

IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF *CLINACANTHUS NUTANS* 80% METHANOL LEAF EXTRACT

I. M Hassan*, B Sa'idu*, J. A Ishaq*, A Dahiru*, N Abdulazeez*, A. A Abubakar**, N. N Pilau*** and ****B. Ibrahim

*Department of Veterinary Physiology and Biochemistry Usmanu Danfodiyo University Sokoto

**Department of Veterinary Surgery and Radiology Usmanu Danfodiyo University Sokoto

***Department of Veterinary Medicine Usmanu Danfodiyo University Sokoto

****Department of Veterinary Physiology and Biochemistry University of Maiduguri

ABSTRACT

The objective of this study was to evaluate the antioxidant effects of *Clinacanthus nutans* leaf. The plant leaf was extracted with 80% methanol and screened for antioxidant effects using free radical scavenging assay (DPPH) and Ferric reducing ability of plasma (FRAP). Results show high activity with significant difference at $p < 0.05$ in FRAP assay. Inhibitory concentration (IC_{50}) of 54.810 ± 0.22 $\mu\text{g/mL}$ with DPPH and 301.189 ± 0.69 $\mu\text{g/mL}$ with FRAP were also calculated. High antioxidant activities of this crude extract may be due to the presence of large quantities of bioactive compounds that may be of therapeutic benefit. There is need for *in vivo* antioxidant study of this plant in mammals to reaffirm its antioxidant effect. Toxicity study to evaluate effects in various body tissues and organs are also recommended. Phytochemical study to identify and or isolate the bioactive compounds with medicinal benefit is strongly recommended.

Key word: antioxidants, DPPH, FRAP, free-radicals, oxidative-stress

INTRODUCTION

Clinacanthus nutans Lindau (Cn) commonly referred to as “Sabah Snake Grass” is widely used as a Thailand traditional medicine, and considered as one of the most valuable plants for primary healthcare by the ministry of health Thailand (Chithra *et al.*, 2016). Several studies reported the traditional use of *C. nutans* in managing various forms of inflammatory conditions such as insect bites, allergic responses, and viral diseases (Kunsorn *et al.*, 2013). Extracts from a different part of *C. nutans* are used as remedies against hepatitis and herpes virus (Ueda *et al.*, 2013). Research has shown that the chlorophyll chloroform extracts alone contain bio-active constituents such as 132-hydroxy-(132-R)-phaeophytin b, 132-hydroxy-(132-S)-phaeophytin a, and 132-hydroxy-(132-R)-phaeophytin which have activities against herpes virus (Azmir *et al.*, 2014). A previous study on phytochemical and chemical investigation of the bio-active compounds reveal the presence and isolation of stigmasterol, lupeol, β -sitosterol, belutin, six known C-glycosyl flavones, vitexin, isovitexin, shaftoside, isomollupentin-7-O- β -glucopyranoside, orientin, isoorientin, five sulphur-containing glycosides, two glycolipids, a mixture of nine cerebrosides and a monoacylmonogalatosylglycerol (Sekar, 2015). With the health benefits of this plant, there is dearth of online literature on its antioxidant effect.

MATERIALS AND METHODS

Clinacanthus nutans (leaves) were collected at University Agriculture Park, Universiti Putra Malaysia. A plant sample was identified at Institute of Bioscience (IBS) by botanists and voucher number of SK2893/15 was allocated to it.

Extraction of leaf was carried out at biotechnology laboratory as reported by Renshaw *et al.* (2015). A total of 100g of semi powdered leaf was soaked in 1000 mL of 80% methanol in 2.5 litres flat bottomed flask and allowed to stand with ceaseless daily shaking for 3 days at 25°C (Wu *et al.*, 2015). Extracts were filtered with What-man filter paper and concentrated with a rotary evaporator at 42°C to semi-solid form. Crude extract was then weighed, transferred to sample bottles and stored at 4°C (Ojinnaka *et al.*, 2016).

