BIOACTIVITY OF *ANTIDESMA BUNIUS* LEAVES (EUPHORBIACEAE) AND THEIR MAJOR PHENOLIC CONSTITUENTS

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**Abstract**

Chromatographic fractionation of the methanol extract of *Antidesma bunius* leaves afforded six polyphenols, namely, corilagin (1), gallic (2), ferrulic (3) and ellagic (4) acids in addition to the flavone vicinin II (5) and the dimmer amentoflavone (6). Their structures were elucidated by NMR and HRESI analysis. This is the first reported occurrence of the biflavone (amentoflavone) and vicinin in this species. The total phenolic content was estimated at 90 mg/ml of gallic acid equivalent (GAE) per 100 g plant extract using the Folin-Ciocalteu method. Antioxidant biochemical assay of the plant methanol extract was carried out using culture medium of hepatocyte cell lines. An increase in glutathione reductase enzyme and a reduction of the nitric oxide level in the cell culture revealed that *A. bunius* leaf extract possesses significant antioxidant activity in comparing with quercetine as a reference. The hepatotoxicity and hepatoprotective activities of *A.bunius* leaf extract and the pure isolated compounds: 1, 2 and 5 were evaluated by adopting the MTT colorimetric assay. Compounds 1 and 2 revealed a margin of safety on the monolayer hepatocyte with an IC$_{50}$ > 1000 µg/ml while for 5, IC$_{50}$ was at 125 µg/ml. The hepatoprotective activities for *A.bunius* leaf extract, 1 and 2 in comparison to Silymarin (50 µg/ml) were at 6.5, <12.5 and 12.5 µg/ml respectively while 5 did not show hepatoprotective activity at the tested concentrations.

**Keywords** : *Antidesma bunius*, Euphorbiaceae, phenolics, amentoflavone, antioxidant effect, hepatotoxicity, hepatoprotection
Introduction

Antidesma bunius (L.) Spreng (family Euphorbiaceae) is a medicinal plant found in many dipterocarp forested areas in Thailand, Philippines, and also throught the region of southeast Asia (Roger, 2004). The plant is commonly known to Thai local people as Mao Luang while in Philippines as Bignay. It is a shrub species with wide-spreading branches forming a dense crown evergreen. The fruits when ripe are traditionally used as a medicinal plant for gastric intestinal problem, e.g. diabetes, dysentery, indigestion and constipation. Its leaves usually used as flavoring when combined with other vegetables while its fruits are processed into jam and jelly and its juices fermented into wine and brandy. Recently, bignay was used to treat different illnesses ranging from colds to cancer (Magsino, 2003).

Previous studies demonstrated cytotoxic activity against brine shrimp (Micor et al., 2005) of A. biunius leaves and fruits. Various alkaloids specially the glycine-derived acetogenic quinoline alkaloïd and antidesmone have been detected in some Antidesma species (Steenkamp et al, 2009; Bringmann et al. 2000; Watt and Breyer-Brandwijk, 1962). Antidesma species are used by the Vhavenda for the treatment of gynaecological complaints (Steenkamp et al, 2009). The root-bark is taken for dysentery and washed in to ease body pain (Palgrave, 2002; Gerstner, 1938). Fruits of A.biunius were analyzed by Butkhup and Samappito (2008) for the contents of catechins and procyanidins qualitatively and quantitatively. Furthermore, this crop plant has been considered to be good source of bioactive phenolics. To our best of knowledge, it could be possibly inferred that our report is the second one concerning isolation and characterization of A. biunius leaves phenolics. The previous phytochemical investigation of its phenolics lead to the isolation of corilagin, gallic and chebulic acid (Hui and Sung, 1968). We aimed to isolate and characterize major phenolic constituents from the methanol extract of the leaves of A. biunius (MEABL), to estimate their total content and evaluate the antioxidant activity of MEABL through biochemical level assays (Lactate dehydrogenase (LDH), nitric Oxide (NO) production) and glutathione reductase (GR) in culture medium of hepatocyte cell lines (Rec.GSSC (DGKC) 1970; Montgomery and Dymock, 1961; Goldberg and Spooner, 1983). In addition, we aimed to evaluate the in vitro hepatotoxicity and hepatoprotective activity of MEABL and isolated pure phenolics (Seglen, 1976; Kiso et al., 1983).

