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Phytochemical Screening, Chemical Composition, and Antimicrobial Activities of *Abutilon pannosum* (Forst.f.) Schlecht. Collected from Shada Mountain, Al- Baha Region, Saudi Arabia

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THIS STUDY was conducted to determine the phytochemical constituents and *in vitro* antimicrobialactivity of *Abutilonn pannosum* (Forst.f.) Schlecht, locally known as 'ragged mallow, leaf extracts growing wildly in Al-Baha region, Saudi Arabia. The plant leaves were collected, air-dried, macerated, and then extracted with ethanol, chloroform and hot water. The phytochemical constituents and antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus*),Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*), and the yeast fungus *Candida albicans* were determined. Results showed that the extracts had saponins, coumarin, alkaloids, tannins, flavonoids, and steroids. GC/MS analysis of ethanol extract revealed 32 compounds, the most important of which were 9-Octadecenoic acid (Z)-, methyl ester, and methyl 10-trans,12-cis-octadecadienoate, while chloroform extract revealed 36 bioactive compounds, the most important of which was phytol, and aqueous extract revealed 43 bioactive compounds, the most important of which was benzyldiethyl (2,6-xylylcarbamoylmethyl) ammonium benzoate. Ethanol extract's antimicrobial activity increased with concentration, from inactive at 25-50mg/mL to moderately active at 100-200mg/mL to active at 300mg/mL. At all concentrations, the activity of chloroform extract was slightly higher, ranging from moderately active to active. The chloroform extract was most effective against all microorganisms tested compared to ethanol and water extracts. This plant can be useful as antimicrobial agent against pathogenic microorganisms, which may be used in food and pharmaceutical industry.

Keywords: *A. pannosum*, Antimicrobial activity, Chemical composition, Phytochemical.

Introduction

Plants are rich source of food supplements, modern medicines, folkmedicines, pharmaceutical intermediates and chemical entities for synthetic drugs due to presence of numerous bioactive phytochemicals, essential mineral elements and other pharmacological properties (Sathish et al., 2021). Plants have proven to be a massive reservoir of biologically active compounds with a diverse spectrum of chemical structures and disease-fighting powers (Kavitha & Satish, 2013).Medicinal plants are used for medicinal purposes practically in every culture, and plant parts such as the bulb, leaves, roots, barks, peels, seeds, and flowers are employed in traditional medicine (Aadesariya et al., 2017c).Plant diversity is important for maintaining and preserving ecological balance and stability around the planet, and diverse plant species have been used in ethnomedicine from ancient times (Aati et al., 2019).

Plants have long been regarded to be a possible source of biologically active medications, and they have served humans in a number of traditional capacities from the dawn of time (Saleem et al., 2020). The presence of numerous phytoconstituents such as alkaloids, tannins,

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flavonoids, and phenolic chemicals gives plants their therapeutic significance (Edeoga et al., 2005). Plants contain a wide range of compounds with antibacterial, anticancer, anti-inflammatory, analgesic, antiviral, and antitumor properties, including phenols, glycosides, flavonoids, phenolics, saponins, cyanogenic glycosides, betalains, amines, alkaloids, tannins, terpenoids, and a few different endogenous metabolites (Aadesariya et al., 2017b; 2019). Plant-derived bioactive non-nutrient compounds that are useful to human health and disease prevention are known as phytochemicals (Bano & Deora, 2019). Alkaloids, cardiac glycosides, and steroids were discovered during a preliminary phytochemical screening of *Abutilon pannosum* (Kamel et al., 2017).

Around 80% of the world's population believes in traditional medicines for primary health care, the majority of which comprise plant extracts (Sandhya et al., 2006). Plant-derived medications have a number of advantages over synthetic drugs, including being safer and providing more therapeutic benefits while also being more inexpensive (Grierson & Afolayan, 2005). Antibiotic abuse has resulted in the rise of multidrug-resistant human bacterial infections (Elabd et al., 2015). Extensively resistant clinical bacterial isolates make it difficult to control and manage human infections (Magiorakos et al., 2012). Antimicrobial resistance has emerged, posing a serious threat to human life (El Sayed et al., 2019).

The Malvaceae family includes *A. pannosum*, which grows in tropical, subtropical, and temperate climates (El Sayed et al., 2019). The leaves of *A. pannosum* are used to cure dehydration, diarrhea, bronchitis, pile grumbles, gonorrhea, reduce fever in diabetics, hemorrhoids, and anemia, treat vaginal infection, and clean wounds and ulcers, as well as adjunct to medicines used for stack complaints (Aadesariya et al., 2017c, d; Mohammed et al., 2021). Due to the presence of a substantial amount of mucilage, the *Abutilon* species are responsible for the treatment of specific health conditions, such as rheumatoid arthritis, and are also employed as a diuretic and demulcent (Ali et al., 2014). This study is conducted to evaluate the chemical, biological and antimicrobial properties of *Abutilon pannosum* medicinal plant widely grown in Shada mountain, Al-Baha area, Saudi Arabia.

