ISSN 0974 – 5211

Journal of Natural Products
Volume 5 (2012)
www.JournalofNaturalProducts.Com

Research Paper

Bioactive compounds from *Phyllanthus atropurpureus*

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(Received 18 July 2011; Revised 21 July-02 Sept. 2011; Accepted 03 September 2011)

ABSTRACT

From the ethyl acetate soluble fraction of the *Phyllanthus atropurpureus* six compounds were isolated. Four of them are isolated for the first time from the plant, the structures were established as di [3, 4, 5- trihydroxy- phenyl] ether, 5, 6, 8, 4'- tetrahydroxy isoflavone, Robustaside A, 6'- (4''- hydroxy cinnamoyl) arbutin, and 6'- (3'', 4''- dihydroxy cinnamoyl) arbutin. The other two isolated compounds are Demethoxysudachitin, and quercetin-7- O-glucoside. Concerning the biological studies, the results revealed that the total extracts can be considered an efficient antimicrobial, when ethyl acetate fraction of leaves shows antibacterial activity against gram –ve (*E. coli)* more than the other tested materials as well as Ampecillin. All tested materials show significant gram +ve antibacterial activity (*S. aureus*) compared to the effect of Gentamycin. Also robustaside A was found to produce a specific strong anti-tumor activity against hepatocellular carcinoma.

Keywords: *Phyllanthus atropurpureus*; *Euphorbiaceae*; Hepatocellular carcinoma.

INTRODUCTION

Genus *Phyllanthus* (Family Euphorbiaceae) is considered one of the important medicinal and ornamental plants. The chemical review on genus *Phyllanthus*, reveals the presence of sterols and/ or terpenes (Lam, et al., 2007; Ndlebe, et al., 2008), lignans (Tuchinda, et al., 2006; Murugaiyah, et al, 2007; Luo, et al., 2009), flavonoids (Shakil, et al, 2008; Than et al, 2006), polyphenolic compounds and tannins (Liu, et al, 2008, Shin, et al., 2005, Zhang, et al., 2001), in addition to minor alkaloids (Houghton et al., 1996).

The natural compounds of genus *Phyllanthus* are characterized by their physiological and medicinal values such as antioxidant (Sabir and Rocha, 2008; Kumar, et al., 2007, Than, et al., 2006) antihepatotoxic (Sabir and Rocha, 2008, Naaz, et al., 2007, Pramyothin, et al., 2007) anticancer (Rajkapoor, et al., 2007, Sureban, et al., 2006) antidiabetic (Ali, et al., 2006) diuretic (Hnatyszyn, et al., 1999) and anti-inflammatory (Yerra, et al., 2006).

It is worthy to be note that there is almost no report on the chemistry of *Phyllanthus atropurpureus* Boj. Hort. Maurit. growing in Egypt as well as biological screening of this plant. In the present study, the aim was describe, isolate and study

the biological effect of the bioactive compounds found in *Phyllanthus atropurpureus* Boj. Hort. Maurit. growing in Egypt.

MATERIALS AND METHODS

***Plant materials*:** *P. atropurpureus* Boj. Hort. Maurit. (Family-*Euphorbiaceae*) (spurge), was collected in the flowering stage from the plant cultivated in medicinal plants garden of Faculty of Science, Ain Shams University, Cairo, Egypt. The identification was kindly verified by Dr. Hesham El-shamy, Professor of medicinal, aromatic and ornamental plants, Horticulture Department, Faculty of Agriculture, Zagazig University, Egypt. A voucher specimen is deposited in Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt. The plant was shade dried and ground by electric mill to moderately fine powder.

