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UPLC-ESI-MS polyphenolic profiling and the antimicrobial potential of *Ailanthus altissima* (Mill.) Swingle leaves growing in Egypt

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ABSTRACT: The need of finding out new and potent antimicrobial agents to combat the multidrug resistance pathogens becomes critical. *Ailanthus altissima* is characterized by its vast and remarkable therapeutic potential as a traditional folk medicine. The current study aims to investigate the polyphenolic profiling and the antimicrobial activity of the methanol extract of *A. altissima* leaves and its derived fractions. The antimicrobial activity was investigated through two *in-vitro* assays; agar well diffusion and broth microdilution against six pathogens while the polyphenolic profiling was characterized through UPLC-ESI-MS analysis. All the test pathogens were susceptible to the test samples except *Mucor circinelloides*. The zone of microbial growth inhibition of the samples was found in 10-40 mm range and the minimum inhibitory values ranged between 3.9 and 1000 μg/mL. The ethyl acetate fraction exhibited significant antimicrobial potential especially against *P. aeruginosa, S. aureus* and *C. albicans* where the diameters of inhibition zones were 40, 30 and 25mm, respectively and their corresponding MIC values were 3.9, 7.8 and 15.6 μg/mL, respectively. The UPLC-ESI-MS analysis of the samples revealed the tentative identification of various phenolic compounds mainly corilagin. *A. altissima* offers a perfect choice to be investigated as a natural and potent antimicrobial agent.

KEYWORDS: *Ailanthus altissima*; antimicrobial; corilagin; UPLC-ESI-MS; phenolics.

1. INTRODUCTION

The ability of some pathogenic microorganisms to develop resistance against the available antibiotics in the market through mutation or through gaining resistance genes from other microbes beside the excessive and the improper use of these antibiotics led to the emergence of microbial strains that are not susceptible to any of the available antimicrobial agents. The development of these multi-drug resistance microorganisms is considered a life threatening problem that creates an urgent demand to find out innovative and equipotent antimicrobial agents to avoid the appearance of untreatable microbial infections [1, 2].

Medicinal plants are considered the best solution for such serious issue due to their richness with variable secondary metabolites that are characterized by their astonishing multiple therapeutic properties [3].

Ailanthus altissima (Mill) Swingle, commonly known as the tree of heaven, belongs to family Simarobaceae [4]. It had been utilized as a traditional herbal remedy of many ailments such as ascariasis, bleeding, diarrhea, dysentery, hemorrhoids beside other gastric and intestinal disorders [5-7]. Many recent researches reported the significant therapeutic potential of *A. altissima* as it possessed remarkable anti-inflammatory, antimalarial, antiplasmodial, antiviral, antimicrobial, cytotoxic and analgesic activities [8-9]. Various secondary metabolites were characterized from the various parts of *A. altissima* including quassinoids, flavonoids, phenolics, alkaloids, sterols, terpenoids, volatile components and lignans [10-13].

The ultra-high performance liquid chromatography (UHPLC) hyphenated with mass spectrometer (MS) is a new advancement of separation and identification techniques which is applied to recognize the major and minor phytochemicals in the various botanical samples. The main purpose of this study is to isolate and

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tentatively identify the various classes of secondary metabolites existed in methanol extract of *A. altissima* leaves and its ethyl acetate and *n*- butanol derived fractions through application of UPLC-ESI-MS analysis. Also, assessing their antimicrobial potential against some pathogenic microbes.

2. RESULTS AND DISCUSSION

2.1. Identification of the major chemical constituents of *A. altissima* using (UPLC-ESI-(-ve)-MS)

The UPLC-ESI-MS was used to identify the phytochemical constituents of the methanol extract of the *A. altissima* leaves as well as its ethyl acetate and *n*-butanol derived fractions and their TICs were exhibited in Figure 1.

