

TOTAL PHENOLIC, FLAVONOID CONTENTS AND ANTIOXIDANT ACTIVITY OF TAMARIND SEED AND PULP EXTRACTS

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Received: 12.9.2021

Accepted: 15.01.2022

SUMMARY

Tamarind (*Tamarindus indica*) has long been known for its high nutrition content and pharmacological potential. However, there is lack of studies on the content of antioxidants, phenolic and flavonoid contents of tamarind seed grown in Vietnam. Thus, the aim of this study was to compare the seeds and pulps of *Tamarindus indica* from three different areas across Vietnam including Son La, Hai Phong and Sai Gon with regard to the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of their water and methanol extracts, as well as their cytotoxicity on a normal BKH-21 cells. TPC and TFC were evaluated by the Folin–Ciocalteu reagent and aluminum chloride, respectively. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays were used to investigate antioxidant capacity. The safety of *T. indica* extracts was assessed by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Our results showed that the methanolic extracts yielded higher TPC (742.919 ± 50.360 mg GAE/g extract), TFC (68.492 ± 0.023 mg QE/g extract) and possessed stronger free radical scavenging activity (IC_{50} of $52.5 \mu\text{g/mL}$) compared to that of water extracts. *T. indica* seeds from all three regions possessed higher TPC, TFC and antioxidant activity than those of pulps. Regarding the safety, *in vitro* analysis showed that tamarind seed and pulp extracts only became toxic to BHK-21 cell line at a very high concentration with IC_{50} values range from $143.77 \mu\text{g/mL}$ to $620.35 \mu\text{g/mL}$. This study revealed that *T. indica* seeds and pulps can serve as functional food as well as potential antioxidants in pharmaceutical products.

Keywords: ABTS, antioxidant, DPPH, *Tamarindus indica*

INTRODUCTION

The accumulation of reactive oxygen species (ROS) with a single unpaired electron can stimulate oxidative stress which participates in the pathogenesis of many physiological

disorders and diseases, including cellular injury, aging, cancer, and hepatic, neurodegenerative, cardiovascular and renal disorders (Alfadda, Sallam, 2012). The human body possesses a variety of endogenous antioxidants such as superoxide dismutase, catalase (CAT) and

glutathione peroxidase. These enzymes neutralize free radicals by giving up some of their electrons, thus maintaining cellular homeostasis (Kurutas, 2016). Nevertheless, endogenous antioxidants alone may be inadequate to deactivate the free radicals in the body, especially during inflammation or oxidative stress (Young, Woodside, 2001).

Research has been on the rise for natural antioxidants from plants with low toxicity and high efficacy since they can provide additional help for the plasma antioxidants in clearing free radical (Ronald, Guohua, 2000). Commonly known antioxidants in plants are phenolic and flavonoid compounds such as tocopherols, carotenoids, phenolic acids (benzoic acid derivatives and cinnamonic acids), flavonoids, and diprenes (Zargoosh *et al.*, 2019). These natural compounds are plant secondary metabolites that hold an aromatic ring with at least one hydroxyl group which are responsible for antioxidant activity because they are good electron donors (Tungmannithum *et al.*, 2018, Bendary *et al.*, 2013). Studies have shown that phenolic compounds possess free radical inhibition capacity, metal inactivation or oxygen scavenging and prevent oxidative disease burden (Babbar *et al.*, 2015).

Tamarind (*Tamarindus indica*) is a fruit plant that belongs to the legume family, grows in tropical and subtropical regions such as Africa, India and Southeast Asia, with ideal average temperature of 25°C (Cardoso *et al.* 2016). Tsuda and colleagues reported four phenolic antioxidants in Indian tamarind seeds: 2-hydroxy-3,4-dihydroxyacetophenone; methyl 3, 4-hydroxybenzoate; 3,4-dihydroxyphenyl acetate and epicatechin (Tsuda *et al.*, 1994). Sudjaroen and colleagues identified the polyphenolics profile of Thailand tamarind pericarp which was dominated by proanthocyanidins in various forms, indicating that tamarind may be an important source of cancer chemopreventive natural products in tropical regions (Sudjaroen *et al.*, 2005). Even though tamarind extracts have been studied for their chemical properties as well as biological

activities in the world, there is very limited study about its extracts in Vietnam. Therefore, further studies on the antioxidative activities and toxicological effect of *T. indica* are required. In addition, chemical properties and biological activities of tamarind fruits could be differed by the collected areas. From these standpoints, it was of great interest to compare the total phenolic (TPC), flavonoid contents (TFC) and cytotoxicity of *T. indica* seed and pulp extracts in water and methanol obtained from three different areas across Vietnam (Son La, Hai Phong and Sai Gon). Moreover, the antioxidant capacity of these extracts was determined using the DPPH and ABTS radical scavenging activity.

MATERIALS AND METHODS

Sample collection and preparation

Fresh tamarind fruits were collected from three different areas across Vietnam (Son La, Hai Phong and Sai Gon) in February 2019 when they were close to ripe and dried at 70°C for 6 hours. The brown peel was then removed, whereas the seeds and pulps were thoroughly separated and dried to constant weight. The samples were then blended to a homogeneous, soft powder and sieved through a 0.18 mm sieve.

Sample extraction

The grounded powders (30 g) of *T. indica* seeds or pulps were immersed in water or methanol (50 mL) over night at room temperature to form water extracts or methanol extracts. The mixture was then sonicated in an ultrasonic bath for 20 minutes to accelerate the extraction process. This process was repeated three times. Next, the extracts were concentrated to dryness in a rotary evaporator under reduced pressure and controlled temperature (50–60°C) to give final residues. All samples were stored at 4°C until further use.

