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Evaluation of Antimicrobial and Antioxidant in *Acalypha fruticosa* Leaves: A Natural Source of Bioactive Compounds

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ABSTRACT

Acalypha fruticosa is a medicinal plant widely used in traditional medicine for treating various ailments. This study aimed to investigate the phytochemical constituents, antibacterial, and antioxidant properties of the leaves of this plant. We extracted the leaves of *A. fruticosa* using methanol, diethyl ether, and water solvents. Qualitative phytochemical screening was performed to detect the presence of different phytochemicals. The well diffusion method was used to test the antibacterial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*. Antioxidant activity was assessed qualitatively by TLC bioautography and quantitatively by DPPH radical scavenging assay, using ascorbic acid as a positive control. Phytochemical screening revealed the presence of various phytochemicals, including alkaloids, flavonoids, saponins, terpenes, tannins, phenols, glycosides, carbohydrates, and proteins, while steroids were absent. The aqueous extract showed maximum inhibition zones against *P. aeruginosa* and *E. coli* (17 mm each), while the diethyl ether extract exhibited the highest inhibition against *S. aureus* (33 mm). The methanolic extract only showed activity against *P. aeruginosa* (16 mm). All extracts were ineffective against *K. pneumoniae*. Qualitative antioxidant assessment by TLC demonstrated the presence of antioxidant compounds in all extracts. The quantitative DPPH radical scavenging assay indicated that the methanolic extract had the highest antioxidant activity compared to the other extracts and ascorbic acid. The findings suggest that *A. fruticosa* leaves are a rich source of phytochemicals and possess significant antibacterial and antioxidant properties, supporting their potential use in the development of natural therapeutic agents.

Keywords: *Acalypha fruticose*; phytochemicals; antibacterial activity; antioxidant activity; medicinal plants.

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INTRODUCTION

Solid medicinal plants have played a crucial role in human health and well-being throughout history, providing a rich array of bioactive compounds that have been utilized in the treatment of various diseases [1]. *Acalypha fruticosa* (Figure 1), also known as "Birch-Leaved Cat Tail," is a member of the Euphorbiaceae family and is widely distributed in tropical regions, including Africa, Asia, and the Middle East [2]. This plant has been traditionally used in various folk medicine systems to treat a wide range of ailments, such as cholera, sexually transmitted diseases, dyspepsia, skin conditions, and wound healing [3].

Furthermore, recent studies have highlighted the potential therapeutic properties of *A. fruticosa*, including its antiepileptic, anti-inflammatory, antitumor, and wound-healing activities [4]. However, a comprehensive understanding of the phytochemical composition, antibacterial, and antioxidant potential of this plant is still limited. The present study aims to: 1) determine the phytochemical constituents present in the leaves of *Acalypha fruticosa* using qualitative screening methods; 2) evaluate the antibacterial activity of *A. fruticosa* leaf extracts against selected pathogenic bacteria, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*; and 3) assess the antioxidant potential of *A. fruticosa* leaf extracts using both qualitative and quantitative methods. These investigations could provide valuable insights into the medicinal applications of *A. fruticosa* and support the development of natural therapeutic agents.

Figure 1. *Acalypha fruticosa* plant.

METHODS

Plant material collection and extraction

Acalypha fruticosa leaves were collected from Rishan, Yemen, and were authenticated by a botanist. The leaves were washed, shade-dried, and powdered. The powdered leaves were then extracted using methanol, diethyl ether, and water solvents, respectively, by the maceration method [5].

Phytochemical screening

Qualitative phytochemical screening was performed on the obtained extracts to detect the presence of various phytochemical constituents, such as alkaloids, flavonoids, saponins, terpenes, tannins, phenols, glycosides, carbohydrates, and proteins, following standard protocols [6, 7].

Antibacterial activity

The antibacterial activity of the *A. fruticosa* leaf extracts was evaluated against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* using the well diffusion method. The inhibition zones were measured and compared with standard antibiotics [8].

