

Effect of Pretreatment with Aqueous Leaves Extract of *Vitex doniana* on Cadmium-Induced Toxicity to Rats

ABSTRACT

Aim: To determine the effect of pretreatment with aqueous leaves extract of *Vitex doniana* on the cytotoxic response of the antioxidant defense systems in the liver and kidneys of rats exposed to mild dose of cadmium.

Materials and methods: Sixteen Male wistar strain rats (180-220 g b.wt) were divided into four groups: Group A served as the control and rats were exposed to and maintained on normal rat diet and tap water throughout the period of study. Group B were maintained on normal rat diet and then exposed to a single daily oral dose of cadmium (3 mg CdCl₂/kg) in drinking water for five days. Groups C were pretreated with 200 mg/kg *Vitex doniana* leaves extract for fifteen days and thereafter received a single daily oral dose of cadmium (3 mg CdCl₂/kg) in drinking water for five days. Group D were exposed to only 200 mg/kg *Vitex doniana* leaves extract for fifteen days. At the end of the experiment membrane lipid peroxidation, glutathione contents and activities of antioxidant enzymes catalase, superoxide dismutase, and cadmium content were determined in the liver and kidney samples

Results: The results showed that pretreatment with aqueous leaves extract of *Vitex doniana* effectively countered Cd-induced membrane lipid peroxidation, depletion of the non-enzymic antioxidants, glutathione, and induction of the antioxidant enzymes catalase and superoxide dismutase in the liver and kidney as well as effectively reduced cadmium accumulation in the liver and kidney and cadmium-induced liver tissue cell injury. The protective effect of aqueous leaves extract of *Vitex doniana* against Cd-induced lipid peroxidation and tissue glutathione depletion was more pronounced in the kidney than in the liver.

Conclusion: The aqueous leaves extract of *Vitex doniana* significantly reversed Cd induced deleterious alterations in the liver and kidney tissue of the rats. The active antioxidant principle was not determined but is presumed to be polyphenols.

Keywords: Cadmium, *Vitex doniana*, polyphenols, pretreatment, endogenous antioxidants, lipid peroxidation.

1. INTRODUCTION

Cadmium, a toxic heavy metal, a common industrial and environmental pollutant, released into the environment from natural and anthropogenic sources has been shown to manifest its toxicity in humans and animals mainly by virtue of its bioaccumulation in target tissues, stimulation of the generation of free radicals and interference with the utilization of essential metals, all of which culminate in oxidative stress [1, 2, 3, 4, 5]. Oxidative stress is a condition associated with an increased rate of cellular damage induced by an imbalance between reactive oxygen species (ROS)

44 formation and scavenging by antioxidants [6, 7]. It has been demonstrated that Cd induces oxidative
45 stress by stimulating the production of free radicals such as hydroxyl radicals [5], superoxide anions,
46 nitric oxide and hydrogen peroxide [8], resulting in membrane lipid peroxidation and oxidative
47 deterioration of proteins and DNA and in the process initiating various pathological conditions in
48 humans and animals [8].

49 Humans become exposed to Cd pollutants through the food chain and the air. Following oral
50 exposure, Cd is absorbed and delivered to the liver by endogenous intestinal protein metallothionein
51 and from the liver it is rapidly redistributed to other organs with the kidney as the main target organ for
52 Cd toxicity [9]. Public health interest in the toxic effects of environmental Cd was awakened by
53 epidemiological evidence linking industrial Cd waste pollution of marine food sources to the outbreak
54 of *itai itai disease* in Japan, a disease characterized by, among others, severe bone disorders and
55 renal tubular lesions [10]. Studies with experimental animals have shown that exposure to Cd results
56 in toxic lesions in many species, with Cd showing various mechanisms of toxicity in particular species
57 under different experimental conditions [8]. Severity of Cd intoxication of target organs is dependent
58 on the route, dose, and duration of exposure and it manifests in various forms ranging from acute
59 toxicosis to cancer. For this reason, it has been postulated that ingestion of antioxidant could counter
60 Cd oxidative damage to target tissue cells. The role of antioxidants is to neutralize the excess of free
61 radicals, to protect the cells from toxic effects and to contribute to disease prevention [7, 11]. This has
62 been demonstrated in experimental cadmium intoxication where certain micronutrients antioxidants
63 notably, vitamin E and selenium, have reportedly reversed or prevented cadmium toxicity to target
64 tissue cells [12, 13, 14, 15, 16, 17, 18].

