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Original Research Article

Antioxidant Activity and Cytotoxicity of the Leaf and Bark Extracts of *Tarchonanthus camphorates*

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Abstract

Purpose: *To investigate the antioxidant potential and cytotoxicity of the leaf and bark extracts of Tarchonanthus campharatus..*
Methods: *The antioxidant activity of the aqueous leaf extract (Aq LF), methanol leaf extract (MET LF), dichloromethane leaf extract (DCM LF), methanol bark extract (MET BK), dichloromethane bark extract (DCM BK), and ethyl acetate bark extract (Et Ac BK) were examined by 1,1-Diphenyl-2-picryl-hydrazil (DPPH), 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$), nitric oxide radical scavenging, reducing power and iron chelating activity methods. Brine shrimp lethality and MTT cytotoxicity tests were used to investigate the cytotoxicity of the extracts.*
Results: *The Aq LF, DCM LF, MET LF and MET BK showed good DPPH, ABTS radical scavenging and total reducing power activities. Total reducing power was high and in the rank order of DCM LF > Aq LF > MET LF > MET BK. All the extracts, however, showed weak nitric oxide scavenging activity as well as weak iron chelating ability. Flavonoids, phenols, tannins and saponins were present in some of the extracts, but alkaloids, terpenoids, cardenolides and cardiac glycosides were absent in all the extracts. All the extracts did not show significant cytotoxic properties ($p \geq 0.05$) with 50% inhibitory concentration IC_{50} values > 30 µg/ml in both cytotoxicity assays.*
Conclusion: *The antioxidant activity and low cytotoxicity of Tarchonanthus camphoratus probably justify its use in folk medicine.*

Keywords: *Tarchonanthus camphoratus, Antioxidant activity, Cytotoxicity*

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INTRODUCTION

In the living system, free radicals are constantly generated and can cause extensive damage to tissues and biomolecules, creating various degenerative diseases [1]. Due to the adverse side effects of synthetic drugs, natural antioxidants from plant origin have drawn much attention in recent years. Plants are known to possess potent bioactive compounds capable of preventing and treating most oxidative-related diseases and these compounds might act as leads in drug development [2].

Tarchonanathus campharatus L,.(family Asteraceae) has many medicinal applications in traditional healing where infusions and tinctures of the leaves are used for stomach trouble,

abdominal pain, headache, toothache, asthma, bronchitis and inflammation; furthermore, smoke generated by burning the fresh or dried plant is inhaled for rheumatism [3]. The plant is also used in the treatment of venereal diseases, indigestion, heartburn, coughs while a strong infusion is used in a hot bath for the management of paralysis and cerebral haemorrhage [4]. The medicinal activity of plants may be attributed to the presence of antioxidants [5].

The potential toxicity of plants is an important consideration when studying the traditional use of plants and also when identifying their potential for other biological properties. No specific report regarding the antioxidant potential and cytotoxicity of the extracts of the leaf and bark of *Tarchonanathus campharatus* has been published. We therefore decided to investigate the antioxidant potential and cytotoxicity of the various extracts of the leaf and bark of *Tarchonanathus campharatus*.

EXPERIMENTAL

Plant materials

Fresh leaves and bark of *Tarchonanthus camphoratus* were collected from Sangoyana in the northern part of Kwa-Zulu Natal province, South Africa, during the month of April 2011. The plant was identified by the local people during the time of collection and later authenticated by Mrs N.R. Ntuli, Department of Botany, University of Zululand. in Kwa-Zulu Natal, South Africa. A voucher specimen, (NSKN 1), was deposited at the University of Zululand herbarium. The plant material was dried at room temperature; the bark was ground to a fine powder and stored in an air tight container until further use. The fresh leaves were air-dried.

Preparation of the extracts

A portion (300 g) of the bark powder was sequentially soaked in hexane, followed by dichloromethane, ethyl acetate, and methanol each for 48 h. The dry ungrounded leaves (400 g) were soaked separately in dichloromethane, methanol for 48 h and in water for 24 h. The extract solution, each case, was filtered through Whatman filter paper no. 1. The hexane, dichloromethane, ethylacetate and methanol extracts were concentrated under reduced pressure at 50 ^{0}C using a rotary evaporator and preserved in a refrigerator pending further use. The aqueous extract was also concentrated to a brown powder in a freeze dryer. FD—Bench top K, VirTis model.

