African Journal of Biochemistry Research Vol. 6(8), pp. 98-104, 30 April, 2012
Available online at http://www.academicjournals.org/AJBR
DOI: 10.5897/AJBR12.001
ISSN 1996-0778 ©2012 Academic Journals

*Full Length Research Paper*

# *In-vitro* assessment of antioxidant potential and phenolic capacity of *Temnocalyx obovatus* leaves

**P. Dzomba*, E. Togarepi and C. Mahamadi**

Chemistry Department, Faculty of Science Education, P. Bag 1020, Bindura, Zimbabwe.

Accepted 4 April, 2012

***Temnocalyx obovatus*** **is widely found in many areas in Zimbabwe, Nigeria and Zambia where it is used to treat a variety of ailments. Phenolic compounds in *T. obovatus* assessed by Folin-Ciocalteu method varied from 1.18 to 2.89 mg tannic acid $g^{-1}$. Acetone extract showed the greatest flavonoids, flavonols and proanthocyanidins content 5.12, 4.13 and 3.07 mg catechin $g^{-1}$ respectively. 2, 2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\cdot}$) scavenging effect, Ferric reducing ability and egg lipid peroxidation inhibition assays were adopted to evaluate antioxidant activity. Acetone extracts exhibited the greatest antioxidant activity for all the assays, followed by methanol extracts. Hexane extracts showed the weakest activity. Correlation studies showed that antiradical activity is explained more by flavonols and proanthocyanidins (73%) and flavonoids (71%) and weakly by phenolic contents (43%). The results revealed significant phenolic capacity and antioxidant potential of *T. obovatus* to warrant its use as a source of antioxidants. It can be added to foods, such as, meat and soups for enhancement of nutritional content and promotion of health.**

**Key words:** Antioxidant activity, phenolics, *T. obovatus*.

## INTRODUCTION

Researches in traditional food and medicinal plants have increased previously (Prasad et al., 2011, 2010; Miliauskas, 2006). This is as a result of the need to find new sources of natural antioxidants which are believed to be safer than their synthetic counterparts. Synthetic antioxidants are cheap and very efficient however they have been implicated in the etiology of cancers such that some countries are now banning or enforcing restrictions to their use (Bergfeld et al., 2005; Wangensteen et al., 2004). Tert-butyl-hydroquinone (TBHQ) a synthetic antioxidant widely used to prevent rancidity in oils and lipid based foods is banned in Japan and certain European countries (Shahidi and Wanasundara, 1997). Since the report by Chipault et al. (1956) on antioxidant activity of 72 different spices, many researches reports the possibility of using plant extracts as potent antioxidants to prevent food rancidification and diseases as a result of oxidative stress. Rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*) and oregano (*Origanum vulgare*) were found to be effective sources of antioxidants resulting in their application in nutraceutical and pharmaceutical industry (Miliauskas, 2006). In Zimbabwe, there are many unstudied traditional food and medicinal plants which can be sources of the increasingly sort natural antioxidants. The advantage of these plants is that they have been used for several years as either medicines to treat ailments or as food sources therefore placing a plus sign to their safety concerns. *Temnocalyx obovatus* (Figure 1) is an example of such plant. The plant is widely found in many areas in Zimbabwe, Nigeria and Zambia. It falls under the family Rubiaceae. It is an under shrub, sometimes in clumps, it is 2 to 3 ft. high, with 3-angled slightly pubescent stems; flowers greenish with darker apex, solitary or few of them are found together in the axils. In many African societies, its leaves are fused to make a particular kind of tea. It is taken as a medication or merely as a beverage. Its leaves are used to treat a variety of ailments such as, boosting the immune system, abdominal pain, including menstrual pain, backache and chest pains, strengthening bones, building stamina, coughs and flu, whooping cough, muscular toning, oedema, constipation, ulcers, asthma, loss of appetite and hypertension. *T. obovatus* is used to

*Corresponding author. E-mail: pdzomba@gmail.com or pdzomba@buse.ac.zw. Tel: +263773474525. Fax: +263 7536.