65 Apart from micronutrient supplements, a number of medicinal plant extracts have been reported to
66 exhibit a protective role against ROS and lipid peroxidation induced by xenobiotics including
67 environmental pollutants like Cd [7, 19, 20, 21, 22]. *Vitex doniana* leaves have been in used for many
68 decades in the treatment of many illnesses, and as food in Nigeria and some part of Africa [23]. This
69 plant is usually common, relatively cheap and with promising health-boosting potentials. There is
70 evidence that *Vitex doniana* is an important source of polyphenols which are known to act as strong
71 antioxidants against ROS induced oxidative stress [7, 24]. The study of the biological effects of
72 polyphenols has become an area of interest in the light of recent advances in the field of nutrition and

73 medical sciences. We report on the protective effect of *Vitex doniana* leaves extract on Cd-induced
 74 oxidative damage to rat hepatic and renal tissues as determined by tissue Cd bioaccumulation
 75 profiles, oxidative stress biomarkers such as membrane lipid peroxidation, antioxidant defense
 76 enzymes activities, non-enzymic tissue antioxidant levels, and biomarkers of tissue cellular damage.

77 **2. MATERIALS AND METHODS**

78 **2.1 Collection of Leaf Samples and Preparation of Extract**

79 Fresh leaves of *Vitex doniana* were obtained in the vicinity of the Faculty of Medical Sciences,
 80 University of Jos, Jos, Plateau State, Nigeria. They were identified and authenticated by Mr Joseph in
 81 Federal College of Forestry Jos, Plateau State. The leaves were washed with distilled water to
 82 remove dust particles and shade dried at room temperature under continuous ventilation for two
 83 weeks and the dried leaves were pounded into fine powder using a pestle and a mortar. Sixty grams
 84 of *Vitex doniana* powder was weighed and soaked overnight in 500 ml of distilled water. The mixture
 85 was shaken on a mechanical shaker for 3 hours and filtered through a Whatman No.1 filter paper.
 86 The resulting aqueous extract of *Vitex doniana* was concentrated and evaporated to dryness using a
 87 Rotary Evaporator at 40°C and stored in the refrigerator pending use. The extract was further
 88 reconstituted in distilled water at appropriate concentration before administering to experimental
 89 animals.

90 **2.2 Animal Treatment**

91 Wistar Strain male rats (b.wt. 180-220 g) obtained from the Animal House Unit, University of Jos,
 92 were used in the study. They were maintained on a standard rat diet, 'Vital Feed' (purchased from
 93 Grand Cereals and Oil Mills Ltd, Kuru, Nigeria) and tap water as drinking water, *ad libitum*. The
 94 respective working doses of *Vitex doniana* extract and Cd (as CdCl₂) administered orally to
 95 experimental animals in this study was first determined in a pilot study. The Cd dose used was the
 96 lowest in the graded concentration of the Cd salt tolerated by the rats with quantifiable tissue
 97 biochemical changes without fatality. On the other hand, the *Vitex doniana* extract dose used was the
 98 concentration that produced significant antioxidant defence capability compared to control rats fed
 99 normal diet alone.

Rats were weighed and distributed evenly, 3 rats/cages into four standard plastic-metal rat cages, labeled A-D, respectively. Rats in the four groups were fed the standard 'Vital feed' rat diet and drinking water *ad libitum*. However, each rat in groups C and D received twice daily, an oral supplement of aqueous extract of *Vitex doniana* at a dose of 200 mg *Vitex doniana* extract/kg b.wt/day, administered by means of a needle-free Syringe. The daily oral *Vitex doniana* supplementation was carried out for 15 days. Thereafter, rats in group B and C were each given one single oral dose CdCl₂ in aqueous solution (3 mg CdCl₂/kg b.wt) daily for 5days.

2.3 Tissue Collection and Preparation

At the end of the feeding experiment, on day 11, rats under anaesthesia were sacrificed by decapitation and, in each case, the liver and kidneys were excised and washed in ice-cold normal saline to remove adhering blood particles. Homogenates of liver and kidney samples of each rat were prepared separately by homogenizing 1 g portion in ice-cold 50 mM Tris-HCl buffer, pH7.4 (1:10, w/v) in an Akia homogenizer. The homogenates were centrifuged at 2,400 xg for 10 min in a refrigerated low-speed centrifuge and the supernatant (S1) fractions were collected with Pasteur Pipette into plastic vials and stored at 2 °C pending biochemical analysis. The rest of the kidney and liver samples were used for determination of Cd content.

