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RESEARCH ARTICLE

Isolation of biologically active metabolites from *Bougainvillea spectabilis* Willd. cultivated in Egypt

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Abstract

Bougainvillea spectabilis Willd. is an ornamental plant cultivated in tropical, subtropical regions and other places as Egypt. The present study aimed to perform bioassay guided fractionation and isolation of some of the bioactive compounds from the Egyptian cultivate. The total ethanol extracts of the leaves (T.ET.L.), stems (T.ET.S.) and flowers (T.ET.F.) were screened for some pharmacological activities viz. in vivo anti-oxidant and anti-hepatotoxic, in addition to in vitro cytotoxic activities. The anti-oxidant effect was assessed by measuring serum glutathione level (GSH) in alloxan-induced diabetic rats. The anti-hepatotoxic activity was evaluated via measuring serum markers level viz. alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in CCl4-induced hepatotoxicity in rats. In vitro cytotoxicity of the different extracts was estimated for liver cancer cell line (HEPG2) adopting Sulforhodamine B stain assay. T.ET.L. exhibited significantly potent anti-oxidant and anti-hepatotoxic activities, while T.ET.S. showed the highest cytotoxic activity. Through biological guided fractionation, leaves and stems were subjected to successive solvent extraction, whereas the leaves ethyl acetate (Et.Ac.L.) and the stems ethanol 70% (Et.70%S.) extracts showed highly potent activities. Thus, different chromatographic techniques were performed on Et.Ac.L. and Et.70%S. extracts leading to the isolation of five bioactive metabolites. Three flavonoids were isolated from Et.Ac.L.; genistein-7-O-rutinoside **(1)**, formononetin-7-O-rutinoside **(2)** and myricetin **(3)**, while orobol-7-O-glucoside **(4)** and hesperidin **(5)** were isolated from Et.70%S. This work demonstrated the importance of the plant as a promising anti-oxidant, anti-hepatotoxic and cytotoxic product for nutraceutical use.

Keywords: antihepatotoxic; antioxidant; Bougainvillea spectabilis Willd; cytotoxic; flavonoids

Introduction

Nyctagineaceae comprises a number of popular flowering ornamentals, including Bougainvil-lea species which occur mainly in tropical and subtropical re-gions [1]. Bougainvillea spectabilis Willd. is one of the most commonly known species of the genus Bougainvillea. Through phytochemical analysis, phytoconstituents including alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids and tannins [2] were detected in the different extracts of leaves, stems and flowers of B. spectabilis Willd. Srinivasan and Subramanian [3] isolated and characterized isorhamnetin and quercetin from fresh flowers with white bracts, while quercetin and caffeic acid were isolated from leaves and stems, respectively [4, 5]. Furthermore, pinitol was reported to be isolated from the leaves [6] and the stem bark [7] of the plant. In a previous communication, the influence of plant growth regulators on calluogenesis and secondary metabolites production in B.

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spectabilis Willd. callus cultures was studied providing various successful cultures that could be a potential source for different metabolites [8].

Pharmacological studies revealed that the leaves and roots of B. spectabilis Willd. possessed anti-viral activity [9], while leaves extracts showed good anti-hyperglycemic activity [10]. Furthermore, the plant extracts showed anti-bacterial [11] and anti-fertility [12] activities. Very few studies were traced concerning the anti-hyperlipidemic [13] anti-oxidant [14], anti-inflammatory [15], anti-hepatotoxic [16] and cytotoxic [17] activities.

As free radicals and oxidative stress are involved in the production of various diseases in human, as hepatotoxiciy and cancer, the authors are concerned with the efficacy of the studied plant in preventing the free radical-induced tissue damage. Therefore, this study was planned to assess anti-oxidant, anti-hepatotoxic and cytotoxic activities for the extracts of leaves, stems and flowers of B. spectabillis Willd. cultivated in Egypt and to determine the biological active extracts in a trial to isolate the main metabolites from them.

Materials and Methods

Plant material

The leaves, stems and flowers (with purple bracts) of Bougainvillea spectabilis Willd. were collected from the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, Egypt, during the flowering stage from March to July (2009-2010). The plant was identified by Mrs. Thérèse Labib, a consultant of Orman Botanical Garden, Giza, Egypt and its identity was verified by Dr. Mohamed El Gebaly (Plant Taxonomy and Egyptian Flora Department, National Research Center, Dokki, Giza). A voucher specimen (# 2982016) has been deposited at the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University.

Preparation of total ethanol extracts

About one kilogram of each of the powdered leaves and stems were separately extracted by ethanol (70 %) by using Soxhlet apparatus [18], till exhaustion, to yield T.ET.L. and T.ET.S., respectively. Fresh flowers (1 Kg) were exhaustively extracted with ethanol (70 %) but by cold maceration (as concluded by the authors, during the course of this work, heating demonstrated degradation for some of their metabolites) to provide T.ET.F. The obtained extracts were completely dried, under vaccum, using rotatory evaporator (Buchi, Germany) at 40°C and stored at low temperature (-4°C).

Preparation of successive extracts

The air dried powdered leaves and stems (800 g, each) were separately extracted by petroleum ether followed by methylene chloride, ethyl acetate and 70 % ethanol using Soxhlet apparatus [19]. The obtained successive extracts were evaporated to dryness, under vaccum, and stored in sealed glass vials at -4°C for biological and phytochemical studies.

