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Research Article

DETERMINATION OF FREE PHENOLIC ACIDS, FLAVONOID CONTENTS AND ANTIOXIDANT CAPACITY OF ETHANOLIC EXTRACTS OBTAINED FROM LEAVES OF MISTLETOE (*TAPINANTHUS GLOBIFERUS*)

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ABSTRACT

In the last few decades, studies have increased in the assessment of relevance of natural antioxidants in the prevention and management of diseases particularly those with generation of free radicals as etiological factor. Antioxidant properties, phenolic and flavonoid contents of *Tapinanthus globiferus* were determined chemically and biologically. Different concentrations of ethanolic extract of *T. globiferus* demonstrated high DPPH radical scavenging and iron chelating activities. Ethanolic extract of *T. globiferus* reduced generation of reactive oxygen species in isolated mitochondria in the presence or absence of hydrogen peroxide. The antioxidant activities were in dose-dependent manner. Analyses by high perfomance liquid chromatography revealed presence of phenolic compounds such as gallic, chlorogenic and caffeic acids; flavonoid compounds present included rutin and quercetin. *T. globiferus* demonstrated high antioxidant activities which may be due to the presence of phenolic and flavonoid contents.

Keywords: Statistical analyses , Wistar rats , phenolic content , Iron- chelating capacity **.**

INTRODUCTION

Cellular damage or oxidative injury arising from free radicals or reactive oxygen species (ROS) now appears the fundamental mechanism associated with a number of human neurodegenerative disorders, diabetes, cardiovascular diseases, inflammation, viral infections, autoimmune pathologies and digestive system disorders. Free radicals are generated through normal metabolism of drugs, environmental chemicals and other xenobiotics as well as endogenous chemicals, especially stress hormones (adrenalin and noradrenalin). Accumulated evidence suggests that ROS can be scavenged through chemoprevention utilizing natural antioxidant compounds present in foods and medicinal plants. Africa continent has enormous biodiversity resources, but plagued with several diseases, including those associated with ROS overproduction as one of the etiological factor. Therefore, therapy using free-radical scavengers (antioxidants) has potential to prevent, delay or ameliorate many of these disorders (Delanty and Dichter, 2000). Over the past two decades, an expanding body of evidence from epidemiological and laboratory studies have demonstrated that some edible plants as a whole, or their identified ingredients with antioxidant properties have substantial protective effects on human carcinogenesis (Surh and Fergusson, 2003; Park and Pezzuto, 2002; Wattenberg, 1996; Greenwald, 2002; IARC, 1996; Fujiki, 1999; Tsao et al., 2004; Kinghorn et al., 2004; Mehta and Pezzuto, 2002). Similar evidence also exist to demonstrate the chemopreventive capacities of ethnobotanicals and components of vegetable diets with free-radical scavenging potential on ulcers (Borreli and Izzo, 2000), diabetes (Sabu and Kuttan, 2002), memory and cognitive function (Howes and Houghton, 2003), Alzheimer's disease (Howes et al., 2003; Perry et al., 1998), age-related neurological dysfunction (Youdim and Joseph, 2001; Delanty and Dichter, 2000), cardiovascular and renal disorders (Anderson et al., 1999; Miller, 1998) and several other human ailments (Scartezzini and Speroni, 2000; Borek, 2001; Craig, 1999; Galvano et al., 2001; Lampe, 2003; Surh, 1999).

Tapinanthus globiferus, popularly called 'afomo' in South Western Nigeria is a parasitic plant known to grow on different trees. There has been no report about its free phenolic acid and flavonoid contents. Furthermore, little is known about the antioxidant potential of this plant. Although *T. globiferus* is being used in folkloric medicine in the management of free radical related disorders, there is little or no information about its phytochemical constituents and antioxidant activities which may be involved in its antihypertensive ability. This study is designed to determine the phenolic acid and flavonoid contents, as well as antioxidant activities of T. globiferus.

Fig. 1: Tapinanthus globiferus.

MATERIALS AND METHODOLOGY

Chemicals

All chemicals used including solvents, were of analytical grade. 1,1-diphenyl-2-picryl hydrazyl (DPPH), Folin Ciocalteu´s phenol reagent, malonaldehyde bis-(dimethyl acetal) (MDA), Thiobarbituric acid (TBA), sodium dodecyl sulfate, ascorbic acid, 2′,7′-dichlorofluorescein diacetate (DCFH-DA), Tris-HCl, ethylene glycol tetraacetic acid (EGTA), quercetin, rutin, chlorogenic acid and gallic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), ferrous sulfate, mannitol, sucrose were obtained from Vetec (Rio de Janeiro, RJ, Brazil.

