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Evaluation Of Anti-Oxidant Activity And Cytotoxicity Of Aqueous Extract From Nipa Palm (Nypa Fruticans Wurmb.) Vinegar

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ABSTRACT

Fermented sap of nipa palm or nipa palm vinegar (NPV) is conventionally used for cooking as sour food seasoning. This study was aimed to determine phenolic compounds and flavonoids and evaluate in vitro antioxidant and cytotoxicity of NPV aqueous extract. NPV aqueous extract was was performed by liquid–liquid extraction. Aqueous extract was determined total phenolic content (TPC) and total flavonoid content (TFC) by Folin-Ciocalteu and aluminum chloride colorimetric assays, respectively. Antioxidant activity was evaluated by scavenging of DPPH, NO radicals and inhibition of lipid peroxidation. Cytotoxicity was evaluated against human skin fibroblasts. The results were represented that TPC and TFC were 1.76±0.02 GAE and 0.81±0.04 QE, respectively. NPV aqueous extract was exhibit DPPH and NO scavenging activities (SC_{50} = 105.23±27.14 and 0.84±0.02). Therefore, the extract was poorly inhibited lipid peroxidation. NPV aqueous extract were non-toxic to human skin fibroblast cells at maximum concentration of assay (1.0 mg/ml) when compared with sodium lauryl sulfate and can be safe when processing it as skin care products. This study was provided useful information on appropriate processing of NPV to health promoting products on different means.

Keywords: anti-oxidant, cytotoxicity, nipa palm vinegar, Nypa fruticans Wurmb.

INTRODUCTION

Oxidant elevation and/or antioxidant reduction can cause oxidative stress in human, which is lead to progression of pathogenic ageing and can cause chronic, inflammatory and degenerative diseases (Candido et al., 2015). Reactive oxygen species such as superoxide, hydrogen peroxide, and hydroxyl radical are affected to lipids, proteins and DNA resulting in accelerated aging, cancer, and brain degenerative disorder (Sies, 2015). Many studies are supported that bioactive compounds from foods may prevent risks of these degenerative diseases by providing an antioxidant effect (Aune, 2019; Fernandez-Mar et al., 2012; Kozłowska and Szostak-Wegierek, 2014; Ramadan and Al-Ghamdi 2012). The antioxidative activities of vinegar are exerted mainly from active constituents i.e., carotenoids, phytosterols phenolic compounds, vitamins C and vitamin E (Charoenkiatkul et al., 2016). It can used to control oxidation and reduced rate of food spoilage. Vinegars are traditionally use for food preservatives in many countries, which can inhibit microbial growth. There are also use as functional food additives on promoting of health benefits (Chen et al., 2016; Finley et

al., 2011; Pooja and Soumitra, 2013). Vinegar is generally used as sour food seasoning and as traditional medicine for relieve obesity (Kim et al., 2013; Beh et al., 2017), hyperlipidemia (Seo et al., 2015), diabetes (Yun et al., 2007), and cancer (Baba et al., 2013). Vinegar has high antioxidant activity, antimicrobial properties, antidiabetic effects and therapeutic properties (Budak et al., 2014), Polyphenolic compounds are quality determinants of vinegar antioxidant, which are also relating to colour and astringent of vinegar (Mas et al., 2014). Nipa palm (Nypa fruticans Wurmb.) is growing in mangrove forest of Thailand and can tolerate in brackish/saline water (Prasad et al., 2013). Utilizations of nipa palm are widely, including coastal-erose prevention, food resources, material providing. Sap from floral and fruit stalk can process as sugar, syrup, alcohol, vinegar, local made beverages and desserts, which are provide people income (Javier and Scott, 2013; Kusmana, 2018; Matsui et al., 2014). Fermented sap of nipa palm is namely nipa palm vinegar (NPV), which conventionally used for cooking as sour food seasoning (Cheablam and Chanklap, 2020). NPV has showed biological activities, such as in vivo antioxidant activity (Beh et al., 2016), vitro antioxidant activity (Chatatikun and Kwanhian, 2020), anti-obesity (Beh et al., 2017), antidiabetic activity (Yusoff et al., 2015; Yusoff et al., 2017), and hepatoprotective effects (Yusoff et al., 2017). NPV aqueous extract had evaluated on anti-diabetic activity (Yusoff et al., 2015; Yusoff et al., 2017), while unextracted NPV had evaluated on anti-oxidant activity (Chatatikun and Kwanhian, 2020; Beh et al., 2016). Hence, antioxidant activity and cyto-toxicity of NPV aqueous extract has not been previously evaluated. This study was aimed to determine phenolic compounds and flavonoids and evaluate in vitro antioxidant and cytotoxicity of NPV aqueous extract.