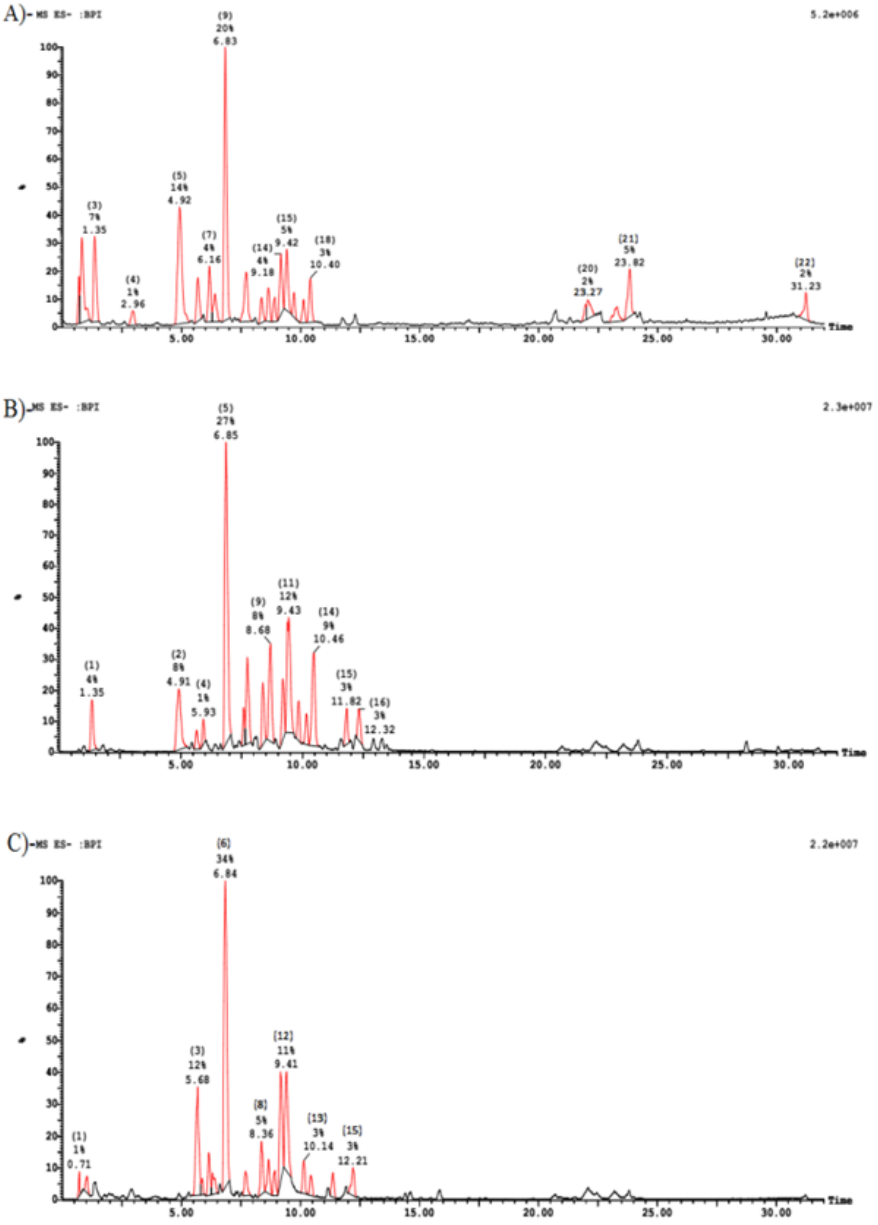

Figure 1. Total ion chromatograms (TIC) of A)-85% MeOH extract, B)- EtOAc fraction and C)- *n*-BuOH fraction of *Ailanthus altissima* leaves, Numbering of peaks refers to compounds tentatively identified in Table (1-3).