Determination of total phenolic content (TPC)

TPC of *T. indica* extracts was measured using Folin–Ciocalteu test referring to the protocol developed by Zargoosh *et al.* (Zargoosh *et al.*, 2019) with some modifications. In brief, each

extract was dissolved in DMSO 99.9% (v/v) in a test tube to yield a stock solution at 5 mg/mL. 20 μ L of the extract (5 mg/mL) was mixed with 50 μ L of the Folin–Ciocalteu reagent (diluted 10-fold with deionized water beforehand). After incubating for 5 minutes at room temperature, the mixture was added 100 μ L of sodium carbonate (Na_2CO_3) along with 230 μ L deionized water to reach a final volume of 400 μ L. After 30 minutes, the absorbance of each mixture was measured using the UV-spectrophotometer at 760 nm against a blank of DMSO.

A serial dilution (0.0125 to 0.4 mg/mL) of gallic acid was prepared to construct a calibration curve of standard reference. The TPC of the gallic acid standards was analyzed in parallel with *T. indica* extracts. The absorbance was measured using the UV-spectrophotometer at 760 nm against a blank of methanol (MeOH). TPC from plant extracts was expressed as mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

Determination of total flavonoid content (TFC)

Total flavonoid content of individual extract was determined following a procedure described by Chang *et al.* (2002) with some modifications. An aliquot of 120 μ L of extract solution (5–100 mg/mL) or quercetin (0.05–1 mg/mL) were mixed with 20 μ L of NaNO_2 10% (w/w). The mixture was incubated for 6 minutes before adding 20 μ L AlCl_3 10% (w/w). After another 6 minutes, 200 μ L of NaOH (1M) and 140 μ L ethanol 30% was added. The final mixture was incubated for 30 minutes at room temperature. Quercetin serial dilution was used to construct the TFC standard curve. The absorbance was then measured at 490 nm against a reagent blank of DMSO (for plant extract) or methanol (for quercetin). The outcome data were expressed as milligrams of quercetin equivalents per gram (mg QE/g) of dry extract.

DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Brand-Williams *et al.*, 1995) was adopted

to measure the free radical scavenging activity (RSA) of *T. indica* extracts. Briefly, 9 μ L of either extract solution (6.25, 25, 100 μ g/mL) or Ascorbic acid (positive control, 1.25, 2.5, 5, 10, 50 μ g/mL) in DMSO was added to 171 μ L of DPPH (0.1 mM) solution. The mixture was incubated for 20 minutes in a dark area. The absorbance was measured at the wavelength of 490 nm using a microplate spectrophotometer (Bio-Rad) against DMSO as negative control. The percentage of inhibition of the DPPH radical was calculated as follows:

$$\% \text{ scavenging of DPPH} \bullet = \left[\frac{\text{control absorbance} - \text{extract absorbance}}{\text{control absorbance}} \right] \times 100$$

A graph of inhibition percentages against extract concentrations was plotted and EC_{50} value (concentration that scavenged 50% of DPPH radical activity) was deduced. All experiments were carried out in triplicate. EC_{50} values were reported as mean \pm SD of triplicates.

ABTS radical scavenging activity

In addition to the DPPH assay, the 2,2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid), commonly called ABTS⁺ scavenging activity, was also implemented. Initially, ABTS 7 mM solution was reacted with potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) 2.45 mM solution and left overnight in a dark room to yield a dark blue solution containing ABTS radical cations (ABTS⁺). The working solution was prepared by diluting the prepared ABTS⁺ solution in ethanol to reach an absorbance of 0.70 ± 0.02 at 750 nm.

ABTS radical scavenging activity was assessed by mixing 9 μ L of either *T. indica* extract (6.25–750 μ g/mL) or Trolox (positive control, 0.625, 1.25, 2.5, 5, 10 μ g/mL) with 171 μ L of ABTS working solution. The mixture was incubated for 10 minutes in a dark area. The absorbance was measured at the wavelength of 750 nm using a microplate spectrophotometer (xMark, Bio-Rad) against DMSO (sample negative control) and absolute ethanol (Trolox negative control). The percentage of inhibition (I%) was calculated as follows:

% scavenging of ABTS = [(control absorbance – extract absorbance) / control absorbance] × 100

A graph of inhibition percentages (I%) against extract concentrations was plotted and EC₅₀ value (the concentration necessary for 50% reduction of ABTS) was constructed. All experiments were carried out in triplicate. Data was reported as mean ± SD of triplicates.

Cytotoxicity evaluation

Toxicological profile of *T. indica* extracts were assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, Baby Hamster Kidney fibroblast (BHK-21) cells were seeded into four 96-well plate at a concentration of 7×10^3 cells/well. After 24 hours, *T. indica* water and methanolic extracts (1, 3, 9, 27, 81, 243, and 729 µg/mL) were treated into the plates and incubated for 48 hours prior to the addition of MTT. The absorbance was measured at 570 nm against untreated control.

Statistical analysis

ANOVA test followed by Tukey's test ($p < 0.05$) was used to analyze the differences among TPC, TFC in two extraction solvents (methanol, water). Paired sample t-test was used to analyze

the cytotoxic effect of different concentrations of tamarind extracts with regards to untreated control. The data were statistically analyzed using IBM SPSS Statistics version 26 (Armonk, NY: IBM Corp). EC₅₀ values (concentration that inhibits 50% of DPPH/ABTS activities) of the extracts were calculated using CurveExpert Professional 2.7 software. A value of $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Total phenolic content

Phenolic compounds in plants have been shown to have redox properties which permit them to act as antioxidants (Soobrattee *et al.* 2005). In principle, total phenolic content (TPC) was measured using the Folin–Ciocalteu reagent in every extract. TPC was calculated from a gallic acid calibration curve ($y = 46.619x + 0.005$, $R^2 = 0.9972$) and expressed in gallic acid equivalents (GAE) per gram extract weight (GAE/g extract) (Figure 1).

According to Table, TPC content in methanolic extracts (ranging from 56.616 ± 0.523 to 742.919 ± 50.360 mg GAE/g extract) is higher than that in water extracts (ranging from 30.555 ± 4.987 to 240.482 ± 3.312 mg GAE/g extract).