***Extraction and chromatography*:** The air-dried powdered leaves of *P. atropurpureus* Boj. Hort. Maurit. (575g) was extracted by cold maceration with 75% ethanol (4 L.) till complete exhaustion. The combined extract was evaporated under reduced pressure at 50°C to give 65.6g of greenish brown residue. To the concentrated ethanolic leaves extract about 500ml of MeOH: H_2O mixture (9:1) was added to dissolve it then extracted with light petroleum, chloroform, and ethyl acetate. The combined ethyl acetate fractions were then washed with distilled water, dried over anhydrous sodium sulphate and then the solvent was distilled off under reduced pressure at 50°C to afford 6.5g of ethyl acetate soluble fraction. The air-dried powdered stem and roots of *P. atropurpureus* were extracted with 75% ethanol and fractionated in the same manner as that of leaf. The chromatoplates revealed that the ethyl acetate fractions of stem and root are similar and in the same time are slightly different from leaf ethyl acetate fraction. The TLC investigation of ethyl acetate soluble fraction of leaf revealed the presence of four major and nine minor spots Table-1.While that of stem and root ethyl acetate fractions revealed the presence of two major and eight minor spots Table- 2.

Column chromatography of leaf ethyl acetate soluble fraction and isolation of the major compounds: 6.5g of the leaf ethyl acetate soluble fraction was dissolved in the least amount of methanol and adsorbed on 15g of silica gel for column and transferred into a silica column (2.5 × 150 cm, 250g) packed with benzene. Elution was carried out starting with benzene and the polarity gradually increased with chloroform and methanol. Fractions (250ml each) were collected, concentrated and examined by TLC using solvent system Ethyl acetate: Acetic acid: Formic acid: H_2O (100: 11: 11: 27), and 50% aqueous sulphuric acid visualizing reagent. The similar fractions were pooled.

Apparatus and Equipments: Melting points were carried out using Melting point apparatus, Digital, electro-thermal LTD (England)**,** U.V. lamp for TLC visualization U.V.P., GL-58 (λ_{max} 254 and 366 nm). For U.V. spectral analysis, Shimadzu U.V.-1700 spectrophotometer (Japan) was used. Infra-red spectra were generally obtained in KBr discs on a pye-Unicam SP 3000 and carried out on IR spectrophotometer, Jasko, FT/IR-460 plus. The ^{1}H and ^{13}C NMR spectra were recorded using JEOL and Varian MAT 500, 300, 125 and 75 MHz respectively, chemical shifts were given in ppm with the TMS as internal standard. The mass spectra were carried on VG-Quattro II waters, Masslynx V_{40} SP_4, 508, Copy rights© 2004 Micromass Ltd.

TLC analyses were carried out on silica gel GF_{245} chromatoplates with the following developing systems: $CHCl_3$ / MeOH (80:20), Ethyl acetate: Acetic acid: Formic acid:

H_2O (100: 11: 11: 27), and Benzene: Ethyl acetate: Formic acid: H_2O (3: 5: 1.4: 0.6). For column chromatography, silica gel 60, 40µm was used.

Biological investigation

Anticancer Activity: Cell Culture*:* Hepatocarcinoma (HepG2) cells were obtained frozen in liquid nitrogen (-180^{o}C) from the American Type Culture Collection (ATCC). The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing. HepG2 cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium). Media were supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamine, containing 100units/ml penicillin G sodium, 100units/ml streptomycin sulphate, and 250ng/ml amphotericin B. Cells were maintained at sub-confluency at 37°C in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested materials were dissolved in dimethyl sulphoxide (DMSO). All cell culture materials were obtained from Cambrex BioScience (Copenhagen, Denmark). All chemicals were from Sigma/Aldrich, USA. All experiments were repeated three times.

Tested materials*:* Material 1: Robustaside A, Material 2: Ethanolic extract of aerial parts of *Phyllanthus atropurpureus*, Material 3: Ethanolic extract of root of *Phyllanthus atropurpureus.*

Methods: Cytotoxicity of tested materials was measured against HepG2cells using Sulphorhodamine-B (SRB) assay (Skehan, et al., 1990).