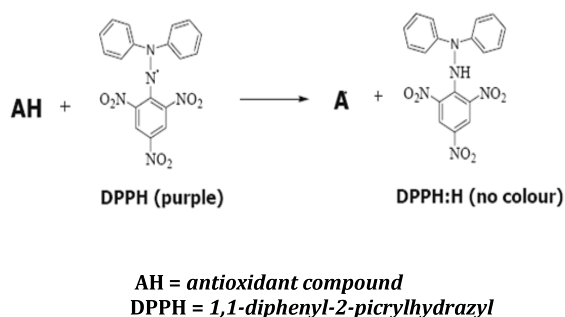
Plants Extract and Extraction procedure

Leaves of *Tapinanthus globiferus* was obtained from Ogbomoso, Nigeria in 2011 and was identified by Dr. Ogunkunle of the Botany Unit, Department of Pure and Applied Biology and were confirmed

with a plant name index. The leaves were dried under room temperature. The dried leaves were grinded into a powdery form and the powder of *T. globiferus* (100 g) was macerated at room temperature with ethanol (70%) and extracted for 72 hours at the Biochemical Toxicological Unit, Department of Biological sciences, Federal University of Santa Maria, Santa Maria RS, Brazil. On the third day, the combined ethanolic extract was filtered and the solvent was fully evaporated under reduced pressure to give a green solid. The ethanolic extract was then diluted in distilled water in order to prepare different concentrations (10, 50, 100, and 250 µg/mL).

DPPH radical scavenging activity

The free radical scavenging activity of *T.globiferus* extract was measured with the stable radical 1,1-diphenyl-2-picryl hydrazyl (DPPH) (Howes et al., 2003) in terms of hydrogen-donating or radicals scavenging activity according to the method described by Bandoniene and Murkovic (2002). A solution of DPPH (0.3 mM) in ethanol was prepared, and 100 µL of this solution was added to 20 µL aquoeus extract at different concentrations (10, 50, 100 and 250 µg/mL).



AH = ***antioxidant compound***
DPPH = ***1,1-diphenyl-2-picrylhydrazyl***

Fig 1: The *In Vitro* chemical representation of DPPH *(1,1-diphenyl-2-picrylhydrazyl)* free radical scavenging assay.

Distilled water and ascorbic acid at the same concentrations used for aqueous extract were used as negative and positive controls respectively. After 30 minutes, absorbances were measured at 548 nm in ELISA plate reader (TP-Reader, China).

The free radical scavenging capacity of the extract is calculated with the equation;

$$\% \text{ inhibition} = [(\text{Control Absorbance} - \text{Test Absorbance})/\text{Control Absorbance}] \times 100$$

Total phenolic content

The total phenolic content of *T.globiferus* was estimated according to the method of Makkar et al. [21] with minor modifications. Samples of the extract (10 - 250 µg/mL) were added to a test tube and the volume was adjusted to 1.4 mL with distilled water. Then, 0.2 mL of Folin-Ciocalteu reagent (diluted 1:1 with water) and 0.4 mL of sodium carbonate solution (7.5%) were added sequentially to the test tube. The tubes were then incubated for 40 min at 45°C and the absorbance was measured at 725 nm in a spectrophotometer (SP-2000UV). The standard curve was prepared using 0, 1, 2.5, 5, 10 and 15 µg/mL solutions of gallic acid (0.1 mg/mL). Total phenol value was calculated and expressed as microgram gallic acid equivalent (µg GAE)/g of dry extract.

Iron-chelating activity

The method described by Benzie and Strain (1996) was adopted for the assay. The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. The reaction containing 40ul of ferric chloride (3.12mM), 740ul of Tris-HCl (0.1M) and different volumes of different concentrations of extract was made up to 2ml with distilled water and was incubated for 5 minutes. The mixture was used to zero the wavelength of the spectrophotometer. Twenty five microliter of ortho-phenanthroline was thereafter added to the mixture in the cuvette and absorbance taken at 510nm. The Fe^{2+} chelating capacity was calculated thus:

$$Fe^{2+} \text{ chelating activity } (\%) = \{(Ac - As)/ Ac\} \times 100$$

Animals

Male Wistar rats weighing 270-320 g and with age from 2.5 to 3.5 months from breeding colony (Animal House-holding, UFSM, Brazil) were used for the study. They were kept in cages with free access to foods and water in a room with controlled temperature (22 ± 3°C) and in 12 h light/dark cycle. The protocol has been approved by the guidelines of the Brazilian association for laboratory animal science (CONCEA).

