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In vitro antioxidant activities and volatile compounds from karanda (*Carissa carandas* L.) fruit wine

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Abstract *Carissa carandas* L. or karanda (in Thai called namdaeng or manaao ho) is widely used as a medicinal plant. Karanda juice was fermented for 22 days to produce alcohol using *Saccharomyces cerevisiae* TISTR5918. Total soluble solids or TSS of karanda juice (approximately 1.0 °Brix) was adjusted to 24 °Brix and samples were collected every 2 days for analysis of total soluble solids (TSS), pH and alcohol content. Yield of alcohol was measured at 12.50±0.35% in the final fermentation. TSS dropped gradually from 24 °Brix to 9.17±0.58 °Brix, whereas pH increased slightly from 2.47±0.06 to 2.80±0.00 (Day 0-22). Volatile compounds in karanda wine (KW) were examined and analyzed using gas chromatography-mass spectrometry (GC-MS). Volatile compounds detected in KW included 3-methyl-1-butanol, butanoic acid, butyrolactone, phenylethyl alcohol, butanedioic acid, benzene ethanol and 2-propenyl ester as a common flavor and aroma in wine. Heavy metals (Pb, As, Cu and Cd) determined by atomic absorption spectroscopy (AAS) were present in lower than their maximum allowed concentrations. Methanol was detected at only 0.005% in KW analyzed by gas chromatography equipped with a flame ionization detector (GC-FID). Antioxidant activities were determined by three methods comprising diphenyl picrylhydrazyl radical scavenging assay (DPPH), radical cation decolorization assay (ABTS) and reducing power. The IC_{50} values of DPPH and ABTS were 0.84±0.03% and 3.28±0.03%, respectively, and the EC_{50} of reducing power was 11.03±0.11%. Total phenolic content, measured according to the Folin-Ciocalteu procedure, was determined as 746.64±3.10 mg GAE/100 ml sample.

Keywords: *Carissa carandas* L.**,** karanda wine**,** antioxidant activities**,** volatile compounds

Introduction

Nowadays, interest in developing functional foods for nutrition and disease prevention has mushroomed, with increased consumer demand for fruits and their products such as wine (Rupasinghe and Clegg, 2007). Wine is

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considered to be one of the oldest alcoholic beverages obtained from fruit juice using yeast such as *Saccharomyces cerevisiae* for fermentation (Mundaragi and Hangadurai, 2017; Reddy *et al.*, 2008). Water makes up 75-90% of wine which also consists of phenolics, organic acids, mineral salts, and pectins at around 15% with the remaining component as ethyl alcohol (8-13% v/v) depending on the wine type and strength (Conde *et al.*, 2006). Generally, the raw materials used for winemaking are grapes (Reddy and Reddy, 2005) but several fruits such as raspberry (Jeong *et al.*, 2010; Duarte *et al.*, 2010), cherry (Sun *et al.*, 2011), custard apple (Jagtap and Bapat, 2015), guava (Sevda and Lambert, 2011), lychee (Alves *et al.*, 2011), papaya (Lee *et al.*, 2010), peach (Davidović *et al.*, 2013) and pomegranate (Mena *et al.*, 2012) were investigated for wine production in previous studies. Fruit wines have recognized health-promoting properties related to the antioxidant activity of their individual fruits (Nuengchamnong and Ingkaninan, 2010). Some antioxidants called polyphenols promote the flow of blood through the body (Bors and Michel, 2002), while de Lorgeril *et al.* (2002) reported that drinking a moderate amount of wine reduced the risk of cardiovascular disease. Furthermore, drinking wine was also considered to reduce the risk of mortality compared to imbibing other forms of alcohol (Klatsky *et al.*, 2003). Apart from antioxidants, wine also contains concentrations of some inorganic minerals which are beneficial to health and play an important role in normal body functioning. Nevertheless, some elements such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are deleterious for consumers and these are frequently detected as the main food and beverage contaminants. Consequently, the metal content of wine is regulated according to legislation laid down by the European Union (Organisation Internationale de la Vigne et du Vin or O.I.V (Klarić *et al.*, 2016).