Chemicals, drugs and biochemical kits

All solvents were of analytical grade; absolute ethanol, petroleum ether (60-80°C), methylene chloride, ethyl acetate, methanol, formic acid, acetic acid and n-butanol. Alloxan and carbon tetrachloride were purchased (Sigma Co., USA); vitamin E was available in the form of gelatinous capsules, each contains 400 mg dl α-tocopherol acetate (Pharco Pharmaceutical Co., A.R.E.); Silymarin (Sedico Pharmaceutical Co., 6 October city, Egypt). Glutathione kits for assessment of antioxidant activity (Wak-Chemie Medical, Germany); transaminase kits for assessment of serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase (Biomerieux Co., France).

Experimental animals

Male mice (20-25 g) and adult male albino rats (Sprauge Dawely strain of 130-150g) were obtained from the National Research Centre, Dokki, Giza, Egypt. The animals were kept under the same hygienic conditions and fed on standard laboratory diet [normal diet consisted of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein 95% pure (10.5%) and starch (54.3%)] and provided with water ad libitum. All animal procedures followed were in accordance with the regulations of the Animal Ethics Committee of the National Research Centre and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Toxicological and Pharmacological evaluation

The following toxicological and pharmacological studies were performed on T.ET.L., T.ET.S., T.ET.F. in addition to the successive extracts (petroleum ether, methylene chloride, ethyl acetate and 70% ethanol extracts) of the selected bioactive organ of B. spectabilis Willd. The median lethal dose (LD_{50}) was estimated by determining the minimal dose that kills all animals and the maximal dose that fails to kill any animal, then several doses were chosen in between these two doses, each was injected subcutaneously in a group of 6 mice which were observed for 24 h for symptoms of toxicity and mortality [20]. Drug therapeutic dose was calculated according to Paget and Barnes [21]. Furthermore, the pharmacological potentialities

were evaluated according to the published procedures including in vivo anti-oxidant [22] which was assessed by measuring serum glutathione (GSH) of alloxan-induced diabetic rats. Additionally, the anti-hepatotoxic activity [23] was evaluated by monitoring serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in CCl_4-induced hepatotoxic rats, before and after induction. The effects produced were, in each case, compared with those of appropriate reference drugs. Results for these biological activites are presented in Table (1) and Figure (1).

Moreover, aliquots (5-50 μg/ml) of DMSO solutions of the total ethanol extracts and the successive extracts of the selected bioactive organ (petroleum ether, methylene chloride, ethyl acetate and 70% ethanol extracts) were separately subjected to evaluation of the cytotoxic activity against liver cancer cell line (HEPG2) [24]. Results are illustrated in Figure 2.

Experimental data were presented as mean $\pm$ standard error and were statistically analyzed using ANOVA and paired samples t-test via SPSS (version 20).

Phytochemical study

General experimental conditions

Solvent systems for paper chromatography (PC) included S_1 (n-Butanol: acetic acid: water; 4: 1: 5v/v/v) (upper phase) using Whatman sheets No. 3 (Whatman Ltd., England). Precoated silica gel plates 60 F_{254} (Reide-de Haen, Germany) were used for thin layer chromatography (TLC) by applying the solvent systems S_2 (ethyl acetate: formic acid: acetic acid: water; 100: 11: 11: 27v/v/v/v) and S_3 (ethyl acetate: methanol: water; 100: 13.5: 10 v/v/v). Melting points for isolated compounds were carried out on an Electrothermal 9100 apparatus (Electrothermal Engineering Ltd, Essex, England). EI-mass spectra were recorded using a Finnigan MAT SSQ 7000 single-stage quadrupole mass spectrometer and electron energy of 70 eV. NMR spectra (chemical shifts in ppm, coupling constants in Hz) were recorded on NMR Joel GLM, Joel TMS route instrument, 500 MHZ (Japan) using DMSO-d_6 as a solvent.

Isolation and identification of the compounds

Biological screening of the investigated extracts revealed that the ethyl acetate extract of the leaves (Et.Ac.L.) possessed potent anti-oxidant and anti-hepatotoxic activities, whereas ethanol 70% extract of the stems (Et.70%S.) showed highest liver cytotoxic activity. Therefore, these extracts were subjected to fractionation for isolation of some of the constituents. Chromatographic fractionation of Et.Ac.L. and Et.70%S. (5g, each) was performed using PC and S_1 as solvent system. Each chromatogram was visualized under UV before and after spraying with1% aluminium chloride or exposure to ammonia vapour. Three major spots were detected (R_f: 0.08, 0.14 and 0.32 in system S_1) in case of Et.Ac.L, while Et.70%S. showed two major spots (R_f : 0.18 and 0.22 in system S_1). Each compound was purified by repeated preparative TLC using S_2 as solvent system and 1% aluminium chloride as spraying reagent. Final purification was performed by recrystallization using methanol to afford three pure compounds **(1-3)** from Et.Ac.L. and two compounds **(4-5)** from Et.70%S.

Results

Soxhlet extraction of the leaves and stems yielded a dark green residue (335 g) and a greenish residue (205 g), respectively. Meanwhile, a purple residue (202 g) from the flowers was afforded. Furthermore, the successive extracts of the leaves were dried to provide petroleum ether (fatty dark green residue, 44.5 g), methylene chloride (dark green residue, 23.1 g), ethyl acetate (yellow residue, 20.8 g) and 70 % ethanol (red residue, 180 g) extracts. Additionally, the stems yielded extracts viz. petroleum ether (fatty dark green residue, 8.4 g), methylene chloride (dark green residue, 16.0 g), ethyl acetate (yellow residue, 20.5 g) and 70 % ethanol (red residue, 130 g) extracts.