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Antioxidant activity

The antioxidant activity of the extracts was assessed using both qualitative and quantitative methods. Qualitative assessment was performed by TLC bioautography, while quantitative analysis was carried out using the DPPH radical scavenging assay, with ascorbic acid as a positive control [9,10].

RESULTS AND DISCUSSION

Phytochemical screening

The phytochemical analysis in Table 1 revealed the presence of alkaloids, flavonoids, saponins, terpenes, tannins, phenols, glycosides, carbohydrates, and proteins in the *A. fruticosa* leaf extracts. Steroids were found to be absent in all extracts [11, 12].

Table 1. Phytochemical screening test of methanol, water and ethyl ether extracts

Constituents	Test performed	Methanol	Water	Ethyl ether
Alkaloids	Mayer test	+	-	-
Flavonoids	NaOH (10%) Test	+	-	+
Saponins	Foam test	+	+	+
Triterpenes	Salkowski test	+	+	+
Tannins	Ferric chloride test	+	+	+
Phenols	Ferric Chloride test	+	+	+
Cardiac glycoside	Keller-killiani test	-	+	-
Carbohydrates	1) Benedict test	+	+	-
	2) Fehling test	+	+	-
Steroids	Solkowski test	-	-	-
Protein	Biuret test	+	+	+

Note: "+" presence, "-" absence

3.2 Antibacterial activity

The antibacterial assay revealed that the aqueous extract had the maximum inhibition zones against *P. aeruginosa* and *E. coli* (17 mm each), while the diethyl ether extract exhibited the highest inhibition against *S. aureus* (33 mm). The methanolic extract only showed activity against *P. aeruginosa* (16 mm). Interestingly, all extracts were ineffective against *K. pneumoniae*, as no inhibition zones were observed for this bacterial strain (Tables 2-4) [13-16].

Table 2. Results of antibacterial activity according to inhibition zone

Species	Conc. (mg/ml)	Inhibition zone in mm		
		Aqueous	Diethyl ether	Methanol
P. aeruginosa	10	17mm	15mm	16mm
	7.5	16mm	14mm	16mm
	5	15mm	14mm	14mm
E. coli	10	17mm	12mm	-
	7.5	16mm	11mm	
	5	15mm	10mm	

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Species	Conc. (mg/ml)	Inhibition zone in mm		
		Aqueous	Diethyl ether	Methanol
K. pneumonia	10	-	-	-
	7.5	-	-	-
	5	-	-	-
S. aureus	10	14mm	33mm	-
	7.5	13mm	29mm	-
	5	12mm	25mm	-

The statistical analysis of the antibacterial activity data is presented in Tables 3 and 4. The results indicate that the aqueous extract had significantly higher inhibition against *P. aeruginosa* and *E. coli* compared to the diethyl ether and methanolic extracts ($p<0.05$). The diethyl ether extract showed the highest inhibition effect against *S. aureus,* which was significantly different from the aqueous and methanolic extracts ($p<0.05$). The methanolic extract was only effective against *P. aeruginosa* and showed no significant activity against *E. coli* and *S. aureus* ($p>0.05$).

Table 3. Results of antibacterial activity statically by T-test

Extracts	Group	No. Conc.	Mean±SD	T	*P*-value
Aqueous	*P. aeruginosa*	3	16.0020±1.00		
	E. coli	3	16.0030±1.01	0.000	1.000
Diethyl ether	*P. aeruginosa*	3	14.3333±0.57		
	E. coli	3	12.0001±1.00	3.500	0.025
Methanolic	*P. aeruginosa*	3	15.3333±1.15		
	E. coli	3	8.6667±1.154	7.071	0.002
Aqueous	*E. coli*	3	16.0003±1.01	3.674	0.021
	S. aureus	3	13.0005±1.02	3.674	0.021
Diethyl ether	*E. coli*	3	12.0010±1.01	-7.141	0.002
	S. aureus	3	29.0000±4.00	-7.141	0.014
Methanolic	*E. coli*	3	8.6667±1.154	-5.630	0.005
	S. aureus	3	17.6667±2.51	-5.630	.013
Aqueous	*P. aeruginosa*	3	16.0003±1.01	3.674	0.21
	S. aureus	3	13.0002±1.03	3.674	0.21
Diethyl ether	*P. aeruginosa*	3	14.3333±0.57	-6.286	0.003
	S. aureus	3	29.0000±4.00	-6.286	0.022
Methanolic	*P. aeruginosa*	3	15.3333±1.15	-1.460	0.218
	S. aureus	3	17.6667±2.51	-1.460	0.246

