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Full Length Research Paper

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

Martin KIENDREBEOGO[1]*, Marie-Geneviève DIJOUX-FRANCA[2], Charles Euloge LAMIEN[1], Aline MEDA[1], Denis WOUESSIDJEWE[3], Odile Germaine NACOULMA[1]

[1]Laboratoire de Biochimie et de Chimie Appliquées, UFR Sciences de la Vie et de la Terre, Université de Ouagadougou, OUAGADOUGOU, Burkina Faso.
[2]Département de Pharmacochimie Moléculaire (DPM), UMR 5063 CNRS-UJF, Equipe de Pharmacognosie, UFR de Pharmacie, Université Joseph Fourier de Grenoble, LA TRONCHE, France.
[3]Département de Pharmacochimie Moléculaire (DPM), UMR 5063 CNRS-UJF, Equipe de Pharmacotechnie, UFR de Pharmacie, Université Joseph Fourier de Grenoble, MEYLAN, France.

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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_{50} of 95.27±2.30 µg/ml) and an insignificant acute toxicity on mice (LD_{50}: 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_{50} 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 (*Sorgum 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.

*Corresponding author. E-mail: martin.k@univ-ouaga.bf, kmartin71@hotmail.com; Tel:(+226)76.61.01.42.

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 Velaskez 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

(methanol) was taken as the activity. The activity, expressed as IC_{50} value (µg/ml: 50% inhibitory concentration) was consistent with the mean value of 3 measurements. Quercetine (IC_{50} 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 essay 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% $AlCl_3$ 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 essayed by the pH differential method as described by Sereme et al. (1993) at pH 0.6 and 3.5 (ΔOD) at 480nm. Using pure apigenidin as the reference, the anthocyanidins content was computed according to the following equation. Appropriate adjustments were made for dilutions.

Apigenidin content (µg/ml) = ΔOD x 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 $[M]^{+\bullet}$ (10.6). D/CIMS m/z (%): 287 $[M+H]^{+\bullet}$ (55.6). UV $\lambda^{MeOH}{}_{nm}$: 349, 291,266; $\lambda^{AlCl_3}{}_{nm}$: 425, 296 (sh), 274; $\lambda^{AlCl_3+HCl}{}_{nm}$: 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 ^1H and 100MHz ^{13}C) (NMR). Chemical shifts are expressed in ppm (δ) relative to the solvent CD_3OD as internal standard.

Toxicity assay

The acute toxicity study was performed as described by Lietchfiel 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_{50} was determined by probit test using percent versus dose's log (Carvalho et al., 1999).

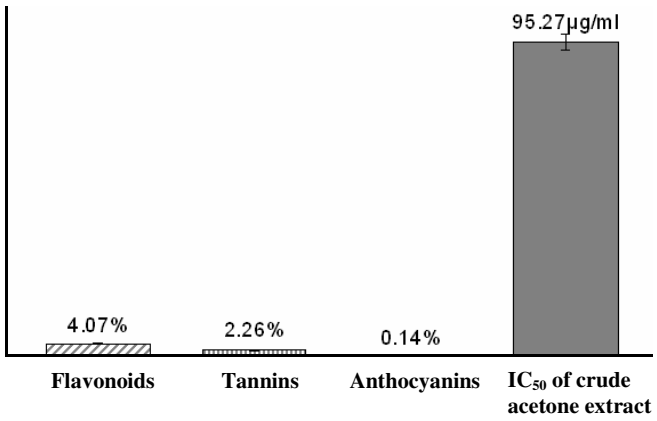

Figure 1. Polyphenolics content (flavonoids, tannins and anthocyanins) and IC_{50} of the crude extract.