Thailand has a tropical climate and is known for the wide variety of fruits that are available throughout the year (Lertputtarak and Sarunya, 2012). Several fruits including mao (Nuengchamnong and Ingkaninan, 2010), longan (Chanrittisen and Chomsri, 2010), banana (Cheirsilp and Umsakul, 2008), mulberry (Butkhup *et al.*, 2011), pineapple (Chanprasartsuk *et al.*, 2010), santol (Jitjaroen *et al.*, 2009), ma-kiang (Chomsri *et al.*, 2012), passion fruit, pineapple and star gooseberry (Chueachot and Chantha, 2014) can be used to make alcoholic beverages such as wine. *Carissa carandas* L., or karanda (in Thai called namdaeng or manaao ho), is a tropical plant that belongs to the Apocyanaceae family and is closely related to *Carissa spinarum* L. (Mundaragi and Thangadurai, 2017). The whole karanda plant can be used as traditional medicine (Pewlong *et al.*, 2014). Noticeable biological properties include antidiabetic (Itankar *et al.*, 2011), antimicrobial (Agarwal *et*

al., 2012), cytotoxicity (Pewlong *et al.*, 2014), hepatoprotective (Hegde *et al.*, 2009), and anti-inflammatory (Bhaskar and Balakrishnan, 2009). Various parts of the plant are used to treat rheumatoid arthritis, piles, indigestion, splenomegaly, anorexia, cardiac diseases, edema, and amenorrhoea with anti-emetic, cardiotonic, anti-bacterial (Kaunda and Zhang, 2017; Verma and Chaudhary, 2011) and antiviral properties (Begum *et al.*, 2013) also reported. Karanda fruit is a good source of vitamin C, phenolic compounds, anthocyanins and minerals especially iron, calcium and phosphorus (Singh *et al.*, 2014; Wani *et al.*, 2013; Sueprasarn *et al.*, 2013). Moreover, volatile compounds detected in karanda fruit consist of 2-phenyl ethanol, linalool, β-caryophyllene, isoamyl alcohol, benzyl acetate, lupeol, oxalic, tartaric, citric, malic, malonic and glycolic acids, glycine, alaline, phenyl alaline, cerine, glucose, galactose and a novel triterpenic alcohol as carissol, an epimer of α-amyrin. Over 150 compounds have also been detected in the aroma including isoamyl alcohol, isobutanol and β-caryophyllene (Kumar *et al.*, 2013). Unripe karanda fruits are used as a thermogenic (Mishra *et al.*, 2012) for the treatment of diarrhea and antidiabetic potential (Itankar *et al.*, 2011), and as an astringent (Sueprasarn *et al.*, 2017; Devmurari *et al.*, 2009), analgesic and anti-inflammatory (Pewlong *et al.*, 2014). At the ripened stage, karanda fruit is sweet, cooling, appetizing and antiscorbutic. It is also useful in bilious complaints, expectorant anorexia, burning sensation, scabies, pruritus and other skin diseases (Kumar *et al.*, 2013). Karanda fruit is a good appetizer and often used to make pickle, jelly, jam, squash, syrup, tarts and chutney (Maheshwari *et al.*, 2012). In Thailand, many local products are made from karanda fruits including juice, jam, and desserts (Sudjaroen and Suwannahong, 2017). Therefore, this study aimed to investigate the physicochemical properties, metal content and antioxidant activity of wine produced from karanda fruits (*Carissa carandas* L.).

Materials and methods

Yeast strain and growth conditions

Saccharomyces cerevisiae TISTR5918, was purchased from the Thailand Institute of Scientific and Technological Research, inoculated in 100 ml of YM medium containing (g/l): 0.3 g of yeast (HiMedia, India), 5.0 g of peptone (HiMedia, India) and 10.0 g of glucose (Ajax Finechem, New Zealand) and incubated at 30 ℃, pH 4.5 for 12-24 h (Mundaragi and Thangadurai, 2017). Yeast cell suspension (about 10^8 CFU/ml) was transferred to a 2 L Erlenmeyer flask containing 1,000 ml of pre-sterilized karanda fruit juice, incubated at 30 ℃ for 12-24 h and kept until required for further experiments.