2.1.1. Compounds tentatively identified in the methanol extract of A. altissima leaves

Twenty phenolic compounds were identified in *A. altissima* methanol extract. The retention time, the molecular weight and the MS fragmentation data of these compounds were presented in **Table 1**. Peak 2, R_t 0.80 min, showed a molecular ion at m/*z* 377 which produced fragment ions at m/*z* 197 (syringic acid), 179 due to loss of hydroxyl radical (18 Da) from the syringic acid and 149. This compound was identified as a derivative of syringic acid [16]. Peak 3, R_t 1.35 min, exhibited a molecular ion at m/*z* 331[M−H]$^-$ which was fragmented producing two base peaks at m/*z* 169 and 125 which are characteristic peaks to gallic acid and formed due to loss of hexoside moiety (162 Da). So, this compound was identified as galloyl hexoside [17]. Peak 4, 6 and 7 showed the same deprotonated molecular ion at m/*z* 353 which produced a fragment ion at m/*z* 191 (quinic acid). So, they were identified as chlorogenic acid isomers [18, 19]. Moreover, two more fragment ions were recorded in MS spectra of peak 4 and 7 at m/*z* 179 and 135 which are characteristic peaks for caffeic acid. Peak 5, R_t 4.92 min, exhibited the deprotonated molecule ion [M − H]$^-$ at *m/z* 183 and a base peak at *m/z* 124 [M − H−44−15]$^-$ due to loss of CO_2 (44 Da) and CH_3 (15 Da). So, this compound was identified as methyl gallate [20]. Peak 8, R_t 6.40 min, exhibited the parent ion at m/*z* 291 and daughter ions at m/*z* 247 corresponding to the loss of CO_2 (44 Da), 191 and 175 which are in full agreement with the MS/MS data of brevifolin carboxylate [21]. Peak 9, R_t 6.83 min, possessed a molecule ion at m/*z* 633 [M − H]$^-$ which produced two fragment ions at m/*z* 463 [M−H−170]$^-$ due to loss of gallate moiety and 301[M−H−170−162]$^-$ due to loss of galloylglucose. Such fragmentation pattern is characteristic to corilagin [21, 22]. Peak 10, R_t, 7.17 min, exhibited [M−H]$^-$ ion at m/*z* 305 and fragment ions at m/*z* 273, 247 and 169. This compound was identified as (epi) gallocatechin [23]. Peak 11, R_t 8.35 min, exhibited a parent peak at m/*z* 785 which produced the following daughter peaks in MS spectrum; 633 [M−H−152]$^-$ due to loss of galloyl moiety, 331[M−2H−152−301]$^-$ due to loss of galloyl and HHDP moieties, respectively, 301 (HHDP) and 169 (gallate moiety). This compound was identified as digalloyl-HHDP-glucose [23]. Peak 12, R_t 8.64 min, showed a deprotonated ion peak at m/*z* 615 in addition to three daughter peaks at m/*z* 463[M−H−152]$^-$ due to loss of galloyl moiety, 301 (quercetin) and 169 (gallate). So, it was identified as quercetin galloyl hexoside [24]. Peak 13, R_t 8.90 min, had a molecular ion peak at m/*z* 555 [M−H]$^-$ and fragment ions at m/*z* 433 [M−H−122]$^-$ corresponding to loss of benzoyl moiety and 300 (quercetin aglycone) [M−H−122−132]$^-$ due to loss of pentose sugar (132 Da) so that, it was identified as quercetin benzoyl pentoside [25, 26]. Peak 14, R_t 9.18 min, presented a molecule ion at m/*z* 895[M−H]$^-$ and fragment ions at 447 [M−H−162−132−154]$^-$ indicating loss of hexosyl, pentosyl and dihydrogalloyl moieties, respectively and 285 corresponding to luteolin aglycone formed as a result of losing one more hexosyl moiety. This compound was identified as luteolin dihydrogalloyl hexosyl pentosyl hexoside [27]. Peak 15, R_t 9.41 min, displayed a molecule ion at m/*z* 609 [M−H]$^-$ and fragment ions at m/*z* 463 [M−H−146]$^-$ corresponding to loss of deoxyhexosyl moiety (146 Da) and 301(quercetin) [M−H−146−162]$^-$ due to loss of hexosyl moiety (162 Da). Based on this MS data, this compound was identified as rutin [28]. Peak 16, R_t 9.72 min, presented a molecule ion peak at m/*z* 599 in addition to three base peaks at m/*z* 447 formed due to loss of galloyl moiety (152 Da), 301 (quercetin) produced due to loss of deoxyhexosyl moiety, 169 (gallate reside). So, this compound was identified as quercetin galloyl deoxyhexoside [24]. Peak 17, R_t 10.12 min, gave a molecule ion at m/*z* 431. The MS fragment ion at m/*z* 269 indicated the loss of hexosyl moiety so that this compound was identified as apigenin hexoside [19]. Peak 18, R_t 10.41 min, showed a deprotonated molecular ion at m/*z* 447 and MS fragment ion at m/*z* 285 indicating the loss of hexosyl moiety. So, this compound was identified as kaempferol hexoside [29]. Peak 19, R_t 21.96 min, showed a molecule ion at m/*z* 593 with fragment ions at m/*z* 577, 473, 353 and 297. The two fragment peaks at m/*z* 473 and 353 were produced due to loss of 120 Da which indicated that the two glycosyl moieties are attached to carbon atoms of flavonoid skeleton. Such c-glycosylation commonly occurs at C-6 and C-8 of flavonoid aglycone. Based on the mentioned MS data, this compound was identified as apigenin-6, 8-di-C-hexoside [30]. Peak 20, R_t 23.27 min, showed a molecule ion at m/*z* 325 and a fragment ion at m/*z* 293. This compound was identified as *p*-coumaroyl hexoside [31]. Peak 22, R_t 31.23 min, had a molecule ion at m/*z* 284 and product ions at m/*z* 217, 199, 134 and 117. This compound was identified as luteolin [17]. From the TIC, it was observed that the three major phytochemicals in the crude methanol extract of *A. altissima* leaves were corilagin, methyl gallate and galloyl hexoside, respectively.