MATERIALS AND METHODS

NPV collection and extraction

NPV was traditional processed and purchased from five local markets from different of locations of Nakhon Si Thammarat, Thailand during November to December 2021. NPV was collected at 4 °C and sent to herbal laboratory unit, Suan Sunandha Rajbhat University, Samut Songkhram, Thailand. Liquid–liquid extraction was performed for NPV aqueous extract as described by Qiu et al. (2010). Briefly, 0.5 L of pooled NPV was concentrated until 0.25 L by rotary evaporator (N-1000, EYELA, USA) with water bath at 37 °C under vacuum. Concentrated NPV was extracted with ethyl acetate (RCI Labscan, Bangkok, Thailand): water (1:1) by separatory funnel. Upper (ethyl acetate) layer was removed and remaining (aqueous) layer was collected. This extraction process was repeated in three times and all of aqueous layers were collected and concentrated again under vacuum at 40 °C. NPV aqueous extract was freeze-dried in lyophilized form and kept at -4 °C.

Total Phenolic Content (TPC)

NPV aqueous extract (1 mg/ml) was dissolved in ethanol (RCI, Labscan, Thailand). Gallic acid (Sigma-Aldrich, USA) was dissolved in dimethyl sulfoxide, DMSO (RCI, Labscan, Thailand) and diluted (0.00012, 0.00024, 0.00049, 0.00098, 0.00195 and 0.00391 mg/ml) for standard curve plotting. 0.1 ml of sample was filled in test tube, and added with 4.6 ml of distilled water and 1 ml of Folin-Ciocalteu reagent (Loba Chemie, India), respectively. Mixture was standing in room temperature for 3 min and added with 3 ml of 2% Na_2CO_3 (w/v) was filled, then centrifuged for 2 h (150 rpm). Absorbance of mixture was determined at 765 nm against gallic acid standard curve. TPC was represented as mg of gallic acid equivalent (GAE) per g (Biju et al., 2014).

Total Flavonoid Content (TFC)

NPV aqueous extract (10 mg/ml) was dissolved in DMSO (RCI, Labscan, Thailand), and quercetin (HWI Analytik GmbH, Germany) was in DMSO (0.0020, 0.0039, 0.0078, 0.0156, 0.0312, 0.0625 and 0.125 mg/ml) dissolved in DMSO for standard curve plotting. Total flavonoid content was determined by aluminum chloride colorimetric assay. Measurement of absorbance was done on mixture in each well of 96-well plate (SPL Life

Sciences, Korea) by microplate reader (BIO-RAD, USA) at 515 nm. Each measurement was repeated in triplicate and the flavonoid content was reported as mg of quercetin equivalents (QE) per g of extract (Kelly et al., 2002).

Antioxidant Tests

DPPH radical scavenging activity

Reduction of 2,2-diphenyl-1-picrylhydrazyl, DPPH (Sigma-Aldrich, USA) radical absorbance was measured in present of NPV aqueous extract (0.01-1 mg/ml) by 96 well microplate reader (BIO-RAD, USA). DPPH radical (6×10^{-5} M) and ascorbic acid were used as negative and positive controls, respectively. Results was calculated and represented as 50% scavenging concentration (SC_{50}) against DPPH radical (Manosroi et al., 2010; Ranasinghe et al., 2012).

NO radical scavenging activity

NPV aqueous extract was dissolved with absolute ethanol and diluted to 0.001, 0.01, 0.1, 1 and 10 mg/ml. Each concentration was determined for NO radical scavenging in Griess reagent. Mixture was incubated in a 96-well plate at room temperature for 10 minutes, and the absorbance was then measured at 540 nm using a microplate reader. Ascorbic acid was used as positive control. Results was calculated and reported as SC_{50} on reduction of NO radical (Aktas et al., 2013).

