Journal of Biochemistry & Molecular Medicine
JBMM, 1(1): 12-17
www.scitcentral.com

Original Research Article: Open Access

Antioxidant and Toxicological Effects of Crude Extracts from *Calotropisprocera* Leaves

Eric Banan-Mwine Daliri[1*], Vincent Appiah[2*] and Alexander Weremfo[3]

[*1]*Department of Food Science and Biotechnology, Kangwon National University, Chuncheon, South Korea*

[2]*West African Centre for Cell Biology of Infectious Pathogens, University of Ghana, Ghana*

[3]*Department of Biochemistry, University of Cape Coast, Cape Coast, Ghana*

Received December 5, 2017; Accepted December 20, 2017; Published March 8, 2018

ABSTRACT

Many plants and plant parts have been used in traditional medicine for centuries, yet their possible toxic effects associated with their use have not been studied. In this study, we tested the *in vitro* antioxidant ability of *Calotropisprocera* leaf extracts by β-carotene bleaching assay, ferric reducing assay andhydrogen peroxide scavenging assay. Methanolic extracts of the leaf showed the highest potency. The toxicity of the methanolic extract was tested in albino rats. All the concentrations administered to the rats showed acute and subacute toxicity manifested by hair and weight loss, sluggishness and significant elevation in serum activities of glutamate oxaloacete transaminase, glutamate pyruvate transaminase, alkaline phosphatase, alanin aminotransferase and serum bilirubin level relative to a control group. These results indicate that, though the crude extracts contain antioxidant factors, they also contain plant toxins that may be injurious when consumed.

INTRODUCTION

Reactive oxygen species (ROS) are considered as toxic molecules and have been associated with pathological conditions including cancer, aging and neurodegenerative disorders [1]. During cellular metabolism, molecular oxygen (O_2) is reduced to superoxide which triggers the generation of other reactive species. Since ROS are highly reactive and unstable, they can easily interfere with other cellular process resulting in protein and DNA destruction, fatty acid oxidation and synaptic plasticity [2]. Though the body naturally reduces ROS, the toxic effects occur when the destructive effects of ROS surpass the ability of the body to neutralize the oxidizing species and repair cellular damage [3].

Plants are important sources of bioactive compounds and have been used in traditional medicine for centuries. The health effects of plant products result from the presence of components such as alkaloids, cardiac glycosides, tannins, sterols, triterpenes, anthocyanins [4], flavonoids [5], and other phenolic compounds [6]. Phenolic compounds have potent antioxidant potency and have been shown to protect against coronary heart disease and cancer. Due to the toxic effects of synthetic antioxidants such as *butylhydroxyanisole* and butylatedhydroxytoluene [7], the search and use of natural antioxidants from plant sources have increased.

Calotropisprocera Linn., a wild growing plant of the family Asclepiadaecae, is known to possess diverse medicinal properties. Crude extracts of different parts of this plant have been used in traditional medicine and has been shown to treat ulcer, diabetes [8], inflammation [9] and diseases of the spleen and liver [10]. However, treating disease with crude plant extracts may not be completely safe. Some extracts, despite their positive effects, may also cause severe side effects such as liver damage. To the best of our knowledge, no study has been done to ascertain the hepatotoxicity of *Calotropis procera* leaf extracts after their antioxidant properties have been determined. Therefore, in this study, we investigated the antioxidant activity of various extracts of *Calotropisprocera* leaves *in vitro*. We then studied the acute toxicity and hepatotoxic effects of the extracts in albino rats.

Corresponding Author: Eric Banan-MwineDaliri, Department of Food Science and Biotechnology, Kangwon National University, Chuncheon, South Korea. Email: ericdaliri@yahoo.com

Citation: Daliri E, Appiah V and Weremfo A. (2018) Antioxidant and Toxicological Effects of Crude Extracts from Calotropisprocera Leaves. J Biochem Mol Med, 1(1): 12-17.