Experimental

Instruments

1D & 2D NMR experiments (1H, 13C and HMBC) were recorded using a Jeol EX-500 spectrometer: 500MHz (1H NMR) 125 MHz (13C NMR) DMSO-d6 and a Brucker spectrometer: 400 MHz (1H NMR), 100 MHz (13C NMR) HMQC and HMBC in CD3OD. The chemical shifts are expressed in
(ppm) and the coupling constants are in Hz. The mass spectra (EIMS) were measured on a Finnigan-Mat SSQ 7000 spectrometer; \( m/z \) (rel. %) while the high resolution mass spectra (HREIMS) were obtained on a VG Auto Spec M series sector (EBE) mass spectrometer. The UV spectra were recorded on UV spectrophotometer (Shimadzu UV-240). Column chromatography was carried out on Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) and Sephadex LH-20 (Pharmacia). The paper chromatography (Whatman No. 1 and 3 MM) was performed using solvent systems: 1) \( \text{H}_2\text{O} \), 2) 15% \( \text{HOAc} \) (\( \text{H}_2\text{O} \)--\( \text{HOAc} \) 85:15), 3) BAW (\( n \)-BuOH--\( \text{HOAc} \)--\( \text{H}_2\text{O} \) 4:1:5, upper layer), 4) (\( \text{C}_6\text{H}_6 \)--\( n \)-BuOH--\( \text{HOAc} \)--\( \text{H}_2\text{O} \)--pyridine 1:5:3:3, upper layer). Solvents 3 and 4 were used for sugar analysis.

**Chemicals and standards**

Folin – Ciocalteu's phenol reagent and acetic acid were from Merck; gallic acid (3,4,5-trihydroxybenzoic) were from Sigma, hydrochloric acid (minimum 37%); sodium chloride, methanol and sodium sulphate were from Readel-de Haën; diethyl ether (anhydrous) was from J.T. Baker; and Amberlite XAD-4 was from FLUKA. The following compounds were purchased: 4-(22-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES 99%) (ACROS Co.), Potassium Chloride (KCl) (S.R.L), D(+) glucose (Merck), Calcium chloride (\( \text{CaCl}_2 \)) (S.D), Fetal Bovine Serum (FBS) (Lonza), Penicillin Streptomycin (PS) (Gibco BRL), Sodium thiopental, Silymarin (Sigma). Sodium Chloride (NaCl), Sodium Phosphate monobasic (\( \text{NaH}_2\text{PO}_4 \)), Sodium Phosphate dibasic (\( \text{Na}_2\text{HPO}_4 \)), Potassium Phosphate monobasic (\( \text{KH}_3\text{PO}_4 \)), Ethylenebis (oxyethylenenitrito) tetra-ethylene glycol bis (2-aminoethyl ether)-N,N,N'N'-tetraacetic acid (EGTA), Collagenase Type IV, Culture medium RPMI-1640, Trypan Blue, Insulin, Dexamethasone, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium-bromide (MTT) and Dimethylsulfoxide (DMSO). (Sigma Co.)

**Extraction and isolation**

Dried powdered leaves of *A.bunius* (500 g) were extracted by maceration using methanol (3L x 2) at room temperature. The extract was filtered and the solvent was removed under vaccum. The crude extract (130 g) was chromatographed on a polyamide 6S column with a gradient elution of MOH: \( \text{H}_2\text{O} \) with increasing polarity. The separation was monitored by PC, and eluted fractions exhibiting similar appearances when combined, yielding 9 fractions. Repeated paper chromatography and purification over Sephadex LH-20 column lead to the isolation of six pure compounds (1-6). Fraction 2 afforded compounds 1 (25 mg) while fraction 2 afforded compound 2 (30mg), 3 (10 mg), 4 (15mg) and 5 (18 mg). Fraction 8 afforded compound 6 (7 mg).
Estimation of the Phenolic Content by the Folin–Ciocalteu Test

The total concentration of phenols in the extract was determined according to the Folin–Ciocalteu method (Waterman and Mole, 1994). The total phenol concentration was calculated from a calibration curve, using gallic acid as a standard, and the results were expressed as mg of gallic acid equivalents (mg GAE) per 100 g fresh fruit.

