

Phytochemical Profiling and Antibacterial Potential of Medicinal Plants from Pandanad, Kerala: A Natural Remedy Exploration

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Abstract

Chemicals present in plants naturally are the phytochemicals. These compounds have medicinal properties and have been used for various diseases. Compared to pharmaceutical chemicals, there is no side effect for phytochemicals. Now a day these phytochemicals are used for the production of medicines as well as other medicated products. In the present study phytochemicals of five different medicinal plants such as *Azadirachta indica* (Neem), *Biophytum sensitivum* (Little tree), *Chromolaena odorata* (Christmas bush), *Mimosa pudica* (Sensitive plant) and *Tagetes erecta* (Marigold) were analysed using various chemical tests. Antibacterial activity was also analyzed against *Bacillus cereus*, *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*. All the five plants contain phytochemicals, but intensity or concentration of these compounds was notably higher in *Tagetes erecta* compared to other plants. Also the results highlight that *Tagetes erecta* exhibited the most potent antioxidant activity among the tested plants. *Tagetes erecta* showed significant antimicrobial activity for all the microorganisms selected compared to other plants because of the presence of phytochemicals and antioxidants. This suggested the potential of *Tagetes erecta* as a promising candidate for therapeutic as well as industrial applications.

Key words: Phytochemicals, Antioxidants, Antimicrobial, Therapeutic, Industrial application

1. Introduction

“Plants have long been recognized as a rich source of bioactive compounds for centuries known as phytochemicals, which possess various medicinal properties (Seow *et al.*, 2011)”. These natural compounds have been utilized in traditional medicine for long years to treat a wide range of ailments, and recent scientific advancements have further highlighted their potential in modern therapeutic practices. It

possessed significant medicinal properties, ranging from antimicrobial and anti-inflammatory effects to antioxidant and anticancer activities. Phytochemicals are considered a safer alternative to synthetic pharmaceuticals, as they often exhibit fewer side effects while providing effective treatment for various diseases (Oladunmoye *et al.*, 2020). In recent years, 'there has been a growing interest in exploring the phytochemical profiles of different plant species', with the goal of identifying new sources for drug development and other medicated products.

Also certain plants exhibits significant antimicrobial activities against microbes include the gram positive bacteria as well as the gram negative bacteria, fungus and some viruses.

This study focuses on the phytochemical composition and antibacterial activity of five plants: *Azadirachta indica* (Neem), *Biophytum sensitivum* (Little Tree), *Chromolaena odorata* (Christmas bush), *Mimosa pudica* (Sensitive plant), and *Tagetes erecta* (Marigold).

***Azadirachta indica* (Neem)** (Figure 1A), known as the "village pharmacy," is famous for its wide range of medicinal uses, including antibacterial, antifungal, and antiviral effects. "It contains a variety of phytochemicals, including flavonoids, terpenoids, and alkaloids, which contribute to its health-promoting properties (Brahmachari, 2004)". Neem is also known for its ability to support immune function, treating skin conditions and combating infections.

***Biophytum sensitivum* (Little Tree)** (Figure 1B) is noted for its adaptogenic and anti-inflammatory properties. It has been traditionally used to treat anxiety, wounds, and fever. The plant's phytochemical composition includes flavonoids, alkaloids, and saponins, which are believed to contribute to its therapeutic effects, including its ability to reduce inflammation and act as a mild sedative (Patel *et al.*, 2017).

***Chromolaena odorata* (Christmas bush)** (Figure 1C), also known as the "Siam weed," has gained attention due to its wide range of medicinal applications, including wound healing, pain relief, and anti-inflammatory effects. Its phytochemical content includes phenolics, flavonoids, and essential oils, which are responsible for its therapeutic properties (Bennet *et al.*, 2012).

***Mimosa pudica* (Sensitive Plant)** (Figure 1D) is widely known for its unique response to touch, but it is also utilized in traditional medicine for treating wounds, inflammation, and anxiety. The phytochemicals found in *Mimosa pudica*, including alkaloids, tannins, and flavonoids, are believed to possess sedative, anti-inflammatory, and antimicrobial properties (Bhardwaj *et al.*, 2012).

***Tagetes erecta* (Marigold)** (Figure 1E) a widely cultivated ornamental plant, well-known for its vibrant flowers has been used for centuries in traditional medicine for its healing properties, particularly in treating wounds, burns, and infections. “The plant contains various phytochemicals, including flavonoids, carotenoids, and essential oils, which contribute to its antioxidant, antimicrobial, and anti-inflammatory activities (Chand *et al.*, 2013)”.