Antimicrobial investigation**:** Cup-plate method (Woods and Washington, 1995) was used to detect the preliminary antimicrobial activity of different fractions including ethyl acetate extract of leaves, roots and stem. The samples were dissolved in dimethyl sulfoxide (DMSO) at concentration of 200mg/ml. The nutrient agar or saburaud's dextrose agar was seeded by about 10^6 microbial cells. Gram +ve bacteria (*Staphylococcus aureus*) and Gram –ve bacteria (*Escherichia coli*) as well as *Candida albicans* as a fungus are used as tested microorganisms. Each cup was filled by about 100µl from each extract (200mg/ml). Penicillin and gentamycin as well as nystatin were used as standards. The plates were incubated overnight at 37°C for bacteria and 30°C for fungus. Zones of inhibition were measured (mm) and recorded in Table 3.

RESULTS AND DISCUSSION

The ethyl acetate-soluble fractions of the ethanol extract of *Phyllanthus atropurpureus* Boj. Hort. Maurit. growing in Egypt on repeated chromatographic purification on a silica gel column and PTLC yielded six compounds (1-4 from leaves and 5,6 from stem and roots, Table 1 and 2). These compounds gave a blue fluoresce with UV lamp (365nm) and yellow colour with NH_4OH which may be indicating the phenolic and flavonoidal properties of these compounds.

Compound 1: was obtained as reddish brown needles (from MeOH), m.p. 141-143°C, It is freely soluble in methanol and ethyl acetate, slightly soluble in chloroform and insoluble in benzene and light petroleum. Compound 1 gave yellow colour with ammonia, brownish orange with 50% aqueous sulphoric acid reagent and blue fluorescence under UV light.

The UV spectroscopic analysis showed absorption peak at λ_{max} (methanol) 292 nm. The IR spectrum showed the following absorption frequencies: $\mathcal{V}_{max}$ (KBr) cm^{-1}: 3621, 3500-3100, 3030, 2929-2800, 1649, 1517, 1465, 1246, 1194, 1095, 1023, 833 and 760.

The IR spectrum of compound 1 showed an absorption frequency at 3621 cm^{-1} attributed to unbounded -OH, broad absorption band at 3500-3100 cm^{-1} for several –OH groups and 1649, 1517, 1465 cm^{-1} for aromaticity in addition to peaks at 1246, 1194, 1095 and 1023 cm^{-1} for C-O stretching (ether linkage).

EI-MS exhibited a molecular ion peak at m/z 266 (M^+) which is compatible with the molecular formula $C_{12}H_{10}O_7$. EI-MS: m/z (%): 266 (M^+, 4.7), 265 (7.47), 248 (0.23), 140 (2.47), 125 (3.3), 124 (1.06), 112 (6.79),109 (10.19), 108 (4.51), 107 (20.84), 99 (12.88), 98 (13.08), 95 (39.08), 84 (20.84), 79 (41.92), 67 (52.58), 66 (4.14), 60 (100).

The ^{1}H-NMR showed a broad singlet at δ 6.615- 6.651 ppm for aromatic protons and singlet signal at δ 3.338 ppm for –OH groups. ^{13}C-NMR spectral data and DEPT 135 experiments showed that compound 1 contains only two types of carbons: =C-H at δ (115.29- 117.30) ppm and C-O at δ (149.87- 150.76) ppm.

From the previously mentioned data, it is assumed that this material is di (3, 4, 5- trihydroxy phenyl) ether (Fig. 1). For our knowledge, this is the first report about the isolation of this compound from nature. Also, this is the first detection of this class of natural product (diphenyl ether) in terrestrial plant while many marine brown algae (Corinne, et al., 1995) contain the phlorotannins.

Compound 2: occurs as reddish brown needles (methanol), with m.p. 141-143°C. It is freely soluble in methanol and ethyl acetate, slightly soluble in chloroform and insoluble in benzene and light petroleum. It gave yellow colour with alkali (T.S) and $AlCl_3$ in addition to blue colour with ferric chloride indicating its phenolic character (Trease and Evans, 2002). It is freely soluble in methanol and ethyl acetate, slightly soluble in chloroform, insoluble in benzene and light petroleum. It gave red colour with nitric acid indicating its isoflavone nature (Harborne, et al., 1975).

