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Antibacterial properties of natural tropical fruit vinegars against *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus* bacteria

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Abstract. Various researchers have found that vinegar has antibacterial effect on different types of pathogenic bacteria which make it useful for a varieties of application in medical, food preservation and as cosmetic ingredient. Many new emerging tropical fruits vinegars were not tested for antibacterial properties especially for skin and acne causing treatment. Therefore, this study aim is to test several tropical fruits vinegar in order to determine their ability against skin and acne causing bacteria ultimately could lead to application of effective vinegars in personal care and cosmetic products. Tropical fruit vinegars such as pineapple, mango, coconut, dokong and rambutan vinegars were tested for the presence of acetic acid, titratable acid, pH and anti-microbial properties against acne causing bacteria *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The presence of acetic acid content in the vinegar samples was determined using HPLC analysis against 100% glacial acetic acid standard and shown to be between 6.444, 6.959, 5.832, 6.484 and 6.373 min. Titratable acidity range lied between $1.14 \pm 0.06\%$ to $3.26 \pm 0.09\%$ of which rambutan vinegar showing highest acetic acid content while pineapple vinegar giving the lowest value. The pH value among five vinegar samples fell within the range of 3.06 to 3.65 of which pineapple vinegar has the lowest value and coconut vinegar had the highest pH. Antimicrobial properties of different tropical fruit vinegar samples with different percentage of acetic acid content in samples comprised of 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0% were determined by antimicrobial disk diffusion assay on *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. Clear zone of inhibition could be observed at 1% acetic acid in all samples, ranging from 7.8 mm to 8.6 mm against main acne causative bacteria, *Propionibacterium acnes* indicating that the vinegars has antibacterial properties against the bacterium

1. Introduction

Vinegar has an old history traced back over 10,000 years (Tan, 2005). Vinegar is produced via a two-step process of fermentation from a sugar to alcohol and converted to an acetic acid which involving yeasts as the primary agent and followed by acetic acid bacteria (Solieri & Giudici, 2009). Apple cider vinegar is made from apple is popular in United States for cooking purpose. Balsamic vinegar is made traditionally from the concentrated white Trebbiano grapes juice and aged in wood barrels is famous in Italy. Malt vinegar is made from malting barley which is a popular beverage in England (Tan, 2005). Fruit vinegars are made from fruit wines commonly without additional flavouring (Chang *et al.*, 2005).



Currently, tropical fruit vinegars are getting popular. Such example are vinegars from Rambutan and Dokong (SI Mokhtar *et al.*, 2016)

Acetic acid is the primary acid in vinegar. Commonly, acetic acid is well known for the purpose of cooking, cleaning and other household uses. Various researchers have found that vinegar has antibacterial effect on different types of pathogenic bacteria which make it useful for a varieties of application. For example, mother of vinegar has been demonstrated to have a therapeutic effect on burns due to antibacterial properties (Budak *et al.*, 2014). Besides, organic acid such as acetic acid are also used to inhibit growth of spoilage bacteria in meat such as beef and poultry (Lingham *et al.*, 2012). The factors that affect the antimicrobial activity of organic acid including the concentration of acid, ionic strength, pH, and temperature. Majority of the organic acid found in fruit and fermented food including acetic acid are not harmful to human health at low level (Budak *et al.*, 2014) usually around 3 percent concentration (Ryssel *et al.*, 2009).

With the emergence of tropical vinegars in the market recently, new ingredient can be utilised for medical, cosmetic and food purposes. Several new market vinegars such as pineapple, mango, coconut, dokong and rambutan vinegars have yet to be tested for the presence of acetic acid, titratable acid, pH and anti-microbial properties against common skin bacteria such as *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. The findings could lead to development of new formulations for cosmetic products using vinegars having anti- bacterial agents against skin and acne causing bacteria.

2. Methodology

2.1. Sample collection and handling

Five different types of fruit vinegars; (i) Pineapple Vinegar (PV) (ii) Mango vinegar (MV) (iii) Coconut Vinegar (CV) were purchased from supermarket while (iv) Rambutan Vinegar (RV) and (v) Dokong Vinegar (DV) were produced in Universiti Malaysia Kelantan. The samples were stored in room temperature (25 to 30°C) and dry place until further analysis.