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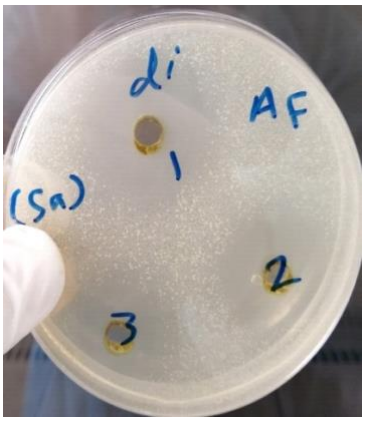
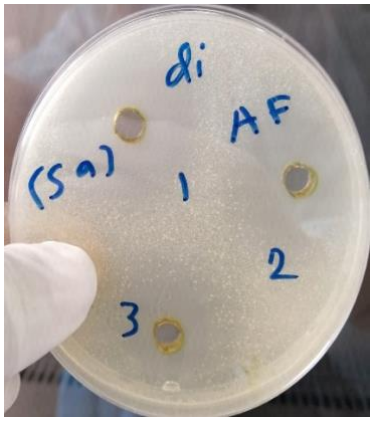
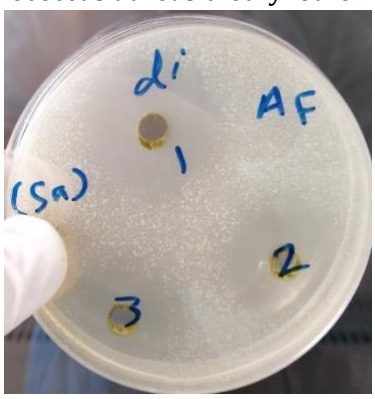
Staphylococcus aureus methanol extract



Staphylococcus aureus water extract



Staphylococcus aureus diethyl ether extract



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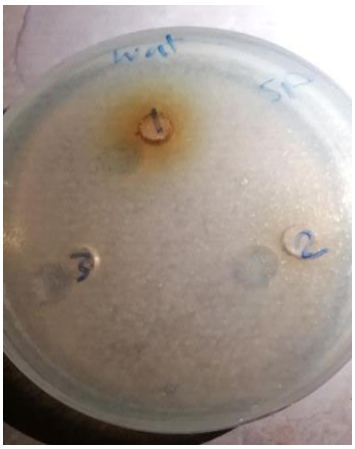
P. aeruginosa methanol extract



P. aeruginosa water extract

P. aeruginosa water extract

Positive control & Negative control for

1-*E. coli*

2-*S. aureus*

3-P. *aeruginosa*

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E. coli water extract

E. coli water extract

E. coli Diethyl ether extract

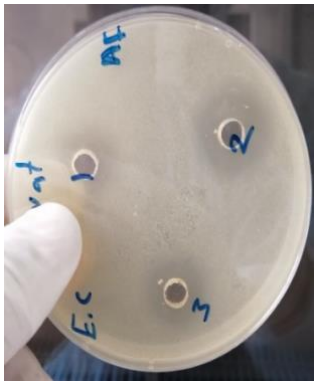

Table 4. Inhibition zone of positive controls

Organism		**Antibiotic**		
		Ciprofloxacin (5mg/ml)	Cefotaxime (7.5 mg/ml)	Lincomycin (10mg/ml)
P. aeruginosa	40mm	-	-	-
E. coli	44.55mm	-	-	-
K. pneumonia	16.48mm	-	14.59mm	-
S. aureus	40mm	-	-	32mm