Table 1. Compounds tentatively identified in 85% methanolic extract of *A. altissima* by UPLC-ESI-(-ve)-MS.

Peak no.	RT (min)	MW	[M-H]$^-$ (m/z)	Fragments (m/z)	Tentative Identification
1.	0.70	290	289	273, 175, 159	Unknown
2.	0.80	378	377	197, 179, 149	Syringic acid derivative
3.	1.35	332	331	169, 125	Galloyl hexoside
4.	2.96	354	353	191, 179, 134	Chlorogenic acid isomer

5.	4.92	184	183	124	Methyl gallate
6.	5.69	354	353	191	Chlorogenic acid isomer
7.	6.16	354	353	191, 179, 173, 135	Chlorogenic acid isomer
8.	6.40	292	291	247, 191, 175	Brevifolin carboxylate
9.	6.83	634	633	463, 331	Corilagin
10.	7.71	306	305	273, 247, 169	(Epi)gallocatechin
11.	8.35	786	785	615, 331, 301, 169	Heterophylliin A (Digallolyl HHDP glucose)
12.	8.64	616	615	463, 301, 169	Quercetin galloyl hexoside
13.	8.90	556	555	433, 300	Quercetin benzoyl pentoside
14.	9.18	896	895	447, 285	Luteolin dihydrogalloyl hexosyl pentosyl hexoside.
15.	9.41	610	609	463, 301	Rutin
16.	9.72	600	599	447, 301, 169	Quercetin galloyl deoxyhexoside
17.	10.12	432	431	269	Apigenin hexoside
18.	10.41	448	447	285	Kaempferol hexoside
19.	21.96	594	593	577, 473, 353, 297	Vicenin -2 (Apigenin-6,8-di-C-hexoside)
20.	23.27	326	325	293	*p*-coumaroyl hexoside
21.	23.82	556	555	---	Unknown
22.	31.23	285	284	217, 199, 134, 117	Luteolin

2.1.2. Compounds tentatively identified in the ethyl acetate fraction derived from the methanol extract of A. altissima leaves

Fifteen phenolic compounds were identified in the *A. altissima* ethyl acetate derived fraction. The retention time, the molecular weight and the MS data of these compounds were presented in **Table 2**. Peak 1, R_t =1.35 min, exhibited a molecular ion peak ion at *m/z* 169 [M − H]-. It was further fragmented in the MS spectrum producing a characteristic product ion at *m/z* 125 corresponding to the loss of CO_2 (44 Da). It was tentatively identified as gallic acid [24]. Peak 2, R_t =4.91 min, exhibited a molecule ion at *m/z* 183 and a fragment ion at *m/z* 124 which are in consistent with methyl gallate that was also mentioned in *A. altissima* MeOH extract **(peak 5)**. Peak 3 and 4 at R_t 5.64 and 5.93 min, respectively displayed a molecular ion peak at m/*z* 353 (caffeoyl quinic acid) and MS fragment ions at m/*z* 191 (quinic acid) and 191, 179 (caffeic acid) and 135 (caffeic acid – CO_2), respectively. So, they were identified as Chlorogenic acid isomers [18, 19]. Peak 5, R_t 6.85 min, possessed a molecule ion at m/*z* 633 and two fragment ions at m/*z* 463 and 301 so that, this compound was identified as corilagin which was also identified in *A. altissima* MeOH extract **(peak 9)**. Peak 7, R_t 7.74 min, the [M−H]–ion at m/z 305 [M−H]-and fragment ions at m/z 273, 247 (brevifolin) [M−44−15]- due to loss of CO2 and CH3, respectively and 217, which suggested a methyl ester derivative of brevifolin carboxylate [32]. Peak 8, R_t 8.38 min, exhibited a parent peak at m/*z* 785 and fragment ions at m/*z* 633, 331, 301 and 169. This compound was identified as digalloyl-HHDP-glucose that was also identified in the *A. altissima* MeOH extract **(peak 11)**. Peak 9, R_t 8.68 min, showed a [M−H]- ion at m/*z* 615 in addition to three fragment ions at m/*z* 463, 301 and 169. Such MS data confirmed that this compound is quercetin galloyl hexoside that was also identified in the *A. altissima* MeOH extract **(peak 12)**. Peak 10, 11, 12, 13 and 14 exhibited the MS fragmentation patterns of luteolin dihydrogalloyl hexosyl pentosyl hexoside, rutin, quercetin galloyl deoxyhexoside, apigenin hexoside and kaempferol hexoside, respectively which were identical to the MS data of the same compounds that are already identified in the MeOH extract of *A. altissima*. Peak 15, R_t 11.82 min, had the [M−H]- ion at m/*z* 523 and fragment ions at m/*z* 301, 179 and 151 which were indicative to quercetin aglycone so that it was identified as a derivative of quercetin [33]. Peak 16, R_t 12.32 min, exhibited a precursor ion at m/*z* 409 and daughter ions at m/*z* 285 and 179. This compound was identified as luteolin derivative [19]. From the TIC, it was observed that the three major phytochemicals in the ethyl acetate fraction were corilagin, rutin and kaempferol hexoside, respectively.