The IR spectrum showed absorption peaks at 3500-3250 cm^{-1} indicating the presence of hydroxyl group; peak at 1733 cm^{-1} for γ - pyrone ring and peaks at 1620 and 1458 cm^{-1} for aromaticity. The UV data showed one major band at λ_{max} 252 nm (band II) and a shoulder at 292 nm (band I) in methanol suggesting an isoflavone material (Mabry et al., 1970). Addition of NaOMe showed a bathochromic shift (+8 and +22 nm) in band I and II respectively indicating the presence of free hydroxyl groups at ring A. $AlCl_3$ produced a bathochromic shift (+20 nm) in both band I and II indicating the presence of ortho-dihydroxy groups in ring A and/ or free 5-OH, this will confirmed upon addition of HCl, where the absorption of $AlCl_3$ was reduced but not return back to methanol spectrum values. NaOAc caused no bathochromic shift in band II indicating the absence of free hydroxyl group at C-7. No bathochromic shift in band I upon the addition of NaOAc/ H_3BO_3.

The MS fragmentation showed parent ion (M^+) at m/z 286 which is in a good accordance with a molecular formula $C_{15}H_{10}O_6$ and fragments at m/z 121, 118 confirmed the presence of one hydroxyl group at ring B.

The ^{1}H-NMR showed singlet signal at 7.416 ppm which was assigned to H-2 and H-7, and two signals at δ 7.40 and 6.74 ppm (each 2H, d, J=8.5) which were assigned to H- 2', 6' and H- 3', 5' respectively (Mabry, et al., 1970).

From the previous spectral analysis; IR, UV, MS and ^{1}H-NMR confirmed that the compound 2 is 5, 6, 8, 4'-tetrahydroxy isoflavone (Fig. 1). For our knowledge this is the first report of isolation of this compound from family Euphorbiaceae.

Compound 3: occurs as buff fine powder (Chloroform-methanol) m.p 144-146°C; It is soluble in methanol and ethyl acetate, slightly soluble in chloroform, and insoluble in benzene. It gives yellow colour with alkali (T.S) and aluminum chloride (T.S).

The UV spectrum showed λ_{max} (methanol) at 299.5 and 313.5 nm which implied the presence of a conjugated chromophore and sodium methoxide which caused 48 nm bathochromic shifts in UV spectrum indicating the presence of free OH group. The IR spectrum showed a hydroxyl stretching band at 3500-3250 cm^{-1}. Also it showed an absorption band at 1690 cm^{-1} for ester carbonyl besides another bands at 1605, 1512 and 1448 cm^{-1} for aromaticity. The ^{1}HNMR showed signals for two sets of aromatic protons (δ 6.81 and 7.45 ppm, J= 8.7 Hz) and (δ 6.65 and 6.94 ppm, J= 8.7 Hz), trans - olefinic protons (J= 15.9) at δ 7.63 and 6.34 ppm, and sugar protons (anomeric proton at δ 4.73 ppm). The ^{13}C-NMR revealed ester carbonyl at δ 169.06 ppm and six carbon signals arising from a monosaccharide moiety whose anomeric carbon signal is at 103.67 ppm indicating the presence of a glucose moiety in the molecule (Pretsch, et al., 2000).

The MS patterns are a suggestive one and showed parent ion peak at m/z 418 corresponding to the molecular formula ($C_{21}H_{22}O_9$), the fragments at m/z 309 $[M-109]^+$ and 147 support the presence of a cinnamoyl group. Finally it was considered to be a trans-cinnamoyl group esterified with a hydroxyl group of the glucose moiety and the fragment at m/z 164 and 147 implied the presence of p-coumaroyl moiety **(**Pretsch, et al., 2000, Birgit and Glombitza, 1999).