Materials and Methods

Collection of plant material

Fresh leaves of *Abutilon pannosum* were collected from Shada mountain, Al-Baha area, Kingdom of Saudi Arabia during the period March-April 2021, and used to prepare the ethanol, chloroform, and aqueous extracts. The plant was taxonomically identified and authenticated by Dr. Entsar Abu Shenab Rizgalla Hammad, Department of Biology, Faculty of Science and Arts, Al-Mikhwah, Al-Baha University. The voucher specimens were identified according to Chaudhary (2001) after comparison with voucher specimens deposited at the Herbaria of King Saud University, Saudi Arabia. A voucher specimen was deposited in the department for further use. The leaves were washed with fresh water to remove soil and dust particles and cut into small pieces, dried under shade for two weeks, macerated with an electric grinder into a fine powder, and stored frozen for further use in the preparation of extracts.

Preparation of plant extract

Preparation of ethanol and chloroform extracts

Extraction was carried out according to Sukhdev et al. (2008). A finely powdered plant sample (200g) was soaked with chloroform and ethanol separately, and the extraction was carried out for three days with daily filtration and evaporation of the solvent under reduced pressure using rotary evaporator apparatus and stored in the refrigerator till used.

Preparation of aqueous extract

Extraction was carried out according to Sukhdev et al. (2008). A finely powdered plant sample (200g) was extracted by soaking in 100mL hot distilled water for four hours with continuous stirring. After cooling, the extract was filtered using a filter paper and stored in the refrigerator till used.

Determination of the yield of plant extract

The sample extract was allowed to air dry in an evaporating dish till complete dryness and the yield was calculated as follows:

(Weight of extract obtained)/(Weight of plant sample) X 100

Preliminary phytochemical screening

The phytochemical analysis was carried out to determine the saponins, coumarin, alkaloids, tannins, flavonoids, and steroids according to the

methods of Njoku & Obi (2009) and Alloui et al. (2020).

Analysis of ethanol, chloroform and aqueous extracts by GC/MS

Various components of ethanol, chloroform, and aqueous extracts were identified and quantified using a gas chromatography/mass spectrophotometer (GC/MS-QP2010-Ultra, Shimadzu Company, Japan). The sample extract (0.1µL) was dissolved in ethanol and injected in a split mode (a ratio of 1:10) into the capillary column (Rtx-5ms-30m×0.25mm×0.25µm), and helium as the carrier gas, passed with a flow rate of 1.61 ml/min. The oven temperature program was started from 60°C with the rate of 10°C/min to 300°C as the final temperature with 6 min holding time. The injection port temperature was 300°C, the ion source temperature was 200°C and the interface temperature was 250°C. The sample was analyzed using the scan mode in the range of m/z 40-500 charges to ratio and the total run time was 30min. The chemical constituents of the extracts were identified by comparing their retention times and mass spectra (MS) with the reference spectra in the mass spectrometry data center of the National Institute of Standards and Technology (NIST), and the results were recorded (Boudjema et al., 2018).

Determination of antimicrobial activity

Antimicrobial activity test

Microorganisms: Bacterial strains of *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853), in addition to the fungal strain *Candida albicans* (ATCC 7596) were used for antimicrobial activity tests.

Preparation of bacterial suspensions: Aliquots (1mL) of a 24h broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°C for 24h. The bacterial growth was harvested and washed off with 100mL sterile normal saline, to produce a suspension containing about 10^8-10^9cfu/mL, which was stored in the refrigerator at 4°C till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique (Miles & Misra, 1938). Serial dilutions of the stock suspension were prepared in a sterile normal saline solution and 0.02mL volumes of the appropriate dilution were transferred by micropipette onto the surface of solidified nutrient agar plates, which were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37°C for 24h. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0.02mL) was multiplied by 50, and by the dilution factor to get the viable count of the stock suspension, expressed as cfu/ml suspension. Fresh stock suspensions were prepared each time. The stability of all the above-mentioned experimental conditions was ensured to obtain suspensions with very close and accurate viable counts.

Preparation of fungal suspension: The fungal cultures were maintained on Sabouraud dextrose agar (SDA) and incubated at 25°C for 4 days. The culture was then harvested, washed with sterile normal saline solution, and stored at 4°C in 100mL sterile normal saline solution until used.

