

Phytochemical Screening and Evaluation of Antibacterial and Antioxidant Properties of *Calotropis procera* and *Argemone Mexicana*

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Abstract: Medicinal plants are sources of various biologically active compounds and have been used in the treatment of numerous diseases for decades. In order to evaluate the phytochemical constituents, antibacterial, and antioxidant activities of the leaves of *C. procera* and *A. mexicana*, the plants were extracted in methanol and petroleum ether. Phytochemical screening and identification of secondary metabolites were performed through qualitative analysis. Antibacterial activities of the plant extracts were evaluated against four selected bacteria, *E. coli*, *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*, via the agar well diffusion method. Antioxidant activity was assessed through the DPPH free radical scavenging activity test.

The extract yields for *C. procera* in methanol and petroleum ether were 4.2% and 4.3%, respectively, while *A. mexicana* had yields of 6.2% and 4.3%, respectively. Primary metabolite analysis was positive for carbohydrate, reducing sugar, glycoside, protein, fat, and fixed oil content. Secondary metabolites tests showed that there were tannins, alkaloids, saponins, flavonoids, steroids, phenols, and coumarins present in the two plant species, but terpenoids were found only in *C. procera*. Antibiotic analysis results showed that there was selective antibiotic action, where *C. procera* was effective only against *E. coli*, while *A. mexicana* had action on *E. coli* and *K. pneumoniae*. The DPPH assay results showed that there was concentration-dependent antioxidant action in the two plants. At the concentration of 150 µg/mL, *C. procera* had an antioxidant capacity of 65.39%, while *A. mexicana* had an antioxidant capacity of 63.55%.

From the results, it can be concluded that the presence of *C. procera* and *A. mexicana* is associated with significant amounts of phytochemicals that show considerable antibacterial and antioxidant activities. The results confirm the use of these plants in traditional medicine and reveal prospects for developing natural antioxidants and antibiotics..

Keywords: *Calotropis procera*, *Argemone mexicana*, phytochemicals, antibacterial activity, antioxidant activity, DPPH test, medicinal plants

INTRODUCTION

Plants have been used for their medicinal properties in many human health care systems for time immemorial and have become one of the major sources for drug discovery and use to date. This is due to their wide range of chemicals, their biological acceptability, and comparatively lesser side effects as compared to their counterparts. As per the World Health Organization (WHO, 2019), a considerable number of people throughout the world make use of herbal medicine as the first choice for primary healthcare.



Of the many medicinal plants found throughout the world, *C. procera* from the Apocynaceae family and *A. mexicana* from the Papaveraceae family are found throughout the tropics and subtropics and have been extensively studied from an ethnopharmacological point of view. *C. procera* has been traditionally used to treat skin diseases, inflammation, wound healing, pain, and respiratory ailments, while *A. mexicana* is mostly used in traditional medicine to deal with microbial infections, digestive issues, and skin diseases (Harborne, 1998). The various pharmacological actions exhibited by these two plants are attributed to a variety of phytochemicals found in them, like alkaloids, flavonoids, tannins, saponins, glycosides, and phenols that are biologically active compounds.

Antimicrobial Resistance (AMR) has been a prevalent problem in recent times that has decreased the efficacy of numerous antibiotics commonly used in clinical practices, making them less effective for treatment (Cowan, 1999). This situation has necessitated the search for novel antimicrobial agents that have potential therapeutic significance. Plants that have antimicrobial properties are seen as one possible solution due to their phytochemical compounds that are capable of inhibiting microorganisms by destroying the cell walls, enzymes, and replication of genetic material (Newman & Cragg, 2016).

In light of this, the present study was conducted with an aim to evaluate the phytochemistry and antimicrobial effects of decoctions of *C. procera* and *A. mexicana*. The objective of the present study was to evaluate their efficacy against bacteria and thus establish scientific proof for their use.

II. MATERIALS AND METHODS

2.1 Sample collection

2.1 Collection of Samples

The freshly harvested samples of *C. procera* and *A. mexicana*, which were free from any diseases, were collected during the study period, i.e., October 2024, from local sites in Rajasthan. The selection of plant samples was based on the criteria of healthiness, being free from any disease, and having appropriate morphology according to botanical descriptions.

Upon collection of plant specimens, they were taken to the laboratory in sterile polythene bags to prevent contamination and retain their attributes. In the first instance, the leaves of plants were washed with running tap water to clean the surface from all impurities. Subsequently, the leaves were washed using distilled water.

The plant specimens were authenticated in the laboratory by Dr. Shahdab Hussain, Assistant Professor of Botany at the Department of Botany, Sangam University, Bhilwara, Rajasthan, using taxonomic keys. Voucher specimens were prepared from collected plant samples and stored in the herbarium of the Department of Botany, Sangam University, Bhilwara, Rajasthan, for documentation and future use. The other parts of the plants were dried in the laboratory at room temperature and stored in sealed bottles after reaching a constant weight.



(B)

Figure 1: (A) *Calotropis Procera* (B) *Argemone Mexicana*



2.2 Leaf extract preparation

The freshly collected plant leaves were washed thoroughly under flowing tap water to clear dust and impurities, followed by treatment with sterile distilled water to ensure proper surface sterilization. The cleaned leaves were shade-dried at ambient temperature to preserve thermolabile phytoconstituents and subsequently processed into a fine powder using a mechanical grinder. The powdered material was stored in airtight containers for extraction. The decoction techniques were employed for the preparation of crude leaf extracts.

Decoction method

The leaf samples, powdered, were then subjected to decoction extraction, in which each sample underwent a separate extraction process using 100 ml of methanol and 100 ml of petroleum ether. This process was carried out till the solvent became clear, meaning complete extraction of the soluble phytochemical compounds (Gul A. et al., 2022). The extracts were then filtered using Whatman No. 1 paper to ensure clarity, followed by evaporation of the solvents at room temperature. The dried crude extracts were then stored at 4 °C for future analysis.