Extensive NMR analysis: ^{1}H-^{1}H COSY, DEPT 135, APT, gHSQCAD and gHMBC spectral data allowed a complete assignment of protons attached to their respective carbons. Only one CH_2 group at 64.74 ppm (C-6') and five quaternary carbon at 169.06, 161.35, 153.9, 152.3, 127.37 ppm (C-9", C-4", C-4, C-1, and C-1" respectively) present in the material and this is confirmed by DEPT 135 and APT. The chemical shift (δ_C 152.3) of one of the aromatic carbons indicated that it was attached to an oxygen atom, and the chemical shift of the sugar anomeric proton [δ_H 4.73 (d, J= 6.9 Hz)] and carbon [δ_C 103.67] signals indicated that the sugar moiety was not attached to the carboxyl group but to this phenolic oxygen through a glycosidic linkage. The gHMBC correlation achieved between the anomeric proton at δ_H 4.73 (H-1') and δ_C 152.3 (C-1) support a C-1 location for the sugar unit in the glucosidic linkage. The existence of cinnamoyl group in the compound, with an ester linkage at C-9", was established by gHMBCAD correlations: H-6' to C- 9"; H-2" and H-6" to C- 7" and C-4"; and H-3" and H- 5" to C-2", C-6" and C-4"

Finally the data confirmed that compound 3 is 6'-(4"-hydroxy- cinnamoyl) arbutin (Fig. 1) which is known as robustaside A **(**Michael and Glombitza**,** 1995) by comparing its UV, ^{1}H-NMR and ^{13}C-NMR spectra with the reported data. This is the first report about isolation of robustaside A from genus *Phyllanthus* and the second from the nature **(**Michael and Glombitza**,** 1995**).**

Compound 4: occurs as pale yellow crystals (Chloroform-methanol) m.p 150-152°C; It is soluble in methanol and ethyl acetate, slightly soluble in chloroform, and insoluble in benzene. It gives yellow colour with alkali (T.S) and aluminum chloride (T.S).

The UV spectrum showed λ_{max} (methanol) at 288 and 324 nm which implied the presence of a conjugated chromophore. Sodium methoxide which caused 50 nm bathochromic shifts in indicating the presence of free OH group. The IR spectrum showed a hydroxyl stretching band at 3600-3100 cm^{-1}. Also it showed an absorption band at 1715 cm^{-1} for ester carbonyl besides another bands at 1606, 1511 and 1430 cm^{-1} for aromaticity.

Finally from the previous data and by comparing data of compound 4 with robustaside A confirmed that compound 4 is 6'- (3", 4"-dihydroxy cinnamoyl) arbutin

or it can be named as 3''-hydroxy robustaside A (Fig. 1). For our knowledge this is the first report about the isolation of this compound from the nature.

Compound 5: was obtained as yellow crystals (MeOH / ethyl acetate), m.p. 250-251°C. It is soluble in ethyl acetate, methanol, insoluble in benzene and chloroform. It developed a yellow colour with ammonia, $AlCl_3$ and showed negative Molisch's and Fehling's tests.

The IR spectrum showed a hydroxyl stretching band at 3500-3100 cm^{-1}. Also it showed an absorption band at 2924 and 2853 cm^{-1} for aliphatic C-H. It showed an absorption band at 1648 cm^{-1} for γ-pyrone ring beside another three bands at 1453, 1386 and 1181 cm^{-1} for -CH_2 bending, -CH_3 bending and C-C respectively. Compound 5 was recognized as a flavone from its UV absorption maxima at 302 nm (band I) and 278 nm (band II). A bathochromic shift (+66 nm) in band I was observed upon addition of NaOMe indicated a free hydroxyl group at C-4'. Bathochromic shift (+42 nm) in band I was observed upon addition of $AlCl_3$ and $AlCl_3$/ HCl indicating the presence of –OH group at C-5 and absence of orthohydroxylation as well. In addition, bathochromic shift of band II was observed by the effect of NaOAc that confirmed the presence of free –OH group at C-7.

The mass spectrum exhibited a molecular ion at m/z 329 (M^+ -1) which is in a good accordance with a molecular formula $C_{17}H_{14}O_7$ for this compound. Fragment at m/z 212 is corresponding to ring A with two methoxyl groups and two hydroxyl groups. Fragment 118 is corresponding to ring B with one hydroxyl group.

