ANTIOXIDANT PROPERTIES OF EXTRACTS FROM *Tacazzea apiculata* OLIV. (PERIPLOCACEAE)

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Abstract: The root bark of *Tacazzea apiculata* Oliv. (Periplocaceae) is used in traditional medicine to treat hemorrhoids, inflammations and cancers. Free radical scavenging effects of the extracts obtained from the root of *T. apiculata* were studied using 2,2-Diphenyl-1-picrylhydroxyl **(**DPPH) radical and 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays with the view to justify the traditional uses of the plant. The results revealed that the root of *T. apiculata* is rich in bioactive compounds. The median effective concentration EC_{50} of the Tacazzea hexane extracts was 1380 µg/ml in the ABTS assay, that of Tacazzea ethyl acetate extract were found to be 125 and 5.9 µg/ml for DPPH and ABTS assays, respectively and Tacazzea methanol extract gave EC_{50} of 75.0 and 6.2 µg/ml DPPH assay and ABTS assays, respectively. These results could serve as bases for the uses of *T. apiculata* in traditional medicine and suggest its potential of as a good source of drugs.

Keywords: Antioxidants, **e**xtracts, DPPH, *Tacazzea apiculata*, periplocaceae

Introduction

The plant *Tacazzea apiculata* Oliv is a woody climber widely distributed in tropical Africa. In South Africa, the twig is powdered and taken in milk or water as "tonic" to improve the general health condition of the body. The leaf is used for skin diseases (Burkill, 1997). The flowers are considered edible (Peters *et al*., 1992). In Nigeria it is known by the Hausa tribe as *yadiyar kada* (meaning: crocodile vine) where the powdered root mixed with milk or honey is taken orally as remedy to relief pains in pile, inflammatory conditions and cancers (Abubakar *et al.,* 2007).

Botanical studies of *Tacazzea apiculata* showed that the leaf has dorsiventral lamina with characteristic anatomical features which include unicellular, uniserrate and non-glandular trichomes, anomocytic stomata on the leaf (Ahmed *et al.,* 2003). Preliminary phytochemical screening of methanol extract of the root bark revealed the presence of triterpenoids/steroids, saponins, and flavonoids in the plant and the extract gave a medium lethal dose (LD_{50}) of 118.3 $mgkg^{-1}$ *i.p* (Ahmed *et al.,* 2006). Ahmed *et al.* (2010) also reported the presence and concentration of some micronutrients, essential elements including Mn (1110±28.4 ppm) in leaves, Cu (28.2±6.47 ppm) in twigs, Fe (1310±26.6 ppm) and Zn (177±6.69 ppm) in root of *T. apiculata*.

The aim of this work is to establish the free radical scavenging effects of the crude methanol extract and to provide a chromatographic profile for the bioactive constituents in the root of the plant with the view to justify its traditional uses as remedy for inflammation and other diseases.

Materials and Methods

Preparation of extracts from T. apiculata root powder

The powdered drugs (1 kg) were extracted sequentially with hexane (3 L), ethyl acetate (3 L) and methanol (3 L) by maceration/electric shaking for a period of 48 hrs in each case. The extracts were concentrated under reduced pressure using rota-vapour. The TLC profiles of the extracts were studied on pre-coated plates (Merck F_{254}) and visualizing with appropriate spray-reagents (Wagner and Bladt 1996; Paschal *et. al.*, 2002).

Solvents/chemicals

General purpose solvents used were obtained from Sigma–Aldrich (St. Louis, MO, USA). Free Radicals and Materials for Antioxidant Studies used were 2, 2-Diphenyl-1-Picrylhydraxyl (DPPH) Sigma–Aldrich (St. Louis, MO, USA). TLC plates (pre-coated silica on aluminium/glass backing) from Merck (Germany). TLC Solvent Systems developed included Hexane: Ethyl acetate (9:1) or (7:3), Ethyl acetate: Methanol: Water (100:13.5:10). While TLC spray reagents used included Anisaldehyde spray (alcohol 90 ml + Conc. H_2SO_4 5 ml + anisaldehyde 5 ml) and DPPH Spray (DPPH 0.2 mg + alcohol 100 ml).

