The Journal of Phytopharmacology 2013; 2(4): 22-29
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2013; 2(4): 22-29

O. Nana
National Advanced School of Agro-Industrial Sciences, P. O. Box 455 Ngaoundere, Cameroon

J. Momeni
Department of Chemistry, Faculty of Science, National Advanced School of Agro-Industrial Sciences, P. O. Box 454 Ngaoundere, Cameroon

R. Nzangué Tepongning
Department of Chemistry, Faculty of Science, University of Ngaoundere, P. O. Box 454 Ngaoundere, Cameroon

M. B. Ngassoum
Department of Biomedical Sciences, Faculty of Science, University of Ngaoundere, P. O. Box 454 Ngaoundere, Cameroon

Correspondence:
O. Nana
National Advanced School of Agro-Industrial Sciences, P. O. Box 455 Ngaoundere, Cameroon
Tell: 0023799487211
E-mail: om_nana@yahoo.fr

Phythochemical screening, antioxydant and antiplasmodial activities of extracts from *Trichilia roka* and *Sapium ellipticum*

O. Nana, J. Momeni, R. Nzangué Tepongning, M. B. Ngassoum*

Abstract

Antiplasmodial and antioxidant activities of extracts derived from *Trichilia roka* (Chiov) root bark and *Sapium ellipticum* (Hochst) Pax root bark were determined respectively in vivo by Peters et al. methods and by DPPH radical-scavenging and β-carotene-linoleate model systems. Of those extracts, the two methanolic extracts revealed a good antioxidant and the antiplasmodial activities. The *T. roka* methanolic extract was more effective than *S. ellipticum* extract concerning antiplasmodial activity and the *S. ellipticum* extract, the most promising one concerning antioxidant activity with IC_{50} 42.18 µg/ml better than BHT value IC_{50} 83.36 µg/ml taking as control. The high activities of these two plant extracts suggest that they are good source of natural antioxidant and may contain new compounds with lead structures for drug development against malaria.

Keywords: *Trichilia roka, Sapium ellipticum,* Antioxidant activity, Antiplasmodial activity.

Introduction

In Africa society, the use of traditional medicinal plants to fight against different pathologies is transmitted orally from generation to generation. To assure the continuity of this precious cultural heritage, the scientist used to explore the vegetal kingdom, sources of many drugs to discover others metabolites with more activities. Malaria is the world's most important parasitic disease especially when *Plasmodium falciparum* is the causative agent.[1] The lack of new therapeutic targets, unavailability and unaffordability of antimalarial drugs, the resistance of the mosquitoes vector to insecticides and other available antimalarials seriously weaken the control approaches [2] so that news molecules with antimalarial activity are always needs.[3] Natural products and their derivatives have traditionally been the most common source of drugs, and still represent more than 30% of current pharmaceutical market.[4] About 80% of African population consults traditional healers and uses folk medicine to treat various pathologies.[5] A scientific evaluation of these plants is necessary for their incorporation into the officinal health care system in Africa.

T. roka and *S. ellipticum* are two medicinal plants used by the Cameroonian population against malaria and internal diseases. *T. roka* belongs to the Meliaceae family which are known to be a rich source of limonoids.[5]

A number of limonoids isolated from several genera of Meliaceae exhibit antimalarial, antimicrobial, anti-inflammatory, antischistosomal, anticonvulsant, anticancer, antitrypanosomal and antimutagenic activities.[5-7] Three seco-limonoids with insect antifeedant were isolated from *T. roka*.[8] From *S. ellipticum* belonging to Euphorbiaceae family, some terpenoids with antifungal activity were isolated.[9] To the best of our knowledge, no research work concerning antiplasmodial and antioxidant has been done on these two medicinal plants. The aim of this work is to study on extracts from *T. roka* and *S. ellipticum* these activities.

Materials and methods

Plant material

The plants samples were collected in Touboro around Ngaoundere in December 2008 and identified by Pr Mapontmesem, a botanist in the Department of Biological Sciences of the University of Ngaoundere. The samples (root and stem bark) were dried and powdered. An amount of respectively 3.25 kg and 5.50 kg for *Trichilia roka*; 5.10 kg and 2.50 kg for *Sapium ellipticum* were obtained.

Extraction

Samples from roots of the above mentioned plants were extracted by mechanical agitation in conical flask for 3 h per 500 g of powder at 25°C using successively hexane, ethyl acetate and methanol/water (4/1 v/v) for *T. roka* in the same order, ethyl acetate, ethanol and methanol for *S. ellipticum*. Organic solvents were removed by evaporation under reduced pressure with rotavapor. The extracts and their yield obtained are given in table 1.

