Mal J Nutr 15(1): 65 - 75, 2009

Nutritional Composition, *in vitro* Antioxidant Activity and *Artemia salina* L. Lethality of Pulp and Seed of *Tamarindus indica* L. Extracts

Khairunnuur FA[1], Zulkhairi A[2], Azrina A[2], Moklas MAM[1], Khairullizam S[1], Zamree MS [3]& Shahidan MA[3]

[1] *Department of Human Anatomy, Division of Physiology, Faculty of Medicine and Health Sciences Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia*

[2] *Department of Nutrition & Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia*

[3] *Herbal Technology Center, Forest Research Institute of Malaysia, Kepong, 52109 Kuala Lumpur Malaysia*

ABSTRACT

This study was designed to examine the nutritional composition, antioxidant activity and medium lethal concentration (LC_{50} value) of *Tamarindus indica* L. pulp and seed extracts *in vitro*. The extraction was set at 40°C, 60°C and 100°C for 12 hours, 6 hours and 15 minutes respectively to determine the optimum extraction parameter whereas the anti-oxidant activity of the extracts was measured using iron (III) reduction (FRAP) assay. Total phenolic content (TPC) of the extracts was estimated as gallic acid equivalent by Folin-Ciocalteau method. Toxicity potential of the extract was assessed *in vitro* by *Artemia salina* lethality test both in seed and pulp samples. The results showed that tamarind seed contained a higher percentage of carbohydrate, protein, fat and energy (15%, 82%, 95% and 33.13% respectively) than the pulp. On the other hand, the pulp demonstrated a high moisture (51.1%) and ash (34.84%) content than the seed. For the mineral analysis, tamarind seed contained higher Ca and C (1.0% and 50.73% respectively) than the pulp (0.27% and 40.40% respectively). No heavy metals were detected in both samples. Seed extracted at 60°C / 6 hours and 100°C / 15 minutes showed the highest TPC value and were significantly different (p<0.05) than the seed extracted at 40°C / 12 hours. Anti-oxidant activity is positively correlated to the TPC value of the extracts (R=0.991). The pulp and seed extracted at 100°C / 15 minutes showed the highest FRAP value among its groups (216.17 ± 14.06 μmol (Fe) / g and 659.74 ± 16.40 μmol (Fe) / g respectively). This study indicates that tamarind pulp and seed extracts possess beneficial antioxidant properties and the optimum extraction parameter is 100°C for 15 minutes. In *Artemia salina* lethality test, tamarind pulp caused significant mortality of the crustacean larvae with LC_{50} in the range of 26-28 μL / mL. Tamarind seed were not toxic to *Artemia salina* since the LC_{50} of the extracts was higher than 1000 μL / mL.

Correspondence author: Dr Zulkhairi Hj Amom; *Email:zakirukm@hotmail.com, fradical@medic.upm.edu.my*

INTRODUCTION

Tamarind *(Tamarindus indica* L.) is a perennial herb belonging to the dicotyledonous family of Leguminosae. Its local names include Indian date (English), *asam jawa* (Malay), *siyambala* (Sinhala), *sampalog* (Philippines) and *puli* (Tamil). The tree averages 20-25 m in height and 1 m in diameter, has a wide spreading crown and a short, stout trunk. It is slow growing, but long lived, with an average life span of 80-200 years. Today, tamarind grows widely in most tropical and subtropical regions of the world. Tamarind is well adapted to semi-arid tropical conditions and also grows well in many humid tropical areas with seasonally high rainfall. Tamarind is grown commercially in plantations and homestead gardens for its product, and along avenues as a ornamental plant in towns and cities. Tamarind has many uses and it is best known for its fruits.

Tamarind pulp is widely consumed in many countries around the world. It is often made into juice, infusion or brine though there are many different recipes. In some African countries, the pulp juice is mixed with wood ash to neutralize the sour taste of the tartaric acid, but the common method is to add sugar to make a pleasantly acid drink. The pulp is often eaten raw and sweetened with sugar (Lotschert & Beese, 1994). In Ghana, a bitter infusion of the pods is used for cooking cereals and is often added to the water in which poisonous yams are soaked to detoxify them. In India, the juice is used to preserve fish, which can be preserved for up to six months when mixed with acetic acid. Tamarind is also used in the same way in Sri Lanka and many other Asian countries (Macmillan, 1943). The juice is also an ingredient of Worcestershire and other barbecue sauces, commonly used in European and North American countries (NAS, 1979).