Doses were prepared by dissolving (100 mg) of crude extract in 1 mL of 100% dimethyl sulfoxide (DMSO) to obtain a stock solution (100 mg/mL). DMSO was used to solubilize the crude extract, further dilution (sub-stocks solution) was carried out by adding distilled water using this formula $M_1V_1=M_2V_2$ in microlitre ($\mu\text{g/mL}$). A two-fold serial dilution was used to prepare the concentration of interest (7.81-1000 $\mu\text{g/mL}$) in 96-well microplate (Sigma Aldrich, USA).

Evaluation of free radical scavenging activity of crude extracts was carried out with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) according to Pavithra and Vadivukkarasi (2015) with some modifications. DPPH 0.0238 g was dissolved in methanol and make up to a final volume of 100 mL (stock solution 0.6 mM). The solution was stored in an amber bottle to protect from the light for 30 minutes. A 10 mL DPPH stock solution was diluted to 90 mL (1/10 dilution) with methanol to make the working solution. Concentrations of Plant samples were also prepared at 7.81-1000 $\mu\text{g/mL}$ in 96 well plates using two-fold serial dilutions. DPPH reagent was added into the sample dilutions in the dark. The mixture was incubated for 30 minutes at room temperature. Absorbance was measured at 517 nm using UV-VIS spectrophotometer (Sigma Aldrich, USA). Trolox and Quercetin were used as standard (REFERENCES).

Evaluation of antioxidant power of crude extract was carried out with a prepared Ferric Reducing Antioxidant Power assay (FRAP). Crude extracts were dissolved in 0.1% DMSO and 100% methanol was added to obtain 1 mg/mL stock solution. Working solution was constituted by adding 300 mM acetate buffer (pH 3.6) to 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ), 40 mM hydrochloric acid and 20 mM ferric chloride at 10:1:1 (v/v/v). Prepared FRAP reagent was then added to crude extract at the concentration of 7.81-1000 $\mu\text{g/mL}$ in 96-well plates. The mixtures were diluted vigorously and incubated at 37°C for 5 minutes (Machán *et al.*, 2016). Measurement of absorbance was carried out using UV-VIS spectrophotometer at 593 nm at the end of the experiment, Trolox and Quercetin were used as standard (REFERENCES).

Statistical analysis

The experiments were repeated three times independently. Data were expressed as mean $\pm$ standard error of mean (mean $\pm$ S.E.M). The IC_{50} was calculated and analyzed using Graph Pad. Antioxidant activities of crude extract using DPPH and FRAP assays were calculated and analyzed using one-way ANOVA followed by Dunnett's post hoc test, where $P < 0.05$ was considered statistically significant between different concentrations and the standard using Graph Pad Prism version 5.0 software (Graph Pad Software, USA).

RESULTS AND DISCUSSION

The result of percentage yields of the plant extract is 30.12 %.