The median lethal dose (LD_{50})

LD_{50} of T.ET.L., T.ET.S. and T.ET.F. were 6.38, 6.20 and 5.75 g/kg b.wt., respectively.

Anti-oxidant activity

The serum glutathione level was greatly reduced after induction of diabetes (Figure 1) which was returned close to its normal level by treatment of animals with vitamin E (% of change=1.10).

Likewise, T.ET.L. extract showed the highest anti-oxidant activity as it restored the level of serum glutathione (% of change=4.14). Furthermore, Et.Ac.L. exhibited the highest activity (% of change=8.60) among the successive leaves extracts.

Anti-hepatotoxic activity

Pretreatment with T.ET.L., T.ET.S. and T.ET.F. (100 mg/kg b.wt.) before liver damage, showed no changes in AST, ALT and ALP serum levels, while the CCl_4 intoxication resulted in a significant increase in their levels in all tested groups.

Treatment with T.ET.L., T.ET.S. and T.ET.F. significantly prevented CCl_4-induced elevation in the serum levels of AST, ALT and ALP compared to the negative control (Table 1). T.ET.L. was the most potent with % of change= 39.60, 27.05

Table 1 Anti-hepatotoxic activity ofthe different extracts of *B. spectabilis* Willd. and Silymarin drug onserum enzymes level (AST, ALT, ALP) in liver damaged rats.

Group	Dose (mg/kg b. wt)	AST(U/L) Zero	7 days (before liver damage)	72 hours (after CCl_4 injection)	7days (after liver damage)	ALT(U/L) Zero	7 days (before liver damage)	72 hours (after CCl_4 injection)	7days (after liver damage)	ALP(KAU) Zero	7 days (before liver damage)	72 hours (after CCl_4 injection)	7days (after liver damage)
Control (negative)	1 ml saline	31.20±1.30	30.60±0.90	146.30±6.20	152.80±7.40 [4.40]	30.90 ±1.20	29.80±0.90	154.30±6.80	165.20±7.40 [7.06]	7.20±0.10	7.10±0.10	61.40±3.20	69.80±1.70 [13.68]
T. ET. L.	100	29.60±0.90	29.10±0.90	**68.40±1.70** ^{a}	**41.30±1.60**^{ab} **[39.60]**	33.10±1.30	32.60±1.10	**62.10±1.4** ^{a}	**45.30±1.10**^{ab} **[27.05]**	7.40±0.10	7.20±0.10	**28.10±0.70** ^{a}	**22.30±0.60**^{a} **[20.64]**
T. ET. S.	100	33.40±1.30	33.10±1.20	81.30±3.40^{a}	46.20 ±2.30^{ab} [43.20]	28.90±0.80	28.50±0.80	78.40±3.20^{a}	52.60 ±2.40^{ab} [32.90]	7.10±0.10	7.20±0.10	36.90±1.30^{a}	25.70±0.50^{a} [30.35]
T. ET. F.	100	30.90±1.30	30.50±0.90	96.20±2.80^{a}	56.70 ±1.10^{ab} [41.10]	29.70±0.80	29.30±0.70	91.20±1.10^{a}	63.40±2.90^{ab} [30.50]	7.20±0.10	7.10±0.10	39.80±1.40^{a}	31.20±1.10^{a} [21.61]
Pet. Et. L.	100	34.20± 1.30	33.70±1.10	86.40±2.40^{a}	51.30±2.20^{ab} [40.63]	31.20±1.30	30.90±1.20	82.70±3.40^{a}	56.70±1.90^{ab} [31.44]	7.10±0.10	7.20±0.10	41.90±1.70^{a}	34.50±1.40^{a} [17.66]
Met. Cl. L.	100	31.90±1.30	31.50± 1.30	106.20±4.30^{a}	93.80±4.20^{a} [11.68]	29.80±0.80	29.50±0.80	93.20±3.70^{a}	88.60±3.90^{a} [4.94]	7.30±0.10	7.50±0.10	46.10±2.90^{a}	41.20±2.10^{a} [10.63]
ET. Ac. L.	100	32.70±1.30	32.20±0.90	61.70±2.90^{a}	**39.30±1.40**^{ab} **[36.30]**	33.60±1.30	34.10±1.20	71.30±2.90^{a}	**47.30±1.60**^{ab} **[33.70]**	7.40±0.10	7.20±0.10	32.20±1.40^{a}	**28.90±0.80**^{a} **[10.25]**
Et.70% L.	100	33.20± 0.90	32.80± 1.10	**53.70± 2.10** ^{a}	**41.20 ± 1.80**^{a} **[23.27]**	28.70± 0.60	28.20±0.70	**48.60±0.7** ^{a}	**35.30± 0.20**^{ab} **[27.37]**	7.20± 0.10	6.90± 0.10	**24.60±0.50** ^{a}	**19.10± 0.40**^{ab} **[22.36]**
Silymarin (reference drug)	25	32.40±1.30	32.20±1.10	46.90±1.80^{a}	30.10±0.90^{ab} [35.82]	33.20±1.30	34.30±1.10	43.10±1.60^{a}	31.70±1.20^{ab} [26.45]	7.30±0.10	7.10±0.10	19.80±0.60^{a}	7.10±0.10^{ab} [64.14]