RESULTS AND DISCUSSION

The antioxidant activity was evaluated with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) essay, using a free radical which shows a characteristic absorption at 517 nm (purple) (Velaskez 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_{50} value of 95 µg/ml. Compared to the ethanolic extract of *S. orobanchioides* which exhibits an IC_{50} 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 tanin 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 ^{13}C, ^1H NMR, UV and MS spectra to the literature confirmed our

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

Positions	RMN ^{1}H (δ_H ppm)		RMN ^{13}C (δ_C ppm)	
	Compound A[a]	Luteolin[b]	Compound A[a]	Luteolin[b]
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

[a]CD_3OD, 400 and 100 MHz, *J*(Hz) are in parenthesis; [b]DMSO_d_6, 700 MHz.

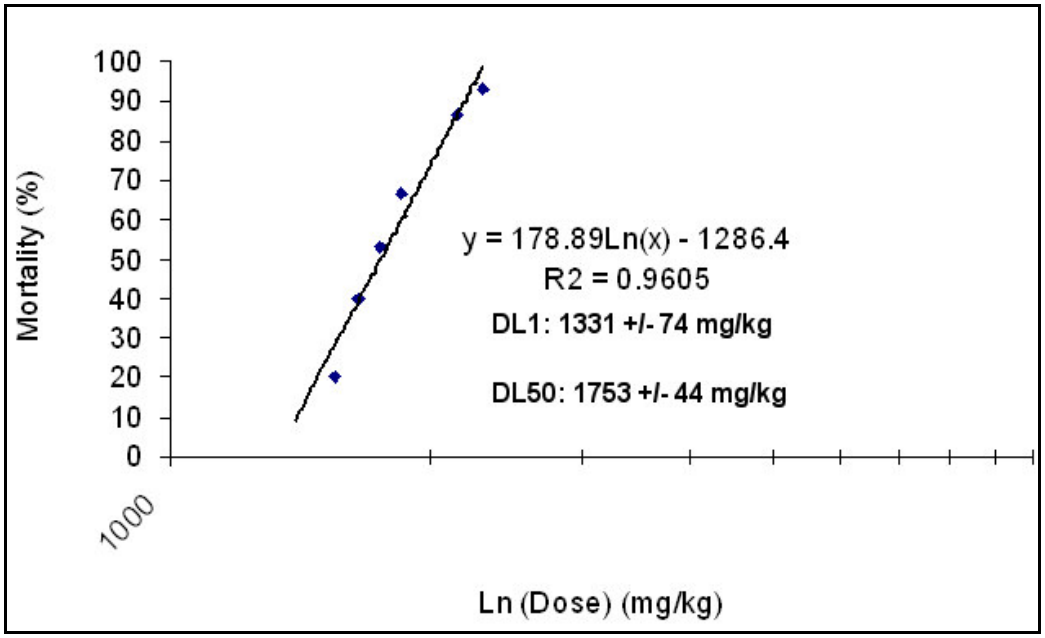

Figure 2. Dose-response curve of acute toxicity determined by probit method.

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 taninns 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_{50} value of 1753 mg/kg (Figure 2) could be qualified as relatively nontoxic to mice when administrated intraperitoneally.

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REFERENCES

Arvouet-Grant A, Vennat B, Pourrat A, Legret P (1994). Standardisation d’un extrait de propolis et identification des principaux constituants. J. Pharm. Belg. 49(6): 462-468.

Badami S, Gupta MK, Surest B (2003). Antioxydant activity of the ethanolic extract of *Striga orobanchioides*. J. Ethnopharmacol. 85(2-3): 227-230.

Bors W, Hester W, Michel C, Saran M (1990). Flavonoids as antioxydants: determination of radical scavenging efficiencies. Methods Enzymol. 186: 343-355.

Carvalho JCT, Sertie JAA, Barbosa MVJ, Patricio KCM, Caputo LRG, Sartio SJ, Ferreira LP, Bastos JK (1999). Antiinflammatory activity of the crude extract from the fruits of *Pterodon emarginatus* Vog. J. Ethnopharmacol. 64: 127-133

Choudhury MK, Marting ZG, Agbaji AS, Nok AJ, Mukhopadyay S (2000). Chemical investigation of the flowers of *Striga senegalensis*. Indian J. Pharm. Sci. 62(5):396-397.