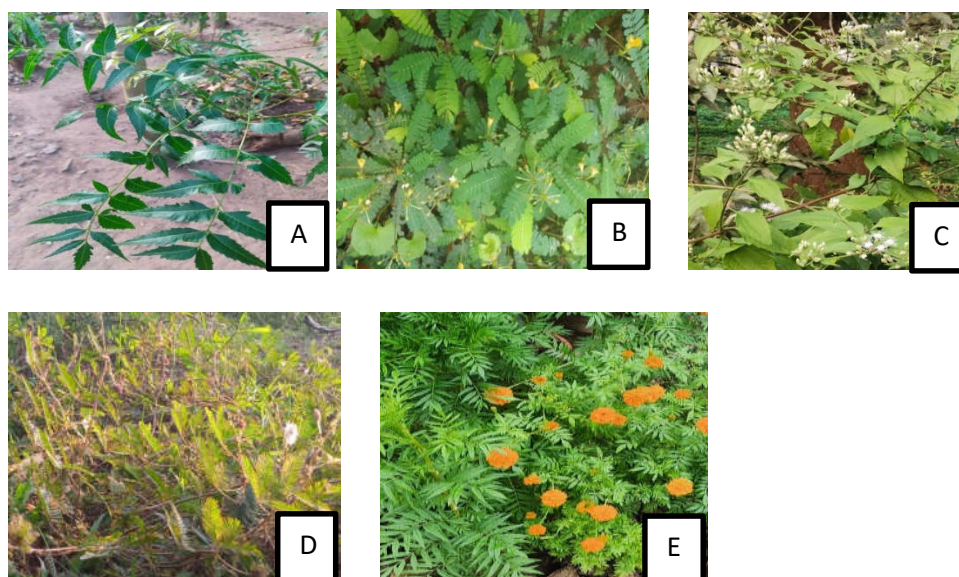


Figure 1. A) *Azadirachta indica* B) *Biophytum sensitivum* C) *Chromolaena odorata* D) *Mimosa pudica* E) *Tagetes erecta*

The study aims to explore the phytochemical profiling, antioxidant property and antimicrobial activity of above mentioned five plants using various chemical tests and well diffusion assay, with the goal of identifying and comparing the intensity of their bioactive compounds as well the antimicrobial property. By understanding the presence and intensity of these bioactive compounds and the antimicrobial property, we can better assess their therapeutic potential and explore their role in the development of alternative medicines or other medicated products.

2. Materials and Methods

2.1 Collection, Authentication and Processing of plants

Leaves of five plants such as *Azadirachta indica*, *Biophytum sensitivum*, *Chromolaena odorata*, *Mimosa pudica* and *Tagetes erecta* were collected from Pandanad, Kerala, India. Authentication was done by submitting the plant samples to the Herbarium at Fatima Mata College, Kollam, for proper identification. The collected leaves were cleaned by washing thoroughly to remove any dirt or impurities. Then shade dried, powdered and stored until used (Sathianarayanan *et al.*, 2010). For extraction, 10 g of the powdered leaf material from each plant was macerated in 100 ml hydro-ethanol solvent (7:3) for 72 hours at room temperature (Perez *et al.*, 1990). The resulting mixture was filtered and the filtrate was stored at 4°C until used.

2.2 Phytochemical tests

Phytochemical screening was performed to qualitatively identify the bioactive compounds present in the plant extracts using standard chemical tests. The methods used to test for various phytochemicals are as follows (Table 1):

Table 1. Qualitative tests for the analysis of phytochemicals present in plant extract

Qualitative test for phytochemicals	Observation
Alkaloid Dragendroff's test 1 ml plant extract + 2 ml Dragendroff's reagent (Harborne, 1998).	Reddish brown colour
Carbohydrate Seliwanoff's test 1 ml plant extract + 3 ml Seliwanoff's reagent heated in a boiling water bath for 1 minute (Seliwanoff, 1939).	Rose red or brown colour
Flavonoid	Yellow precipitate