Tamarind pulp has been reported to be used in the treatment of a number of ailments, including the alleviation of sunstroke, *Datura* poisoning (Gunasena & Hughes, 2000), and the intoxicating effects of alcohol and cannabis. It can be gargled for sore throats, dressing of wounds (Chaturvedi, 1985) and is said to aid the restoration of sensation in cases of paralysis. Tamarind pulp is also said to aid in the cure of malarial fever (Timyan, 1996). The fruits are reported to have anti-fungal and anti-bacterial properties (John, Joy & Abhilash, 2004). The pulp extract has also been shown to enhance the bioavailability of ibuprofen in humans (Garba *et al*., 2003).

Numerous studies on aqueous extracts of tamarind seeds have shown a strong anti-diabetic effect in rats (Maiti *et al*., 2004; Osawa *et al.*, 1994). In Cambodia and India, it has been reported that the seed extracts is used to treat boils and dysentery (Rama Rao, 1975; Jayaweera, 1981). Boiled, pounded seeds are reported to treat ulcers and bladder stones whereas powdered seed husks are used to treat diabetes (Rama Rao, 1975). The anti-oxidative activity of tamarind seed was investigated by Osawa *et al*. (1994). They found that an ethanol extract prepared from the seed coat exhibited anti-oxidative activity as measured by the thiocyanate and thiobarbituric (TBA) method. Ethyl acetate extracts prepared from the seed coat also possess a strong antioxidant activity and was confirmed by Luengthanaphol *et al*. (2004). This data suggests that tamarind seed coat, a byproduct of the tamarind gum industry, could be used as a safe and low-cost source of anti-oxidant, although other herbals may be more effective (Ramos *et al*., 2003).

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism. At low-to-moderate concentrations, they possess various physiological roles ranging from cellular signal transduction to defence against pathogens (Valko *et al*., 2007). However, during oxidative stress, there is an overproduction of ROS and RNS on one

side and a deficiency of enzymatic and non-enzymatic antioxidant defence system on the other, resulting in degradation of cellular components, DNA, carbohydrates, proteins and lipids. This will eventually lead to cellular dysfunction and cell death. The involvement of free radicals in biological systems suggests that oxidative stress plays a cardinal role in the pathogenesis of many diseases as well as in the ageing process (Valko *et al*., 2007).

Recently there has been a growing interest in the search for natural antioxidants for three principal reasons (Dastmalchi *et al*., 2007): (i) numerous clinical and epidemiological studies have demonstrated that consumption of fruits and vegetables is associated with reduced risks of developing chronic diseases such as cancer, cardiovascular disorders and diabetes; (ii) safety considerations regarding the potential harmful effects of the chronic consumption of synthetic antioxidants in foods and beverages; and (iii) the public's perception that natural and dietary antioxidants are safer than synthetic analogues. These have resulted in increased interest in spices, aromatic and medicinal plants as sources of natural antioxidants.

The aim of the present work is to evaluate the nutritional composition of *Tamarindus indica* seed and pulp as well as its *in vitro* antioxidant activities in addition to its total phenolic contents. The antioxidant activity of the extracts was tested, using ferric reducing / antioxidant power (FRAP) assay and compared to that of BHT. In spite of their beneficial medicinal uses, many plants are known to be toxic. For this reason, the lethality of the extracts was also determined using Brine Shrimp Lethality Test (BSLT). *Artemia salina* L. (Artemiidae), the brine shrimp, is an invertebrate component of the fauna of saline aquatic and marine ecosystems. It plays an important role in the energy flow of the food chain (Sanchez Fortun, Sanz-Barrera & Barahona-Gomariz, 1995) and can be used in a laboratory bioassay in order to determine lethality through the estimation of the medium lethal concentration (LC_{50} values) (Lewan, Andersson & Morales-Gomez, 1992), which has been reported to be useful for a series of toxins and plants extracts previously (Meyer *et al*., 1982).

MATERIALS AND METHODS

Chemicals

Butylated hydroxytoluene (BHT), ferric chloride, hydrochloric acid, ferrous sulphate, acetic acid, sodium acetate and gallic acid were purchased from Sigma Chemical Co. (USA). Folin-Ciocalteu and sodium carbonate were from Merck (Germany). 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) was from Fluka (Switzerland).