Phytochemical screening

Phytochemical screenings of these extracts were performed using standard procedures.[10]

Alkaloids test

Each fraction of each specimen (0.5g) was dissolved in 10 ml of distilled water. Then 1 ml of sulphuric acid 2% and 4 ml of Draggendorf reactive was added. The formation of an insoluble brownish substance (crystal) shows the presence of alkaloids.

Flavonoids test

To a test tube containing 0.5 g of each extracts was added 0.5 ml of methanol, two to three pieces of magnesium and 3 ml of concentrated hydrochloric acid drop after drop. The mixture changed from light yellow to brick-red colour, this with effervescence indicated the presence of flavonoids.

Phenols and polyphenols test

In to a test tube containing 2 ml of water, 0.5 g of each fraction was introduced, and then, 3 ml of iron chloride 1%. After having homogenised, some two or three drops of potassium hexacyanoferrate 1% were added. The solution changed from yellow to green indicated the presence of phenol and from yellow to blue the presence of polyphenolic compounds.

Saponins test

Zero point five gram of each extract was dissolved in 5 ml of distilled water. The mixture was vigorously shake and heated until boiling point. Persistent foam indicates the presence of saponins.

Sugar test

Each extracts of 0.5 g was dissolved in 5 ml of distilled water. 5 ml of α-naphtol 1% was added to the preceding solution. To this, 1 ml of concentrated sulphuric acid drizzled down the test tube. The formation of ringlet between the two phases indicated the presence of sugar.

Triterpenoids test

Four or five drops of Liebermann Burchard reagent constituted with: 1 ml of concentrated sulphuric acid, 20 ml of acetic anhydride, and 50 ml of chloroform mixed and shake very well were added to a solution obtained by dissolving 0.5 g of extract in 1 ml methanol, a violet coloration indicate the presence of triterpenoids.

Terpenoids test

To a solution prepared by dissolving 0.5 g of extracts in 5 ml methanol, 5 drops of concentrated sulphuric acid were added, the brick-red coloration indicate the presence of terpenoids.

In vivo antiplasmodial activity

Strain conditioning

Parasites (*Plasmodium berghei*) obtained from the veterinary laboratory of the University of Zaria Nigeria were inoculated to healthy mice in an appropriate laboratory in the following proportion 0.5 ml of contaminated blood. These mice served as bearers of parasites and whenever it is needed, they are killed and their blood is kept in tube containing 0.1 g of powdered heparin and 1 ml of physiological water. These tubes were kept at 4°C in a refrigerator until use.

Treatment of rodents

The two plants extracts were administrated separately to mice during five days from the third day post infection. Tree doses of extract, 500, 250 and 100 mg /kg; were administrated one time daily at eleven o'clock. The concentration of extract was determined based on the weight of the animal, taking into consideration that to a 20 g animal must be administrated 0.2 ml of solution by gavage with a gastric prod.[11] Calculation of the parasite densities was carried out using the OMS (1982) formula as follow: D = 50* number of parasites/number of read scapes for the full bloated parasitary densities.

Qualitative DPPH assay using thin layer chromatography (TLC)

The qualitative test to DPPH consisted of using a chromatographic plate on which spots of the two plant extracts were fixed and develop in ethyl acetate/CH3OH (5/95, v/v). But the *Trichilia roka* hexane extract was instead developed in a solution of hexane/ ethyl acetate (3/2, v/v). Chromatographic plates were then spread with 0.2% DPPH solution and kept in the dark for 30min. Spots initially purple became yellow, thus indicating the antioxidant character of different compounds present in each extract. The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution was decolourised as the colour changes from purple to light yellow. The degree of reduction in absorbance measurement was indicative of the radical scavenging power of the extract. The fast colour change was an indication of the high antioxidant power of the extract.[12]