Phytochemical screening of the plant extracts

The extracts were screened for phytochemicals using standard procedures [6].

Antioxidant screening of the plant extracts

1,1-Diphenyl-2-picryl-hydrazil (DPPH) radical scavenging assay

The radical scavenging activity of the extracts was performed using DPPH spectrophotometric method [7]. One millilitre of 0.3mM DPPH methanol solution was added to 2.5 ml solution of the extract or standard (25 - 250 µg/ml) and allowed to react at room temperature in the dark for 30 min. The absorbance was measured against a corresponding blank at 517 nm. Methanol (1.0 ml) plus extract solution (2.5 ml) was used as the blank. BHT was used as a positive control and all measurements were done in triplicate. Inhibition (%) of free radical DPPH was calculated as in Eq 1.

Inhibition (%) = {(As – Ac)/As}100 …… (1)

where As is the absorbance of blank solution and Ac the absorbance of the extract solution.

ABTS radical cation scavenging assay

The pre-formed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$) was generated according to the modified method of Re *et al* [8] by oxidation of ABTS solution (7mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 16 h in the dark at room temperature. One millilitre of ABTS was added to 1 millilitre of various extract concentrations (25 - 250, µg/ml) and the absorbance of the mixture measured against a corresponding blank at 734 nm after 6 min. BHT was used as the positive control and all measurements were in triplicate. Inhibition (%) was determined as in Eq 1.

Nitric oxide (NO) radical inhibition assay

Nitric oxide scavenging activity of the test compounds was determined using sodium nitroprusside (SNP) generating NO system. NO generated from SNP in aqueous solution at physiological pH reacts with oxygen to produce nitrite ions which react with Greiss reagent [9]. The reaction mixture containing 2 ml sodium nitroprusside (10mM), 0.5 ml phosphate buffered saline (pH 7.4) and 0.5 ml of various concentrations of the extracts (25 - 250 µg/ml) or standard solution (ascorbic acid, 0.5 ml) was

incubated at 25 ºC for 150 min. After incubation, a 1 ml aliquot of the reaction mixture was removed and diluted with 1 ml Greiss reagent. The absorbance of the solution was measured at 540 nm against the corresponding blank solution at 540 nm. The same reaction mixture without the extract but with an equivalent amount of water served as the control. Percent nitric oxide inhibition was computed as in Eq 1.

Chelating effect of ferrous ions

The chelating effect was determined according to the method of Dinis *et al* [10]. Briefly, 2 ml of various concentrations (25 - 250 µg/ml) of the extract in methanol was added to a solution of 2mM $FeCl_2$ (0.05 ml). The reaction was initiated by addition of 5Mm ferrozine (0.2 ml). The mixture was then shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The control consisted of $FeCl_2$ and ferrozine, complex molecules. Na_2EDTA was used as positive control.The chelating effect of ferrous ions was evaluated as in Eq 2.

Fe^{2+} chelating effect (%) = {(As – Ac)/Ac}100 ….. (4)

where As is the absorbance of blank solution and Ac the absorbance of the extract solution.

Determination of total reducing power

Reducing power was determined according to the method of Oyaizu [11]. Each extract (25 - 250 µg/ml) in methanol or water (2.5 ml) was mixed with 2.5 ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1 % potassium ferricyanide, and the mixture was incubated at 50 ^{o}C for 20 min. Thereafter, 2.5 ml of 10 % trichloroacetic acid was added and the mixture was centrifuged at 1000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml of deionised water and 0.5 ml of 0.1 % ferric chloride, and its absorbance measured spectrophotometrically at 700 nm against blank. Ascorbic acid was used as the reference standard.