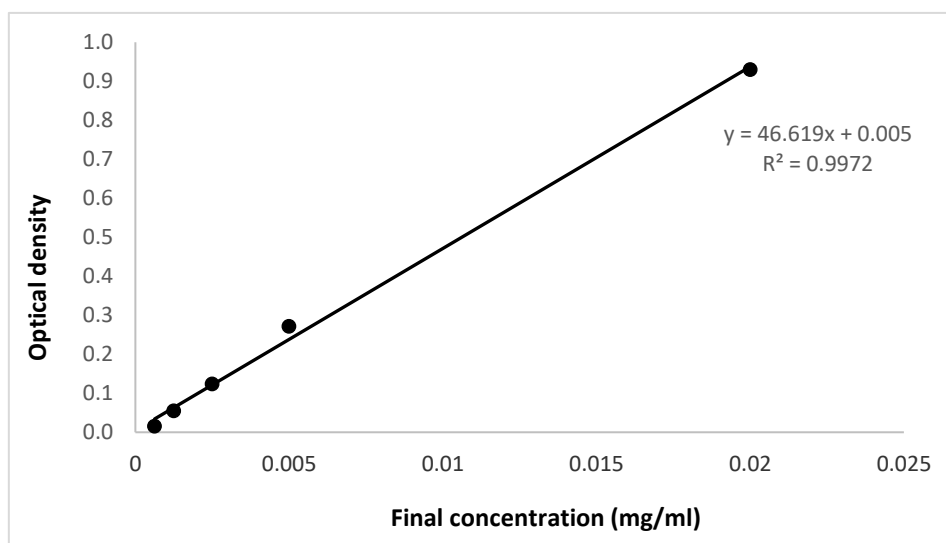


Figure 1. Gallic acid calibration curve. The experiment was carried out in triplicate.

Regarding the plant origin, the seeds of *T. indica* from Sai Gon exhibit the highest TPC, both in water (240.482 ± 3.312 mg GAE/g) and methanolic (742.919 ± 50.360 mg GAE/g) extracts, as compared with seeds from the other two regions. On the other hand, the pulps of *T. indica* from Son La contained the highest TPC, as seen in both water (35.184 ± 3.526 mg GAE/g) and methanolic (82.612 ± 2.888 mg GAE/g) extracts.

Sai Gon methanolic seed extract yielded even higher TPC than those in previous studies. The highest polyphenolic content obtained from the Malaysian *T. indica* methanolic seed extract was 572 ± 3.78 mg GAE/g (Razali *et al.* 2015). In the other hand, the highest polyphenolic content obtained from the Egypt *T. indica* seeds in n-butanol fraction was 378 ± 11.7 mg GAE/g (Guneidy *et al.* 2020). The variability can be caused from their distinct geographical origins or different extraction methods.

Table 1. Total phenolic content of *T. indica* in water and methanolic extracts (mg GAE/g).

Site	Part of the plant	Water extracts (mg GAE/g)	Methanolic extracts (mg GAE/g)
Sai Gon	Seed	(H1) 240.482 ± 3.312	(M1) 742.919 ± 50.360
	Pulp	(H2) 30.555 ± 4.987	(M2) 56.616 ± 0.523
Son La	Seed	(H3) 174.527 ± 7.887	(M3) 666.082 ± 35.248
	Pulp	(H4) 35.184 ± 3.526	(M4) 82.612 ± 2.888
Hai Phong	Seed	(H5) 119.647 ± 2.963	(M5) 673.927 ± 36.114
	Pulp	(H6) 33.128 ± 2.802	(M6) 57.045 ± 0.142

Total flavonoid content

Conventionally, total flavonoid contents in plant extracts were quantitatively determined using aluminum chloride in a colorimetric method. In this study, TFC results were derived from the calibration curve ($y = 2.9776x + 0.0172$, $R^2 = 0.9934$) of quercetin (0.05- 1 mg/mL) and expressed in quercetin equivalents (QE) per gram dry extract weight (mg QE/g extract) (Figure 2). TFC in methanolic extracts widely ranged from 6.420 ± 0.007 to 68.492 ± 0.023 (mg QE/g extract), indicating a ten-fold variation. TFC in water extracts ranged approximately six-fold variation (from 3.652 ± 0.315 to 19.084 ± 0.115 mg QE/g extract). Of note, methanolic extracts yields a significantly higher TFC than water extracts ($p < 0.05$). Methanol was considered as the most effective solvent to extract bioactive compounds from plants (Truong *et al.* 2019). This is because methanol contains both polar (hydroxyl, -OH) and non-polar (methyl, -CH₃) groups which facilitates the extraction of many polar and non- polar phenolic compounds from the plants. As previously

reported, high flavonoids were also observed for fraction of n-butanol (83 ± 6 mg rutin/g) from Egyptian *T. indica* seeds (Guneidy *et al.* 2020).

DPPH radical scavenging activity

The DPPH radical scavenging activities of *T. indica* seeds and pulps water extracts are presented in Figure 3. All the extracts exhibited concentration-dependent DPPH radical scavenging activities which were in the following order: H1 > H3 > H5 > H6 > H2 > H4. Given the range of extract concentrations (6.25, 25, 100 µg/mL) and ascorbic acid (1.25, 2.5, 5, 10, 50 µg/mL), only EC₅₀ of ascorbic acid (11.6 µg/mL) and H1 (64.4 µg/mL) were found. Thus, H1 (Sai Gon seed water extract) exhibited the strongest DPPH radical scavenging activity compared to the other *T. indica* water extracts but weaker than that of Ascorbic acid positive control.