2.2 Determination of pH value

pH value of each sample was identified using the pH meter. Distilled water was used to rinse the electrode of pH meter. The electrode was placed in pH 4 and pH 7 of buffer solutions for calibration of pH meter. The electrode was dipped in each samples for determining their pH values. Analysis was run in triplicate and the data was recorded.

2.3 Detection of acetic acid presence by High-performance liquid chromatography analysis

The five vinegar samples were analysed for their content in acetic acid. Before analysis, each sample was diluted 8:2 with deionized water and filtered through a 0.45- μ m membrane filter. Standard acetic acid solution was prepared by diluting 100% glacial acetic acid with deionized water and 20% of standard acetic acid solution was used for the HPLC analysis. All standard solution were stored at 4 °C. The methanol (700 mL) and distilled water (300 mL) were measured in a measuring cylinder and then mix thoroughly to prepare 1 L solution as a mobile phase. The acetic content of each samples was analysed by using high performance liquid chromatography (HPLC). A Shimadzu HPLC Series SIL-20AC apparatus was equipped with an isocratic pump, a Shimadzu SPD-M20A HPLC Photodiode Array

(PDA) Detector was set at 254 nm for acid determinations. The samples were separated isocratically using a Hypersil GOLD™ C18 column (150 x 4.6 mm i.d.). The oven temperature was set at 50 °C. The run time was 15 minutes and 20 µL injection volume was used. The mobile phase of 70:30 methanol-water was used at a flow rate of 0.6 mL min⁻¹.

2.4 Estimation of acetic acid concentration by Titratable Acidity method

0.1 M sodium hydroxide (NaOH) solution was prepared by mixing 1 g of NaOH pellets (M=40 gmol⁻¹) with 250 mL of distilled water and used as standardized solution. The burette was filled with 50 mL 0.1 M NaOH solution. The volume of 1 mL of vinegar sample was pipetted into conical flask and 150 mL distilled water was added into the flask and mixed well. Two drops of phenolphthalein indicator was added as an indicator. A sheet of white paper was placed beneath the receiving flask to more easily observe the endpoint. 0.1 M NaOH was slowly added drop by drop to the sample. The flask was swirled continuously while adding NaOH until the indicator changed colour, indicated the endpoint for titration. The final burette reading was noted down and the volume of NaOH used was recorded. Each samples was titrated three times to get the average value. The experiment was repeated with pineapple, mango, coconut, dokong and rambutan vinegar samples. The percentage of acetic acid in the vinegar samples was calculated as the formula of titratable acidity (TA) as shown below.

$$\% \text{ TA (acetic acid)} = \frac{\text{N of NaOH} \times \text{mL of NaOH} \times 60.05}{\text{Sample weight} \times 10} \quad (\text{Equation 1.0}) \text{ (Tan, 2005)}$$

2.5 Antimicrobial test of the fruit vinegar samples

P. acnes and *S. epidermis* used in this study were obtained from Dental Department of Hospital Universiti Sains Malaysia, Kubang Kerian, Kelantan, while *S. aureus* obtained from culture collection Faculty of Agro-based Industry, Universiti Malaysia Kelantan, Jeli Campus. *P. acnes* bacteria was sub-cultured on brain heart infusion medium agar. Single colony of the bacterium was inoculated in 10mL of brain heart infusion medium broth and incubated at temperature of 37 °C for 48 hours in an anaerobic jar with Oxoid AnaeroGen compact pouch. *S. aureus* and *S. epidermis* were sub-cultured on nutrient agar and single colony of the subculture bacteria was inoculate in 10 ml nutrient broth and incubated 24 and 72 hrs respectively at temperature of 37 °C. All the stock cultures were kept at 4 °C for the assay.

Antimicrobial testing of the different fruit vinegar samples with different acetic acid percentage (0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0%) were performed using disk diffusion method. Each agar plate was divided into sections. 100 µL of the fresh culture suspension of tested bacteria was spread evenly throughout the plate using a glass rod. Any extra liquid was allowed to dry on the plate. 5 µL of the sample to be tested was transferred to each of the filter paper disks (5.5 mm) on the control plate. The disks were left to fully dry before placing on the agar plates using a pair of sterile forceps. One disk with samples was placed in correspond section. The disk was placed gently on top of the agar and was lightly pressed down with the forceps. The plates was incubated at 37 °C, 24 hours for *S. aureus*, 48 hours for *P. acnes* and 72 hours for *S. epidermidis*. The area of inhibited bacterial growth was measured with a calliper. Tetracycline dissolved in DMSO was used as a positive control.