Production of karanda wine (KW)

Carissa carandas L., or karanda fruits were collected from the local Si Mum Mueang Market (Rangsit), Pathum Thani, Thailand. The fruits were washed and those with good appearance (without physical injury, decay or visual contamination) were selected prior to fermentation to reduce the initial microbial count. Karanda juice was extracted manually using a blender. Water was added to the juice in the ratio of 1:1 (w/v) and the mixture was adjusted to 24.0 °Brix (from 1.0 °Brix) by addition of sucrose, before boiling at 100°C for 15 min to inhibit the growth of microorganisms. Subsequently, 10% wine yeast was inoculated into the karanda juice and incubated at room temperature. Fermentation was carried out in a sanitized glass carboy for 22 days.

Physicochemical analysis

Analytical methods

Samples were collected every 2 days for analysis of total soluble solids (TSS), pH and alcohol content. The pH value was measured using a pH meter (Suntex, Taiwan), while TSS was determined by a hand-held digital refractometer (ATAGO, Japan) as percent Brix, and alcohol content was measured using an ebulliometer (Laboratoires Dujardin-Salleron, France). Data were presented as mean values of triplicate experiments. Measurement methods were modified according to Sevda and Lambert, (2011) and Buechsenstein and Ough, (1978).

Gas chromatography-mass spectrometry (GC-MS) analysis

The gas chromatography-mass spectrometry (GC-MS) technique was carried out at the RSU Scientific and Technological Research Equipment Center (RSU-STREC), Rangsit University, Thailand to determine the chemical compounds in karanda wine (KW). GC-MS analysis was performed on an Agilent Technologies model 7890A coupled with a Mass Selective Detector model 5975C Inert XL High Performance Turbo Pump. Compounds were separated on a Mega-5MS (30 m×0.25 mm, 0.25 µm film thickness). Mass detector conditions were electronic impact (EI) mode at 70 eV. Oven temperature was set a 40°C for 2 min and 1 µl of sample was injected by splitless sample injection technique.

Qualitative analysis of alcohols

Qualitative analysis of the alcohols was carried out using a gas chromatograph equipped with a flame ionization detector or GC-FID (Agilent

Technologies, USA). Detector and injector temperatures were set at 250°C and 150°C, respectively. A 500 µl sample was injected, while oven temperature was programmed identically as for the GC-MS analysis described earlier. Qualitative analysis was carried out at the RSU Scientific and Technological Research Equipment Center (RSU-STREC), Rangsit University, Thailand.

Metal content analysis

Various metal contents including lead (Pb), arsenic (As), copper (Cu) and cadmium (Cd) were determined by atomic absorption spectroscopy or AAS (Thermo Scientific™ iCE™ 3500, USA). This technique was carried out one element at a time using a hollow-cathode lamp for the specific wavelength at the RSU Scientific and Technological Research Equipment Center (RSU-STREC), Rangsit University, Thailand. Data were compared with the Thai Industrial Standards Institute (TISI) limits.

Determination of total phenolic content (TPC)

Total phenolic content was measured according to the Folin-Ciocalteu procedure of Siddiqui *et al.* (2017). In brief, 1 ml of KW was mixed with 4.5 ml distilled water and 0.5 ml of 2 N Folin-Ciocalteu reagent was added. The mixture was shaken vigorously before adding 4 ml of sodium carbonate (7.5%). Then, the mixture was vortexed, followed by incubation for 60 min in the dark at room temperature, and centrifuged at 6,000 rpm for 5 minutes at 25°C. Absorbance was recorded at 734 nm by a spectrophotometer (Shimadzu model UV-1601, Japan). Finally, TPC was expressed as mg of gallic acid equivalent per 100 ml of sample (mg GAE/100 ml).

Determination of in vitro antioxidant activity

Free radical scavenging activity (DPPH) assay

Free radical scavenging activity or DPPH assay was modified according to the procedure described by Marković *et al.* (2015). Briefly, 2 ml of various KW concentrations were added to 2 ml of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl or DPPH radical (Sigma-Aldrich, Germany) solution, with 2 ml of KW used instead of 2 ml of ethanol as control. The mixture was then shaken vigorously and allowed to stand at room temperature in the dark for 30 min. The standard used in this study was ascorbic acid. Absorbance was determined at 515 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The radical stock solution was freshly prepared each day. Antioxidant activity was

defined as the inhibition percentage of the initial concentration of DPPH radical caused by each diluted wine sample according to the equation below:
% Inhibition = $[(A_{control} - A_{sample}) / A_{control})] \times 100$

