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Phytochemical Profile and Antimicrobial Assessment of *Abutilon fruticosum* Guill. & Perr. Growing in Gebel Elba, Egypt

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Abstract

Abutilon fruticosum Guill. & Perr. (Family Malvaceae) is distributed throughout a number of arid and semi-arid regions and has been used in traditional and folk medicine. The current study was conducted to estimate the phytochemical profiling and antimicrobial activity of *Abutilon fruticosum* Guill. & Perr. growing in *Gebel Elba*, Egypt against some respiratory tract infections isolates (Gram-negative bacteria: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter cloacae*, and *Acinetobacter baumannii*; Gram-positive bacteria: *Staphylococcus aureus, Streptococcus epidermidis, and Enterococcus faecalis;* and yeast: *Candida tropicalis*). Phytoconstituents determination of *Abutilon fruticosum* was carried out using the high resolution ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC/qTOF-MS/ MS); which yielded the determination of thirty constituents including phenolic acids, alkaloids and the majority of flavonoids. *Abutilon fruticosum* extracts were tested for their *in vitro* antimicrobial activity using the disc diffusion method. Plant extracts exhibited potent antimicrobial activity against the tested isolates. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for the most suitable successive fractions were determined. Butanol fraction showed the lowest MIC (25 mg/ ml) against all the tested organisms. While; ethyl acetate fraction exhibited the lowest MIC (12.5 mg/ ml) against *Klebsiella pneumoniae*. *Abutilon fruticosum* Guill. & Perr. extracts are enriched in phytoconstituents which are likely attributed to their potential antimicrobial activity.

Keywords*: Abutilon fruticosum*; Malvaceae; UPLC/qTOF-MS/MS; phytoconstituents; antimicrobial; respiratory tract infections.

1. Introduction

Abutilon fruticosum Guill & Perr. is one such important medicinal plant belonging to family Malvaceae, growing naturally at Gebel Elba National Park, in the southeastern corner of Egypt along the Egyptian-African Red Sea coast. Traditionally; *A. fruticosum* Guill & Perr. has been used for the treatment of some illnesses, as dysentery, eye irritation, diuretic, fever treatment, cure of stomach diseases [1] and applied for the treatment of wounds [2]. Flowers, leaves, and seeds are used for the treatment of acne, cold, and bronchitis [3]. Respiratory tract infections are serious health problems causing increased morbidity and mortality rates. The high proportion of respiratory infections is due to aerobic respiratory bacteria like *Pseudomonas aeruginosa* which infects patients with chronic obstructive pulmonary disease; *Staphylococcus aureus* which is the major cause of hospital-acquired pneumonia [4] and *Klebsiella pneumoniae* which is an opportunistic pathogen with highly antimicrobial resistant [5]. The ethnobotanical approach attracts the attention of many researchers to discover new drugs from plants that are being used in folk as well as modern medicinal alternative healthcare systems; in the hope of finding novel antimicrobial compounds safe with lower or no side effects [6]. Medicinal plants are a source of bioactive phytoconstituents which may be used as a precursor for several drugs [7]. Therefore; in this study, we aimed to determine the phytoconstituents and evaluate the antimicrobial activity of *Abutilon fruticosum* against some respiratory tract infections microorganisms (Gram-negative bacteria, Gram-positive bacteria, and yeast).

2. Materials and methods

2.1. Plant materials

Aerial parts of *Abutilon fruticosum* were collected from Wadi Kanssisrob, Gebel Elba National Park, at the southeastern corner of Egypt along the Egyptian-African Red sea coast at spring 2019; and scientifically identified at Plant Taxonomy Unit, Desert Research Centre, Egypt. Where, CAIH-1020-R voucher number was placed in Desert Research

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Centre herbarium. Plant samples were cleaned, freed from dirt particles, shade dried till constant weight, and ground to a fine powder. Extraction of the plant material was performed at room temperature by maceration with ethanol 70% as a solvent. The combined extracts are then fractionated successively using a separating funnel apparatus in order of increasing polarity. The obtained residue from each solvent was dried.