**Figure 1.** Scientific name: *Temnocalyx obovatus* (family Rubiaceae).

treat diarrhoea in chickens and stomach disorders in turkeys, goats and cows (Mahamadi et al., 2011). It is also used as an antidote for poisoning which includes snakebites (Mahamadi et al., 2011). These properties are akin to the presence of polyphenols (Maestri et al., 2006), thus this study sought to investigate the antioxidant potential and phenolic capacity of *T. obovatus.*

## MATERIALS AND METHODS

### Plant material

The leaves were collected in April, June and July 2010 from Mashonaland East in Chivhu, Zimbabwe. The plants were identified by their characteristic leaves and validated by a taxonomist at Harare Botanical Garden specimen 2010/2011 and was deposited in the Chemistry Department (Natural Product Section) of Bindura University for future reference.

### Sample preparation

Plant leaves were air-dried at room temperature for three weeks to obtain a constant mass. The dried leaves were later ground two times into powder using a wooden mortar and pestle, followed by sieving through a laboratory king test sieve of 0.75 µm. The fine material which passed through the sieve was collected. The remaining residues were then discarded. A quantity of 10 g of the ground material was extracted continuously with cold organic solvents 100 ml; acetone, methanol, ethanol, hexane, petroleum ether and diethyl ether (Merck Co. Germany) for 5 h on a shaker at room temperature followed by filtration through Whatman no. 1 filter paper. The residues were re-extracted under the same treatment and the filtrates combined. The extracts were then centrifuged at 1536 × g for 20 min at 20°C. The supernatants were taken into 100 ml flasks and then evaporated in a Bucchi rotary evaporator at 40°C. The extracts obtained after evaporation were weighed to determine the yield and re-suspended in 100 ml of each solvent and stored in a deep freeze before use.

### Determination of total phenolics

Total phenolic compounds in plant extracts were determined by Folin-Ciocalteu method as reported by Wolfe et al. (2003) with minor modifications. For the preparation of the calibration curve, 1 ml aliquots of 0.1, 2.0, 4.0 and 6.0 mg/L tannic acid solutions in each respective solvent: methanol, ethanol, petroleum ether, hexane, diethyl ether and acetone were mixed with 5 ml of Folin-Ciocalteu reagent (diluted ten-fold) and 4 ml of sodium carbonate solution (75 g/L). The absorbance was read after 30 min at 20°C and at 725 nm and the calibration curves were constructed. A volume of 1 ml of each plant extract (0.1 mg/ml) was mixed with the same reagents as described previously, and after 30 min the absorbance was measured for the determination of total plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in plant extracts in tannic acid equivalents (TAE) was calculated by the following formula:

$$T = \frac{c \times v}{m}$$

where; T is total content of phenolic compounds in mg/g plant extract, in TAE; C is the concentration of tannic acid established from the calibration curve in mg/L; V is the volume of extract in ml; and m is the weight of plant solvent extract in gram.

### Determination of total flavonoid content

The method of Ordon et al. (2006) was employed with slight modifications. This was done by adding 0.5 ml of 2% $AlCl_3$ ethanol solution to 0.5 ml plant sample (0.1 mg/ml). Absorbance of the mixture was measured after one h at 425 nm. Total flavonoids content was evaluated as mg/g quercetin equivalents by a calibration method of 0, 0.5, 1.0, 1.5 and 2.0 mg/ml quercetin.

### Determination of total flavonols

The amount of flavonols was evaluated using the method of Kumaran and Karunakaran (2007). A volume of 2.0 ml of 2% ethanolic $AlCl_3$ and 3 ml (50 g/L) sodium acetate solutions was mixed with 2.0 ml of the sample extract of concentration 0.1 mg/ml. The absorbance was measured at 440 nm after standing for 2 to 5 h at 20°C. Total flavonols was estimated as mg/g of quercetin equivalent using a calibration method of 0, 0.5, 1.0, 1.5 and 2.0 mg/ml quercetin.

### Determination of total proanthocyanidins

The total proanthocyanidin compounds of the samples were estimated using UV-spectrometry as reported by Sun and Ho (2005) by adding 0.5 ml of 0.1 mg/ml of the extract solution to 3 ml of 4% vanillin methanol solution and 1.5 ml hydrochloric acid, and the mixture was to stand for fifteen minutes. The absorbance was measured at 500 nm and the results expressed as catechin equivalents using a calibration method of 0, 0.5, 1.0 1.5 and 2.0 mg/ml catechin.

