



Chemical constituents, antioxidant and antimicrobial activities of *Pterygota alata* (Roxb.) leaves extracts grown in Egypt

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Abstract

Overproduction of reactive species and microbial resistance to the existent antibiotics are still great health challenges. The current study aimed to evaluate the antioxidant and antimicrobial activities of *Pterygota alata* leaves extracts, in addition to isolation and identification of their chemical constituents. *In vitro* antioxidant activity was explored using 2,2'-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH), whereas the *in vitro* antimicrobial activity was evaluated using the disc agar plate method. Chemical constituents of the leaves extracts were isolated through column chromatography, and then identified using ¹H, ¹³C-NMR and IR spectroscopic tools. In the DPPH assay; the half maximal inhibitory concentration (IC₅₀) value of the total extract was 72.4 ± 1.4 µg/ ml, relative to 11.2 ± 0.6 µg/ ml of the standard ascorbic acid. On the other hand; the antimicrobial results revealed that the ethyl acetate extract showed the strongest inhibitory potential against most of the tested microbes with inhibition zones of 16.8- 22.8 mm, followed by petroleum ether with inhibition zones between 13.4- 19.6 mm, and n-BuOH with inhibition zones ranging from 14.9-19.3 mm. These results were compared with the standard antibiotics such as; Amphotericin β, Ampicillin, and Gentamycin. Furthermore; chromatographic isolation of the different solvents extracts resulted in the isolation of five phytoconstituents, their chemical structures were assigned as; β-sitosterol (1), apigenin-7-β-D-glycoside (2), gallic acid (3), luteolin-7-β-D-glucoside (4), and 4'-methoxy myricetin-3-β-D-glucoside (5). Current findings suggested that leaf extracts of *P. alata* could be used for the development of natural drugs; to treat microbial infections and reactive oxygen species (ROS) associated disorders.

Keywords: *Pterygota alata* (Roxb.), Phenolics, Antioxidant, Antimicrobial activities

1. Introduction

Reactive oxygen species (ROS) and/or reactive nitrogen species (RNS) are highly energetic molecules carrying single electrons able to penetrate cells and tissues. Over-production of these reactive species led to the generation of a highly risk phenomenon known as oxidative stress. This phenomenon was accompanied by several health disorders such as; inflammations and cancer diseases. Naturally occurring antioxidant compounds were able to diminish such harmful effects, due to their high capabilities to act as free radical scavengers as reported by Ghareeb *et al.*, (2018a, b, c); Sobeh *et al.*, (2018); Bakchiche *et al.*, (2019).

Infectious diseases remain a major challenge worldwide and are considered as great health issue. Ghareeb *et al.*, (2018d); Hamed *et al.*, (2019) pointed that there is high resistance of the pathogenic microbial strains towards the existing antibiotics. Therefore, best efforts are being done to isolate and identify alternative naturally occurring antimicrobial compounds.

The plant genus *Pterygota* Schott & Endl. (Sterculiaceae) comprises approximately fifteen to twenty species distributed in tropical Asia and Africa. *P. alata* (Roxb) R. Br. is a large deciduous tree known as Budah coconut tree. This plant was distributed in Asia especially in China; Vietnam, India, Philippines and Myanmar (Lin *et al.*, 2010). *P. alata* has a long history in the folk medicine for the treatment of many diseases including; hemorrhoids, dropsy, swelling oedema, gout, leprosy, pain and skin diseases (Prasad *et al.*, 2008; El-Sherei *et al.*, 2018).

Previous phytochemical investigations on various extracts of this plant led to the identification of several secondary metabolites including; non alkaloidal nitrogenous bases namely uracil, 1-methyl uracil, 3-methyl uracil, and adenine (El-Sherei *et al.*, 2018); flavonoids (Lin *et al.*, 2010; El-Sherei *et al.*, 2018); phenylpropanoids, triterpenes, lignans,

steroids, and anthraquinones (Lin *et al.*, 2010). From the biological point of view; *P. alata* showed numerous biological activities such as; antioxidant (Jahan *et al.*, 2014); anti-hyperglycemic (El-Sherei *et al.*, 2018); and anti-oxidative stress (El-Sherei *et al.*, 2018). Therefore; the current study aimed to investigate the chemical profile of *P. alata*, and to explore its *in vitro* antimicrobial and antioxidant potentials.