MATERIALS AND METHODS

Leaves

Leaves of *Calotropisprocera* were collected from the University of Cape Coast-Ghana, School of Biological Sciences botanical garden and authenticated by the herbarium. The leaves were washed and air-dried for one week.

Preparation of extracts

The dried leaves were ground into powder (177g) and divided into three equal parts. One part (59.14g) was extracted with 250 mL of 70% methanol (Zayo-Sigma Chemicals Ltd.-Nigeria) using a Soxhlet extractor for 48 hours. The other parts of the powdered samples were extracted with either distilled water or ethyl acetate. The extracts were heated at 60 ºC to expel the solvents and stored at 4°C till use.

In vitro Antioxidant Activity

β-carotene bleaching assay

The β-carotene bleaching inhibitory assay was carried out according to a method reported earlier [11]. Briefly, β-carotene solution was prepared by dissolving 2 mg of β-carotene (Selleck Chemicals- South Krea) in 10 mL chloroform (Zayo-Sigma Chemicals Ltd.-Nigeria) and 1.0 mL of this solution was then pipetted out into a flask containing 20 mg of linoleic acid (Zayo-Sigma Chemicals Ltd.-Nigeria) and 200 mg of Tween 40 emulsifier (Zayo-Sigma Chemicals Ltd.-Nigeria). One mL of the solution was then pipetted into a flask and chloroform was completely evaporated using a water bath at 40 ^{0}C. Fifty mL of distilled water was then added. Aliquots of 5 mL of this emulsion were transferred into a series of tubes containing various concentrations of the extracts (50-250 µg/mL) or α tocopherol (control). The absorbance of the fractions and the control were measured immediately (t=0). The tubes were incubated at 50°C in a water bath and the absorbance was measured at 20 min interval at 470 nm till 120 min (t=120) using a spectrophotometer (Bio-Rad SmartSpec 3000 UV/Vis).

Ferric reducing ability

The reducing ability of the leaf extracts were determined by the potassium ferricyanide-ferricchloride method reported earlier [12]. One mL of the different dilutions of extracts (50-250 µg/mL) was added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide (The Science Company, Lakewood). The mixtures were incubated at 50 °C for 20 min, after which 2.5 mL trichloroacetic acid (10%) was added. An aliquot of the mixture (2.5 mL) was taken and mixed with 2.5 mL water and 0.5 mL 1% $FeCl_3$ (The Science Company, Lakewood). The absorbance at 700 nm was measured after allowing the solution to stand for 30 min.

Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentrations of the leaf extracts (50-250 µg/mL) in distilled water were added to 0.6 mL of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the fractions was determined and compared with the standard, α-tocopherol [13].

Toxicological Studies

Animals

Albino rats (180–220 g) of either sex were obtained from the animal house, School of Biological Sciences, University of Cape Coast, Ghana. The rats were housed in proper cages with adequate lighting and ventilation at an ambient temperature of 28-30ºC. The rodents were fed with standard rodent diet and with water ad libitum. Approval from the University of Cape Coast animal ethical committee for the usage of animals in the experiments was obtained.

Acute toxicity

The rats were randomly divided into three groups each containing 5 rats. Doses of extract (0.5, 1 and 2g/kg body weight) were given orally, a dose to each group, and the effects observed. The overall behaviour exhibited by the animals was recorded using a checklist, which included urinary frequency, defecation, changes in locomotory activity, convulsion, lacrimation, and salivation, within the first 6 hours after extract administration. The animals were observed for 48 hours during which time the number of death were recorded.

Subacute toxicity

Rats of either sex weighing between 180 and 220g were randomised into four groups each containing 5 rats. The first, second and third groups were orally given 0.667, 0.33, and 0.167g/kg body weight respectively of the plant extract daily for 28 consecutive days while rats in the fourth group (control) were given 1mL of distilled water daily for 28 consecutive days. After 28 days, blood samples were collected by cardiac puncture and then centrifuged at 2000g for 10 min to separate the serum for the various biochemical analyses. Biochemical parameters (serum glutamate oxaloacete transaminase (AST), serum glutamate pyruvate transaminase (GGT), serum alkaline phosphatase (ALP), alanine aninitransferase (ALT) and serum bilirubin were determined at 29th day after completion of treatment.