Table 2. Compounds tentatively identified in EtOAc fraction derived from methanolic extract of *A. altissima* leaves by UPLC-ESI-(-ve)-MS.

Peak no.	RT (min)	MW	[M-H]- (m/*z*)	Fragments (m/*z*)	Tentative Identification
1.	1.35	170	169	125	Gallic acid
2.	4.91	184	183	124	Methyl gallate
3.	5.64	354	353	191	Chlorogenic acid isomer
4.	5.93	354	353	191, 179, 135	Chlorogenic acid isomer
5.	6.85	634	633	463, 301	Corilagin
6.	7.58	398	397	365, 319, 275	Unknown
7.	7.74	306	305	273, 247, 217	Methyl brevifolin carboxylate
8.	8.38	786	785	633, 331, 301, 169	Digalloyl-HHDP-glucose

9.	8.68	616	615	463, 301, 169	Quercetin galloyl hexoside
10.	9.20	896	895	447, 285	Luteolin dihydrogalloyl hexosyl pentosyl hexoside.
11.	9.43	610	609	463, 301	Rutin
12.	9.85	600	599	447, 301, 169	Quercetin galloyl deoxyhexoside
13.	10.16	432	431	269	Apigenin hexoside
14.	10.46	448	447	285	Kaempferol hexoside
15.	11.82	524	523	301, 179, 151	Quercetin derivative
16.	12.32	410	409	285, 179	Luteolin derivative

2.1.3. Compounds tentatively identified in the n-butanol fraction derived from the methanol extract of A. altissima leaves

Fourteen phenolic compounds were identified in the *A. altissima n*-butanol fraction. The retention time, the molecular weight and the MS fragmentation data of the compounds tentatively identified in the *n*-butanol fraction derived from the methanol extract of *A. altissima* were presented in **Table 3**. Peak 2, 5, 7 and 10 exhibited identical MS data of galloyl hexoside, brevifolin carboxylate, (epi)gallocatechin and quercetin benzoyl pentoside, respectively. These compounds were already identified in *A. altissima* MeOH extract. Moreover, peak 3, 4, 6, 9, 11, 12 and 13 were identified as chlorogenic acid, cryptochlorogenic acid, corilagin, quercetin galloyl hexoside, luteolin dihydrogalloyl hexosyl pentosyl hexoside, rutin and apigenin hexoside, respectively based on the MS fragmentation data. These compounds were also identified in both *A. altissima* MeOH extract and its ethyl acetate derived fraction. The MS data of both peak 8 and 15 was consistent with MS data of digalloyl HHDP glucose and luteolin derivative, respectively which were also identified in the ethyl acetate derived fraction of *A. altissima*. Peak 14, R_t 10.44 min, showed a molecular ion peak at m/*z* 593 which was further fragmented into two base peaks at m/*z* 447 $[M-H-146]^-$ formed as a result of loss of deoxyhexosyl residue and 285 (kaempferol/luteolin aglycone) $[M-H-146-162]^-$ formed due to subsequent loss of hexosyl moiety. This compound was identified as Kaempferol-3-*O*-rutinoside/luteolin-7-*O*- rutinoside [16]. From the TIC, it was observed that the three major phytochemicals in the *n*-butanol fraction were corilagin, chlorogenic acid isomer and rutin, respectively.