Figure 4 showed the DPPH radical scavenging activities of *T. indica* seeds and pulps methanolic extracts which were in the following order: M3 > M5 > M1 > M4 > M2 > M6. Given the

range of extract concentrations (6.25, 25, 100 $\mu\text{g/mL}$), only EC_{50} values of M3 was deduced (52.5 $\mu\text{g/mL}$). In general, the seeds of *T. indica* from three areas exhibit higher antioxidative activities than their pulps.

Cardoso and colleagues (2016) reported that the EC_{50} values of Brazilian tamarind seeds, sweet variety ranged widely from 8.92 (CO_2 -50% ethanol as extraction solvent) to 370.82 $\mu\text{g/mL}$ (CO_2 -10% ethanol as extraction solvent). In another study, EC_{50} values using DPPH scavenging activity showed that n-butanol fraction of Egyptian *T. indica* seeds has a powerful antioxidant capacity ($2.1 \pm 0.08 \text{ mg/g DW}$) (Guneidy *et al.*, 2020). Even though the samples originated from the same geographical regions (Brazilian tamarind seeds), differences in extraction methods caused wide variability in the results of antioxidant capacities, let alone different geographical locations.

ABTS radical scavenging activity

The obtained data indicated that *T. indica* water extracts scavenged ABTS radical in a dose-dependent manner (6.25-100 $\mu\text{g/mL}$) (Figure 5). ABTS radical scavenging ability of these samples can be ranked as $\text{H3} > \text{H1} > \text{H5} > \text{H4} > \text{H6} > \text{H2}$.

The ABTS radical scavenging activity of *T. indica* methanolic extracts were also expressed in a dose-dependent manner (6.25-750 $\mu\text{g/mL}$) (Figure 6). IC_{50} of Trolox (6.2 $\mu\text{g/mL}$), M3 (225 $\mu\text{g/mL}$), M5 (378.4 $\mu\text{g/mL}$) and M1 (471.6 $\mu\text{g/mL}$) were calculated from this assay. Even though ABTS radical scavenging activity of the extracts was lower than that of Trolox reference compound, their antioxidant activity could be considered good. These data indicated that Tamarin seeds can be very potential natural antioxidants.

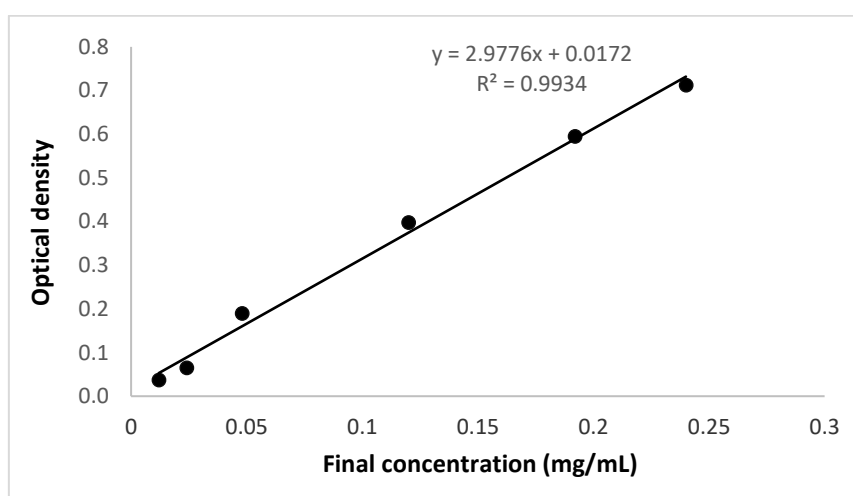


Figure 2. Quercetin calibration curve. The experiment was carried out in triplicate.

Table 2. Total flavonoid content of *T. indica* in water and methanolic extracts (mg QE/g).

Site	Part of the plant	Water extracts (mg QE/g)	Methanolic extracts (mg QE/g)
Sai Gon	Seed	(H1) 6.163 ± 0.025	(M1) 41.811 ± 0.621
	Pulp	(H2) 5.550 ± 0.027	(M2) 6.420 ± 0.007
Son La	Seed	(H3) 3.652 ± 0.315	(M3) 41.764 ± 0.015
	Pulp	(H4) 6.672 ± 0.009	(M4) 15.849 ± 0.710
Hai Phong	Seed	(H5) 19.084 ± 0.115	(M5) 68.492 ± 0.023
	Pulp	(H6) 6.128 ± 0.015	(M6) 21.107 ± 1.169