### Antioxidant activity determination

Antioxidant test systems differ significantly such that the results of a single assay can give just a limited assessment of the antioxidant properties of a sample (Dejian et al., 2005; Gianni et al., 2005). As such, in the present research, total antioxidant activity was

**Table 1.** Total phenolic, flavonoids, flavonols and proanthocyanidins content of the different solvents.

| Sample | Solvent | Average ± SD | | | |
|---|---|---|---|---|---|
| | | TP | TFD | TF | TPR |
| Powdered leaves | Methanol | 2.89 ± 0.03 | 2.08 ± 0.01 | 3.84 ± 0.02 | 2.25 ± 0.01 |
| | Ethanol | 1.82 ± 0.01 | 1.00 ± 0.01 | 1.45 ± 0.01 | 1.93 ± 0.01 |
| | Acetone | 1.67 ± 0.01 | 5.12 ± 0.01 | 4.13 ± 0.01 | 3.07 ± 0.01 |
| | Hexane | 1.46 ± 0.02 | 0.86 ± 0.02 | 0.25 ± 0.03 | 1.68 ± 0.01 |
| | Petroleum ether | 1.31 ± 0.01 | 1.46 ± 0.01 | 0.90 ± 0.01 | 1.87 ± 0.01 |
| | Diethyl ether | 1.18 ± 0.01 | 0.91 ± 0.01 | 0.87 ± 0.01 | 1.69 ± 0.02 |

DW, Dry weight; TP, total phenols (mg tannic acid $g^{-1}$ DW); TFD, total flavonoids (mg quercetin $g^{-1}$ DW); TF, total flavonols (mg quercetin $g^{-1}$ DW); TPR, total proanthocyanidins (mg catechin $g^{-1}$ DW).

evaluated through three assays, DPPH· scavenging activity, the ferric reducing ability of plasma (FRAP) and inhibition of egg lipid peroxidation.

### *DPPH· radical scavenging assay*

The DPPH· radical scavenging activity was evaluated according to a slightly modified method described by Pothitirat et al. (2009) and Masuda et al. (2007). The reagent (1 ml of $1\times10^{-3}$ $molL^{-1}$) DPPH· solution in methanol was mixed with 1 ml of plant extract, butylated hydroxytoluene (BHT), ascorbic acid and catechin [standard antioxidants (Sigma Chemical Co. St Louis, USA)] of different concentrations. The reaction mixture was incubated in the dark for 30 min and there after the optical density was recorded at 517 nm. For the control, 1 ml of DPPH· solution in methanol was mixed with 1 ml of each solvent without extract and optical density of solution was recorded after 30 min. The decrease in optical density of DPPH· on addition of test samples in relation to the control was used to calculate the antioxidant capacity as percentage of inhibition (% I) of DPPH radical:

$$\% I = \frac{Ac - As}{Ac} \times 100$$

where; $A_s$ is the absorbance with extract/standards after 30 min and $A_c$ is the absorbance without the extract after 30 min. Each assay was carried out in triplicate.

### *Total antioxidant power (FRAP)*

This was done as reported by Benzie and Strain (1996) with slight modifications. Fresh working solutions consisting of 25 ml acetate buffer, 3.1 g sodium acetate, 16 ml acetic acid, and pH 3.6 were used for this analysis. A volume of 2.5 ml of 2, 4, 6-tripydal-s-triazine solution in 40 ml HCl (10 Mm) and 20 Mm $FeCl_3.6H_2O$ solution were mixed at 37°C. Extracts, catechin and ascorbic acid of different concentrations were allowed to react with the mixture for 30 min in the dark and absorbance was measured at 563 nm. Total antioxidant power to reduce Fe(III) to Fe(II) was determined by the change in absorbance which was then calculated and related to absorbance change of an Fe(II) standard solution.

### *Inhibition of lipid peroxidation*

The assessment was performed following a method reported by Zhang et al. (1996). An equal volume 2 ml of egg yolk was added to 0.1 $mol/dm^3$ phosphate buffer solution (PBS) (pH 7, 45). The mixture was stirred magnetically for 10 min and then diluted with 24 ml of phosphate buffer solution. The homogenized yolk (1 ml), extract, ascorbic acid, catechin (0.5 ml) of different concentrations, PBS 1 ml and $25 \times 10^{-3}$ mol $dm^{-3}$ (1 ml) iron sulphate were mixed and shaken at 37°C for 15 min. The reaction was stopped by the addition of trichloroacetic acid and the mixture was centrifuged. Then 1 ml of 8 $g/dm^3$ thiobarbituric acid was added to 3 ml of the supernatant. The solution was heated at 10°C for 10 min and then absorbance was measured at 523 nm. Percentage lipid peroxidation inhibition was calculated as follows.