Quantification of phenolics and flavonoids compounds by HPLC-DAD

Free phenolic and flavonoid contents were determined using High performance liquid chromatography (HPLC-DAD). This was performed with the HPLC system (Shimadzu, Kyoto, Japan), Prominence Auto Sampler (SIL-20A), equipped with Shimadzu LC-20AT reciprocating pumps connected to the degasser DGU 20A5 with integrator CBM 20A, UV-VIS detector DAD (diode) SPD-M20A and Software LC solution 1.22 SP1. Briefly, reverse phase chromatographic analyses were carried out under gradient conditions using C_{18} column (4.6 mm x 250 mm) packed with 5µm diameter particles; the mobile phase was water containing 2% acetic acid (A) and methanol (B), and the composition gradient was: 5% of B until 2 min and changed to obtain 25%, 40%, 50%, 60%, 70% and 100% B at 10, 20, 30, 40, 50 and 65 min, respectively, following the method described by Laghari et al. (2011) with slight modifications. The extracts of T. globiferus were analyzed, at a concentration of 5 mg/mL. The presence of six phenolics compounds was investigated, namely, gallic, chlorogenic and caffeic acids and the flavonoids quercetin, rutin and kaempferol. Identification of these compounds was performed by comparing their retention time and UV absorption spectrum with those of the commercial standards. The flow rate was 0.6 ml/min, injection volume 40 µl and the wavelength were 254 nm for gallic acid, 325 nm for caffeic and chlorogenic acids, and 365 nm for quercetin, rutin and kaempferol. All the samples and mobile phase were filtered through 0.45 µm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.031 – 0.250 mg/ml for kaempferol, quercetin and rutin; and 0.006 – 0.250 mg/ml for gallic, caffeic and chlorogenic acids. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 500 nm). Calibration curve for gallic acid: Y = 11611x + 1468.8 (r = 0.9999); chlorogenic acid: Y = 14762x + 1257.5 (r = 0.9997); caffeic acid: Y = 11526x + 1293.1 (r = 0.9995); rutin: Y = 13035x – 1045.9 (r = 0.9998); quercetin: Y = 15105x – 1192.3 (r =0.9998) and kaempferol: Y = 15223x – 1303.9 (r = 0.9999). All chromatography operations were carried out at ambient temperature and in triplicate.

Isolation of rat liver mitochondrial

Rat liver mitochondrial was isolated as previously described by Puntel et al. [2010] with some modifications. The livers were rapidly removed (within 1 min) and immersed in ice-cold `` isolation buffer I´´ containing 225 mM manitol, 75 mM sucrose, 1 mM K^+-EGTA and 10 mM K^+-HEPES, pH 7.2. The tissue was minced using surgical scissors and then extensively washed. The tissue was then homogenized in a power-driven, tight-fitting Potter-Elvehjem homogenizer with Teflon pestle. The resulting suspension was centrifuged, for 7 min at 2000 g in Hitachi CR 21E centrifuge. After centrifugation, the supernatant was centrifuged for 10 min at 12000g. The pellet was resuspended in ´´ isolation buffer II´´ containing 225 mM manitol, 75 mM sucrose, 1 mM K^+-EGTA, and 10mM K^+-HEPES, pH 7.2, and recentrifuged at 12,000g for 10min. The supernatant was decanted, and the final pellet was gently washed and resuspended in respiration buffer containing 100 mM sucrose, 65 mM KCl, 10 mM K^+-HEPES and 50 µM EGTA, pH 7.2, to a protein concentration of 0.6 mg/mL.

Generation and measurement of reactive oxygen species (ROS)

ROS production in isolated mitochondria was measured using a 2′,7′-dichlorofluorescein diacetate (DCFH-DA) fluorescence probe. Mitochondrial suspensions (0.25 mg/mL) in respiration buffer containing 100 mM sucrose, 65 mM KCl, 10 mM K^+-HEPES and 50 μM EGTA, pH 7.2 were incubated with different concentrations of the extract (10-250 μg/mL) in the presence or absence of 12mM of H_2O_2. Then, 3.33 μM of DCFH-DA; then, 10, 50, 100, 250 μg/mL of ethanolic extract [Ketson and Brandt, 1965; Kinghorn et al., 2004; Lampe, 2003] were added to the solution. The formation of the oxidized fluorescent derivative (DCF) was monitored using a spectrofluorimeter (Shimadzu RF-5301) with excitation and emission wavelengths of 488 and 525 nm respectively and with slit widths of 1.5 nm.

Statistical analysis

Values were expressed as mean ± SEM (standard error of mean). Statistical analyses were performed by one way ANOVA, followed by Duncan's multiple range tests. The results were considered statistically significant for $p < 0.05$.

RESULTS

Iron- chelating capacity

Iron binding of ethanolic extract of *T. globiferus* at different concentrations was determined and the values were summarized in the table 1. The iron-chelating activity of T. globiferus was dose-dependent with IC_{50} at 247.120± 0.914.

Table 1: Effects of ethanolic extract of the leaves of *T. globiferus* on iron chelation.

