

Full Length Research Paper

## Acute toxicity and antioxydant property of *Striga hermonthica* (Del.) Benth (Scrophulariaceae)

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Plants containing flavonoids have been reported to possess strong antioxidant properties. The aqueous acetone extract of *Striga hermonthica* was further separated into aqueous and ethyl acetate fractions and assayed for their *in vitro* antioxidant properties using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The crude extract exhibits a weak antioxidant activity (IC<sub>50</sub> of 95.27±2.30 µg/ml) and an insignificant acute toxicity on mice (LD<sub>50</sub>: 1753±44 mg/kg). Luteolin was isolated and identified by mean of its spectral data as the main DPPH radical scavenger of the ethyl acetate fraction, exhibiting an IC<sub>50</sub> value of 6.80±1.46 µg/ml. These results suggest an antioxidant potential for *S. hermonthica*.

**Key words:** *Striga hermonthica*, luteolin, antioxidant, acute toxicity.

### INTRODUCTION

*Striga hermonthica* (Del.) Benth (Scrophulariaceae) is a semi-parasitic plant growing in millet (*Pennisetum americanum*) and sorghum (*Sorghum bicolor*) fields. It is widespread in West and East Africa (Musselman et al., 1991; Mohamed, 1994). In Burkina Faso, the plant is traditionally used in dermatosis, leprosy and jaundice treatments (Nacoulma, 1996). Antibacterial (Hussain and Deeni, 1991), contraceptive (Choudhury et al., 1998) and a weak antiplasmodial (Okpako and Ajaiyeoba, 2004) activities of *S. hermonthica* have been reported. The flavones apigenin and 5-hydroxy-6,8-dimethoxyflavone-7,4'-O - diglucoside have been previously isolated from the whole plant extracts (Khan et al., 1998; Choudhury et al.,

2000). Plants containing flavonoids have been reported to possess antioxidant properties (Bors et al., 1990). Hence, in the present study, the aqueous acetone extract of *S. hermonthica* was screened for its main antioxidant flavonoid and for its acute toxicity on mice.

### MATERIALS AND METHODS

**Extraction** was performed twice using aqueous 80% acetone for 24 h with agitation at room temperature. 200 g of dry powered material (leaves, flowers, and stem) collected at Gampela (east of Ouagadougou, Burkina Faso) was used to get 36.5 g of crude extract.

**DPPH radical scavenging activity** was measured as described by Velasquez et al. (2003). A test sample solution in methanol (0.75 ml) was added to 1.5 ml of 20 mg/ml DPPH methanol solution. After shaking, the mixture was incubated for 15 min in darkness at room temperature and then absorbance was measured at 517 nm. The difference in absorbance between a test sample and a control

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(methanol) was taken as the activity. The activity, expressed as IC<sub>50</sub> value (µg/ml; 50% inhibitory concentration) was consistent with the mean value of 3 measurements. Quercetin (IC<sub>50</sub> 3.60µg/ml) served as positive control. A 2 mg/ml crude extract solution was used for tannin, flavonoids and anthocyanidins quantification (µg/ml). Concentrations were expressed in percentage (µg compounds for 100 µg of dry extract). Each assay was conducted 3 times.

**Flavonoids** were estimated using Dowd's method according to Arvouet-grant (1994). Test sample solution (1 ml) was mixed to 1 ml of 2% AlCl<sub>3</sub> methanol solution and incubated for 10 min. The difference in absorbance between test sample and control measured. Quercetin was used as standard.

**Tannins** were quantified by the E.E.C method as described by Sereme et al. (1993). Test sample solution (1 ml) was mixed in a first tube with 6 ml of water and 1 ml of ammoniac solution (8 g/l), and in a second tube 1 ml of sample was added to 5ml of water and 1 ml of ammonium ferric citrate solution (28%, 3.5g/l) prepared 24 h before the experiment. 10 min after the completion of these operations the difference of absorbance between the 2 tubes were measured at 525 nm. Tannic acid was used as standard.

**Anthocyanins** were assayed by the pH differential method as described by Sereme et al. (1993) at pH 0.6 and 3.5 (OD) at 480nm. Using pure apigenidin as the reference, the anthocyanidins content was computed according to the following equation. Appropriate adjustments were made for dilutions.

$$\text{Apigenidin content (}\mu\text{g/ml)} = \text{OD} \times 66.99$$

### Isolation procedure

Crude aqueous acetone extract was partitioned between water and ethyl acetate. Ethyl acetate fraction (1 g) was chromatographed on silica gel using a gradient of hexane, ethyl acetate and methanol. Fractions were submitted to bio-guided TLC to check their antioxidant status; 2% DPPH in methanol was sprayed as reagent. The most antioxidant fractions (122 mg) were subjected to MPLC on RP18 column using a linear gradient of methanol in aqueous ammonium acetate to yield 46.9 mg of mixed compounds. Successive preparative TLC with dichloromethane/methanol 19/1, dichloromethane/methanol 11/1 and dichloromethane/methanol 13/1 to obtain compound A (3.6 mg).