Lead acetate test 1 ml plant extract + 1 ml lead acetate solution (Trease & Evans, 1989).	
• Phenolics Ferric chloride test 1 ml plant extract + 1 ml 5% ferric chloride solution (Singleton & Rossi, 1965).	Dark blue or green colour
• Protein and amino acid Xanthoproteic test 1 ml plant extract + 1 ml concentrated nitric acid (HNO ₃) (Miller, 1985).	Dark yellow colour
• Quinone Concentrated HCl test 1 ml plant extract + concentrated HCl (Harborne, 1998).	Green colour
• Saponin Foam test 10 mg plant extract + 10 ml distilled water, shaken well for 1 minute (Obadoni & Ochuko, 2001).	Formation of foam
• Steroids Salkowski's test 1 ml plant extract + few drops of concentrated sulfuric acid, shaken well and allowed to stand (Meyer <i>et al.</i> , 1999).	Red colour in the lower level
• Tannins HCl test 2 ml plant extract + 1 ml 1% HCl (Harborne, 1998).	Yellow or greenish colour
• Terpenoids	Reddish brown or golden yellow colour

Salkowski’s test Plant extract + few drops of concentrated sulfuric acid (Meyer <i>et al.</i> , 1999).	
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2.3 Antioxidant assay

1. DPPH radical scavenging activity

‘The antioxidant activity of the plant extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method’ as described by Blois (1958). 1 g of the standard ascorbic acid was made up to 100 ml with distilled water in a standard flask. Take different concentrations from 0.2 to 1 ml in different test tubes. 1 ml of the each extracts were made up to 100 ml and prepared different concentrations (0.2 – 1 ml) in different test tubes. Make up the test tubes into 5 ml and added 1 ml DPPH reagent. Kept for incubation in dark for 1 hour. The absorbance was read at 517 nm by using a UV-visible spectrophotometer.

Radical scavenging activity of each sample is calculated using the formula

Radical Scavenging Activity = $\frac{A(\text{control})-A(\text{sample})}{A(\text{control})} \times 100$

Where, A(control) – Absorbance of control

A(sample) – Absorbance of sample

The concentration of the extract required to scavenge 50% of the DPPH radicals (IC₅₀) was determined by plotting a dose-response curve of radical scavenging activity versus concentration.

2.4 Antibacterial activity by well diffusion assay (AATCC TM100)

The antibacterial activity of five plant extracts was done by well diffusion assay against *Bacillus cereus*, *S. aureus*, *E. coli* and *Pseudomonas aeruginosa*. where Muller Hinton Agar plates prepared and cut the wells. Lawn cultures of selected bacterial species were made by using cotton swab. After that all the extracts with concentrations of 25, 50, 75 and 100 were added to the respective wells. Plates were then kept at 37°C for 24 hrs. Then the zone of inhibition was measured.

3. Results and Discussion

In this study, plant leaves of *Azadirachta indica* (Neem), *Biophytum sensitivum* (Little Tree), *Chromolaena odorata* (Christmas bush), *Mimosa pudica* (Sensitive plant), and *Tagetes erecta* (Marigold) were collected, prepared the extracts and then the phytochemical analysis was done. The phytochemical screening was carried out qualitatively by certain chemical tests.

3.1 Qualitative phytochemical analysis

Hydro ethanolic extracts of the samples were used for analyzing the phytochemicals. All the samples exhibited the existence of bioactive compounds such as alkaloids, carbohydrates, flavonoids, phenols, proteins and amino acids, quinones, saponins, steroids, tannins and terpenoids. The results of the phytochemical screening for *Tagetes erecta* (TE), *Mimosa pudica* (MP), *Chromolaena odorata* (CO), *Biophytum sensitivum* (BS), and *Azadirachta indica* (AI) are summarized in Table 2. Figure 2 (A-I) showed the colour changes of each phytochemical tests. The presence and intensity of various bioactive compounds are represented using a semi-quantitative scale, where:

- -: Absent
- +: Low intensity
- ++: Moderate intensity
- +++: High intensity
- ++++: Very high intensity

Table 2. Phytochemical analysis of selected plants

Experiment	Observation	Inference				
		TE	MP	CO	BS	AI
Alkaloids	Reddish brown colour	-	+++	-	++	-
Carbohydrates	A rose red colour	-	++++	-	+++	++
Flavonoids	An intense yellow colour	++	+	+	+	+++
Phenols	Dark green colour	++++	+	++	+++	++
Protein and	Dark yellow colour	+++	+++	+++	+++	++++

amino acids						
Quinones	A green colour	+++	+	++	+	+
Saponins	Formation of foam persist for 5 minutes	-	-	+	+	-
Steroids	Red colour in lower level	++	++	++	+++	+
Tannins	Yellow colour	++++	+++	++	++	+++
Terpenoids	Golden yellow colour	++++	+++	+	+	++