Agar disc diffusion method: The disc diffusion method was used to test the antibacterial and antifungal activities of the extracts on Mueller Hinton agar (MHA) and SDA media, respectively (Alves et al., 2000; Mukhtar & Ghori, 2012; CLSI, 2017). Bacterial and fungal suspensions were diluted with a sterile physiological solution to 10^8cfu/mL (Turbidity= McFarland standard 0.5). One hundred microliters (100µL) of bacterial and fungal suspensions were swabbed uniformly on the surface of MHA and SDA, and the inoculums were allowed to solidify for 5min. Sterile filter paper discs (Whatman No.1, 6mm in diameter) soaked with 20µL of a solution of each plant extract, were placed on the surface of the MHA and SDA. The inoculated plates were incubated at 37°C for 24h in an inverted position. After incubation, the diameter (mm) of the inhibition zone was measured, and the antimicrobial activity was determined based on the diameter of the inhibition zone as follows: <9 mm zone (resistant strain); 9-12mm (partially sensitive strain); 13-18 mm (sensitive strain); >18 mm (very sensitive strain).

Statistical analysis

The statistical analysis was carried out using Statistical Analysis Systems (SAS, 2002). The general linear model (GLM) procedure was used. Duncan's multiple range test was conducted for

mean separation between treatments (P≤0.05). The data were reported as mean ± standard deviation of triplicate determination.

Results and Discussion

Yield of A. pannosum leaf extracts

Solvents with varying polarity, such as ethanol, chloroform, and hot water, were used to extract *A.pannosum*. The type of solvent had a significant (P<0.001) impact on percentage yield, with chloroform extract yielding the highest (9.00±0.08%), followed by aqueous (6.69±0.004%) and ethanol (5.80±0.01%) extracts (Table 1). The yield of ethanol soluble and water soluble extracts of *A. pannosum* leaves in this investigation is lower than that reported by Saini et al. (2014), who reported yields of 21.5% and 24.0%, respectively. The difference could be due to the species employed in the study, since they looked at the yield of *A. indicum* while I looked at the yield of *A. pannosum*.

Phytochemical screening of A. pannosum leaf extracts

Saponins, coumarin, alkaloids, tannins, flavonoids, and steroids were found in the extracts of *A.pannosum* leaves, while steroids were absent in the ethanol extract, alkaloids were absent in the aqueous extract, and alkaloids and flavonoids were absent in the chloroform extract (Table 2). Previous research found flavonoids, tannins, sterols/triterpenes, and volatile oils in aerial portions of *A.pannosum* gathered from several regions of Saudi Arabia and extracted using ethanol and chloroform (Akbar & Al-Yahya, 2011). Alkaloids, sterol lipids, glycerophospholipids, fatty acid, steroid glycoside, antioxidant, and heterocyclics compounds were found in dichloromethane leaf extracts of *A. pannosum* (Aadesariya et al., 2017a,d), whereas the methanolic extract contained steroids, flavonoids, alkaloids, photo proteins, and antibiotics (Aadesariya et al., 2019).

Chemical composition of A. pannosum leaf extracts

Table 3 shows that 32 bioactive compounds were detected in ethanol extract, with the highest peak area being of 9-octadecenoic acid (Z)-, methyl ester (19.33%) with a retention time of 17.64 min and the lowest peak area was of n-tridecylcyclohexane (0.20%) with a retention time of 10.19 min. The other main components were methyl 10-trans,12-cis-octadecadienoate (10.85%), 1-nonadecene (10.76%), gamma-sitosterol (10.67%), and benzyldiethyl (2,6-xylyl-carbamoyl-methyl)-ammonium benzoate (7.30%). In chloroform extract, 36 bioactive compounds were identified, with phytol having the highest peak area (16.63%) with a retention time of 17.78 min and 4,6-Dimethyldodecane having the lowest peak area (0.29%) with a retention time of 7.80 min, and the other major compounds being 1-nonadecene (7.66%), phytol acetate (6.13%), squalene (6.07%) (Table 4).

TABLE 1. Yield (%) of *Abutilon pannosum* leaves extracted with different solvents (mean±SD)

Extract	Weight of the plant powder (g)	Weight of the crude extract obtained (g)	Yield (%)
Ethanol extract	200	11.60±0.02^{c}	5.80±0.01^{c}
Chloroform extract	200	18.00±0.17^{a}	9.00±0.08^{a}
Aqueous extract	200	13.39±0.01^{b}	6.69±0.004^{b}
SL	-	***	***

Means in each column bearing similar superscripts are not significantly different (P>0.05)
*** = P<0.001
SD = Standard deviation

TABLE 2. Phytochemical Screening of *Abutilon pannosum* leaves extracted with different solvents

Phytochemical components	Extract		
	Ethanol	Chloroform	Aqueous
Saponins	+	+	+
Coumarin	+	+	+
Alkaloids	+	-	-
Tannins	+	+	+