The main phenolic compound in the methanol extract of *A. altissima* leaves and its ethyl acetate and *n*-butanol derived fractions was corilagin which is an ellagitannin reported to possess cytotoxic, anti-inflammatory, antimicrobial, antioxidant, antiatherogenic, antihypertensive and hepatoprotective activities [6, 34, 35]. Corilagin was previously separated from *A. altissima* fruit [6] but it is the first time to be reported from the plant leaves.

Table 3. Compounds tentatively identified in *n*-BuOH fraction derived from methanolic extract of *A. altissima* leaves by UPLC-ESI-(-ve)-MS.

Peak no.	RT (min)	MW	$[M-H]^-$ (m/*z*)	Fragments (m/*z*)	Tentative Identification
1.	0.71	290	289	273, 243, 175	Unknown
2.	1.02	332	331	169	Galloyl hexoside
3.	5.68	354	353	191	Chlorogenic acid
4.	6.15	354	353	191, 179, 173	Cryptochlorogenic acid
5.	6.31	292	291	247, 191, 175	Brevifolin carboxylate
6.	6.84	634	633	463, 301, 169	Corilagin
7.	7.70	306	305	169	(Epi)gallocatechin
8.	8.36	786	785	463, 331, 301, 169	Digalloyl-HHDP-glucose
9.	8.66	616	615	463, 300, 169	Quercetin galloyl hexoside
10.	8.92	556	555	433, 300	Quercetin benzoyl pentoside
11.	9.16	896	895	447, 285	Luteolin dihydrogalloyl hexosyl pentosyl hexoside.
12.	9.41	610	609	463, 301	Rutin
13.	10.14	432	431	269	Apigenin hexoside
14.	10.44	594	593	447, 285	Kaempferol-3-*O*-rutinoside/luteolin-7-*O*-rutinoside
15.	12.21	410	409	285, 179, 135	Luteolin derivative

2.2. Antimicrobial activity

2.2.1. Agar well diffusion assay

The antimicrobial activity of the crude methanol extract of *A. altissima* leaves as well as its ethyl acetate and *n*-butanol derived fractions was investigated against six microbial strains using agar well diffusion assay **(Table 4)**. In particular, all the test samples exerted no antimicrobial activity against the pathogenic yeast *Mucor circinelloides* (AUMC 6696). Moreover, the ethyl acetate derived fraction exhibited the most potent antimicrobial activity against the other test microorganisms followed by the *n*-butanol fraction then the crude methanol extract. The ethyl acetate fraction exerted potent antibacterial activity against *P. aeruginosa* where the inhibition zone diameter was 40 mm. It also exerted strong antimicrobial activity against *S. aureus* and *C. albicans* where the inhibition zone diameter was 30 mm and 25 mm, respectively. It also exhibited moderate antibacterial potential against *B. subtilis* and *E. coli* with inhibition zone of 20 mm diameter. Furthermore, the antimicrobial response of the ethyl acetate derived fraction against *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* was stronger than that of the used positive control (gentamycin) tested at the same concentration and conditions. The *n*-butanol fraction exerted strong antifungal potential against *C. albicans* (inhibition zone = 25 mm diameter) which was stronger than the response exerted by gentamycin. It also exerted moderate antibacterial activity against *B. subtilis* and *S. aureus* and weak activity against *E. coli* and *P. aeruginosa* where the inhibition zone diameter was 20 mm, 17 mm, 15mm and 11 mm, respectively. The crude methanol extract showed moderate antimicrobial activity against *B. subtilis* and *C. albicans* and weak activity against *S. aureus*, *E. coli* and *P. aeruginosa* where the inhibition zone diameter was 18 mm, 20 mm, 11 mm, 15mm and 10 mm, respectively.

