Nat. Volatiles & Essent. Oils, 2021; 8(4): 16369-16375

NVEO
Natural Volatiles & Essential Oils

Anti-Inflammatory And Anti-Microbial Activities Of Aqueous Extract From Nipa Palm (Nypa Fruticans Wurmb.) Vinegar

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ABSTRACT

Nipa palm vinegar (NPV) is coming from sap fermentation, which conventionally used for cooking as sour food seasoning, which is exhibit wide range of health promotions. This study was conducted on screening active phytochemical constituents of NPV aqueous extract including, alkaloids, anthraquinones, carotenoids, glycosides, tannins, xanthones, triterpenes and steroids by colour tests, and anti-microbial and anti-inflammatory activities of NPV aqueous extract were also evaluated. NPV aqueous extract was evaluated anti-microbial activity on human skin pathogenic bacteria (Staphylococcus aureus and Cutibacterium acnes)/yeast (Candida albicans and Malassezia furfur), including by disc diffusion method; anti-inflammation by inhibition NO production of LPS-induced macrophages. The finding was show that extract gave negative results on all qualitative phytochemical screenings, which due to very low concentration of active constituents. It also was lack of anti-microbial activity against all skin pathogens. Therefore, this extract (1 mg) was preferable anti-inflammatory agent, which was 0.92 time of triamcinolone acetonide at the same concentration. Lack of anti-microbial activity of extract was related on lose of vinegar acidity. However, anti-inflammatory activity of aqueous extract still exhibited strong activity. Thus, this study was concluded that acidity of NPV was affected to anti-microbial activity especially aqueous extract, however, there was not affect to anti-inflammatory activity and other biological activity, such as, anti-diabetic. Our finding can useful on appropriate matching of NPV preparation with biological assays.

Keywords: anti-inflammation, anti-microbial, nipa palm vinegar, Nypa fruticans Wurmb.

INTRODUCTION

Nipa palm (Nypa fruticans Wurmb.) is growing in mangrove forest especially in southern region of Thailand and can tolerate in flooded wetlands commonly with brackish water. Various benefits of this mangrove palms, such as, prevention of coastal erosion, food production and material sources (Cheablam and Chanklap, 2020). Floral and fruit stalk sap can produce syrup, sweet, alcohol, beverage and vinegar as well as its fruits can made local desserts, which are gain income of community. (Javier and Scott, 2013; Kusmana, 2018; Matsui et al., 2014). Fermented sap is named nipa palm vinegar (NPV), which conventionally used for cooking as sour food seasoning. NPV are exhibit wide range of health promotions, including antioxidant, anti-diabetes, liver protection, anti-obesity, anti-lipidaemia, anti-inflammation and immunomodulant (Beh et al., 2016; Beh et al., 2017; Chatatikun and Kwanhian, 2020; Yusoff et al., 2015; Yusoff et al., 2017). Moreover,

NPV is possess anti-bacterial activity by can inhibited human pathogenic enterobacteria including Vibrio cholera, V. parahemolyticus, Salmonella enterica serovar Typhimurium, S. paratyphi A, Shigella sonnei, Staphylococcus aureus and Escherichia coli. Brackish and saline water NPV are more potent growth-inhibitory and bactericidal effects rather than fresh water NPV. Thus, anti-bacterial activity of NPV is depended on salinity of soil where palm grow up (Senghoi and Klangbud, 2021). Recently, plant-origin vinegars are trend to favor as health-promoting product; and consumers are pay attention on functional food as (Chen et al., 2016). Acetic acid and other organic acids are primary constituent of vinegar, which can kill or growth-inhibit bacteria (Nada, 2013); and NPV is also an effective anti-microbial agent (Myint, 2011). Herbal plants can be protecting, treating and minimizing skin infections with lower side effects when compared to modern medicines (Ahuja et al., 2021). NPV aqueous extract had also reduced blood glucose and improved insulin level in animal model (Yusoff et al., 2015; Yusoff et al., 2017). Hence, we were interested to screening another active phytochemical constituent in aqueous extract of NPV, such as, alkaloids, anthraquinones, carotenoids, glycosides, tannins, xanthones, triterpenes and steroids. We were also evaluating biological activities of NPV aqueous extract including, anti-microbial activity on human skin pathogenic bacteria/yeast by disc diffusion method and anti-inflammation by inhibition NO production of LPS-induced macrophages. This finding can be providing data on appropriate extraction and formulation on health promoting products.