Biochemical analysis

The collected blood samples were used for the analysis of biochemical markers AST [14], ALT [15], SGGT [16], ALP [17], biluribin [10] levels.

Statistical analysis

The results were expressed as means ± S.E.M, and analyzed for statistical significance using Student's test. P values< 0.05 were considered significant

RESULTS AND DISCUSSION

Antioxidant assay

It is difficult to measure the many different antioxidants that may be present in plants and hence several techniques have been established to assess the antioxidant activates of different substances. Among them, β-carotene bleaching assay, ferric reducing ability and hydrogen peroxide scavenging assay are commonly used [18]. We therefore chose these methods to assess the antioxidant activities of *C. procera* leaf extracts. In this study, we determined the ability of *C. procera* leaf extracts to prevent β-carotene bleaching (**Figure 1**). The methanolic fraction of *C. procera* showed comparable inhibition of α-tocopherol on β-carotene bleaching with concentrations ranging from 50 to 250 µg/mL. The water and ethyl acetate fractions also inhibited β-carotene bleaching but to a lesser extent. Methanol is a polar organic solvent and might have extracted both polar and organic antioxidant molecules in the leaves thereby expressing the highest activity.

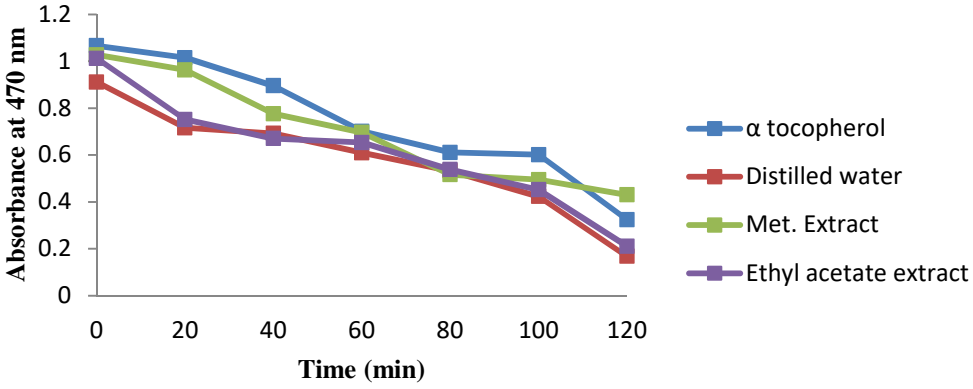

Figure 1. β-Carotene bleaching assay of each extract of *Calotropisprocera* leaves at 470 nm. Addition of the fractions of *C. procera* reduced the discolouration of β-carotene thereby preventing its bleaching.

The transformation of Fe^{3+} into Fe^{2+} in the presence of various fractions was measured to determine the reducing power ability. The reducing ability of a compound generally depends on the presence of reductones (antioxidants), which exert the antioxidant activity by breaking the free radical chain by donating a hydrogen atom [19]. In this method, antioxidant compounds form a colored complex with potassium ferricyanide, trichloroacetic acid and ferric chloride that was measured at 700 nm. Increase in absorbance of the reaction mixture indicates the increase in the reducing power of the sample. There was a concentration-dependent increase in the absorbance of reaction mixture for all the extracts and standard (**Figure 2**). Methanolic fraction showed the highest absorbance and hence the highest reducing power among the fractions. The reducing activities of the other fractions were in the order α-tocopherol> methanol > ethyl acetate >water fractions. Our results are in agreement with that reported by Tadhani et al. [18] which found that methanolic plant extracts had stronger antioxidant activities than other extracts.