Tagetes erecta (TE) displayed the highest intensity of phenols, tannins, and terpenoids among the tested plants. Manimegalai *et al.*, 2023 detected various phytochemicals in *Tagetes erecta*. *Mimosa pudica* (MP) exhibited the highest carbohydrate content along with notable tannins and proteins. Ahuchaogu *et al.*, 2017 screened the extract of the whole *M. pudica* plant and reported the presence of phytoconstituents. The ethanol extract consisted of 'alkaloids, flavonoids, steroids, phenols, tannins, cyanogenic glycosides and anthocyanins'. *Chromolaena odorata* (CO) had moderate levels of phenols, quinones and proteins with a positive test for saponins. Budha *et al.*, 2023 showed the presence of phenols, flavonoids, quinones, terpenoids, and coumarins as secondary metabolites. *Biophytum sensitivum* (BS) showed moderate to high levels of steroids, phenols, and carbohydrates. Pawar *et al.*, 2014 revealed 'the presence of flavonoids, saponins, tannins, terpenes, steroids, amino acids, essential oil, polysaccharides and pectin'. *Azadirachta indica* (AI) was rich in flavonoids, proteins and amino acids but had no presence of alkaloids and saponins. Sandhir *et al.*, 2021 has shown the presence and concentration of phytochemicals of neem plant.