Biochemical Assays For total methanol extract of A. bunius leaves

Lactate dehydrogenase (LDH)

LDH leakage from cell cytosol to the culture medium has been generally used as an indicator of cell integrity. Primary culture of rat hepatocytes were incubated for 2 hours with ascending concentrations (125, 250, 500, 1000 µg) of different tested samples/mL culture medium. LDH was measured in culture medium to reflect cell viability. The LDH activity was measured by an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie (Rec. GSCC( DGKC),1970).

Determination of Nitric Oxide (NO) production

Nitric oxide is very specific to liver injury and is almost always produced during liver inflammation. To test for the possible hepatoprotective effects of tested silymarin samples, different concentrations (12.5, 25, 50, 100 µg/mL) of tested samples were added to culture medium of primary hepatocyte culture that has been subjected to paracetamol injury and incubated for 2 hours. Culture medium was used for measurements of nitrite (a stable metabolite of NO) to reflect NO production by Griess method (Montgomery and Dymock, 1961). Decrease levels of NO production are indicative of hepatoprotection.

Glutathione Reductase (GR)

Reduced glutathione is the main non enzymatic antioxidant within the cell and plays an important role in the defense against oxidative stress. It is assumed that glutathione reductase depletion reflects intracellular oxidation. On the contrary, an increase in glutathione reductase concentrations could be expected to prepare the cell against a potential oxidative insult.

The content of reduced glutathione was quantified by the fluorometric assay of Goldberg and Spooner (1983). The method takes advantage of the reaction of reduced glutathione with N-(1-naphthyl) ethylenediamine. Addition to the cell culture evoke a dramatic decrease of cytosolic GR which have been pretreated for 2 hours with different concentrations (12.5, 25, 50, 100 µg/mL) of tested samples.

NB: Quercetin was used as a positive control; paracetamol (25 mM/mL) was used to induce liver injury. Each concentrations was placed in three wells so that mean of triplicates and standard deviation was used for statistical analysis.
Concentration of samples used: 100 µg sample

Statistical analysis: All data presented are based on means of triplicate absorbance determinations. Experiments were repeated twice for all samples.

In Vitro Bioassay on Primary Culture of Rat Hepatocytes Monolayer:

Animals: Wistar male rats (250-300 g), obtained from the animal house of the NRC (National Research Center, Cairo).

Isolation and Preparation of Rat Hepatocytes Monolayer Culture:

A primary culture of rat hepatocytes was prepared according to (Seglen, 1976) method, which was modified by (Kiso et al., 1983) using a waster male rat (250-300 gm) The rat was obtained from the animal house of the NRC (National Research Center, Cairo). Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Guide, 1985).

IC50 Determination on Rat Hepatocytes Monolayer Culture:

After 22-24 hours, the rat hepatocyte monolayer was washed twice with Phosphate Buffer Saline (PBS). In order to determine IC50, different concentrations were prepared for each sample (100 – 1000 µg /mL). After two hours of cells incubation with the extract, cell viability was determined using the MTT assay. The assay was performed according to the method of (Mosmann, 1983) modified by Carmichael et al., (1987). Absorbance of formasan crystals produced by viable cells was read at 540 and 630 nm dual wave-length using the Automatic Kinetic Microplate Reader (Labsystems Multiskan RC reader). Each experiment was repeated three times and the mean absorption of each concentration was calculated. A graph plotted with X-axis showing the different concentrations of the extract used and the y-axis showing the absorbance percentage of viable cells. The IC50 was graphically determined from the concentration that yielded an absorption coinciding with the 50% of cells that received no extract.