Lipid peroxidation inhibition activity

NPV aqueous extract was dissolved with absolute ethanol and diluted to 0.001, 0.01, 0.1, 1 and 10 mg/ml. Each concentration was evaluated for inhibitory lipid peroxidation activity by measurement of ferric iron-thiocyanate complex. Absorbance of iron-thiocyanate was monitored by microplate reader at 490 nm, and positive control was α-tocopherol. Results was calculated and reported as 50% inhibitory peroxidation concentration (IPC_{50}) on reduction of lipid peroxidation (Manosroi et al., 2012).

Cytotoxicity Test

Human skin fibroblast cell (passage 64) was obtained from Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. Cell culture was maintained in Dulbecco's modified Eagle's medium containing 10% foetal bovine serum, 1% penicillin/streptomycin at 37 °C in a humidified incubator containing 5% CO_2. NPV aqueous extract was dissolved with 10% DMSO in culture medium and filtered with 0.2 μm filter-membrane and diluted by ten-fold serial dilution (0.0001, 0.001, 0.01, 0.1 and 1.0 mg/ml). After overnight culture in a 24-well plate (1×10^5 cells/well, 500 μL medium/well), the cells were pre-treated with different concentration of extract. Cytotoxicity was performed by sulforhodamine B (SRB) staining on cell viability and reported as percentage of cell survival when compared with sodium lauryl sulfate (SLS) as control (ISO 10993-5, 2009; Vichai et al., 2006).

Statistical Analyses

Phytochemical content and biological properties of NPV aqueous extract were represented as mean and standard deviation, which were calculated from triplicate-repeated measurements.

RESULTS AND DISCUSSION

TPC and TFC contained in NPV aqueous extract were 1.76±0.02 GAE and 0.81±0.04 QE, respectively. NPV aqueous extract was exhibit DPPH and NO scavenging activities (SC_{50} = 105.23±27.14 and 0.84±0.02). Therefore, the extract was poorly inhibited lipid peroxidation (Table 1). NPV aqueous extract were non-toxic to human skin fibroblast cells at maximum concentration of assay (1.0 mg/ml) when it was compared with sodium lauryl sulfate (Table 2). The extract was strongly scavenged NO was related to anti-inflammation activity. The previous studies had reported was anti-inflammatory activity of unextracted NPV with NO scavenge action by reduced production of NO from LPS induced-macrophage cells (Beh et al., 2017; Senghoi

and Klangbud, 2021). We were suggested that unextracted NPV and NPV aqueous extract were similar anti-oxidant and anti-inflammation activities, which were not affected from changing of vinegar acidity. There were similar result as anti-diabetic activities at in vitro and in vivo model that were evaluated NPV aqueous extract (Yusoff et al., 2015; Yusoff et al., 2017). Poor lipid peroxidation inhibition of NPV aqueous extract was due to low solubility in non-polarity environment of assay. There was implied that it was active in aqueous phase and not affect by loss of acidity, such as liver protection, anti-obesity and anti-lipidaemia (Beh et al., 2016; Beh et al., 2017; Chatatikun and Kwanhian, 2020). However, only anti-microbial assay was concerned on vinegar acidity (Senghoi and Klangbud, 2021). In addition, this extract was non-toxic to human skin cells and can be safe when processing it as skin care products. This study was provided useful information on appropriate processing of NPV to health promoting products on different means.

Table 1 Active constituents and antioxidant activity of NPV aqueous extract

Test / Assay	TPC	TFC	DPPH	NO	LPI
Aqueous extract	1.76±0.02	0.81±0.04	105.23±27.14	0.84±0.02	>1000
α-tocopherol	-	-	-	-	0.12±0.06
Ascorbic acid	-	-	0.04±0.01	0.04±0.01	-

DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; NO = nitric oxide radical scavenging activity; LPI = lipid peroxidation inhibition activity; ND = not determined

Table 2 Cytotoxicity of NPV aqueous extract

Sample (mg/ml)	Human skin fibroblast cell viability (%)				
	0.0001	0.001	0.01	0.1	1.0
Aqueous extract	95.2±2.57	93.4±3.46	91.3±3.10	88.4±3.76	85.±2.73
Sodium lauryl sulfate [a]	93.5±1.75	92.0±3.89	88.6±5.60	46.3±3.44	24.1±1.61

[a] Positive control on cytotoxicity at 0.1 to 1.0 mg/ml

CONCLUSION

NPV aqueous extract were exhibit DPPH and NO scavenging activities with non-toxic against to human skin fibroblast cells.