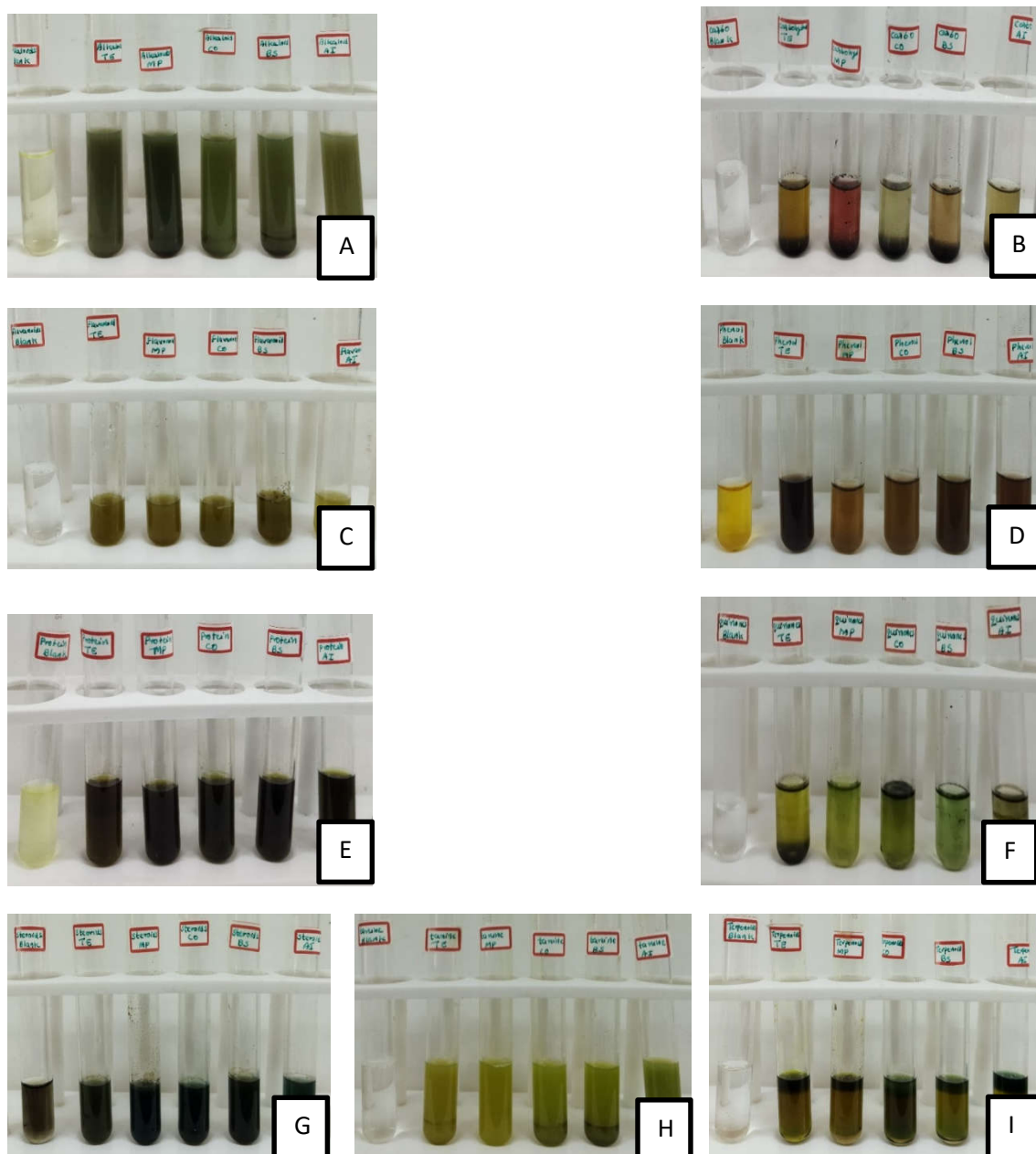


Figure 2. Colour changes during phytochemical tests. A) Alkaloids B) Carbohydrates C) Flavonoids D) Phenols E) Proteins F) Quinones G) Steroids H) Tannins I) Terpenoids

3.2 Antioxidant assay

The antioxidant assay was performed to evaluate the free radical scavenging activity of the selected plants (*Azadirachta indica*, *Biophytum sensitivum*, *Chromolaena odorata*, *Mimosa pudica* and *Tagetes erecta*) using IC_{50} values as a measure of efficacy (Table 3). A line graph illustrating the percentage of free

radical inhibition versus the concentration of each plant extract and the standard was plotted to visualize the scavenging efficiency (Figure 3).

Table 3. IC₅₀ Values of Selected Plants and Standard in Antioxidant Assay

Sample	IC ₅₀ Value (µg/ml)	Observation
Ascorbic Acid (Standard)	0.6539	Highest antioxidant activity
Tagetes erecta (TE)	1.1887	Most potent among the extracts
Mimosa pudica (MP)	4.315	Low antioxidant activity
Chromolaena odorata (CO)	4.4579	Lowest antioxidant activity
Biophytum sensitivum (BS)	1.6953	Moderate antioxidant activity
Azadirachta indica (AI)	3.9192	Comparatively lower antioxidant activity