TABLE 3. Chemical composition of *Abutilon pannosum* leaf ethanol extract

Sr. no.	RT (min)	Name of compound	Molecular formula	Molecular weight	Peak area (%)
1	6.58	Cyclooctane,1,2-diethyl-	$C_{12}H_{24}$	168	0.98
2	6.80	Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl	$C_{16}H_{30}$	222	0.33
3	7.26	Dodecane,4-cyclohexyl-	$C_{18}H_{36}$	252	1.86
4	9.33	E-14-Hexadecenal	$C_{16}H_{30}O$	238	0.36
5	9.39	1-Tridecene	$C_{13}H_{26}$	182	0.46
6	10.19	n-Tridecylcyclohexane	$C_{19}H_{38}$	266	0.20
7	10.59	Cycloheptasiloxane, tetradecamethyl-	$C_{14}H_{42}O_7Si_7$	518	0.39
8	11.13	Phenol, 2,4-bis-(1,1-dimethylethyl), TMS	$C_{14}H_{22}O$	206	0.63
9	11.61	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-	$C_{15}H_{26}O$	222	0.26
10	11.69	Cyclohexanemethanol, 4-ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)-	$C_{15}H_{26}O$	222	0.32
11	11.93	Pentadecane, 1-methoxy-13-methyl-	$C_{17}H_{36}O$	256	1.28
12	12.00	1-Heptadecene	$C_{17}H_{34}$	238	2.85
13	12.16	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	2.62
14	13.04	2-Naphthalenemethanol, decahydro-α,α,4a-trimethyl-8-methylene-	$C_{15}H_{26}O$	222	2.75
15	14.36	1-Nonadecene	$C_{19}H_{38}$	266	3.80
16	14.89	Phytolacetate	$C_{22}H_{42}O_2$	338	0.52
17	14.99	2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-alpha,alpha,4a,8-tetramethyl-	$C_{15}H_{26}O$	222	1.13
18	15.85	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.96
19	16.50	1-Nonadecene	$C_{19}H_{38}$	266	4.75
20	17.59	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	10.85
21	17.64	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	19.33
22	17.86	Methylstearate	$C_{19}H_{38}O_2$	298	2.60
23	17.98	l-Norvaline, N-(2-methoxyethoxycarbonyl)-, undecyl ester	$C_{20}H_{39}NO_5$	415	1.92
24	18.46	1-Tetracosanol	$C_{24}H_{50}O$	354	3.98
25	19.50	Methyl cis-11-eicosenoate	$C_{21}H_{40}O_2$	324	1.88
26	19.72	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$	326	1.51
27	20.25	1-Heptacosanol	$C_{27}H_{56}O$	396	2.56
28	21.15	1-Heptacosanol	$C_{27}H_{56}O$	396	1.88
29	21.74	Benzyldiethyl (2,6-xylyl-carbamoyl-methyl)-ammonium benzoate	$C_{28}H_{34}N_2O_3$	446	7.30
30	21.91	1-Heptacosanol	$C_{27}H_{56}O$	396	1.28
31	28.36	Stigmasterol	$C_{29}H_{48}O$	412	1.82
32	29.17	Gamma-sitosterol	$C_{29}H_{50}O$	414	10.67
					100.00

