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Phytochemical and Biological Studies on Isoflavonoids from *Dalbergia paniculata* Cultivated in Egypt

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Abstract: Background: *Dalbergia paniculata* Roxb. (Fabaceae) is a tree that is widely distributed throughout Sourthern and Central India. It is known to possess antimicrobial, antioxidant, anti-inflammatory and antidiarrhoeal activities. No scientific evidence is available for the biological effects of this plant. This study aimed to isolate the isoflavonoids in the stem bark and leaves of *D. paniculata* and evaluates the antioxidant activity of the hydro-alcoholic extract and isolated compounds. **Results and Conclusion:** Four isoflavonoids were isolated from the chloroform-soluble part of the defatted ethanolic extract of the stem bark and leaves of *D. paniculata*. The structures of the isolated compounds were elucidated by chromatographic and spectroscopic means. The compounds were identified as biochanin A (1), genistein (2), dalpatein (3) and formononetin (4). Total flavonoid content was determined in the leaves (1.51±0.05), stem bark (0.83±0.08) and seeds (0.68±0.03). An RP-HPLC method using an isocratic conditions has been developed for analysis of the isolated isoflavonoids in different parts of *D. paniculata*. The alcoholic extracts of the leaves and stem bark as well as the isolated isoflavonoids exhibited significant *in vitro* antioxidant activities in 1, 1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical scavenging assay. *In vivo* antioxidant activities of leaves, stem bark extracts and biochanin A were evaluated in alloxan-induced diabetic rats. There was a significant increase in blood glutathione level induced by leaves extract at the tested dose (100 mg kg^{-1} body weight).

Key words: *Dalbergia paniculata*, Fabaceae, isoflavonoids, biochanin A, antioxidant

INTRODUCTION

The genus *Dalbergia* (Fabaceae) comprises about 159 species, native to the tropical regions of Central and South America, Africa, Madagascar and Southern Asia. The plants of this genus are known for their deeply pigmented heartwoods of varying colors, often valued for their uses in wooden crafts. The heartwoods are used in traditional medicine and have antibiotic and cytotoxic activities (Barragan-Huerta *et al*., 2004). *Dalbergia* species have been reported to contain flavonoids, furans, benzophenones, styrenes and terpenoids (King *et al*., 1972). Some *Dalbergia* species are known to possess antimicrobial, antioxidant, anti-inflammatory and antidiarrhoeal activities (Jurd *et al*., 1971; Cheng *et al*., 1998; Hajare *et al*., 2001; Mujumdar *et al*., 2005). *Dalbergia paniculata* Roxb. is a tree of yellowish white flowers that is widely distributed throughout Southern and Central India. Its heartwood is used for construction of buildings and musical instruments.

Isoflavonoids are one of the most important and distinctive classes of naturally occurring flavonoids that occur in plants belonging to the subfamily Papilionoideae of family Fabaceae (Mabry *et al*., 1970). Genistein, daidzein and glycitein from soy and red clover and biochanin A and formononetin from red clover are among the well-known isoflavonoids. Continued interest in isolation of isoflavonoids from plant sources arises due to the diverse range of biological activities they possess. These include antimicrobial, oestrogenic and insecticidal activities (Miadokova, 2009; Birt *et al*., 2001; Akhter *et al*., 2008; Boland and Donelly, 1998; Cherdshewasart and Sutjit, 2008; Orgaard and Jensen, 2008). Isoflavonoids have been classically defined as dietary antioxidants, i.e., compounds that may protect against oxidative stress linked to inflammation and the risk of macromolecules damage by free radicals. They protect the body against hormone-related cancers, like breast, endometrial and prostate cancer.

In the present study, we describe the isolation of isoflavonoids from the stem bark and leaves *D. paniculata*, spectrophotometric and HPLC determination of isolated isoflavonoids and evaluation of their *in vitro* and *in vivo* antioxidant activity.