2.4 Phytochemical Analysis

Phytochemical screening of the leaves of *Vitex doniana* was done using standard method of Harborne [25].

2.5 Biochemical Analysis

Membrane lipid peroxidation, non-enzymic tissue antioxidants (ascorbic acid and glutathione) concentration and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase) activity were determined in the liver and kidney supernatant fractions and red blood cells. Lipid peroxidation measured by malondialdehyde (MDA) content was assayed by the thiobarbituric acid reaction according to the method described by [26]. The concentration of reduced glutathione (GSH) in the supernatant fraction of the tissue homogenates were assessed by the Ellman reaction [27] using the method described by [28] Catalase (CAT) activity was determined by measuring the rate of decomposition of hydrogen peroxide at 570 nm as described by [29]. Superoxide dismutase (SOD)

activity was assayed as described by [30]. Tissue cell injury enzyme biomarkers; serum alkaline phosphatase (ALP), alanine aminotransferases (ALT) and aspartate aminotransferases (AST) were determined according to the method of [31].

2.6 Tissue Cadmium Determination

The cadmium contents of the liver and kidney tissue were estimated with inductively couple plasma optical emission spectrophotometer (ICP OES) optima 2000DV after wet digestion. 1g portion of the tissue was digested with 20 ml HNO₃-HClO₄ mixture (1:4 v/v) at 100 °C and the resultant digest diluted to 100 ml with deionized water [32].

2.7 Statistical Analysis

Statistical analysis of numerical data (expressed as mean ± SD) was done using the statistical package for the social sciences software (SPSS) programme. One way analysis of variance (ANOVA) with post hoc analysis was used to assess the differences between the experimental groups and statistical significance was considered at p<0.05.

3. RESULTS

3.1 Tissue Cadmium Content

The results of tissue cadmium determination are summarized in Table1. It can be observed from the table that cadmium was detected in both the liver and kidney of both the control and treated rats, but the mean cadmium contents of both the liver and kidney of rats exposed to Cd alone was significantly higher (p<0.05) than those of the corresponding tissues in both the control and extract treated groups. However, the liver and kidney of rats exposed to Cd following pretreatment with aqueous extract of *Vitex doniana* (group C) had a significantly lower (p<0.05) Cd content than the corresponding tissues of rats exposed to Cd alone (group B). This suggests that pretreatment with aqueous extract of *Vitex doniana* impaired Cd bioaccumulation in the liver and kidney. Also, it can be observed from the table that the mean kidney tissue Cd content of rats is significantly higher (p<0.05) than the liver, suggesting that the kidney is more active in bioaccumulation of Cd than the liver.

Table 1 Effect of pretreatment with aqueous leaves extract of *Vitex donian* on cadmium content of rat liver and kidneys

Group	Treatment	Cadmium concentration ($\mu\text{g/g}$ tissue)	
		Liver	Kidney
A	Control	0.14 $\pm$ 0.30	0.19 $\pm$ 0.10
B	Cd	1.60 $\pm$ 0.29 ^a	2.60 $\pm$ 0.25 ^a
C	VDE + Cd	0.42 $\pm$ 0.10 ^{ab}	0.65 $\pm$ 0.24 ^{ab}
D	VDE	0.12 $\pm$ 0.10 ^a	0.16 $\pm$ 0.10 ^{ab}

Data are expressed as mean $\pm$ SD, n= 4 for each group. VDE = *Vitex doniana* extract

^a values are significantly different from control (p<0.05)

^b values are significantly different from the group treated with Cd alone (p<0.05)

3.2 Membrane Lipid Peroxidation

The results of the effect of aqueous leaves extract of *Vitex doniana* on lipid peroxidation is summarized in Table 2. It can be observed from the table that there was a significant increase (P < 0.05) in the mean concentration of malondialdehyde (MDA) in rats given Cd alone (group C) when compared with the control (group A), suggesting that ingestion of Cd induced lipid peroxidation as determined by MDA. However, pretreatment with aqueous leaves extract of *Vitex doniana* leads to a significant reduction (P < 0.05) in MDA level in the tissues examined. The mean MDA level is generally higher in the kidney than in the liver except in group A, where the reverse is the case.