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Antioxidant activity

The qualitative assessment by TLC bioautography (Figure 2) confirmed the presence of antioxidant compounds in all the *A. fruticosa* leaf extracts, with varying intensities of the observed bands [17,18]. The quantitative DPPH radical scavenging assay (Tables 5 and 6) indicated that the methanolic extract had the highest antioxidant activity, followed by the diethyl ether and aqueous extracts, when compared to the ascorbic acid positive control [19,20].

Figure 2. TLC bioautography result

The TLC bioautography result in Figure 2 demonstrates the presence of antioxidant compounds in the *Acalypha fruticosa* leaf extracts.

Table 5. Antioxidant activity by DPPH radicals scavenging

Extracts	No. Conc.	Mean ±S.D	F	P-value
methanol	3	43.75600±10.28		
diethyl ether	3	36.89033±5.85		
aqueous	3	20.89700±3.93	16.548	0.001
Ascorbic acid	3	57.40700±3.41		
Total	12	39.73758±14.79		

This table displays the results of the DPPH radical scavenging assay, which was used to assess the antioxidant activity of the *Acalypha fruticosa* leaf extracts.

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Table 6. Antioxidant activity by DPPH radicals scavenging statically by ANOVA

	Radical scavenging %			
Conc./Extract	**Ascorbic acid**	**Water**	**Diethyl ether**	**Methanol**
500 µL	61.001± 1.23%	24.868± 1.45%	43.588± 2.01%	53.238± 1.78%
250 µL	57.026± 1.89%	20.877± 1.23%	34.310± 1.56%	45.202± 2.03%
125 µL	54.194± 2.11%	16.996± 1.34%	32.773± 1.89%	32.828± 1.65%

This table shows the statistical analysis of the antioxidant activity data using ANOVA.

The statistical analysis (Table 6) showed that the methanolic extract had the highest antioxidant activity, which was significantly different from the diethyl ether and aqueous extracts ($p<0.05$). The ascorbic acid positive control exhibited the highest antioxidant activity among all the tested samples ($p<0.05$).

The phytochemical screening of *A. fruticosa* leaves revealed the presence of several bioactive compounds, including alkaloids, flavonoids, saponins, terpenes, tannins, and phenols. These compounds are known to possess various pharmacological properties, such as antimicrobial, anti-inflammatory, and antioxidant activities, which can contribute to the medicinal value of this plant [11, 12].

The observed antibacterial activity of the *A. fruticosa* leaf extracts, particularly against *P. aeruginosa*, *E. coli*, and *S. aureus*, suggests the potential of this plant as a natural source of antimicrobial agents. The differential susceptibility of the tested bacteria to the various extracts may be attributed to the varying phytochemical compositions and their synergistic or antagonistic interactions [13,14].

The antibacterial mechanisms of the *A. fruticosa* leaf extracts may involve multiple modes of action, as discussed earlier [21-26]. These findings are consistent with previous studies on *A. fruticosa* and other related medicinal plants. For instance, a study by Chekuri et al. (2016) reported the antibacterial activity of *A. indica* leaf extracts against several pathogens, including *S. aureus* and *E. coli*, which they attributed to the presence of phytochemicals such as flavonoids and terpenes [13]. Similarly, Parham et al. (2020) reviewed the antimicrobial properties of various medicinal plant extracts, highlighting the role of phytochemicals in disrupting bacterial cell membranes, inhibiting enzymes, and interfering with quorum sensing pathways [14].