Concentration	% Fe^{2+} chelation
5 μg/mL	21.045 ± 0.324
10 μg/mL	24.564 ± 0.30
20 μg/mL	26.47 ± 0.443
50 μg/mL	29.689 ± 0.612
100 μg/mL	37.760 ± 0.964
250 μg/mL	53.620 ± 0.809
IC_{50} (μg/mL)	247.120± 0.914

DPPH Assay

DPPH color was quenched by vitamin C (Table1 or Figure x) and in a concentration dependent manner by alcholic extract from *T. globiferus* and a maximal inhibitory effect of about 90% was obtained with the highest concentration tested (250 g/ml).

Table 2: Percentage free radical inhibition by the ethanolic extract of *Tapinanthus globiferus* (DPPH).

Concentrations	10μg/ml	50μg/ml	100μg/ml	250μg/ml
Ascorbic Acid	75.35±3.18%	78.43±2.43%	80.40±2.22%	80.76±2.40%
T. globiferus	22.95±1.83%	60.89±2.20%	78.23±4.78%	88.75±1.20%

Total phenolics

The amount of total phenolic of ethanolic extract of leaves of *T. globiferus* estimated by Folin-Ciocalteau method was 2.77±1.24 mgGAE/g of dry extract.

Determination of phenolic acid and flavonoid contents in T. globiferus

HPLC fingerprinting of extract revealed the presence of the phenolic acids such as gallic acid (t_R = 12.19 min), chlorogenic acid (t_R = 21.58 min), and caffeic acid (t_R = 24.97 min), while the flavonoid contents included rutin (t_R = 38.03 min) and quercetin (t_R = 45.11 min)(Fig. 1 and Table 3). The highest of the estimated phenolic acids in the ethanolic extract of T. globiferus was chlorogenic acid (5.81±0.05) while the least was caffeic acid (1.77±0.01). The predominant of the estimated flavonoid contents is rutin (8.12±0.09).

Table 3 – Free phenolic and flavonoid compositions of *Tapinanthus globiferus* extracts by HPLC/DAD.

Compounds	TC	
	Mg/g	%
Gallic acid	2.09±0.13a	0.20
Chlorogenic acid	5.81 ± 0.05 b	0.58
Caffic acid	1.77 ± 0.01 a	0.18
Rutic	8.12 ± 0.09 c	0.81
Quercetin	7.03 ± 0.02 c	0.70
Kaempferol	-	-

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters in the column differ by Tukey test at p < 0.05.

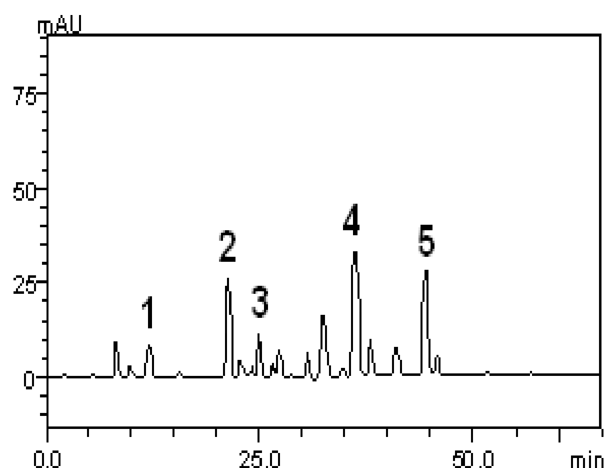


Figure 4 – Representative high performance liquid chromatography profile of *Tapinanthus globiferus* extract. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rutin (peak 4) and quercetin (peak 5).

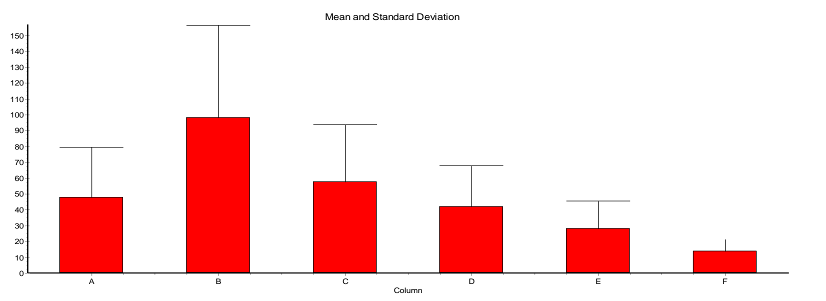


Fig 5: Graph showing the dose-dependent ROS production inhibitory ability of T. globiferus.

A & B= +ve & -ve controls respectively; C, D, E & F = ROS production at concentrations of 10μg/ml, 50μg/ml, 100μg/ml, & 250μg/ml respectively.