Determination of cytotoxicity of the extracts

Brine shrimp lethality test

Brine shrimp lethality test was performed on brine shrimp *nauplii* using Meyer method [12]. The eggs of the brine shrimp were collected from a local fish farm and hatched and kept in artificial seawater (3.8 % NaCl solution) for 48 h to give mature shrimp called *nauplii*. For each extract, 2 mg was dissolved in 1 ml of 1 % DMSO. From this solution, 2.5, 1.25, 0.25 and 0.025 ml were transferred into separate test tubes and made up to 5 ml using sea water to give corresponding concentrations of 1000, 500, 100 and 10 µg/ml. Ten (10) brine shrimps (*nauplii*) were transferred into each of the test tubes using a Pasteur pipette. After 24 h, the vials were inspected using a magnifying glass and the number of surviving *nauplii* in each vial was counted, and lethality (%) of the brine shrimp *nauplii* calculated at each extract concentration. Potassium dichromate was used as a positive control and each test was in triplicate.

MTT assay

MTT assay is based on the reduction of the yellow coloured 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT), by mitochondrial dehydrogenases of metabolically active cells to purple formazan. The intensity of the purple colour of formazan is quantified spectrophotometrically at 570nm and is proportional to the number of live cells present.

Human embryonic kidney (HEK293) and human hepatocellular carcinoma (HepG2) cells were all grown to confluency in 25 cm^2 flasks. This was then trypsinized and plated in 48 well plates at specific seeding densities. The cells were incubated overnight at 37 ^{o}C. The medium removed, and fresh medium Minimal Essential Medium (MEM + Glutmax + (100 µg/ml penicillin G and 100µg/ml streptomycin sulphate) was added. 200 µl of the extract (50 - 350 µg/ml) was then added and incubated for 4 h. Thereafter, the medium was removed and replaced with complete medium (MEM + Glutmax + antibiotics +10 % fetal bovine serum). After 48 h, the cells were evaluated by the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [13]. Briefly, the medium was removed from the cells and 200 µl of 5 mg/ml MTT in phosphate buffered saline (PBS pH 7.4) as well as 200 µl of medium were added to each well containing cells. The multiwell plate was incubated for 4 h and thereafter the medium and MTT were removed and 200 µl of DMSO added to each well, and incubated at 37 ^{o}C for 10 min. The control consisted of 200 µl of the medium only. All determinations were in triplicate. The absorbance of the dissolved solutions were read using a Mindray Plate Reader at 570 nm. Percent cell survival was evaluated as in Eq 3.

Percent cell survival = (Ab /Ao)100 ---------- (3)

where Ab is the absorbance of the sample and Ao is the absorbance of the control.

Determination of total flavonoid content

Colorimetric aluminium chloride method was used for flavonoid determination [14]. Quercetin was used to construct the calibration curve. Ten milligrams of quercetin was dissolved in 80 % ethanol and then diluted to 25, 50 and 100 µg/ml. The diluted standard solution (0.5 ml) was mixed with 1.5 mL of methanol, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured spectrophotometrically (Perkin Elmer UV/Visible spectrophotometer USA) at 415 nm. The amount of 10 % aluminium chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 ml of the extract (100 µg/ml) was reacted with aluminium chloride for the determination of flavonoid content as described above. The total flavonoid content was calculated as mg quercetin equivalents/g of extract powder, by reference to the calibration curve ($y = 0.005x + 0.062$, $R^2 = 0.998$).

Determination of total phenolic content

Total phenolic content was assessed as described by Gulcin *et al* [15]. One millilitre (1 ml) of the extract or standard solution of gallic acid (10, 20, 30, 40, 50 and 100 mg/L) was added to 9 ml of water. One millilitre of Folin Ciocalteu's reagent was added to the mixture and vortexed. After 5 min, 10 ml of 7 % sodium carbonate was added to the mixture and incubated for 90 min at 25 ^{o}C. The absorbance against a reagent blank was determined spectrophotometrically at 750 nm. A reagent blank was prepared and the amount of phenolic compound in the extract was determined as gallic acid equivalent. Total phenol content was obtained from the regression equation (R) for the calibration curve of standard gallic acid ($y = 0.005x + 0.015$, $R^2 = 0.991$) and reported as gallic acid equivalent (GAE)/g of extract powder.