Percentage yield calculation

The extraction yield was calculated by applying the formula suggested by Parveen et al. (2014). **Percent yield (%) = (Weight of dried crude extract / Weight of initial dried plant material) × 100**

2.3 Phytochemical screening

The crude leaf extracts, prepared by means of decoction and maceration, were analyzed through preliminary qualitative phytochemical screening using standardized methods as explained by Harborne et al. (1998), Gul A. et al. (2022), and Dubale et al. (2023). The screening included analysis to detect various primary and secondary plant metabolites such as carbohydrates, proteins, glycosides, amino acids, fats, alkaloids, flavonoids, saponins, terpenoids, tannins, phenolic compounds, steroids, coumarins, and anthraquinones. The qualitative phytochemical screening revealed preliminary details about the phytochemicals contained in the *C. procera* and *A. Mexicana* leaf extracts that were useful for antimicrobial screening.

2.3.1 Qualitative phytochemical tests

The primary metabolic products were detected through the use of standard biochemical techniques, including the Molish test, Barfoed test, Fehling test, Benedict test, Biuret test, Ninhydrin test, and Saponification test. The secondary plant metabolites were tested using methods like Ferric Chloride test, Dragendorff's test, Foam test, Liebermann-Burchard test, Alkaloid reagent test, and Anthraquinone tests using standard plant phytochemical techniques. Adapted from (Harborne et al., 1998; Gul A. et al., 2022)

Table 1. Qualitative phytochemical screening of *C. Procera* and *A. Mexicana* leaf for primary metabolites

Metabolites	Test	Procedure	Positive Result
Carbohydrate	Molisch test	1 mL extract + few drops of alcoholic α -naphthol solution + concentrated sulfuric acid	Violet ring formation
	Barfoed test	1 mL extract + 1 mL Barfoed reagent	Red precipitate
Reducing sugar	Fehling test	1 mL extract + 1 mL Fehling A + 1 mL Fehling B	Bricks' red colour appears.
	Benedict test	1 mL extract + a few drops of Benedict reagent	Red colour
Non-reducing polysaccharide	Iodine test	1 mL extract + a few drops of dilute iodine solution	Blue colour
Glycoside	Alkaline test	0.5 mL extract + NaOH + 1	Yellowish colour layer



		mL distilled water	formation
Amino acids and proteins	Biuret test	2 mL extract + 2 mL NaOH (4%) + few drops of CuSO ₄ (1%)	Violet or pink colour
	Ninhydrin test	1 mL extract + 3 drops Ninhydrin solution	Purple-blue colour
Fat and fixed oil	Saponification test	1 mL extract + few drops alcoholic KOH + one drop phenolphthalein	Soap formation

Modified from (Harborne et al., 1998; Gul A. et al., 2022; Dubale et al., 2023)

Table 2. Qualitative phytochemical screening of *C. Procera* and *A. Mexicana* leaf for secondary metabolites

Metabolites	Test	Procedure	Result
Alkaloids	Dragendorff's test	2 mL extract + a few drops of Dragendorff's reagent	Orange-brown precipitate
Saponins	Foam test	1 mL extract + 10 mL distilled water, shaken for 15 min	Stable foam formation
Flavonoids	Alkaline reagent test	1 mL stock solution + 1 mL dilute NaOH	Yellow-green colour
Steroids	Liebermann–Burchard test	1 mL crude extract + 10 mL chloroform + concentrated sulfuric acid	Bilayer formation (upper red, lower greenish)
Terpenoids	Salkowski test	0.5 mL crude extract + 2 mL chloroform + 3 mL sulfuric acid	Reddish-brown colour
Phenols	Ferric chloride test	2 mL extract + a few drops of 5% ferric chloride	Blue-black precipitate
Coumarins	Alkaline reagent test	2 mL extract + 1 mL 10% NaOH, shaken for 5 min	Yellow-green colour
Anthraquinones	Ammonia test	2 mL extract + 0.5 mL chloroform + 1 mL ammonia	Pink colour

2.4 Antimicrobial assay

2.4.1 Bacterial strains

Reference bacterial cultures of *Staphylococcus aureus* (MCC2043T), *Escherichia coli* (MCC3671T), *Klebsiella pneumoniae* (MCC2451T), and *Pseudomonas aeruginosa* (MCC2080T). The bacterial strains were procured from the National Centre for Microbial Resources. The cultures were stored at 4 °C and periodically sub-cultured to maintain viability. For antimicrobial assessment, fresh inocula were developed through incubating the bacterial strains for 24 h at 37 °C to obtain actively growing cultures.

2.4.2 Preparation of experimental extract and control samples

The crude methanolic leaf extract of *C. procera* and *A. mexicana* was mixed in 50% dimethyl sulfoxide (DMSO), and a Stock solutions were formulated at a 50 mg/mL concentration. Commercially available kanamycin discs were employed as the positive control, while 50% Dimethyl sulfoxide served as the solvent control during all antibacterial assays.



2.4.3 Determination of antibacterial activity using agar well diffusion

Antibacterial potential of crude leaf extract was tested by using the agar well diffusion method in Mueller-Hinton agar as mentioned by Naushad et al. (2012), with slight modifications. Firstly, Mueller Hinton agar was sterilized and then heated up to 45-50 °C and then used for inoculation with standardized bacterial suspension and then was poured onto sterile Petri plates to get a consistent thickness of medium.

When the medium was solidified, 3 wells having a diameter of 6mm each were prepared using a sterile cork borer. Wells were loaded with the amounts of 2.5 mg/mL and 5.0 mg/mL of plant extract, respectively. Kanamycin was used as a reference antibacterial drug. The same volume of 50% of dimethyl sulfoxide, used for preparing the extract, was used as a blank. Plates were kept at room temperature for 2 hours to let the extract diffuse in the medium. Then, after the process of diffusion, the plates were sealed and kept at 37°C in an incubator for 24 hours. Antibacterial activity was measured by taking the diameter of inhibition zones in the form of millimeters using an antibiotic zone reader, as mentioned by Sharmeen et al. (2012).