TABLE 4. Chemical composition of *Abutilon pannosum* leaf chloroform extract

Sr. no.	RT (min)	Name of compound	Molecular formula	Molecular weight	Peak area (%)
1	3.87	1-Decene	$C_{10}H_{20}$	140	0.57
2	3.96	Oxirane,diethylboryloxymethyl-	$C_7H_{15}BO_2$	142	0.38
3	6.57	1-Dodecene	$C_{12}H_{24}$	168	2.15
4	6.67	Dodecane	$C_{12}H_{26}$	170	0.55
5	7.80	4,6-Dimethyldodecane	$C_{14}H_{30}$	198	0.29
6	9.39	1-Tetradecene	$C_{14}H_{28}$	196	3.88
7	11.22	Phenol, 2,4-bis-(1,1-dimethylethyl), TMS	$C_{14}H_{22}O$	206	4.89
8	12.00	1-Heptadecene	$C_{17}H_{34}$	238	4.34
9	14.36	1-Nonadecene	$C_{19}H_{38}$	266	3.91
10	14.88	Phytol acetate	$C_{22}H_{42}O_2$	338	6.13
11	15.16	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296	0.61
12	15.36	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	$C_{20}H_{40}O$	296	2.72
13	15.85	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.46
14	16.50	1-Nonadecene	$C_{19}H_{38}$	266	3.75
15	17.59	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	2.12
16	17.63	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	5.46
17	17.78	Phytol	$C_{20}H_{40}O$	296	16.63
18	18.46	1-Nonadecene	$C_{19}H_{38}$	266	2.11
19	19.50	Methyl cis-11-eicosenoate	$C_{21}H_{40}O_2$	324	1.51
20	20.07	1,1'-Biphenyl]-4,4'-diol, 3,3',5,5'-tetrakis(1,1-dimethylethyl)-tetrakis(1,1	$C_{28}H_{42}O_2$	410	2.37
21	20.25	1-Heptacosanol	$C_{27}H_{56}O$	396	2.19
22	20.75	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	340	0.69
23	21.02	1,3,5-Trisilacyclohexane	$C_3H_{12}Si_3$	132	1.79
24	21.16	Floxuridine	$C_9H_{11}FN_2O_5$	246	4.40
25	21.43	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	1.60
26	21.74	Benzyldiethyl (2,6-xylyl-carbamoyl-methyl)-ammonium benzoate	$C_{28}H_{34}N_2O_3$	446	0.36
27	21.91	1-Heptacosanol	$C_{27}H_{56}O$	396	0.97
28	22.08	Acetic acid, 13-hydroxy-4,4,6a,6b,8a,11,11,14b-octamethyldocosahydropicen-3-yl ester	$C_{32}H_{54}O_3$	486	0.91
29	22.72	Tetracontane	$C_{40}H_{82}$	562	0.76
30	23.77	Squalene	$C_{30}H_{50}$	410	6.07
31	24.20	Tetracontane	$C_{40}H_{82}$	562	2.91
32	24.98	Hentriacontane	$C_{31}H_{64}$	436	2.07
33	25.86	Tetracontane	$C_{40}H_{82}$	562	2.32
34	26.61	VitaminE	$C_{29}H_{50}O_2$	430	3.46
35	28.35	Stigmasterol	$C_{29}H_{48}O$	412	2.06
36	29.15	Gamma-sitosterol	$C_{29}H_{50}O$	414	1.61
					100.00

The aqueous extract identified 43 bioactive compounds, with benzyldiethyl (2,6-xylyl-carbamoyl-methyl)-ammonium benzoate having the maximum peak area (10.85%) with a retention time of 21.75min and β-D-Glucopyranose, 4-O-β-D-galactopyranosyl- having the lowest peak area (0.07%) with retention time of 4.65 min. The remaining major compounds were 9-Octadecenoicacid,methyles ter,(E)- (7.26%), hexadecanoicacid,methylester (5.89%), and phytol (5.54%) (Table 5). Phytol, a diterpene possessing antimicrobial, anticancer, antiinflammatory and diuretic properties (Islam et al., 2015; Bano & Deora, 2019), which is believed to be a precursor of vitamins E and K (Ogunlesi et al., 2009; Satyal et al., 2012), is reported in this study. *A. pannosum* plant leaves have the potential to play a significant role in medication formulation by assisting in the formation of secondary metabolites such as steroids, alkaloids, flavonoids, and phenols, which can be used to treat a variety of ailments and enhance human health (Sejalsadhu et al., 2016). In a dichloromethane extract of *A. pannosum*, Aadesariya et al. (2017a) found 10 types of bioactive chemicals in the order of (22E)-26,26,26,27,27,27-hexafluoro-25-hydroxy- 22,23-didehydrovitamin D3 (20.41%), (6R)-vitamin D2 6,19-sulfur dioxide adduct (15.16%), (-)- Sedamine (14.78%), 3'-N-Acetyl-4'-O-(9-octadecenoyl) fusarochromanone (13.13%), N-methyl-(R,S)-tetrahydrobenzylisoquinoline (12.27%), 1-(9Z-nonadecenoyl)-glycero-3-phospho-(1'-myo-inositol) (5.67%), furfural diethyl acetal (4.11%), trolox (2.62%) and yamogenin 3-O-neohes-peridoside (2.16%). In n-butanol leaf extract of *A. pannosum,* 11,14,17-eicosatrienoic acid (21.05%), 9,12,15-octadecatrien-1-ol (21.03%), 9,12,15-octadecenoic acid (21.01%), pentadecanoic acid (16.81%), citronellylvalerate (11.08%), 9-eicosyne (11.06%), phytol (11.04%), neophytadiene (11.02%), α-tocopherol (4.48%), n-hexadecanoic acid (2.16%), squalene (2.75%) were identified (Aadesariya et al., 2018). In the petroleum ether extract, 31 compounds were identified, with n-nonadecane (8.28%), n-eicosane (7.63%), n-octadecane (7.31 percent), n-heptadecane (7.29%), and n-hexadecane (6.54 percent) as the major components, while in the methylene chloride extract, 29 compounds were identified, with phytol (31.69%), squalene (15.76%), neophytadiene (5.58%), and n-hexadecanoic acid (4.97%) as the major components (Kamel et al., 2017). Triterpenes, fatty acids, monoterpenes, aromatic flavoring agents, esters, n-alkanes, saturated aliphatic hydrocarbons, diterpenes, olefins, monoglycerides, monoterpenoid phenols, poly unsaturated poly acids, and Vitamin E were found in the acetonitrile leaf extract of *A.pannosum* by GC/MS analysis (Sejalsadhu et al., 2016). Twelve phenolic acids were tentatively identified from methanolic extracts of *A. pannosum* leaves, accounting for 9.03%, with protocatechuic acid being the major phenolic acid, 21 flavonoids accounting for 53.78%, with Kaempferol 3-O- [2-p-coumaroyl-D-glucopyranoside] being the major flavonoid glycoside, and luteolin (3.68%) was the major identified flavonoid glycoside. *A. pannosum* methanol extracts of aerial parts were high in phenolic content, with the highest concentration of flavonoids and the lowest concentration of phenolic acids (Mohammed et al., 2021). In a GC/MS examination of *A. indicum* leaves, 25 bioactive phytochemical compounds were discovered, with phytol (53.3%), hexadecanoic acid, ethyl ester (12.21%), Benzofuran, 2,3-dihydro- (5.78%), and 9, 12, 15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (5.61%) in the ethanol leaves extract (Radhakrishnan et al., 2017).