Statistical analysis

The results obtained were expressed as mean ± standard error of mean (SEM) of three determinations. The results of antioxidant activity and cytotoxicity are presented as mean ± SD (n = 3). The data were evaluated through regression analysis using QED statistics program PISCES software (PISCES conservation Ltd, 2007, England). The IC_{50} values, where applicable, were determined by linear regression. Means between treatments were compared by Tukey's Studentized Range Test using one-way analysis of variance (ANOVA).

RESULTS

Antioxidant activity

DPPH radical and $ABTS^{\bullet+}$ radical cation are reactive towards most antioxidants and their decolourization reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate the radical species [8,16].

The IC_{50} values of the extracts, for both DPPH radical scavenging and $ABTS^{\bullet+}$ radical cation scavenging are shown in Table 1. The trend in activity of the extracts in both assays was similar. DCM LF extract had the lowest IC_{50} value in both DPPH and $ABTS^{\bullet+}$ radical scavenging assays and hence the highest radical scavenging activity while DCM BK and Et Ac BK extracts showed very poor radical scavenging activities with $1C_{50}$ values > 1000 µg/ml. There was significant difference ($p \leq 0.05$) in ABTS and DPPH scavenging activity among the extracts. There was no significant difference ($p \geq 0.05$) in the scavenging activity of DPPH and ABTS.

Table 1: The IC_{50} values for the DPPH and $ABTS^{\bullet+}$ radical scavenging activities of the leaf and bark extracts of *Tarchonanthus camphoratus*.

Sample extract	DPPH radical scavenging IC_{50} (µg/ml)[a]	ABTS radical scavenging IC_{50} (µg/ml)[b]
Aq LF	49.03 ± 6.87	48.26 ± 13.26
MET LF	41.70 ± 3.93	60.51 ± 6.42
DCM LF	17.72 ± 5.96	22.27 ± 3.09
MET BK	65.44 ± 7.87	69.45 ± 1.74
DCM BK	>1000	>1000
Et Ac BK	>1000	>1000

[a]*IC_{50} for BHT was 10.88 µg/ml.* [b]*IC_{50} for BHT was 14.54 µg/ml.*

In the reducing power assay, the presence of antioxidants in the extracts led to the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Figure 1 shows dose-response plot for the reducing power of the extracts. DCM LF extract exhibited the strongest reducing ability which was even higher than that of the reference standard, ascorbic acid. There were significant differences between the treatments at $p \leq 0.05$.

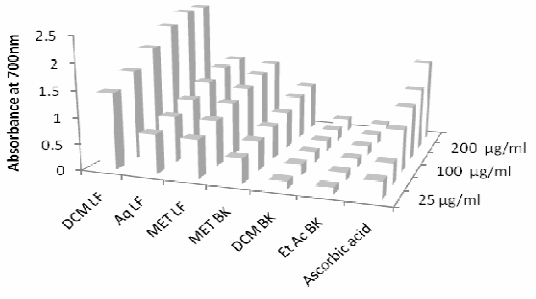

Figure 1: Effects of reducing power on extracts of *Tarchonanthus camphoratus*

Phytochemical analysis

The preliminary phytochemical analysis of the extracts revealed the presence of flavonoids in all the extracts;;saponins were absent only in Aq LF, while phenols were present in all extracts except DCM BK. No tannins were found in the DCM LF and DCM BK; furthermore, alkaloids, terpenoids, cardenolides and cardiac glycosides were absent from all the extracts (Table 2).

All the extracts showed very poor nitric oxide scavenging activity (Table 3) and iron chelating ability (Table 4).

Total phenol and flavonoid contents of the extracts

The extracts had varying amounts of total flavonoids and phenols contents (Table 5). MET LF and DCM LF extracts had the highest flavonoid and phenolic content with the former higher than the latter. The rank order of total flavonoid content was MET LF > DCM LF > Et Ac BK > Aq LF > MET BK > DCM BK while that of total phenol content was MET LF > Aq LF > DCM LF > MET BK > Et Ac BK > DCM BK.