MATERIALS AND METHODS

NPV collection and extraction

NPV was processed from nipa palm sap by traditional method (Chatatikun and Kwanhian, 2020), which was purchased from local markets from different of locations of Nakhon Si Thammarat, Thailand during November to December 2021. NPV (N = 5) was cooling transported to herbal laboratory unit, Suan Sunandha Rajbhat University, Samut Songkhram, Thailand. NPV aqueous extract was prepared by liquid–liquid extraction (Qiu et al., 2010). Pooled NPV (500 ml) was concentrated to 250 ml as final volume under vacuum by rotary evaporator (N-1000, EYELA, USA) with water bath at 37 °C. 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 liquid–liquid extraction was repeated in three times and collected aqueous layers were pooled and concentrated again under vacuum at 40 °C. Pooled aqueous extract was freeze-dried in lyophilized form and kept at -4 °C.

Qualitative phytochemical screening

Alkaloids: NPV aqueous extract (0.025 g) was added with 2N HCl (Merck, Darmstadt, Germany), warmed in water bath. and centrifuged. Then supernatant was separated and reacted with Dragendorff reagent. Result was found orange precipitation when compared with 0.01 g quinine sulfate (Sigma-Aldrich, Fluka, Germany) as positive control (Firdouse and Alam, 2011; Sakunpak et al., 2014).

Anthraquinones: NPV aqueous extract (0.025 g) was added with benzene (Sigma-Aldrich, Fluka, Germany) and centrifuged. Then supernatant was separated and reacted with 10%(v/v) ammonia (Northern chemical, Thailand). Result was found pinkish-red solution when compared with 0.01 g of Cassia fistula extract as positive control (Sakunpak et al., 2014; Sakulpanich and Grissanapan, 2008).

Carotenoids: NPV aqueous extract (0.025 g) was added with chloroform (RCI Labscan, Bangkok, Thailand), sonicated and centrifuged. Then supernatant was separated and reacted with 96%(v/v) sulfuric acid (Merck, Darmstadt, Germany). Result was found greenish-blue or blue solution when compared with 0.01 g of β-carotene (Sigma-Aldrich, Germany) as positive control (Lako et al., 2007).

Flavonoids: NPV aqueous extract (0.025 g) was added with methanol (RCI Labscan, Bangkok, Thailand), warmed in water bath. and centrifuged. Then supernatant was separated and reacted with magnesium

ribbon (Labchem, NSW, Australia) and 37%(v/v) HCl (Merck, Darmstadt, Germany). Positive results were exhibited colour change of solution including, yellow (flavone), red to darkended red (flavonol), darkended red to violet-red (flavanone) and red (flavanonol). 0.01 g of quercetin (HWI, Analytik GmbH, Germany) was used as positive control (Sakunpak et al., 2014).

Glycosides: NPV aqueous extract (0.025 g) or positive controls (0.01 g for each) including fructose (UNIVAR, Australia), glucose (Loba Chemie, India) and sucrose (Fisher Scientific, UK) were added with water, warmed in water bath. and centrifuged. Then each supernatant was spotted in TLC plate silica gel 60 (Merck, Darmstadt, Germany) and spotted plate was dipped in tank contained mobile phase (butanol/acetic acid/diethyl ether/water with ratio 9:6:1:3). Waiting for mobile was proper moved, leaved plate and air dried. Dried plate was sprayed with 10%(v/v) sulfuric acid (Merck, Darmstadt, Germany) and warmed on hot plate. Observation of sample spot gave brown colour and compared with position of positive controls (Hussein et al., 2010).

Tannins: NPV aqueous extract (0.025 g) was added with 98% (v/v) ethanol (Liquor Distillery Organization, Chachoengsao, Thailand), stirred with vortex mixer and centrifuged. Then supernatant was reacted with 10%(v/v) $FeCl_3$ (Carlo Erba, Val de Reuil, France). Positive result was exhibited blueish-green or blackish-green solution (Sakunpak et al., 2014), which compared to 0.01 g of tannic acid (Sigma-Aldrich, Fluka, Germany).