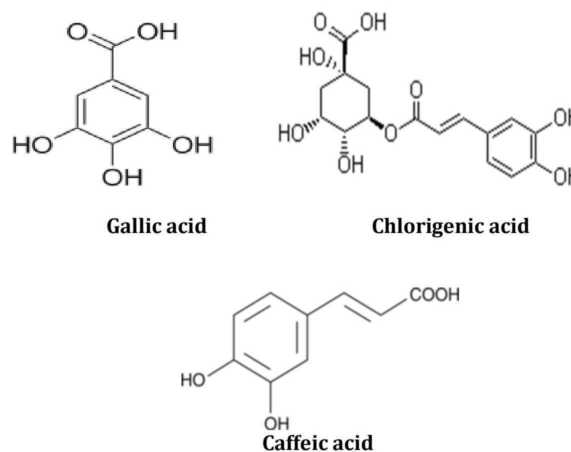
DISCUSSION

Over the decades, there has been tremendous focus on the relevance of natural antioxidants in the prevention and management of different ailments. This study assessed in-vitro, the antioxidative potentials of *T. globiferus* and its phenolic and flavonoid contents. Total phenolic as determined by Folin-Ciocalteu method revealed that ethanolic extract of *T. globiferus* contain considerable quantity of phenolic contents. However, literature have debated that this method overestimate the contents of phenolic compound,, primarily because other agents such as sugar, amino acid e.t.c. interfere with the procedure (Singleton and Rossi, 1965: Vinson et al., 2001). Phenolic compounds have been revealed to possess antioxidant activities.

Antioxidative ability of *T. globiferus* was estimated by assessing its iron chelating capacity. This method is based on the fact that 1,10-phenanthroline will chelate iron II ion to form a complex. However, any other chelator if present in the medium will interfere with the formation of iron II complex by 1, 10-phenanthroline. Measurement of the rate of reduction of the colour allows estimation of the iron chelating ability. The presence of *T. globiferus* extract in the reaction mixture interferes with this by chelating the iron II ion thereby forming complex with the iron II ion. Even at concentration as low as 5µg/ml, ethanolic extract of the plant demonstrated high capacity for iron chelation. This shows that *T. globiferus* possesses iron chelating capacity which increases with increasing concentrations of *T. globiferus*. Although iron is essential to life because of its requirements in various physiological and biochemical processes such as oxygen transport, respiration and its involvement in enzymatic activities, however, it has been implicated in the oxidative damages in lipids, proteins and other cellular components leading to occurrence of diseases such as cardiovascular and neurodegenerative diseases. Hence its control is important for normal functioning of the body. There have been reports that chelating agents which form O-bonds with a metal ion are effective as secondary antioxidants because they reduce the redox potential thereby stabilising the oxidized form of the metal ion (Gordon, 1990). This data showed that *T. globiferus* may be a good antioxidant due to its demonstration of high iron chelating capacity.

The ability of ethanolic extract of *T. globiferus* to scavenge free radicals formed was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). This was compared with a standard (ascorbic acid). Ethanolic extract of *T. globiferus* demonstrated high capacity for scavenging free radicals as shown by the data by reducing the stable radical DPPH to the yellow coloured diphenylpicryl hydrazine and this capacity increases with increasing concentration. Furthermore, comparison with ascorbic acid (standard), showed *T. globiferus* to compete favourably. This demonstrates its high anti-oxidative activity with its IC50 at 38.59µg/ml. The scavenging ability of the *T. globiferus* extract may be due to its biocompositions such as phenolic acids and flavonoid. However, presence of other biomolecules in its extract may contribute to its free radical scarvenging activity.

High performance liquid chromatographic determination (HPLC) showed presence of phenolic acids such as gallic acid, chlorogenic acid and caffeic acid in the ethanolic extract of *T. globiferus* while the flavonoid contents included rutin and quercetin. The highest of the estimated phenolic acids in the ethanolic extract of *T. globiferus* was chlorogenic acid (5.81±0.05) while the least was caffeic acid (1.77±0.01). One of the phenolic constituents of *T. globiferus* is gallic acid. Gallic acid is commonly used in the pharmaceutical industry because many in vivo and in vitro studies in humans, animals, and cell culture have provided evidence for the following actions of gallic acid: (1) it shows cytotoxicity against cancer cells, without harming healthy cells (Elvira et al., 2006); (2) it can be used to treat albuminuria and diabetes(Chiu-Lan et al., 2007); (3) it seems to have antifungal and antiviral properties (Misao et al., 2007); (4) used as an antioxidant and helps to protect human cells against oxidative damage (Jittawan and Sirithon, 2008; Afef et al., 2007); (5) it can be used as a remote astringent in cases of internal hemorrhage (Hurrell et al., 1999);8 (6) used to treat psoriasis and external hemorrhoids containing gallic acid (Cook et al., 1995).