Evaluation of Hepatoprotective Activity:

The primary rat hepatocyte monolayer was prepared as above. Different concentrations were prepared from phospholipids fraction (12.5-100 µg /mL) using serial dilutions technique by dissolving in DMSO (1% maximum concentration). For each concentration, three replicates were carried out; in addition to positive control that was 50 µg/ mL Silymarin. The plate was incubated for 2 hrs at 37 ºC and 5% CO2, then washed twice with PBS. A 200 µL of 25 mM paracetamol was added to each well. After one hour of cells incubation with the paracetamol, cell viability was determined using the MTT assay. The concentration of the extract that was able to
protect the cells from the hepatotoxic effect of paracetamol by hundred percent was considered hepatoprotective.

**Results and discussion**

Column fractionation of the methanol extract from the leaves of *A. bunius* on a polyamide 6S using a water- methanol step gradient elution, repeated paper chromatography and purification on Sephadex LH 20 afforded six compounds (1-6). Compound 1 gave chromatographic, UV, hydrolytic data, $^1$H and $^{13}$C NMR spectral data closely similar to those reported to coraligin (Nawwar *et al.* 1994). While compounds 2, 3, 4 and 5 gave chromatographic, UV, and $^1$H NMR data typical gallic acid, ferulic acid, ellagic acid and Vicinin II respectively (Chanwitheesuk *et al.* 2007; Rajput A.P. and Patel M. K., 2001; Osman *et al* 2009; Nawwar *et al* 1994; Ye *et al* 2007; Xie *et al* 2003).

Compound 6 was obtained as yellow amorphous powder. It showed chromatographic properties (dark purple on PC under UV light, turning yellow when fumed with ammonia vapor, high $R_f$ values in organic solvents, and no development in aqueous solvents), hydrolytic experiment (2N HCl, 1hr, 100 °C) and UV spectral analysis (Experimental) characteristic for a flavone aglycone. In the negative ion HRESI-MS, a peak corresponding to [M-H] $^-$ was observed at $m/z$ 537.077521, showing the molecular formula of 6 to be C$_{30}$ H$_{18}$ O$_{10}$. $^1$H NMR spectrum (CD$_3$OD) showed two signals at $\delta_H$ 13.09 and 12.96 due to chelated phenolic hydroxyls and two doublets at $\delta_H$ 7.52 ($d$, 2H, $J = 8.7$ Hz, H-2'/H-5'); 6.78 ($d$, 2H, $J = 8.7$ Hz, H-3'/H-5') assigned to an aromatic system. Signals of a trisubstituted aromatic ring containing one oxygenated carbon were observed at $\delta_H$ 7.95 ($d$, 1H, $J = 2.3$ Hz, H-2'), 7.12 ($d$, 1H, $J = 8.7$ Hz, H-5') and 7. 90 ($dd$, 1H, $J = 8.7$ and 2.3 Hz, H-6'), along with doublets of a tetrasubstituted aromatic ring containing three oxygenated carbons at $\delta_H$ 6.2($d$, 1H, $J = 2.0$ Hz, H-6) and 6.40 ($d$, 1H, $J = 2.0$ Hz, H-8).The signals at $\delta_C$ 102.7(C-3), 101.5. (C-3''), 97.8 (C-8) and 119.0 (C-3'') in the $^{13}$C NMR spectra, were compatible with the structure of two flavone moieties with the link C-8→C-3''. (Markhan *et al.*, 1987). These data and the additional signals of quaternary carbons detected in the $^{13}$C NMR spectra were compared with previous report of the biflavone amentoflavone (Yue and Kang, 2011). Analysis of the HSQC spectra of 6 permitted to verify correlations which were important to distinguish the carbons and the respective hydrogens. So, this spectra was important in the identification of the aromatic carbons of 1,4-disubstitued system of B ring of Unity I. In the same way it contributed to recognize the resonances of aromatic carbons of B ring of Unity II. Analysis of the HMBC correlation spectra are in agreement to those of amentoflavone (Figure 1) (Markham *et al.*, 1987). Here we report on the first isolation of the biflavonoid
amentoflavone and the flavone viccinin from *A.bunius* species (Tchinda et al., 2006).