Table 2 Effect of pretreatment with aqueous leaves extract of *Vitex donian* on cadmium- induced lipid peroxidation in the liver and kidneys of rat

Group	Treatment	Malondialdehyde concentration (nmol/g tissue)	
		Liver	Kidney
A	Control	28.53 $\pm$ 2.82	24.12 $\pm$ 1.29

B	Cd	63.62±1.66 ^a	73.81±1.89 ^a
C	VDE + Cd	38.04±1.90 ^{ab}	57.40±1.65 ^{ab}
D	VDE	19.35±1.91 ^a	15.75±1.260 ^{ab}

169 Data are expressed as mean ± SD, n= 4 for each group. VDE = *Vitex doniana* extract

170 ^a values are significantly different from control (p<0.05)

171 ^b values are significantly different from the group treated with Cd alone (p<0.05)

172

173 3.3 Non-enzymic Tissue Antioxidants

174 The result of tissue glutathione determination is summarized in Tables 3. The level of glutathione was
 175 significantly much lower in the liver and kidney of rats exposed to Cd alone (group B) than in the
 176 corresponding tissue of the control (group A), suggesting that exposure to Cd markedly depleted
 177 glutathione stores of the liver and kidney. However, pretreatment with aqueous leaves extract of *Vitex*
 178 *doniana* had a sparing effect on tissue glutathione and inhibited its depletion by Cd. The mean
 179 glutathione content of the liver is generally higher than that of the kidney, suggesting that the liver has
 180 higher glutathione reserves than the kidney.

181 Table 3. Effect of pretreatment with aqueous leaves extract of *Vitex donian* on tissue glutathione
 182 content of rats exposed to Cd.

Group	Treatment	Glutathione concentration (µmol/g tissue)	
		Liver	Kidney
A	Control	2.88±0.82	1.24±0.29
B	Cd	1.09±0.63 ^a	0.70±0.10 ^a
C	VDE + Cd	1.92±0.19 ^{ab}	0.90±0.15 ^{ab}
D	VDE	2.90±0.70 ^b	1.50±0.12 ^{ab}

Data are expressed as mean $\pm$ SD, n= 4 for each group. VDE = *Vitex doniana* extract

^a values are significantly different from control (p<0.05)

^b values are significantly different from the group treated with Cd alone (p<0.05)

3.4 Antioxidant Enzymes

The results of the antioxidant enzymes enzyme catalase and superoxide dismutase in the liver and kidney are summarized on Table 4. In both the liver and kidneys, the mean activity of each of the antioxidant enzymes was significantly higher (p<0.05) in rats exposed to cadmium alone (group B) than in the control rats (group A). This would imply that exposure to cadmium induced the activity of each of the two antioxidant enzymes, catalase and superoxide dismutase in the liver and kidney. However, pretreatment with aqueous leaves extract of *Vitex doniana* moderated the antioxidant enzyme- inducing effect of cadmium on catalase and superoxide dismutase in both tissues examined. Catalase and superoxide dismutase activities in the liver and kidney of rats pretreated with aqueous leaves extract of *Vitex doniana* were significantly enhanced.

Table 4. Effect of pretreatment with aqueous leaves extract of *Vitex donian* on the activities of antioxidant enzymes catalase and superoxide dismutase in the liver and kidney of rats exposed to cadmium

Group	Treatment	Catalase activity ($\mu\text{mol/g}$ tissue)		SOD activity ($\mu\text{mol/g}$ tissue)	
		Liver	Kidney	Liver	Kidney
A	Control	6.10 $\pm$ 0.71	7.64 $\pm$ 0.66	9.23 $\pm$ 1.23	44.58 $\pm$ 3.1.81
B	Cd	12.45 $\pm$ 0.73 ^a	17.21 $\pm$ 0.75 ^a	55.05 $\pm$ 0.38 ^a	75.56 $\pm$ 1.81 ^a
C	VDE + Cd	8.20 $\pm$ 0.61 ^{ab}	14.54 $\pm$ 0.26 ^{ab}	36.76 $\pm$ 0.97 ^{ab}	62.55 $\pm$ 1.20 ^{ab}
D	VDE	5.10 $\pm$ 0.15 ^{ab}	5.23 $\pm$ 0.55 ^{ab}	8.02 $\pm$ 1.21 ^{ab}	33.94 $\pm$ 2.40 ^{ab}