2.2. Preliminary phytochemical screening

The presence of phytochemicals in *A. fruticosum* extracts was evaluated using standard procedures as follow: fresh plant was subjected to distillation for volatile oils extraction [8], tests for saponins and alkaloids (Dragendorff's test) were carried out according to the methods described by Adegoke *et al*., [9]. Moreover; tests for glycosides and/or carbohydrates (Molish's test), flavonoids and tannins were carried out based on the methods described by Auwal *et al*., [10]. Furthermore; tests for steroids and/or terpenes (Salkwski's reaction and Libermann-Burchard's test) were performed according to methods outlined by Auwal *et al*., [10] and Malik *et al*., [11], respectively.

2.3. Antimicrobial activity

2.3.1. Testing microbes

Nine microbial isolates (Gram-negative bacteria: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Acinetobacter baumannii*; Gram-positive bacteria: *Staphylococcus aureus*, *Streptococcus epidermidis*, and *Enterococcus faecalis*; and yeast: *Candida tropicalis*), were used to evaluate the antimicrobial activity of *A. fruticosum* extracts. Microbial isolates were collected and identified from clinical materials (sputum, endotracheal tube (ETT), nasal swab, and laryngeal swab (from respiratory tract infections patients– from Giessen University Clinic - Germany) [12].

2.3.2. Antimicrobial susceptibility test

Disk diffusion method was used for antimicrobial susceptibility determination. Briefly, 10 mm in diameter paper discs were separately soaked with 50 µL of the 100 mg/mL of plant extracts (dissolved in dimethyl sulfoxide DMSO) and placed on the Muller Hinton Agar (MHA) plates inoculated with a 24-h culture of the isolates tested. Plates were incubated at 37 ºC for 18-24 hours. Inhibition zone was observed and measured in millimeters (mm) for each microorganism [13]. The antimicrobial test was carried out in triplicates; the results were expressed as mean ± SD.

2.3.3. Determination of minimum inhibitory concentration (MIC)

Butanol, hexane, and ethyl acetate fractions were chosen for the investigation of their MIC against the tested strains. Where 100 µl of the fractions were diluted with a series of tubes containing Mueller Hinton broth (Accumix – Verna, India) to obtain final dilutions of 100, 75, 50, 25, 12.5 6.25, and 3.12 mg/ml v/v [14]. Standard microbial inoculums of the isolated microorganisms were inoculated into all dilutions of different fractions. The inoculated tubes were overnight incubated at 37°C. The highest dilution of the tested extracts of *Abutilon fruticosum* that inhibit microbial growth (no turbidity in the tube) was considered as the MIC value [15].

2.3.4. Determination of minimum bactericidal concentration (MBC)

100 µl from all tubes showed no obvious signs of growth or turbidity were inoculated in sterile plates of Mueller Hinton agar (Accumix – Verna, India) by streak plate method. The plates were then incubated at 37°C overnight. The MBC values were determined as; the lowest concentration of extracts that did not show any growth of tested microbes [15]. Amoxicillin, ceftazidime, erythromycin, and gentamicin were applied as standard commercial drugs for the treatment of respiratory tract infections. Control positive was all strains of tested microbes, while control negative was media with different concentrations of different fractions only.

2.4. UPLCqTOF-MS/MS analysis of active constituents of *Abutilon fruticosum*

Phytoconstituents profiling of *Abutilon fruticosum* extract was analyzed at the Proteomics and Metabolomics lab, Cancer Children Hospital 57357, Cairo, Egypt using UPLC/Q-TOF-MS MS in positive mode. 10 g of plant powder was extracted with 70% methanol and filtered to remove plant debris. 50 mg of the methanolic extract was dissolved in 1 mL of reconstitution solvent (deionized water: methanol: acetonitrile in a ratio of 50:25:25, respectively). The mixture was vortexed for 2 min followed by ultra-sonication for 10 min. and centrifuged for 5 min. at 10,000 rpm. 10 µL of the sample was injected. Chromatographic separations were performed on Sciex ExionLC (High flow LC) coupled with TripleTOF 5600+ equipped with Xbridge C18 column (2.5 µm, 2.1x150 mm; Waters). Gradient elution at a Flow rate of 0.3 mL/min using mobile phases: solvent A (5 mM ammonium format buffer

