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Antioxidant Activity and cytotoxicity study of *Vangueria madagascariensis* Leaf, bark and seed cake Methanolic Extracts

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ABSTRACT

Natural antioxidant plays a vital role in regulating free radicals from the body .So it prevents damage to cell which can be as a result of free radicals. This study was carried out to determine in vitro antioxidant potential, cytotoxicity study in leaf, bark and seed cake of *Vangueria madagascariensis*. Antioxidant activity of the examined extract was analyzed by DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay and oxygen radical absorbance capacity (ORAC). Methanolic leaf extract shows high potential free radical scavenging activity with IC_{50} value of 7.8 µg/ml. Compare to methanolic seed extract that showed IC_{50} value of 62.5 µg/ml. The (ORAC) assay revealed a higher antioxidant activity of Methanolic leaf extract 72.71± 0.89 (µM of Trolox) compared with the positive control; Quercetin (58.97± 0.02). MTT [3-(4,5-dimethylthiazole-2-yl)2,5-diphenyltetrazolium bromide] assay was used to evaluate the cytotoxic activity of the examined extracts. Only *Vangueria madagascariensies* leaf extract was found effective. Total Phenolic content of the examined extracts were measured by the Folin –Ciocalteau procedure and HPLC-DAD. The highest phenolic and flavonoid content were observed from bark and leaf extract which were 170.4 mg/g plant extract as GAE and 298.8 RE/g of extract respectively.The result proves that, *Vangueria madagascariensies* is an excellent source of natural antioxidants that can be used to decrease the effect of oxidative damage. *Vangueria madagascariensies* leaf Extract contain high amount of polyphenols, flavonoids and exhibit antioxidant activities .So this plant could be exploited for medicinal and food application.

INTRODUCTION

Free radicals and its adverse effects were revealed in the last decade (Sen *et al*., 2010). Extreme addition of free radicals results in oxidative stress. This is one of the main causes of creation and progress of diseases and early aging (Oluwafemi *et al.*, 2016). Antioxidants are substances capable to get rid of free radicals and prevent its harm (Chew *et al*., 2008 and Ying *et al*., 2010). Flavonoids, phenols, tannins, and alkaloids are active ingredients in medicinal plant that are related to antioxidant activities (Abdulkadir, *et al*., 2015). The phytochemical constituents such as flavanoids and terpenoids are the major components which are responsible for the potential cytotoxic activity (Prema et.al,2012 and Esmaeili,*et al*.,2014).

Vangueria madagascariensis (VM) J.F. Gmelin. (Rubiaceae) is a native medicinal food plant from Africa, is used as a biomedicine for control of diabetes , bacterial infections in Africa and has many traditional medicine use in various countries (Mahomoodally and Dilmohamed, 2015). It is wide spread in tropical Africa,

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Madagascar and Sudan (AbdelRahman 2011;Orwa *et al*., 2009 and Fentahum and Hager , 2009). Initial phytochemical of *Vangueria madagascariensis* leaves and stems revealed the occurrence of alkaloids, terpenes, cyonogenetic heterosides as well as phenols, tannins, and saponosides (Ramalingum and Mahomoodally, 2014). The aim of this study was to determine the total phenolic content of the methanolic extracts of different parts of Vangueria *madagascariensis* and evaluate its antioxidant activities and cytotoxicity study by using a series of in vitro tests .

MATERIALS AND METHODS

Plant Material and Chemicals:

Different parts from *Vangueria madagascariensis* tree, under investigation were collected in May 2011 from Alfola Agricultural Area, Sudan. Specimens identified by taxonomist in medicinal and aromatic plant national center for research Institute (MAPRI) Khartoum, Sudan. The plants were dried in shades finely powdered with electric mill and kept for the extraction process. All solvents were of analytical grade.

Extraction of Phenolic Compounds:

Dried powdered (20g) of Vangueria madagascariensis leaf (VML), bark (VMB), and seed cake (VMC) were extracted with 80% methanol by sonication (Hwasin Technology, Seoul, Korea) to gain methanolic extract with solid to solvent ratio of 1:10 (w/v) at room temperature for 1 hour.. The methanolic extracts were filtered through filter paper Whatman No. 1. Then solvents were removed by using rotary evaporator (Buchi, Flawil, Switzerland). The yield of each extract was measured then kept at -80oC for further analysis.

Total Phenolic Content:

Standard Folin–Ciocalteu method followed by Noor *et al*. (2014) was used to determine the total phenolic contents (TPC).

HPLC–DAD System for Analysis of Phenolic Compounds:

HPLC analysis was performed using Agilent G1310A pumps with diode array detector and chromatographic separations were performed on a LUNA C-18 column. The composition of solvents and used gradient elution conditions were described formerly by chirinos (2009).