Compound A: EIMS m/z (%): 286 [M]<sup>+</sup> (10.6), D/CIMS m/z (%): 287 [M+H]<sup>+</sup> (55.6), UV<sub>MeOH</sub> nm: 349, 291, 266; AlCl<sub>3</sub> nm: 425, 296 (sh), 274; AlCl<sub>3</sub>+HCl nm: 388, 364, 296 (sh), 274.

Data were compared to literature (Mabry et al., 1970).

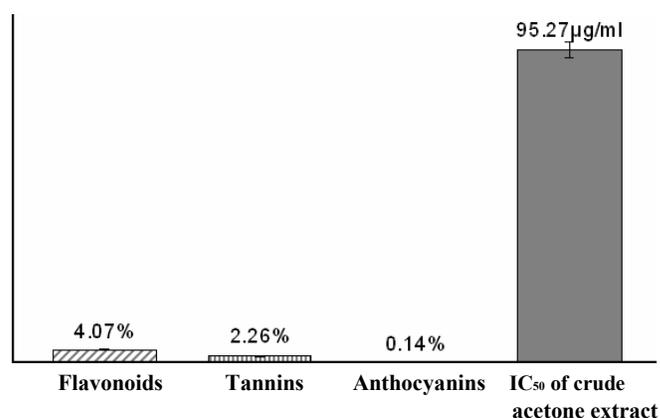
### Structure identification

Spectroscopic data were recorded with the following instruments: CECIL CE 2041 spectrometer (UV/VIS), quadripolar R 210C coupling with IPC (P2A) MSCAN WALLIS computer (MS), BRUKER Avance 400 (400MHz <sup>1</sup>H and 100MHz <sup>13</sup>C) (NMR). Chemical shifts are expressed in ppm (δ) relative to the solvent CD<sub>3</sub>OD as internal standard.

### Toxicity assay

The acute toxicity study was performed as described by Lietchfil and Wilcoxon (1949). Adults NMRI female mice were divided into 18 groups each containing 5 animals. The mice were fasted for 18

h with water *ad libitum*. Crude extract was dissolved in 0.1% Tween 80 aqueous solution. Test solution was administrated intraperitoneally at 6 different doses of 1550, 1650, 1750, 1850, 2150 and 2300 mg/kg body weight, respectively, to different groups of mice. A separate set of control mice were given the vehicle only (0.1% tween 80 aqueous solution). The animals were observed for 24 h and the LD<sub>50</sub> was determined by probit test using percent versus dose's log (Carvalho et al., 1999).