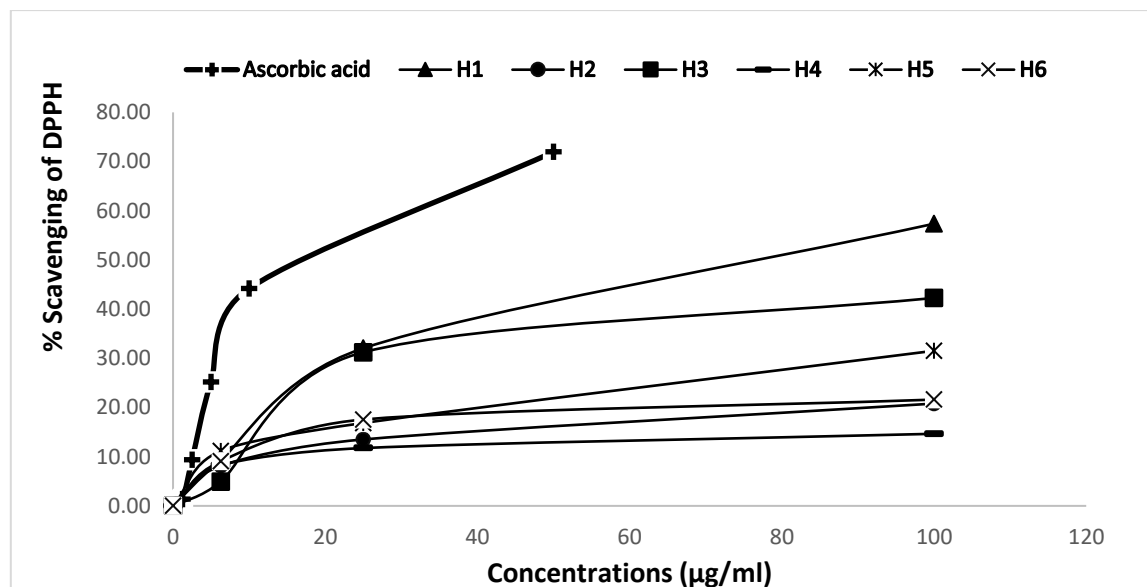


Figure 3. DPPH radical scavenging activity of *T. indica* water extract and ascorbic acid standard at different concentrations. *Abbreviation:* H1, Sai Gon seed water extract; H2 Sai Gon pulp water extract; H3, Son La seed water extract; H4, Son La pulp water extract; H5, Hai Phong seed water extract; H6, Hai Phong pulp water extract.

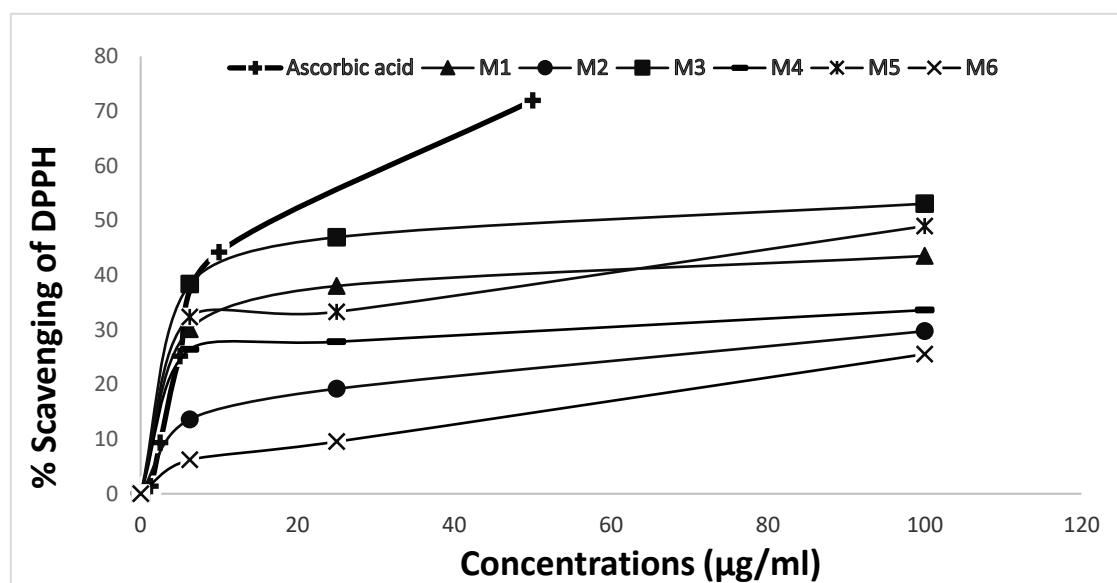


Figure 4. DPPH radical scavenging activity of *T. indica* methanolic extract and Ascorbic acid standard at different concentrations. *Abbreviation:* M1, Sai Gon seed methanolic extract; M2 Sai Gon pulp methanolic extract; M3, Son La seed methanolic extract; M4, Son La pulp methanolic extract; M5, Hai Phong seed methanolic extract; M6, Hai Phong pulp methanolic extract.

In this study, the radical scavenging activities of *T. indica* extracts were increased in a dose-dependent manner but only in a limited range of concentrations. Above this concentration range,

the radical scavenging activities were decreased in a non-specific manner (data not shown). This can be explained by the fact that beside the antioxidant compounds, there were many other

unknown substances exist in the same extract. When increasing the extract concentration, the concentration of other substances in *T. indica* extracts were also increased which might

interfere with the radical scavenging capacities of antioxidant compounds and led to the decrease in the scavenging capacity of total extracts.

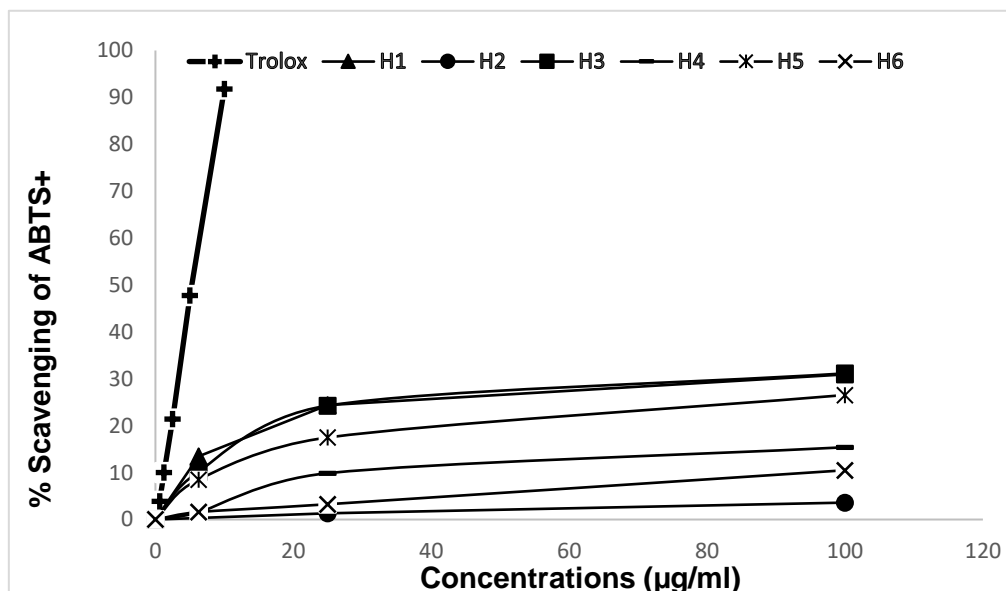


Figure 5. ABTS radical scavenging activity of *T. indica* water extracts and Trolox standard at different concentrations. *Abbreviation:* H1, Sai Gon seed water extract; H2 Sai Gon pulp water extract; H3, Son La seed water extract; H4, Son La pulp water extract; H5, Hai Phong seed water extract; H6, Hai Phong pulp water extract.