Total Flavonoid Content:

The total flavonoid content (TFC) in each sample was measured by the following method of Zhishen *et al*. (1999).

Antioxidant Capacities Measurement:

The antioxidant Capacities of phenolic extracts from *Vangueria madagascariensis* leaves (VML), bark (VMB), and cake (VMC) was measured following Gordon *et al*., (2001) by means of the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH).

ORAC Antioxidant Activity Assay:

The oxygen radical absorbance capacity ORAC is performed according to the procedure described by Perez-Jimenez and Saura-Calixto, (2006)with slightly modified.

MTT Assay and Cellular Viability:

Diverse cell types (A549, PC-3, MCF-7, HepG2, HT-29, WRL-68 and WI-38) were used to determine the inhibitory effect of VML, VMB and VMC on cell growth using the MTT [3-(4,5-dimethylthiazole-2-yl)2,5-diphenyltetrazolium bromide] assay described by Alesiani *et al*., 2010. For measurement of cell viability, cells were seeded at a density of 1 x 105 cells/ml in a 96-well plated and incubated for 24 hours at 37°C, 5% CO2. Next day, cells were treated with the test agents and incubated for another 24 hours. After 24 hours, MTT solution at 2 mg/ml was added for 1 hour. Absorbance at 570 nm were measured and recorded. Results were expressed as a percentage of control giving percentage cell viability after 24 hours exposure to test agent. The potency of cell growth inhibition for each test agent was expressed as an EC_{50} value.

Statistical Analysis:

Two methods of Statistical analysis were performed using the analysis of variance (ANOVA; $P < 0.05$) and the SPSS of the windows statistical package (Release 8.0).

RESULTS AND DISCUSSION

Total Phenolic Content (TPC):

Total phenols were measured by the Folin –Ciocalteau procedure. Figure 1 shows the total phenol of the methanolic extracts of *Vangueria madagascariensis* bark (VMB), leaves (VML), and seed cake (VMC). The elevated contents were 170.4 and 169.5 mg/g plant extract as, GAE for VMB and VML methanolic extract respectively. The lowest concentrations were found in the methanolic VMC extract at112.5 mg/g plant extract as GAE. These findings agree with the earlier studies carried out by (Ramalingu and Mahomoodally, 2014) who reported that leaf extract contains highest amount of total phenolic content than other parts of *Vangueria madagascariensis.*

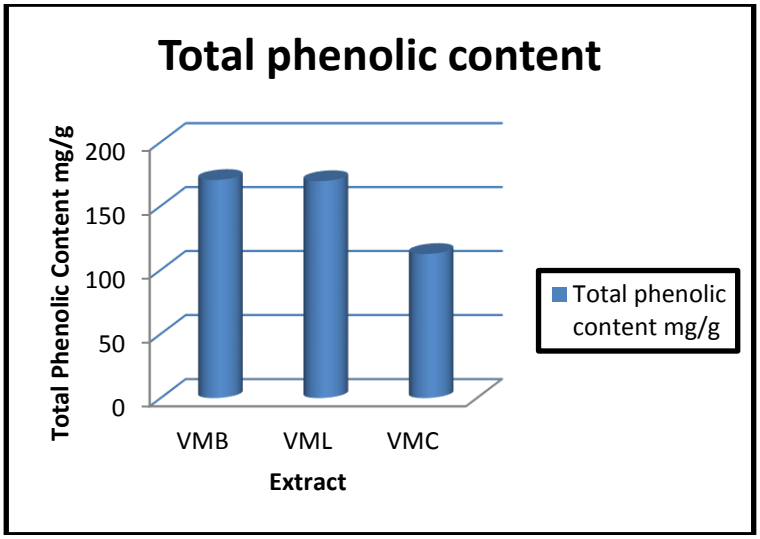

Fig. 1: Total phenolic content of *Vangueria madagascariensis* extracts.

Identification of Phenols by HPLC-DAD:

HPLC-DAD (high-performance liquid chromatography, with diode array detection (DAD) and mass spectrometry (MS) detection. HPLC-DAD was used to identify and distinguish what are the responsible active ingredients in the crude methanolic extracts of VML, VMB and VMC. Table (1) shows that the examined extracts of VML, VMB, and VMC contain syringic, gallic acid, P-hydroxybenzoic, chlorogenic, vanillin, ferulic and p-cumeric acids. Chlorogenic acid was detected to be the main phenolic constituent in all the examined extracts, and it is higher in VML and VMC presenting the levels of 1.027 and 1.226 mg/100 g dry weight contributing about 81.6 and 89.3 % to the total amount, respectively.