TLC profiling of the crude extracts

Chromatographic analysis of the three extracts Tacazzea hexane extract (TZHE), Tacazzea ethyl acetate extract (TZEE) and Tacazzea methanol extract (TZME) that were carried out on TLC plate (silica gel) using appropriate solvent systems *i.e.* hexane: ethyl acetate (9:1) or (7:3) and ethyl acetate: methanol: water (100:13.5:10). The plates were sprayed with anisaldehyde/sulphuric acid as detecting reagent followed by heating at 110^0 C. Chromatograms was scanned accordingly.

Results and Discussion

Chromatograms of different extracts of Tacazzea apiculata Oliv.

The chromatogram (Plate I) of the three extracts using hexane: EtOAc (9:1) and EtOAc: MeOH: H_2O (100:13.5:10) as solvent systems with anisaldehyde/sulphuric acid as spray reagent revealed that the hexane: EtOAc (9:1) is suitable for Extract TZHE (Plate Ia). The plate shows abundant spots which are purple in colour indicating the presence of terpenoids/steroids (Wagner and Bladt, 1996; Eloff *et al.,* 2011). The TZEE and TZME are better separated using EtOAc: MeOH: H_2O (100:13.5:10) (Plate Ib). The spots in these extracts are greenish or greenish blue in colour with few that were light brown in colour. These colours are attributed to phenolic compounds (Paschal *et al.,* 2002) which are also expected in due to the moderately and high polarities of the two extracts, respectively. Both TZEE and TZME exhibited the capacity to bleach DPPH (Plate II) which can serve as a good preliminary result for antioxidant potential of the extracts/ compounds (Oke & Hamburger, 2002; Budzianowski & Budzianowska, 2006). The TLC-DPPH antioxidant assay could also serve as a good guide for monitoring isolation of antioxidant compounds from crude extracts/fraction using preparative TLC or column chromatography.

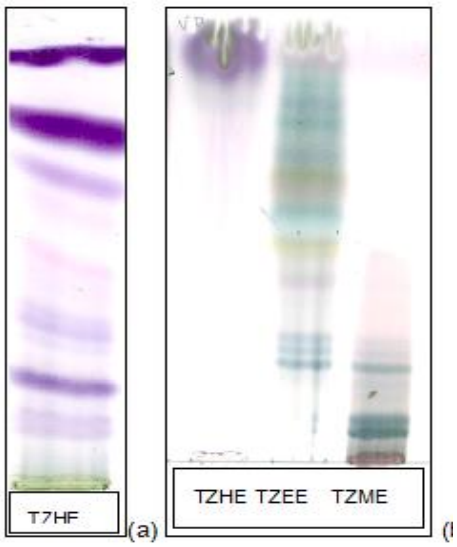


Plate I: TLC profile of *Tacazzea* hexane fraction (TZHE), ethyl acetate fraction (TZEE) and methanol fraction (TZME) with solvent systems (a) Hexane:EtOAc (9:1) and (b) EtOAc:MeOH:H_2O 100:13.5:10. Detected with anisaldehyde spray