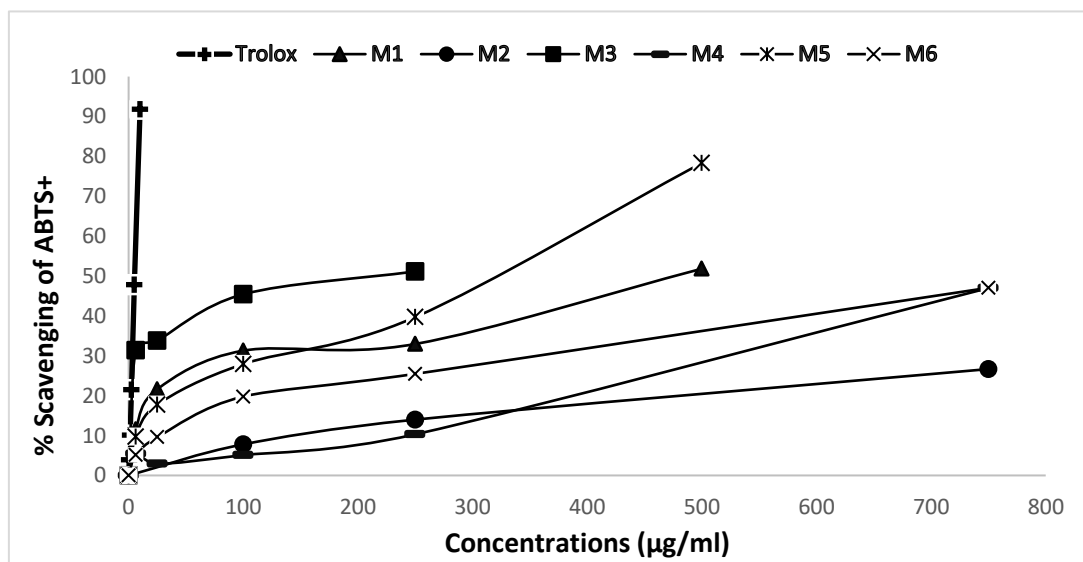


Figure 6. ABTS radical scavenging activity of *T. indica* methanolic extracts and Trolox standard at different concentrations. *Abbreviation:* M1, Sai Gon seed methanolic extract; M2 Sai Gon pulp methanolic extract; M3, Son La seed methanolic extract; M4, Son La pulp methanolic extract; M5, Hai Phong seed methanolic extract; M6, Hai Phong pulp methanolic extract.

Previous studies have shown that the antioxidative capacity is greatly correlated with the total flavonoid and total phenolic content of the plant leaves' crude extract (Sim *et al.* 2010; Mustafa *et al.* 2010). Since we were not able to calculate the EC₅₀ values of all the extracts, it was difficult to understand if TPC and TFC were linearly correlated with antioxidant activities. Nevertheless, it was noticeable that M1, M3, M5 (*T. indica* seeds in methanolic extracts) which contained the highest TPC, TFC also exhibited the strongest DPPH and ABTS scavenging activities.

Cytotoxicity effect

Cytotoxicity effect of *T. indica* water and methanolic extracts (at 1, 3, 9, 27, 81, 243, and 729 µg/mL) on BHK-21 cells lines were illustrated in figure 7 and figure 8, respectively. Table 3 revealed that most *T. indica* extracts started to exert a significant toxicological effect

on BHK-21 cell lines from the concentration of 81 µg/mL compared to the control. Given the concentration range, we could find the IC₅₀ values of H2 (143.77 µg/mL), H3 (400.29 µg/mL), H5 (620.35 µg/mL), M3 (297.94 µg/mL) and M6 (694.713 µg/mL).

In a previous study which assessed the cytotoxic capacity of n-butanol *T. indica* fraction for breast cancer cell line, MCF-7, the IC₅₀ value is 68.5 µg/mL (Guneidy *et al.* 2020). Regarding the cytotoxic effects of the crude methanol seed extract of Malaysian *T. indica* in liver cancer cell line, HepG2, the IC₅₀ value was 104.71 ± 0.07 µg/mL (Razali *et al.* 2015). Given the differences in the cell lines, the treated concentration range and the extraction methods, the cytotoxicity of *T. indica* seed extracts varied between studies. Nevertheless, given the lowest IC₅₀ of 143.77 µg/mL, the extracts from Vietnamese *T. indica* seeds and pulps could still be considered as safe.