2. Material and methods

2.1. Equipment

The NMR spectra were recorded at 400 (^1H) and 100 (^{13}C) MHz on a Varian Mercury 400 NMR spectrometer, and δ values were reported in ppm relative to TMS in the convenient solvent. Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and silica gel G60-120 mesh (Merck, Germany) were used for column chromatography. However for paper chromatography; Whatman No. 1 sheets (Whatman Ltd., England) were used, while silica F254 plates (Merck) were used for Thine layer chromatography (TLC).

2.2. Plant material

Fresh leaves of *P. alata* (Roxb.) were collected from Prince Mohamed Ali Palace, Cairo, Egypt, during July, (2012). The identity of this plant was investigated by Dr. Threase Labib; Consultant in Orman Botanical Garden and National Gene Bank, Egypt. Plant material was air-dried and kept in tightly closed container till use.

2.3. Extraction and fractionation

The shade dried plant was powdered into 3.0 kg and then extracted by percolation in 5 liters of methanol. This extract was concentrated to syrupy consistency under reduced pressure, and then allowed to dry in a desiccator over anhydrous CaCl_2 to a constant weight (491.1 g). The dried extract was dissolved in small amounts of methanol; diluted with 500 ml water in a separating funnel, and then extracted

successively with petroleum ether (4× 1 liter); methylene chloride (CH₂Cl₂) (5× 1 liter), and ethyl acetate (4× 1 liter) in reference to Ghareeb *et al.*, (2017). These exhausted extracts were evaporated to dryness under reduced pressure, and preserved for further investigation.

2.4. Preliminary phytochemical screening tests

The dried powdered leaves extracts of *P. alata* (Roxb.) were subjected to the following phytochemical screening tests according to the conventional standard procedures reported by Salkowski, (1972); Trease and Evans, (1983), (1989); Harborne, (1993); Sofowara, (1993); Edeoga *et al.*, (2005), to determine the presence or absence of the different phyto-constituents. These phytochemicals include; alkaloids (Mayer's and Dragendorff's tests); flavonoids (Shinoda test, Aluminum chloride and Potassium hydroxide tests); steroids and terpenoids (Salkowski and Libarman-Burchard's tests); tannins (Ferric chloride and Gelatin tests); saponins (Frothing and Hemolytic tests); anthraquinones (Borntrager's test); carbohydrates (Molisch's and Barfoed's tests); and coumarins (Sodium hydroxide test). Results of these assays were evaluated by visual inspection, observed as change in color and/or by precipitation.

2.5. Evaluation of antioxidant activities of the solvent extracts using DPPH assay

The antioxidant activity of the different extracts was determined by using DPPH assay. The assay was based on the decrease in absorption of the DPPH solution after addition of the antioxidant measured at 515 nm. The antioxidant reacts with DPPH and reduces it to DPPH-H, and as a consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compound or extracts in terms of hydrogen donating ability (Mosquera *et al.*, 2007). Briefly; freshly prepared (0.004% w/v) methanol solution of 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared, and then stored at 10°C in the dark. A methanol solution of the tested extract fractions was

prepared, and then 40 µl aliquot of this methanol solution was added to 3 ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer. The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min. intervals until the absorbance stabilized (16 min.). The absorbance of the DPPH radical without antioxidant (control), and the reference compound ascorbic acid were also recorded. All determinations were performed in triplicates and then averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the following formula of Yen and Duh, (1994):

$$PI = \left[\frac{(AC - AT)}{AC} \right] \times 100$$

Where; AC= Absorbance of the control at t = 0 min., AT = absorbance of the sample + DPPH at t = 16 min.

2.6. Evaluation of the *in vitro* antimicrobial activities of the tested extracts

The *in vitro* antimicrobial activity of the extracts was determined using disc agar plate method according to Abdel-Aziz *et al.*, (2018); Ghareeb *et al.*, (2019). All the extract fractions were tested *in vitro* for their antifungal activity against some American Type Culture Collection (ATCC) fungal strains including; *Aspergillus fumigatus*, *Candida albicans*, *Geotrichum candidum*, *Syncephalastrum* sp., and for their antibacterial activity against; Gram positive bacteria such as: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus subtilis*; and Gram negative bacteria including: *Neisseria gonorrhoeae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*.

Using nutrient agar and sabouraud dextrose agar media, Ampicillin, Gentamycin and Amphotericin β were used as standard drugs against Gram positive, Gram negative bacteria, and against fungi, respectively. Dimethyl sulfoxide (DMSO) was used as a control solvent. The extract fractions were tested at a concentration of 1 mg/ml against both bacterial and fungal strains.