### Statistical analysis

All analyses were performed in triplicate and data reported as mean ± standard deviation (SD). The data was subjected to analysis of variance (ANOVA) ($p < 0.05$). Results were processed by Excel and SPSS Version 16.0 (SPSS Inc., Chicago, IL, USA). Least significant difference (LSD) tests and T test was applied to separate the means and to compare antioxidant activity of extracts and that of standards respectively.

## RESULTS AND DISCUSSION

### Total phenolic content

The concentration of phenolic compounds in *T. obovatus* varied in different extracts ranging from 1.18 to 2.89 mg tannic acid $g^{-1}$ dry weight and they were in the order methanol > ethanol > acetone > hexane > petroleum ether > diethyl ether (Table 1). The results revealed that methanol and ethanol were better compared to other solvents in extracting phenolic compounds (ANOVA and LSD test analysis $p < 0.05$). This is because they have higher polarity and favourable solubilities for phenolic compounds from plants (Kequan and Liangh, 2006). The less polar solvents such as petroleum ether, diethyl ether and hexane revealed less capability of extracting phenolic compounds. Stecher et al. (2003) observed that low-polarity solvents yield more lipophilic components, while alcoholic solvents give a larger spectrum of apolar

and polar material. In comparison to the food taken daily in diets, total phenolic compounds in *T. obovatus* was lower than that obtained from tomatoes (2.29 to 5 mg

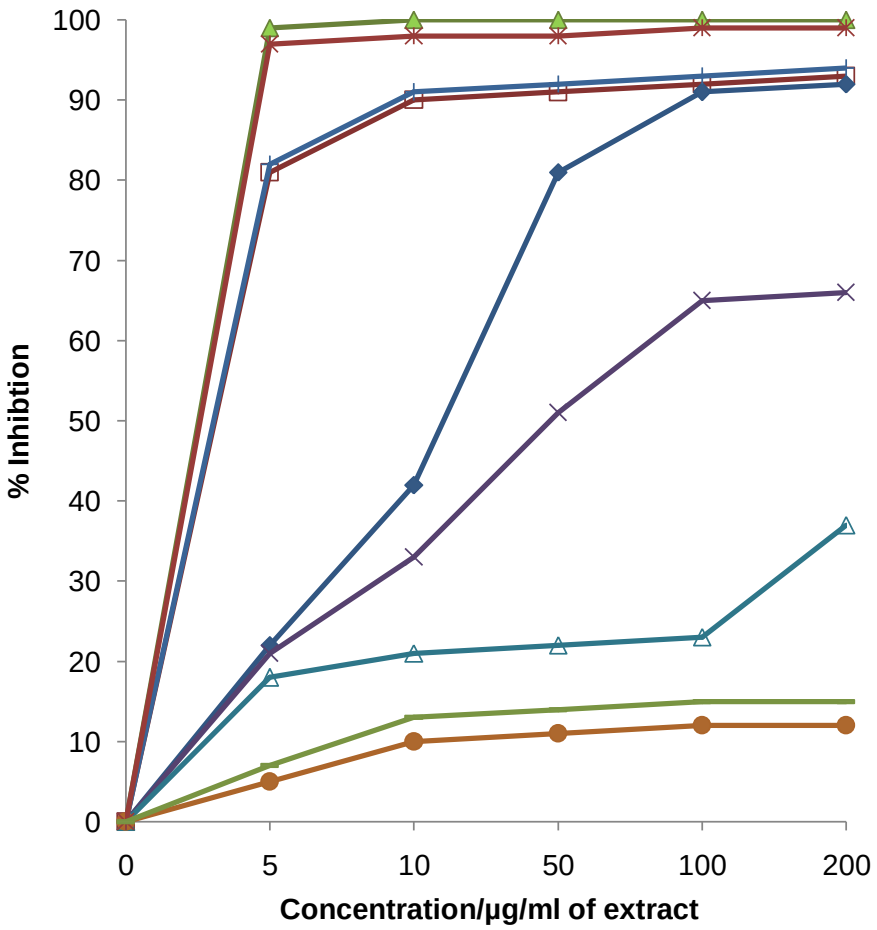


**Figure 2.** DPPH radical scavenging assay.

gallic $g^{-1}$) (Wolfe et al., 2003) but higher than that found in carrot (1.52 mg gallic acid $g^{-1}$) (Kequan and Liangli, 2006) and onion (2.5 mg gallic acid $g^{-1}$) (Kahkonen et al., 1999).