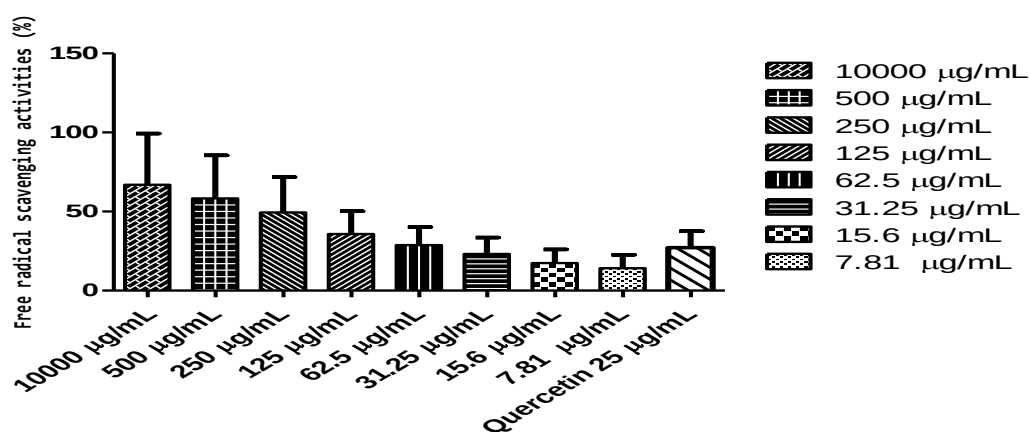


Figure 1: Evaluation of antioxidant effect of the crude extract at different concentrations (7.81-100 µg/mL using DPPH assay. High activity was recorded by comparing all concentrations with quercetin (REFERENCE) (25 µg/mL). The percentage free radical scavenging activity was shown versus the concentration of the crude extract. The values represent mean $\pm$ SEM from three independent experiments. There is no significant difference among the standard and all the crude extract concentrations.

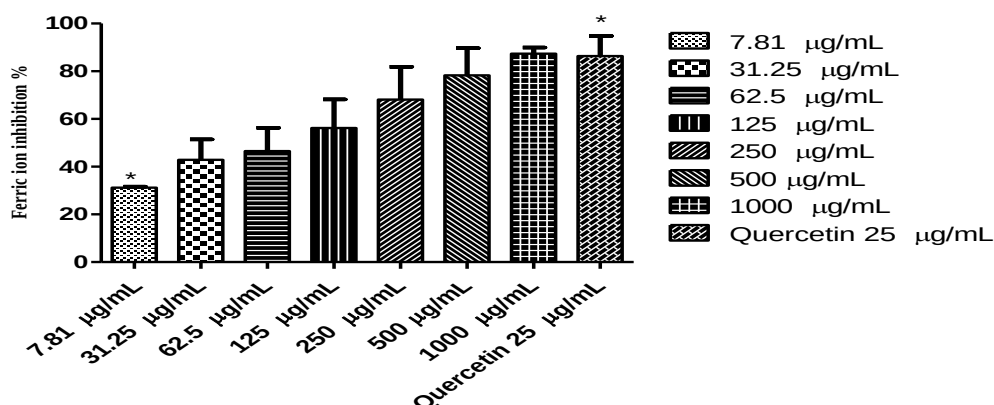


Figure 2: Evaluation of antioxidant effect of the crude extract at different concentrations (7.81-100 µg/mL using FRAP assay. High activity was recorded by comparing all concentrations with quercetin (REFERENCE) (25 µg/mL). The percentage ferric ion inhibition power was shown versus the concentration of the crude extract. The values represent mean $\pm$ SEM from three independent experiments. * $P < 0.05$ represented significant difference between 7.81 µg/mL of the crude extract and 25 µg/mL of quercetin.

It has been reported that the quantity of yield produced by a particular plant sample is proportional to the availability of bioactive compound present, but climate, temperature, drying/storage environment and extraction method may affect the yield in all plant (Fariás *et al.*, 2015). Some reports show high yield and phytochemical constituent in leaf followed by stem and then rhizome of *Zingiber officinale* (Ghasemzadeh *et al.*, 2015).

Several literature also show that, flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins with multiple effects including antioxidant activity have been identified in this plant extract (Skrovankova *et al.*, 2015). Increased antioxidant activities of plant samples were reported to be associated with the qualities and quantities of bioactive compound present in the crude extracts (Casuga *et al.*, 2016). Previous studies on the role of dietary supplements with antioxidant disease prevention and health promotion recorded positive result with this plant (Bjelakovic *et al.*, 2015).

CONCLUSION AND RECOMMENDATION

The crude extract shows high antioxidant activity with DPPH and FRAP assay. Further research using *in vivo* model is needed to reaffirm its antioxidant effect. Phytochemical studies and isolation of bioactive compounds from crude extract is strongly recommended. This study also revealed the high benefit of this plant as natural medicine/supplement.

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