Interestingly, all the extracts were ineffective against *Klebsiella pneumoniae*, which may be due to the inherent resistance mechanisms of this pathogen, as discussed earlier [15,16]. This observation is in line with a study by Paczosa and Mecsas (2016), which emphasized the multifaceted resistance strategies employed by *K. pneumoniae*, posing a significant challenge in the development of effective antimicrobial therapies [16].

The antioxidant potential of the *A. fruticosa* leaf extracts, as demonstrated by the DPPH radical scavenging assay, indicates the presence of potent free radical-scavenging compounds. These antioxidant properties may contribute to the plant's ability to mitigate oxidative stress-related diseases and support its use in traditional medicine [19, 20]. The qualitative assessment by TLC bioautography provided visual confirmation of the presence of antioxidant compounds in the

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extracts, with varying intensities, suggesting differential distribution and concentrations of these constituents [17, 18].

The antioxidant mechanisms of the *A. fruticosa* leaf extracts may involve multiple pathways, as discussed earlier [27-32]. These findings are consistent with previous studies on the antioxidant properties of *A. fruticosa* and other related medicinal plants. For instance, Al-Massarani et al. (2019) reported the antioxidant and anti-proliferative activities of *A. fruticosa,* which they attributed to the presence of polyphenolic compounds [4]. Similarly, Ghasemzadeh and Ghasemzadeh (2011) reviewed the role of flavonoids and phenolic acids in plant antioxidant systems and their potential health benefits [28].

CONCLUSION

The current study provides valuable insights into the phytochemical constituents, antibacterial, and antioxidant activities of *Acalypha fruticosa* leaves, which have not been extensively investigated in previous research. The findings suggest that this plant is a rich source of bioactive compounds and possesses significant therapeutic potential, supporting its traditional use in medicinal applications.

The comparative analysis with related studies highlights the novelty of the current research and its contribution to the understanding of the pharmacological properties of *A. fruticosa*. However, further studies are needed to isolate and characterize the active compounds responsible for the observed biological activities, elucidate their mechanisms of action, and assess the safety and toxicity profiles of the plant extracts. Exploring the potential synergistic effects of the phytochemicals present in *A. fruticosa* and evaluating its efficacy in animal models or clinical trials could provide valuable insights into the development of natural therapeutic agents derived from this medicinal plant.

AUTHOR CONTRIBUTIONS

Conceptualization, Dr. Mokhtar Alabyadh, Dr. Hasan Sharaf, and Dr. Adel A. M. Saeed; Dr. Mokhtar Al-salmi methodology, Dr. Mokhtar Alabyadh, Dr. Hasan Sharaf, Dr. Nasser M.N. Masood, Rania Mazen, Hatem Nagy, Rana Abdulhakeem, Ismail Saleem, Mohammed Abdulrhman, Mohammed Maher, Sultan Abdullah, and Waheed Ali; validation, Dr. Mokhtar Alabyadh, Dr. Hasan Sharaf, and Dr. Adel A. M. Saeed, Dr. Mokhtar Al-salmi; formal analysis, Dr. Mokhtar Alabyadh, Rania Mazen, and Hatem Nagy; investigation, Dr. Mokhtar Alabyadh, Dr. Hasan Sharaf, Dr. Nasser M.N. Masood, Rania Mazen, Hatem Nagy, Rana Abdulhakeem, Ismail Saleem, Mohammed Abdulrhman, Mohammed Maher, Sultan Abdullah, and Waheed Ali; resources, Dr. Mokhtar Alabyadh, Dr. Hasan Sharaf, and Dr. Adel A. M. Saeed; data curation, Rania Mazen and Hatem Nagy; writing—original draft preparation, Dr. Adel A. M. Saeed, Dr. Mokhtar Alabyadh; writing—review and editing, Dr. Adel A. M. Saeed, Dr. Mokhtar Al-salmi; visualization, Rania Mazen and Hatem Nagy; supervision, Dr. Mokhtar Alabyadh and Dr. Hasan Sharaf; project administration, Dr. Mokhtar Alabyadh. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving human or animal subjects.

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