Lethality and cytotoxicity of the extracts

The IC_{50} lethality values of the extracts, i.e., the concentration causing 50% mortality among brine shrimps, are shown in Table 6. DCM BK, MET BK and standard potassium dichromate had IC_{50} values < 100 µg/ml whereas DCM LF, Aq LF and Et Ac BK had IC_{50} values in the range of 100 - 1000 µg/ml; only MET LF extract had an IC_{50} value > 1000 µg/ml.

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Table 2: Phytochemical constituents of the studied extracts of *Tarchonanthus camphorates*

Phytochemical	Aq LF	MET LF	DCM LF	MET BK	DCM BK	Et Ac BK
Alkaloids	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Saponins	-	+	+	+	+	+
Phenols	+	+	+	+	_	+
Tannins	+	+	_	+	_	+
Terpenoids	-	-	-	-	-	-
Cardenolides	-	-	-	-		-
Cardiac glycosides	-	-	-	-	-	-

Note: *+ = presence; - = absence; Aq LF = Aqueous leaf extract: MET LF = methanol leaf extract: DCM LF = dichloromethane leaf extract: MET BK = methanol bark extract: DCM BK : dichloromethane bark extract: Et Ac BK = ethyl acetate bark extract*

Table 3: Nitric oxide inhibition of the leaf and bark extracts of *Tarchonanthus camphorates*

Conc	Nitric oxide inhibition (%)						
	[a]*Aq LF*	[b]*MET LF*	[c]*DCM LF*	[d]*MET BK*	[e]*DCM BK*	[f]*Et Ac BK*	[g]*Vit C*
25	0.53 ± 0.6	0.2 ± 0.4	1.23 ± 0.7	0	0.26 ± 0.3	0.2 ± 0.2	29.8 ± 1.3
50	0.93 ± 0.8	0.65 ± 0.7	2.67 ± 2.0	0. 17 ± 0.3	0.47 ± 0.4	0.27 ± 0.3	41.2 ± 3.0
100	2.03 ± 0.5	2.67 ± 0.3	3.77 ± 1.3	1.83 ± 0.3	1.53 ± 0.5	0.7 ± 0.5	48.7 ± 2.2
150	4.23 ± 1.0	3.47 ± 0.5	7.43 ± 2.8	2.17 ± 0.1	1.97 ± 0.2	1.13 ± 0.2	61.3 ± 2.3
200	6.13 ± 0.5	5.7 ± 0.3	8.6 ± 2.6	3.6 ± 0.7	3.03 ± 0.9	1.23 ± 0.1	68.4 ± 3.3
250	6.63 ± 0.5	6.26 ± 0.2	9.7 ± 0.9	4.2 ± 0.4	4.4 ± 0.7	1.5 ± 0.2	77.1 ± 1.7

Conc = concentration (µg/ml); Vit C = ascorbic acid Data are mean ± SD values of triplicate determinations.

Table 4: Iron chelating activity of the leaf and bark extracts of *Tarchonanthus camphoratus*.

Conc	Iron chelating activity (%)						
	[a]*Aq LF*	[b]*MET LF*	[c]*DCM LF*	[d]*MET BK*	[e]*DCM BK*	[f]*Et Ac BK*	[g]*Na_2EDTA*
25	6.6 ± 2.4	5.5 ± 1.42	6.4 ± 3.7	3.8 ± 0.8	1.22 ± 0.2	8.3 ± 0.7	14.6 ± 3.4
50	9.2 ± 0.3	5.8 ± 1.2	9.3 ± 2.4	9.5 ± 0.5	3.6 ± 0. 4	10.7 ± 1.8	21.1 ± 1.9
100	11.1 ± 2.9	8.2 ± 0.1	9.4 ± 2.4	12.3 ± 0.9	3.8 ± 0.1	14.6 ± 3.5	35.0 ± 0.6
150	10.8 ± 0.3	8.7 ± 0.8	11.4 ± 2.1	12.9 ± 0.5	4.3 ± 0.4	16.2 ± 1.3	39 0 ± 2.9
200	13.6 ± 0.8	9.3 ± 0.4	14.8 ± 1.4	13.8 ± 1.0	4.6 ±0.5	17.2 ± 0.8	60.2 ± 1.1
250	14.7 ± 0.2	11.8 ± 1.7	17.9 ± 3.9	14.1 ± 1.3	5.1 ± 0.2	17.6 ± 0.8	72.0 ± 0.9

Conc = concentration (µg/ml): Data are mean ± SD values of triplicate determinations.