Values are expressed as mean ± Standard Error(S.E.); n=6/group;^{a} Statistically significant from control group in the same interval at p < 0.01 using ANOVA followed by Dunette's test;^{b}Statistically significant from 72h after CCl_4 at p < 0.01 using paired t-test; [ ]: values in parenthesis are % of change calculated as regards of 72 hours after CCl4 administration. AST: aspartate aminotransferase, ALT: alanine aminotransferase, ALP: alkaline phosphatase, T.ET.L: Total ethanol leaves extract, T.ET.S: Total ethanol stems extract, T.ET.F: Total ethanol flowers extract, Pet.Et. L: Petroleum ether leaves extract, Met. Cl. L.: Methylene chloride leaves extract, ET.Ac.L.: Ethyl acetate leaves extract, Et.70% L: 70% Ethanol leaves extract

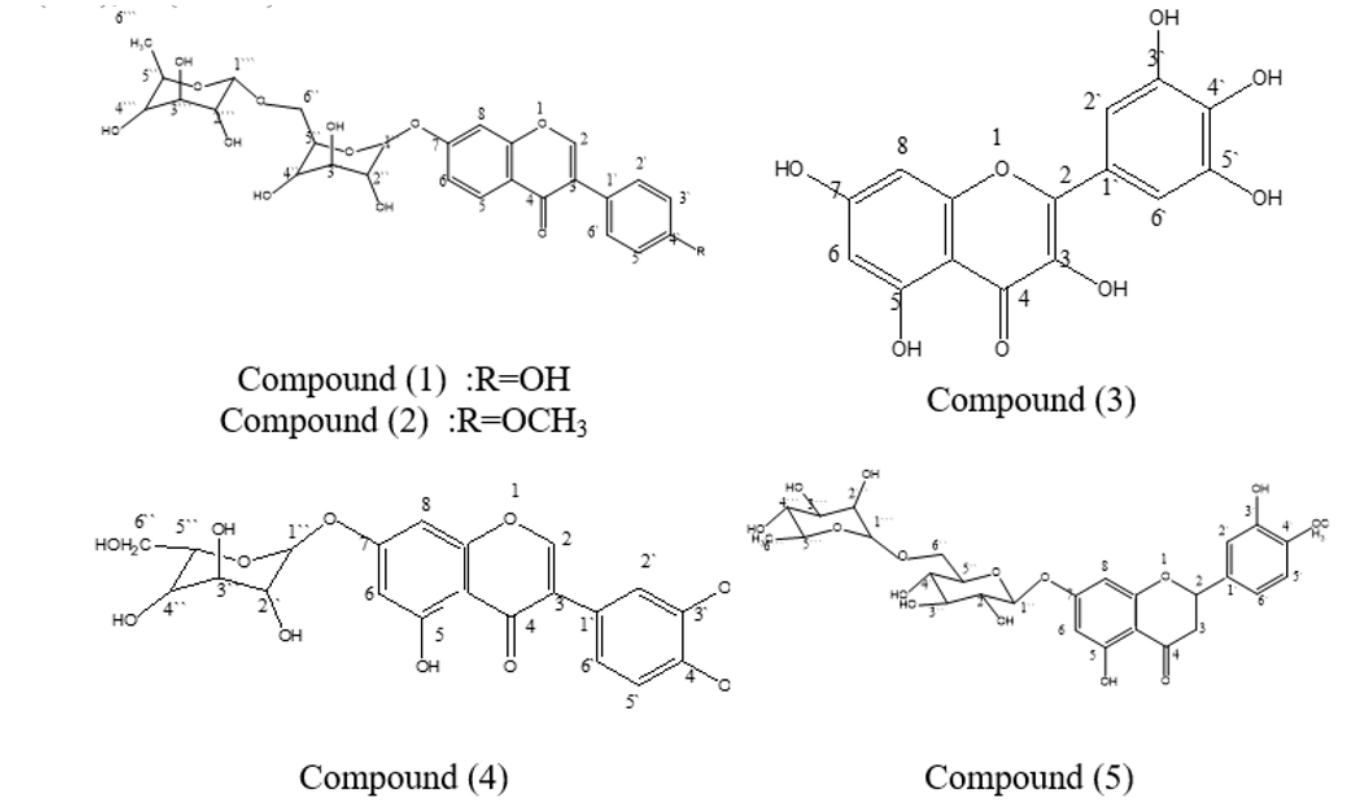

Diagram 1: Structure of Different compounds

and 20.64% for AST, ALT and ALP, respectively, compared to the reference drug (35.82, 26.45 and 64.14%, respectively). Likewise, the successive leaves extracts showed marked activity and restored the levels of AST, ALT and ALP; the most powerful being Et.Ac.L. which showed % of change= 36.30% (AST), 33.70% (ALT) and 10.25% (ALP).

Cytotoxic activity

The investigated extracts of *B. spectabilis* Willd. exhibited variable activities against liver cancer cell line (HEPG2) compared to the reference anticancer drug; doxorubicin (Figure 2). T.ET.S. appeared to be the most active (IC_{50} = 5.4 μg/ml), being almost comparable to doxorubicin (IC_{50} = 4 μg/ml), while T.ET.L. and T.ET.F. showed weak activity (IC_{50}=31.4 and49.5 μg/ml,respectively). Furthermore, investigation of successive stems extracts revealed that Et.70%S. exhibited the highest activity (IC_{50} = 19.1 μg/ml), while petroleum ether and ethyl acetate extracts showed moderate activity (IC_{50} = 21.3 and 23.3 μg/ml, respectively) and the methylene chloride extract showed very weak activity.

Isolated compounds

Five compounds (**1-5**) were isolated from leaves and stems of B. spectabilis Willd.

