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HPLC Phenolic Profiling of Methanol Extracts of Some Species belonging to Genus *Abutilon*

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ABSTRACT

Objectives: This study was aimed to analyzing the phenolic constituents of the 80% methanol extracts of three *Abutilon* species (*A. pannosum*, *A. hybridum* and *A. hirtum*) (family Malvaceae). **Methods**: Defatted 80% methanol extracts of *A. pannosum*, *A. hybridum* and *A. hirtum* aerial parts (leaves and stems) were, each, analyzed using high performance liquid chromatographic (HPLC) technique against external standard calibration at λ 280 and 330 nm. **Results:** Twelve phenolic acids were tentatively identified from *A. pannosum*, *A. hybridum* and *A. hirtum* representing 9.03, 12.23 and 20.51%, respectively. The major identified phenolic acids peaks were that of protocatechuic acid in *A. pannosum* (1.75%), ferulic acid in *A. hybridum* (2.09%) and ellagic acid in *A. hirtum* (4.0%). On the other hand, 21 flavonoids were tentatively identified from *A. pannosum* representing (53.78%), in comparison with 20 identified from *A. hybridum* (42.12%) and 19 identified from *A. hirtum* (35.68%). Kaempferol 3-*O*- [2$^{\prime\prime\prime}$-*p*-coumaroyl-*β*-D-glucopyranoside] was the major identified flavonoid glycoside in *A. pannosum* and *A. hybridum* methanolic extracts (13.27 and 5.13%, respectively) while quercitrin was the major flavonoid glycoside identified in *A. hirtum* (3.21%). The major flavonoid aglycones identified were luteolin (3.68%) in *A. pannosum*, apigenin (9.46%) in *A. hybridum* and quercetin (5.85%) in *A. hirtum*. **Conclusion:** The present study revealed that the investigated extracts are rich in phenolic contents; *A. pannosum* has the highest concentration of flavonoids and the least concentration of phenolic acids, where *A. hirtum* has the highest concentration of phenolic acids, showing promising phenolic content for further study of their isolation, identification as well as evaluation of their biological activity.

Keywords: *Abutilon pannosum; A. hybridum; A. hirtum; Phenolic constituents; HPLC.*

INTRODUCTION

Natural products have played an important role in treating and preventing human ailments. Over 50% of all drugs (and their derivatives and analogs) in clinical use, are estimated higher to be plant derived natural products[1]. The importance of medicinal plants have massively increased due to reorganization of natural products and process in sustaining human and environment health in the economic environment[2]. Flavonoids, phenolics, phenolic acids, coumarins, lignans, lignins and tannins and other forms of phenolic compounds were found to have strong antiallergic, anti-inflammatory, antimicrobial and antioxidant activities[3]. Members of family Malvaceae are widely distributed in tropical and temperate regions[4]. *Abutilon* is the vast genus from the family Malvaceae that contains almost 150 annual or perennial herbs or small trees. It is usually

found in the tropical and subtropical regions of Africa, America, Asia and Australia[5]. Variant biological activities as analgesic, antibacterial, antifungal, anti-hyperglycemic, anti-inflammatory, antioxidant, antipyretic, cytotoxic, gastroprotective, hepatoprotective and wound healing activities have been reported from different *Abutilon* species, owing to the presence of various phytoconstituents such as flavonoids, phenolic acids, sterols, triterpenes, quinones, coumarins, alkaloids and other secondary metabolites. Flavonoids or bioflavonoids are the main secondary metabolites in the genus *Abutilon*[6].

Among this, *Abutilon pannosum* (Forst.f) Schltdl., is an under shrub and is distributed in India, Pakistan, Tropical Africa, China and Arabia. The leaves of *A. pannosum* were used as adjunct to medicines used for stack complaints. The plant contains mucilage, tannins, gallic acid and sequiterpens[7]. *Abutilon hirtum* (Lam.) Sweet is a perennial herb or shrub, 0.5-2.5m in height [Synonym: *A. graveolens* (Roxb. Ex Hornem.) Wight & Arn. Lam.]. Traditionally, the roots are used as antipyretic, antitussive and as analgesic in toothaches while the leaves or flowers are applied to abscesses[8]. While *Abutilon hybridum* is commonly known as the "flowering maple" and is known to originate in South America[9], no previous chemical or biological activities were reported on *A. hybridum*.