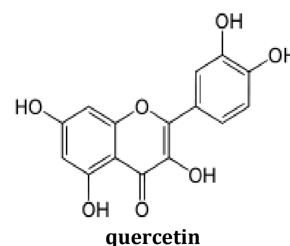


Gallic acid **Chlorigenic acid**

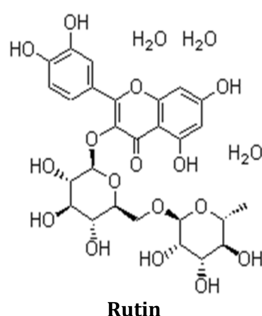
Caffeic acid

Caffeic acid has been shown to inhibit carcinogenesis, although other experiments show possible carcinogenic effects. It is also known as an antioxidant in vitro and also in vivo (Olthof et al., 2001). Caffeic acid also shows immunomodulatory and antiinflammtory activities. Caffeic acid and its derivative, Caffeic acid phenethyl ester (CAPE) have shown tumor-shrinking properties. The subcutaneous and oral administrations of caffeic acid and CAPE significantly reduced liver metastasis (Chung et al., 2004). A study using the caffeic acid phenethyl ester (CAPE) showed a positive effect on reducing carcinogenic incidence. It is known to have antimitogenic, anticarcinogenic, anti-inflammatory, and immunomodulatory properties (Natarajan et al., 1996). Another study also showed that CAPE suppresses acute immune and inflammatory responses and holds promise for therapeutic uses to reduce inflammation (Orban et al., 2000).

The predominant of the estimated flavonoid contents is rutin (8.12±0.09). The relationship between the chemical structure of flavonoids and their radical-scavenging activities was analyzed by Bors, Heller, Michael, and Saran (1990). Quercetin has a catechol structure in ring B, as well as a 2, 3-double bond in conjunction with a 4-carbonyl group in ring C, allowing for delocalization of the phenoxyl radical electron to the flavonoid nucleus. The combined presence of a 3-hydroxy group with a 2, 3-double bond additionally increases the resonance stabilization for electron delocalization; hence it has a higher antioxidant value. This chemical structure and its antioxidative relevance might have contributed to the overall antioxidant activity of *T. globiferus*. Rutin is a glycoside of the flavonoid quercetin. As such, the chemical structures of both are very similar, with the difference existing in the Hydroxyl functional group. Both quercetin and rutin are used in many countries as medications for blood vessel protection, and are ingredients of numerous multivitamin preparations and herbal remedies. In humans, it attaches to the iron ion Fe^{2+}, preventing it from binding to hydrogen peroxide, which would otherwise create a highly-reactive free radical that may damage cells. It is also an antioxidant. Furthermore, it has been shown to inhibit *in vitro* the vascular endothelial growth factor (Haitao et al., 2008) in subtoxic concentrations, so acts as an inhibitor of angiogenesis. This finding can be potentially relevant for the control of some cancers. Also there has been report that rutin inhibits ovariectomy-induced trabecular bone loss in rats, both by slowing down resorption and increasing osteoblastic activity (Horcajada et al., 2000).



quercetin



Rutin

Under normal physiological conditions there is equilibrium between reactive oxygen species (ROS) generated and antioxidants presents. The ROS generated is kept in check by antioxidant defense cascade consisting of enzymatic and non-enzymatic components. One specific ROS, hydrogen peroxide (H_2O_2), which is generated by mitochondrial respiration through a specialized enzyme, is a potent inducer of oxidative damage and mediators of ageing. Here, oxidative damage was stimulated by H_2O_2. DCFH-DA was first described as a probe to evaluate H_2O_2 (Keston and Brandt, 1965); subsequently, it has been suggested that increases in DCF fluorescence actually reflect the overall cellular oxidative stress (Wang and Joseph, 1999), since others forms of free radicals such as peroxyl radical, peroxinitrite, nitric oxide can oxidize DCFH. Our results indicated that H_2O_2 caused a significant increase in ROS production and that ethanolic extract of *T. globiferus* was able to prevent significantly ROS production stimulated by H_2O_2 in a concentration-dependent manner. This effect may be attributed to the activities of quercetin and rutin found in plant extract. In fact, recently, our laboratory have reported that quercetin and its glycoside analog, rutin, prevents against methylmercury-induced ROS production in rat brain slices (Wagner et al., 2010).

These results show possible therapeutic potential of the plant *T. globiferus.* In conclusion, ethanolic extract of *T. globiferus* contains compound(s) that may be capable of scavenging free radicals and, at the same. These results suggest that ethanolic extract of *T. globiferus* could be a good raw material for the development of drugs useful for the treatment of chronic inflammatory diseases.