The ^{1}H-NMR spectrum of this material confirmed the presence of flavone through the arise of an olefinic proton singlet signal localized at δ 6.7 ppm assigned for H-3. The spectrum also showed two broad doublets: one at δ 7.99 (2H, J= 8.75 Hz, H-2', 6') and another one at δ 7.1 (2H, J= 8.75 Hz, H-3', 5') that are characteristic for 4'-substituted B-ring (Surendra, et al., 2009).

Finally, the data confirmed that compound 5 is 5, 7, 4'- trihydroxy- 6, 8-dimethoxy -flavone (Fig.1). Evidently, it was tentatively identified as demethoxysudachitin by comparing its ^{1}H-NMR with the reported data (Alam and Gomes 2003). This represents the first time of isolation of this compound from the genus *Phyllanthus* and from *Phyllanthus atropurpureus* as well.

Compound 6: occurs as yellow crystals, m.p. 254-255°C. It is soluble in ethyl acetate, methanol, insoluble in benzene and chloroform. It dissolves in dilute solution of alkalis producing intense yellow colour, indicating the flavonoidal nature of the material. Alcoholic solution developed a yellow colour when a few drops of 0.1 M $AlCl_3$ solution was added and gives bluish green colour with neutral ferric chloride solution indicating the presence of phenolic group. It gives positive Molisch's test and reduces Fehling's solution after acid hydrolysis. The physical characters, colour reaction and UV absorption of compound 6 suggest the presence of a flavonoidal glycoside skeleton.

The IR showed absorption at 3500-3100 together with peak at 1120 cm^{-1} indicating the presence of several hydroxyl groups. Also it showed the presence of carbonyl group at 1651 cm^{-1}. Compound 6 was recognized as a flavonol from its UV absorption maxima at 256 nm (band II) and 354 nm (band I). A bathochromic shift (+ 52 nm) in band I upon addition of NaOMe is indicating the presence of free hydroxyl group at C-4'. Addition of $AlCl_3$ produced a bathochromic shift (+66 nm) in band I indicating the presence of ortho-dihydroxy groups and/ or free 5-OH this will confirmed upon addition of HCl, where the absorption of $AlCl_3$ was reduced indicating the presence of free 5-OH. This also confirmed by a bathochromic shift in

band I (+ 17 nm) upon the addition of NaOAc/ H_3BO_3 while band II was unaffected by the use of NaOAc indicating substituted OH at C-7. The mass spectrum (FAB-MS) exhibited a molecular ion at m/z 465 $[M+1]^+$ which is in a good accordance with a molecular formula $C_{21}H_{20}O_{12}$ for this compound. EI-MS spectrum exhibited fragments a m/z 302 corresponding to M^+-glu., m/z 152 corresponding to ring A with two hydroxyl groups and m/z 134 corresponding to ring B with two hydroxyl groups.

The ^{1}H-NMR spectrum of compound 6 showed two doublets at 6.09, 6.28 ppm with meta coupling (J= 2 Hz) assigned to H-6 and H-8, respectively, two doublets at δ 6.77(J=9 Hz), 7.61(J= 2Hz) ppm which were assigned to H-5' and H-2', respectively. Proton at δ 7.49 (dd, J= 9, 2 Hz) was assigned to H-6' this pattern is typical for quercetin. The sugar moiety was confirmed to be glucose by the appearance of an anomeric proton signal at δ 5.13 (J =7.5 Hz) characteristic for glucose in β-glucosidic linkage confirmed by ^{13}C-NMR at δ 103.01 ppm. The effect of NaOAc on band II (no bathochromic shift) indicated glycosylation at C-7.

The ^{13}C-NMR spectrum of this material was compared to that of quercetin -7-O- glucoside (Harborne, et al., 1975, Mabry, et al., 1970), and the signals were similar. Extensive analysis of the ^{1}H-^{1}H and ^{1}H -^{13}C correlation spectral data confirmed the assignment of this compound to be quercetin -7- O- glucoside. The glycosidic linkage at C-7 was suggested through the chemical shift of the glucose anomeric proton [δ_H 5.13 (d, J= 7.5 Hz)] and carbon [δ_C 103.01] signals. The gHMBCAD correlation achieved between the anomeric proton at δ_H 5.13 (H-1") and δ_C 160 (C-7) support a C-7 location for the sugar unit in the glucosidic linkage.