Compound (1): 70 mg, yellow crystals; R_f in S1=0.08; m.p.=250-251° C; (EI/MS) 577(M-H), 431, 270(100%);^{1}H-NMR (500 MHz, DMSO): δ 8.3 (1H, s,H-2), 7.66 (2H, m, H-2´& 6´), 7.00 (2H, m, H-3´& 5´), 6.6(1H, d, H-8), 6.2(1H, d, H-6), 5.15(1H, d, H-1´´), 4.32(1H, s, H-1´´´), 3.7-3.1(m, remaining rutinoside protons), 0.99(d, protons of CH_3-6´´´). ^{13}C-NMR (500 MHz, DMSO): δ 180(C-4), 165(C-7), 162(C-4´), 157(C-5), 155(C-9), 154(C-2), 128(C-2´&6´), 122(C-1´), 121(C-3), 115(C-3´&5´), 102(C-10), 101(C-1´´), 98(C-1´´´& 6), 92(C-8), 76.5(C-3´´), 75.5(C-5´´), 72.4(C-2´´& 4´´´),72.1(C-3´´´), 71.6(C-2´´´), 70.9(C-4´´), 69.8(C-6´´), 18(C-6´´´).

Compound **(2):** 50 mg, yellow amorphous powder; R_f in S_1 =0.14; m.p.=230-231° C; ^{1}H-NMR (500 MHz, DMSO) : δ 9.2 (1H, s, H-2), 7.998 (1H, m, H-5), 7.793 (2H, d, H-2´&6´), 7.196 (1H, m, H-6), 6.864(2H, d, H-3´&5´), 6.8 (1H, m, H-8), 5.3(1H, m, H-1´´), 4.4(1H, s, H-1´´´), 3.691(s, protons of O-CH_3), 3.5-3.1(m, remaining rutinoside protons), 0.80(d, protons of CH_3-6´´´).^{13}C-NMR (500 MHz, DMSO): δ 174(C-4), 161(C-7), 159(C-4´), 157(C-9), 153(C-2), 130(C-2´&6´), 126.668(C-5), 124(C-1´), 123(C-3), 119(C-10), 114(C-6),

114(C-3´&5´), 103(C-8), 101.915(C-1´´), 98(C-1´´´), 76.5(C-3´´), 75.293(C-5´´), 72.321(C-2´´&4´´´),72.1(C-3´´´), 71.851(C-2´´´), 70.913(C-4´´), 70.052(C-6´´), 59.479(O-CH_3) and 18(C-6´´´).

Compound **(3):** 60 mg, yellow amorphous powder; R_f in S_1= 0.32; m.p.=357-360° C; (EI/MS) 317(M-H); ^{1}H-NMR (500 MHz, DMSO): δ 7.3 (2H, s, H-2´and H-6), 6.37 (1H, d, H-8), 6.18 (1H, d, H-6). ^{13}C-NMR(500 MHz, DMSO): δ 175(C-4), 163(C-7), 160(C-5), 155(C-9), 146(C-2), 145(C-3´&5´), 131(C-3 &4´), 120(C-1´), 107(C-2´&6´), 102(C-10), 96(C-6), 92(C-8).

Compound **(4):** 20 mg, yellow amorphous powder (MeOH); R_f in S_1 =0.18; m.p.=180-181° C; ^{1}H-NMR (500 MHz, DMSO): δ 9.2(1H, s, H-2), 7.672 (1H, d, H-2´), 7.2(1H, m, H-6´), 7.018(1H, d, H-5´), 6.5 (1H, br. s, H-8), 6.2(1H, br. s, H-6), 5.17 (1H, dd, H-1´´), 3.5 (m, protons of glucose).

Compound **(5):** 40 mg, yellow amorphous powder; R_f in S_1= 0.22; m.p.= 251-254° C; ^{1}H-NMR (500 MHz, DMSO): δ 6.9(2H, d, H-2´&6´), 6.7(1H, d, H-5´), 6.4(1H, d, H-8), 6.24(1H, d, H-6), 5.2 (1H, dd, H-2), 4.824 (1H, d, H-1´´), 4.2 (1H, d, H-1´´´), 3.81 (s, protons of O-CH_3), 3.1(1H, m, H-3), 0.8 (d, protons of CH_3-6´´´).^{13}C-NMR (500 MHz, DMSO): δ 208(C-4), 166(C-7), 162(C-5&9), 148(C-3´), 146(C-4´), 130(C-1´), 118(C-6´),114(C-2´), 110(C-5´), 104.135(C-10), 101.896(C-1´´), 99(C-1´´´), 96.803(C-6), 95.89(C-8), 76.621(C-3´´), 75.266(C-5´´), 72.306(C-2´´& 4´´´), 72.268(C-3´´´), 71.829(C-2´´´), 71.828(C-2), 70.894(C-4´´),70.025(C-6´´), 59.468(O-CH_3), 40.281(C-3), 18(C-6´´´).

Discussion

The total ethanol extracts of the leaves , stems and flowers could be considered non-toxic according to Lorke [25]. T.ET.L. extract exhibited the highest anti-oxidant activity and that agreed with Sandeep et al. [11] who reported the methanol and aqueous extracts of B. spectabilis Willd. leaves to have potential in vitro antioxidant activity. Meanwhile, T.ET.L. and T.ET.S. extracts displayed the highest activities as anti-hepatotoxic and cytotoxic, respectively.