Quantitative antioxidant test using DPPH radical-scavenging activity

The antioxidant activity of the plants extract using the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical was determined by the method described by Brand.[13] The capturing of free element of a solution of DPPH is observed by the disappearance of the purple colour. That reaction of decolouration, depending on the concentration of the antioxidant substances in the milieu can be followed by spectrophotometry. The protocol of evaluation of these activities is described as follow: The solution of DPPH was prepared by dissolving 4 mg in homogeneous manner in 100 ml of methanol. The fractions were added to DPPH so as to have 1 ml of solution of 0.5000, 0.2500, 0.1250, 0.1000, 0.0500, 0.0250, 0.0125 mg/ml of concentration in the Spectrophotometric curves. These curves were introduced in the spectrophotometer and the optical densities read at 517 nm after 30 min of incubation. The negative witness is a solution of DPPH at 10 % in the methanol and the positive witness is the BHT (Butylhydroxytoluene) were submitted to the same analysis and rigorously in the same conditions with the same concentration as the plants fractions. Inhibition of free radical by DDPH in percent (I %) was calculated by using the formula below:

$$I\% = (A_{blank} - A_{sample})/A_{blank} * 100$$

Where A blank is the absorbance of the control reaction and A sample is the absorbance of the test compound. The optical densities obtained helped to draw the graphic according to the inhibition concentrations and the concentration in inhibitors and to deduct the IC_{50} values.

Quantitative antioxidant test using β-carotene-linoleic acid

In this assay, antioxidant capacity was determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation.[14] A stock solution of β-carotene-linoleic acid mixture was prepared by dissolving 5 mg of β-carotene in 10 ml of chloroform and 250 µl of linoleic acid and 2 g of tween 40 were added. Chloroform was completely evapored using a vacuum evaporator. Then, 1 L of aerated distilled water was added with vigorous shaking to form an emultion. Aliquots (1.5 ml) of this emulsion were then transferred into different test tubes containing different concentrations (0.100, 0.050, 0.025, 0.0125, 0.010 mg/ml) of the different extracts. As soon as the emulsion was added to each tube, the zero time

absorbance was measured after 5 minutes in a water bath at 490 nm using Metertech Germany Spectrophotometer UV/vis sp 8001. Thereafter, the tubes were placed at 50°C in a bath water for a period of 02 h before re-measuring. A blank treatment served as the control for the Spectrophotometric readings. The same procedure was repeated with the synthetic antioxidant BHT, as positive control. Antioxidant activity (AOA) was calculated using the following equation: %AOA = (A2H/Ai)*100; with % AOA, antioxidant activity; A2H, β-carotene content after 2 h of assay; Ai, initial β-carotene content.[15]

Results and discussion

The extraction yield and the result of the phythochemical screening of different extracts from *S. ellipticum* and *T. roka* are furnished in Table 1–2. Extracts obtained are constituted of compounds belonging to different classes of secondary metabolites where the major constituents are flavonoids, polyphenols, terpenoids and triterpenes. The two plants did not reveal significant differences in their constituents. This result also shows that *S. ellipticum* extract contained others families compounds than triterpenoids obtained by Kisangau.[6]

Table 1: Extracts from *Sapium ellipticum* and *Trichilia roka* root bark

Plants	Weight of plant	Extraction solvent	Extract's weight of (g)	Yield (%)	IC_{50} (µg/ml)
T. roka (Chiov)	500 g	Hex (a)	8.65	1.73	$7\ 10^{-2}$
	500 g	EtOAc (b)	192.70	38.55	$7\ 10^{-2}$
	500 g	MeOH(c)	194.00	38.80	$4\ 10^{-2}$
S. ellipticum (Hochst.)	500 g	EtOAC	97.74	19.55	$2\ 10^{-2}$
	500 g	EtOAc (EtOH) d	178.53	35.70	$1.2\ 10^{-2}$
	500 g	MeOH	58.51	11.70	$8.4\ 10^{-3}$

Values of IC50 are expressed as mean; n = 4.
a: hexane; b: ethyl acetate; c: Methanol; d: ethyl acetate extract from obtain from ethanol extract