Table 1: Phenolic compounds Content mg/100 g dry weight in *Vangueria madagascariensis* methanolic extracts

Compounds	VMB*	VMC*	VML*
Syringic acid	0.007±0.35	0.021±0.22	0.018±0.11
p-Hydroxybenzoic acid	0.00	0.052±0.12	0.03±0.21
Gallic acid	0.004±0.8	0.061±0.22	0.014 ±0.11
Chlorogenic acid	0.00	1.226±0.56	1.027±0.67
Vanillin	0.015±0.1	0.050±0.21	0.026±0.34
p-coumaric	0.00	0.031±0.23	0.005 ±0.01
Ferulic acid	0.00	0.062±0.18	0.030 ±0.11

*Values are means ± SD (n = 3), and they are given as mg/100 g dry weight of examined extract.

Total Flavonoids:

The total flavonoids of *Vangueria madagascariensis* leaf, bark, and seed cake were determined Fig 2. *Vangueria madagascariensis* leaf had highest amounts of flavonoid content 298.8 expressed as mg of rutin equivalents (RE)/ g of extract. *Vangueria madagascariensis* bark showed medium concentration as 123.6 mg/g of extract. The seed cake extracts of *Vangueria madagascariensis* recorded very low amount of flavonoid 23.1mg/g of extract. These results are agree with the previous studies done by (Ramalingu and Mahomoodally, 2014) who reported that leaf extract contains highest amount of total Flavonoids than other parts of *Vangueria madagascariensis.*

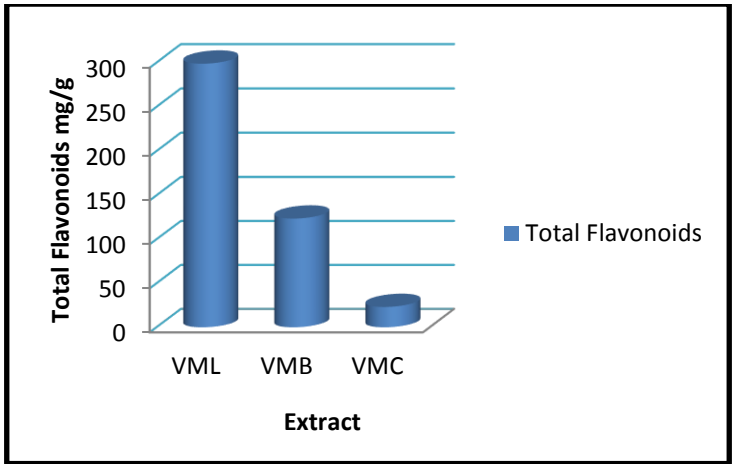

Fig. 2: The total flavonoids of *Vangueria madagascariensis* extracts.

Antioxidant Capacity Assays:

The antioxidant Capacity of *Vangueria madagascariensis* bark, leaf, and seed cake extracts was measured by DPPH assay (Table 2). *Vangueria madagascariensis* leaf, seed cake and bark extracts showed IC50 of 7.81, 31.3 and 62.5µg/ml, respectively. Lower IC50 value reflects better DPPH radical scavenging activity (Mariod *et al*.,2012).The current study showed that *Vangueria madagascariensis* leaf; contained a high level of phenolic compounds and antioxidants potential, which promote its uses as a natural antioxidant. Also these results agree with the previous studies who reported that there is a positive relationship between antioxidant activity potential and amount of phenolic compounds of the crude extracts (Ruiz-Terán *et al*., 2008, Oluwafemi *et al*., 2016 and El Sohaimy *et al*., 2015). These results are in good agreement with that of (Ramalingu and Mahomoodally, 2014) who found that leaf extract show lower IC50 value than other parts of *Vangueria madagascariensis.*

Table 2: Results of DPPH (IC_{50}) of *Vangueria madagascariensis* examined extracts

Extract	IC_{50} µg /ml*
VML	7.81± 0.1
VMB	62.5± 0.5
VMC	31.3± 0.3
Ascorbic Acid	3.13± 0.1

*Values are mean of triplicates ±standard deviation

ORAC Antioxidant Activity Assay:

The oxygen radical absorbance capacity (ORAC) was used to assess the antioxidant activity of *Vangueria madagascariensis* methanolic ,extracts three samples VML,VMC and VMB were compared with the positive control; Quercetin. The assay has been widely used in many recent studies related to plant (Atala, 2009).ORAC results are presented in (Table 3) VML display a higher level of antioxidant activity (72.72 µM of Trolox)than Quercetin at 5 µg/ml (58.97 ±0.02 µM of Trolox) while VMB and VMC showed lower level of antioxidant (47.08, 44.94, and 29.60 µM of Trolox, respectively) than Quercetin at 5 µg/ml (58.97±0.02 µM of Trolox).