### Total flavonoids and flavonols content

Flavonoids and flavonols exhibit a wide spectrum of biological and chemical activities including antiradical activities. Because of this, all extracts were assayed for total flavonoids and flavonols content. Acetone extracts showed the greatest yield for both flavonoids and flavonols (5.12 and 4.13 mg quercetin $g^{-1}$ respectively). The least yield was obtained with hexane (0.84 mg quercetin $g^{-1}$ for flavonoids and 0.27 mg querceting$^{-1}$ for flavonols) (Table 1).

### Total proanthocyanidins content

Among the tested solvents, acetone was the most efficient solvent in extracting proanthocyanidins (3.07 mg catechin $g^{-1}$) followed by methanol (2.25 mg catechin $g^{-1}$) and then ethanol (1.94 mg catechin $g^{-1}$) (Table 1). The results disagree with the results obtained by Lapornik et al. (2005) that described methanol and ethanol as the best extractors of anthocyanins from red and black current. In the studies of Zhao et al. (2006), Kequan and Liangli (2004) and Tabart et al. (2007), similar results were obtained as reported in this study. Acetone (80%) showed the highest extraction capacity for (+) catechin, caffeic, vanillic and coumaric acid from barley. Kequan and Liangli (2004) found out that 50% acetone exhibited superior extraction efficiency of anthocyanins from wheat based foods.

## ANTIOXIDANT ACTIVITY DETERMINATION

### DPPH$^{\cdot}$ scavenging assay

The DPPH$^{\cdot}$ antiradical activity of different solvents shown in Figure 2 generally show that the percentage inhibition was concentration dependent. All extracts exhibited antioxidant activity with acetone showing the