Reviewing the available literature for the three species; (*A. pannosum*, *A. hybridum* and *A. hirtum*) no data was reported for their lipoidal matter content, thus in our previous report[10] a comparative study of their lipoidal content of their aerial parts was done. Also, there are no data could be traced concerning the constituents of *Abutilon hybridum*, on the other hand, apigenin 7-*O*-*β*-D (6$^{\prime\prime}$-*p*-coumaroyl) glucopyranoside[6], apigenin 7-*O*-*β*-D-6$^{\prime\prime}$-*p*-hydroxycinnamoyl glucopyranoside and kaempferol 4′-*O*-(6$^{\prime\prime}$-*O*-E-*p*-coumaroyl)-*β*-D-glucopyranoside were identified from *Abutilon pannosum*[11-12] and one study could be traced concerning the flavonoids and phenolic acids of the aerial parts of *Abutilon hirtum* showed the presence of kaempferol, quercetin, rutin, gallic, *p*-coumaric and caffeic acids[13]. Basing on these data, the present study was aimed to analyze the phenolic compounds present in the aerial parts of three *Abutilon* species (*A. pannosum*, *A. hybridum* and *A. hirtum*) using HPLC technique.

MATERIAL AND METHODS

Plant material

The aerial parts (Leaves and stems) of *A. hybridum* and *A. hirtum* were collected in February and March 2015 from El-Orman Botanical Garden, Giza, Egypt, while the aerial parts of *A. pannosum* were collected from Eltaif road, Saudi Arabia in March 2015. The taxonomic authentication of the three plants was performed by Dr. Thérèse Labib, Head of the Taxonomists at Al-Orman Botanical Garden, Giza, Egypt. Voucher specimens (01Apa, 02Ahy, and 03Ahi /2015) for *A. pannosum*, *A. hybridum* and *A. hirtum* respectively, were deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Ain-Helwan, Cairo, Egypt.

Chemicals

HPLC grade of methanol, ethanol, acetic acid and acetonitrile from Merck were supplied by Food Chemistry Laboratory, Agricultural Research Center, Food Technology Research Institute, Giza, Egypt.

Authentic phenolic acids

4-amino-benzoic, benzoic, caffeic, chlorogenic, ellagic, ferulic, gallic, isoferulic, *p*-coumaric, *p*-hydroxy benzoic, protocatechuic, vanillic and rosmarinic acids moreover catechol and pyrogallol were supplied by Food Chemistry Laboratory, Agricultural Research Center, Food Technology Research Institute, Giza, Egypt.

Authentic flavonoids

Luteolin-6-*C*-glucoside-8-*C*-arabinoside, apigenin 6-*C*-*β*-D-arabinoside-8-*C*-*β*-D-galactopyranoside, apigenin-6-*C*-glucoide-8-*C*-rhamnoside, luteolin-7-*O*-*β*-D- glucopyranoside, catechin, epicatechin, luteolin, naringin, rutin, hesperidin, apigenin-7-*O*-neohespiroside, kaempferol-3,7-*O*-dirhamnoside, apigenin-7-*O*-*β*-D-glucopyranoside, quercitrin, quercetin, kaempferol 3-*O*-[2$^{\prime\prime}$-*p*-coumaroyl-*β*-D-glucopyranoside], naringenin, hesperetin, kaempferol, rhamnetin, apigenin and acacetin were supplied by Food Chemistry Laboratory, Agricultural Research Center, Food Technology Research Institute, Giza, Egypt.