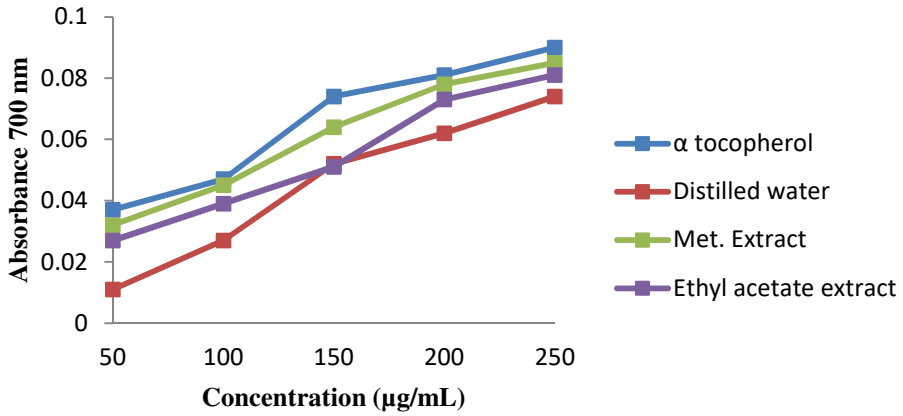

Figure 2. Ferric reducing ability of α-tocopherol and different concentrations of extracts of *Calotropisprocera* leaves.

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radicals which is very toxic to cells [20]. Thus, scavenging of H_2O_2 is a measure of the antioxidant activity of the fractions. The methanolic extract had a lower absorbance value among the extracts and hence maximum antioxidant activity. The antioxidant activity of other extracts was in the order α-tocopherol> methanol > water > ethyl acetate extracts (**Figure 3**). All the fractions of *C. procera* scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it to form water.

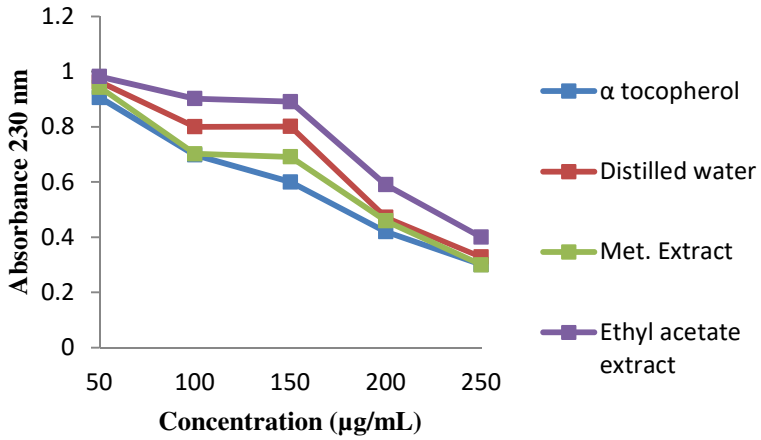

Figure 3. Hydrogen peroxide scavenging activity of α-tocopherol and different concentrations of extracts of *Calotropisprocera* leaves. All fractions of *C. procera* scavenged hydrogen peroxide in a concentration-dependent manner.

Acute toxicity

Since the methanolic extract showed the maximum antioxidant activities, we tested its toxicity.

In the first 24 h, no mortality was observed in rats treated with 0.5 and 1g/kg body weight of the extract. Rats that received higher doses (2g/kg) became sluggish and did not respond to external stimuli. No changes were however observed in urinary frequency, salivation, and diarrhoea. The loss of external stimuli and sluggish movement may be due to the presence of alkaloids [21] which might have affected the nervous systems of the rodents [22]. However the LD_{50} was not determined in this study.