Data are expressed as mean $\pm$ SD, n= 4 for each group. VDE = *Vitex doniana* extract

^a values are significantly different from control (p<0.05)

^b values are significantly different from the group treated with Cd alone (p<0.05)

3.5 Tissue Cell Injury Enzyme Biomarkers

The results are summarized on Table 5. The mean ALT, AST and ALP activities in the liver of rats exposed to cadmium alone was significantly higher ($p<0.05$) when compared to the control rats. This would imply that exposure to Cd caused serious tissue cell damage; hence, more of these cellular enzymes are released. However, pretreatment with aqueous leaves extract of *Vitex doniana* significantly lower ($p<0.05$) the activities of cell injury biomarker enzymes in the tissues examined when compared to rats exposed to cadmium alone. The results suggest that the use of *Vitex doniana* extract caused a reduction in the level of normal metabolic oxidative damage to liver cells.

Table 5: Activities of tissue marker enzymes aspartate and alanine aminotransferases, and alkaline phosphatase in the liver of rats exposed to cadmium following pretreatment with aqueous leaves extract of *Vitex doniana*

Group	Treatment	Liver enzyme activities ($\mu\text{g tissue}$) (umol/g tissue)		
		AST	ALT	ALP
A	Control	133.50 $\pm$ 1.19	123.00 $\pm$ 0.83	81.20 $\pm$ 2.56
B	Cd	176.50 $\pm$ 6.64 ^{ab}	128.50 $\pm$ 3.70 ^b	160.10 $\pm$ 7.97 ^{ab}
C	VDE + Cd	167.50 $\pm$ 4.30 ^a	121.50 $\pm$ 2.64 ^a	91.10 $\pm$ 5.94 ^a
D	VDE	131.53 $\pm$ 3.46 ^b	121.10 $\pm$ 2.89 ^{ab}	101.20 $\pm$ 4.62 ^{ab}

Data are expressed as mean $\pm$ SD, n= 4 for each group. VDE = *Vitex doniana* extract

^a values are significantly different from control ($p<0.05$)

^b values are significantly different from the group treated with Cd alone ($p<0.05$)

3.6 Phytochemical Constituents of *Vitex doniana*

The results of phytochemical screening of *Vitex doniana* are summarized in Table 6. The signs +, ++ and +++ represents the presence of phytochemicals in the plant extract in trace, moderate and abundance respectively. It can be observed from the table that phytochemicals were detected at varying quantity in the aqueous leaves extract of *Vitex doniana*. The screening revealed that alkaloids, saponin, tannins, and cardiac glycosides were moderately present while flavonoids and phenols are abundantly present in the plant extract

Table 6 Results of phytochemical screening of *Vitex doniana*

Phytochemical	Alkaloids	Cardiac glycosides	Flavonoids	Phenols	Saponins	Tannins
VDE	++	++	+++	+++	+	++

VDE = *Vitex doniana* extract, + = present in trace, + = moderately present, +++ = abundantly present

4. DISCUSSION

Cadmium is a toxic metal that promotes early oxidative stress in animals and humans and afterward contributes to the development of serious pathological conditions because of its long retention in some tissues [33, 34]. In the current study, cadmium administration to rats leads to a significantly increased accumulation of cadmium in the liver and kidneys of rats treated with cadmium. The generally higher concentration of Cd in the kidney than in the liver is consistent with its being the major target organ for cadmium toxicity [9]. The presence of cadmium in trace quantities in the liver and kidney of rats on the control diet has also been observed by other workers [14, 32, 35]. It is a testimony of the ubiquity of cadmium in the food chain and the environment. However, pretreatment with aqueous leaves extract of *Vitex doniana* alters the pattern of Cd disposition and markedly reduced the level of bioaccumulation of Cd in the rat liver and kidney. This is in agreement with the previous studies which showed that oral intake of cadmium induces its accumulation in these tissues [17]. The decreased cadmium concentration in the liver and kidneys could be explained by its redistribution to other tissues and organs as well as by formation of cadmium-extract complexes or by interfering with free radical chain initiation and progression of Cd-induced oxidative damage. The decreased accumulation of cadmium in liver and kidneys after pretreatment with aqueous leaves extract of *Vitex doniana* prior to exposure to cadmium, indicate that extract of *Vitex doniana* diminished the toxic effects of cadmium.