**Figure (1):** Compound 6 (Amentoflavone).

**Spectral data of compounds (1-6) isolated from *Antidesma bunius* leaves**

**Corilagin** (1-O-galloyl 3,6-O-hexahydroxydiphenoyl-β-glucopyranoside) (1)

1, was obtained as white amorphous powder of Rf values (x 100): 55 (H2O), 59 (AcOH-6), 32 (BAW). It showed a Mr of 633 in negative ESI-MS, corresponding to a molecular ion [M-H] at m/z =633. UV Spectral Data λ<sub>max</sub> (MeOH): 218 nm 1H- NMR spectral data (DMSO-d<sub>6</sub>) δ (ppm), β glucose moiety: 6.17 (d, J=7.3 Hz, H-1), 3.85 (dd, J=7.3, 2.3 Hz, H-2), 4.56 (s, H-3), 4.19 ((s, H-4), 4.21 (t, J=7.3 Hz, H-5), 4.32 (dd, J=11.2; 6.2 Hz, H-6), 3.94 (dd, J=11.2; 6.2 Hz, H-6)'), 6.98 (s of galloyl protons), 6.46, 6.53 (s of hexahydroxydiphenoyl protons). 13C-NMR spectral data (DMSO-d<sub>6</sub>) δ (ppm) β-B<sub>1,4</sub> glucose moiety: 118.7 (C-1),71.7 (C-2), 77.7 (C-3), 64.0( C-4), 76.4 (C-5), 62.2 (C-6),galloyl : 94.42 (C-1), 109.0 (C-2,6), 145.6 (C-3,5), 139.0 (C-4), 164.9 (C=O) hexahydroxydiphenoyl carbons: 123.0, 123.9(C-1,1′), 106.1, 106.9 (C-2, 2′), 143,9, 144.2, 144.8, 144.9 (C-3,3′, 5,5′) 135.4, 135.6 (C-4, 4′), 115.6, 115.8 (C-6,6′), 166,8, 167.3(C=O)).

**Gallic acid** (3, 4, 5-trihydroxybenzoic) (2)

2, was isolated as needle crystals of Rf values (x 100): 0 (H2O), 9(AcOH-6), 48 (BAW). UV Spectral Data λ<sub>max</sub> (MeOH): 273. 1H- NMR (DMSO-d<sub>6</sub>) δ (ppm): 6.87(s, H-2 and H-6).

**Ferrulic acid** (3)

3, isolated as colourless cubic crystals of Rf values (x 100): (H2O), (AcOH-6), (BAW). UV Spectral Data λ<sub>max</sub> (MeOH): 320.0, a shoulder at 293.1 and 232.6nm). The 1H-NMR spectral data of (3) (Acetone-d<sub>6</sub>, room temp.) δ (ppm): 7.5 (d, J = 16 Hz, H-α), 7.15 (br s, ∆ν<sub>1/2</sub> = 4 Hz, H-2), 7.05
(dd, J = 8 Hz and J = 2 Hz, H-6), 6.94 (d, J = 8 Hz, H-5), 6.36 (d, H-β) and 3.84 (s, OMe)].

**Ellagic acid (4)**

4, was isolated as amorphous powder of Rf values (x 100): 44 (H2O), 55 (AcOH-6), 72 (BAW). UV Spectral Data λmax (MeOH): 255, 362. 1H NMR (DMSO-d6) δ (ppm): 7.48 (s, H-5 and H-5').