pH 3 containing 1% methanol), and solvent B (100% acetonitrile) were applied as follow; (0–1 min) isocratic 90% solvent A, 10% solvent B; (1–21 min) linear from 10–90% B; (21–25 min), isocratic 90% B; finally (25.01 –28 min) isocratic 10% B. MasterView software (SCIEX, USA) was used for peaks extraction from total ion chromatogram (TIC), detecting the retention time and masses of the molecules; MS-DIAL 3.52 software was used for data analysis. Many databases such as HMDB (Human Metabolome Database) and NIST (National Institute of Standards and Technology) libraries were used as references for the tentative identification of compounds. The identification was based on comparing their masses and fragmentation patterns with published compounds in the literature.

3. Results

3.1. Preliminary phytochemical screening

Presence of flavonoids, alkaloids, carbohydrates and/or glycosides, sterols and/or terpenes and tannins in the 70% alcholic extract of *A. fruticosum* has been detected (table 1). Carbohydrates and/or glycosides were found in ethyl acetate and butanol fractions. While; flavonoids were present in ether, ethyl acetate, and butanol fractions. Elsewhere; tannins were observed in butanol fraction and as traces in ethyl acetate. Sterols and/or terpenes were detected in hexane and as traces in diethyl ether fractions. Alkaloids were detected in the ethyl acetate fraction. While absence of volatile oil in the plant.

Table 1: Preliminary phytochemical screening of *A. fruticosum* extracts.

Test for	Ethanol 70%	Hexane	Diethyl Ether	Ethyl Acetate	Butanol
Carbohydrates and/or glycosides	+	-	-	+	+
Flavonoids	+	-	±	+	+
Tannins	+	-	-	±	+
Sterols and/or terpens	+	+	±	-	-

Where: + = present; - =absent; ± = traces

3.2. Antimicrobial activity of *Abutilon fruticosum*

Ethanol 70% extract exhibited potent antimicrobial activity against all the tested isolates; with the highest inhibition zone of 18.5, 17, and 16.5 mm against *S. aureus*, *E. faecalis*, and *E. coli*, respectively. The successive fractions; butanol, ethyl acetate, and hexane showed potent activity on most isolates; while ether exhibited mild activity against three isolates (figure 1). Butanol extract showed the highest inhibition zone of 12.5, 13 and 13.5 mm against *E. cloacae* and, *P. aeruginosa* and *A. baumannii*, respectively. While; ethyl acetate showed high antimicrobial activity against *S. epidermidis*, *K. pneumoniae* and *C. tropicalis* with a zone of inhibition valued 14, 14, and 15 mm, respectively.

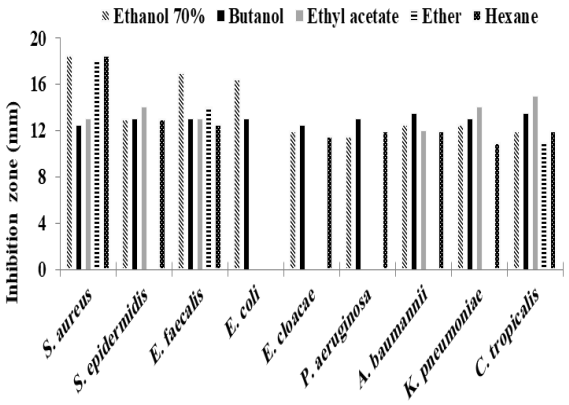

Figure 1: Agar diffusion method of *A. fruticosum* extracts against respiratory tract infections isolates

3.2.1. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The most suitable successive fractions of *Abutilon fruticosum* with the lowest MIC values were butanol followed by ethyl acetate then hexane on Gram-negative bacteria: *K. pneumoniae*, gram-positive bacteria: *Staphylococcus epidermidis* and *Enterococcus faecalis*, and yeast: *Candida tropicalis* (table 2). Butanol showed the lowest MIC values against all the tested microorganisms (25 mg/ml). While; ethyl acetate showed the lowest MIC value (12.5 mg/ml) against Gram-negative bacteria: *K. pneumoniae*.