REFERENCES

1. Afef A, Ines B, Ines S, Kita V, Malika K, Pascal G, Régine S, Anne-Marie M, Kamel G, François L, Marie-Geneviève D, Leila C. (2007): Study of antimutagenic and antioxidant activities of Gallic acid and 1,2,3,4,6-pentagalloylglucose from *Pistacia lentiscus*: Confirmation by microarray expression profiling. Chemico-BiologicalInteractions. Vol. 165,(1), 1-13.
2. Anderson JW, Smith BM, Wasnock CS (1999). Cardiovascular and renal benefits of dry bean and soyabean intake. Amer. J. Clin. Nutr. 70(3Supl):464s-474s.
3. Borek C (2001). Antioxidant health effects of aged garlic extract. J Nutr. 131(3s):1010S-5S.
4. Borelli F, Izzo AA (2000).The plant kingdom as a source of anti-ulcer remedies Phytother. Res. 14(8): 581-591.
5. Bors W, Heller W, Michael C, Saran, M (1990). Radical chemistry of flavonoids antioxidants. Advances in Experimental Medicine and Biology, 264, 165–170.
6. Bravo, L., 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56, 317–333.
7. Chiu-Lan H, Yuh-Charn L, Gow-Chin Y, Hui-Yin C (2007): Preventive effects of guava (Psidium guajava L.) leaves and its active compounds against R-dicarbonyl compounds-induced blood coagulation. *Food Chem.* **2007**, *103*, 528–535.
8. Chung TW, Moon SK, Chang YC, *et al.* (2004). Novel and therapeutic effect of caffeic acid and caffeic acid phenyl ester on hepatocarcinoma cells: complete regression of hepatoma growth and metastasis by dual mechanism. FASEB J. **18** (14):
9. Cook JD, Reddy MB, Hurrell RF. (1995): The effect of red and white wines on nonheme-iron absorption in humans. *Am. J. Clin. Nutr. 61*, 800–804.
10. Craig WJ (1999). Health-promoting properties of common herbs. Amer. J. Clin. Nutr. 70(3 Suppl):491S-499S.
11. Delanty N, Dichter Ma (2000). Antioxidant therapy in neurologic diseases. Arch. Neurol. 57(9):1265-1270.
12. Elvira GM, Chandra S, MarcoVinicio RM, Wenyi W (2006): Catalytic inhibition of human DNA topoisomerase by phenolic compounds in Ardisia compressa extracts and their effect on human colon cancer cells. *Food Chem. Toxicol. 44*, 1191–1203.
13. Fujiki H (1999). Two stages of cancer prevention with green tea. J.Res. Clin. Oncol. 125(11):589-97.
14. Galvano F, Piva A, Ritieni A, Galvano G (2001).Dietary strategies to counteract the effects of mycotoxins: a review. J. Food Prot. 64(1):120-31.
15. Greenwald P (2002). Science, Medicine and the future of Cancer Chemoprevention. Brit. Med. J. 324:714 – 718.
16. Gordon MH (1990): The mechanism of antioxidant action in vitro. In food Antioxidants, Ed., Hudson, B.J.F., Elsevier Applied Science, London, pp 1-18.
17. Haitao L., Bing-Hua J., Sarah M. K., Yi C. C. (2008): Inhibition of Cell Growth and VEGF Expression in Ovarian Cancer Cells by Flavonoids. Nutrition and Cancer. Vol. 60, (6), 800-809.
18. Horcajada-Molteni MN, Crespy V, Coxam V, Davicco MJ, Rémésy C, Barlet JP. (2000): Rutin inhibits ovariectomy-induced osteopenia in rats. J Bone Miner Res.15(11):2251-8
19. Howes MJ, Houghton PJ (2003). Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacol. Biochem. Behav. 75(3):513-27.
20. Howes MJ, Perry NS, Houghton PJ (2003). Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. Phytother. Res.17 (1):1-18.
21. Hurrell RF, Reddy M, Cook JD. (1999): Inhibition of non-heme iron absorption in man by polyphenolic-containing beverages. *Br. J. Nutr. 81*, 289–295.
22. IARC (1996). Preamble to the IARC handbook of cancer chemoprevention. In principles of chemoprevention. Stewart BW, Mc Gregor, Keihues P (eds). IARC Scientific Publication, No139 p1-12. Intl. Agency for Res. Cancer, Lyon, France.
23. Jittawan K, Sirithon S (2008): Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. *Food Chem. 110*, 881–890.
24. Puntel RL, Roos DH, Folmer V, Nogueira CW, Galina A, Aschner M, Rocha JBT (2010) Mitochondrial dysfunction induced by different organochalchogens is mediated by thiol oxidation and is not dependent of the classical mitochondrial permeability transition pore opening. Toxicol Sci 117:133-143.
25. Ketson AS, Brandt R. (1965). The fluorometric analysis of ultra micro quantities of hydrogen peroxide. Analytical Biochemistry 11, 1-5.
26. Kinghorn AD, Su BN, Jang DS, Chang LC, Gu JQ, Carcache-Blanco EJ, Pawlus AD, Lee SK, Park EJ, Cuendet M, Gills JJ, Bhat, HS, Meta Greenwood E, Song LL, Jang M, Pezzuto JM (2004). Natural inhibitors of carcinogenesis. Planta Med. 70(8):691-705.
27. Lampe JW (2003). Spicing up a vegetarian diet: chemopreventiveeffects of phytochemicals. Amer. J. Clin. Nutr. 78(3 Suppl):579S-583S.
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265-275.
29. Mehta RG, Pezzuto JM (2002). Discovery of cancer preventive agents from natural products: from pplants to prevention. Curr. Oncol. Rep. 4(6):478-486.
30. Miller AL (1998). Botanical influences on cardiovascular disease. Altern Med. Rev. 3(6):422-31.
31. Misao U, Hisashi Y, Yukiko K, Masanori H, Tomihiko H, Koyama A (2007): Antiviral effect of octyl gallate against DNA and RNA viruses. *AntiViral Res. 73*, 85–91.
32. Natarajan K, Singh S, Burke TR, Grunberger D, Aggarwal BB (1996). Caffeic acid phenethyl ester is a potent and specific