The isolated compounds were identified based on their UV, EI/MS and NMR (^{1}H and ^{13}C) spectra and comparison with the published data as: **(1)** genestein 7-O- rutinoside [26], **(2)** formononetin-7-O-rutinoside [27], **(3)** myricetin [28] **, (4)** orobol-7-O-glucoside [29] and **(5)** hesperidin [30]. To the best of our knowledge, this is the first report for the isolation of formononetin 7-O-rutinoside, genestein 7-O-rutinoside and myricetin from leaves in addition to orobol 7-O-glucoside and hesperidin from the stems of Bougainvillea spectabilis Willd..

Alloxan and carbon tetrachloride were used in this study as free radicals and oxidative stress inducers. During the course of this work, the studied plant extracts were shown to act as radical scavengers. Additionally, it revealed the inclusion of various flavonoids. Because of the high reactivity of the hydroxyl groups incorporated in the structure of the flavonoids, free radicals are made inactive. Flavonoids as antioxidants may offer resistance against oxidative stress by scavenging free radicals and thus preventing the disease [31]. The anti-oxidant activity of the extracts could be attributed to the presence of myricetin [32]**,** formononetin-7-O-rutinoside [33] and genestein 7-O-rutinoside [34]. This agreed with Mishra et al. [16] who reported that the anti-hepatotoxic activity of the studied plant is due to the availability of sufficient amounts of flavonids and phenolic compounds included in its extracts. Thus, such bioactive metabolites contribute to the anti-hepatotoxic activity through their antioxidative effect which is claimed to be one of the mechanisms of hepatoprotection [35]. Moreover, the potent cytotoxic activity of T.ET.S. on liver cell line may be attributed to the presence of hesperidin [36] and other flavonoids. It has been stated that flavonoids, as antioxidants, can inhibit carcinogenesis through damage of reactive oxygen species which is proposed to be involved in carcinogenesis [37], although the antitumor activity of flavonoids is still a point of discussion and the antioxidant activity is frequently inadequate.

From the findings of this study, it is obvious that the total ethanol leaves extract of B. spectabilis Willd. exerted significant anti-oxidant and anti-hepatotoxic activities, while the total ethanol stems extract exhibited a potent liver cytotoxic activity. Therefore, it could be used as a successful candidate for herbal formulations prescribed for complementary treatment of a wide variety of ailments such as liver toxicity, as well as, liver cancer. However, further laboratory and clinical trials should be carried out to complete the profile of the plant in order to introduce it as a neutraceutical drug**.**

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References

[1] Singh KK. A review: Multiplication of Bougainvillea species through cutting. Int J Chem Stud. 2018;6(2):1961–1965.

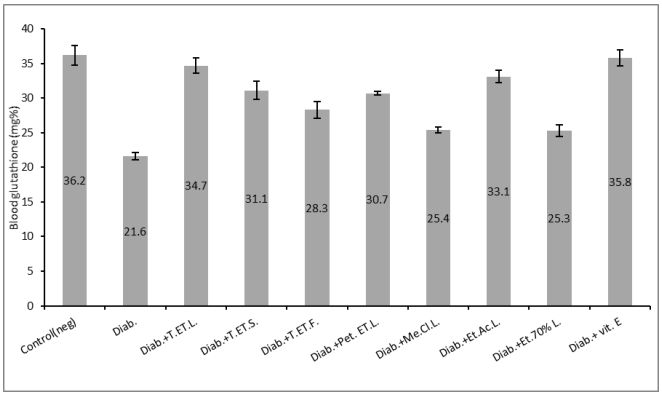

Figure 1 Anti-oxidant activity of the different extracts of *B.spectabilis* Willd. (dose= 100 mg/kg b. wt.)and vitamin E drug in male albino rats (neg: negative, Diab.: diabetic, T. ET. L: Total ethanol leaves extract, T. ET. S: Total ethanol stems extract, T. ET. F: Total ethanol flowers extract, Pet. ET. L: Petroleum ether leaves extract, Met. Cl. L.: Methylene chloride leaves extract, ET. Ac. L.: Ethyl acetate leaves extract, Et.70% L: 70% Ethanol leaves extract, *: significantly different from the control at $p < 0.01$ using ANOVA followed by Dunette's test)

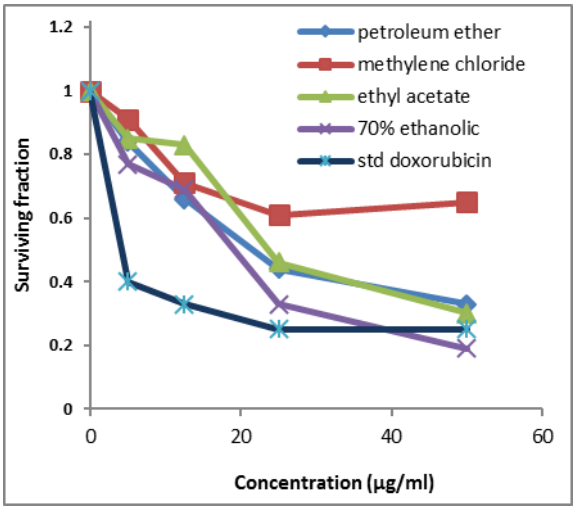

Figure 2 Cytotoxic activity of total ethanol extracts of leaves, stems and flowers

[2] Farzana R, Nadia S, Ijaz A, Saima S, Fakher UN, Shagufta N. Phytochemical analysis and inhibitory activity of ornamental plant (Bougainvillea spectabilis). Asian J Plant Sci Res. 2013;3(2):1–5.