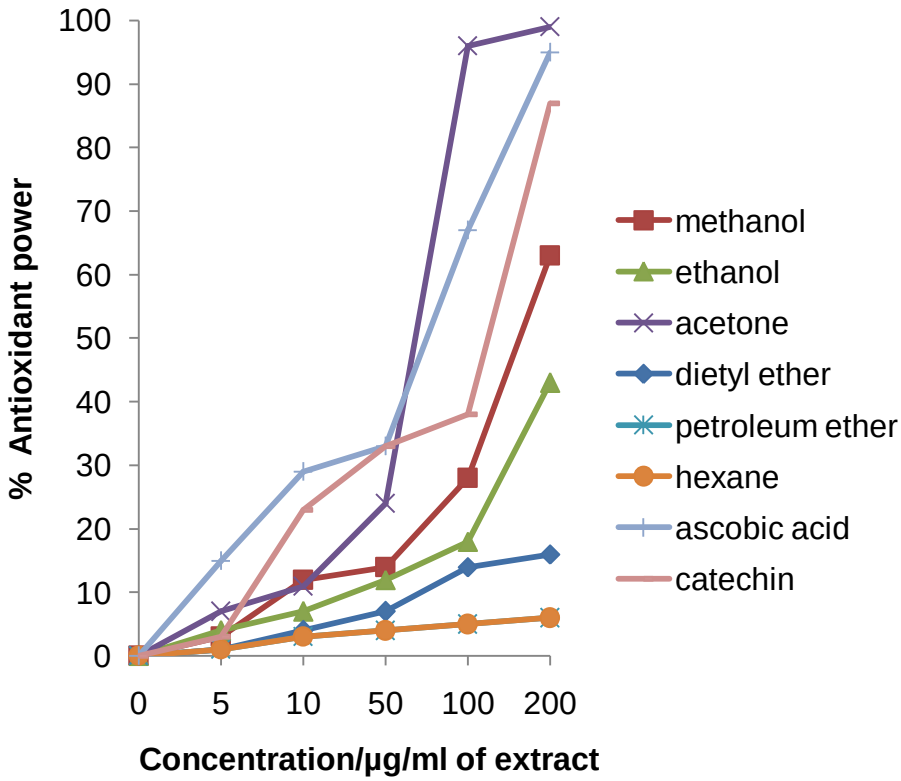


**Figure 3.** Total antioxidant power *(FRAP).*

greatest activity profile just above that of ascorbic acid. It should be noted that the reaction of acetone extract and DPPH· radical was very rapid in all replicas and was completed within 0.5 to 1 min. Methanol extracts were second with activity slightly below that of standard antioxidant BHT. At a concentration of 10 µg/ml, the scavenging activity of ascorbic acid, methanol, and acetone extracts were 99.6, 89.7 and 100% respectively. Hexane extracts showed the weakest radical scavenging activity just like catechin. At 10 µg/ml the percentage inhibition was 10.4 for hexane extracts and 12.8% for catechin. The concentration of antioxidant activity required to decrease the initial DPPH· concentration by 50% ($IC_{50}$) is a parameter widely used to measure antioxidant activity. The lower the $IC_{50}$ value the higher is the antioxidant activity. The antioxidant activity was in the following order: acetone $IC_{50,}$ < 5, methanol < 5, ethanol, 15, diethyl ether 30, petroleum ether > 200 and hexane > 200 µg/ml. Significant difference was observed in the results, though ANOVA analysis showed that $p < 0.05$ at concentrations of 5, 10 and 50 µg/ml.

## FRAP assay

In the present study, acetone extract exhibited the greatest reducing power followed by methanol. However, the results were lower than those of ascorbic acid and catechin at concentration below 50 µg/ml. Above 50 µg/ml, acetone extract showed better antioxidant activity than catechin and ascorbic acid. This may indicate a possibility of competitive kinetics (Figure 3). Diethyl ether, petroleum ether and hexane extracts were poor reducing agents. The results were compared to those reported by Zhao et al. (2006). The highest reducing power was found in acetone extracts as compared to ethanol and methanol using barley as the sample. When the test was performed with plasma in the absence of Fe(III) added to the reaction mixture, it was observed that no color developed. This indicated that there was no detectable free Fe(II) in plasma, and that, there was no detectable agent in normal plasma that reacts directly with 2, 4, 6-tripydal-s-triazine (TPTZ) to form the blue chromogen. Monitoring complete reagent, that is reagent containing TPTZ and Fe(III) but with no sample addition, it also showed that no color developed.

## Inhibition of lipid peroxidation

This assay makes use of the ability of egg yolk lipids to undergo a fast non enzymatic oxidation in the presence of iron sulphate (Watson et al., 2006). The results for lipid peroxidation shown in Figure 4 revealed that, acetone extract was the best egg lipid peroxidation inhibitor followed by methanol and then ethanol extracts.