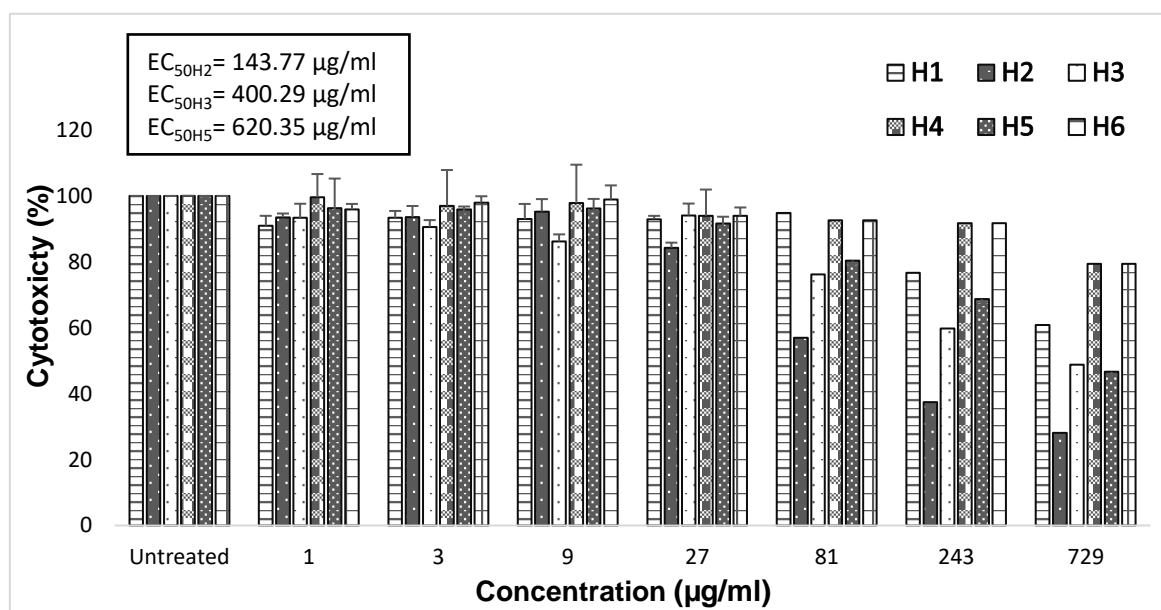


Figure 3. Determination of the cytotoxic activity of *T. indica* water extracts at different concentrations on BHK-21 cells. Abbreviation: H1, Sai Gon seed water extract; H2 Sai Gon pulp water extract; H3, Son La seed water extract; H4, Son La pulp water extract; H5, Hai Phong seed water extract; H6, Hai Phong pulp water extract.

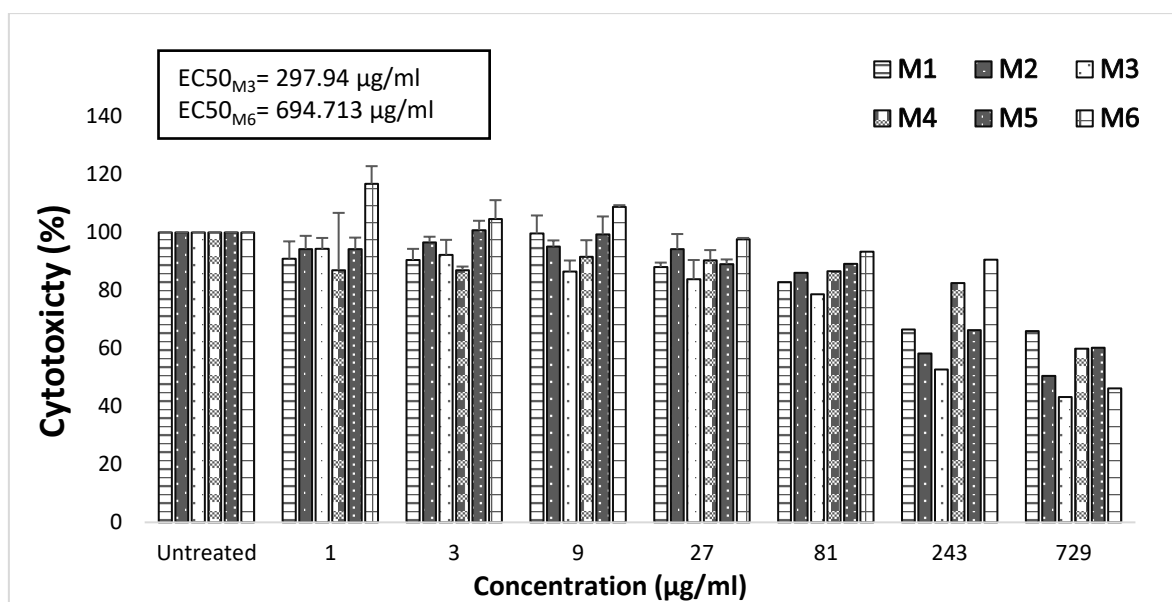


Figure 4. Determination of the cytotoxic activity of *T. indica* methanolic extracts at different concentrations. Abbreviation: M1, Sai Gon seed methanolic extract; M2 Sai Gon pulp methanolic extract; M3, Son La seed methanolic extract; M4, Son La pulp methanolic extract; M5, Hai Phong seed methanolic extract; M6, Hai Phong pulp methanolic extract.

Table 3. Cytotoxic effect (% cell availability) of *T. indica* extracts on BHK-21 cells.

Extract	Concentration of the extract						
	1 µg/ml	3 µg/ml	9 µg/ml	27 µg/ml	81 µg/ml	243 µg/ml	729 µg/ml
H1	91.0 ± 1.6	93.35 ± 2.11	93.0 ± 3.1	92.9 ± 5.6	94.9 ± 3.8	76.7 ± 2.8 *	60.8 ± 2.4 *
H2	93.5 ± 4.2	93.6 ± 3.9	95.3 ± 2.0	84.3 ± 2.5	57.0 ± 4.2 *	37.4 ± 0.9 *	28.1 ± 3.0 *
H3	93.4 ± 2.3	90.6 ± 2.4	86.2 ± 2.6	94.1 ± 0.01	76.2 ± 2.0 *	59.8 ± 2.0 *	48.8 ± 2.0 *
H4	99.6 ± 7.8	97.0 ± 10.9	97.9 ± 12.1	94.0 ± 6.9	92.6 ± 3.1	91.7 ± 15.5	79.4 ± 0.8 *
H5	96.3 ± 3.3	95.9 ± 0.9	96.2 ± 1.3	91.6 ± 3.7	80.4 ± 9.4 *	68.7 ± 4.2 *	46.6 ± 0.4 *
H6	95.9 ± 3.3	98.0 ± 1.9	98.9 ± 1.9	94.0 ± 6.9	92.6 ± 3.1	91.7 ± 15.5	79.4 ± 0.8 *
M1	90.9 ± 1.5	90.5 ± 3.9	99.6 ± 3.9	88.1 ± 2.8	82.9 ± 8.9 *	66.6 ± 2.8 *	66.0 ± 6.4 *
M2	94.3 ± 2.7	96.6 ± 7.5	95.1 ± 0.2	94.2 ± 7.5	86.1 ± 3.0	58.2 ± 6.1 *	50.5 ± 2.6 *
M3	94.3 ± 7.9	92.3 ± 4.8	86.5 ± 11.4	83.8 ± 1.4	78.7 ± 4.0 *	52.7 ± 4.7 *	43.2 ± 6.3 *
M4	87.0 ± 1.5	87.0 ± 1.5	91.5 ± 0.01	90.3 ± 17.9	86.7 ± 0.7	82.5 ± 0.8 *	59.9 ± 0.2 *
M5	94.2 ± 8.3	100.8 ± 3.0	99.3 ± 2.9	89.1 ± 6.7	89.1 ± 9.9	66.3 ± 5.9 *	60.3 ± 2.0 *
M6	116.8 ± 1.2	104.6 ± 6.5	108.8 ± 10.1	97.6 ± 3.8	93.4 ± 1.6	90.6 ± 6.2	46.2 ± 3.2 *