Subacute toxicity

The experimental group of rats was examined for weakness, weight loss and loss of hair on the 29th day. Rats that received 667 mg/Kg body weight of methanolic extracts were found to be weak, lost weight and also lost their hair. These observations might have been caused by the presence of plant toxins in the crude methanolic extracts. Results from liver functional tests showed that all extracts significantly ($P>0.05$) increased the levels of serum ALP, ALT, AST, GGT, and bilirubin relative to the control **(Table 1).**

An increase in these biochemical parameters gives information on whether the disorder is hepatitic or cholestatic in origin [23]. In this study, the raised levels of ALP, GGT as well as bilirubin could indicate biliary obstruction. Also, in such situations, ALP is usually higher than ALT during biliary obstruction as seen in this study. Although serum ALP cannot be used to assess acute liver damage or even cirrhosis, it is an excellent indicator of space-occupying lesion in liver primarily because of destruction of biliary canaliculi within the liver. When the liver is damaged, it is unable to conjugate and excrete bilirubin and therefore the increase in serum bilirubin can also be attributed to the damage of the liver. Damage to the structural integrity of the liver is also indicated by increase in the level of serum aminotransferases (ALT and AST) and GGT as these are cytoplamic in location and are released into circulation after cellular damage [24].

CONCLUSION

From this study, we have shown that methanolic extracts of *C. procera* leaf have strong antioxidant activity, reducing power ability and H_2O_2 scavenging activity. As the various fractions of *C. procera* exhibited different reactive oxygen species scavenging activities, there may be different percentages of phytochemical constituents present in the fractions.

Although the LD_{50} of the extract could not be determined, the toxic symptoms and the high serum levels of the biochemical parameters measured indicate that the extracts were toxic. Further studies to isolate and purify the antioxidant components in the extracts are warranted.

Table 1. Effect of different concentrations of extract on serum biochemical parameters in rats after 28 days of oral administration.

	EXPERIMENTAL GROUP			
Parameters	**Control**	**667 mg/Kg**	**333 mg/kg**	**167 mg/kg**
AST (U/L)	23.07 ± 1.73	53.13 ± 0.03*	50.53 ± 0.07*	45.90 ± 1.90*
ALT (U/L)	26.77 ± 0.71	62.27 ± 0.07*	55.13 ± 1.58*	47.67 ± 0.78*
ALP (U/L)	61.73 ± 1.91	77.13 ± 0.03*	73.73 ± 0.88*	66.65 ± 0.85
GGT (U/L)	46.27 ± 1.99	68.93 ± 0.37*	66.60 ± 0.51*	57.70 ± 2.76*
Bilirubin (mg/dL)	0.43 ± 0.09	1.73 ± 0.03*	1.37 ±0 .07	1.00 ± 0.12