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MATERIALS AND METHODS

Plant material: The stem bark and leaves of *D. paniculata* were collected from Giza Zoo, Giza, Egypt. Plants were identified by Dr. Mohamed Abdelhalim, Department of Plant Systematic, Agricultural Research Center, Egypt. A voucher specimen was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Beni-Sueif University, Egypt. The dried stem bark and leaves were pulverized then stored in dry place at room temperature until use.

Chemicals: Authentic references: biochanin A and formononetin (E. Merck, Darmstadt, Germany). Material for chromatography: Silica gel 60 cc precoated TLC silica gel 60 GF_{254} from E. Merck, Darmstadt, Germany. Solvent systems: S1 (Benzene-ethyl acetate 8:2 v/v), S2 (Chloroform-Methanol 9:1 v/v), S3 (Chloroform-Methanol 7:3 v/v), spraying reagents: $AlCl_3$ 2% w/v in methanol, p-anisaldehyde/sulfuric acid. 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma-Aldrich (Steinheim, Germany). α-tocopheryl acetate was obtained from Pharco Pharmaceutical Co., Egypt. DPPH stock solution was prepared by dissolving 125 mg DPPH in methanol (100 mL) and sonicated for 10 min. DPPH working solution: The stock solution was diluted 1:10 (v/v) in methanol. Stock solutions in methanol: ascorbic acid (1.25 mg mL^{-1}), ethanol extracts of the bark, leaves and seeds (5 mg mL^{-1} each), pure isolated compounds (2 mg mL^{-1} each).

Apparatus: Rotary evaporator (Shimadzu, Germany), Koffler's heating stage microscope for determination of melting points, Jeol mass spectrophotometer, 70 eV. (Finnigan mat SSQ 7000), UV-visible spectrophotometer: Shimadzu UV 240 (P/N 204-58000), NMR Jeol GLM: Jeol TMS route instrument (^{1}H-NMR, 500 MHz).

Extraction and isolation: The dried stem bark and leaves of *D. paniculata* were pulverized to a fine powder (2.3 and 3.0 kg, respectively) and extracted with 70% aqueous ethanol by percolation till exhaustion. The collected hydroalcoholic extract was evaporated under reduced pressure to give a resinous residue (29.8 and 18.0 g, respectively). The residues were suspended in water and successively fractionated using petroleum ether, chloroform, ethyl acetate and n-butanol.

The chloroformic fraction (4.2 g) of the bark was applied onto a silica gel column (200 g, 150× 3.5 cm), using hexane as a solvent and the polarity was increased by $CHCl_3$ (5% increments) then MeOH (1% increments) and fractions (100 mL) each were collected. The obtained fractions were concentrated and monitored by TLC using S_2 and S_3 solvent systems and $AlCl_3$ or p-anisaldehyde/sulphuric acid as spray reagents. Fractions (9-19) Fraction A_1 (500 mg) eluted with chloroform-methanol (9.5: 0.5), showed one major spot with R_f value 0.5 in solvent system S_3 and gives yellow color with $AlCl_3$. These fractions were collected together, solvent evaporated and recrystallized from acetone to yield 400 mg yellow needles (Compound 1).

Fractions (35-42) Fraction B_1 (0.5 g) eluted with chloroform-methanol (7:3) were found to contain major spot in addition to other minor spots. Fraction B_1 was re-chromatographed on silica gel column (25 g, 1.2×50 cm), elution was carried out beginning with chloroform and increasing polarity with successive addition of methanol to yield 50 fractions (20 mL each). The solvent evaporated and the fractions were TLC monitored. Sub-fractions (13-18) eluted with 30% methanol in chloroform contained one spot with R_f value 0.3 in solvent system S_3 they were combined and re-crystallized from acetone to yield 8 mg yellowish white needles (Compound 2).

Five grams of the chloroform-soluble part of the leaves extract were dissolved in ethyl acetate and upon addition of diethyl ether; a white powder (50 mg) was precipitated which upon examination on TLC using system S_2 gave two major spots. This powder was applied on silica gel column (25 g, 1.2×70 cm). Gradient elution was carried out using chloroform and increasing polarity by methanol in 2% increments. Fractions were collected 10 mL each. The obtained fractions were separately concentrated to small volume, monitored by TLC using S_2 and similar fractions were pooled together to give two main fractions A and B.