2.5 Evaluation of antioxidant activity

The antioxidative potential of the selected medicinal plant leaf extracts was determined by the DPPH free radical scavenging capacity assay technique, according to the procedure mentioned by Rosa Tundis et al. (2014). The DPPH solution was mixed with the various solvent extracts of the selected medicinal plants, and the reactions were incubated in the dark at room temperature. The absorbance value was noted using the UV-visible spectrophotometer, and the percentage scavenging activity was calculated. % DPPH Scavenging Activity = $\frac{Ac - As}{Ac} \times 100$

Ac = Absorbance of control

As = Absorbance of sample

2.6 Statistical evaluation of experimental data

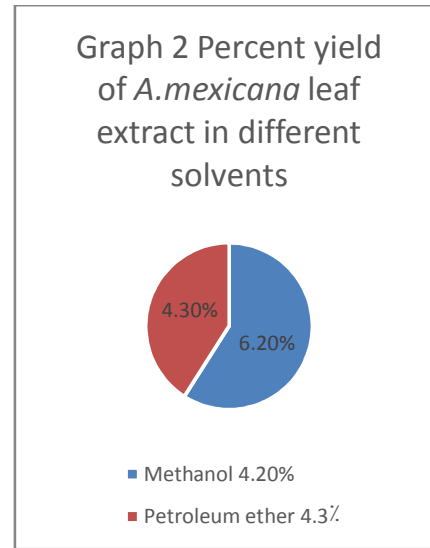
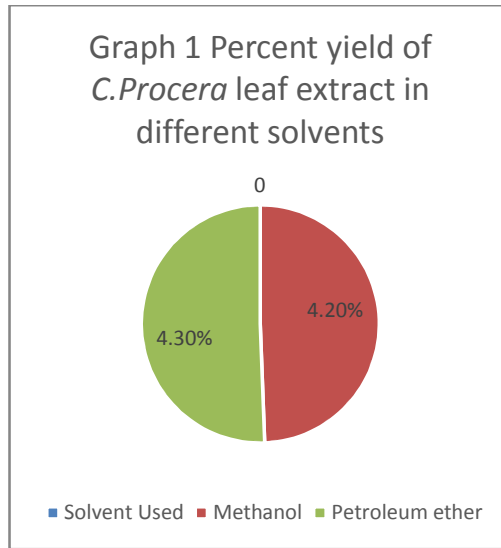
Statistical analysis of the experimental findings was executed using SPSS version 16.0, with triplicate values shown as mean ± standard deviation. Treatment differences were examined by applying one-way ANOVA in a completely randomized design, and further comparison among means was carried out using Tukey's honestly significant difference test. Significance was calculated at $p < 0.05$

III. RESULTS

3.1 Percent yield of crude extract

The percentages obtained in terms of the yields differed between various plant materials. In case of *C. procera*, for example, the methanolic decoction extract gave a yield of 4.2 % crude extract having a light green color, whereas in the petroleum ether decoction extract, a yield of 4.3% was obtained. Similarly, in the case of *A. mexicana*, the methanolic decoction extract gave a yield of 6.2%, but the petroleum ether decoction extract had a slightly lower yield of 4.3 % (Graph 1&2).





3.2 Phytochemical constituents identified in the extract

3.2.1 Primary metabolites

The preliminary phytochemical analysis of methanolic and petroleum ether extracts of the leaves of *C. procera* and *A. mexicana* indicated that these plants contained certain vital primary metabolites. Carbohydrates were observed in both plant species. Methanolic extract of *C. procera* exhibited a highly positive result in the Molisch test, suggesting a very high carbohydrate level. In contrast, the methanolic extract of *A. mexicana* had a moderate positive result. Both petroleum ether extracts had a slightly positive result. Barfoed's test was positive only for the petroleum ether extract of *C. procera*, but negative for other extracts.

For the presence of reducing sugars, Fehling's test and Benedict's test were done for the methanol extracts of *C. procera* and *A. mexicana*. For the absence of reducing sugars, the test was done on the petroleum ether extracts of *C. procera* and *A. mexicana*. Non-reducing polysaccharides were determined in the methanolic extract of *A. mexicana*, with a positive result on the basis of the iodine test. Other extracts gave negative results for non-reducing polysaccharides. Glycosides were detected in both plants. The methanolic extracts of *C. procera* and *A. mexicana* showed a positive reaction with some intensity. This indicated that glycosides were present in fairly large quantities in these samples. The petroleum ether extract of *A. mexicana* was positive but with some weak intensity, while *C. procera* was negative. Protein analysis using the Biuret test confirmed the presence of proteins in all extracts.