2.2.2. Broth microdilution assay

Broth microdilution technique was performed to calculate the MICs values of the test samples against the susceptible test pathogens. Results were displayed in **Table 5**. The test sample was considered potent microbial inhibitor if it possessed MIC value of equal or lower than 500 μg/mL [15]. Based on that, the ethyl acetate fraction could be considered as a potent antimicrobial agent against all the test microorganisms where its MIC ranged between 3.9 and 62.5 μg/mL. It also possessed remarkable antimicrobial potential against *P. aeruginosa*, *S. aureus* and *C. albicans* where its MIC values were 3.9, 7.8 and 15.6 μg/mL, respectively. The MIC values of the *n*-butanol fraction against the test microbes ranged between 15.6 and 1000 μg/mL. It possessed the same MIC value against *C. albicans* as the ethyl acetate fraction. The MIC values of the methanol extract against the test microbes ranged between 62.5 and 1000 μg/mL. It possessed the same MIC value against *B. subtilis* as the ethyl acetate fraction. Both the methanol extract and the *n*-butanol fraction showed strong antimicrobial activity against all the test microorganisms except for *P. aeruginosa* where their MIC values against it was 1000 μg/mL.

Table 4. Antimicrobial activity via well diffusion assay of the methanol extract, the ethyl acetate and the *n*-butanol derived fractions of *A. altissima* leaves.

Sample	Crude MeOH extract	EtOAc derived Fraction	*n*-BuOH derived Fraction	Gentamycin
	Zone of growth inhibition (mm)			
Pathogenic microorganism				
Gram positive bacteria				
Bacillus subtilis (ATCC 6633)	18	20	20	25
Staphylococcus aureus (ATCC 6538)	11	30	17	19
Gram negative bacteria				
Escherichia coli (ATCC 8739)	15	20	15	17
Pseudomonas aeruginosa (ATCC 90274)	10	40	11	22
Fungal strains				
Candida albicans (ATCC 10221)	20	25	24	21
Mucor circinelloides (AUMC 6696)	NA	NA	NA	16

Results are expressed as zone of growth inhibition (mm), NA: no growth inhibition.

Table 5. Minimal inhibitory concentrations (MIC) of the methanol extract, the ethyl acetate and the *n*-butanol derived fractions of *A. altissima* leaves against tested microorganisms.

Sample	Crude MeOH extract	EtOAc derived Fraction	*n*-BuOH derived Fraction
	Minimum inhibitory Conc. (μg/mL)		
Pathogenic microorganisms			
Bacillus subtilis (ATCC 6633)	62.5	62.5	31.25
Staphylococcus aureus (ATCC 6538)	500	7.8	250
Escherichia coli (ATCC 8739)	500	62.5	125
Pseudomonas aeruginosa (ATCC 90274)	1000	3.9	1000
Candida albicans (ATCC 10221)	125	15.6	15.6

3. CONCLUSION

The crude methanol extract of *A. altissima* leaves as well as its ethyl acetate and *n*- butanol derived fractions exhibited remarkable antimicrobial activity against the test pathogenic microorganisms. The most promising antimicrobial potential was displayed by the ethyl acetate derived fraction. So that, the ethyl acetate fraction derived from the methanol extract of *A. altissima* leaves could considered as a potent antimicrobial candidate. Also, various phenolic compounds were tentatively identified in the three test samples via UPLC-ESI-MS. Moreover, corilagin was the main phenolic constituent in the three test samples. The leaves of *A. altissima* have been rich source with bioactive phenolics especially corilagin which supports their application in pharmaceuticals as drug leads or food industries as ingredients of functional foods.

4. MATERIALS AND METHODS

4.1. Plant material

The leaves of *Ailanthus altissima* were collected, in September 2017, from Orman Garden, Giza, Egypt. A plant specimen was identified by Mrs. Treaze Labib (a consultant of plant taxonomy at Agriculture Ministry and the ex-director of Orman Garden) and Mrs. Rehab Mohamed Eid (a botanist at Orman Garden Herbarium). A voucher specimen (No. 198 AC) was deposited in Orman Garden Herbarium. The collected fresh leaves were dried at ambient temperature away from the sunlight and were then ground to fine powder using electric mixer.

4.2. Extraction and Fractionation Processes

The plant leaves dry powder (200 g) was extracted with 85% aqueous methanol at room temperature. The filtrate was further concentrated at reduced temperature and pressure via rotary evaporator (BUCHI, Germany) and the extraction process was repeated three times affording crude extract of uniform weight with yield 23.50%. The crude methanol extract (47.00 g) was successively fractionated by partition using different organic solvents; petroleum ether, dichloromethane, ethyl acetate and *n*-butanol which were also evaporated under vacuum till complete dryness yielding 0.20, 3.82, 6.36, 16.20& 14.30 g corresponding to petroleum ether, dichloromethane, ethyl acetate, *n*-butanol and aqueous fractions, respectively.