Fraction A_2 (8-10) on TLC using S_2 and after spraying with $AlCl_3$ and p-anisaldehyde showed only one spot (R_f = 0.8, S_2). These fractions upon concentration and cooling gave 7 mg white needle crystals (Compound 3).

Fraction B_2 (13-16), eluted with 14% methanol in chloroform, showed one major spot in addition to other minor spots. Fraction B was re-chromatographed on silica gel column (15 g), elution was carried out beginning with chloroform and increasing polarity with successive addition (5%) of methanol. Fractions were collected 10 mL each. The solvent evaporated and the fractions were TLC monitored. Sub-fractions (11-15) eluted with 20% methanol in chloroform contained one spot with (R_f=0.7, S_2). They were combined and re-crystallized from acetone to yield 20 mg white needle crystals (Compound 4):

- **Compound 1:** Yellow needles (400 mg), m.p. 210-213, UV λ_{max} nm (MeOH) 261 and 330sh, (NaOMe) 273 and

Table 1: ^{1}H-NMR data (500 MHz, $CDCl_3$) of compounds 1, 2, 3 and 4

	Compound			
Proton	1	2	3	4
H-2	8.32, s	8.31, s	8.10, s	8.32, s
H-5	-	-	7.35, s	7.97, d (9.2)
H-6	6.40, d (2.3)	6.37, d (1.5)	-	6.93, d (9.2)
H-8	6.24, d (2.3)	6.21, d (1.5)	6.90, s	6.86, s
H-2',6'	7.50, dd (6.6, 2.3)	7.35, d (8)	6.75, s, H-6'	7.00, dd (6.6, 2.3)
H-3',5'	7.00, dd (6.6, 2.3)	6.79, d (8)	6.85, s, H-3'	7.50, dd (6.6, 2.3)
6- OCH_3			3.85, s	
2'-OCH_3			3.60, s	
4'-OCH_3-O	3070, s	-		3.74, s
OCH_2			5.90, s	

329, ($AlCl_3$) 273, 310sh and 375, ($AlCl_3$/HCl) 273, 310sh and 373, (NaOAc) 272 and 326, (NaOAc/H_3BO_3) 262 and 330sh, ^{1}H-NMR: Table 1

- **Compound 2:** yellow needles (7 mg), m.p. 297-298, UV λ_{max} nm (MeOH): 261 and 328 sh, (NaOMe) 275 and 327sh, ($AlCl_3$) 272, 307sh and 374, ($AlCl_3$/HCl) 273, 308sh and 373, (NaOAc) 271 and 326, (NaOAc/H_3BO_3) 262 and 336sh, ^{1}H-NMR: Table 1
- **Compound 3:** White crystals (7 mg), m.p. 253-254, freely soluble in acetone, R_f = 0.8 (S_2), UV λ_{max} nm (MeOH): 248, 261, 268 and 311, (NaOMe) 252, 268, 301 and 345, ($AlCl_3$) 248, 268 and 311, ($AlCl_3$/HCl) 248, 268 and 311, (NaOAc) 253, 268, 301 and 347, (NaOAc/H_3BO_3) 253, 261, 268 and 310
- **Compound 4:** Yellow crystals (15 mg), m.p. 220-223, UV λ_{max} nm (MeOH): 248, 258sh and 301, (NaOMe) 255 and 335, ($AlCl_3$) 248, 261sh and 301, ($AlCl_3$/HCl) 248, 261sh and 302, (NaOAc) 254, 313sh and 335, (NaOAc/H_3BO_3) 264sh and 303, ^{1}H-NMR: Table 1