Radical cation decolorization assay ($ABTS^{\bullet+}$)

The ABTS assay was modified from the method of Re *et al.* (1999). A stock solution of $ABTS^{\bullet+}$ (2,2'-azino-bis[3-ethylbenzthiazoline6-sulfonic acid]) was prepared by mixing 7 mM $ABTS^{+}$ aqueous solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12-16 h at room temperature in the dark before use. Thereafter, this solution was diluted with 95% ethanol at the ratio 1:80 (sample solution: 95% ethanol) and incubated at room temperature to give an absorbance of 0.70±0.02 at 734 nm. Afterward, 3 ml of diluted $ABTS^{\bullet+}$ solution was added to 300 µl of KW and incubated at room temperature for 6 min. In this experiment, ascorbic acid was used as the standard. Absorbance was measured at 734 nm using a spectrophotometer (Shimadzu model UV-1601, Japan). The scavenging activity of KW against ABTS radical cation decolorization was exhibited as percentage of inhibition by the following equation:
% Inhibition = $[(A_{control} - A_{sample}) / A_{control})] \times 100$

Reducing power ability

The method of Chu *et al.* (2000) was modified to determine the reducing power of KW. The sample was diluted with distilled water, then 1 ml was transferred to 200 mM phosphate buffer (2.5 ml, pH 6.6), mixed with potassium ferricyanide (2.5 ml, 1% w/v) in a test tube and incubated in a water bath at 50°C for 20 minutes. Subsequently, 2.5 ml of trichloroacetic acid (10% w/v) was added and the mixture was centrifuged at 3,000 rpm, 25°C for 10 min. The supernatant (2.5 ml) was collected and diluted with distilled water (2.5 ml). Finally, freshly made ferric chloride (0.5 ml, 0.1%w/v) was added and mixed thoroughly. Ascorbic acid was used as the standard in this study. Absorbance was measured at 700 nm using a spectrophotometer.

Results

Physicochemical parameters of karanda wine

Measured physicochemical characteristics during karanda fruit fermentation are shown in Table 1. The pH value increased slightly from 2.47±0.06 at day 0 to 2.80±0.00 after fermentation (day 22). Similarly, alcohol content increased from 0% at day 0 to 12.50±0.35% at the end of the

fermentation process, while total soluble solids (TSS) dropped gradually from 24 °Brix to 9.17±0.58 °Brix at the end of the process.

Table 1. Mean values of the parameters measured during karanda fruit fermentation

Fermentation time (days)	TSS (%Brix)	pH	Alcohol content (%v/v)
0	24.00±0.00	2.47±0.06	0.00
2	20.00±0.00	2.40±0.00	2.20±0.10
4	18.00±0.00	2.40±0.00	4.97±0.40
6	16.00±0.00	2.50±0.00	6.73±0.31
8	13.53±0.50	2.50±0.00	8.97±0.23
10	12.17±0.29	2.50±0.00	10.03±0.60
12	11.00±0.00	2.50±0.00	11.00±0.17
14	10.00±0.00	2.60±0.00	11.77±0.42
16	9.83±0.29	2.60±0.00	12.10±0.40
18	9.33±0.76	2.67±0.06	12.30±0.35
20	9.17±0.58	2.87±0.06	12.63±0.31
22	9.17±0.58	2.80±0.00	12.50±0.35

Methanol content was measured using gas chromatography equipped with a flame ionization detector (GC-FID). After the fermentation was completed, KW contained higher methanol at 0.005±0.00033% (v/v). Furthermore, volatile compounds in KW were studied using gas chromatography-mass spectrometry (GC-MS) (Fig 1). A qualitative data analysis indicated the presence of 7 volatile compounds in KW including 3-methyl-1-butanol, butanoic acid, butyrolactone, phenylethyl alcohol, butanedioic acid, benzene ethanol and 2-propenyl ester. The peak area of phenylethyl alcohol was the highest, followed by the other peaks as shown in Table 2.