4.3. UPLC-ESI-MS analysis

The metabolic profile of the plant crude methanol extract as well as its ethyl acetate and *n*-butanol derived fractions was determined using 100 μg/mL of each sample which were previously dissolved in HPLC analytical grade MeOH and filtered via a membrane disc filter (0.2 μm). Samples (10 μL) were then injected into the UPLC instrument; Waters XEVO TQD UPLC- ESI-MS (MA01757, Milford, USA) equipped with

ACQUITY UPLC- BEH reverse phase C-18 (1.7: 2.1 μm× 50 mm) column. The elution of the test samples was carried out using gradient mixture of filtered and degassed water (A) and methanol (B) both acidified with 0.1% formic acid at a flow rate of 0.2 mL/min and the single run took 32 min. The gradient elution was set as follows: 0–5 min, 10%–30% B; 5–15 min, 30%–70% B; 15–25 min, 70%–90% B, 25–32 min, 90%–100% B.

The parameters for MS analysis were carried out using negative ion mode as follows: source temperature 150°C, cone voltage 60 eV, capillary voltage 3 kV, desolvation temperature 440°C, cone gas flow 50 L/h, and desolvation gas flow 900 L/h. Mass spectra were detected in the ESI between *m/z* 100–1000. The peaks and spectra were processed using the Masslynx 4.1 software. The tentative identification process was performed through comparing the retention time (R_t) of each peak and the corresponding molecular ion peak, together with the major fragments recorded in the MS spectra with the previously published data.

4.4. Antimicrobial Activity

The antimicrobial potential of the methanol extract of *A. altissima* leaves as well as its ethyl acetate and *n*-butanol derived fractions were assessed against six pathogenic microbes which are; two gram positive bacteria; *Bacillus Subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 6538), two gram negative bacteria; *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 90274) in addition to two pathogenic fungi; *Candida albicans* (ATCC 10221) and *Mucor circinelloides* (AUMC 6696). The microbial strains were inoculated in non-supplemented Mueller-Hinton agar or routine bacteriology laboratory Mueller-Hinton agar plates (pH, 7.2–7.4 after gelling) and incubated overnight at 37°C. Gentamycin was used as a positive control. The susceptibility of the microbial strains toward the test samples was investigated through two antimicrobial techniques which are agar well diffusion and broth microdilution.

4.4.1. Agar well diffusion assay

Agar well diffusion method is widely used to evaluate the antimicrobial activity of the plant extracts. Briefly, the agar plates' surfaces were inoculated with the test microbes by spreading a volume of the microbial inoculum (10^8 CFU/mL) over its entire surface. Then, wells of 6 mm diameter were punched aseptically with a sterile cork borer and a volume of 100 μL of the antimicrobial agent (gentamycin) or solution of test samples at concentration of 10 mg/mL (methanol) were loaded into the wells. Then, the agar plates were incubated for 24 h at 37°C for bacteria or 48 h at 37°C for yeast. The test samples as well as the positive control diffused in the agar medium and inhibit the growth of the microbial strains tested. The antimicrobial activity is measured as a zone of microbial growth inhibition in mm [1]. The test sample exerted potent antimicrobial activity when the diameter of the growth inhibition zone is greater than 30 mm, strong antimicrobial response when the growth inhibition zone diameter ranged between 30 and 21 mm, moderate response when the growth inhibition zone diameter ranged between 20 and 16 mm, weak potential when the growth inhibition zone diameter ranged between 15 and 10 mm and little or no activity if the inhibition zone diameter is less than 10 mm [14].

4.4.2. Broth Microdilution Assay

Broth microdilution test was performed to calculate the minimum inhibitory concentration (MIC) which is the lowest concentration of the antimicrobial agent that inhibits the visible growth of the test microorganism using 96-well microtiter plates. Briefly, pure cultures of the test microorganisms were grown overnight and diluted in Muller Hinton broth to a concentration between 10^5 CFU/mL to 10^6 CFU/mL. A stock solution of the test samples was then prepared by dissolving 10 mg of each sample in 10 mL distilled water (1000 ppm). Further serial two fold dilutions of the test samples was made in 96-well microtiter plates. All the test samples were inoculated with 10 μL of the previously prepared microbial suspensions. A positive and a negative control wells were included for every test microorganism to demonstrate adequate microbial growth over the course of the incubation period and media sterility, respectively. The microtiter plates were then incubated for 24 h at 37°C. Turbidity indicates the microbial growth and the MIC is the lowest concentration where no visible growth is observed [15].

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