Antimicrobial activity of A. pannosum leaf extract

Plants are a rich source of antimicrobial compounds that can be used to treat a variety of illnesses, and their antimicrobial constituents can be extracted using a variety of organic solvents (Aadesariya et al., 2017c). The antimicrobial activity of *A. pannosum* leaf extracts in the three solvents (ethanol, chloroform, and water) against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* NCTC 8236, and *Candida albicans* ATCC 7596 was determined using the agar disk diffusion method, with the results shown in Tables 6-9. The activity of the ethanol extract increased when the concentration of the extract was raised. At a concentration of 25mg/mL, the extract was inactive against all microorganisms tested, partially active at 200-300mg/mL, and active against all microbes except *E. coli* at a dose of 300mg/mL (Table 6). For all microorganisms tested, the activity of all chloroform extract concentrations ranged from partially active to active (Table 7), while the activity of aqueous extract ranged from partially active to active in all concentrations except the 25mg/mL concentration (Table 8).

TABLE 5. Chemical composition of *Abutilon pannosum* leaf aqueous extract

Sr. no.	RT (min)	Name of compound	Molecular formula	Molecular weight	Peak area (%)
1	4.65	β-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	0.07
2	6.57	Cyclooctane, 1,2-diethyl-	$C_{12}H_{24}$	168	0.42
3	7.29	Hexylcyclohexane	$C_{12}H_{24}$	168	1.23
4	9.32	1-Tetradecene	$C_{14}H_{28}$	196	1.51
5	9.39	1-Tetradecene	$C_{14}H_{28}$	196	2.87
6	11.92	3,7,11,15-Tetramethyl-2-hexadecene	$C_{20}H_{40}$	280	1.97
7	12.00	1-Heptadecene	$C_{17}H_{34}$	238	4.90
8	12.08	Hexadecane	$C_{16}H_{34}$	226	1.13
9	12.15	Diethylphthalate	$C_{12}H_{14}O_4$	222	4.70
10	12.67	Hexadecamethylcyclooctasiloxane	$C_{16}H_{48}O_8Si_8$	592	1.66
11	14.29	Tridecane, 3-methylene-	$C_{14}H_{28}$	196	1.04
12	14.36	1-Nonadecene	$C_{19}H_{38}$	266	4.70
13	15.32	8-Octadecanone	$C_{18}H_{36}O$	268	0.41
14	15.76	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.30
15	15.85	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	5.89
16	16.21	Ethyliso-allocholate	$C_{26}H_{44}O_5$	436	0.09
17	16.51	1-Nonadecene	$C_{19}H_{38}$	266	2.23
18	17.49	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	0.99
19	17.55	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296	1.42
20	17.59	Methyl10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294	4.83
21	17.64	9-Octadecenoicacid,methylester,(E)-	$C_{19}H_{36}O_2$	296	7.26
22	17.78	Phytol	$C_{20}H_{40}O$	296	5.54
23	17.87	Methylstearate	$C_{19}H_{38}O_2$	298	1.69
24	17.99	l-Norvaline, N-(2-methoxyethoxycarbonyl)-, undecyl ester	$C_{15}H_{29}NO_5$	303	4.02
25	18.46	n-Tetracosanol-1	$C_{24}H_{50}O$	354	0.93
26	20.23	OleicAcid	$C_{18}H_{34}O_2$	282	0.14
27	21.03	1,3,5-Trisilacyclohexane	$C_3H_{12}Si_3$	132	2.17
28	21.17	2-Ethylbutyric acid, eicosyl ester	$C_{26}H_{52}O_2$	396	2.87
29	21.25	13-Docosenoicacid,methylester,(Z)-	$C_{23}H_{44}O_2$	352	2.28
30	21.29	13-Docosenoicacid,methylester,(Z)-	$C_{23}H_{44}O_2$	352	1.39
31	21.43	Hexadecanoicacid,2-hydroxy-1-(hydroxyme	$C_{19}H_{38}O_4$	330	2.39
32	21.75	Benzyldiethyl (2,6-xylyl-carbamoyl-methyl)-ammonium benzoate	$C_{28}H_{34}N_2O_3$	446	10.85
33	22.09	Acetic acid, 13-hydroxy-4,4,6a,6b,8a,11,11,14b-octamethyldocosahydropicen-3-yl ester	$C_{32}H_{54}O_3$	486	1.25
34	22.65	1,3,5-Trisilacyclohexane	$C_3H_{12}Si_3$	132	0.64
35	23.48	Cyclononasiloxane,octadecamethyl-	$C_{18}H_{54}O_9Si_9$	666	1.39
36	23.59	Acetic acid, 13-hydroxy-4,4,6a,6b,8a,11,11,14b-	$C_{32}H_{54}O_3$	486	0.58
37	23.77	Squalene	$C_{30}H_{50}$	410	1.19
38	23.87	cis-13-docosenoylchloride	$C_{22}H_{41}ClO$	356	0.93
39	24.25	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	1.49
40	25.87	Tetrapentacontane	$C_{54}H_{110}$	758	0.95
41	26.19	Spirost-5-en-3-ol, (3beta,25R)-	$C_{27}H_{42}O_3$	456	2.42
42	26.44	Stigmast-5-en-3-ol, oleate	$C_{47}H_{82}O_2$	678	3.12
43	26.63	VitaminE	$C_{29}H_{50}O_2$	430	1.14
					100.00