Table 2. Retention times and peak areas of volatile compounds in karanda wine

Volatile compound	Peak number	Retention time (min)	Peak area (%)
3-methyl-1-butanol	1	3.199	15.72
Butanoic acid	2	8.143	1.07
Butyrolactone	3	14.815	10.36
Phenylethyl alcohol	4	17.739	69.63
Butanedioic acid	5	20.599	0.37
Benzene ethanol	6	31.793	1.79
2-propenyl ester	7	32.144	0.31

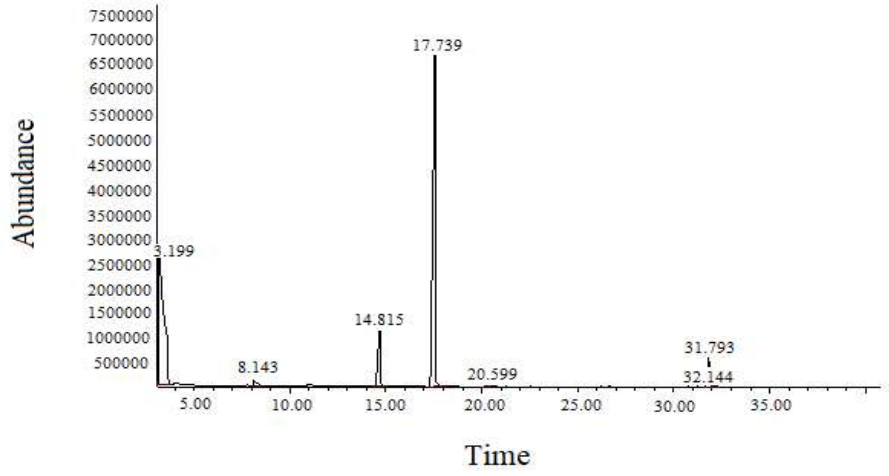

Figure 1. A GC-MS chromatogram showing volatile compounds analyzed in karanda wine

For quality control, the heavy metal contents of Cu, Pb and As in KW were compared to local Thai fruit wine under Thai Industrial Standards Institute (TISI) legislation. Heavy metals in KW were analyzed by AAS and results showed that 4 elements as Pb, As, Cu and Cd were detected at 7.9 x 10^{-6}, 5.3 x 10^{-7}, <1.0 x 10^{-3} and <1.0 x 10^{-5} mg/ml, respectively. These trace element amounts were less than the index value of TISI legislation, except for Cd which was not indicated in TISI legislation. Results are summarized in Table 3.

Table 3. Measurement of various metal concentrations in KW using AAS

Heavy metal	KW (mg/ml)	TISI* index (mg/ml)
Pb	7.9×10^{-6}	$< 2 \times 10^{2}$
AS	5.3×10^{-7}	$< 1 \times 10^{2}$
Cu	$<1.0 \times 10^{-3}$	$< 5 \times 10^{3}$
Cd	$<1.0 \times 10^{-5}$	-

*TISI = Thai Industrial Standards Institute

Total phenolic content (TPC)

The TPC of the extract was determined by the Folin-Ciocalteu method and reported as gallic acid equivalent of 746.64±3.10 mg GAE/100 ml sample.

Evaluation of antioxidant activity in KW

The *in vitro* antioxidant activities of KW are demonstrated in Table 4. A lower IC_{50} value of DPPH corresponds to a higher antioxidant activity. The percentage of IC_{50} obtained from KW was approximately 0.84±0.03, while the IC_{50} value of L-ascorbic acid (standard) exhibited at 0.0032±0.03%, lower than the KW sample. Moreover, L-ascorbic acid gave a higher value of IC_{50} (0.002±0.11%) than the IC_{50} value (3.28±0.03%) of KW when evaluated by $ABTS^{\bullet+}$assay. Reducing power was measured by the direct reduction of Fe^{3+} to Fe^{2+}. Results exhibited that L-ascorbic acid displayed the highest activity (EC_{50}: 0.005±0.05%) while the sample of KW showed the lowest activity (EC_{50}: 11.03±0.11) in reducing power.