Xanthones: NPV aqueous extract (0.025 g) was added with methanol (RCI Labscan, Bangkok, Thailand), warmed in water bath and centrifuged. Then supernatant was reacted with 5%(w/v) KOH. Positive result was exhibited cleared-yellow precipitation. 0.01 g of xanthone (Sigma-Aldrich, Germany) was positive control (Firdouse and Alam, 2011).

Triterpenes: NPV aqueous extract (0.025 g) was added with dichloromethane (RCI Labscan, Bangkok, Thailand) and centrifuged. Then supernatant was reacted with 96%(v/v) sulfuric (Merck, Darmstadt, Germany) and positive result was observed brownish-ring between solution layers (Firdouse and Alam, 2011). 0.01 g of stigmasterol (Sigma-Aldrich, Germany) was positive control.

Steroids: NPV aqueous extract (0.025 g) was added with chloroform and centrifuged. Then supernatant was reacted with 96%(v/v) sulfuric (Merck, Darmstadt, Germany) and 99.8% (v/v) acetic acid (RCI Labscan, Bangkok, Thailand). Positive result was exhibited green solution. 0.01 g of triamcinolone acetonide (Sigma-Aldrich, Germany) was positive control (Firdouse and Alam, 2011).

Antimicrobial test

Microbials: Skin pathogenic bacteria including Staphylococcus aureus and Cutibacterium acnes were provided from Thailand Institute of Scientific and Technological Research (TISTR) and Faculty of Medicine, Chiang Mai University, Thailand. Skin pathogenic yeasts including Candida albicans and Malassezia furfur were provided from Faculty of Medicine, Chiang Mai University, Thailand.

Culture media and Materials: Bacterial and yeast cultures were performed by brain heart infusion, BHI (HiMedia Laboratories, India) and yeast malt (HiMedia Laboratories, India), respectively. 6 mm filter paper disc (Macherey-Nagel, Germany), petri dishes (Union Science, Thailand), laminar flow biohazard class II (Renovation Technology, Thailand) and soft incubator SLIO-600ND (EYELA, Tokyo, Japan) were used in this test. 0.015 mg of erythromycin disc and 0.002 mg of clindamycin disc (Oxoid Ltd., UK) were positive antibacterial control for S. aureus and P. acnes, respectively. 0.025 mg of fluconazole and 0.2 mg of ketoconazole were positive anti-yeast control for C. albicans and M. furfur, respectively.

Disc diffusion method: Each bacterium and yeast isolated colonies were inoculated in BHI and yeast malt plates, respectively. Aqueous extract (8 mg) was dissolved with 95% ethanol (sterile) and diluted (100 mg/ml). 10 μl of undiluted extract was dropped to filtered-paper disc on medium. Then diluted extract was applied

for 1 2 and 3 times (0.1, 1.0 and 10.0 mg), respectively. Tested disc, standard antibiotic disc and negative control disc (95% ethanol) were applied within same medium plate. Incubation condition was 37±1 °C for 24-48 h. Interpretation of results was evaluated by measuring diameter of inhibition zone (mm) surrounded each disc. (Bauer et al., 1966; Clinical and Laboratory Standards Institute, 2016).

Anti-inflammatory test

NPV was screened on anti-inflammation by monitoring of nitric oxide (NO) reduction, which was produced from lipopolysaccharide (LPS)-induced macrophage cells. Briefly, macrophage cell (RAW264.7) was obtained from TISTR, Pathum Thani, Thailand. Cell was cultured in high-glucose Dulbecco's modified Eagle's medium containing fetal bovine serum (1%) and penicillin/streptomycin (1%) at 37 °C in a humidified incubator containing 5% CO_2. After overnight culture in a 24-well plate (1×10^5 cells/well, 500 µL medium/well), the cells were pre-treated with NPV (dissolved in 10%v/v DMSO) for 1 h and LPS for an additional 24 h, the culture supernatant from each well was collected at the end of scheduled experiments and used to measure NO production. 50 µl of culture medium and 100 µl of Griess reagents were added and 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. Triamcinolone acetonide (0.1 mg/ml) was used as positive control (Torres-Rodriguez et al., 2016).

Statistical Analyses

Phytochemical screening of NPV aqueous extract was represented as qualitative results. Mean and standard deviation of anti-microbial and anti-inflammatory activities were calculated from triplicate and four-time repeated measurements, respectively.