Preparation of tamarind pulp and seed extracts

Fresh matured tamarind fruits were collected from Universiti Putra Malaysia (UPM) herbal centre after being identified and confirmed by a plant taxonomist. The fruits were peeled and the pulps were separated from the seed. A quantity of 10% aqueous pulp extract was prepared by soaking 100 g of the fresh pulp (equivalent to 25 tamarind pods) in 1L of distilled water and mixed thoroughly. The mixture was incubated in shaking water bath at various temperatures and time setting: 40 °C / 12 hours, 60 °C / 6 hours and 100°C / 15 minutes. Once filtered, the filtrates were freeze dried and kept at -20°C until used. The variation of temperature and incubation time was proposed with the goal of optimising the yield of potential biological compounds from the extract since no data on the optimisation of the extraction procedure of this plant has been reported. For the seed extraction, the seeds were rinsed, thoroughly dried and ground before use. Fifty pods of tamarind fruit were used to produce 100 g of grounded seeds. A similar procedure of extraction was applied to tamarind seed.

Proximate and mineral analysis

The total ash, moisture, crude protein, fat and carbohydrate were determined in accordance with AOAC methods (AOAC, 1995). All the proximate analyses were carried out in triplicate and reported in percentage. The minerals including both macro- and micro-elements were determined by scanning electron microscopy attached with energy dispersive X-ray (SEM-EDX) technique (Japan).

Determination of total phenolic content

The concentration of total phenolic was based on the method described by Velioglu *et al.* (1998) with some modification. Briefly, an aliquot (2 mL) of appropriately diluted extracts were mixed with 1 mL of 1 N Folin–Ciocalteu reagent in 10 mL volumetric flasks. After 5 minutes, 4 mL of saturated sodium carbonate solution was added. The volume was then made up to 10 mL with distilled water and mixed thoroughly. The absorbance of the reaction mixtures was measured at 760 nm against a blank after 2 hours. Gallic acid (GA) was used to construct a standard curve (0–50 mg / L). Measurements of every sample were taken in triplicate and the results are expressed as milligram gallic acid equivalents (GAE) / g dried weight.

Ferric reducing/antioxidant power (FRAP) assay

The ferric reducing ability of the plant materials was assessed using the method described by Benzie & Strain (1996). Briefly, the FRAP reagent containng 2.5 mL of 10 mM of 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3 M acetate buffer, pH 3.6 was freshly prepared and warmed to 37ºC prior to the analysis. 100 μL of sample (studied extracts) were mixed with 300 mL distilled water and 3 mL of FRAP reagent was added. The absorbance was measured every 10 seconds and the reaction was followed until it reached the plateau. Values were calculated according to the calibration curve with aqueous solutions of $FeSO_4 \cdot 7H_2O$ in the range of 100–1000 μM. The final results were expressed as the concentration of antioxidants having ferric reducing ability equivalent to that of 1 mM FeSO4, particularly expressed as mol Fe(II) equivalent / g sample in dry weight.

***Artemia salina* lethality test**

Dried *Artemia salina* cysts were hatched in filtered and sterilised artificial seawater (1 g cyst per liter) at 28°C, under conditions of strong aeration and continuous light regime (Vanhaecke *et al.*, 1981). Approximately 24 hours after hatching, the phototropic nauplii were collected with a pipette and concentrated in a small vial. Various concentrations of extracts ranging from 0μL / mL to 1000 μL / mL were prepared in 6x4 cell culture well plates. The concentrations were obtained by transferring the corresponding volume of the extracts from the stock solution to different wells and added with artificial seawater to make up the final volume of 1 mL. The wells were then gently shaken to ensure that the compounds diffused adequately in the aqueous solution. Three replicates were used for each treatment and control.

An average of 10 to 15 nauplii was transferred to each well using a pipette. Each test consisted of exposing groups of 10 to 15 *Artemia* aged 24 hours to various concentrations of the extracts tested. The lethality was determined after 24 hours of exposure where the larvae were observed under stereo microscope. The number of survivors were counted and percentages of deaths were calculated. Larvae were considered dead if they did not exhibit any movement during observation.

Statistical analysis

All experiments were conducted in triplicate and statistical analysis was done using the

Statistical Package for Social Sciences (SPSS) version 14 programme. Results were expressed as a mean of three determinations ± SEM. A value of $p<0.05$ was used to denote statistical significance.