Table 4. Values of reducing power, free radical scavenging activity and radical cation decolorization in karanda wine

Sample	Method		
	Reducing power EC_{50} (%)	**DPPH IC_{50} (%)**	**$ABTS^{+}$ IC_{50} (%)**
KW	11.03±0.11	0.84±0.03	3.28±0.03
Standard			
L-ascorbic acid	0.005±0.05	0.0032±0.03	0.002±0.11

IC_{50} is the concentration of antioxidants which can inhibit the free radicals at 50%
EC_{50} is the concentration that has the ability to reduce free radicals at 50%
± Standard deviation of 3 samples (repeat the experiment)
IC_{50} and EC_{50} values correspond to higher antioxidant activity

Discussion

Winemaking is one of the most ancient human technologies and has been performed since the beginning of civilization (Jagtap and Bapat, 2015). Generally, the process of fruit wine production is similar to making wine from grapes (Kosseva *et al.*, 2016). Major requirements for wine fermentation are pH, sugar concentration, temperature and acidity of fruit juice (Dias *et al.*, 2007). Thus, to produce a high percentage of ethanol, concentration of sugar was deliberately increased because sugar concentration of karanda juice is very low (approximately 1 °Brix). The TSS value is directly related to the final alcoholic content of wine (Conde *et al.*, 2006). Nevertheless, a too high concentration of sugar in a fermentation medium can inhibit the growth of yeast and impact on ethanol production (Cheng *et al.*, 2009). High sugar concentration inhibits the growth and multiplication of yeast in the process of fruit wine fermentation. Increasing initial sugar concentration from 200 to 300 g/L resulted in a significant decrease in fermentation efficiency and yeast

viability (Gavimath *et al.*, 2012). Yeast strains used for fermentation have adapted to diverse sugar compositions in different fruits (Duarte *et al.*, 2010). High ethanol concentration also reduces cell vitality and increases cell death because ethanol is an inhibitor of yeast growth (Stanley *et al.*, 2010). In our study, TSS of karanda juice fermentation decreased from 24 °Brix to 9.17 ±0.58 °Brix over 22 days. Gavimath *et al.* (2012) reported that TSS values of papaya, banana, orange and lime wine fermentations performed for 22 days decreased from 24 °Brix to 10, 8, 12 and 12°Brix, respectively.

Seven volatile compounds were deleted in KW by GC-MS analysis comprising 3-methyl-1-butanol, butanoic acid, butyrolactone, phenylethyl alcohol, butanedioic acid, benzene ethanol and 2-propenyl ester. These volatile compounds are usually detected in fruit wine fermentations (Xu *et al.*, 2017; Musyimi *et al.*, 2014; Oliveira *et al.*, 2011; Pantelić *et al.*, 2014). Normally, volatile compounds are present in considerable amounts in alcoholic beverages such as wine and they enhance the final quality of the product. These volatile aromatic compounds are not only present in fruit juices but also synthesized by wine yeast during fermentation (Molina *et al.*, 2007). Several volatile compounds including alcohols, aldehydes, esters, acids and other minor components are already present in fruit wine fermentations (Verzera *et al.*, 2008). For example, acetaldehydes, alcohols, acids, esters, and lactones were detected in mango, cagaita, cherry, banana and grape wine, respectively. Phenyl ethanol, which has been detected in wine is a compound alcohol with an aroma similar to roses (Wondra and Berovic, 2001; Musyimi *et al.*, 2014). Butyrolactone as one of the main lactones involved in wine fermentation contributes aromas related to caramel and coconut indicators. This volatile compound showed a behavior similar to the higher alcohols (Caldeira *et al.*, 2010). Esters often have fruity aromas and are commonly present in unaged wine. Levels of esters increase with aging, and wine which was aged in barrels showed the highest significant levels. Isovaleric and butanoic acids have aromas which are similar to butter and cheese (Petropulos *et al.*, 2014). Pozo-Bayón *et al.* (2005) reported the detection of benzene ethanol in sherry wine as the only higher alcohol that produces pleasant sweet odors like roses. Benzene ethanol is derived from L-phenylalanine through the metabolic reaction of *S. cerevisiae.*

Concentrations of heavy metals (lead, arsenic, copper and cadmium) usually result from the residues of agrochemical products including insecticides and fungicides used for plant cultivation. Furthermore, heavy metal contamination might occur during wine production and the packaging process (Klarić *et al.*, 2011). Metals such as lead, cadmium and arsenic are toxic to human cells, whereas high copper levels cause deterioration through

hematopoiesis, bone metabolism, and disorders of the digestive, cardiovascular, and nervous systems (Duarte *et al.*, 2010). Dakuzaku *et al.* (2001) reported detection of the heavy metal arsenic in grape wine at 0.00-0.02 mg/ml for 60 samples. Moreover, De Nisco *et al.* (2013) detected lead, cadmium and copper in berry wine at 0.06496, 0.0002 and 0.0016 mg/ml, respectively, while in our study 4 heavy metals as Pb, As, Cu and Cd were detected in KW at 7.9×10^{-6}, 5.3×10^{-7}, $<1.0 \times 10^{-3}$ and $<1.0 \times 10^{-5}$ mg/ml, respectively. However, these heavy metal contents, as mentioned before, were lower than their maximum allowed concentrations.