Data is represented as mean ± SD (n = 3). *, p < 0.05

CONCLUSION

In this study, assessment of total phenolic and flavonoid content as well as free radical scavenging activity showed that the seeds and

pulps from *T. indica* can be the potent source for natural antioxidants. The methanolic extracts yielded the highest TPC (742.919 ± 50.360 GAE/g extract), TFC (68.492 ± 0.023 mg QE/g extract) and possessed highest free radical

scavenging capacity (EC_{50} of 52.5 $\mu\text{g/mL}$) compared to water extracts. The variability in phytochemical properties of these extracts could be explained by the difference in their geographic origins (Sai Gon, Son La or Hai Phong) as well as what type of plant components they were (*T. indica* seeds possessed higher TPC, TFC and antioxidant activity than those of pulps). Regarding the safety, *in vitro* analysis showed that the extracts from Vietnamese *T. indica* seeds and pulps were considerably safe (lowest IC_{50} = 143.77 $\mu\text{g/mL}$). In general, tamarind seeds possessed the highest phenolic and flavonoid contents, exhibited the strongest antioxidative capacities, and only became toxic to BHK-21 cell line at very high concentrations (IC_{50} values ranging from 400.29 $\mu\text{g/mL}$ to 620.35 $\mu\text{g/mL}$). Regarding the safety of these extracts, *in vitro* analysis showed that they were not very toxic (cell viability > 80%). While further toxicological assays are in need, the data of this study provided evidences for the use of tamarind in ethnomedicine and their therapeutic potential in modern medicine.

Acknowledgments: *This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106.02-2018.24.*

REFERENCES

- Alfadda AA., Sallam RM (2012) Reactive oxygen species in health and disease. *J Biomed Biotechnol* 2012: 936486.
- Babbar N, Oberoi HS, Sandhu SK (2015) Therapeutic and nutraceutical potential of bioactive compounds extracted from fruit residues. *Crit Rev Food Sci Nutr* 55: 319-337.
- Bendary E, Francis RR, Ali HMG, Sarwat MI, El Hady S (2013) Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. *Annals of Agricultural Sciences* 58: 173-181.
- Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci Technol* 28: 25-30.
- Cardoso Lima Reis PM, Dariva C, Barroso Vieira GA, Hense H (2016) Extraction and evaluation of antioxidant potential of the extracts obtained from tamarind seeds (*Tamarindus indica*), sweet variety. *J Food Eng* 173: 116-123.
- Chang CC, Yang MH, Wen HM, Chern CJ. (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 10: 178-182.
- Kurutas EB (2016) The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J* 15: 71.
- Mustafa RA, Hamid AA, Mohamed S, Bakar FA (2010) Total phenolic compounds, flavonoids, and radical scavenging activity of 21 selected tropical plants. *J Food Sci* 75: C28-35.
- Razali N, Junit SM, Ariffin A, Ramli NS, Aziz AA (2015) Polyphenols from the extract and fraction of *T. indica* seeds protected HepG2 cells against oxidative stress. *BMC Complement Altern Med* 15: 438.
- Ronald LP, Guohua C (2000) Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications. *HortSci* 35: 588-592.
- Sim K, Malek SNA, Wahab N (2010) Phenolic content and antioxidant activity of crude and fractionated extracts of *Pereskia bleo* (Kunth) DC. (Cactaceae). *Afri J Pharm Pharmacol* 9: 001-0094.
- Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T (2005) Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat Res* 579: 200-213.
- Sudjaroen Y, Haubner R, Wurtele G, Hull WE, Erben G, Spiegelhalder B, Changbumrung S, Bartsch H, Owen RW (2005) Isolation and structure elucidation of phenolic antioxidants from Tamarind (*Tamarindus indica* L.) seeds and pericarp. *Food Chem Toxicol* 43: 1673-1682.
- Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC (2019) Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *severinia buxifolia*. *J Food Qual* 2019: 8178294.
- Tsuda, T., M. Watanabe, K. Ohshima, A. Yamamoto, S. Kawakishi and T. Osawa (1994) Antioxidative components isolated from the seed of tamarind (*Tamarindus indica* L.). *J Agric Food Chem* 42: 2671-2674.

Tungmunnithum D, A Thongboonyou, A Pholboon, A Yangsabai (2018) Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. *Medicines (Basel)* 5.
Young IS, JV Woodside (2001) Antioxidants cin

health and disease. *J Clin Pathol* 54: 176-186.

Zargoosh Z, M Ghavam, G Bacchetta, A Tavili (2019) Effects of ecological factors on the antioxidant potential and total phenol content of *Scrophularia striata* Boiss. *Sci Rep* 9: 16021.