RESULTS AND DISCUSSION

NPV aqueous extract was very low concentration of active constituents and show negative results on all qualitative phytochemical screenings. Moreover, it also was lack of anti-microbial activity against skin pathogens including S. aureus, C. acnes, C. albicans and M. furfur by disc diffusion method (Table 1). Therefore, this extract (1 mg) was strongly anti-inflammatory agent by reduced NO production of LPS-induced macrophage cells. This activity was preferable, which was 0.92 time of triamcinolone acetonide at the same concentration (Table 2). Our finding was contrasted with previous study that use of unextracted NPV directly on anti-microbial activity against food-borne pathogens. Unextracted vinegar was still acidity and can inhibited Vibrio cholera, V. parahemolyticus, S. enterica serovar Typhimurium, S. paratyphi A, S. sonnei, S. aureus and E. coli by broth microdilution method. Thus, anti-microbial activity of NPV was depended on acidity rate of vinegar. In addition, NPV acidity is also depended on salinity of soil where nipa palm growth (Senghoi and Klangbud, 2021).

Table 1 Anti-microbial activity of NPV aqueous extract *

Pathogen /Sample	Amount (mg)	Diameter of inhibition zone (mm)			
		Plate 1	Plate 2	Plate 3	Average
S. aureus					
NPV aqueous extract	0.1-10.0	ND	ND	ND	ND
95% Ethanol	7	ND	ND	ND	ND
Erythromycin	0.015	26.26	24.76	23.97	23.0±1.16
C. acnes					
NPV aqueous extract	0.1-10.0	ND	ND	ND	ND
95% Ethanol	7	ND	ND	ND	ND
Clindamycin	0.002	50.38	51.3	50.84	50.87±0.51
C. albicans					

NPV aqueous extract	0.1-10.0	ND	ND	ND	ND
95% Ethanol	7	ND	ND	ND	ND
Fluconazole	0.025	22.52	20.10	20.34	20.98±1.34
M. furfur					
NPV aqueous extract	0.1-10.0	ND	ND	ND	ND
95% Ethanol	-	ND	ND	ND	ND
Ketoconazole	0.2	28.98	30.56	30.20	29.91±0.83

* Concentration of extract was 0.1, 1.0 and 10.0 mg; ND = not detected

However, anti-inflammatory activity of NPV was not related to acidity and this aqueous extract still exhibited strong activity (Beh et al., 2017) when evaluated with similar method (Senghoi and Klangbud, 2021). In previous studies, NPV aqueous extract were also active anti-diabetic activities at in vitro and in vivo model. It is reduced carbohydrate absorption by inhibit intestinal glucose transporters and reduce postprandial hyperglycaemia (Yusoff et al., 2015; Yusoff et al., 2017). Aqueous extract may also give similar actions without acidity relation on liver protection, anti-obesity and anti-lipidaemia (Beh et al., 2016; Beh et al., 2017; Chatatikun and Kwanhian, 2020). This study was demonstrated negative anti-microbial activity of NPV aqueous extract depended on acidity, and strong anti-inflammatory activity that unrelated on vinegar acidity. Our finding can useful on appropriate matching of NPV preparation with biological assays.

Table 2 Anti-inflammatory activity of NPV aqueous extract was represented by inhibition of NO production from LPS-induced macrophage cells

Sample/Conc. (mg/ml)	**Inhibition of NO production (%)**				
	0.0001	**0.001**	**0.01**	**0.1**	**1.0**
Aqueous extract	ND	17.3±2.7	24.6±4.2	25.0±4.2	25.1±3.0
Triamcinolone acetonide	16.4±3.9	20.0±3.1	23.7±3.1	23.7±3.1	27.3±3.2

CONCLUSION

NPV aqueous extract was lack of anti-microbial activity against skin pathogens including S. aureus, C. acnes, C. albicans and M. furfur by disc diffusion method. The extract was strongly anti-inflammatory agent by reduced NO production of LPS-induced macrophage cells when compared with triamcinolone acetonide at the same concentration (1 mg/ml)

ACKNOWLEDGEMENTS

We would like to sincerely thank to academic staffs from Samut Songkhram Education Center, Suan Sunandha Rajabhat University for research assistants. We are grateful to Institute of Research and Development, Suan Sunandha Rajabhat University, Bangkok, Thailand for partial funding support.

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.

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