**Figure 1.** Polyphenolics content (flavonoids, tannins and anthocyanins) and IC<sub>50</sub> of the crude extract.

## RESULTS AND DISCUSSION

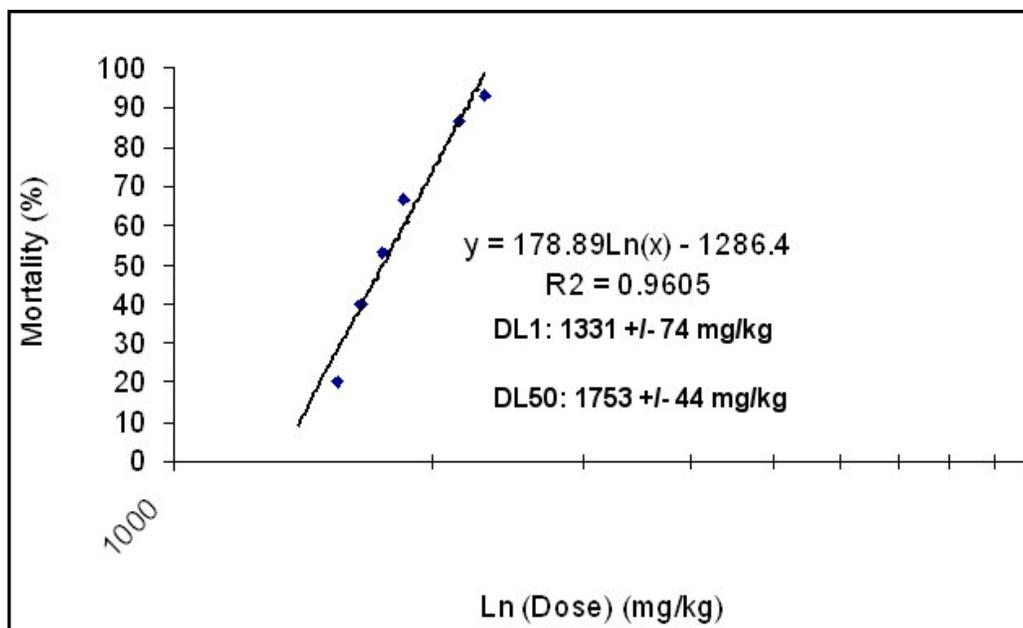
The antioxidant activity was evaluated with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, using a free radical which shows a characteristic absorption at 517 nm (purple) (Velasquez et al., 2003). The purple color rapidly faded when DPPH encountered any radical scavengers. Crude aqueous acetone extract shows a weak DPPH radical scavenging activity with an IC<sub>50</sub> value of 95 µg/ml. Compared to the ethanolic extract of *S. orobanchioides* which exhibits an IC<sub>50</sub> value of 18.65µg/ml (Badami et al., 2003), *S. hermonthica* is a relatively weaker anti-DPPH scavengers source. The crude aqueous acetone extract contained polyphenolics (Figure 1); mainly flavonoids (4%), tannins (2.26%) and anthocyanins (0.14%). These compounds are known for their antioxidant potentialities (Bors et al., 1990; Yokozawa et al., 1998; Einbond et al., 2004).

Radical scavengers were partitioned through liquid/liquid extraction between ethyl acetate and water. Depending of their polarity, flavonoidic aglycones were concentrated in the apolar fraction when tannin and anthocyanins were encountered in the aqueous fraction. Bio-guided fractionation led to the main radical scavenging flavonoid of *S. hermonthica* in the ethyl acetate fraction. This flavonoid was identified as luteolin. Its structure was completely assigned by means of its spectral data (Table 1). Comparison of <sup>13</sup>C, <sup>1</sup>H NMR, UV and MS spectra to the literature confirmed our

**Table 1.** Spectral data of isolated compound compared to luteolin.

Positions	RMN <sup>1</sup> H (δ <sub>H</sub> ppm)		RMN <sup>13</sup> C (δ <sub>C</sub> ppm)	
	Compound A <sup>a</sup>	Luteolin <sup>b</sup>	Compound A <sup>a</sup>	Luteolin <sup>b</sup>
2			164.9	164.4
3	6.48 (s)	6.65	102.4	103.3
4			182.5	182.1
5			161.8	61.91
6	6.14 (d, 2)	6.18	98.7	99.3
7			164.6	164.5
8	6.37 (d, 2)	6.43	93.6	94.3
9			158.0	157.7
10			103.9	104.2
1'			122.3	122.0
2'	7.31 (br s)	7.38	112.7	113.8
3'			145.6	146.2
4'			149.6	150.1
5'	6.84 (d, 8)	6.88	115.4	116.5
6'	7.32 (dd, 2, 8)	7.40	118.9	119.4

<sup>a</sup>CD<sub>3</sub>OD, 400 and 100 MHz, *J*(Hz) are in parenthesis; <sup>b</sup>DMSO-d<sub>6</sub>, 700 MHz.



**Figure 2.** Dose-response curve of acute toxicity determined by probit method.

assignment (Nissler et al., 2004; Mabry et al., 1970; Harborne, 1994). Luteolin is one of the most potent DPPH radical scavengers beyond flavones (Yokozawa et al., 1998). Apigenin and 5-hydroxy-6,8-dimethoxyflavone-7,4'-O-diglucoside, previously isolated from *S. hermonthica* (Khan et al., 1998; Choudhury et al., 2000) could also contribute to its total antioxidant activity together with tannins and anthocyanins. Luteolin has been shown to be absorbed from intestine and to be present in human serum (Shimoi et al., 1998) where it may act as antioxidant by scavenging free radicals as well as inhibiting nitric oxide synthetase (Middleton et al., 2000).

According to the acute toxicity scale of Hodge and Sterner (1943), the crude acetone extract of *Striga hermonthica* which exhibited a LD<sub>50</sub> value of 1753 mg/kg (Figure 2) could be qualified as relatively nontoxic to mice when administrated intraperitoneally.

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