Determination of total flavonoid content in *D. paniculata*: A spectrophotometric method was used to determine total flavonoid content in different organs of *D. paniculata*. To 0.6 g of the alcoholic extract of each organ, 1 mL of 0.5% (w/v) hexamine solution in water, 20 mL acetone and 2 mL HCl (25%) were added and boiled under reflux for 30 min. The mixture was filtered while hot through a piece of cotton into a measuring flask (100 mL). The residue and cotton were re-extracted two times under reflux, each with 20 mL acetone, for 10 min then filtered while hot into the measuring flask, cooled to room temperature and completed to the volume with acetone. Twenty milliliters of the extract of each organ were diluted with 20 mL water into a separating funnel and shaked with 10 mL ethyl acetate (3 times). The combined ethyl acetate extract was washed with water (x 2, 50 mL), transferred to a 50 mL measuring flask and completed to the volume with ethyl acetate. Ten milliliters of each of the purified flavonoidal fraction were mixed with one ml $AlCl_3$ reagent and completed to 25 mL with 5% methanolic acetic acid. A blank experiment was carried out, at the same time using 10 mL ethyl acetate instead of the purified flavonoid fraction. After 30 min, the absorbance of the test solutions was measured at 425 nm against blank solution.

HPLC determination of individual isoflavonoids in *D. paniculata*: Powdered plant organs (0.5 g) were placed in a 250 mL flask along with 50 mL ethanol, 20 mL de-ionized water and 8 mL conc. HCl. The mixture was refluxed for 2 h and then filtered and diluted to 100 mL volume. Each hydrolyzed sample was filtered through 0.45 µm filters and directly injected into HPLC. HPLC was carried out with a Shimadzu liquid chromatograph equipped with LC-10 AD pump, SPD-10A UV detector, Phenomenex Luna® C18 column (5 µm, 4.6× 250 mm) column for analytical purposes. An aliquot of 20 µL were injected. Ambient temperature was used. The flow rate was 0.8 mL min^{-1}. Isocratic elution with 0.5% ortho-phosphoric acid aqueous solution: methanol (60:40) mixture were used. The assay was done in triplicate for each case. Isoflavonoids were quantified at 280 nm using peak area by comparison to a calibration curve derived from biochanin A.

Determination of *in vitro* antioxidant activity: The hydrogen atom donation abilities of the ethanolic extracts of stem bark and leaves and the isolated compounds were measured from the bleaching of a purple-colored ethanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent. An aliquot of the sample (10 µL) was added to DPPH working solution (190 µL). A blank was prepared by adding 10 µL of ethanol to 190 µL of DPPH working solution. The mixture was incubated at (30±2°C) for 30 min and then the absorbance was measured at 517 nm. Ascorbic acid was used as a reference. The radical scavenging activities of samples, expressed as percentage inhibition of DPPH, were calculated according to the formula:

$$\% \text{ of Inhibition} = 1 - \frac{\text{Sample A517}}{\text{Blank A517}} \times 100$$

Determination of *in vivo* antioxidant activity

Animals: Albino mice (25-30 g body weight) were used for the toxicological study. Adult male albino rats of Sprauge Dawley strain (130-140 g body weight) were used for antioxidant screening. Animals were kept in standard environmental conditions, fed with standard laboratory diet consisting of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein-95% pure (10.5%), starch (54.3%) and water *ad libitum*. All of the methods used in the present study were approved by the ethics committee of National Research Centre, Giza, Egypt.

Toxicological study: Determination of the LD_{50} of the stem bark extract, leaves extract and biochanin A isolated from *D. paniculata* was estimated according to published procedure (Karber, 1931). Experiments were done to determine the minimal dose that kills all animals (LD_{100}) and the maximal dose that fails to kill any animal. Several doses at equal logarithmic intervals were chosen in between these two doses, each dose was injected in a group of 6 animals by subcutaneous injection. The animals were then observed for 24 h and symptoms of toxicity and mortality rates in each group were recorded and the LD_{50} were calculated.