RESULTS AND DISCUSSION

Proximate analysis and mineral contents

The proximate and minerals composition of tamarind fruit pulp and seed are presented in Table 1. Protein content of tamarind seed was about 13.35%, which is about seven times higher than tamarind pulp which contained only 2.40% of protein. Tamarind seed contained 2.90% of fat, whereas tamarind pulp showed an inappreciable amount of the total fat which was 0.14%. Total carbohydrate of tamarind seed was 61.15% whilst tamarind pulp contained 51.50% of total carbohydrate. The level of total ash in tamarind seed and pulp were quite similar at 2.15% and 3.30% respectively. Moisture content formed the bulk of tissue weight in the fresh tamarind seed and pulp with mean values of 20.45% and 42.70% respectively. Total energy content in 100 g of tamarind seed and pulp were 324.5 Kcal and 217.0 Kcal respectively.

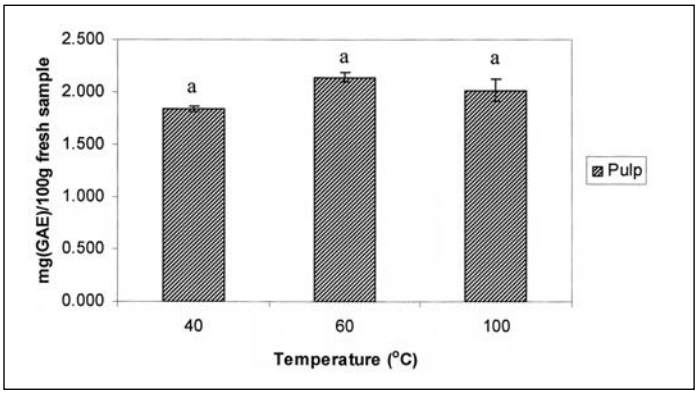
As shown in Table 1, both tamarind fruit pulp and seed possessed carbon and oxygen at a high percentage. Both of these elements are more or less present in equal ratios. Pulp samples contained a higher amount of oxygen than carbon (57.85% and 40.40% respectively) while seed samples contained higher amounts of carbon than oxygen (50.73% and 46.82% respectively). Both of these samples showed significant differences in their carbon and oxygen values. These two elements along with

Table 1. Proximate and minerals composition of *Tamarindus indica* pulp and seed

Constituents	*Pulp*	*Seed*
Carbohydrate, %	51.50	61.15
Protein, %	2.40	13.35
Fat, %	0.14	2.90
Ash, %	3.30	2.15
Moisture, %	42.70	20.45
Energy, Kcal/100g	216.86	324.10
Carbon, mg/100g sample	40.40[a]	50.73[b]
Oxygen, mg/100g sample	57.85[c]	46.82[d]
Magnesium, mg/100g sample	0.15	0.16
Phosphorus, mg/100g sample	0.16	0.15
Potassium, mg/100g sample	1.16	0.67
Calcium, mg/100g sample	0.27[e]	1.0[f]
Aluminium, mg/100g sample	ND	0.21
Sulfur, mg/100g sample	ND	0.15
Bromide, mg/100g sample	ND	0.39
Molybdenum, mg/100g sample	ND	0.40

Values within the same row with different superscripts are significantly different from each other at 5% statistical level. Energy was calculated by summation of (fat x 9 kcal) + (protein x 4 kcal) + (carbohydrate x 4 kcal). ND: Not detected; 1% = 10,000 ppm.

a)



b)

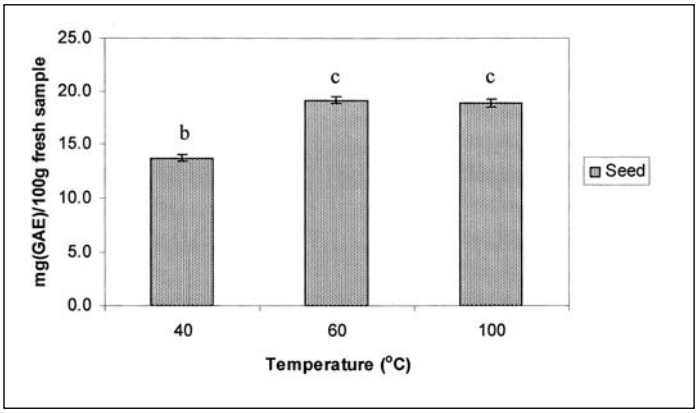


Figure 1. Total phenolic content of tamarind (a) pulp and (b) seed extracted at various temperatures. Data expressed as mean ± SEM. Bars with same alphabets are not significantly different (p>0.05)