Table 5: Total phenol and flavonoid contents of the extracts of *Tarchonanthus camphorates*

Extract	Flavonoid content (mg/g)	Phenol content (mg/g)
Aq LF	7.8 ± 2.9	22.7 ± 1.6
MET LF	57.4 ± 4.7	26.9 ± 2.1
DCM LF	42.6 ± 1.1	21.7 ± 1.3
MET BK	6.1 ± 3.3	16.7 ± 0.5
DCM BK	4.9 ± 2.9	5.5 ± 0.1
Et Ac BK	9.0 ± 5.4	12.5 ± 1.8
Et Ac BK	9.0 ± 5.4	12.5 ± 1.8

Data are mean ± SD values of triplicate determinations.

The MTT cytotoxicity results for the extracts are summarised in Table 6. All the extracts showed low toxicity against human embryonic kidney cells and human hepatocellular carcinoma cells with high IC_{50} values (> 30 µg/ml). There was no significant difference between the IC_{50} values of various the treatments in both cytotoxicity assays ($p \geq 0.05$).

DISCUSSION

Phenols and polyphenolic compounds, such as flavonoids, are widely distributed in plant, and they have been shown to possess significant antioxidant activities [17]. The MET LF, DCM LF, Aq LF and MET BK showed high DPPH and $ABTS^{\bullet+}$ radical scavenging activities and high total reducing power which suggests presence of electron donating phytochemicals in the extracts. The antioxidant activity of phenols is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [18]. Flavonoids, on the other hand, exhibit their antioxidative action through scavenging or chelating processes [19]. The high phenolic and flavonoid contents of MET LF, DCM LF, Aq LF and MET BK extracts may be responsible for their high total reducing power, DPPH and $ABTS^{\bullet+}$ radical scavenging activities. A linear correlation between the total content of phenolics and antioxidant capacity has been reported [20]. In this study, however, the DCM LF extract which showed the highest antioxidant activity did not have the highest total phenolic contents (Table 4). Lack of correlation between phenolics and antioxidant activity in certain medicinal plants has also been observed [21, 22]. Beside the total phenol and total flavonoids, other phytochemicals like phenolic acid, ascorbic acid and pigments acting individually or synergetically may contribute to the high antioxidant activity [21]. The extracts, however, showed poor nitric oxide inhibition and poor iron chelating ability. Variations in the antioxidant activities of the extracts in the different antioxidant assays may be due to the diversity in the basic chemical structures of their phytoconstituents which possess different degrees of antioxidant activity against free radicals.

The brine shrimp lethality test has been used to determine the cytotoxicity of plant extracts. However, some plants which are known to be toxic to livestock have been found to be non-toxicity to brine shrimps [23]. Cell-line toxicity assessment was therefore carried out alongside brine shrimp assay in the present work for better assessment of cytotoxicity of the plant extracts.. The results in both cytotoxic assays showed that all the extracts had IC_{50} values of > 30 µg/ml and hence the extracts are considered non-toxic according to the criteria of the American National Cancer Institute [21]. On the other hand, Meyer *et al* [12] classified crude extracts and pure substances as toxic if IC_{50} values < 1000 µg/ml and non-toxic if IC_{50} values >1000 µg/ml for assays carried out with brine shrimp.

Based on the foregoing, the extracts evaluated are therefore considered to be potent, non-toxic bioactive compounds.