Apparatus

HPLC identification of polyphenolic compounds (phenolic acids/flavonoids) was handled at Food Chemistry Laboratory, Agricultural Research Center, Food Technology Research Institute, Giza, Egypt on Agilent 1200 series chromatograph coupled to a quaternary pump (DE 62975591) and UV detector (DE 82800737) and C18 reverse phase (BDS 5 µm, Labio, Czech Republic) packed stainless-steel column (4 × 250 mm, I.D.). The chromatographic system was equipped with a quaternary pump (water 2695 alliance, Milford MA, USA), degasser, auto injector, and diode array (DAD) detector.

Methods

Preparation of the plant extracts

Fine air-dried powdered (2 g) of aerial parts of the three *Abutilon* species were individually extracted with 80% aqueous methanol till exhaustion (4 × 250 mL)

Table 1. HPLC phenolic profiling of 80% methanol extracts of the aerial parts of *A. pannosum, A. hybridum and A. hirtum*

Tentative identified phenolic compounds	*A. pannosum*		*A. hybridum*		*A. hirtum*	
	R_t(min)	Area %	R_t (min)	Area %	R_t (min)	Area %
Pyrogallol	3.90	1.44	3.94	1.54	3.71	3.15
Gallic acid	5.36	0.16	5.40	0.14	5.46	0.13
4-Amino-benzoic acid	6.17	0.26	6.08	0.40	6.14	0.38
Catechol	6.65	0.41	6.68	0.15	6.71	0.79
Catechin	7.07	0.26	7.12	0.19	7.01	1.00
Chlorogenic acid	7.30	0.70	7.29	0.51	7.31	1.02
p-Hydroxy benzoic acid	7.87	0.39	7.86	0.75	7.72	2.27
Epicatechin	8.15	0.49	8.12	0.64	8.16	1.54
Vanillic acid	8.33	0.30	8.39	0.75	8.34	1.47
Caffeic acid	8.46	0.32	8.59	0.92	8.50	0.88
Protocatechuic acid	8.99	1.75	8.90	1.16	8.95	2.29
p-Coumaric acid	9.64	0.72	9.74	2.08	9.66	1.94
Ferulic acid	9.86	1.18	9.98	2.09	9.84	1.70
Isoferulic acid	10.17	0.79	10.14	0.44	10.02	2.21
Rosmarinic acid	10.29	0.73	10.27	1.91	10.32	2.22
Luteolin-6-*C*-glucoside-8-*C*-arabinoside	10.47	1.44	10.46	0.99	10.50	1.48
Ellagic acid	11.31	1.73	11.24	1.08	11.34	4.00
Apigenin-6-*C*-arabinoside-8-*C*-galactoside	11.48	1.34	11.44	1.32	11.49	2.72
Apigenin-6-*C*-glucoside-8-*C*-rhamnoside	11.91	2.32	11.88	1.27	11.93	2.37
Luteolin-7-*O*-glucoside	-	-	12.05	0.89	-	-
Luteolin	12.20	3.68	12.20	0.43	12.25	3.78
Naringin	12.39	2.75	12.49	1.27	12.45	0.87
Rutin	12.62	2.44	12.69	0.60	12.64	2.99
Hesperidin	12.94	3.32	-	-	12.92	1.68
Apigenin-7-*O*-neohespiroside	13.15	3.49	13.22	1.69	13.21	1.75
Kaempferol-3,7-*O*-dirhamnoside	13.37	3.03	13.36	1.38	13.43	0.83
Apigenin-7-*O*-*β*-D-glucopyranoside	13.89	2.16	13.73	0.45	-	-
Quercitrin	14.01	0.87	-	-	14.06	3.21
Quercetin	14.32	2.24	14.33	6.19	14.40	5.85
Kaempferol 3-*O*-[2\\\-*p*- coumaroyl- *β*-D - glucopyranoside]	14.77	13.27	14.77	5.13	14.86	2.31
Naringenin	14.97	2.64	15.01	3.39	15.05	1.25
Hesperetin	15.25	1.56	15.25	1.26	15.35	0.89
Kaempferol	16.10	1.50	16.23	3.68	16.28	0.44
Rhamnetin	16.36	2.25	16.54	1.24	-	-
Apigenin	17.27	1.44	17.28	9.46	17.31	0.65
Acacetin	18.75	1.29	18.71	0.65	19.03	0.07
Total phenolic acids identified	**9.03 %**		**12.23 %**		**20.51%**	
Total flavonoids identified	**53.78 %**		**42.12 %**		**35.68 %**	
Other phenolics identified	**1.81 %**		**1.69 %**		**3.94%**	
Total Identified compounds.	**64.62%**		**56.04%**		**60.13%**	
Total Non-identified compounds	**35.38%**		**43.96%**		**39.87%**	