By comparing the MS, ^{1}H- and ^{13}C- NMR data of this compound with the reported data (Harborne, et al., 1975), can be concluded that this material is 5, 3', 4'-trihydroxy flavonol-7-O-glucoside (Fig.1). Evidently, compound 6 was tentatively identified as quercetin-7-O-glucoside or Quercimeritrin.

This represents the first time of isolation of quercetin-7-O- glucoside from the genus *Phyllanthus*.

Anti-tumor activity: By using SRB assay, the effect of the three materials on the proliferation of HepG2 cell line was studied after 48 hrs of incubation. As shown in Table 4, the treatment of HepG2 cells with the material 1 (robustaside A) lead to high inhibition in the cell proliferation as concluded by the low IC_{50} values 4.93µg/ml, This revealed a specific strong anti-tumor activity of the compound against hepatocellular carcinoma. Increasing the concentrations of either material 2 (ethanolic extract of aerial parts) or material 3 (ethanolic extract of root) in the culture medium of HepG2 cells reduced the cell proliferation in a dose dependant manner. Maximum concentration of ethanolic extract of aerial parts and ethanolic extract of root (10µg/ml) exhibited only 25% cell mortality.

Anti-microbial activities: As shown in Table 3, the given results revealed that: ethyl acetate fraction of leaves shows significant gram –ve antibacterial activity (*E .coli)* than the other materials as well as Ampecillin. All materials show significant antibacterial activity against gram +ve bacteria (*S. aureus*) compared to the effect of Gentamycin.

The results from the anti-tumor activity revealed a specific anti-tumor activity of the robustaside A against hepatocellular carcinoma. This effect may be mainly due to the phenolic characters of the tested material (Rajkapoor, et al., 2007). As will the Anti-microbial activities may be due to the presence of flavonoids and phenolic compounds in the ethyl acetate fractions of roots and aerial parts.

Acknowledgments: The authors express deep feelings of gratitude to Prof. Dr. Fathy Serry, Professor of Microbiology, and Ahmed Olwan, Assistant Lecturer of Microbiology, Faculty of Pharmacy, Zagazig University for carrying out the antimicrobial activity. Our thanks also go to Dr. Dalia Hamdan, Lecturer of Pharmacognosy, Faculty of Pharmacy, Zagazig University for her kind help in carrying out the spectral analysis of some of the isolated compounds.

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Table- 1: The elution of the major compounds from leaf ethyl acetate soluble fraction.

Eluent	Fr. No.	Fr. wt. (g)	Isolated compounds
Chloroform: methanol 99.5: 0.5	69- 73	0.55	**compound 1**
Chloroform: methanol 96: 4	97-99	0.10	**compound** 2
Chloroform: methanol 92: 8	114-122	1.04	**compound** 3
Chloroform: methanol 84: 16	123-125	0.15	**compound** 4

Table- 2: The elution of the major compounds from steam and root ethyl acetate soluble fractions.

Eluent	Fr. No.	Fr. Wt. (g)	Isolated compounds
Chloroform: methanol 92: 8	79-82	0.150	**compound** 5
Chloroform: methanol 84: 16	83-88	0.170	**Compound 6**

Table- 3: Anti-microbial activities of different fractions of *P. atropurpureus.*

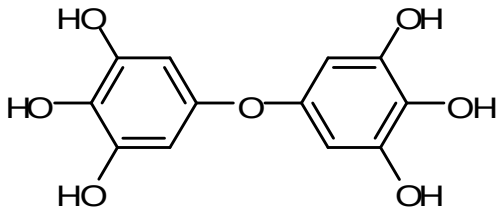
Material	Zone of inhibition (mm)		
	Gram –ve bacteria	Gram +ve bacteria	Fungi
	E. coli	***S. aureus***	***C. albicans***
1-Ethyl acetate fraction of leaves	14	25	10
2-Ethyl acetate fraction of stem	10	18	12
3-Ethyl acetate fraction of root	10	16	10
Ampecillin (10µg/well)	14	30	-
Gentamycin (10µg/well)	14	14	-
Nystatin (30µg/well)	-	-	16

- = No zone of inhibition.
- 200 mg/ml DMSO from plant fractions concentration were used. 100µl solutions were applied.