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Conflict Of Interest And Ethical Clearance

Ethics Committee of Suan Sunandha Rajabhat University was exempted this research protocol (COE.1-001/2022). The authors were confirmed that there are no conflicts of interest on this study.

REFERENCE

Aktas N, Genc Y, Gozcelioglu B, Konuklugil B, Harput US. 0Radical scavenging effect of different marine sponges from mediterranean coasts. Records of Natural Products, 2013, 7(2), 96- 104.

Aune D. Plant Foods, Antioxidant Biomarkers, and the Risk of Cardiovascular Disease, Cancer, and Mortality: A Review of the Evidence. Advance Nutrition, 2019, 10(Suppl4), S404-S421.

Baba N, Higashi Y, Kanekura T. Japanese black vinegar "Izumi" inhibits the proliferation of human squamous cell carcinoma cells via necroptosis. Nutrition and Cancer, 2013, 65(7), 1093-1097.

Beh BK, Mohamad NE, Yeap SK, Ky H, Boo SY, Chua JYH, Tan SW, Ho WY, Sharifuddin SA, Long K, Alitheen NB. Anti-obesity and anti-inflammatory effects of synthetic acetic acid vinegar and Nipa vinegar on high-fat-diet-induced obese mice. Scientific Reports, 2017, 7(1), 6664.

Beh BK, Mohamad NE, Yeap SK, Lim KL, Ho WY, Yusof HM, Sharifuddin SA, Jamaluddin A, Long K, Alitheen NB. 2016. Polyphenolic profiles and the in vivo antioxidant effect of nipa vinegar on paracetamol induced liver damage. RSC Advances, 2016, 6(68), 63304–63313.

Biju J, Sulaiman CT, Satheesh G, Reddy VRK. Total phenolics and flavonoids in selected medicinal plants from Kerala. International Journal of Pharmacy Pharmaceutical Science 2014, 6, 406-408.

Budak NH, Aykin E, Seydim AC, Greene AK, Seydim ZBG. Functional properties of vinegar. Journal of Food Science, 2014, 79(5), 757–764.

Candido TLN, Silva MR, Agostini-Costa TS. Bioactive compounds and antioxidant capacity of buriti (Mauritia flexuosa L.f.) from the Cerrado and Amazon biomes. Food Chemistry, 2015, 177, 313-319.

Charoenkiatkul, S., Thiyajai, P., & Judprasong, K. (2016). Nutrients and bioactive compounds in popular and indigenous durian (Durio zibethinus murr.). Food Chemistry, 193, 181-186.

Chatatikun M, Kwanhian W. Phenolic profile of nipa palm vinegar and evaluation of its antilipidemic activities. Evidence-Based Complementary and Alternative Medicine, 2020, 2020, 6769726.

Cheablam O, Chanklap B. Sustainable Nipa Palm (Nypa fruticans Wurmb.) Product Utilization in Thailand. Scientifica (Cairo), 2020, 2020, 3856203.

Chen HY, Chen T, Giudici P, Chen F. Vinegar functions on health: constituents, sources, and formation mechanisms. Comprehensive Reviews in Food Science and Food Safety, 2016, 15(6), 1124–1138

Fernandez-Mar MI, Mateos R, Garcıa-Parrilla MC, Puertas B, Cantos-Villar E. Bioactive compounds in wine: resveratrol, hydroxytyrosol and melatonin. A review. Food Chemistry, 2012, 130, 797-813.

Finley JW, Kong AN, Hintze K J, Jeffery EH, Ji LL, Lei XG. Antioxidants in foods: state of the science importnt to the food industry. Journal of Agricultural and Food Chemistry, 2011, 59(13), 683-6846.