2.6 Statistical analysis

Triplicate results are expressed as the mean \pm standard deviation (SD). Statistical analyses were carried out using one way ANOVA where significant value is $p < 0.05$ using Excel software version 2016.

3. Results and Discussion

The presence of acetic acid content in the samples was determined by comparing their retention time in HPLC analysis to the known concentration of 100% glacial acetic acid standard. The refraction index trace was characterized by the largest eluting peaks which corresponded to acetic acid and was eluted within the range of 5.8 min to 7.0 min. Table 1 show the retention time of highest peak for PV, MV, CV, DV, and RV were 6.444, 6.959, 5.832, 6.484 and 6.373 min. The average value of highest peak retention time among vinegar samples was 6.42 min which considered closer to the retention time of standard solution, 5.53 if comparing with other peaks. Vinegar contains plenty of organic acids which contributes to its flavour such as acetic, citric, gluconic, lactic, malic, succinic and tartaric acids but usually acetic acid concentration is highest (Sanarico *et al.*, 2003).

Table 1. Retention time of highest peak for different fruit vinegars samples.

Samples	Peak #	Retention time	Area
Pineapple vinegar (PV)	5	6.444	73471574
Mango vinegar (MV)	3	6.959	70644820
Coconut vinegar (CV)	3	5.832	12590643
Dokong vinegar (DV)	6	6.484	11799028
Rambutan vinegar (RV)	3	6.373	57024122
20% acetic acid solution	1	5.533	1582714

Table 2 show percentage of acetic acid and pH value of five (5) types of fruit vinegars. The percentage of acetic acid in vinegar samples was determined by titratable acidity using titration method. According to the data from the table, it shown that the range of titratable acidity lied between 1.14 ± 0.06 % to 3.26 ± 0.09 % which rambutan vinegar showing highest acetic acid content while pineapple vinegar giving the lowest value. Titratable acidity of acetic acid for PV, MV, CV, DV, and RV were 1.14%, 1.20%, 2.43%, 3.49 % and 3.26 % respectively. There was significant different in the acetic acid content between the samples ($p < 0.5$). Acetic acid gives vinegar its characteristic sour taste and pungent odour. The optimum acetic acid content in vinegar range between 4% and 5% by weight (Lerona & Amibahar). According to Nandasiri (2012), fruit vinegar beverages are classified into two different types based on their acetic acid concentration. They are fruit vinegar beverage which is low in acetic acid (less than 3% v/v) and concentrated fruit vinegar beverage which is high in acetic acid (5 - 7% v/v). The concentrated fruit vinegar beverage usually has less than 3% (v/v) of the total sugar content and around 5 to 7% (v/v) of the acidity level.

In addition, the pH value among five vinegar samples fell within the range of 3.06 to 3.65 which pineapple vinegars gave the lowest value and coconut vinegars had shown the highest pH value. According to S.I. Mokhtar *et al.* (2016) the differences of pH value between local fruits vinegars with the apple cider vinegar were not obvious which local fruits vinegar range from 3.48 to 3.88 while apple cider is 3.10 and nipa vinegar is 2.86.

Table 2. Illustrated the titratable acidity percent of acetic acid for each vinegar samples and the relative pH values.

Samples	TA % (acetic acid)	pH
Pineapple vinegar (PV)	1.14 ± 0.06 ^a	3.06 ± 0.01 ^a
Mango vinegar (MV)	1.20 ± 0.00 ^a	3.09 ± 0.01 ^b
Coconut vinegar (CV)	2.43 ± 0.05 ^b	3.65 ± 0.00 ^c
Dokong vinegar (DV)	3.49 ± 0.03 ^c	3.39 ± 0.00 ^d
Rambutan vinegar (RV)	3.26 ± 0.09 ^d	3.32 ± 0.00 ^c

The value were mean ± standard deviation (n=3). The values followed by different superscript letters (a, b, and c) in the same row within the column of each individual portion are significantly different (p<0.5) by Duncan's multiple range tests.