**Vicenin II (Apigenin-6, 8-di-C-β-D-glucopyranoside) (5)**

5, was obtained as yellowish white amorphous powder of Rf values (x 100): 34 (H2O), 48 (15% AcOH), 13 (BAW). It showed a Mr of in negative ESI-MS, corresponding to a molecular ion [M-H]- at m/z = . UV Spectral Data λmax (MeOH): 270 nm, 351 nm, + NaOMe: 274 nm, 404 nm, + NaOAc: 274 nm, 342 nm, 381 nm, + NaOAc + H3BO3: 272 nm, 349 nm, 351 nm, +Al Cl3: 273 nm, 349 nm, 445 nm, +Al Cl3 + HCl: 273 nm, 349 nm, 403 nm. 1H NMR Spectral Data (DMSO-d6) δ (ppm), apigenin moiety: 6.79 (s, H-3), 7.98 (d, J= 8.5 Hz,H2',6'), 6.85 (d, H-3',5'), glucose moieties: 4.73 (d, J = 8.69 Hz, H-1''), 4.66 (d, J = 8.0 Hz, H-1'''), 3-4 multiplet. 13C-NMR Spectral Data (DMSO-d6) δ (ppm) apigenin moiety: 164.0 (C-2), 102.5 (C-3), 180.2 (C -4), 158.0 (C-5), 108.2 (C-6), 161.1 (C-7), 105.1 (C-8), 155.0 (C-9), 121.5 (C-1'), 129.0 (C-2'), 115.8 (C-3'), 160.8 (C-4'), 115.8 (C-5'), 129.0 (C-6'), glucose moieties: 73.6 (C-1''), 73.2 (C-2''), 79.1 (C-3''), 70.6 (C-4''), 81.8 (C-5''), 61.2 (C-6''), 74.5 (C-1'''), 69.7 (C-2'''), 78.8 (C-3'''), 70.8 (C-4'''), 79.1 (C-5'''), 60.8 (C-6''').

**Amentoflavone (3′, 8′′- Biapigenin) (6)**

6, was isolated as amorphous yellow powder. Negative ESI MS (M-H) m/z= 537.077521 corresponding to the chemical formula: C30 H18 O10. UV MeOH : 269, 291 (s) ,332; MeOH + NaOMe 275, 295 (s), 379; MeOH + NaOAc : 274 , 380; NaOAc+ H3BO3:270 , 339 ; MeOH + AlCl3 ; 279,303 345; MeOH + AlCl3 + HCl : 279, 303 , 345, 384. 1H NMR (400 MHz in MeOD): Unity I: δ 6.46 (s, 1H, H-3); 6.41 (s, 1H, H-6); 7.52 (d, 2H, J = 8.7 Hz, H-2'/H-5'); 6.78 (d, 2H, J = 8.7 Hz, H-3'/H-5'); 7.12 (d, 1H, J = 8.7 Hz, H-5'); 7.90 (dd, 1H, J = 8.7 and 2.3 Hz, H-6'). 13C NMR (400MHz in CDCl3): Unity I: δ 163.4 (C-2); 100.8 (C-3); 181.5 (C-4); 160.8 (C-5); 97.3 (C-6); 163.4 (C-7); 97.8(C-8); 156.9 (C-9); 102.8 (C-10); 120.7 (C-1'); 126.7 (C-2'/C-6'); 114.3 (C-3'/C-5'); 160.0 (C-4'); Unity II: 163.7 (C-2); 101.5 ( C-3); 181.8 (C-4); 160.0 (C-5); 97.6(C-6); 163.6 (C-7); 92.5 (C-8); 156.9 (C-9); 102.81 (C-10); 120.8(C-1'); 126.4 (C-2'); 119.0 (C-3'); 158.4 (C-4'); 114.8 (C-5'); 130.2 (C-6').

The total phenolic content of *A. biunius* leaves was estimated at 90 mg/ml of gallic acid equivalent (GAE) per 100 g plant extract using the Folin-Ciocalteu method.
Antioxidant Biochemical Assays for Total Methanol Extract of *A. biunius* Leaves:

**Hepatoprotective effect against Paracetamol:** The sample showed the highest therapeutic index of 80 similar to that reference control of quercetin (Table1.).