Cd-induced oxidative stress is characterized by increased lipid peroxidation and/or altered nonenzymatic and enzymatic antioxidant system. The results of this study showed that exposure to cadmium causes a significant increase in membrane lipid peroxidation in the liver and kidneys of rats but pretreatment with aqueous leaves extract of *Vitex doniana* was effective in the prevention of oxidative damage induced by cadmium, which resulted in significantly lower degree of lipid

peroxidation in the liver and kidneys. This is consistent with the previous studies in which natural plant products effectively reduced lipid peroxidation induced in response to various toxicants [36, 37, 38]. This can be explained by the important role of polyphenols in preventing lipid peroxidation and in protection of integrity and functioning of tissues and cells. This is in accord with reported protective effects of antioxidant nutrients against Cd-induced oxidative stress and lipid peroxidation in the liver and kidney [12, 15, 17].

Reduced glutathione (GSH) is an essential constituent of the endogenous antioxidant defence system and it functions as a direct free-radical scavenger as well as reduces intracellular reactive oxygen species [39]. It is usually used up in the course of destroying the oxy-radicals leading to the depletion of its tissue reserves in the process [16]. In the present study, exposure to cadmium induced a significant depletion of glutathione in the liver and kidney of rat which was effectively prevented by pretreatment with aqueous leaves extract of *Vitex doniana*. The observed sparing effect of aqueous leaves extract of *Vitex doniana* on Cd-induced depletion of glutathione in the rat liver and kidney is consistent with a protective role for aqueous leaves extract of *Vitex doniana* against Cd toxicity. This would appear to suggest that sparing effect of aqueous leaves extract of *Vitex doniana* pre-supplementation or tissue glutathione was most effective in the kidney. The diminished level of liver GSH in Cd-treated rats in the present study might be due to its reductive defence role in maintaining an oxidant/antioxidant balance during cadmium-toxicity

There is an increasing body of evidence suggesting that cadmium induces alterations in the activities of endogenous antioxidant enzymes with catalase and superoxide dismutase (SOD) being among the first enzymatic antioxidant defences for the body against oxidant-induced cytotoxic challenge [5, 40]. Upon ingestion, many xenobiotics, including prooxidants, such as 2, 4- dinitrophenyl hydrazine [41], induce the enzymes that metabolize them. Furthermore, when cells are oxidatively challenged, antioxidant enzymes levels increases as a protective mechanism [18]. In the current study the results obtained regarding the activities of SOD and catalase in the liver and kidney clearly indicate that exposure to mild doses of cadmium significantly induced enzyme activity which was effectively reversed by pre-treatment with aqueous leaves extract of *Vitex doniana*. This is in accord with reported protective effects of antioxidant nutrients against Cd-induced oxidative stress and alteration

of levels of antioxidant enzymes in the liver and kidney [16, 17, 18, 42]. The overall result suggests that the extract acts in a similar mode to that of Livolin forte [43].

Measurement of activities of “marker” enzymes or biomarkers in tissues and body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organs/tissues long before it is revealed by histological techniques [44, 45]. In this study, a marked increase in the activities of aminotransferases and alkaline phosphatase in the liver tissue of Cd-exposed rats are indications that the liver has been injured. However, pretreatment with aqueous leaves extract of *Vitex doniana* led to a marked decrease in the activities of these marker enzymes suggesting a marked reduction in the degree and rate of tissue cell injury. This is in accord with reported protective effects of protective effect of carrot juice against cadmium-induced toxicity in the liver [18].

5. CONCLUSION

From the findings of this study on the effect of pretreatment with *Vitex doniana* leaves extract on all the indicators of cytotoxic response to cadmium exposure, it can be concluded that an organism well nourished with the natural antioxidant components in the plant *Vitex doniana* at the time of Cd exposure has a more effective defence capability against Cd-induced oxidative damage to tissue than otherwise as determined by the various parameters. Although the study did not specifically identify the active principle but based on the well reported antioxidant properties of *Vitex doniana* due to the presence of polyphenols, polyphenols are presumed as the active antioxidant principle.

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