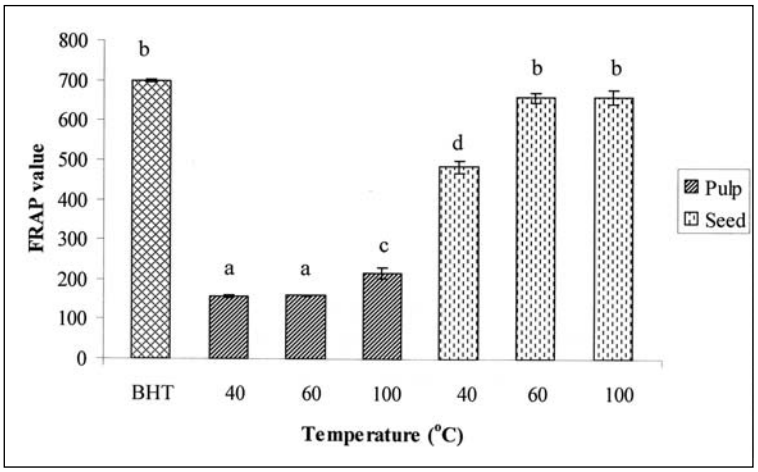


Figure 2. Ferric reducing / antioxidant power (FRAP) of tamarind pulp and seed extracted at various temperatures. Data expressed as mean ± SEM. Bars with same alphabets are not significantly different (p>0.05). BHT: Butylated hydroxytoluene

magnesium, phosphorus, potassium and calcium were the common elements for both the tamarind samples. Both of these samples possessed almost similar amounts of magnesium and phosphorus at 0.15% and 0.16% in pulp and 0.16% and 0.15% in seed respectively. Potassium contents of tamarind pulp and seed varied, 1.16% and 0.67% respectively whereas tamarind pulp contained a higher value. In contrast, tamarind seed contained a significantly higher amount of calcium than tamarind pulp (1.0% and 0.27% respectively). Aluminium, sulphur, bromide and molybdenum were only common to tamarind seed (0.21%, 0.15%, 0.39% and 0.40% respectively) while tamarind pulp was devoid of these elements. This study shows that the nutritional status of tamarind seed is better than tamarind pulp as it contains more beneficial minerals. The analysis of the samples showed that heavy metals like lead and arsenic that are toxic to the body tissue were not detected in both of the samples. This is a clear indication that both tamarind pulp and seed can be used as valuable sources of minerals for human and animal consumption.

Total phenolic content

Phenolic compounds are commonly found in plants and have been reported to have several biological activities including anti-oxidant properties. It may be a useful indicator of potential nutritional benefit. The total phenolic content in the extracts was determined according to the colorimetric Folin–Ciocalteu method with gallic acid as a standard compound ($R^2 = 0.97$, $y = 13.89x$) (data not shown). A wide range of total phenolic content was found in the plant materials studied as shown in Figure. 1. Their contents ranged from 1.83 ± 0.02 to 19.21 ± 0.29 mg gallic acid equivalent (GAE) / 100g of dried samples, with an average of 9.64 mg(GAE) / 100g fresh sample.

Among the six samples, the tamarind seed extracted at 60°C / 6 hours demonstrated the highest phenolic content (19.21 ± 0.29 mg(GAE) / 100g fresh sample), followed by tamarind seed extracted at 100°C / 15 minutes (18.91 ± 0.36 mg(GAE) / 100g fresh sample), tamarind seed extracted at 40°C / 12 hours (13.72 ± 0.30 mg(GAE) / 100g fresh sample), tamarind pulp extracted at 60°C / 6 hours (2.14 ± 0.05 mg(GAE) / 100g fresh sample), tamarind pulp extracted at 100°C / 15 minutes (2.02 ± 0.10 mg(GAE) / 100g fresh sample) and tamarind pulp extracted at 40°C / 12 hours (1.83 ± 0.02 mg(GAE) / 100g fresh sample). One-way ANOVA showed significant differences ($p<0.05$) in total phenolic content among all the tamarind pulp extracts compared with the tamarind seed extracts at all temperatures. No significant difference was observed in the phenolic content of all the tamarind pulp extracts extracted at three different temperatures (40ºC, 60ºC and 100ºC). However, for the seed extracts, samples extracted at 60°C / 6 hours and 100°C / 15 minutes were significantly higher in total phenolic content compared to tamarind seed extracted at 40°C / 12 hours.