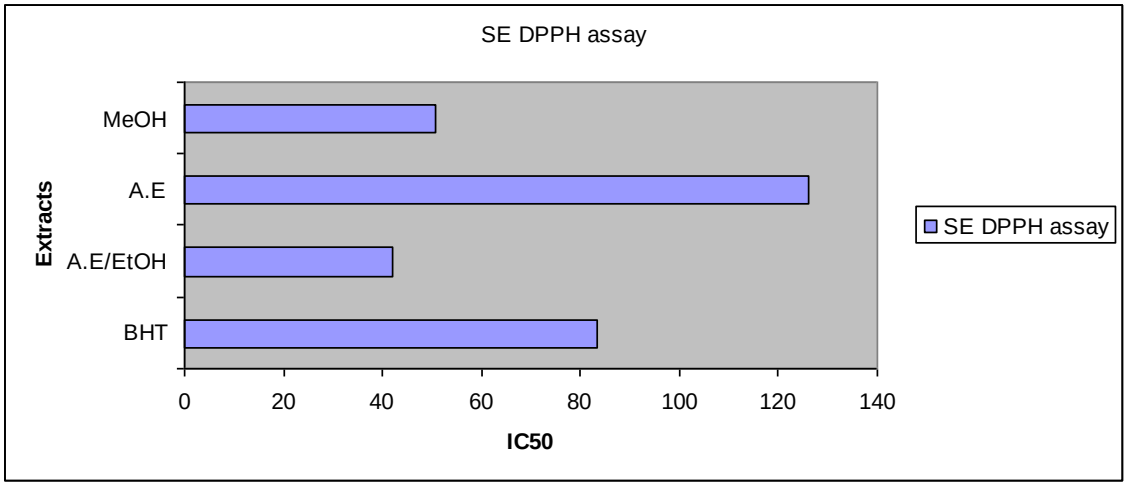
Table 2: Phythochemical constituents of different extracts from *S. ellipticum* and *T. roka*

Chemical components	Plants					
	S. ellipticum			*T. roka*		
Extract codes	EtOAC	EtOH (EtOAc)	MeOH	Hex	EtOAc	MeOH
Alkaloids	-	-	-	-	-	-
Flavonoids	+	++	++	+	+	++
Phenols / polyphenols	+++	++	++	+	+	++
Saponins	-	-	-	-	-	-
Sugars	-	-	-	-	-	-
Terpenoids	+	+++	++	++	++	++
Triterpenoids	+	++	++	+	+	+

MeOH: methanolic extract; EtOAc: ethyl acetate extract ; Hex : hexane extract; EtOAc (EtOH) : ethyl acetate extract from obtain from ethanol extract; - : not detected; + : minor presence; ++ : presence; +++: abundent presence

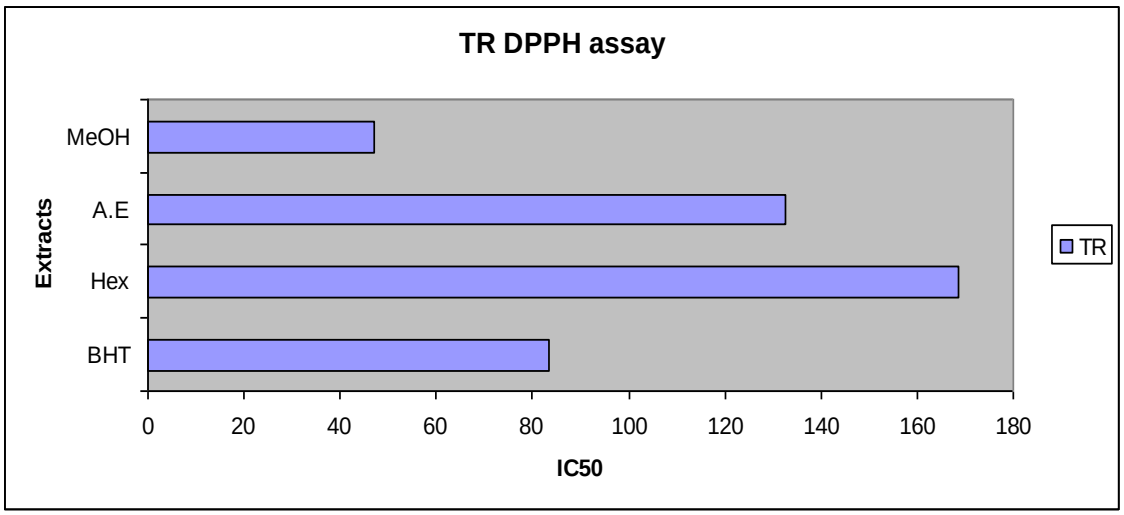
The extracts were subjected to antioxidant activities screening using two complementary test systems namely DPPH free radical scavenging and β-carotene/linoleic acid. Figures 1 and 2 shows the results of free radical scavenging capacities of the extracts which shows that, ethyl acetate fraction for S. ellipticum and hexane fraction for *T. roka* were more powerful than BHT taking as control. The addition of the two extracts and BHT at various concentrations prevented the bleaching of β-carotene to different degrees. The β-carotene in this model system undergoes rapid discoloration in the absence of an antioxidant. This is because of the couple oxidation of β-carotene and linoleic acid, which generates free radicals.