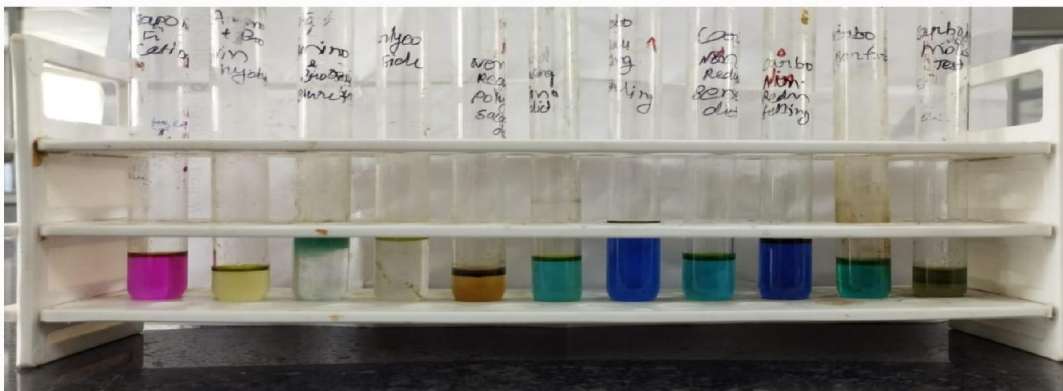
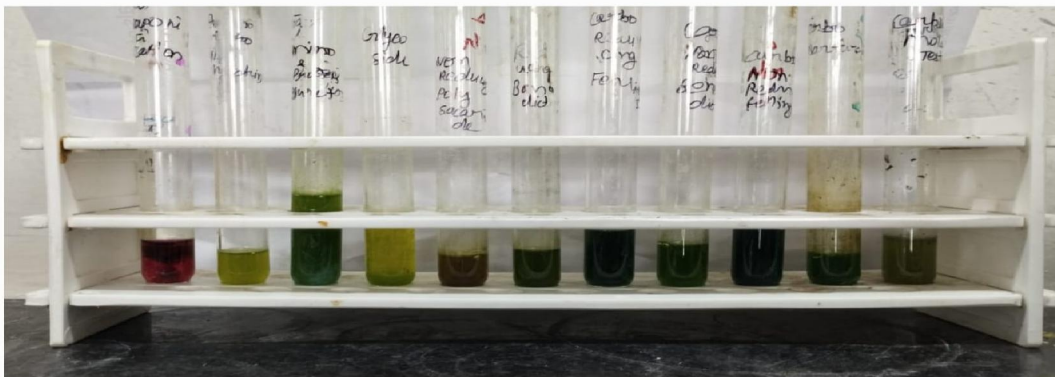
The methanolic extract of *C. procera* gave a moderate positive result, whereas the rest of the extracts revealed a weak positive response. Only amino acids were found to be present in the methanolic extract of *A. mexicana*, which gave a moderate positive result in the Ninhydrin test. In contrast, all other extracts were negative for amino acids. According to the saponification test, fats and fixed oils were found in the plants. Methanolic extracts were found to give a moderate positive response, indicating the high percentage of fats and fixed oils, whereas petroleum ether extracts gave a weak positive response. As a conclusion, it could be said that methanolic extracts of both *C. procera* and *A. mexicana* are richer in phytochemicals as compared to petroleum ether extracts (Table 3; Figure 2, 3)



Table 3. Qualitative phytochemical analysis of primary metabolites in leaf extracts of *C. Procera* and *A. Mexicana*, (+) = Presence of phytochemicals, (-) = Absence of phytochemicals, M - Methanol, P- Petroleum ether

S. No.	Primary Metabolites	Test Name	Result			
			<i>C. procera</i>		<i>A. Mexicana</i>	
			M	P	M	P
1.	Carbohydrate	Molisch	+++	+	++	+
		Barfoed	-	+	-	-
2.	Reducing Sugar	Fehling	+	-	+	-
		Benedict	+	-	+	-
3.	Non Reducing Polysaccharide	Iodine test	-	-	+	-
4.	Glycosides	Alkaline test	++	-	++	+
5.	Protein	Biuret	++	+	+	+
6.	Amino Acid	Ninhydrin	-	-	++	-
7.	Fat and fixed oil	Saponification	++	+	++	+

(A)

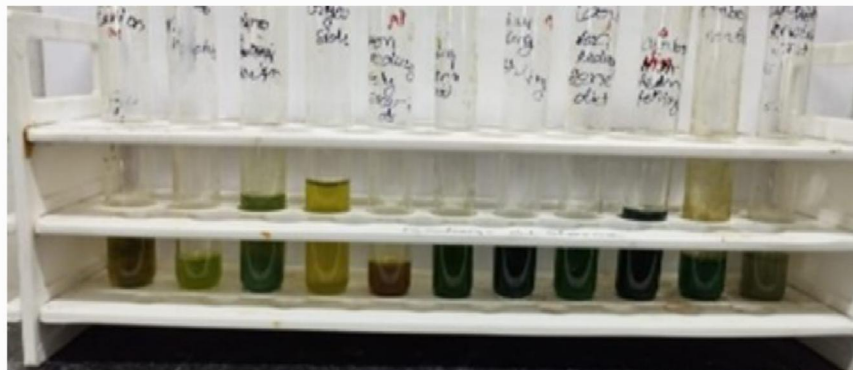


(B)

Figure 2: Qualitative phytochemical analysis of *C. procera* leaf for primary metabolites.



Methanol solvent (B) Petroleum ether solvent



(A)



(B)

Figure 3: Qualitative phytochemical analysis of *A. mexicana* leaf for primary metabolites. (A) Methanol solvent (B) Petroleum ether solvent

3.2.2 Secondary metabolites

Phytochemical analysis of the methanolic and petroleum ether extracts of leaves from *C. procera* and *A. mexicana* identified the existence of various secondary metabolites present in quantity. Tannins were present in both types of plants. Tannins in *C. procera* had a strong positive reaction in the ferric chloride test, thus indicating their higher presence than that in its petroleum ether extract, which had a moderate positive reaction. On the other hand, in *A. mexicana*, tannins in the methanolic extract had a moderate positive reaction, while none were present in its petroleum ether extract. Alkaloids were identified in all extracts through Dragendorff's test.

The methanolic extract of *C. procera* gave a moderate positive result, whereas the petroleum ether extract gave a weak positive result. The weak positive results were recorded for methanolic and petroleum ether extracts of *A. mexicana* as well, signifying the presence of alkaloids in them. All plant extracts, i.e., both plant species, gave positive results for the presence of saponins as determined by the foam test; however, the weak positive result indicates that there is a low amount of these substances in the extracts. Flavonoids were present in all extracts based on the alkaline reagent test. Steroids were found in both plant species.