R_t = retention time Area% = peak area

then filtered to remove free unextractable substances. The methanol (MeOH) filtrates were concentrated using rotary vacuum evaporator at 40-50ºC, each was defatted with petroleum ether to yield 370, 310 and 290 mg of defatted 80% methanol extract of *A. pannosum*, *A. hybridum* and *A. hirtum*, respectively, and the dried residues were preserved at 4-5ºC for further process[14].

HPLC Analysis

From the 80% methanol extract of each, of the aerial parts of *A. pannosum*, *A. hybridum* and *A. hirtum*, 150 mg was extracted individually with 10 mL methanol. Then, the sample was centrifuged for 7 min at 4200 rpm. The supernatant was filtered through polyamide filter Chromafil AO-45/25 and kept in a dry vial. About 1-3 mL was collected in separate vials for injection into reversed phase HPLC equipped with diode array (DAD) detector; the temperature was maintained at 35°C, with auto-sampling injector. The flow rate of the mobile phase was 1 mL/min with linear gradient elution and the injection volume was 10µLof the standards and extracts with mobile phase of water/acetic acid (98:2 v/v, solvent A) and methanol/acetonitrile (50:50, v/v, solvent B), starting with 5% B and increasing B to levels of 30% at 25 min, 40% at 35 min, 52% at 40 min, 70% at 50 min, 100% at 55 min. The polyphenolic compounds were assayed by external standard calibration at λ 280 nm and 330 nm[15-16].

RESULTS AND DISCUSSION

HPLC is a highly reproducible and precise quantitative analysis with automated operation that is nowadays a premier analytical technique used for the quality assessment of plant extract[17]. The identified compounds were deduced by comparing their retention times with that of the available reference standard phenolics that were injected under the same conditions. The quantitative estimation of each identified component depends on the relative measurements of the percentage of the area under the peak. The results recorded in **Table 1** and **Figures (1, 2, 3)** showed the types and concentrations of flavonoids and phenolic acids present in the 80% MeOH extract of the aerial parts of *A. pannosum*, *A. hybridum* and *A. hirtum*, respectively. It revealed that; twelve phenolic acids were tentatively identified from *A. pannosum*, *A. hybridum* and *A. hirtum* representing 9.03, 12.23 and 20.51%, respectively. The major identified phenolic acids peaks were that of protocatechuic acid in *A. pannosum* (1.75%), ferulic acid in *A. hybridum* (2.09%) and ellagic acid in *A. hirtum* (4%). On the other hand, 21 flavonoids were tentatively identified from *A. pannosum* representing (53.78%), 20 from *A. hybridum* (42.12%) and 19 from *A.hirtum* (35.68%). In addition to two other phenolics; pyrogallol and catechol were tentatively identified in the three *abutilon* species.