**Concentration of Glutathione Reductase Activity:** The sample induced significant endogenous intracellular production of GR (67.88/10⁶ cells) (1.00µg/mL) into culture medium which reflects antioxidant capacity that differs with the reference control quercetin (Table1.).

**Effect of the sample on the inhibition of Nitric Oxide:** Results revealed dose dependant decrease in the levels of NO in culture medium of hepatocytes exposed to injury. The maximal effect was achieved at 100µg/mL, the maximum tested concentration (Table1.).

<table>
<thead>
<tr>
<th>Sample</th>
<th>LDH</th>
<th>HP</th>
<th>TI %</th>
<th>GR</th>
<th>NO</th>
</tr>
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<tbody>
<tr>
<td>MEABL</td>
<td>&gt;1000</td>
<td>&lt;12.5</td>
<td>&gt;80</td>
<td>67.88</td>
<td>7.95±2.2</td>
</tr>
<tr>
<td>Quercetin</td>
<td>1000</td>
<td>12.5</td>
<td>80</td>
<td>80.51</td>
<td>11.11±1.7</td>
</tr>
<tr>
<td>Silymarin</td>
<td>500</td>
<td>50</td>
<td>10</td>
<td>62.52</td>
<td>10.97±1.9</td>
</tr>
</tbody>
</table>

**LDH:** Lactate dehydrogenase leakage after 2h (µg/mL).
**HP:** Hepatoprotection (µg/mL).
**TI %:** Therapeutic index %.
**GR:** Glutathione reductase /10⁶ cells (1.00µg/mL)
**NO:** Nitric Oxide (µmol nitrite/10⁶cells) (100µg/mL).

Results obtained in the hepatocyte culture model presented in this study revealed:

1) A comparative analysis of the potential hepatoprotective effect assumed.
2) An exploration of the preventive effect against oxidative stress by GR assay as an intracellular screening system for testing antioxidant properties.
3) The role of Ant. Sample MEABL in protecting hepatocytes from nitric oxide, a specific factor of liver injury, by inhibiting the production of nitrite, a stable metabolite of NO.

**Hepatotoxicity**

IC₅₀ of MEABL, 1 and 2 were higher than 1000 µg/mL. This result revealed that the samples exhibited high margin of safety after 2hrs treatment of the monolayer of rat hepatocites. Sample 5 exerted hepatotoxic effects on the hepatocytes revealed by an IC₅₀ of 125 µg/mL as shown in figure (2).
Figure (2): Viability of Monolayer of Rat Hepatocytes after 2 hrs Treatment with Different Concentrations of MEABL and Compounds 1, 2 and 5 using MTT Colorimetric Assay. Each Point Represents the Mean (n=3).

Hepatoprotective Activity

Sample 5 did not show any hepatoprotective activity on the hepatocyte cells against the toxic effect induced by the paracetamol. For samples 1, 2 and MEABL their hepatoprotection were shown at 6.5, 12.5 and <12.5 µg/mL respectively, as shown in figure 3.

Figures (3): Viability of Monolayer of Rat Hepatocyte after 2 hrs Treatment with Different Concentrations of MEABL and Compounds 1, 2 and 5 Followed by Treatment with 25 mM Paracetamol for 2hr. in Comparison with 50 µg/ml Silymarin as Control Using MTT Colourimetric Assay. Each Point Represents the Mean (n=3).

Conclusion

In this study, we report the isolation and identification of six phenolic compounds. Among them, the biflavone amentoflavone and the C glycoside viccinin II are isolated for the first time from A. b luminus species. In vitro antioxidant biochemical assays of the leaves extract revealed a relatively high antioxidant potency expressed by increased LDH leakage from cell cytosol and GR and high depression of NO level in comparison with quercetin. The study showed that A. b luminus leaves in addition to compound 1
and 2 have no toxicity on the hepatocytes and possess high hepatoprotective activity in the in vitro assay. *A. bunius* bioactivity could be attributed to its estimated high phenolic content (90 mg/ml of gallic acid equivalent (GAE) per 100 g plant extract).

**References:**