For the case of *C. procera*, there was a highly positive response for the methanol extract in Liebermann-Burchard's test, implying that it contained more steroids. Conversely, the petroleum ether extract exhibited a weakly positive response. Similarly, for *A. mexicana*, the methanol extract produced a moderate positive response, while the petroleum ether extract showed a weakly positive response. Terpenoids were found to be present only in the methanol extract of *C. procera*, which gave a weak positive result in the Salkowski test. All other extracts did not give positive results in the terpenoid test. Phenolics were detected in both plants. Methanolic extracts gave moderately positive results,

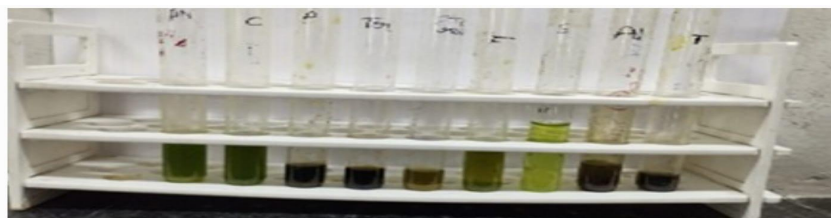


whereas petroleum ether extracts gave a weakly positive result, suggesting the presence of phenolics in lesser quantity. Coumarins were present in all the extracts, as observed by a weakly positive result obtained from each sample after adding the alkaline reagent test. Anthraquinones were not present in either of the two plants since all extracts gave negative results in the ammonia test.

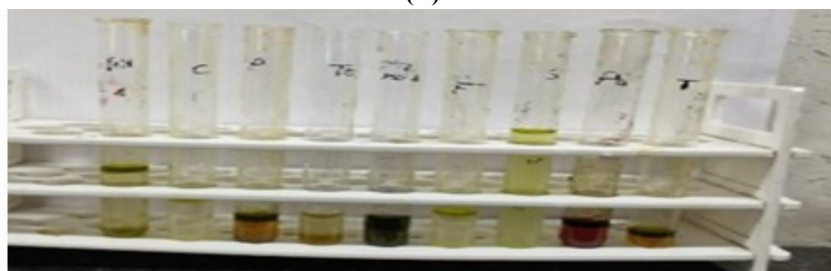
Generally, the extracts from both *C. procera* and *A. mexicana* showed more amounts of secondary metabolites as opposed to those that were extracted using petroleum ether. The compounds identified included tannins, steroids, phenols, alkaloids, flavonoids, saponins, and coumarins, but there was no presence of anthraquinones in any extract (Table 4, Figures 4 & 5).

Table 4. Qualitative phytochemical evaluation of secondary metabolites in leaf extracts of *C. Procera* and *A. Mexicana*, (+) = Presence of phytochemicals, (-) = Absence of phytochemicals, M -Methanol, P- Petroleum ether

S.No.	Secondary Metabolites	Test Name	Result			
			<i>C. procera</i>		<i>A. Mexicana</i>	
			M	P	M	P
1.	Tannin	Ferric Chloride	+++	++	++	-
2.	Alkaloids	Dragendorff's test	++	+	+	+
3.	Saponin	Foam test	+	+	+	+
4.	Flavonoids	Alkaline reagent test	+	+	+	+
5.	Steroids	Liebermann Burchard	+++	+	++	+
6.	Terpenoids	Salkowski reaction	+	-	-	-
7.	Phenol	Ferric Chloride	++	+	++	+
8.	Coumarin	Alkaline Reagent	+	+	+	+
9.	Anthraquinone	Ammonia test	-	-	-	-



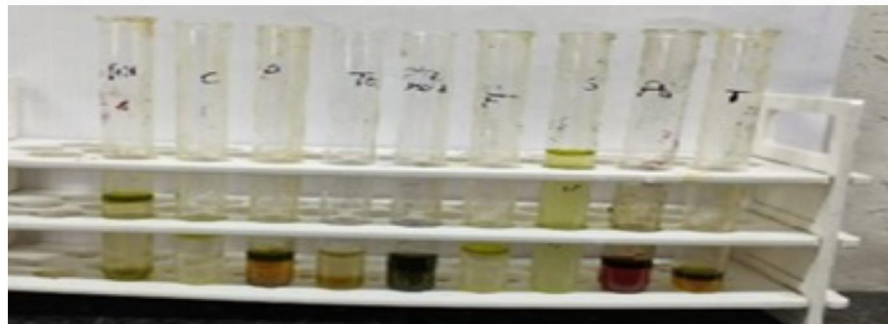
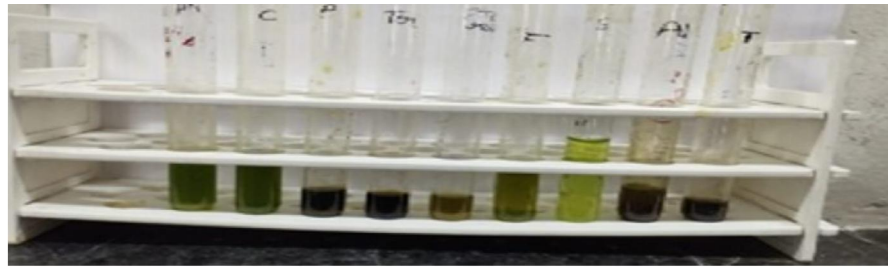
(A)



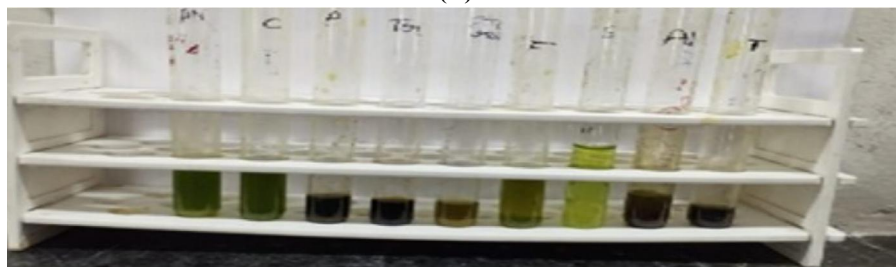
(B)