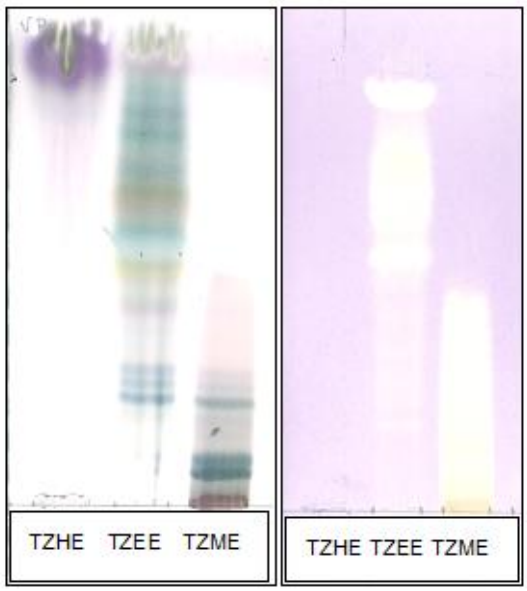
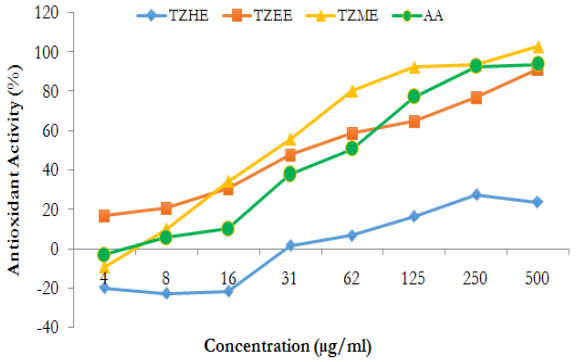


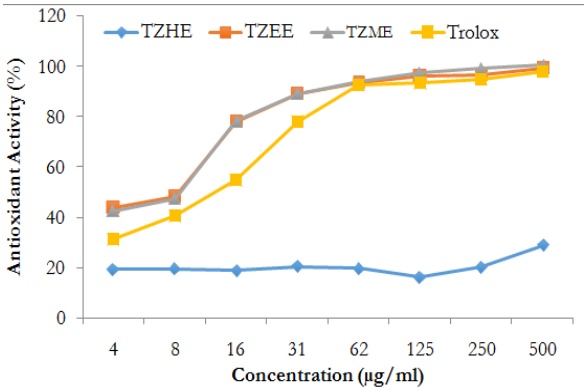
Plate II: TLC profile of *Tacazzea* hexane fraction (TZHE), ethyl acetate fraction (TZEE) and methanol fraction (TZME) with solvent systems EtOAc:MeOH:H_2O (100:13.5:10) sprayed with anisaldehyde spray reagent compared to DPPH/methanol spray reagent

Effects of T. apiculata Crude Extracts on DPPH

The antioxidant capacity or free radical scavenging activity of *T. apiculata* was studied because free radicals and oxidative stress are known to be associated with inflammations (Winrow *et al.*, 1993). Moreover, free radical scavengers have been reported to suppress upregulation of cyclooxygenase (COX) and subsequently reduce inflammation (Feng *et al.*, 1995; Kumagai *et al.*, 2000). The ethyl acetate extract and methanol extract of *T. apiculata* exhibited significant dose dependent scavenging of DPPH activity ($P \leq 0.05$), with a median effective concentration (EC_{50}) of Ethyl acetate (TZEE) and methanol extract (TZME) to be 125 and 75 µg/mL, respectively against ascorbic acid 35.3 µg/mL. Fig. 1 presents the antioxidant activity of the three crude extracts of *T. apiculata.*



TZHE: Tacazzea hexane extract, TZEE: Tacazzea ethyl acetate extract, TZME: Tacazzea Methanol extract
Fig. 1: Effects of *T. apiculata* crude extracts on DPPH



TZHE: Tacazzea hexane extract, TZEE: Tacazzea ethyl acetate extract, TZME: Tacazzea Methanol extract
Fig 2: Effects of *Tacazzea* crude extracts on ABTS radicals

Effects of Tacazzea apiculata crude extracts on ABTS radicals

The ethyl acetate extract and the methanol extracts of *T. apiculata* also inhibited the ABTS• radical (Fig. 2). The median effective concentration (EC_{50}) of the ethyl acetate extract was 5.9 µg/mL and that of the methanol extracts was 5.9 µg/mL.