Antioxidant activity of the tamarind extract

FRAP assay was used to assess the free radical scavenging capacities and the reducing potentials of the antioxidant constituents of the tamarind extracts. This experiment demonstrated that both tamarind seed and pulp extracts possessed strong antioxidative properties. The seed extracts were collectively found to be higher in FRAP values in contrast to the pulp extracts whereas seed extracted at 100°C / 15minutes contained significantly high FRAP value (659.74 ± 16.40 μmol (Fe) / g) than the pulp extracted at the same extraction parameter ($p<0.05$). Extraction at 100°C / 15 minutes contained highest antioxidant potential with FRAP value of 216.17 ± 14.06 μmol (Fe) / g followed by the pulp extracted at 60°C / 6 hours (159.16 ± 2.86 μmol (Fe) / g) and at 40°C / 12 hours (157.09 ± 3.39 μmol (Fe) / g) (Figure 2). The seed extracts had the

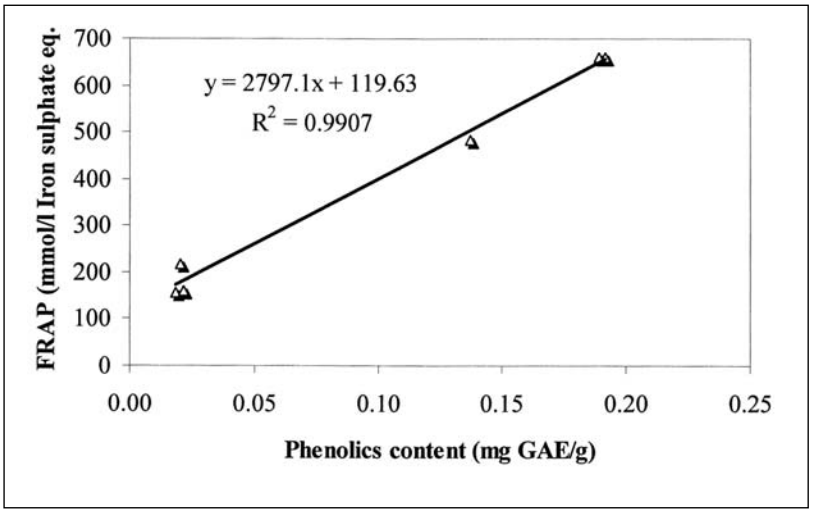


Figure 3. Correlation between total phenolic content and FRAP. Values are mean of three determinations ± SEM. GAE: gallic acid equivalent

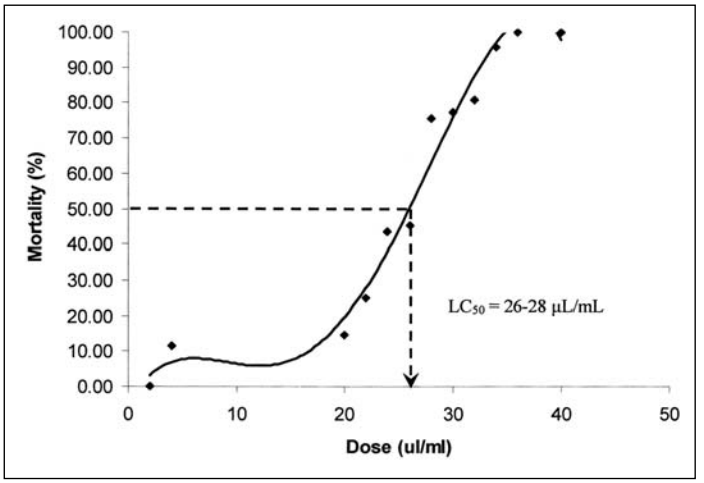


Figure 4. Dose-response relationship of *Tamarindus indica* pulp extract

highest anti-oxidant propensities compared to the pulp extracts. In the tamarind seed extracts, the order of efficacy was 100°C / 15 minutes > 60°C / 6 hours > 40°C / 12 hours with FRAP value of, 659.74 ± 16.40, 658.70 ± 11.18 μmol (Fe) / g and 484.22 ± 14.45 μmol (Fe) / g respectively.