Figure 4: Qualitative phytochemical analysis of *C.procera* leaf for secondary metabolites (A) Methanol solvent (B) Petroleum ether solvent





(A)



(B)

Figure 5: Qualitative phytochemical analysis of *A. mexicana* leaf for secondary metabolites. (A) Methanol solvent (B) Petroleum ether solvent

3.3 Evaluation of antibacterial effect of methanolic leaf extract

The antibacterial effects of the leaf extract from *C. procera* and *A. mexicana* were tested on selected bacteria strains using the agar well diffusion technique, and the findings were presented as the zone of inhibition (mm). The bacterium *S. aureus* displayed no significant inhibition (NA) for the extracts from both *C. procera* and *A. mexicana* at the two concentrations tested (2.5 mg/mL and 5.0 mg/mL). On the other hand, the drug kanamycin produced an extensive

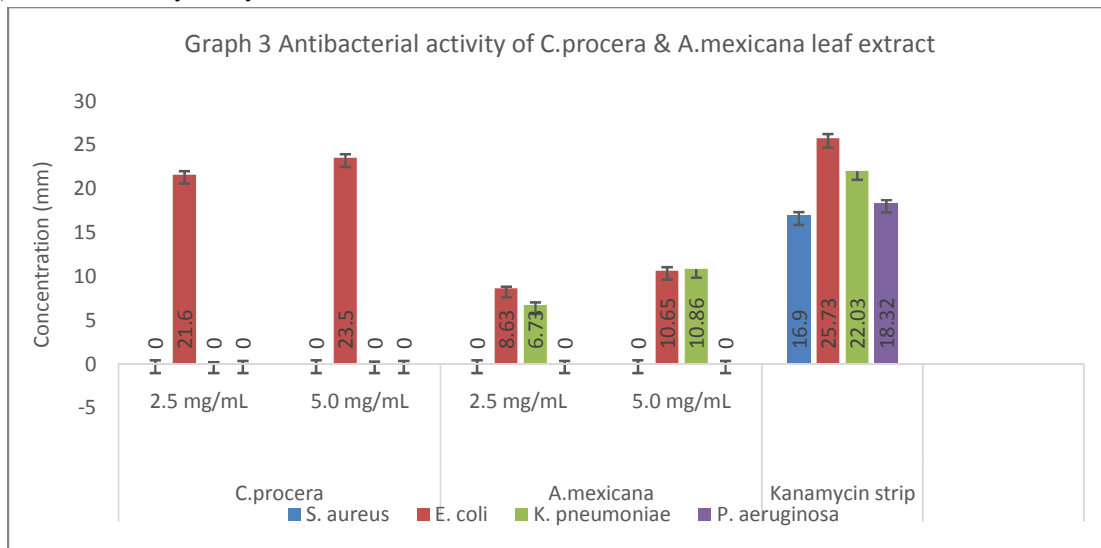


inhibition zone of 16.9 ± 0.45 mm, while DMSO showed no antibacterial effects. For the bacteria *E. coli*, *C. procera* was shown to be a highly active agent, with inhibition zones of 21.6 ± 0.43 mm and 23.50 ± 0.45 mm at concentrations of 2.5 mg/mL and 5.0 mg/mL, respectively. The *A. mexicana*, however, did not show high activity, with the following data for the zone: 8.63 ± 0.23 mm and 10.65 ± 0.43 mm. The standard antibiotic, Kanamycin, demonstrated the highest value, with the value for the zones being 25.73 ± 0.53 mm, while DMSO had no value. In the case of *K. pneumoniae*, there was no antibacterial activity in *C. procera* extract against the bacteria at the two concentrations. In this respect, *A. mexicana* had moderate antibacterial activity in relation to the zones obtained of 6.73 ± 0.26 mm at 2.5 mg/mL and 10.86 ± 0.31 mm at 5.0 mg/mL. As regards Kanamycin, the zones were 22.03 ± 0.33 mm, while DMSO had none. In regard to *P. aeruginosa*, there was no activity of the *C. procera* and *A. mexicana* extracts against the bacteria at both concentrations. In this respect, Kanamycin yielded a zone of 18.32 ± 0.40 mm, while DMSO yielded none. Generally, *C. procera* showed great antibacterial effects on *E. coli*, whereas *A. mexicana* demonstrated moderate effects on both *E. coli* and *K. pneumoniae*. Neither of the plant extracts was inhibitive to the growth of *S. aureus* and *P. aeruginosa*. Antibacterial effects were proportional to increasing concentrations, but generally Kanamycin proved more effective than both samples (Table 5; Graph 3; Figure 6,7).

Table 5 Comparative antibacterial activity of leaf extracts of selected medicinal plants against pathogenic bacterial strains.

Bacterial strains	Zone of inhibition (mm)					
	<i>C. procera</i>		<i>A. mexicana</i>		Kanamycin strip	DMSO
	2.5 mg/mL	5.0 mg/mL	2.5 mg/mL	5.0 mg/mL		
<i>S. aureus</i>	NA	NA	NA	NA	16.9 ± 0.45^a	NA
<i>E. coli</i>	21.6 ± 0.43^b	23.50 ± 0.45^a	8.63 ± 0.23^b	10.65 ± 0.43^a	25.73 ± 0.53^a	NA
<i>K. pneumoniae</i>	NA	NA	6.73 ± 0.26^b	10.86 ± 0.31^a	22.03 ± 0.33^a	NA
<i>P. aeruginosa</i>	NA	NA	NA	NA	18.32 ± 0.4^a	NA

Values are given as mean \pm SD of three replicates, and distinct superscript letters denote significant differences ($p < 0.05$) as determined by Tukey's HSD test.