Table (2): Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *Abutilon fruticosum* extracts against respiratory tract infections isolates.

Strains	Extracts					
	MIC			MBC		
	Butanol (mg/ml)	Ethyl acetate (mg/ml)	Hexane (mg/ml)	Butanol (mg/ml)	Ethyl acetate (mg/ml)	Hexane (mg/ml)
S. aureus	25	50	75	75	75	75
S. epidermidis	25	50	50	75	50	75
E. faecalis	25	50	50	75	50	75
E. coli	25	-	-	75	-	-
E. cloacae	25	-	75	75	-	100
P. aeruginosa	25	-	75	75	-	100
A. baumannii	25	50	75	75	75	100
K. pneumoniae	25	12.5	50	50	25	50
C. tropicalis	25	50	50	75	75	100

3.3. Phytochemical profiling of *Abutilon fruticosum* using high-resolution UPLCqTOF-MS MS analysis

Profiling of phytoconstituents of *A. fruticosum* extract revealed the identification of 30 bioactive compounds including flavonoids (flavonols, flavones, and anthocyanins) in addition to alkaloids and phenolic acids (figure 2; table 3). The results showed that most of the identified compounds were flavonoids named: Okanin-4'-*O*-glucoside **7**, Quercetin-3,4'-O-di-beta-glucopyranoside **9**, Quercetin **10**, Rutin **12**, Diosmin **13**, Kaempferol-3-Glucuronide **15**, Isorhamnetin-3-*O*-rutinoside **16**, Kaempferol-3-*O*-glucoside **17**, Kaempferol **18**, Apigenin 7-*O*-neohesperidoside (Rhoifolin) **19**, Apigenin-7-*O*-glucoside **20**, Baicalein-7-*O*-glucuronide **21**, Kaempferol-3-*O*-(6""-*p*-coumaroyl)-glucoside **24**, Quercetin-3-glucuronide **25** Acacetin-7-*O*-rutinoside **26**, Naringenin **28**, and Apigenin **29**. Another group detected in *A. fruticosum* extract was acids named: *Trans*-cinnamic acid **4**, Sinapic acid **8**, Ferulic acid **11** and *p*-Coumaric acid **27**. Beside determination of two aromatic aldehydes: Coniferaldehyde **23** and Benzaldehyde **30**. Moreover; three anthocyanins were identified as: Cyanidin-3, 5-di-*O*-glucoside **6**, Cyanidin-3-glucoside **14** and Cyanidin-3-*O*-rutinoside **22**. Elsewhere; nitrogenous compounds named: 6-Hydroxynicotinic acid **1**, Trigonelline **2**, Harmaline **3**, and 3-Formylindole **5** were tentatively identified by comparison with previously published literature.

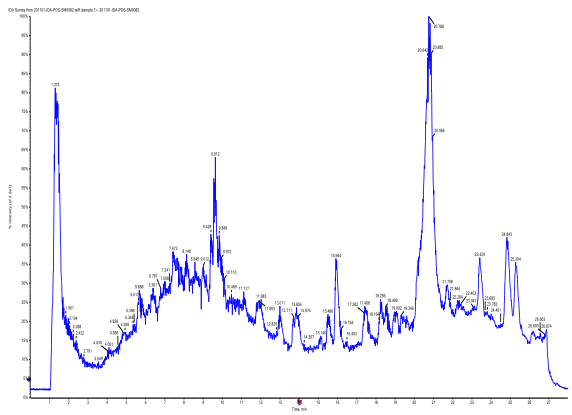

Figure 2: Total ion chromatogram of *Abutilon fruticosum* in positive mode.