Table 1: Summary of median effective concentrations (EC_{50}) of *T. apiculata*

Sample	EC_{50} (µg/ml)	
	DPPH assay	ABTS assay
TZHE	-	1380
TZEE	125	5.9
TZME	75.0	6.2
Ascorbic	35.3	-
Trolox	-	1.8

TZHE: Tacazzea hexane extract, TZEE: Tacazzea ethyl acetate extract, TZME: Tacazzea Methanol extract

Table 2: Trolox equivalent antioxidant capacity (TEAC) values of *T. apiculata*

Sample	Slope	TEAC	r^2
TZHE	0.025	0.00	0.804
TZEE	1.719	0.21	0.875
TZME	1.761	0.22	0.869
Trolox	8.129	1.00	0.911

TZHE: Tacazzea hexane extract, TZEE: Tacazzea ethyl acetate extract, TZME: Tacazzea Methanol extract, r^2: regression coefficient

Trolox equivalent antioxidant capacity of T. apiculata

The trolox equivalent antioxidant capacity (TEAC) measures antioxidant activity in relation to trolox, a water-soluble vitamin E analogue. The TEAC assay involves prior generation of the free radical through reaction between 2, 2´-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and potassium sulphate ($K_2S_2O_8$) to form a mono-cation which is a blue/green chromophore $ABTS^{\bullet+}$. This radical is reduced by antioxidants to a colourless ABTS, a reaction that depends on the concentration of the antioxidant. The extent of decolourisation as percentage antioxidant activity of sample was calculated relative to the reactivity of trolox under the same conditions (Re *et al*., 1999).

The trolox equivalent antioxidant capacity (TEAC) was calculated as a ratio of the gradient on extract equation to the gradient on trolox equation. The extract has equal capacity with trolox if its TEAC value is equal to 1. From Table 2, the extracts have TEAC less than 1 (as in Trolox).

Free radical scavenging activity of T. apiculata

One difficulty in assessing antioxidant activity is the option of which method to use. Different methods seem to give different values and methods have their advantages and disadvantages. To measure the antioxidant capacities of *T. apiculata* extracts, the TLC-DPPH assay was first carried out as preliminary test which was followed by two different *in vitro* assays ($DPPH^{\cdot}$, TEAC /$ABTS^{\cdot+}$). These procedures measure consumption of a stable free radical (DPPH· or $ABTS^{\cdot+}$) following addition of the tested compound. Owing to their advantage of being very simple to carry out, they are widely used in the assessment of radical scavenging activity in biological samples and both of them are characterized by excellent reproducibility under certain assay conditions. However, DPPH· and TEAC/$ABTS^{\cdot+}$ assays may show significant differences in their response to antioxidants. The TEAC has the major advantage that it is applicable to both aqueous and lipophilic systems (Re *et al.*, 1999).

The preliminary antioxidant screening of *T. apiculata* using TLC-DPPH method indicated that the ethyl acetate extract TZEE and the methanol extract TZME possess the potential to scavenge free radicals (Plate II). From the results (Fig. 1), the activities of the ethyl acetate extract may be attributed to presence of bioactive compounds such as phenolics which are very good scavengers of free radicals. The EC_{50} were obtained by linear regression for the ethyl acetate extract and methanol extracts to be 74 and 9.2 µg/ml, respectively. Even 500 µg/ml of the hexane extract did not scavenge 50% of the radical hence EC_{50} was not calculated for the extract. The chromatogram (Plate II) of the TLC-DPPH assay suggested that the methanol may contain high concentrations of polyphenolic compounds. In the ABTS assay, the antioxidant activity of *T. apiculata* was presented as trolox equivalent antioxidant capacity (TEAC).

Conclusion

The results of these studies have provided some scientific bases for the use of *Tacazzea apiculata* in traditional medicine for the treatment of inflammations and other diseases. The plant can be a good candidate for isolation of bioactive compounds.

Acknowledgement

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References

Abubakar MS, Musa AM, Ahmed A & Hussaini IM 2007. The perception and practice of traditional medicine in the treatment of cancers and inflammation by Hausa and Fulani tribes of Nigeria. *J. Ethnopharm.*, 111: 625-629.