Briefly, 20 ml of sterilized media was poured onto each Petri dish and allowed to solidify. Wells of 6 mm in diameter were made in the solidified media with the use of sterile borer. A sterile swab was used to evenly distribute each microbial suspension over the surface of solidified media, and then 0.2 ml of the tested extracts dissolved in DMSO (1 mg/ ml) was added to each well. Plates were incubated at 37°C for 24 h in case of detecting antibacterial activity, and for 48 h at 25°C for antifungal activity. This assay was carried out in triplicates, and zones of inhibition were measured in mm using a ruler.

2.7. Chromatographic separation of petroleum ether, methylene chloride and ethyl acetate extracts

According to Ghareeb *et al.*, (2017); Saad *et al.*, (2017b), the petroleum ether plant extract (64 g) was applied onto the top of a silica gel column (300 g, 100× 4.7 cm) previously packed in petroleum ether. The column was gradually eluted with petroleum ether containing increasing proportions of ethyl acetate. The collected fractions were concentrated and monitored by silica gel TLC using petroleum ether: EtOAc (80: 20) as developing solvent system, and vanillin/ H₂SO₄ acid as spraying reagent, to afford compound 1 (20 mg), which was isolated as colorless crystalline needles. Chromatographic isolation of methylene chloride extract (60 g) on silica gel column chromatography eluted with methanol led to the isolation of a yellow powder of chloroform/ methanol compound 2 (10 mg). The ethyl acetate extract (19.19 g) was applied into the top of a silica gel column (350 g, 100 × 45 cm) previously packed in ethyl acetate. The column was gradually eluted with ethyl acetate containing increasing proportion of methanol. The effluent was collected in 200 ml fractions, each fraction was concentrated to a small volume and monitored by silica gel TLC GF₂₅₄ plates, using chloroform methanol ingredient manner as a developing system, and vanillin/ H₂SO₄ acid as spraying reagent.

Similar fractions were pooled together and evaporated to dryness to afford three major fractions.

Fractions (4-8) were monitored on TLC silica gel GF₂₅₄ plates using chloroform: methanol 95: 5 as a developing system, and vanillin/ H₂SO₄ acid as spraying reagent to afford compound 3 (20 mg). Fractions (9-25) were well collected and evaporated under reduced pressure into the top of Sephadex LH-20 column (7 g, 10× 1cm) pre-packed in methanol and eluted by methanol: water (7: 3).

Sub-fractions were collected together, where evaporation of the solvent produced yellow amorphous powder of compound 4 (25 mg). While, fractions (26-39) were applied into the top of silica gel column eluted with chloroform: methanol 8.5: 1.5. The sub-fractions (81-100) were applied into the top of Sephadex LH-20 column (10 g, 10× 1cm) pre-packed in methanol, and eluted by methanol: water (8: 2). All these sub fractions were collected together, where evaporation of the solvent yielded yellow amorphous powder of compound 5 (9 mg).

2.8. Acid hydrolysis

Five mg of each compound was separately refluxed with 2M HCl in MeOH (5 ml) at 80°C for 5 h in a water bath (Agrawal, 1989). The reaction mixture was evaporated; the hydrolysate was diluted with 10 ml of H₂O, and then extracted with CHCl₃ (3 × 10 ml). The CHCl₃ extracts were evaporated to afford the aglycones identified as; apigenin, luteolin and myricetin for compounds 2, 4 and 5; respectively, compared with authentic samples. The aqueous layer was neutralized with Na₂CO₃ and then concentrated to 1 ml under reduced pressure. The residue was compared with standard sugars by silica gel TLC [(CHCl₃-MeOH-H₂O: 30: 12: 4), 9 ml of lower layer and 1 ml of HOAc], where the sugars were identified to be glucose for compounds 2, 4 and 5 (Mohammed *et al.*, 2019).

2.9. Statistical analysis

Data of the antimicrobial and antioxidant activities were presented as mean ± standard deviation. Assays were carried out in triplicates (n= 3), using SPSS 13.0 program (SPSS Inc. USA).