inhibitor of activation of nuclear transcription factor NF-kappa B. Proc. Natl. Acad. Sci. U.S.A. **93** (17): 9090–5.

33. Olthof MR, Hollman PC, Katan MB (2001). Chlorogenic acid and caffeic acid are absorbed in humans. J.Nutr. **131** (1): 66–71.
34. Orban Z, Mitsiades N, Burke TR, Tsokos M, Chrousos GP (2000). Caffeic acid phenethyl ester induces leukocyte apoptosis, modulates nuclear factor-kappa B and suppresses acute inflammation. Neuroimmunomodulation **7** (2): 99–105
35. Perry EK, Pickering AT, Wang WW, Houghton P, Perry NS (1998). Medicinal plants and Alzheimer's disease: Integrating ethnobotanical and contemporary scientific evidence. J. Altern. Complement Med. 4(4):419-28.
36. Park EJ, Pezzuto JM (2002). Botanicals in cancer chemoprevention. Cancer Metastasis Rev. 31(3-4):231-255.
37. Roos DH, Puntel RL, Farina M, Aschner M, Bohrer D, Rocha JBT, de Vargas Barbosa NB (2011) Modulation of methylmercury uptake by methionine: Prevention of mitochondrial dysfunction in rat liver slices by a mimicry mechanism. Toxicol Appl Pharm 252:28-35.
38. Sabu MC, Kuttan R (2002). Antidiabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethnopharmacol, 81:155 – 160.
39. Scartezzini P, Speroni E (2000).Review on some Indian traditional medicine with antioxidant activity. J.Ethnopharmacol. 71(1-2):23-43.
40. Singleton VL, Rossi JA (1965): Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144-153.
41. Surh Y (1999). Molecular mechanisms and chemopreventive effects of selected dietary and medicinal phenolic substances. Mut. Res. 428(1-2):305-327.
42. Surh YZ, Ferguson LR (2003). Dietary and medicinal antimutagens and anticarcinogens: molecular mechanisms and chemopreventive potential-highlight of a symposium.
43. Tsao AS, Kim ES, Hong WK (2004). Chemoprevention of Cancer. CA Cancer J. Clin.54:150 – 180.
44. Vinson JA, Su X, Zubik L, Bose P (2001): Phenol antioxidant quantity and quality in foods: fruits. J. Agric. Food Chem. 49, 5315-5321.
45. Wattenberg LW (1996). Chemoprevention of Cancer. Preventive Med.25:44 – 45.
46. Wagner C, Vargas AP, Roos DH, Morel AF, Farina M, Nogueira CW, Aschner M, Rocha JB (2010) Comparative study of quercetin and its two glycoside derivatives quercitrin and rutin against methylmercury (MeHg)-induced ROS production in rat brain slices. Arch Toxicol 84:89-97.
47. Wang H, Joseph JA. (1999). Quantifying cellular oxidative stress by a dichlorofluorescein assay using microplate reader. Free Radical Biology and Medicine 27, 612-616.
48. Youdim KA, Joseph JA (2001). A possible emerging role of phytochemicals in improving age-related dysfunction: a multiplicity of effects. Free Radic. Biol. Med. 30(6):583-594.
49. Laghari, A.H, Memon, S., Nelofar, A., Khan, K.M., Yasmin, A., 2011. Determination of free phenolic acids and antioxidant activity of methanolic extracts obtained from fruits and leaves of *Chenopodium album*. Food Chem. 126, 1850–1855.