TABLE 6. Inhibition zones (mm) of different concentrations of *Abutilonn pannosum* leaf ethanol extract against microorganisms tested

Microorganisms	Extract concentration (mg/mL)				
	25	50	100	200	300
Escherichia coli	8	8	9	10	10
Pseudomonas aeruginosa	8	9	9	10	13
Staphylococcus aureus	8	8	9	10	13
Bacillus cereus	8	9	10	11	13
Candida albicans	0	8	10	12	15

TABLE 7. Inhibition zones (mm) of different concentrations of *Abutilonn pannosum* leaf chloroform extract against microorganisms tested

Microorganisms	Extract concentration (mg/mL)				
	25	50	100	200	300
Escherichia coli	10	11	11	13	11
Pseudomonas aeruginosa	9	11	11	15	13
Staphylococcus aureus	10	15	13	15	15
Bacillus cereus	12	13	15	15	13
Candida albicans	11	12	15	15	17

TABLE 8. Inhibition zones (mm) of different concentrations of *Abutilonn pannosum* aqueous leaf extract against microorganisms tested

Microorganisms	Extract concentration (mg/mL)				
	25	50	100	200	300
Escherichia coli	8	9	10	11	13
Pseudomonas aeruginosa	8	9	9	11	10
Staphylococcus aureus	8	9	9	10	11
Bacillus cereus	8	9	9	10	11
Candida albicans	8	9	9	9	10

TABLE 9. Antimicrobial activity of *Abutilon pannosum* leaf extract (inhibition zone in mm)

Organisms	Extract			SL
	Ethanol	Chloroform	Aqueous	
Escherichia coli	9.0±0.93^{c}	11.2±1.01^{a}	10.2±1.78^{b}	***
Pseudomonas aeruginosa	9.8±1.78^{b}	11.8±2.11^{a}	9.4±1.06^{b}	***
Staphylococcus aureus	9.6±1.92^{b}	13.6±2.03^{a}	9.4±1.06^{b}	***
Bacillus cereus	10.2±1.78^{b}	13.6±1.24^{a}	9.4±1.06^{b}	***
Candida albicans	9.0±5.24^{b}	14.0±2.27^{a}	9.0±1.06^{b}	***

Values are expressed as mean±SD
N = 3
*** = P<0.001

Except for *E. coli*, chloroform extract had the maximum activity (P<0.001) against all microorganisms, while aqueous extract had the lowest activity (P<0.001) (Table 9). The activity of the ethanol, aqueous, and methanol extracts of *A.pannosum* leaves against *Staphylococcus aureus* ATCC 33591, *Proteus mirabilis* ATCC 43071, *Escherichia coli* ATCC 2592, and *Pseudomonas aeruginosa* ATCC 27853 was mild to very strong (Rayes, 2012). El Sayed et al. (2019) found that the water extract of *A. pannosum* leaves had a strong inhibitory effect against *E. coli* and *E. faecalis*, a moderate inhibitory effect against *S. aureus* and *P.*