3. Results and Discussion

3.1. Preliminary phytochemical screening of different solvent extracts of *P. alata*

Results of preliminary phytochemical screening of different solvent extracts of *P. alata* (Roxb.) revealed that the main organic constituents of *P. alata* (Roxb.) were; carbohydrates and/or glycoside, tannins; flavonoids, unsaturated sterols and/or triterpenes in the methanol extract, and few nitrogenous bases in ethyl acetate and methanol extracts. Sterols and/or triterpenes were present in petroleum ether, methylene chloride and ethyl acetate extracts. Flavonoids, carbohydrates and/or glycoside were present in methylene chloride, ethyl acetate and methanol extracts. However, tannins were present only in ethyl acetate and methanol extracts. Numerous plant extracts belonging to different species were screened for their phytochemical constituents, which contributed to their demonstrated bioactivities such as; antioxidant, antimicrobial and cytotoxic activities (Ghareeb *et al.*, 2015a, b; Ghareeb *et al.*, 2016; Ghareeb *et al.*, 2017; Hamed *et al.*, 2017a, b; Madkour *et al.*, 2017; Saad, 2017a, b).

3.2. Biological investigations

3.2.1. Antioxidant activity of total extract of *P. alata* (Roxb.) using DPPH assay

Reviewing of previous literature revealed that the genus *Pterygota* gave markedly strong antioxidant activity (Jahan *et al.*, 2014). In the present study, the radical scavenging activity of total extract of *P. alata* was estimated using DPPH assay. This radical scavenging activity was compared to ascorbic acid as a standard. The tested extract possessed variable antioxidant scavenging affinity against DPPH radical. According to the IC_{50} ; (IC_{50} of total extract = 72.4 ± 1.4 μ g/ml, IC_{50} of ascorbic acid = 11.2 ± 0.6 μ g/ml), total extracts had scavenging effects on the DPPH radical of about 30% of the standard ascorbic acid (Fig. 1).

The relative antioxidant potential of the total extract could be attributed to its phenolic contents. In a

previous study of Jahan *et al.*, (2014), different solvent extracts of *P. alata* leaves grown in Bangladesh were evaluated for their DPPH free radical scavenging activity. IC_{50} recorded values were; 60.12 mg/ml, 76.61 mg/ml, 16.13 mg/ml, 21.35 mg/ml, and 86.25 mg/ml; for ethanol, n-hexane, chloroform, ethyl acetate, and aqueous extracts, respectively. According to Rice-Evans *et al.*, (1996); certain structural criteria were required for the tested phenolic compound to give optimum antioxidant activities including; the presence of heavy hydroxylation/methoxylation patterns, and conjugated aromatic system.

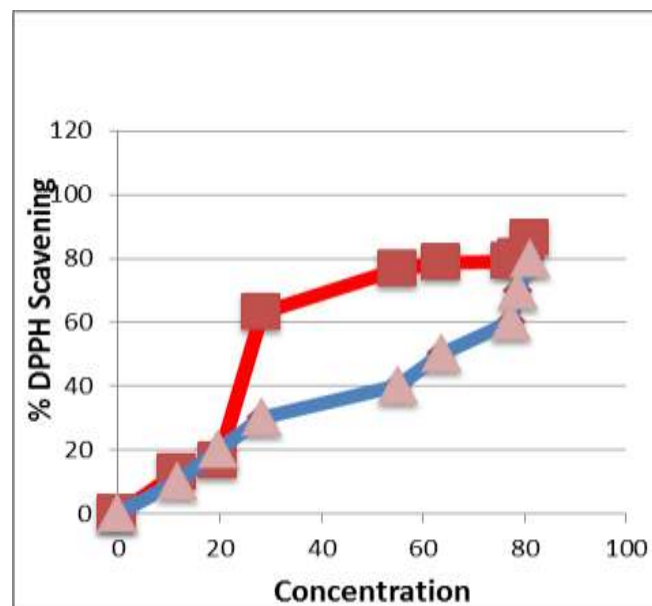


Fig. 1: Free radical scavenging activity of the total extract of *P. alata*

3.2.2. Evaluation of the antimicrobial activities of *P. alata* extracts

Results presented in Table 2 showed the antimicrobial activities of different extracts of *P. alata* against different test microbes belonging to fungi; Gram positive bacteria, and Gram negative bacteria, observed through measuring the diameter of inhibition zones in mm. The butanol extract didn't exhibit any antifungal potential against all tested fungi; whereas, ethyl acetate extract showed antifungal potency against *A. fumigatus* (20.2 mm), *C. albicans* (16.8

mm) and *G. candidum* (22.8 mm), however, no activity was detected for ethyl acetate extract against *Syncephalastrum* sp. On the other hand; petroleum ether extract showed antifungal activities against *A. fumigatus* (17.4 mm), *C. albicans* (15.6 mm) and *G. candidum* (18.1 mm), but *Syncephalastrum* sp. didn't show any sensitivity to petroleum ether extract. All previous results were compared with those of the standard antifungal antibiotic (Amphotericin B).