Table (3): Major constituents of alcholic extract of *Abutilon fruticosum*

No.	R.T. (min.)	Precursor m/z	MS/MS	Proposed compound	Formula
1	1.318	140.07	96, 140	6-Hydroxynicotinic acid	$C_6H_5NO_3$
2	1.417	138.05	79	Trigonelline	$C_7H_7NO_2$
3	2.120	215.01	70, 155, 215	Harmaline	$C_{13}H_{14}N_2O$
4	4.055	149.06	121, 149	*Trans* Cinnamic acid	$C_9H_8O_2$
5	4.268	146.06	118, 146	3-Formylindole	C_9H_7NO
6	5.075	611.16	287, 449, 611	Cyanidin-3, 5-di-*O*-glucoside	$C_{27}H_{31}O_{16}$
7	5.260	451.12	289	Okanin-4'-*O*-glucoside	$C_{21}H_{22}O_{11}$
8	5.340	225.08	119, 147, 192, 207	Sinapic acid	$C_{11}H_{12}O_5$
9	5.586	627.15	303, 465	Quercetin-3,4'-*O*-di-beta-glucopyranoside	$C_{27}H_{30}O_{17}$
10	5.612	303.04	153, 201, 303	Quercetin	$C_{15}H_{10}O_7$
11	6.342	195.07	145, 177	Ferulic acid	$C_{10}H_{10}O_4$
12	6.692	611.16	302, 611	Rutin	$C_{27}H_{30}O_{16}$
13	6.789	609.14	463	Diosmin	$C_{28}H_{32}O_{15}$
14	7.074	449.11	287	Cyanidin-3-glucoside	$C_{21}H_{21}O_{11}$
15	7.349	463.09	287	Kaempferol-3-Glucuronide	$C_{21}H_{18}O_{12}$
16	7.425	625.18	317, 479	Isorhamnetin-3-*O*-rutinoside	$C_{28}H_{32}O_{16}$
17	7.668	449.11	287	Kaempferol-3-*O*-glucoside	$C_{21}H_{20}O_{11}$
18	7.681	287.05	121,153, 287	Kaempferol	$C_{15}H_{10}O_6$
19	7.708	579.17	271, 579	Apigenin 7-*O*-neohesperidoside (Rhoifolin)	$C_{27}H_{30}O_{14}$
20	7.869	433.11	271	Apigenin-7-*O*-glucoside	$C_{21}H_{20}O_{10}$
21	8.139	447.09	271, 447	Baicalein-7-*O*-glucuronide	$C_{21}H_{18}O_{11}$
22	8.346	595.15	287	Cyanidin-3-*O*-rutinoside	$C_{27}H_{31}O_{15}$
23	8.554	179.07	119, 147	Coniferaldehyde	$C_{10}H_{10}O_3$
24	8.621	595.15	147, 287	Kaempferol-3-*O*-(6""-p-coumaroyl)-glucoside	$C_{30}H_{26}O_{13}$
25	8.981	479.08	303	Quercetin-3-glucuronide	$C_{21}H_{18}O_{13}$
26	9.281	593.18	285, 447	Acacetin-7-*O*-rutinoside	$C_{28}H_{32}O_{14}$
27	9.570	165.05	119, 147	*p*-Coumaric acid	$C_9H_8O_3$
28	11.074	273.08	153	Naringenin	$C_{15}H_{12}O_5$
29	11.158	271.05	119, 121, 153	Apigenin	$C_{15}H_{10}O_5$
30	20.616	107.05	51, 79, 107	Benzaldehyde	C_7H_6O