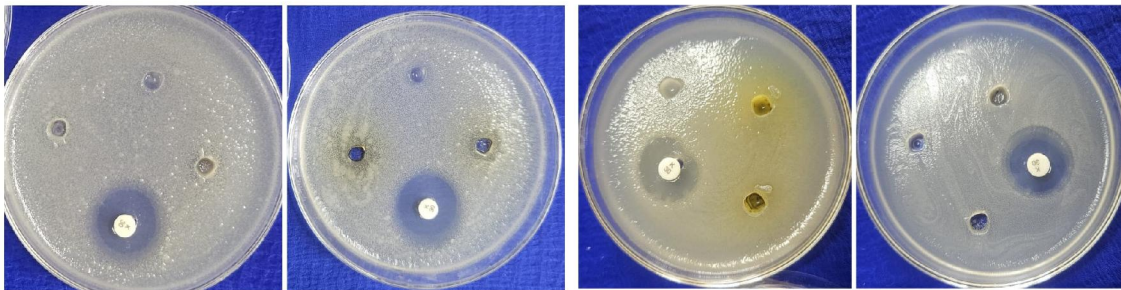
(A)



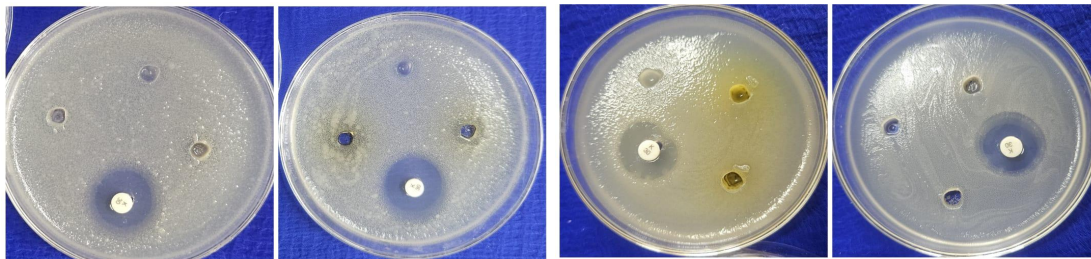


(C)

Figure 6. Antibacterial activity of methanolic leaf extracts of (A) *C. procera* against *E. coli*, (B) *A. mexicana* against *E. coli*, and (C) *A. mexicana* against *K. pneumoniae*.



(B)



(C)

(D)

Figure 7 Zone of inhibition produced by Kanamycin (positive control) against (A) *S. aureus*, (B) *E. coli* (C), *K. pneumoniae* (D), and *P. aeruginosa*.

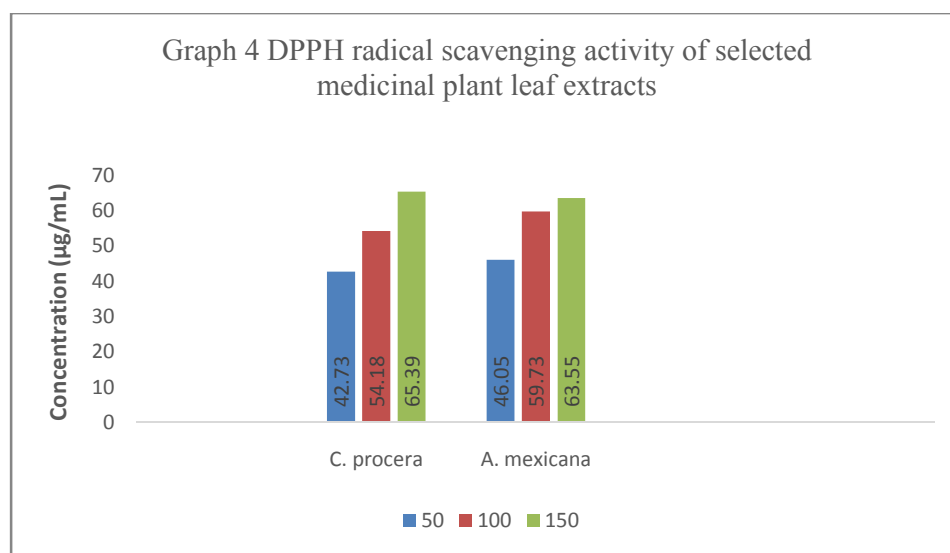
3.4 Antioxidant activity analysis

The antioxidant capacity of *C. procera* and *A. mexicana* methanolic extracts was measured using the DPPH free radical scavenging assay at concentrations of 50, 100, and 150 $\mu\text{g/mL}$. There was an evident concentration-dependent increase in the antioxidant capacity of both plant samples. For *C. procera* extract, scavenging activities increased from 42.73% at 50 $\mu\text{g/mL}$ (0.465 absorbance value) to 54.18% at 100 $\mu\text{g/mL}$ (0.372 absorbance value) and further increased to 65.39% at 150 $\mu\text{g/mL}$ (0.281 absorbance value). This was compared to the control absorbance value of 0.812. On the other hand, methanolic extracts of *A. mexicana* showed scavenging activities of 46.05%, 59.73%, and 63.55% at concentrations of 50, 100, and 150 $\mu\text{g/mL}$, with respective absorbance values of 0.438, 0.327, and 0.231. Based on this information, one can conclude that both samples were antioxidants with greater antioxidant capacity in *C. procera* compared to *A. mexicana* (Table 6; Graph 4)



Table 6 Antioxidant activity of selected medicinal plant extracts by DPPH assay

S.No.	Plant Name	Menstruum	Concentration (µg/mL)	Absorbance of Sample (As)	Absorbance of Control (Ac)	% DPPH Scavenging Activity
1	<i>C. procera</i>	Methanolic	50	0.465	0.812	42.73
			100	0.372	0.812	54.18
			150	0.281	0.812	65.39
2	<i>A. Mexicana</i>	Methanolic	50	0.438	0.812	46.05
			100	0.327	0.812	59.73
			150	0.231	0.812	63.55



IV. DISCUSSION

This study showed that both *C. procera* and *A. mexicana* contain substantial amounts of phytochemicals. The results of extractions show higher extraction yields using methanol than petroleum ether, especially in *A. mexicana*. This suggests that methanol was more effective in extracting phytochemicals, especially those that are polar. Phytochemical analysis indicated the presence of various primary and secondary metabolites such as carbohydrates, glycosides, proteins, tannins, alkaloids, saponins, flavonoids, phenols, steroids, and coumarins. Terpenoids were found only in *C. procera*. These phytochemicals prove that the plant species have medicinal properties, as reported by other studies (Moustafa et al., 2010; Kumar et al., 2023 ; Malik et al., 2023).