***In vivo* antioxidant activity:** *In vivo* antioxidant activity was determined by measuring glutathione level in blood of alloxan-induced diabetic rats according to Beutler *et al.* (1963) using α-tocopherol as a positive control (Beutler *et al*., 1963). Animals were randomly divided into 6 groups, 6 rats each. The first group was kept as negative control while for other groups; diabetes mellitus was induced according to Eliasson and Samet (1969). A single dose of 150 mg alloxan/kg body weight was injected intraperitoneally in each animal followed by an overnight fast. The second group of diabetic rats was kept untreated; the third group received the reference drug α-tocopherol 7.5 mg kg^{-1} body weight; the fourth group was orally administered hydro-alcoholic extract of stem bark using a dose of 100 mg kg^{-1} body weight; the fifth group was orally administered hydro-alcoholic extract of leaves using a dose of 100 mg kg^{-1} body weight and the sixth group was orally administered biochanin A using a dose of 10 mg kg^{-1} body weight. The rats were kept for one week before the determination of glutathione in blood. Fresh blood samples (0.1 mL) were taken from the retro orbital venous plexus of the rats for the estimation of blood glutathione level which was measured using the bio-diagnostic kit and the absorbance was measured at 405 nm using the following equation:

$$\text{GSH blood} = A_{sample} \times 66.66 \text{ mg day}^{-1}$$

where, A_{sample} is the absorbance of each sample at 405 nm.

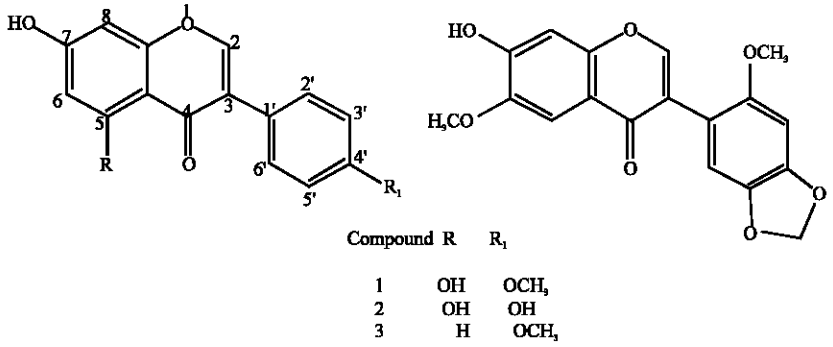
Statistical analysis: The results are expressed as mean±SE (n = 6). Statistical significance was determined by ANOVA with $p<0.01$ considered significant.

RESULTS AND DISCUSSION

The chloroform-soluble part of the defatted ethanolic extract of the stem bark of *D. paniculata* afforded two Compounds 1 and 2. The chromatographic properties of the isolated compounds have confirmed their aglycone nature. The identification of these compounds was based on co-chromatography, m.p., UV and ^{1}H NMR spectra which were compared with the published data.

Compound 1 was isolated as yellow crystals. The EI MS spectrum showed a molecular ion peak at *m/z* 284.07 in addition to the following peaks at *m/z* 269.03 (M^+- CH_3), *m/z* 132.04 and *m/z* 152.02, derived from the retro-Diels-Alder fragmentation, suggesting that ring A of the isoflavone skeleton had two hydroxyl groups and ring B had one methoxy group. The UV spectrum (λ_{max} in MeOH), (261 and 330 nm sh) showed the characteristic absorption of an isoflavone (Mabry *et al*., 1970). A bathochromic shift (273, 310sh and 375 nm) occurred upon addition of $AlCl_3$. No change upon addition of HCl. This suggested the presence of free 5-OH group. A bathochromic shift (270, 330 nm) upon addition of sodium acetate suggested the presence of free 7-OH group. The ^{1}H-NMR data of compared 1 are shown in Table 1. A are shown in Table 1. From the previous data, melting point and co-TLC with authentic reference compound, compound 1 could be identified as biochanin A (Fig. 1). This compound was previously isolated from *D. paniculata* stem bark (Narayanan and Seshadri, 1971).