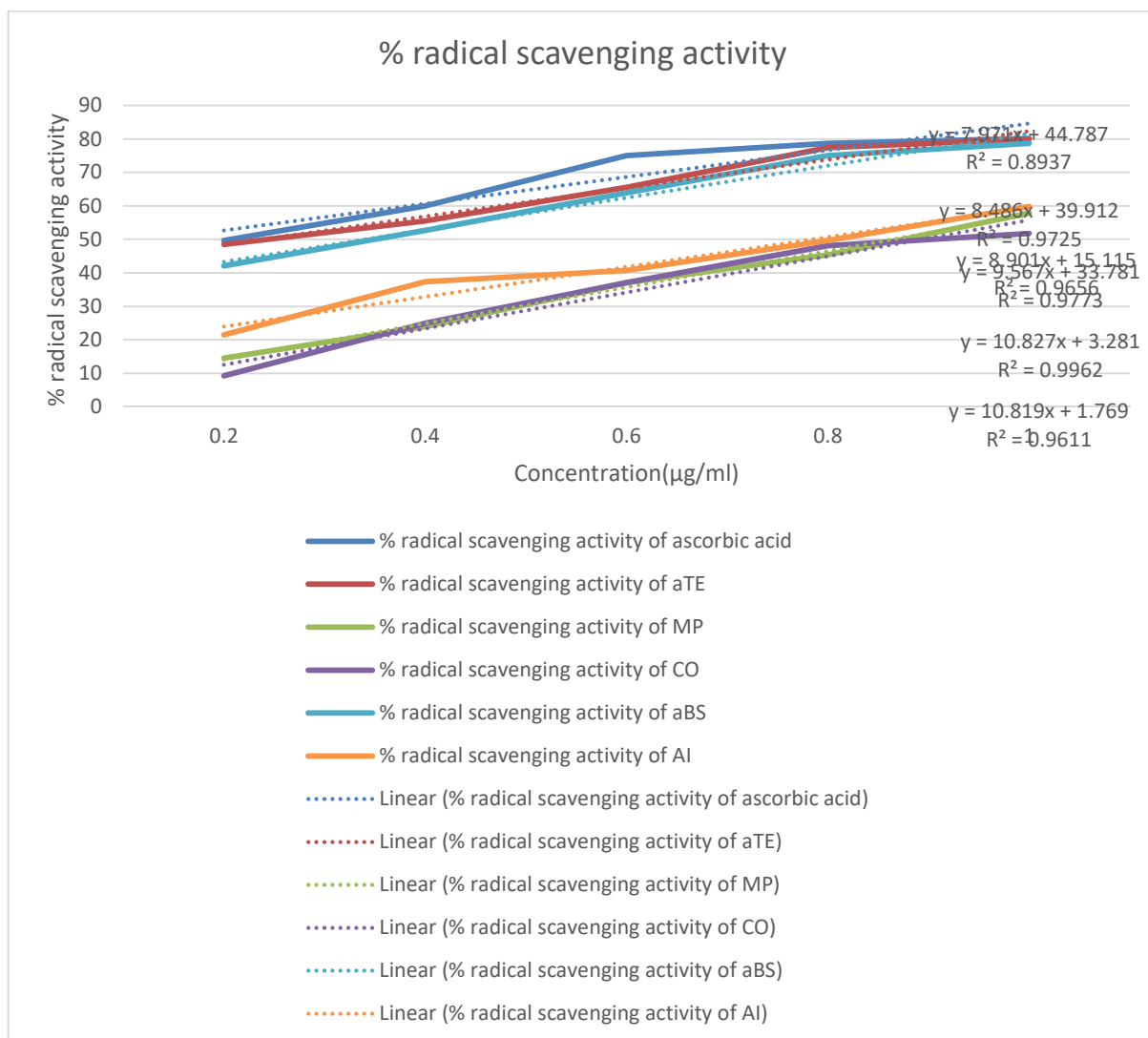


Figure 3. Antioxidant activity of selected plant extracts

These results indicate that all the plants possess free radical scavenging potential, with *Tagetes erecta* showing the strongest antioxidant activity among the plant samples. The IC_{50} value of *Tagetes erecta* is closer to that of the standard (ascorbic acid), suggesting a high level of efficacy in neutralizing free radicals. Kumar *et al.*, 2020 provides an in-depth analysis of the antioxidant properties of *Tagetes erecta* and highlights its potential in the nutraceutical and pharmaceutical industries. Overall, the results demonstrate that the plants are directly influenced by their phytochemical composition. Future studies should focus on isolating the active compounds from *Tagetes erecta* and other potent plants to further explore their antioxidant mechanisms and potential commercial applications.

3.3 Antibacterial activity of selected plant extracts

The antibacterial activity of plant extract with different concentrations like 25, 50, 75 and 100 was checked against Gram positive (*Bacillus cereus* and *S. aureus*) and Gram negative (*E. coli* and *Pseudomonas aeruginosa*) bacteria by well diffusion assay. Zone of microbial inhibition was measured and depicted in Table 4 (Figure 4 is the graphical representation).

Table 4. Antibacterial activity of the plant extracts against selected bacterial species

Plant species	Zone of inhibition (mm)															
	Gram positive bacteria								Gram negative bacteria							
	<i>Bacillus cereus</i>				<i>S. aureus</i>				<i>E. coli</i>				<i>Pseudomonas aeruginosa</i>			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
<i>Azadirachta indica</i>	0	10	11	14	0	0	10	11	0	10	12	11	0	10	11	13
<i>Biophytum sensitivum</i>	10	11	13	15	0	0	10	12	0	0	12	14	0	10	11	11
<i>Chromolaena odorata</i>	11	12	14	17	0	10	10	10	0	0	11	12	0	0	10	10
<i>Mimosa pudica</i>	0	10	12	13	0	0	10	10	0	10	12	12	0	10	17	17
<i>Tagetes erecta</i>	10	11	11	17	10	10	13	15	0	10	12	13	0	10	11	13