The antibacterial tests revealed selectivity on the part of the methanolic extracts. Whereas *C. procera* showed growth inhibition effects against *E. coli* bacteria, *A. mexicana* was active against both *E. coli* and *K. pneumoniae* bacteria. These antibacterial actions could be attributed to the presence of such active ingredients as phenolics, flavonoids, coumarins, among others, which have been shown to inhibit bacterial growth (Saddiq et al., 2022; El-Sherbiny et al., 2024). It has been established that both plant extracts possess concentration-dependent antioxidant activities, with increasing extract concentrations resulting in an increased ability to neutralize free radicals. In comparison with one another, *C. procera* proved to be slightly more effective as regards antioxidant actions. This antioxidant activity could be attributed to the presence of such active ingredients as phenolic and flavonoid compounds (Ahmad Nejjad et al.,



2023; Zhang et al., 2015). In summary, both tested plants possessed certain phytochemicals, along with having both antibacterial and antioxidant properties.

V. CONCLUSION

In conclusion, the current study found that the *C. procera* and *A. mexicana* plant extracts are good sources of bioactive chemicals. Both had antibacterial activity and antioxidative potentials, where *C. procera* had a relatively stronger antioxidative potential compared to *A. mexicana*. This could be attributed to the presence of phytochemicals such as flavonoids, phenols, tannins, and alkaloids in both plants. Further research is needed to identify the active ingredients in the two plants.

REFERENCES

- [1]. Ahmad Nejjhad, A., Alizadeh Behbahani, B., Hojjati, M., Vasiee, A., & Mehrnia, M. A. (2023). Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of *Calotropis procera* leaf aqueous extract. *Scientific Reports*, *13*(1), 14716. <https://doi.org/10.1038/s41598-023-42086-1>
- [2]. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*(4), 564–582. <https://doi.org/10.1128/CMR.12.4.564>
- [3]. Dubale, S., et al. (2023). Phytochemical screening and biological activities of medicinal plants: A review. *Journal of Medicinal Plants Research*, *17*(2), 45–60.
- [4]. El-Sherbiny, G. M., Alluqmani, A. J., Elsehemy, I. A., & Kalaba, M. H. (2024). Antibacterial, antioxidant, cytotoxicity, and phytochemical screening of *Moringa oleifera* leaves. *Scientific Reports*, *14*, 30485. <https://doi.org/10.1038/s41598-024-80700-y>
- [5]. Gul, A., et al. (2022). Standard methods for phytochemical analysis of medicinal plants. *International Journal of Pharmacognosy and Phytochemical Research*, *14*(3), 120–130.
- [6]. Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Chapman & Hall.
- [7]. Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., et al. (2023). *Major phytochemicals: Recent advances in health benefits and extraction method*. *Molecules*, *28*(2), 887. <https://doi.org/10.3390/molecules28020887>
- [8]. Malik, C., Mohanty, J. P., Pradhan, S., & Sharma, C. (2023). Phytochemistry and pharmacology of *Argemone mexicana* Linn—An Indian medicinal plant. *Research Journal of Pharmacognosy and Phytochemistry*, *15*(1), 27–32. <https://doi.org/10.52711/0975-4385.2023.00005>
- [9]. Moustafa, A. M. Y., Ahmed, S. H., Nabil, Z. I., Hussein, A. A., & Omran, M. A. (2010). Extraction and phytochemical investigation of *Calotropis procera*: Effect of plant extracts on the activity of diverse muscles. *Pharmaceutical Biology*, *48*(10), 1080–1090. <https://doi.org/10.3109/13880200903490513>
- [10]. Naushad, M., et al. (2012). Antibacterial activity of plant extracts using the agar well diffusion method. *Journal of Applied Microbiology Research*, *6*(2), 55–62.
- [11]. Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs. *Journal of Natural Products*, *79*(3), 629–661. <https://doi.org/10.1021/acs.jnatprod.5b01055>
- [12]. Parveen, S., et al. (2014). Extraction yield and phytochemical analysis of medicinal plants. *Asian Journal of Plant Science and Research*, *4*(3), 1–6.
- [13]. Saddiq, A. A., Tag, H. M., Doleib, N. M., Salman, A. S., & Hagagy, N. (2022). Antimicrobial, antigenotoxicity, and characterization of *Calotropis procera* and its rhizosphere-inhabiting actinobacteria: In vitro and in vivo studies. *Molecules*, *27*(10), 3123. <https://doi.org/10.3390/molecules27103123>
- [14]. Sharmeen, S., et al. (2012). Evaluation of antibacterial activity using the disc diffusion method. *Journal of Pharmaceutical Sciences and Research*, *4*(8), 1875–1880.
- [15]. Trease, G. E., & Evans, W. C. (2009). *Pharmacognosy* (16th ed.). Saunders Elsevier.



- [16]. Tundis, R., Loizzo, M. R., & Menichini, F. (2014). Evaluation of antioxidant activity of selected plant extracts: A DPPH assay. *Journal of Natural Products*, 77(7), 1549–1557.
- [17]. World Health Organization. (2019). *WHO global report on traditional and complementary medicine*. WHO Press.
- [18]. Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., & Li, H. B. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. *Molecules*, 20(12), 21138–21156. <https://doi.org/10.3390/molecules201219753>

