM]	15.945 µg/ml
Methanol- Hexane mixture extract of Psidium guajava [PgMH]	15.383 µg/ml
Ethyl acetate extract of Psidium guajava[PgET]	17.146 µg/ml
Chloroform extract of Psidium guajava[PgCF]	17.266 µg/ml

Fig 2: DPPH Assay of different solvent extracts of *Psidium guajava*



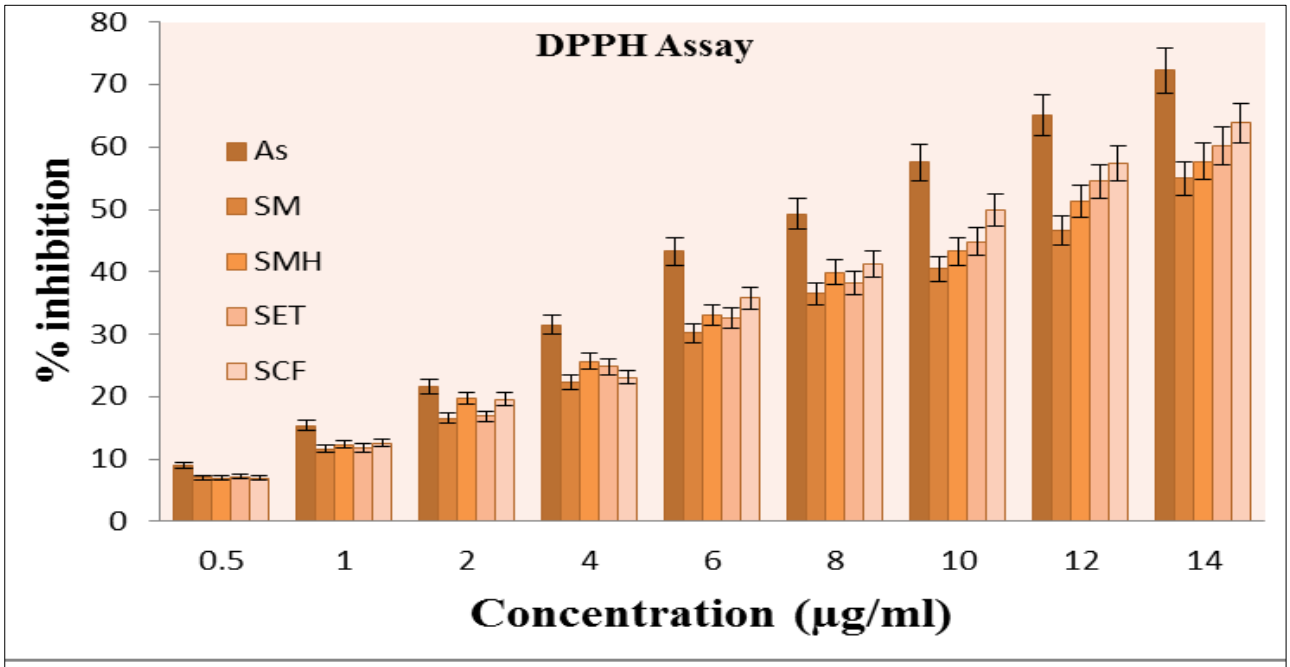
Sample	IC_{50} Value
Standard Ascorbic acid[As]	12.035 µg/ml
Methanol extract of Basella alba [BM]	16.195 µg/ml
Methanol-Hexane mixture extract of Basella alba [BMH]	18.400 µg/ml
Ethyl acetate extract of Basella alba [BET]	18.213 µg/ml
Chloroform extract of Basella alba [BCF]	17.275 µg/ml