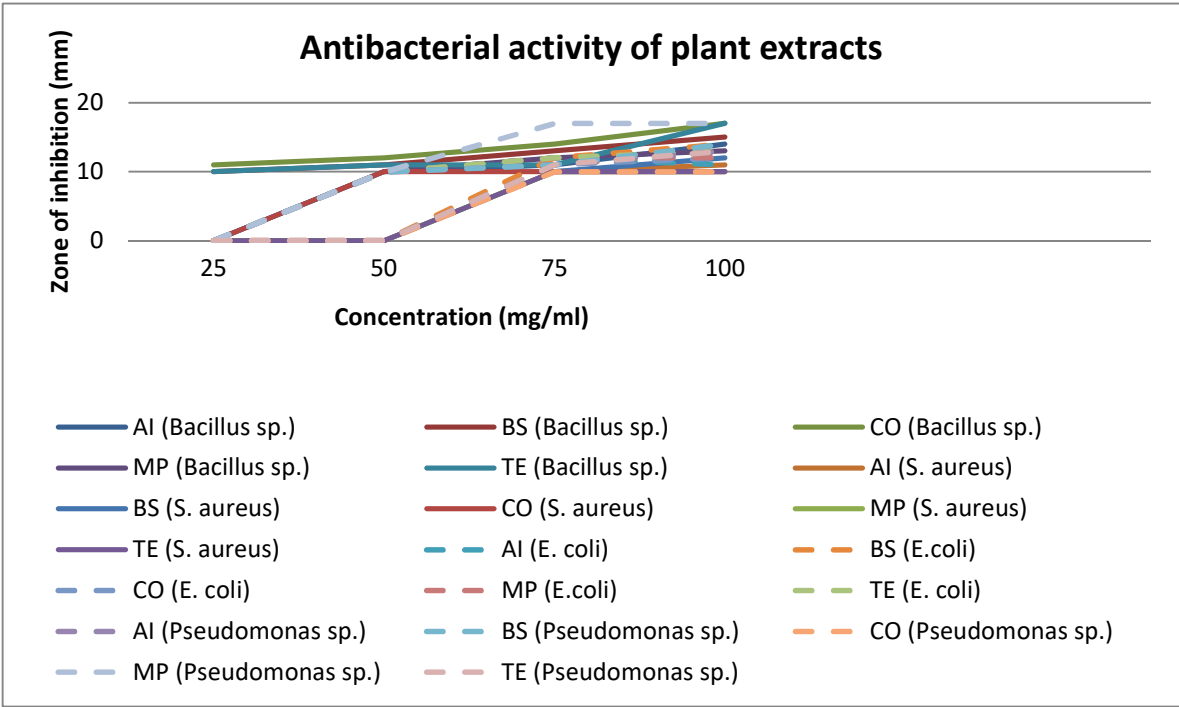


Figure 4. Antibacterial effect of plant extracts on gram positive and gram negative bacteria