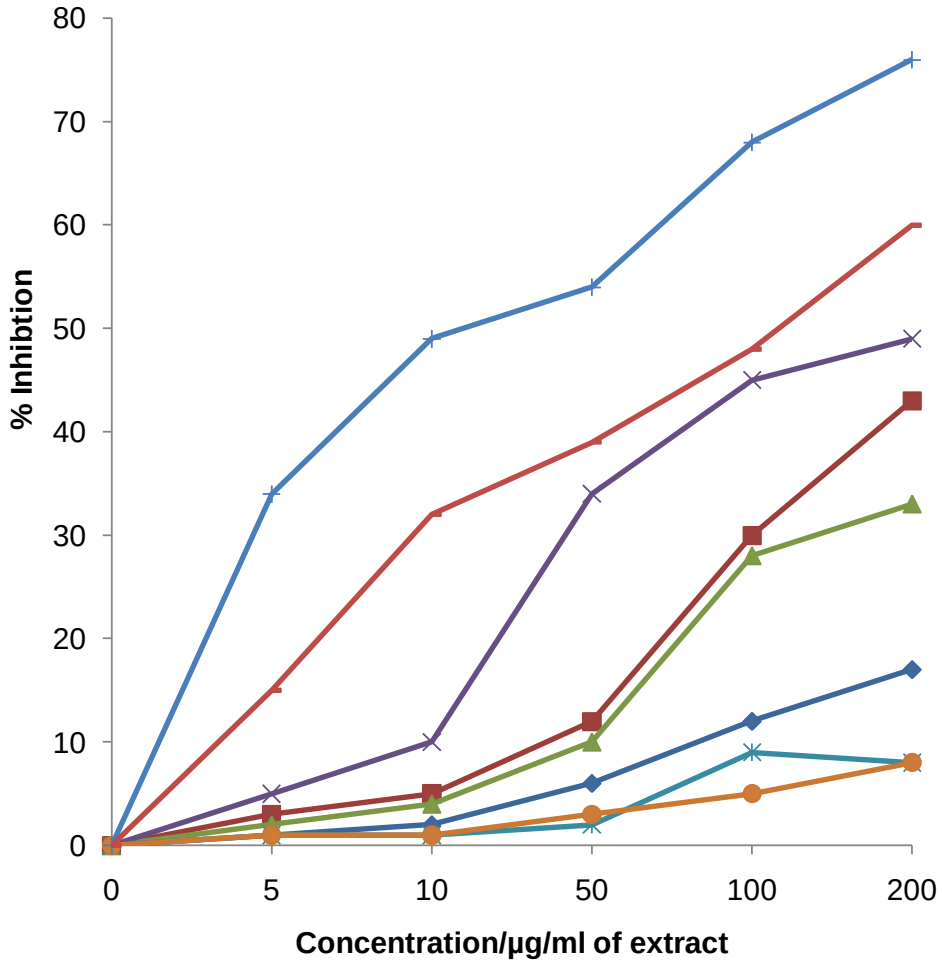


**Figure 4.** Inhibition of lipid peroxidation.

Compared to ascorbic acid and catechin, acetone was significantly inferior [*t*-test $p < 0.05$ at concentrations, 5; 10 and 50 (µg/ml)]. The less polar solvent extracts (diethyl ether, petroleum ether and hexane) were observed with poor lipid peroxidation inhibitors even at concentrations above 200 µg/ml. Lipid peroxidation inhibition of the extracts was also concentration dependent.

## Relationship between yield, phenolic, flavonoids, flavonols and proanthocyanidins with antioxidant activity

This study's analysis showed that there was good correlation between yields ($R^2$ = 0.901), flavonols ($R^2$ = 0.729), proanthocyanidins ($R^2$ = 0.729), flavonoids ($R^2$ = 0.708) and antioxidant activity, but weak correlation in phenolic compounds ($R^2$ = 0,426). Similar results were reported by Chung et al. (1999). These results suggested that the antioxidant capacity of extracts was largely due to the contribution of flavonoids, flavonols and proanthocyanidins.

## Conclusion

The results indicate that extraction solvents have a significant impact on antioxidant activity as well as selectivity for different polyphenols. Basing the employed models for antioxidant activity test, acetone proved to be the best solvent for extracting polyphenols from *T. obovatus.* The antioxidant activity of *T. obovatus* can be explained significantly in terms of flavonols, proanthocyanidins and flavonoids. *T. obovatus* consists of significant antioxidant activity and some amounts of flavonoid, flavonols and proanthocyanidins compounds to warrant its use as a source of natural antioxidants which can be used to promote health and delay of food rancidification. *T. obovatus* can also be used as alternative medicine for treatment of ailments therefore authenticating its use in traditional medicines.

## ACKNOWLEDGEMENTS

The authors would like to express sincere gratitude to Professor M. F. Zaranyika and Mr .Dotito (University of Zimbabwe), Mr. Makahamadze and Mr. Mapfaka (Bindura University) for their kind supports.

### REFERENCES

Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma as a measure of antioxidant power: the FRAP assay. Anal. Biochem., 239: 70-76.

Bergfeld WF, Belsito DV, Marks JGJ, Andersen FA (2005). Safety of ingredients used in cosmetics. J. Am. Acad. Dermatol., 52: 125-132.

Chipault JR, Mizuno GR, Lundberg WO (1956). The antioxidant properties of spices in Foods. Food Technol., 10: 209-210.

Chung HYT, Soung DY, Kye IS, No JK, Baek BS (1999). Peroxynitrite-scavenging activity of green tea tannin. J. Agric. Food Chem.. 46: 4484-4486.

Gianni S, Silvia M, Mariavittoria M, Martina S, Stefano M, Matteo R (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. Food Chem., 91: 621-632.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS (1999). Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food Chem., 47: 3954-3962.