Fig 3: DPPH Assay of different solvent extracts of *Basella alba*



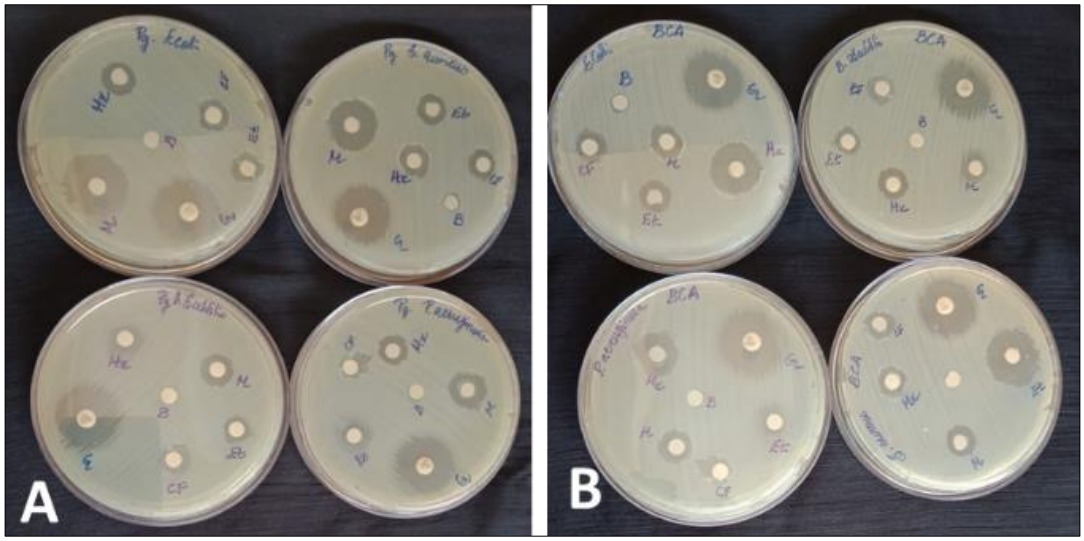
Sample	IC_{50} Value
Standard Ascorbic acid [As]	13.891 µg/ml
Methanol extract of Centella asiatica [CM]	16.553 µg/ml
Methanol-Hexane mixture extract of Centella asiatica [CMH]	15.668 µg/ml
Ethyl acetate extract of Centella asiatica [CET]	15.268 µg/ml
Chloroform extract of Centella asiatica [CCF]	17.194 µg/ml

Fig 4: DPPH Assay of different solvent extracts of *Centella asiatica*



Sample	IC_{50} Value
Standard Ascorbic acid [As]	13.552 µg/ml
Methanol extract of Solanum torvum [SM]	17.595 µg/ml
Methanol-Hexane mixture extract of Solanum torvum [SMH]	17.106 µg/ml
Ethyl acetate extract of Solanum torvum [SET]	15.284 µg/ml
Chloroform extract of Solanum torvum [SCF]	14.334 µg/ml

Fig 5: DPPH Assay of different solvent extracts of *Solanum torvum*



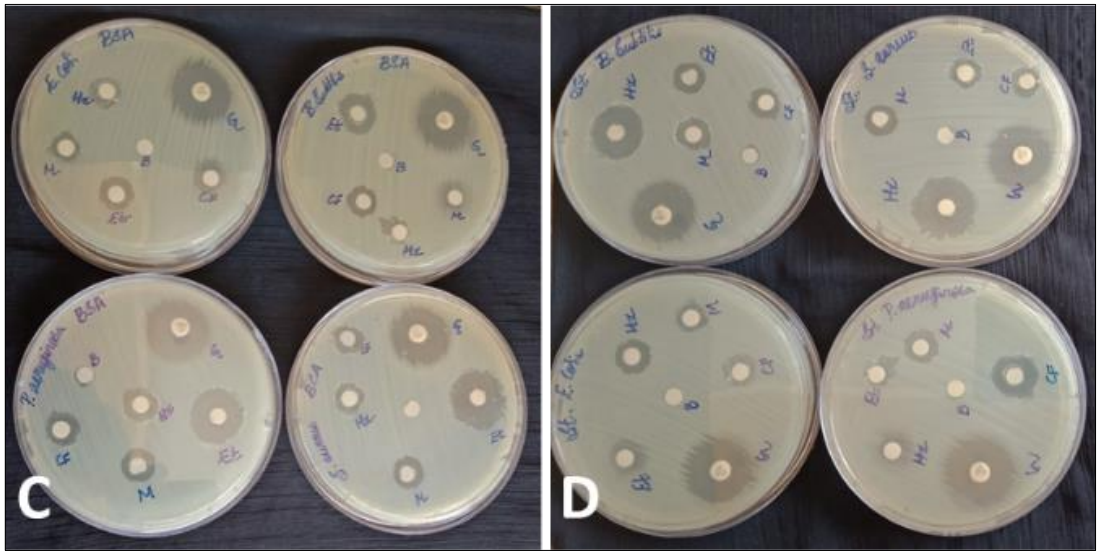


Fig 6: Disc diffusion assay: *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa.* [A-*Pisidium guajava*, B: *Basella alba*, C: *Centella asiatica*, D: *Solanum torvum* Extracts of Methanol-M, MH-Methanol-Hexane mixture, Et-Ethyl acetate, CF-Chloroform, B-Blank/Control, G-Gentamicin]

Conclusion

In this study, phytochemical analysis of extracts from *Psidium guajava*, *Basella alba*, *Centella asiatica* and *Solanum torvum* revealed the presence of secondary metabolites, along with antioxidant and antimicrobial potential. These findings highlight the significance of these plant extracts as valuable microbial resources for producing bioactive compounds with potential applications in medical, pharmaceutical, industrial, and agricultural sectors. Further investigations are warranted to identify and isolate potent therapeutic bioactive compounds from these extracts, paving the way for the development of novel drugs and agricultural products.

Conflict of Interest: None to declare.

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