For Gram positive bacteria; all extracts didn't exhibit any antibacterial activities against *S. pyogenes*. Meanwhile, Ethyl acetate extract presented antibacterial potential against Gram positive bacteria, i.e., *S. aureus* (18.2 mm), *S. epidermidis* (20.3 mm), and *B. subtilis* (22.6 mm). In addition; petroleum ether extract showed antibacterial potencies (following ethyl acetate and butanol extracts) against Gram positive bacteria, i.e., *S. aureus* (15.2 mm), *S. epidermidis* (13.4 mm), and *B. subtilis* (13.4 mm). Butanol extract exhibited such activities against Gram positive bacteria (following ethyl acetate) with inhibition zones values of; 19.3 mm for *S. aureus*, 17.1 mm for *S. epidermidis*, and 17.1 mm for *B. subtilis*.

For Gram negative bacteria; both *N. gonorrhoeae* and *P. aeruginosa* showed resistance to all tested extracts. Ethyl acetate extract demonstrated higher antibacterial activities against Gram negative bacteria with inhibition zones values of 22.4 mm (*K. pneumoniae*), and 20.4 mm (*E. coli*). Petroleum ether followed ethyl acetate extract in its antibacterial potentials against *K. pneumoniae* (19.6 mm) and *E. coli* (17.1 mm). Finally; butanol extract exhibited antibacterial activities against Gram negative bacteria lower than those of the previous two extracts, i.e. *K. pneumoniae* (17.1 mm), and *E. coli* (14.9 mm). These activities were compared with those of the standard antibacterial antibiotics, i.e. Ampicillin and Gentamycin.

3.3. Characterization of the isolated compounds from different solvent extracts

Phytochemical investigation of the different solvent extracts of *P. alata* (64.67g of petroleum ether, 60.03 g of methylene chloride, and 19.4 g of ethyl acetate extracts) resulted in the isolation of five compounds mainly; β -sitosterol (1), apigenin-7- β -D-glycoside (2), gallic acid (3), luteolin-7- β -D-glucoside (4), and 4'-methoxy myricetin-3- β -D-glucoside (5). Their chemical structures were elucidated using chemical tests; spectroscopic tools (^1H & ^{13}C -NMR and IR), and via comparison with the previous literatures (Fig. 2).

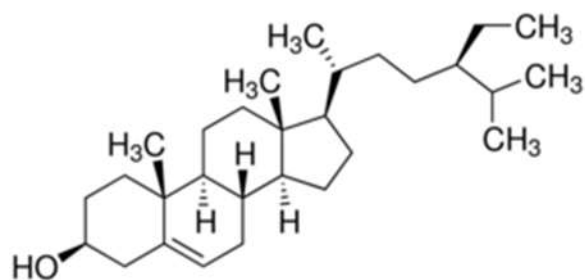
Compound 1 (20 mg) was isolated as colorless crystalline needles, m.p. 281-283°C, Rf 0.39 (CHCl_3 : MeOH; 9: 1; TLC) and 0.54 (Pet. ether/ EtOAc: 80: 20; TLC). It is soluble in chloroform and methylene chloride but insoluble in methanol. It gave a red color with Salkowski test, also it gave a violet ring superimposed by a green color with Liberman-Burchard's; test suggested its steroidal nature. It showed a single spot on TLC which attained a violet color with pinkish ting after spraying with 10% v/c sulphuric acid. The IR (KBr, V_{max}) spectrum showed absorption bands at 3448 cm^{-1} (OH stretching), at 2926 cm^{-1} (=CH stretching), at 2855 cm^{-1} (-CH stretching), at 1707 cm^{-1} (-C=C stretching), at 1461 cm^{-1} (methylene), at 1377 cm^{-1} (-CH₃ bending), at 1057 cm^{-1} (C-O stretching), and at 963 cm^{-1} (C-bending). Therefore, compound (1) could be identified as β -sitosterol via co-chromatography with authentic sample in reference to El-Sayed *et al.*, (2010).