4. Discussion

Traditionally, rural communities using herbal medications as an alternative treatments for many decades till now based on their abundance, low cost and less or no side effects. Antimicrobial agents of plant origin have various therapeutic potentials in the treatment of infectious diseases. The phytochemical screening of alcholic extract of *A. fruticosum* showed existence of various phytoconstituents as flavonoids, alkaloids, carbohydrates and/or glycosides, sterols and/or terpenes and tannins which may be related to its strong antimicrobial activity (figure 1). The most suitable successive fractions with the lowest MIC and MBC values were butanol and ethyl acetate; this may

be attributed to the presence of tannins, and flavonoids in these extracts which were reported to inhibit microbial growth. This result is in accordance with Musheerul *et al*., [7] who revealed that; the alcoholic stem extract of *Abutilon theophrasti* showed antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumonia.* Our findings were also consistent with the results obtained from the previous study on *Abutilon indicum* extracts which found to exhibit potent antibacterial and antifungal activities against *Staphylococcus Aureus*, *E. coli*, and *Candida albicans* [16]. In agreement with another report by Gomaa *et al*., [17]; the successive extracts of *A. indicum* leaves showed significant antibacterial activity against *S. aureus*, *E. coli*, and *Klebsiella sp*.

For the first time; the phytoconstituents profiling of *A. fruticosum* using UPLCqTOF-MS/MS technique was carried out; and showed that the major constituents identified were flavonoids. Quercetin, kaempferol, and apigenin and their derivatives were the most abundant flavonoids detected. Flavonoids are one of the most abundant and widespread class of natural components found in a variety of plant parts that inhibit or kill many bacterial strains [18]. Many studies supposed the main antimicrobial mechanisms of flavonoids as inhibition of nucleic acid synthesis, reduction in cell attachment and biofilm formation [19], deformation of the protein on the cell membrane, alteration of the membrane permeability [20] and decrease of the pathogenicity [21]. The results are agreed with Gomaa *et al*., [17] who reported that; quercetin, kaempferol and their glycosides are the most common flavonoids isolated from different *Abutilon* species, in addition to luteolin and apigenin. Kaempferol and its glycosides process great antimicrobial activity [22]. Quercetin also, is a potent antibacterial molecule and has been shown to be effective against *Staphylococcus aureus*, as well as *Staphylococcus epidermidis* [23]. Likewise; apigenin was reported to have antibacterial activity against *P. aeruginosa*, and *E. coli* [24]. Also; the flavanone naringenin was shown to have potent activity against *E. coli*, *S. aureus*, and *Enterococcus faecalis* [25]. Phenolic acids are also determined in phytoconstituents analysis of *A. fruticosum* that could contribute to its antimicrobial activity. Another class identified was alkaloids; which act as defensive mechanism against predators and pathogens in many plants [26]. Harmaline, an indole alkaloid, found in a variety of medicinal plants in Malvaceae family such as *Grewia bicolor* [27] and is reported to possess antimicrobial activity in addition to number of biological characteristics including antioxidant and anti-inflammatory activity [28]. Moreover; trigonelline was reported to found in family Malvacaea in *Abutilon hybridum* [29] and *G. barbadense* [30]. Plants containing trigonelline have been reported to have many therapeutic effects including antibacterial and antiviral activities [26]. In summary; our study revealed that *A. fruiticosum* Guill. & Perr. has potent antimicrobial activity against the tested respiratory tract infections isolates, which is most likely due to its enrichment with active compounds as flavonoids, phenolic acids and alkaloids that have been shown to possess good biological activities, particularly antimicrobial activity, suggesting that it could be a good source of antimicrobial agents from natural resources.

5. Conclusion

Our study showed that *A. fruticosum* Guill. & Perr. extracts possess potent antimicrobial activity against some microorganisms involved in respiratory tract infections. The phytochemical profiling of the plant revealed the identification of active constituents including flavonoids, phenolic acids, and alkaloids which are probably related to its antimicrobial activity. Thus; further studies are needed, particularly chemical tests for isolation and purification of the active compounds attributed to the antimicrobial activity; as well as *in vivo* tests in order to discover new antimicrobial agents from the natural products derived from *A. fruticosum* Guill. & Perr.

Conflicts of interest

There are no conflicts to declare.

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