The pulp extracted at 40°C / 12 hours and at 60°C / 6 hours did not show any significant difference when compared but both showed significant differences compared to pulp extracted at 100°C / 15 minutes, BHT and all of the seed extracts. BHT and the seed extracted at 60°C / 6 hours and at 100°C / 15 minutes did not show any significant difference among them but showed significant difference to seed extracted at 40°C / 12 hours and to all of the pulp extracts.

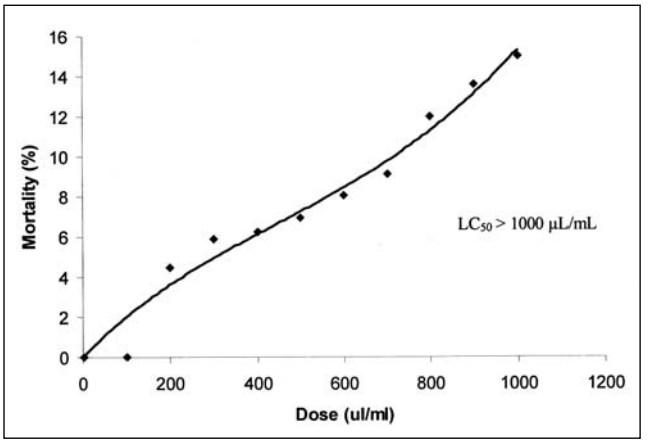


Figure 5. Dose-response relationship of *Tamarindus indica* seed extract

Total phenol content versus the radical scavenging activity

In this analysis, the correlation between the radical scavenging activity and phenol content of the six samples was studied using a linear regression analysis. As demonstrated in Figure 3, the correlation coefficient between total phenolics and FRAP values (R^2 = 0.99, y = 2797.1x + 119.63) was found to be very strong, more than 0.5. These strong correlation values between total phenols and the antioxidative activity suggests that the major antioxidant compounds in studied extracts might be phenolics.

Brine shrimp test

The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. In addition, the method is rapid, simple, reproducible and economical. A wide variety of biologically active chemical compounds, in particular cytotoxic agents, are toxic to brine shrimp; the death of this organism when exposed to varying concentrations of these compounds forms the basis of a toxicity test. Bioactive compounds are nearly always toxic in high concentrations and, as toxicology can be described as pharmacology at higher doses, this premise has been applied to the screening of medicinal plant extracts in the brine shrimp toxicity test. The test has also been used for monitoring mycotoxins, wastewater and marine pollutants, detergents and surfactants, petroleum products, food dyes, anti-fouling paints for ships, heavy metals and pesticides (Sam, 1993).

The results of *Artemia salina* testing are summarised in Figures 4 and 5. The results revealed that seed of *Tamarindus indica* was not toxic to *Artemia salina* since the LC_{50} of the extract was higher than 1000 μL / mL. Pulp of *Tamarindus indica* exhibited weak toxicity with the LC_{50} of the extract ranging from 26-28 μL / mL.

CONCLUSIONS

This study reveals that tamarind pulp and seed contain nutritionally useful quantities of macro- and micro-nutrients such as protein, carbohydrate, calcium, potassium and magnesium and our bodies should receive significant amounts of each of these nutrients for normal body functions. In addition, stress and exposure to environmental pollution may raise our requirements for minerals, especially calcium. Therefore, tamarind pulp and seed can be used as alternative sources of

nutrients to alleviate malnutrition and to improve nutritional status in humans and animals.

Both the tamarind pulp and seed extracts may also be rich in several phytonutrients that act as powerful dietary antioxidants. The extracts that were extracted at different temperatures demonstrated varying degrees of activity in the ferric reducing / antioxidant power (FRAP) assay. The seed at 100°C / 15 minutes showed the highest FRAP value among the different sample groups. The antioxidative data presented in this study suggest that the optimum temperature of tamarind pulp and seed extraction is at 100°C for 15 minutes. Total phenolic content and antioxidant capacity have different values among the samples and there is a strong correlation between phenolic concentration and antioxidant potency which indicate that the phenolic content is likely to contribute to the antioxidant activity of the extracts. Brine shrimp results show that the seed extract is virtually non-toxic to the shrimps, with LC_{50} value greater than 1000 μL / mL. On the other hand, the pulp extract exhibited low toxicity with LC_{50} ranging from 26 μL / mL to 28 μL / mL. However, further investigation and experiments such as *in vivo* studies are needed to confirm the toxicity potential of these extracts.