[3] Srinivasan KK, Subramanian SS. Flavonoids of Bougainvillea spectabilis. Arogya. 1983;9(2):176–178.

[4] Chang W, Lee Y, Lu F, Chiang H. Inhibitory effects of flavonoids on xanthine oxidase. Anticancer Res. 1993;13(6A):2165–2170.

[5] Chang WS, Chang YH, Lu FJ, Chiang H. Inhibitory effects of phenolics on xanthine oxidase. Anticancer Res. 1994;14(2A):501–506.

[6] Narayanan CR, Joshi DD, Mujumdar AM, Dhekne VV. Pinitol a new antidiabetic compound from the leaves of Bougainvillea spectabilis. Curr Sci. 1987;56(3):139–141.

[7] Jawla S, Kumar Y, Khan MSY. Isolation of Antidiabetic Principle from Bougainvillea spectabilis Willd (Nyctaginaceae) Stem Bark. Tropical Journal of Pharmaceutical Research. 2013;12(5):761–765. Available from: https://dx.doi.org/10.4314/tjpr.v12i5.15.

[8] Eid HH, Deeb KSE, Metwally GF. Abdel Halim MB. Influence of plant growth regulators on calluogenesis and secondary metabolites production in Bougainvillea spectabilis Willd. cultures. World J Pharm Res. 2015;4(6):296–313.

[9] Balasaraswathi R, Sadasivam S, Chitra H, Raja J. Inhibition of in-vitro translation and cleavage of rRNA by Bougainvillea antiviral protein. Indian J Agric Biochem.

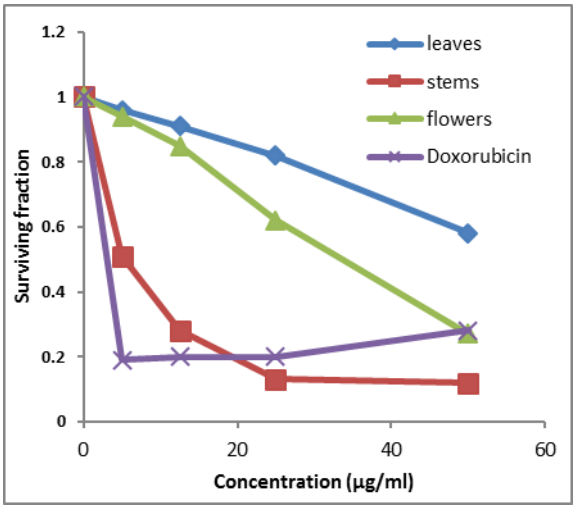

Figure 3 Cytotoxic activity of petroleum ether,methylene chloride, and ethyl acetate extracts of stems of B. spectabilis Willd on liver cell line(HEPG2).

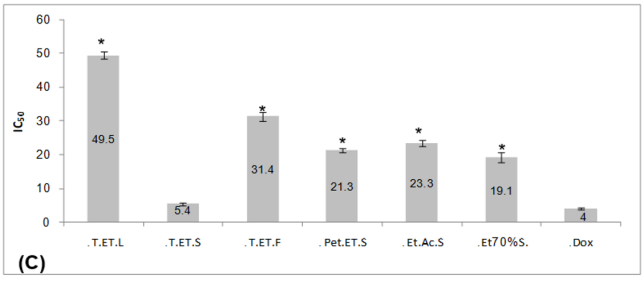

Figure 4 IC_{50} of the different extracts on liver cell line (HEPG2) (T. ET. L.: Total ethanol leaves extract, T. ET. S.: Total ethanol stems extract, T. ET. F.: Total ethanol flowers extract, Pet. ET. S.: Petroleum ether stems extract, ET.Ac.S.: Ethyl acetate stems extract, Et.70% S: 70% Ethanol stems extract,Dox.: Doxorubicin , *: significantly different from Dox. at $p < 0.01$using ANOVA followed by Dunette's test.)

2001;14(1&2):67–68.

[10] Bhat M, Kothiwale SK, Tirmale AR, Bhargava SY, Joshi BN. Antidiabetic Properties ofAzardiracta indicaand-Bougainvillea spectabilis:In VivoStudies in Murine Diabetes Model. Evidence-Based Complementary and Alternative Medicine. 2011;2011:1–9. Available from: https://dx.doi.org/10.1093/ecam/nep033.

[11] Sandeep D, Mamta S, Sonam R, Meenakshi B, Manish K, Anil KC. Evaluation of antimicrobial and antioxidant activities of Bougainvillea spectabilis. Int J Pharm Pharm Sci. 2013;5(3):178–182.

[12] Hembrom AR, Shail P, Singh VN. Anti-fertility effects of aqueous leaf extract of Bougainvillea spectabilis on seminal LDH isozymes in mice. J Appl Biosc. 2013;39(1):60–62.

[13] Saikia H, Lama A. The effect of Bougainvillea spectabilis leaves on serum lipids in albino rats with high fat diet. Int J Pharm Sci Drug Res. 2011;3(2):141–145.

[14] Chauhan P, Mahajan S, Kulshrestha A, Shrivastava S, Sharma B, Goswamy HM, et al. Bougainvillea spectabilisExhibits Antihyperglycemic and Antioxidant Activities in Experimental Diabetes. Journal of Evidence-Based Complementary & Alternative Medicine. 2016;21(3):177–185. Available from: https://dx.doi.org/10.1177/2156587215595152.