Kequan Z, Liangli Y (2006). Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT – Food Sci. Technol., 39: 1155-1162.

Kequan Z, Liangli Y (2004). Effects of extraction solvents on wheat bran antioxidant activity estimation. LWT – Food Sci. Technol., 37(7): 717-721.

Kumaran A, Karunakaran RJ (2007). *In vitro* antioxidant activities of methanol extracts of phyllanthus species from India. Lebens-Wiss Tech., 40: 344-352.

Lapornik B, Prosek M, Wondra GA (2005). Comparison of extracts prepared from plant by products using different solvents and extraction time. J. Food Eng., 77(2): 214-222.

Maestri DM, Nepote V, Lamarque AL, Zygadlo JA (2006). Natural products as antioxidants. Res. signpost., 37: 105-135.

Mahamadi C, Togarepi E, Dzomba P (2011). Extraction of phenolic compounds from *Temnocalyx obovatus*. A. J. Biotech., 10(54): 11245-11248.

Masuda T, Yonemori S, Oyama Y, Takeda Y, Tanaka T, Andoh T (2007). Evaluation of the antioxidant activity of environmental plants: activity of leaf extracts from seashore plants. J. Agric. Food Chem., 47: 1749-1754.

Miliauskas G (2006). Screening, isolation and evaluation of antioxidative compounds from *Geranium macrorrhizum, Potentilla fruticosa* and *Rhaponticum carthamoides*. Europian Food Res. Technol., 218: 253-261.

Ordon EAA, Gomez JD, Vattuone MA, Isia M (2006). Antioxidant activities of *Sechium edule* Jacq Swart extracts. Food Chem., 97: 452-458

Prasad KN, Fouad AH, Yang B, Kong KW, Ramannan RN, Azrina A, Amin I (2011). Response surface optimization for the extraction of phenolic compounds and antioxidant capacities from *Mangifera pajang* Kosterm. peels. Food Chem., 128: 1121-1127

Prasad KN, Chew LY, Khoo HE, Kong KW, Azlan A, Amin I (2010). Antioxidant capacities of peel, pulp and seed fractions of *Canarium odontophyllum* Miq. fruit. J. Biomed. Biotech., pp. 1-8.

Pothitirat W, Chomnawang M T, Supabphol R, Gritsanapan W (2009). Comparison of bioactive compounds content, free radical scavenging and anti-acne inducing bacteria activities of extracts from the Mangosteen fruit rind at two stages of maturity. Fitoterapia, 80: 442-447.

Shahidi F, Wanasundara UN (1997). Measurement of lipid oxidation and evaluation of antioxidant activity, in natural antioxidants, chemistry, health effects, and applications. AOCS Press. Champaign, pp. 379-396.

Stecher G, Huck CW, Stoggl WM, Bonn GK (2003). Phytoanalysis: a challenge in phytomics. Trends Anal. Chem., 22: 1.

Sun T, Ho C (2005). Antioxidant activities of buckwheat extracts. Food Chem., 90: 743-749.

Tabart J, Kevers C, Sipel A, Pincemail J, Defraigne JO, Dommes J (2007). Optimisation of extraction of phenolics and antioxidants from black currant leaves and buds and of stability during storage. Food Chem., 3: 1268-1275.

Wangensteen H, Samuelsen AB, Malterud KE (2004). Antioxidant activity in extracts from coriander. Food Chem., 88: 293-297.

Watson J, Arnold M, Ho YS, O` Dell T (2006). Age-dependent modulation of Hippocampal long term potentiation by antioxidant enzymes. J. Neurosci. Res., 84: 1564-1574.

Wolfe K, Wu X, Liu RH (2003). Antioxidant activity of apple peels. J. Agric. Food Chem., 51: 609-614.

Zhang EX, Yu LJ, Zhou YL, Xiao X (1996). Studies on the peroxidation of polyunsaturated fatty acid from lipoprotein induced by iron and the evaluation of the anti-oxidative activity of some natural products. Acta Biochem. Biophys. Sin., 28: 218-222.

Zhao H, Dong J, Lu J, Chen J, Li Y, Shan L, Lin Y, Fan W, Gu G (2006). Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley *(Hordeum vulgare L.).* J. Agric. Food Chem., 54(19): 7277-7286.