ACKNOWLEDGEMENTS

The study was supported by Research University Grant Scheme (RUGS) Universiti Putra Malaysia grant number 91135. The authors wish to acknowledge the Bioscience Institute and Anatomy Laboratory of Universiti Putra Malaysia for offering their facilities for the analysis.

REFERENCES

AOAC (1995). Official Methods of Analysis. 16th edn. Association of Official Analytical Chemists, Washington, DC.

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

Chaturvedi AN (1985). Firewood farming on the degraded lands of the Gangetic plain.U. P. *Forest Bulletin* No.50. Lucknow, India Government of India Press 1: 286.

Dastmalchi K, Dorman HJD, Kos-ar M & Hiltunen R (2007). Chemical composition and in vitro antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L) extract. *Food Sci Tech* 40: 239–248.

Garba M, Yakasai IA, Bakare MT & Munir HY (2003). Effect of *Tamarindus indica* L on the bioavailability of ibuprofen in healthy human volunteers. *Eur J Drug Metab Pharmacokinet* 28(3): 179-184.

Gunasena HPM & Hughes A (2000). Tamarind, *Tamarindus indica L.*, International Centre for Underutilised Crops, Southampton, UK.

Jayaweera DMA (1981). Medicinal plants (indigenous and exotic) used in Ceylon. Part 111. *Flacourtiaceae-Lytharaceae*. A publication of the National Science Council of Sri Lanka. pp. 244-246.

John J, Joy M & Abhilash EK (2004). Inhibitory effects of tamarind (*Tamarindus indica* L.) on polypathogenic fungi. *Allelopath J* 14(1): 43-49.

Lewan L, Andersson M & Morales-Gomez P (1992). The use of *Artemia salina* in toxicity testing. *Altern Lab Animals* 20: 297-301.

Lotschert W & Beese G (1994). Tropical plants. Collins Photo Guide. Harper Collins Publishers. p. 223.

Luengthanaphol S, Mongkholkhajornsilp D, Douglas S, Douglas PL, Pengsopa L & Pongamphai S (2004). Extraction of antioxidants from sweet Thai tamarind seed coat: preliminary experiments. *J Food Eng* 63(3): 247-252.

Macmillan H.F (1943). Handbook of tropical plants. 362. Medicinal plants. Anmol Publications, New Delhi, India.

Maiti R, Jana D, Das UK & Ghosh D (2004). Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* in streptozotocin-induced diabetic rats, *J Ethnopharmacol* 92(1): 85-91.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE & Mc Laughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica* 45: 31-34.

NAS (1979). Tropical legumes: resources for the future. Washington DC. pp.117-121.

Osawa T, Tsuda T, Watanabe M, Ohshima K & Yamamoto A (1994). Antioxidative components isolated from the seeds of tamarind (*Tamarindus indica* L). *J Agric Food Chem* 42(12): 2671-2674.

Rama Rao M (1975). Flowering plants of Travancore. Bishen Singh Mahendra Pal Singh, Dehra Dun India. p. 484.

Ramos A, Visozo A, Piloto J, Garcia A, Rodriguez CA & Rivero R (2003). Screening of antimutagenicity via antioxidant activity in Cuban medicinal plants. *J Ethnopharmacol* 87(2&3): 241-246.

Sam TW (1993). Toxicity testing using the brine shrimp; *Artemia salina*. *Bioactive Nat Prod*: 441-457.

Sanchez-Fortun S, Sanz-Barrera F & Barahona-Gomariz MV (1995). Acute toxicities of selected insecticides to the aquatic arthropod *Artemia salina*. *Bull Environ Contam Toxicol* 54(1): 76-82.

Timyan J (1996). Important trees in Haiti. Southeast Consortium for International Development, 1634, 1 Street N.W. Suite 702, Washington DC 20006.

Vanhaecke P, Persoone G, Claus C & Sorgeloos P (1981). Proposal for a short-term toxicity test with *Artemia nauplii*. *Ecotoxicol Environ Saf* 5: 382–387.

Valko M, Leibfritz D, Moncol J & Cronin MTD (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Intl J Biochem Cell Biol* 39: 44–84.

Velioglu YS, Mazza G, Gao L & Oomah BD (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *J Agric Food Chem* 46: 4113-4117.