Based on the Table 3, the zone of inhibition of all vinegar samples against three acne causing bacteria increased as the percentage of acetic acid content increased. Minimum inhibitory concentration of all the vinegars are at 0.5% acetic acid concentration however 1 % of acetic acid content in the vinegars exhibited the minimum optimum efficiency in inhibiting three acne causing bacteria. The bacteria inhibition zone of the acetic acid percent below 1 % did not show significant impact of the bacteria growth. For 1 % of acetic acid content, the zone of inhibition against *P. acnes* for PV, MV, CV, DV, and RV were 7.8 mm, 7.4 mm, 8.3 mm, 8.1 mm, and 8.6 mm. Rambutan vinegar (RV) shown the biggest zone of inhibition than other samples against *P. acnes*, *S. aureus*, and *S. epidermis*. The minimum dilution of Apple Cider Vinegar required for growth inhibition varied for each microbial species (Darshna Yagnik *et al.*, (2018). Adnyani, Ni Made Dwi (2019) reported that apple cider vinegar had antibacterial effect to *Propionibacterium acnes* showing average diameter of inhibition zone in acetic acid concentration of 0.625%, 1.25%, 2.5%, and 5% is 8,1 mm, 12,1 mm, 14, 9 mm, 18,9 mm using disc diffusion method.

Table 3. Zone of inhibition (mm) of different fruit vinegar samples with different percentage of acetic acid against acne causing bacteria using disk diffusion method.

% samples	Acne causing bacteria	Samples					
		T	PV	MV	CV	DV	RV
0.125	<i>S. aureus</i>	17.7	ND	ND	ND	ND	ND
	<i>S. epidermidis</i>	12.0	ND	ND	ND	ND	ND
	<i>P. acnes</i>	14.2	ND	ND	ND	ND	ND
0.25	<i>S. aureus</i>	23.9	ND	ND	ND	ND	ND
	<i>S. epidermidis</i>	14.0	ND	ND	ND	ND	ND
	<i>P. acnes</i>	17.2	ND	ND	ND	ND	ND
0.50	<i>S. aureus</i>	26.8	8.3	7.4	8.3	8.1	8.6
	<i>S. epidermidis</i>	15.2	5.8	ND	5.9	6.1	6.2
	<i>P. acnes</i>	19.5	7.0	7.0	6.8	6.6	7.3
1.00	<i>S. aureus</i>	TBTM	8.9	8.0	9.9	9.9	10.4
	<i>S. epidermidis</i>	TBTM	6.4	6.2	7.0	7.3	7.5
	<i>P. acnes</i>	TBTM	7.8	7.4	8.3	8.1	8.6
1.50	<i>S. aureus</i>	TBTM	Na	Na	10.8	11.3	13.5
	<i>S. epidermidis</i>	TBTM	Na	Na	8.0	8.2	8.6
	<i>P. acnes</i>	TBTM	Na	Na	10.0	10.2	10.7
2.00	<i>S. aureus</i>	TBTM	Na	Na	12.2	13.9	15.9
	<i>S. epidermidis</i>	TBTM	Na	Na	8.6	9.1	10.2
	<i>P. acnes</i>	TBTM	Na	Na	11.3	10.9	12.0

2.50	<i>S. aureus</i>	TBTM	Na	Na	Na	15.5	17.0
	<i>S. epidermidis</i>	TBTM	Na	Na	Na	9.4	11.3
	<i>P. acnes</i>	TBTM	Na	Na	Na	11.2	13.9
3.00	<i>S. aureus</i>	TBTM	Na	Na	Na	18.4	20.3
	<i>S. epidermidis</i>	TBTM	Na	Na	Na	11.4	12.9
	<i>P. acnes</i>	TBTM	Na	Na	Na	13.5	15.1

Legend: TBTM- too big to measure; ND -Not detected; Na -Not applicable; T – tetracycline in DMSO; PV - pineapple vinegar; MV -mango vinegar; CV -coconut vinegar; DV -dokong vinegar; RV -rambutan vinegar.

Conclusion

According to the result obtained from HPLC analysis, the existing of acetic acid in vinegar samples could be confirm within retention time range from 5.8 min to 7.0 min by comparison with 20% glacial acetic acid standard. Titratable acidity have shown that the rambutan vinegar had the highest acetic acid content, $3.26 \pm 0.09\%$ while pineapple vinegar giving the lowest value, $1.14 \pm 0.06\%$. From disc diffusion assay, 1% of the acetic acid in the vinegars and above was shown effective against acne causing bacteria for most vinegars. There was potential for vinegar samples to be develop into acne control product due to their presence of antimicrobial activity.

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