Reddy *et al.* (2008) noted that concentrations of methanol in wine did not normally exceed 100 mg/l, indicating an improvement in the product. Soufleros *et al.* (2001) reported that levels of methanol at 100-300 mg/ml were not potentially injurious to health, while Reddy *et al.* (2009) reported that the human oral lethal dose for methanol was estimated at about 340 mg/kg body weight. Therefore, the methanol content in KW was not harmful to humans.

Wide-ranging spectrophotometric assay methods are now used to investigate antioxidants in various food and beverages (Sreeramulu and Raghunath, 2010). Several methods such as 2,2-azino-bis-3-ethylbenzthiazoline -6-sulfonic acid or ABTS assay, 1,1-diphenyl-2-2picrylhydrazyl or DPPH assay and reducing power ability are extremely popular because these methods are rapid, sensitive, reproducible, and require simple conventional laboratory equipment (Scartezzini *et al.*, 2006). For the ABTS assay, a blue/green ABTS $^{\bullet+}$ is degenerated by the antioxidants, whereas purple DPPH is reduced to 1,1-diphenyl-2-picryl hydrazine for the DPPH assay, with reducing power ability different for both methods. There are no free related radicals but the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) was observed (Sreeramulu and Raghunath, 2010). The IC_{50} values of DPPH and ABTS assay were also analyzed. The low IC_{50} values in KW indicated high antioxidant activity but lower than the activity of L-ascorbic acid in our experiment. Likewise, the reducing power assay exhibited a low EC_{50} value in KW as shown in Table 4. The IC_{50} value represents the concentration of the sample required to inhibit 50% of the radical (Thinh *et al.*, 2017). The EC_{50} value can be defined as the concentration of the sample leading to 50% transformation of Fe^{3+} to Fe^{2+} by donating an electron (Iancu *et al.*, 2016). A lower IC_{50} and EC_{50} indicate a higher antioxidant activity of a compound (Govindan and Muthukrishnan, 2013). Kosseva *et al.* (2016) reported the IC_{50} of DPPH assay and the EC_{50} value of peach wine at 1.55 ± 0.09 and 3.01 ± 0.12 mM TE/L, respectively, while the IC_{50} of ABTS assay for grape wine analysis was 11.4 ± 0.40 mM TE/L (Pinto *et al.*, 2005).

Several polyphenols and other bioactive compounds are released into aqueous ethanolic solution during the winemaking process (Jagtap and Bapat, 2015; Shahidi, 2009). The phenolics are usually present in wines but their concentrations and compositions depend on the source of fruit and the method of winemaking. Elderberry, blueberry and blackcurrant wine recorded total phenolic compounds at 175.3, 167.6 and 150.9 mg GAE/100 ml sample, respectively (Pinto *et al.*, 2005).

It concluded that some physicochemical properties of wine fermented from karanda fruit including TSS, pH, alcohol content, volatile compounds, heavy metals and methanol content. Alcohol was obtained at 12.50±0.35% after fermentation for 22 days and 7 volatile compounds as 3-methyl-1-butanol, butanoic acid, butyrolactone, phenylethyl alcohol, butanedioic acid, benzene ethanol and 2-propenyl ester were found in the wine. These major components are normally detected in fruit wines and they contribute to the aroma. Heavy metal contents were lower than their maximum allowed concentrations, and methanol was detected at only 0.005%. Antioxidant activity was determined as DPPH, ABTS and reducing power assay. The IC_{50} and EC_{50} of these methods exhibited high activity, and total phenolic content in KW was high. Therefore, some characteristics of karanda wines were similar to other beverages. Results demonstrated that karanda fruit has the potential to produce fermented beverages.

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