Values represent mean ± S.E.M. (n = 4). Values in rows with (*) are significantly different (P<0.05) when compared with control.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Beckhauser TF, Francis-Oliveira J, De Pasquale R (2016) Reactive oxygen species: physiological and physiopathological effects on synaptic plasticity. J Exp Neurosci 10: 23.
2. Radak Z, Marton O, Nagy E, Koltai E, Goto S: The complex role of physical exercise and reactive oxygen species on brain. J Sports Health Sci 2: 87-93.
3. Ali S, Ahmed M, Sakandar S, Khan S (2017) Antioxidants and free radicals in human diseases: current status and future prospectives. Eur J Pharm Med Res 4: 171-180
4. Cortez R, Luna-Vital DA, Margulis D, Mejia E (2017) Natural Pigments: Stabilization Methods of Anthocyanins for Food Applications. Compr Rev Food Sci Food Safety 16:180-198.
5. Salvamani S, Gunasekaran B, Shaharuddin NA, Ahmad SA, Shukor MY (2014) Antiartherosclerotic effects of plant flavonoids. BioMed Res Int
6. Działo M, Mierziak J, Korzun U, Preisner M, Szopa J, Kulma A (2016) The potential of plant phenolics in prevention and therapy of skin disorders. Int J Mol Sci 17: 160.
7. Nieva-Echevarría B, Manzanos MJ, Goicoechea E, Guillén MD (2015) 2, 6-Di-tert-butyl-hydroxytoluene and its metabolites in foods. Compr Rev Food Sci Food Safety 14: 67-80.
8. Rahmatullah M, Sultan S, Toma T, Lucky S, Chowdhury M, et al. (2010) Effect of Cuscutareflexa stem and Calotropisprocera leaf extracts on glucose tolerance in glucose-induced hyperglycemic rats and mice. African Journal of Traditional, Complementary and Alternative Medicines 7: 109-112.
9. Kumar V, Basu N (1994) Anti-inflammatory activity of the latex of Calotropisprocera. Journal of Ethnopharmacology 44:123-125.
10. Setty SR, Quereshi AA, Swamy AV, Patil T, Prakash T, Prabhu K, Gouda AV (2007) Hepatoprotective activity of Calotropisprocera flowers against paracetamol-induced hepatic injury in rats. Fitoterapia 78: 451-454.
11. Jayaprakasha GK, Jena BS, Negi PS, Sakariah KK (2002) Evaluation of antioxidant activities and antimutagenicity of turmeric oil: a byproduct from curcumin production. Zeitschriftfür Naturforschung C 57: 828-835.
12. Yoon NY, Xie C, Shim K-B, Kim Y-K, Lee DS, Yoon HD (2011) Antioxidant and Cholinesterase Inhibitory Activities of Antarctic Krill Eupausiasuperba. Fisheries Aquat Sci 14: 289-293.
13. Tchimene MK, Nwaehujor CO, Ezenwali M, Okoli CC, Iwu MM (2016) Free radical scavenging activity of lupeol isolated from the methanol leaf extract of CratevaadansoniiOliv.(Capparidaceae). Int J Pharmacogn Phytochem Res 8: 419-426.
14. Bailey WC, DeRouen TA, Ziskind MM, Greenberg HB (1975) Autoanalytic (colormetric) determinations of

SGOT in isoniazid recipients are reliable. American Review of Respiratory Disease 111: 237-238.

15. Matsuzawa T, Katunuma N (1966) Colorimetric assays for serum alanine transaminase and lactic dehydrogenase using diazonium zinc salt. Anal Biochem 17:143-153.
16. Pham NM, Zhenjie W, Morita M, Ohnaka K, Adachi M, et al. (2011) Combined effects of coffee consumption and serum γ-glutamyltransferase on serum C-reactive protein in middle-aged and elderly Japanese men and women. Clin Chem Lab Med 49: 1661-1667.
17. McComb RB, Bowers GN (1972) Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum. Clin Chem 18: 97-104.
18. Tadhani M, Patel V, Subhash R (2007) In vitro antioxidant activities of Stevia rebaudiana leaves and callus. J Food Compost Anal 20: 323-329.
19. Kumar RS, Rajkapoor B, Perumal P (2012) Antioxidant activities of IndigoferacassioidesRottl. Ex. DC. using various in vitro assay models. Asian Pac J Trop Biomed 2: 256-261.
20. Phaniendra A, Jestadi DB, Periyasamy L (2015) Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 30: 11-26.
21. Doshi H, Satodiya H, Thakur MC, Parabia F, Khan A (2011) Phytochemical screening and biological activity of Calotropisprocera (Ait). R. Br.(Asclepiadaceae) against selected bacteria and Anopheles stephansi Larvae. Int J Plant Res 1: 29-33.
22. Yagiela JA, Dowd FJ, Johnson B, Mariotti A, Neidle EA (2010) Pharmacology and therapeutics for dentistry-E-Book. (6th edn), Elsevier.
23. Hall P, Cash J (2012) What is the real function of the liver 'function'tests? Ulster Med J 81:30.
24. Giannini EG, Testa R, Savarino V (2005) Liver enzyme alteration: a guide for clinicians. Can Med Assoc J 172: 367-379.