Ahmed A, Abdurahman EM, Abubakar MS & Yusuf MK 2003. Botanical studies on the leaves of *Tacazzea apiculata* Oliv. *Nig. J. Phytomed. and Therap.*, 8: 16-19.

Ahmed A, Abdurahman EM, Ilyas N & Yaro AH 2004. Antinociceptive and anti-inflammatory activities of ethanolic extract of the root bark of *Tacazzea apiculata* Oliv. (Periplocaceae). *A paper presented at the 8th Annual Scientific Conference of the Neuroscience Society of Nigeria.* Zaria 22nd -26th November 2004: p. 30.

Ahmed A, Yaro AH & Akpjeshi AM 2006. Determination of the anticonvulsant activity of the methanolic extract of the root bark of *Tacazzea apiculata* Oliv. (Periplocaceae). *2nd Annual Scientific Conference of the International Research and Development Institute* (IRDI). Nsukka: p30.

Ahmed A, Ilyas N, Ibrahim H, Musa KY & Yaro AH 2007. Analgesic effects of *Tacazzea apiculata* Oliv. *Nig. J. Pharm. Sci.*, 6(2): 136-140.

Ahmed A, Ibrahim H, Ilyas N, Musa KY & Saidu MU 2010. Essential and trace elements of *Tacazzea apiculata* Oliv. (Periplocaceae). *Int. J. Pharm. Res. & Innovs.*, 1: 1-3.

Budzianowski J & Budzianowska A 2006. Chromatographic and spectrophotometric analyses of the DPPH Free radical scavenging activity of the fractionated extracts from *Lamium album* L., *Lamium purpureum* L. and *Viscum album* L. *Herba Polonica*, 52(1/2): 51-57.

Burkill HM, 1997. *The Useful Plants of West Tropical Africa.* 2nd edn. Vol 4 Royal Botanical Gardens Kew, p. 430.

Eloff JN, Ntloedibe DT & van Brummelen R 2011. A simplified but effective method for the quality control of medicinal plants by planar chromatography. *Afr. J. Trad. Compl. Altern. Med*., 8(S): 1-12.

Fayed SA 2009. Antioxidant and anticancer activities of *Citrus reticulate* (Petitgrain Mandarin) and *Pelargonium graveolens* (Geranium) essential oils. *Res. J. Agric & Biol. Sci*., 5: 740-747.

Kumagai T, Kawamoto Y, Nakamura Y, Hatayama I, Satoh K, Osawa T & Uchida K 2000. 4-Hydroxy-2-nonenal, the end product of lipid peroxidation, is a specific inducer of cyclooxygenase-2 gene expression. *Biochem. and Biophys. Res. Comm.*, 273: 437–441.

Oke JM & Hamburger MO 2002. Screening of Some Nigerian Medicinal Plants for Antioxidant Activity Using 2, 2, Diphenyl-Picryl-Hydrazyl Radical. *Afr. J. Biomed. Res.* 5: 77 – 79.

Paschal ME, Correetero ME, Sloving KV & Villar A 2002. Simplified screening by TLC of plant drugs. *Pharm Biol.*, 40(2): 139-143.

Peters CR, O'Brien EM & Drummon RB 1992. *Edible Wild Plants of Sub-Saharan Africa.* Royal Botanic Gardens Kew, p. 67.

Re R, Pellegrini N, Proteggente A, Pannala A &Yang M 1999. Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Rad. Biol. and Med.*, 26: 1231-1237.

Wagner H, & Bladt S *1996. Plant Drug Analysis. A Thin Layer Chromatography Atlas*. 2nd edn., Springer Verlag, Berlin Heidelberg New York, pp. 16-67.

Winrow VR, Winyard PG, Morris CJ & Blake DR 1993. Free radicals in inflammation: second messengers and mediators of tissue destruction. *British Med. Bull.*, 49: 506–522.