CONCLUSION

The potential antioxidant capability and weak cytotoxicy of *T. camphoratus* has been established in this study. It's apparent that *T. camphoratus* contains compounds that could quench free radicals and convert them into stable

Table 6: Brine shrimp lethality and MTT cytotoxicity of the extracts of *Tarchonanthus camphorates*

Extract/Standard	Brine shrimp test IC_{50} (µg/ml)	MTT assay IC_{50} (µg/ml)	
		Human embryonic kidney cells	***Human hepatocellular carcinoma cells***
DCM BK	94.1	572.2	1039.6
MET BK	73.7	422.6	1033.1
Et Ac BK	161.0	385.7	629.1
DCM LF	205.0	291.0	856.8
Aq LF	565.1	899.1	700.8
MET LF	1165.6	320.1	469.3
Potassium dichromate	3.75×10^{-1}		

compounds. Although, the detailed mechanisms of their effectiveness in treating cerebral haemorrhage, inflammation and rheumatism are not known, it seems that the antioxidant properties of *T. camphoratus* and its relatively weak toxic activity justify its use in traditional medicine. More detailed chemical and pharmacological understanding of the leaves and bark of the plant is necessary.

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REFERENCES

1. *Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408: 239-247.*
2. *Farombi EO, Fakoya A. Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of Hibiscus sabdarrifa. Mol Nutr Food Res 2005; 49: 1120-1128.*
3. *Hutchings A, Van Staden J. Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1. Plants used for headaches. J Ethnopharmacol 1994; 43: 89-124.*
4. *Anthony CD. Consultant, July Monthly Column for Soap, Perfumery and Cosmetics. 1999 [cited 2011 Feb 26]. Available from: http://www.dweckdata.com/published.*
5. *Milner JA. Functional foods and health promotion. J Nutrition 1999; 129: 7.*
6. *Harbone JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd edn, London; Chapman and Hall: 1998.*
7. *Mensor LI, Menezes FS, Leitao GG, Reis AS, Santos TC, Coube CS, Leita SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res. 2001; 15: 127-130.*
8. *Re RN, Pellegrinni A, Proteggente A, Pannala M, Rice EC. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med. 1999; 26: 1231-1237.*
9. *Marcocci L, Maguier JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract Egb761. Biochem. Res Commun 1994; 201: 748-755.*
10. *Dinis TCP, Madeira VMC, Almeida M. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid-peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 1994; 315: 161-169.*
11. *Oyaizu M. Antioxidant activities of browning reaction prepared from glucosamine. Jnp J Nutr 1986; 44: 307-315.*
12. *Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 1982; 45: 31-34.*
13. *Mosmann T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. J Immunol Methods 1983; 65: 55-63.*
14. *Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 Citrus specis peels and tissues. Pak J Pharm Sci 2009; 22: 277-281.*
15. *Gülçin I, Oktay M, Kıreçci E, Küfrevio lu I. Screening of antioxidant and antimicrobial activities of anise (Pimpella anisum L.) seed extracts. Food Chem 2003; 83: 371-382.*
16. *Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohoni DP, Biyani MK, Mohan H. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 2003; 63: 97-104.*
17. *Van Acker SA, Van Den Berg DJ, Tromp MN, Griffioen DH, Van Bennekom WP, Van Der Vijgh WJ, Bast A. Structural aspects of antioxidant activity of flavanoids. Free Rad Biol Med 1996; 20: 331-342.*
18. *Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem 2001; 49: 5165-5170.*
19. *Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. J Pharm Pharmacol 2003; 55: 131-142.*
20. *Cai YZ, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004; 74: 2157-2184.*
21. *Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, Ercisli S. Total phenolics content,antioxidant and antimicrobial activities of some medicinal plants. Pak J Pharm Sci 2009;22: 102-106.*
22. *Bajpai M, Pande A, Tewari SK, Prakash D. Phenolic contents and antioxidant activity of some food and medicinal plants. Int J Food Sci Nutr 2005; 56: 287-291.*
23. *McGaw LJ, Eloff JN. Screening of 16 poisonous plants for antibacterial, anthelmintic and cytotoxicity activity invitro. S Afr J Botany 2005; 71: 302-306.*
24. *Suffness M, Pezzuto JM. Methods in Plant Biochemistry: Assays for Bioactivity Academic Press, London 1990; pp 71–133.*