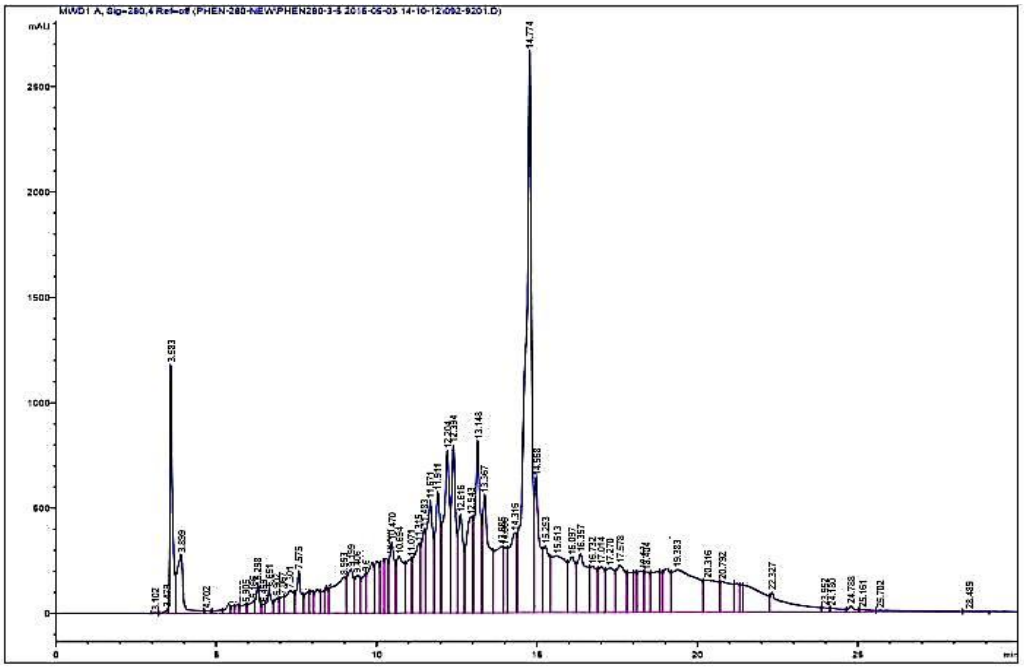

Figure 1. HPLC phenolic profile of the 80% methanol extract of *A. pannosum.*

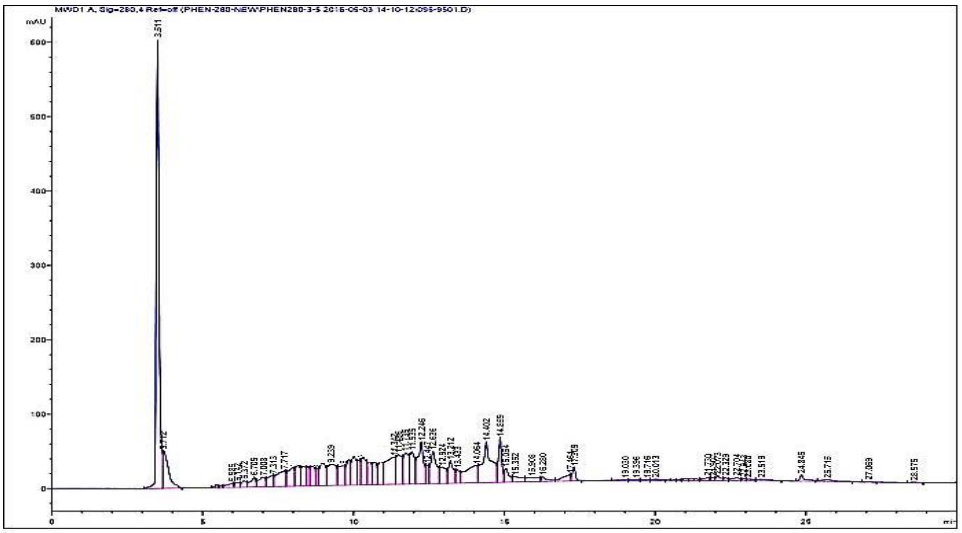

Figure 2. HPLC phenolic profile of the 80% methanol extract of *A. hybridum.*

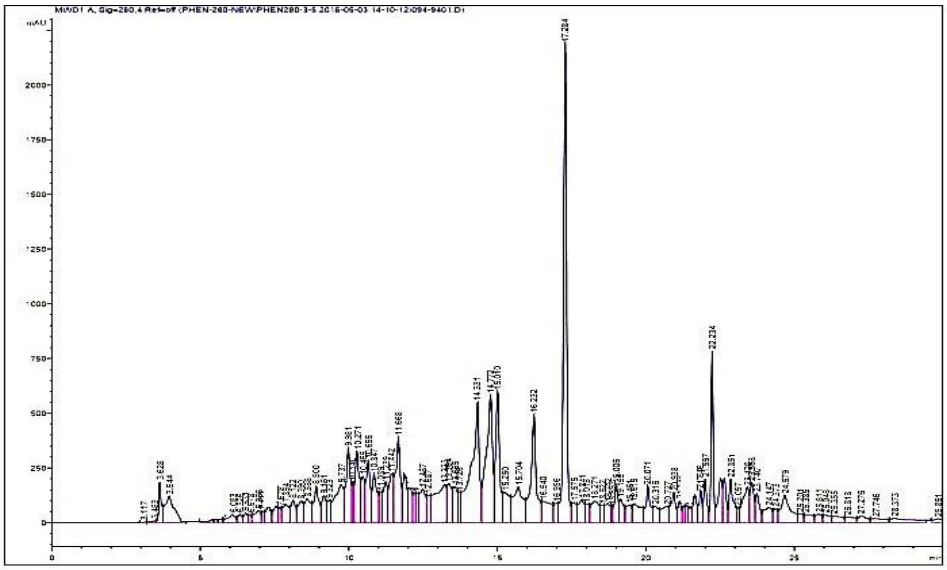

Figure 3. HPLC phenolic profile of the 80% methanol extract of *A. hirtum.*

Kaempferol 3-*O*-[2\\-*p*-coumaroyl-*β*-D-glucopyranoside] was the major identified flavonoid glycoside in *A. pannosum* and *A. hybridum* methanolic extracts (13.27 and 5.13%, respectively) while quercitrin was the major flavonoid glycoside identified in *A. hirtum* (3.21%). The major flavonoid aglycone in *A. pannosum* was luteolin (3.68%) which is useful in protection against cardiovascular diseases[18]. Apigenin in *A. hybridum* (9.46%) has strong antioxidant and anti-inflammatory activities and was reported as a cancer chemopreventive agent[19], while quercetin was the major aglycone identified in *A. hirtum* (5.85%). Quercetin, the most powerful antioxidant flavonoid was found also beneficial as antiallergic and has an important role in reducing risk for cancers as well as cardiovascular health improvement[20]. For the best of our knowledge, these identified phenolic compounds were tentatively identified for the first time from *A. pannosum* and *A. hybridum*. In accordance with the previous reported data[13], kaempferol, quercetin, rutin as well as, gallic, *p*-coumaric and caffeic acids were also identified from aerial parts of *A. hirtum* in addition to 27 other phenolic compounds tentatively identified for the first time from *A. hirtum*.

Previous studies on other *Abutilon* species also showed they are rich in phenolics as in *A. mauritianum*,

the HPLC analysis of the leaves methanol extract showed that *A. mauritianum* is rich in catechins, which was expected to be responsible for the antiproliferative activity of the methanol extract[21].

CONCLUSION

Based upon the HPLC fingerprints, the present study, conducted on three methanol *Abutilon* extracts, revealed that they are rich in phenolic contents with great similarity in their constituents. *A. hirtum* showed the highest concentration of phenolic acids followed by *A. hypridum* then *A. pannosum*, while *A. pannosum* showed the highest flavonoids contents followed by *A. hypridum* then *A. hirtum*. Therefore, it is worthwhile to proceed on study to evaluate their biological activity, therapeutic efficacy and toxicity as well as on the isolation and identification of the active compounds present in these valuable medicinal plants.

Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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