aeruginosa, and no activity against *S. enterica*, *K. pneumoniae* ATCC 13883, and *K.pneumoniae* ATCC 700603. The ethanolic extract of *A. pannosum* leaves had the highest antibacterial activity ($P<0.001$) when compared to penicillin potassium and streptomycin sulphate, and it inhibited *Pr. vulgaris* with a 23.5mm maximum diameter inhibition zone (Survase et al., 2013). The antimicrobial activity indexes of methanol extract, methyle chloride extract and petroleum ether extracts of *A. pannosum* leaves against *Escherichia coli* were found to be 66.7%, 33.3%, and 0.0%, respectively, while the activity indexes of methylene chloride, methanol and petroleum ether extracts against *Staphylococcus aureus* were 63.6%, 59.1% and 9.1%, respectively, and against *Candida albicans*, their activity indexes were 38.5%, 34.6%, and 11.5%, respectively (Kamel et al., 2017). Saini et al. (2014) found that the alcoholic leaf extract of *A. indicum* had a positive and good response against the fungi *Microsporum gypseum* and *Penicelliium chrysogenum*, whereas the aqueous leaf extract showed no activity against the fungi under study.

Conclusion

The current study is an extensive and exhaustive investigation into the phytochemical characterization, antioxidant, and antimicrobial inhibitory actions of *A. pannosum* carried out in Shada mountain, Al-Baha area, Saudi Arabia. The phytochemicals investigated in ethanol, chloroform, and aqueous extracts were discovered in the plant. The extracts contained different chemical compounds in different concentrations, according to GC/MS analysis, with the major compounds being 9-octadecenoic acid (Z)- methyl ester and methyl 10-trans,12-cis-octadecadienoate in ethanol extract, phytol in chloroform extract, and benzyldiethyl(2,6-xylylcarbamoylmethyl) ammonium benzoate in aqueous extract. These compounds have antimicrobial characteristics, and despite the fact that *A. pannosum* grows wild in Al-Baha region, no research has been done to establish its use as a medicinal plant. As a result, the findings of this study may provide future insight into the plant's possible use in food and pharmaceutical industries for human welfare.

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الفحص الكيميائي النباتي، والتركيب الكيميائي، والأنشطة المضادة للميكروبات لنبات (*Abutilon pannosum*, Forst.f. Schlecht) تم جمعها من جبل شدا بمنطقة الباحة بالمملكة العربية السعودية

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تم إجراء هذه الدراسة لتحديد المكونات الكيميائية النباتية والنشاط المضاد للميكروبات في المختبر لمستخلصات اوراق نبات *Abutilonn pannosum* (Forst.f.) Schlecht، المعروف محليًا باسم «خبيثة ممزقة» التي تنمو بريا في منطقة الباحة بالمملكة العربية السعودية. تم جمع أوراق النبات وتجفيفها بالهواء وتنعيمها ثم استخلاصها بالإيثانول والكلوروفورم والماء الساخن. تم تحديد المكونات الكيميائية النباتية والنشاط المضاد للميكروبات ضد البكتيريا موجبة الجرام (*Staphylococcus aureus*، *Bacillus cereus*)، والبكتيريا سالبة الجرام (*Escherichia coli*، *Pseudomonas aeruginosa*)، وفطر الخميرة *Candida albicans*. أظهرت النتائج أن المستخلصات تحتوي على صابونين وكومارين وقلويدات وتانين وفلافونويد وستيرويدات. أظهرتحليل كروماتوغرافيا الغاز/مطياف الكتلة (GC/MS) لمستخلص الإيثانول 32 مركبًا، أهمها (Octadecenoic-9 acid (Z)-, methyl ester, and methyl 10-trans,12-cis-octadecadienoate)، بينما أظهر مستخلص الكلوروفورم 36 مركبًا نشطًا بيولوجيًا أهمها الفايتول، وأظهر المستخلص المائي 43 مركباً نشطاً حيوياً، أهمها بنزوات الأمونيوم (2،6-زيل كاربامويل ميثيل). زاد النشاط المضاد للميكروبات لمستخلص الإيثانول مع زيادة لتركيز، من غير نشط عند 50-25 مجم/مل إلى نشط بشكل معتدل عند 200-100 مجم/مل إلى نشط عند 300 مجم/مل. في جميع التراكيز، كان نشاط مستخلص الكلوروفورم أعلى قليلاً، حيث تراوح من نشط معتدل إلى نشط. كان مستخلص الكلوروفورم أكثر فاعلية ضد جميع الكائنات الحية الدقيقة المختبرة مقارنة بمستخلصات الإيثانول والماء. يمكن أن يكون هذا النبات مفيدًا كعامل مضاد للميكروبات ضد الكائنات الدقيقة المسببة للأمراض، والتي يمكن استخدامها في صناعة الأغذية والأدوية.