Compound 2 (10 mg) was isolated as a yellow powder. It showed a dark purple spot upon PC under UV lamp; and acquired yellow color after spraying with 5% AlCl_3 spraying reagent, indicating its flavonoid nature. Also, it gave positive Molisch's test indicating its glycosidic nature. Acid hydrolysis afford apigenin (organic phase) and glucose (aqueous phase). ^1H -NMR spectrum (400 MHz, MeOD); showed the presence of characteristic signals at δH (ppm): 7.49 (2H, d, J=8.8 Hz, H-2' and H-6'), 7.82 (2H, d, J=8.8Hz, H-3' and H-5'), 6.71 (1H, s, H-3), 6.68 (1H, d, J= 2.0 Hz, H-8), 6.49 (1H, d, J=2.0 Hz, H-6), 5.5 (1H, H-1''), 3.61-3.20 (sugar protons).

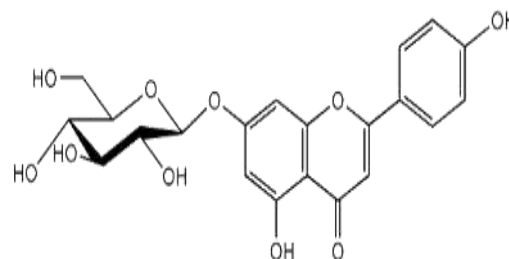
Table 1: Antimicrobial potential of different solvent extracts of *P. alata*

Microorganisms tested	Leaves extracts			Standard
	Clear Inhibition zone (mm) ^b			
	Ethyl acetate	Pet. ether	Butanol	
<u>Fungi</u>				Amphotricin β^d
<i>A. fumigatus</i> (RC MB 02564)	20.2 \pm 1.5 ^c	17.4 \pm 1.2	NA ^a	23.7 \pm 0.63
<i>C. albicans</i> (RCMB05035)	16.8 \pm 0.63	15.6 \pm 2.1	NA	21.9 \pm 0.58
<i>Geotrichum candidum</i> (RCMB 05096)	22.8 \pm 0.58	18.1 \pm 0.63	NA	26.4 \pm 1.2
<i>Syncephalastrum</i> sp. (RCMB06324)	NA	NA	NA	25.4 \pm 0.72
<u>Gram-positive bacteria</u>				Ampicillin^e
<i>Staph. aureus</i> (RCMB 010027)	18.2 \pm 0.63	15.2 \pm 1.5	19.3 \pm 0.58	28.9 \pm 0.63
<i>Staph. epidermidis</i> (RCMB010024)	20.3 \pm 0.72	13.4 \pm 0.63	17.1 \pm 0.63	25.4 \pm 0.58
<i>S. pyogenes</i> (RCMB 010015)	NA	NA	NA	22.8 \pm 1.2
<i>B. subtilis</i> (RCMB010067)	22.6 \pm 0.35	13.4 \pm 0.25	17.1 \pm 0.72	27.8 \pm 0.63
<u>Gram-negative bacteria</u>				Gentamycin^f
<i>Neisseria gonorrhoeae</i> (RCMB 010076)	NA	NA	NA	19.9 \pm 0.58
<i>P. aeruginosa</i> (RCMB010085)	NA	NA	NA	2.34 \pm 0.72
<i>K. pneumoniae</i> (RCMB 0010093)	22.4 \pm 0.63	19.6 \pm 0.58	17.1 \pm 0.15	26.3 \pm 0.63
<i>E. coli</i> (RCMB010056)	20.4 \pm 1.5	17.1 \pm 1.2	14.9 \pm 0.15	25.3 \pm 1.5

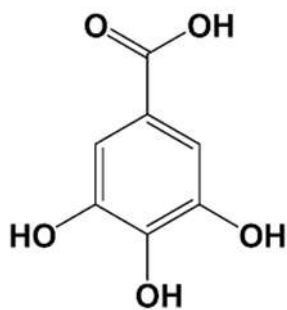
Where; ^aNA: No activity; ^bInhibition zones diameter (mm); ^cMean \pm SD, (n= 2); ^dAmphotricin β : was used as a positive antifungal control (100 μ g/ disc); ^eAmpicillin: was used as antibacterial (Gram +ve) control (100 μ g/ disc); ^fGentamycin was used as an antibacterial (Gram -ve) control (100 μ g/ disc); SD: Standard deviation.