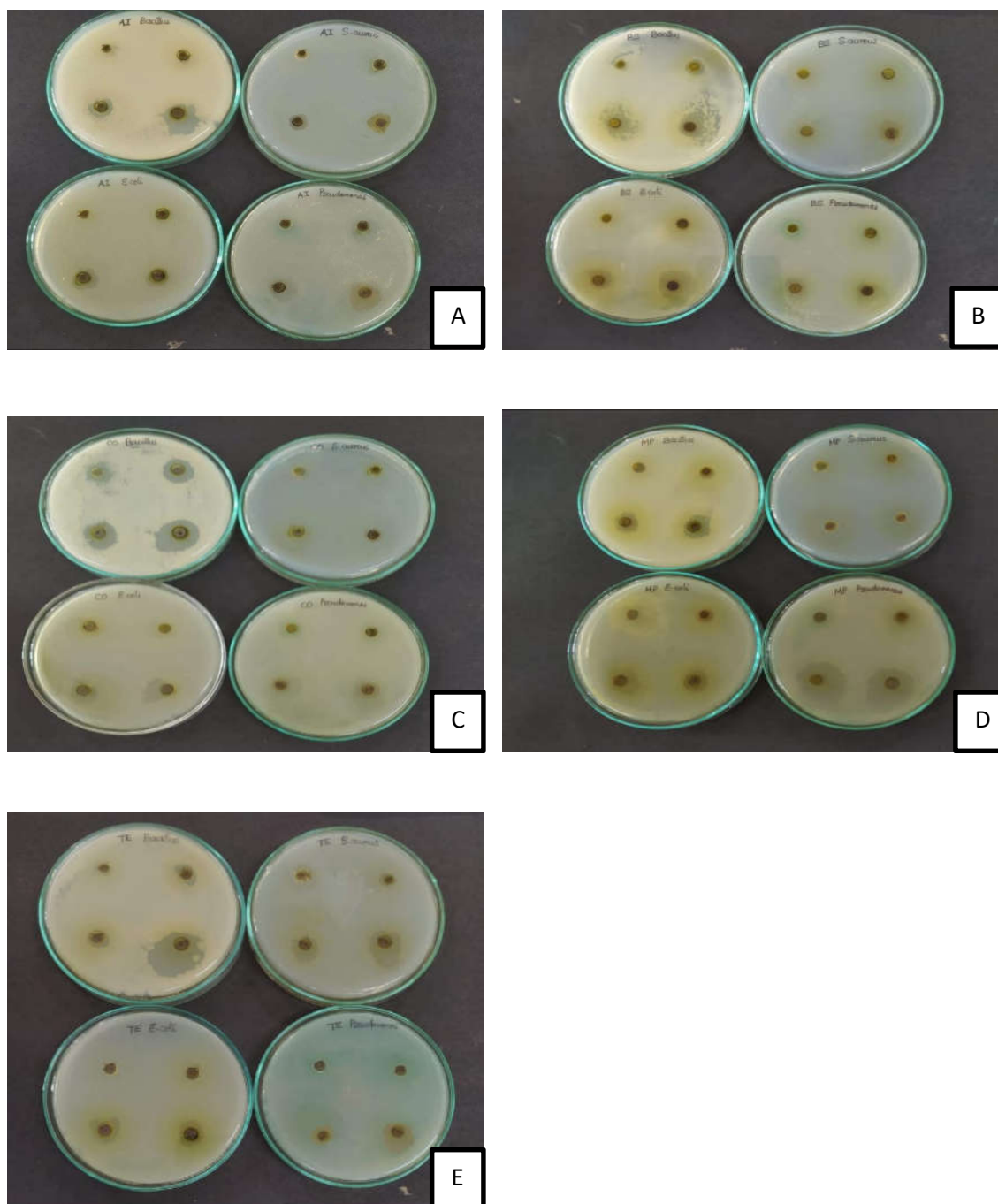


Figure 5. Antibacterial activity of the plant extracts against *Bacillus cereus*, *S. aureus*, *E. coli* and *Pseudomonas aeruginosa* with concentrations of 25, 50, 75 and 100. A) AI B) BS C) CO D) MP and E) TE

The results showed the antibacterial property of selected plants. For most plant extracts, the zone of inhibition increases with an increase in concentration suggesting a dose dependent antibacterial effect.

Among them considerable notification was exhibited by *Tagetes erecta* which showed zone of inhibition on all bacterial isolates taken (Figure 5). The phytochemicals present in the plants were useful in their antimicrobial activity against the pathogens (Karpagam *et al.*, 2019). Jafari *et al.*, 2017 reported the antimicrobial properties of *Tagetes erecta* against various infectious microorganisms.

4. Conclusion

The phytochemical profiling of the five selected plants such as *Azadirachta indica*, *Biophytum sensitivum*, *Chromolaena odorata*, *Mimosa pudica* and *Tagetes erecta* demonstrated the occurrence of diverse bioactive compounds such as alkaloids, carbohydrates, flavonoids, quinones, phenols, steroids, tannins, saponins, terpenoids, protein and aminoacids with variations across each samples. However, the concentration or intensity of these compounds was significantly greater in *Tagetes erecta* compared to the other plants. The antioxidant assay showed free radical scavenging activity of all the selected plants indicating its possibility as a natural basis of antioxidants. It indicates the capacity of plants to combat oxidative stress. The results highlight that *Tagetes erecta* exhibited the most potent antioxidant activity among the tested plants. Antimicrobial activities of the five plants were also done by well diffusion assay. The results showed that all the plants have antimicrobial property upon various concentration but *Tagetes erecta* found to be more significant that suggesting a dose dependent antibacterial effect. From these findings *Tagetes erecta* found to be a promising candidate for the development of health supplements, pharmaceuticals, and other antioxidant-based products because of the presence of phytochemicals and antioxidants contributing to its antimicrobial efficacy. Future areas of investigation ought to concentrate on isolating and characterizing particular active constituents, determining their efficacy, and exploring their scalability for product development in nutraceuticals, skincare, and plant-based therapeutics.

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Conflict of interests

All authors have no conflicts of interest to disclose.

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