Table 3: Results of ORAC (µM of Trolox) of *Vangueria madagascariensis* extract

Extract	ORAC (µM of Trolox)*
VML	72.71± 0.89
VMB	47.08± 0.23
VMC	44.94± 0.34
Quercetin	58.97± 0.02

*Values are mean of triplicates ±standard deviation.

Cytotoxic activity:

MTT assay was used, to assess the cytotoxic activity of extracts from different parts of *Vangueria madagascariensis* .Crude extracts of VML, VMB and VMC were tested with a series of different doses on A549, PC-3, MCF-7, HepG2, HT-29, WRL- -68 and WI-38, respectively, and After 24 hours, cell viability was determined by the MTT assay, only VML was found effective Figure 3.Test agents induced cell cytotoxicity in a concentration dependent manner. These dose titration curves allowed determining of EC50 for the test agents towards different cell lines (Table 4).The curves present the growth of melanoma cancer cells which inhibited in a dose-dependent manner.

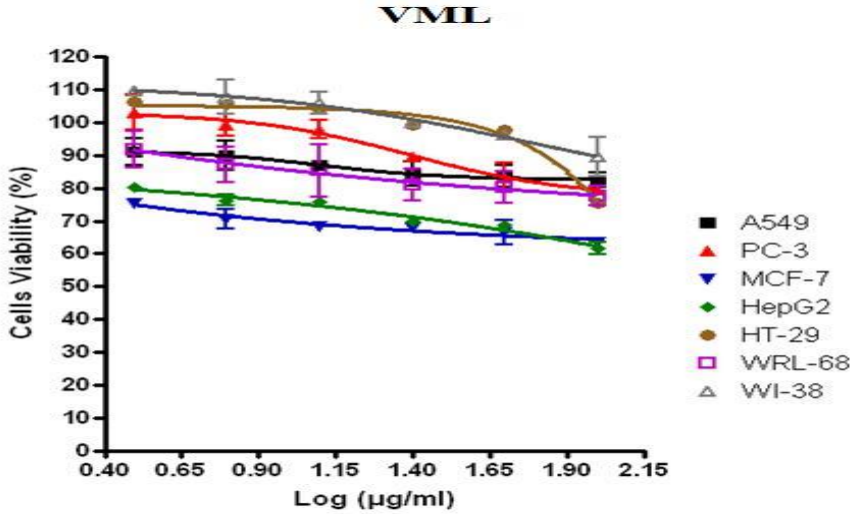

Fig. 3: Response curve of extract (VML) in MTT assays

Table 4: The Effect of *Vangueria madagascariensis* leaf extract (VML) on various cells type measured by MTT assay

MTT assay (Test agent)	EC_{50} ± S.D (µg/ml)*
	VML*
non- small cell lung cancer cells (A549)	42.54 ± 2.32
prostate adenocarcinoma cells (PC-3)	34.42 ± 2.97
Human breast carcinoma cells (MCF-7)	22.75 ± 1.98
Human hepatocellular carcinoma cells (HepG2)	28.40 ± 3.54
Human colon adenocarcinoma cells (HT-29)	53.20 ± 4.24
normal hepatic cells.(WRL-68)	44.47 ± 1.27
Normal lung fibro blast cells.(WI-38T)	64.74 ± 2.92

*Values are mean of triplicates ±standard deviation

Conclusion:

Vangueria madagascariensis can be considered as a promising medicinal plant that deserves to be further explored for the control of many diseases .The extracts of *Vangueria madagascariensis* leaf, bark and seed cake contain high levels of phenols, total flavonoids and antioxidant activities. All these constituents assist extracts of *Vangueria madagascariensis* to be as an active natural antioxidant since the leaf has a role in the food manufacturing and pharmaceutical purposes. In vitro cytotoxic activity against A549, PC-3, MCF-7, HepG2, HT-29, WRL-68 and WI-38, a cell line at different concentrations were evaluated, only the leaf extract were found effective. Also these results confirm the traditional use of this plant as control of a wide range of disease. Further studies are needed to isolate and purify active ingredients of this plant, to investigate In vivo antioxidant properties of *Vangueria madagascariensis*, to identify its main components and developed novel drugs from the *Vangueria madagascariensis* plant. Since it is well recognized that some synthetic antioxidants have side effects that can cause health problems. This study has provided an opportunity to create valuable main information on the bioactivity of *Vangueria madagascariensis* and has opened new perspectives for further pharmacological study.

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