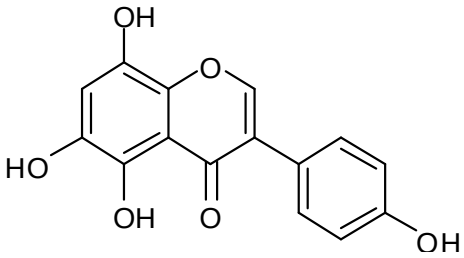
Table- 4: Anti-tumor activity of *Phyllanthus atropurpureus* against HepG2 cell line.

Concentration of tested materials (µg /ml)	HepG2cells viability (% of Control)		
	Material 1	Material 2	Material 3
0	100	100	100
1	94.8	98.7	98.6
2.5	71.3	97.8	97.7
5	48.6	82.9	84.9
10	34.5	75.3	73.9

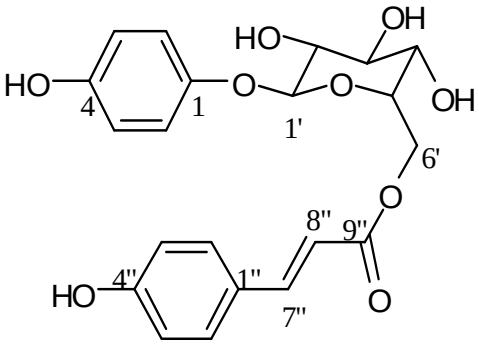
- Material 1: Robustaside A.
- Material 2: Ethanolic extract of aerial parts.
- Material 3: Ethanolic extract of root**.**



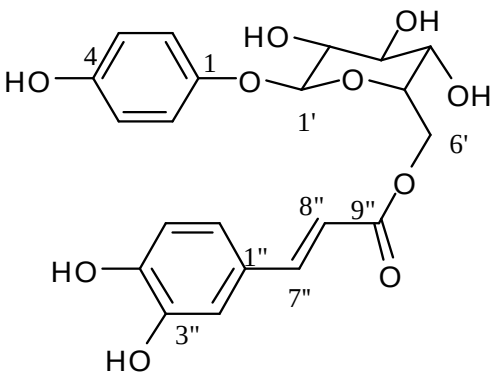
Compound 1
Di (3, 4, 5- trihydroxy phenyl) ether



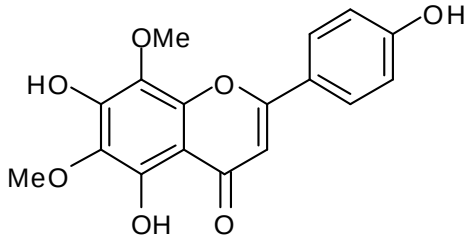
Compound 2
5, 6, 8, 4'-tetrahydroxy isoflavone



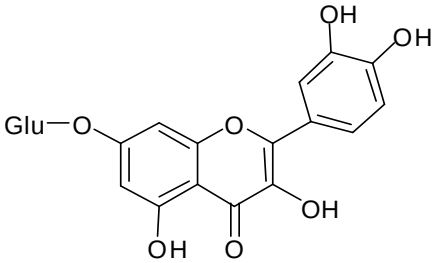
Compound 3
6'-(4"-hydroxy- cinnamoyl) arbutin



Compound 4
3"-hydroxy robustaside A



Compound 5
Demethoxysudachitin



Compound 6
Quercetin-7-O-glucoside

Figure- 1: The suggested structures of the isolated compounds from *Phyllanthus atropurpureus*.