Choudhury MK, Phillips AL, Mustapha A (1998). Pharmalogical studies of *Striga senegalensis (S*crophulariaceae) as an abortifacient. Phytother. Res. 12(2): 141-143

Einbond LS, Reynertson KA, Luo XD, Basile MJ, Kennelly EJ (2004). Anthocyanin antioxidants from edible fruits. Food Chem. 84: 23-28.

Harborne JB (1994) The Flavonoids – Advances in Research since 1986. Chapman and Hall, London. pp. 450-451.

Hodge HC, Sterner JH (1943). Determination of substances acute toxicity by DL_{50}. American Industriel Hygien Association. 10: 93-96.

Hussain HS, Deeni YY (1991). Plants in Kano ethnomedicine; screening for antimicrobial activity and alkaloids. Int. J. Pharmacognosy. 29(1): 51-56

Khan IZ, Aqil M, Kolo B (1998). 5-Hydroxy-6,8-dimethoxyflavone 7,4'-*O*-diglucoside from *Striga hermonthica* (DEL) Benth. Ultra Scientist of Physical Sciences 10(2): 278-280.

Lietchfiel JT, Wilcoxon FA (1949). A simplified method of evaluationg dose effect experiments. J. Pharmacol. Exp. Ther. 95: 99-113.

Marbry TJ, Markham KR, Thomas MB (1970). The systematic Identification of Flavonoids. Springer-Verlag. Berlin. p. 354.

Middleton E, Kandaswami C, Theoharides TC (2000). The effect of plant flavonoids on mammalian cells: Implications for inflammation, heart disease and cancer. Pharmacol. Rev. 52: 673-751.

Mohamed KI (1994). Biosystematics and diversification in the genus *Striga* Lour. (Scrophulariaceae) in Africa. Ph.D thesis, Old Dominion University, Norfolk. p. 170.

Musselman LJ, Bharathalasksmi SB, Knepper DA, Mohamed KI, White CI (1991) Recent research on the biology of *Striga asiatica*, *S. gesnerioides* and *S. hermonthica*. In: S.K. Kim, ed. Combating *Striga* in Africa. Proceedings, International Workshop, Ibadan, Nigeria, 1988. IITA, Ibadan. pp. 31-41.

Nacoulma OG (1996). Plantes médicinales et pratiques médicales traditionnelles au Burkina-Faso: cas du plateau central. Thèse d’état Université de Ouagadougou, Ouagadougou, Burkina-Faso. p. 261.

Nissler L, Gebhardt R, Berger S (2004). Flavonoid binding to a multi-drug-resistance transporter protein: an STD-NMR study. Anal. Bioanal. Chem. 379: 1045–1049.

Okpako LC, Ajaiyeoba EO (2004). In vitro and in vivo antimalarial studies of *Striga hermonthica* and *Tapinanthus sessilifolius* extracts. Afr. J. Med. Med. Sci. 33(1): 73-75.

Sereme A, Kouda-Bonafos M, Nacro M (1993). Phenolic compounds in *Sorghum caudatum* tissues during plant development. Biomass and Bioenergy 4(1): 69-71.

Shimoi K, Okada H, Furugori M, Goda T, Takase S, Suziki M, Hara Y, Yamamoto H, Kinae N (2003). FEBS Lett. 438: 220-224.

Velaskez E, Tournier HA, Mordujovichole BP, Saavedra G, Scinella GR (2003) antioxidant activity Paraguyan plant extracts. Fitoterapia 74: 91-97.

Yokozawa T, Chen CP, Dong E, Tanaka T, Nonaka GI; Nishioka I (1998) Study on the inhibitory effect of tannins and flavonoids against the 1,1 diphenyl-2-picrylhydrazyl radical. Biochem. Pharmacol. 56: 213-222.