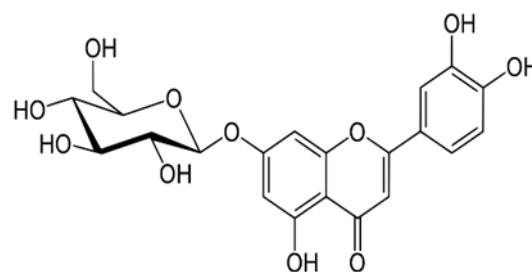
β -sitosterol



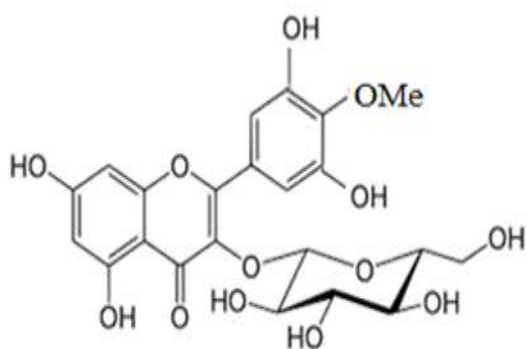
Apigenin-7-O- β -D-glucoside



Gallic acid



Luteolin-7-O- β -D-glucoside



4'-methoxy-myricetin-3-O- β -D-glucoside

Fig. 2: Chemical structures of the compounds isolated from *P. alata* leaves extracts

¹³C-NMR spectrum (100 MHz, MeOD), also revealed the presence of carbon signals which were resonated at δ C (ppm): 181.0 (C-4), 167.13 (C-2), 166.52 (C-7), 165.66 (C-5), 160.16 (C-4'), 159.88 (C-9), 125.78 (C-2' and C-6'), 125.63 (C-1'), 117.17 (C3' and C-5'), 113.47 (C-10), 112.96 (C-3), 100.35 (C-1''), 94.48 (C-6), 94.34 (C-8), 76.45 (C-5''), 74.94 (C-3''), 94.84 (C-2''), 69.92 (C-4''), and 62.98 (C-6''). ¹H-NMR spectrum of compound (2) showed the presence of one ortho aromatic doublet at δ 7.82 corresponding to H-3' and H-5', one ortho aromatic doublet at δ 7.49 corresponding to H-2', H-6', two meta coupled aromatic doublets at 6.49 & 6.68 corresponding to H-6 and H-8; respectively, and one singlet at 6.71 corresponding to H-3. ¹³C-NMR spectrum of compound (2) showed the presence of fifteen aromatic carbons in addition of signals at 100.53, 74.84, 69.92, 76.45 and 62.98 ppm, which confirmed the presence of sugar moiety (glucose). Therefore, compound (2) was identified as apigenin-7- β -D-glycoside. It was confirmed by co-chromatography with authentic sample, and was consistent with previous published data of Mabry *et al.*, (1970); Markham (1982); Salama *et al.*, (2011). This is the first report of the isolation of apigenin-7-O- β -D-glycoside from *P. alata* extracts.

Compound 3 (20 mg) was obtained as off-white amorphous powder, m.p. 251–253°C. Rf 0.56 (chloroform: methanol; 9.5:0.5; TLC), 0.79 (BAW; PC) and 0.51 (15% AcOH; PC). It showed shiny violet fluorescence under short UV light which turned to deep blue color with FeCl₃ spraying reagent. ¹H-NMR (400 MHz, DMSO-d₆): showed a characteristic signal in the aromatic region for two identical aromatic protons at δ H 7.83 (2H, s, H-2 and H-6). ¹³C-NMR (100 MHz, DMSO-d₆): δ ppm 100.70 (C-2, C-5), 164.90 (-CO). The NMR and chemical data were in agreement with the reported data of 3,4,5-trihydroxybenzoic acid (gallic acid) (Chanwitheesuk *et al.*, 2007). This compound was previously isolated from *P. alata*.