[15] Chatterjee C, Chatterjee M, Mandal G. Evaluation of anti-inflammatory activity of methanolic extract of leaves of Bougainvillea spectabilis in experimental animal models. Pharmacognosy Research. 2015;7(1):18–18. Available from: https://dx.doi.org/10.4103/0974-8490.147194.

[16] Mishra N, Tandonn VL, Dhama K, Khandia R, Munjal A. Does Bougainvillea spectabilis protect swiss albino mice from aflatoxin-induced hepatotoxicity? Adv Anim Vet Sci. 2016;4(5):250–257.

[17] Do LT, Aree T, Siripong P, Vo NT, Nguyen TT, Nguyen PK. Tip-pyang S. Cytotoxic flavones from the stem bark of Bougainvillea spectabilis willd. Planta med. 2018;84(02):129–134.

[18] Hasmida MN, Syukriah N, Liza AR, S M, Azizi M, Y C. Effect of different extraction techniques on total phenolic content and antioxidant activity of Quercus infectoria galls. Int Food Res J. 2014;21(3):1075–1079.

[19] Agrawal M, Agrawal Y, Itankar P, Patil A, Vyas J, Kelkar A. Phytochemical and HPTLC studies of various extracts of Annona squamosa (Annonaceae). Int J Pharm Tech Res. 2012;4(1):364–368.

[20] Karber G. Determination of LD50. Arch Exp Pathol Pharmacol. 1931;162:480–480.

[21] Paget GE, Barnes JM. Toxicity tests. In: DR L, AL B, editors. Evaluation of drug activities pharmacometries. Academic Press; 1964. p. 135–135.

[22] Beutler E, Duron O, Kelly B. Improved Method for the Determination of Blood Glutathione. J Lab Clin Med. 1963;61:882–883.

[23] Klassen CD, Plaa GL. Comparison of the biochemical alteration elicited in liver of rats treated with CCl4 and CHCl3. Toxic Appl Pharmacol. 1969;18.

[24] Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, et al. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. JNCI Journal of the National Cancer Institute. 1990;82(13):1107–1112. Available from: https://dx.doi.org/10.1093/jnci/82.13.1107.

[25] Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54(4):275–287. Available from: https://dx.doi.org/10.1007/bf01234480.

[26] Marzouk MSA, Ibrahim MT, El-Gindi OR, Bakr MSA. Isoflavonoid Glycosides and Rotenoids from Pongamia pinnata Leaves. Zeitschrift für Naturforschung C. 2008;63(1-2):1–7. Available from: https://dx.doi.org/10.1515/znc-2008-1-201.

[27] Mabry TJ, Markham KR, Thomas MB. The Scientific Identification of Flavonoids. NewYork (USA: Springer Verlag; 1996.

[28] He D, Gu D, Huang Y, Ayupbek A, Yang Y, Aisa HA, et al. Separation and Purification of Phenolic Acids and Myricetin from Black Currant by High-Speed Countercurrent Chromatography. Journal of Liquid Chromatography & Related Technologies. 2009;32(20):3077–3088. Available from: https://dx.doi.org/10.1080/10826070903320756.

[29] Choi HJ, Bae EY, Song JH, Baek SH, Kwon DH. Inhibitory effects of orobol 7-O-D-glucoside from banaba (Lagerstroemia speciosa L.) on human rhinoviruses replication. Lett Appl Microbiol. 2010;51(1):1–5.

[30] Maltese F, Erkelens C, van der Kooy F, Choi YH, Verpoorte R. Identification of natural epimeric flavanone glycosides by NMR spectroscopy. Food Chemistry. 2009;116(2):575–579. Available from: https://dx.doi.org/10.1016/j.foodchem.2009.03.023.

[31] Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997;2(4):152–159. Available from: https://dx.doi.org/10.1016/s1360-1385(97)01018-2.

[32] Ong KC, Khoo HE. Biological effects of myricetin. General Pharmacology: The Vascular System. 1997;29(2):121–126. Available from: https://dx.doi.org/10.1016/s0306-3623(96)00421-1.

[33] Mu H, Bai YH, Wang ST, Zhu ZM, Zhang YW. Research on antioxidant effects and estrogenic effect of formononetin from Trifolium pratense (red clover). Phytomedicine. 2009;16(4):314–319. Available from: https://dx.doi.org/10.1016/j.phymed.2008.07.005.

[34] Han RM, Tian YX, Liu Y, Chen CH, Ai XC, Zhang JP, et al. Comparison of flavonoids and isoflavonoids as antioxidants. J Agric Food Chem. 2009;57(9):3780–3785.

[35] Nwaehujor CO, Ode JO, Nwinyi FC, Asuzu OV. Mechanism of action involved in the hepatoprotective activities of methanol extract of Cassytha filiformis L. aerial parts in CCl4-induced liver damage. Comparative Clinical Pathology. 2014;23(6):1749–1755. Available from: https://dx.doi.org/10.1007/s00580-014-1997-4.

[36] Al-Ashaal HA, El-Sheltawy ST. Antioxidant capacity of hesperidin fromCitruspeel using electron spin resonance and cytotoxic activity against human carcinoma cell lines. Pharmaceutical Biology. 2011;49(3):276–282. Available from: https://dx.doi.org/10.3109/13880209.2010.509734.

[37] Stefani ED, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Carzoglio JC, Ronco A, et al. Dietary Antioxidants and Lung Cancer Risk: A Case-Control Study in Uruguay. Nutrition and Cancer. 1999;34(1):100–110. Available from: https://dx.doi.org/10.1207/s15327914nc340114.