Figure 1: Free radical scavenging capacities of the *S. ellipticum* extracts using DPPH assay



SE: *S. ellipticum*; MeOH: *S. ellipticum* methanolic extract; A.E: *S. ellipticum* ethyl acetate extract; A.E/EtOH: *S. ellipticum* ethyl acetate extract obtain from ethanol extract; BHT: Standard butylhydroxytoluene

Figure 2: Free radical scavenging capacities of the *T. roka* extracts using DPPH assay



TR: *T. roka*; MeOH: *T. roka* methanolic extract; A.E: *T. roka* ethyl acetate extract; Hex: *T. roka* hexane extract; BHT: butylhydroxytoluene

According to this statement, *S. ellipticum* and *T. roka* extracts prevented discoloration of the couple β-carotene and linoleic acid. Table 3 below show the values of IC_{50} for different extracts obtained by using the curve of the variation of inhibition percentage in function of concentration. The free radical scavenging activity of MeOH extract was the highest one compare to all other extracts (IC_{50}= 0.0008 µg/ml) for *S. ellipticum* and IC_{50} = 0.007 µg/ml for *T. roka* extract. But these result were lower than the BHT activity with the IC_{50} = 2.2 10-10 µg/ml taking as control. Polar extracts exhibited stronger activity than non polar extracts. These activities could be related to theirs phenolic constituents. Antiplasmodial test result is given in Table 4. Decrease in parasites from the first to the fifth day of treatment, indicates the methanolic extract of *T. roka* was potentially active against *P. berghei* and this activity grows with concentration. The 500 mg/kg, was deathly to the mice. So this dose could be potentially toxic for the mice. Further studies would be carrying out for toxicity activity.

Table 3: Inhibition percentage in function of the concentration for *Sapium ellipticum* and *T. roka* by using β carotene and linoleic acid system

	***Sapium ellipticum* extracts**					
	AE/EtOH		**AE**		**MEOH**	
Conc. (µg/ml)	**Inhibition percentage**	**IC_{50}**	**Inhibition percentage**	**IC_{50}**	**Inhibition percentage**	**IC_{50}(µg/ml)**
0,1000	66.32	$1.2\ 10^{-2}$	85.85	$2\ 10^{-2}$	76.57	$8.4\ 10^{-3}$
0,0500	65.02		75,57		76.83	
0,0250	51.60		63.90		61.33	
0,0125	48.68		54.05		56.93	
0,0100	37,04		42.30		49.70	
	***Trichilia roka* extracts**					
	HEX		**AE**		**MEOH**	
Conc. (µg/ml)	**Inhibition percentage**	**IC_{50}**	**Inhibition percentage**	**IC_{50}**	**Inhibition percentage**	**IC_{50}(µg/ml)**
0,1000	65.87	$7\ 10^{-2}$	66.84	$7\ 10^{-2}$	66.53	$4\ 10^{-2}$
0,0500	52.04		60.42		63.53	
0,0250	49.65		56.49		61.21	
0,0125	41.32		49.49		55.02	
0,0100	41.04		48,65		48.45	
BHT						
						IC_{50}(µg/ml)
Conc. (µg/ml)	**0.1000**	**0.0500**	**0.0250**	**0.0125**	**0.0100**	$2.2\ 10^{-10}$
Inhibition percentage	82.20	80.87	80.42	79.10	78.10	

Values of Inhibition percentage are expressed as mean; **n** = 3; **A.E/EtOH** : *S. ellipticum* ethyl acetate extract from obtain from ethanol extract; **MeOH**: *S. ellipticum* methanolic extract; **A.E** : *S. ellipticum* ethyl acetate extract; **Hex** : *T. roka*; hexane extract; **AE** : *T. roka* ethyl acetate extract ; **MeOH**: *T. roka* methanolic extract; **BHT**: Butylhydroxytoluene; Concentration is given in µg/ml.

Table 4: In vivo antiplasmodial test result of parasitemia and parasites density during the five days of treatment

	Dose	**Days of treatment**	**Parasitemia ±SEM**	**Parasites density ±SEM**
Methanolic extract	100 mg/kg	J1	36.3±3.2	181.4±16.3
		J2	18.5±4.0	92.7±20.0
		J3	12.5±2.3	62.4±11.3
		J4	5.6±2.0	28.2±10.0
		J5	1.6±0.8	7.8 3±3.8
	250 mg/kg	J1	54.0±1.5	269.9±7.5
		J2	28.3±2.8	141.5±13.8
		J3	14.1±3.0	70.6±15.0
		J4	4.6 1±3.0	22.8±5.3
		J5	1.0 0.±0.0	5.0±0.0

Values are expressed as mean ± SEM; **n** = 3. **J1**: Day one of treatment; **J2**: Day two of treatment; **J3**: Day three of treatment; **J4**: Day four of treatment and **J5**: Day five of treatment.