Compound 4 (25 mg) was obtained as yellow amorphous powder. Rf 0.45 (chloroform: methanol; 9:1). It showed deep purple fluorescence under UV light which changed to yellow with ammonia vapor. It gave positive Molisch's test indicating its glycosidic nature. Acid hydrolysis: yield luteolin (organic phase) and glucose (aqueous phase). ¹H-NMR (400 MHz, MeOD): δ ppm 12.95 (1H, s, 5-OH), 7.60 (1H, dd, J=8.4, 2.0 Hz, H-6'), 7.46 (1H, d, J=2.0 Hz, H-2'), 7.07 (1H, d, J=8.4 Hz, H-5'), 6.97 (1H, d, J=2.0 Hz, H-8), 6.49 (1H, s, H-3), 6.70 (1H, d, J=2.0 Hz, H-6), 5.6 (H-1''), and 3.60-3.30 (m, sugar protons). ¹³C-NMR (100 MHz, MeOD): δ ppm 189.0 (C-4), 166.99 (C-2), 163.39 (C-7), 161.59 (C-5), 157.40 (C-9), 150.81 (C-3'), 144.24 (C-4'), 124.81 (C-1'), 120.12 (C-6'), 117.24 (C-5'), 113.57 (C-2'), 108.76 (C-10), 108.09 (C-5'), 101.64 (C-1''), 99.54 (C-6), 92.80 (C-8), 74.94 (C-5''), 72.62 (C-3''), 71.92 (C-2''), 70.94 (C-4''), and 61.49 (C-6''). ¹H-NMR spectrum of compound (4) showed the presence of two meta-coupled aromatic doublets at 6.70 & 6.97 ppm corresponding to H-6 and H-8 respectively, one ortho-aromatic doublet at 7.07 ppm corresponding to H-5', one meta-aromatic doublet at 7.46 ppm corresponding to H-2', one doublet at 7.60 ppm corresponding to H-6' confirming 3',4' Dihydroxy pattern in B-ring, and singlet at 6.94 ppm corresponding to H-3. ¹³C-NMR spectrum of compound 4, showed the presence of fifteen aromatic carbons. The glycoside moiety was confirmed as β -glucopyranoside depending on the characteristic down field location of C-1'' at 101.64 ppm assignable to anomeric sugar carbon which identified to be β -glycoside. The conformation of glucopyranoside was determined as ⁴C₁, from previous resonance data and J₁₂ value vicinal coupled proton. Based on the above data and via comparing with previous literatures, compound 3 could be identified as luteolin-7- β -D-glucoside (Mabry *et al.*, 1970; Salama *et al.*, 2011).

Compound 5 (9 mg) was obtained as amorphous powder, it showed yellow fluorescence spot under

UV light which turned to yellowish orange with naturstoff reagent, and faint blue with FeCl₃. Its R_f was 0.46 (chloroform: methanol; 8.5: 1.5) using TLC, and; it gave positive Molisch's test. Acid hydrolysis yield Myricetin (organic phase) and glucose (aqueous phase). ¹H-NMR (400 MHz, MeOD): δppm 7.9 (2H, H-2'/6'), 6.73 (1H, S, H-8), 6.67 (1H, S, H-6), 3.47 (-OMe), 4.79 (H-1''), 3.30-3.35(m-sugar).

¹³C-NMR (100MHz, MeOD): δppm 177.4 (C-4), 164.0 (C-7), 161.2 (C-5), 153.0 (C-9), 149.29 (C-2), 147.96 (C-3'/5'), 134.15 (C-3), 141.6 (C-4'), 120.20 (C-1'), 108.8 (C-2'/6'). 103.91 (C-10), 98.6 (C-6), 93.3 (C-8), 55.43 (-OMe), 103.11(C-1''), 71.51 (C-2''), 76.40 (C-3''), 69.88 (C-4''), 76.91 (C-5''), 61.21 (C-6''). The ¹H-NMR spectrum of this compound showed two meta-coupled protons at δppm 6.76 (H-6) and 6.75 (H-8) of A-ring, and one singlet at δppm 7.9 (H-2'/6'), corresponding to equivalent proton of 3', 4', 5'- substituted B-ring. The ¹³C-NMR spectrum of compound 5 revealed the presence of 13 carbon signals, from which two signals were corresponding to two carbon 147.96 (C-3'/5') and 108.8 (C-2'/6') & H- 3.74, and carbon signal at 55.43 which indicated the presence of methoxy group. The down field of C-4' (141.6) indicated the attachment of methoxyl group to C-4' (Markham *et al.*, 1982); in addition, the signals at δppm 4.79 (H-1''), and signals at δppm 103.11, 71.51, 76.40, 69.88, 76.91 and 61.12 revealed the presence of 3-β-D-glucopyranoside. The ¹H-NMR and ¹³C-NMR spectral data of compound 5, were consistent with those reported for myricetin (Markham *et al.*, 1982). Therefore, compound 5 was identified as 4'-methoxy myricetin-3-β-D-glucopyranoside. This is the first report of isolating this compound from *P. alata* extracts.

Conclusion

The current study presented the isolation and characterization of five compounds (three flavonoids glycosides, one phenolic acid and one sitosterol), from the leaves extracts of *P. alata* (Roxb.) grown

in Egypt. Moreover; this study also demonstrated the antimicrobial efficacy of the different solvent extracts of *P. alata* against some pathogenic strains, in addition to the antioxidant potential of these extracts against DPPH radical.

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Conflict of interests

The authors declare no conflict of interests regarding this article.

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