Compound 2 was isolated as white crystals. The EI MS showed molecular ion peak at *m/z* 270.24. The spectrum also showed the fragment ions of *m/z* 153.46 and *m/z* 118.24 derived from the retro-Diels-Alder fragmentation, suggesting that ring A of the isoflavone skeleton had two hydroxyl groups and ring B had one hydroxyl group. The UV spectrum (λ_{max} in MeOH), (261 and 328sh) showed the characteristic absorption of an isoflavone. A bathochromic shift (272, 307sh and 374 nm) upon addition of $AlCl_3$ and no change upon addition of Hcl suggested the presence of free 5-OH



Compound	R	R_1
1	OH	OCH_3
2	OH	OH
3	H	OCH_3

Fig. 1: Chemical structures of compounds 1-4

group. A bathochromic shift (271, 326 nm) upon addition of sodium acetate suggested the presence of free 7-OH group. The ^{1}H-NMR data of Compound 2 are shown in Table 1. From the previous data, Compound 2 could be identified as genistein (Fig. 1). This Compound is first time isolated from *D. paniculata*.

The chloroform-soluble fraction of the defatted ethanolic extract of the leaves afforded two Compounds 3 and 4. The chromatographic properties of the isolated compounds have confirmed their aglycone nature.

Compound 3 was isolated as white crystals. The EI MS spectrum showed a molecular ion peak at *m/z* 342.07 in addition to the following peaks at *m/z* 313.05 (M^+- CO), *m/z* 264.01 and *m/z* 209.03. The UV spectrum (λ_{max} in MeOH), (256 and 312 nm) showed the characteristic absorption of an isoflavone. No UV bathochromic shift was observed with $AlCl_3$ suggesting the absence of free 5-OH group. A bathochromic shift (252 and 348 nm) upon addition of sodium acetate suggested the presence of free 7-OH group. The ^{1}H-NMR data of compound 3 are shown in Table 1. From the previous data, compound 3 could be identified as Dalpatein (Fig. 1). This compound was previously isolated from *D. paniculata* flowers (Adinarayana and Rajasekhara, 1973). It is first time to isolate dalpatein from the leaves of the plant.

Compound 4 was isolated as yellowish white crystals. The EI MS spectrum showed a molecular ion peak at m/z 268.24. The UV spectrum (λ_{max} in MeOH) (249, 262sh and 296 nm) showed the characteristic absorption of an isoflavone. No UV bathochromic shift was observed with $AlCl_3$ suggesting the absence of free 5-OH group. A bathochromic shift (256, 276sh, 334 nm) upon addition of sodium acetate suggested the presence of free 7-OH group. The ^{1}H-NMR data of compound 4 are shown in Table 1. From the previous data, the Compound 4 could be identified as formononetin (Fig. 1). This compound was previously isolated from *D. paniculata* bark (Narayanan and Seshadri, 1971).

Table 2: HPLC determination of isolated isoflavonoids from *D. paniculata*

	Leaves	Stem bark	Seeds
Biochanin A	-	1.07±0.01	1.04±0.00
Formononetin	0.53±0.01	0.39±0.00	0.36±0.00
Genistein	0.27±0.01	0.35±0.00	0.30±0.01
Dalpatein	0.48±0.01	-	-

Table 3: Results of *in vitro* antioxidant activity of leaves and stem bark extracts and isolated isoflavonoids from *D. paniculata*

Sample	Percent inhibition
Stem bark extract	68.1±0.9
Leaves extract	68.5±0.5
Biochanin A	30.4±1.6
Formononetin	19.3±3.6
Genistein	34.3±5.8
Dalpatein	65.8±5.7
Ascorbic acid	68.7±0.3

Colorimetric determination of flavonoid content in plant material based on color reaction with $AlCl_3$ reagent is well-known method to determine total flavonoid content. Determination of total flavonoid content in different organs of *D. paniculata* was carried out using biochanin A as a standard. The leaves were found to contain the highest amount of flavonoids (1.51±0.05 g%), followed by the stem bark (0.83±0.08 g%) then the seeds (0.68±0.03 g%). An isocratic HPLC analysis method was used to determine the content of individual isoflavonoids in different organs. Results are shown in Table 2. The bark contains the highest amount of biochanin A and genistein. Formononetin was higher in the leaves. Dalpatein was present only in the leaves.