ISO 10993-5: 2009. Biological evaluation of medical devices. Part 5: Tests for in Vitro Cytotoxicity. International Organization for Standardization, 2009, Geneva: Switzerland.

Kelly EH, Anthony RT, Dennis JB. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. Journal of Nutritional Biochemistry, 2002, 13, 572-584.

Kim JY, Ok E, Kim YJ, Choi K-S, Kwon O. Oxidation of fatty acid may be enhanced by a combination of pomegranate fruit phytochemicals and acetic acid in HepG2 cells. Nutrition Research and Practice, 2013; 7(3), 153-159.

Kozłowska A, Szostak-Wegierek D. Flavonoids--food sources and health benefits. Roczniki Panstwowego Zakladu Higieny, 2014, 65(2), 79-85.

Kusmana C. Mangrove plant utilization by local coastal community in Indonesia. Earth and Environmental Science, 2018, 196(1), 012028.

Manosroi A, Kumguan K, Chankhampan C, Manosroi W, Manosroi J. Nanoscale gelatinase A (MMP-2) inhibition on human skin fibroblasts of Longkong (Lansium domesticum Correa) leaf extracts for anti-aging. Journal of Nanoscience and Nanotechnology, 2012, 12(9), 7187-7197.

Manosroi A, Ruksiriwanich W, Abe M, Sakai H, Manosroi W, Manosroi J. Biological activities of the rice bran extract and physical characteristics of its entrapment in niosomes by supercritical carbon dioxide fluid. Journal of Supercritical Fluids, 2010, 54, 137-144.

Mas A, Torija MJ, García-Parrilla MC, Troncoso AM. Acetic acid bacteria and the production and quality of wine vinegar. The Scientific World Journal 2014, 2014, 1-6.

Matsui N, Okimori Y, Takahashi F, Matsumura K, Bamroongrugsa N. Nipa (Nypa fruticans Wurmb) Sap collection in Southern Thailand I. Sap production and farm management. Environment and Natural Resources Research, 2014, 4(4), 75.

Prasad N, Yang B, Kong KW, Khoo HE, Sun J, Azlan A, et al. Phytochemicals and antioxidant capacity from Nypa fruticans Wurmb Fruit. Evidence-Based Complementary and Alternative Medicine, 2013, 2013(4), 154606.

Ramadan MF, Al-Ghamdi A. Bioactive compounds and health-promoting properties of royal jelly: a review. Journal of Functional Foods, 2012, 4, 39-52

Ranasinghe P, Premakumara G, Wijayarathna C, Ratnasooriya W. Antioxidant Activity of Caryota urens L. (Kithul) Sap. Tropical Agricultural Research, 2012, 23(2), 117-125.

Senghoi W, Klangbud WK. Antioxidants, inhibits the growth of foodborne pathogens and reduces nitric oxide activity in LPS-stimulated RAW 264.7 cells of nipa palm vinegar. Peer Journal, 2021, 9, e12151.

Seo H, Jeon B-D, Ryu S. Persimmon vinegar ripening with the mountain-cultivated ginseng ingestion reduces blood lipids and lowers inflammatory cytokines in obese adolescents. Journal of Exercise Nutrition and Biochemistry, 2015, 19(1), 1–10.

Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biology, 2015, 4, 180-183.

Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nature Protocols, 2006, 1, 1112–1116.

Yun SN, Ko SK, Lee KH, Chung SH. Vinegar processed ginseng radix improves metabolic syndrome induced by a high fat diet in ICR mice. Archives of Pharmacal Research, 2007, 30(5), 587-595.

Yusoff NA, Lim V, Al-Hindi B, Abdul Razak KN, Widyawati T, Anggraini DR, Ahmad M, Asmawi MZ. Nypa fruticans Wurmb, Vinegar's aqueous extract stimulates insulin secretion and exerts hepatoprotective effect on STZ-induced diabetic rats. Nutrients, 2017, 9(9), 925.

Yusoff NA, Yam MF, Beh HK, Abdul Razak KN, Widyawati T, Mahmud R, Ahmad M, Asmawi MZ. Antidiabetic and antioxidant activities of Nypa fruticans Wurmb, vinegar sample from Malaysia. Asian Pacific Journal of Tropical Medicine, 2015 8(8), 595–605.