Conclusion

T. roka and *S. ellipticum* are known as medicinal plants that are use in various ways in West Africa countries. *T. roka* is a rich source of limonoids and seco-limonoids. The present work indicates that methanol extract of *Trichilia roka* root bark are potentially active against *P. berghei*. *T. roka* root bark and *S. ellipticum* extracts have also proved antioxidant activities supporting traditional uses of these plants by the populations against malaria and internal infections and suggest flavonoïds, polyphenols, terpenoids or triterpenoids present in these plant extracts could be the active compounds. Further studies will be conducted to isolate and purify active compounds from these extracts and the study of toxicity of *T. roka* root bark methanol extract 500 mg/kg dose.

References

1. Fisher P.R. and Biakek R.. Prevention of malaria in children. Clin. Infect. Dis.2002;34:493-498.

2. El Tahir A., Satti G.M.H, Khalid S.A.. Antiplasmodial activity of selected Sudanese medical plants with emphasis on Maytenus senegalensis (Lam.) Exell. J. Ethnopharmacol.1999;64:227-233.

3. Soh P.N., Benoit-Vical F.. Are West African plants a source of future antimalarial drugs? J. Ethnopharmacol.2007;114:130-140.

4. Kirk P.. Stitching together naturally. Nature 2002;1:748.

5. Komane B.M., Olivier E.I., Viljoen A.M.. Trichilia emetica (Meliaceae)-A review of traditionnal uses, biological activities and phytochemistry. Phytochem. Lett. 2011;4:1-58.

6. Germano M.P., D'Angelo V., Sanogo R., Catania S., Alma R, DePasquale R., Bisignano G.. Hepatoprotective and antibacterial effects of extracts from Trichilia emetica Vahl.(Meliaceae). J. Ethnopharmacol. 2005;96:227-232.

7. Germano M.P., D'Angelo V., Sanogo R., Catania S., Alma R., DePasquale R., Bisignano G.. Evaluation of the antioxidant properties and bioavaibility of free and bound phenolic acids from Trichilia emetica Vahl. J. Ethnopharmacol. 2006;105:368-375.

8. Nakatani M., Niyako O., Takashi T., kosei M., Hideo N., Tsunao H.. Isolation and structures of three seco-limonoids, insect antifeedants from Trichilia roka (Meliaceae). Heterocycle 1984;22(10).

9. Kisangau D.P., Hosea K.M., Lyaruu H.V.M., Joseph C.C., Mbwambo Z.H., Masimba P.J., Devkota K.P. and Sewald N.. Screening of traditionally used Tanzanian medicinal plants for antifungal activity. Pharm. Biol. 2009;47(8):708-716.

10. Trease, G.E. and Evans W.C.. Pharmacognosy. 13th edn. Baillière Tindall, London, 1989.

11. Prozesky E.A., Meyer J.J.M., Louw A.I.. In vitro antiplasmodial activity and cytotoxicity of

ethnobotanically selected South African plants. J. Ethnopharmacol. 2001;76:239-245.

12. Miller H.E.. A simplified method for the evaluation of antioxidant. J. Am. Oil Chem. Soc. 1971; 18:439-452.

13. Brand-William W., Cuvelier M.E., Berset C., Wiss L.. Use of a free radical method to evaluate antioxidant activity. Food Science Technol. 1995;28:25-30.

14. Dapkevicius A., Venskutonis R., Van Beek T.A., Lenssen P.H.. Antioxidant activity of extracts obtained by different isolation procedures from some aromatics herbs growing in Lithuania. J. Sci. Food Agric. 1998;77:140-146.

15. Avlessi F., Dangou J., Wotto V.D., Alitonou G.A., Sohounhloue D.K., Menut C.. Propriétés antioxydantes de l'huile essentielle des feuilles de Clausena anisata (Wild) Hook. C. R. Chimie 2004;7:1057-1061.

16. Tepe B., Dafetera D., Sokmen A., Sokmen M., Polissiou M.. Antimicrobial and antioxydant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae). Food Chem. 2005;90:333-340.

17. Tepe B., Dafetera D., Sokmen A., Sokmen M., Polissiou M.. Antimirobial and antioxidant activities of the essential oil and various extracts of Cyclotrichiun origanifolium (Labill.). J. food Eng. 2005;69:335-342.