Flavonoids act as antioxidant agents by direct free radical scavenging, transition metal chelation and maintenance of endogenous antioxidants such as the glutathione and superoxide dismutase systems (Tourino *et al*., 2005). The antioxidant activity of the ethanolic extracts of the stem bark and leaves of *D. paniculata* as well as the isolated isoflavonoids were determined. *In vitro* antioxidant activity was tested using 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical scavenging assay. Results are shown in Table 3. Radical-

Table 4: Results of *in vivo* antioxidant activity of leaves extract, stem bark extract and biochanin A isolated from *D. paniculata*

Treatment	Blood glutathione (mg%)	Percent change from control
Control (1 mL saline)	36.3±1.4	-
Diabetic	21.7±0.6	40.2
Diabetic + vitamin E	35.9±1.2	1.1
Diabetic + stem bark extract	31.2±0.8	14.1
Diabetic + leaves extract	34.6±0.9	4.7
Diabetic + biochanin A	35.1±0.7	3.3

scavenging activity of the ethanolic extract of the leaves showed the highest inhibition in DPPH activity (68.5%). Dalpatein (3) isolated from this extract, exhibited significant antioxidant activity (65.8%).

Based on our results, it appears that the most effective radical scavengers are isoflavonoids with the 3,4-dihydroxy substitution pattern on the B-ring as represented by the high antiradical activity of dalpatein having dioxy ether linkage in the 3 and 4 positions (65.8%). The presence of o-dihydroxy structure on the B-ring confers a higher degree of stability on the isoflavonoid phenoxyl radical by participating in electron delocalization and is an important feature for the antiradical potential. Intramolecular rearrangement may take place when the 5-OH group is present, giving a catechol-like structure in ring C (Amic *et al*., 2003). This was ascertained by the higher antiradical activity of genistein (34.3%) and biochanin A (30.4%) than formononetin (19.3%) that lacks the 5-OH group. Moreover, methoxy groups introduce unfavorable steric effects and produced drastic reduction in antioxidant activity. This was obvious in the lower activity of biochanin A, possessing 4'-methoxy group, than genistein.

In acute toxicity study, the LD_{50} of the stem bark and leaves extracts were found to be 5.0 and 5.2 g kg^{-1} body weight, respectively. This LD_{50} is categorized as unclassified according to the ranking system of European Economic Community (EC Directive 83.467.EEC, 1983). *In vivo* antioxidant activity of extracts and biochanin A isolated from *D. paniculata* was determined by measuring blood glutathione level in alloxan-induced diabetic rats. Blood glutathione level in the diabetic rats was increased by treatments with α-tocopherol or *D. paniculata* stem bark or leaves extracts and biochanin A as indicated by ANOVA ($p<0.01$). Results of the *in vivo* antioxidant activity are shown in Table 4. Antioxidant activity has been reported from other *Dalbergia* spp. Butein isolated from *D. odorifera* inhibited iron-induced lipid peroxidation in rat brain homogenate in a concentration-dependant manner (Cheng *et al*., 1998). Antioxidant activity of phenolic compounds extracted from *D. odorifera* were compared with butylated hydroxytoluene and α-tocopherol. The results showed that 2, 4-dihydroxy-5-methoxyflavone, daidzein, vestitol and medicarpin had moderate antioxidant activity while 2, 3, 7-trihydroxy-4-methoxyisoflavone and 4, 5, 7-trihydroxy-3-methoxyflavone had strong antioxidant activity (Wang *et al*., 2000). The potent protective effect of compounds isolated form *D. odorifera* heartwood on glutamate-induced oxidative injury in HT22 cells was reported (An *et al*., 2008).

CONCLUSION

It could be concluded that *D. paniculata* stem bark and leaves can be used as a source for isoflavonoids especially biochanin A. Stem bark and leaves of this plant can be used as an antioxidant agent. More extensive toxicological study is needed to evaluate its potential cytotoxic activity.

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