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Physicochemical and Antibacterial Activity of Soap Enriched with Harumanis Pruned Leaves Extract

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Abstract. The aim of this study was to formulate antibacterial soap from Harumanis pruned leaves extract. The antibacterial properties of Harumanis leaves extract were tested using disc diffusion test against five bacteria i.e. *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas putida*, *Bacillus megaterium* and *Bacillus licheniformis*. Methanol, ethanol and distilled water were used to extract the Harumanis pruned leaves. Ethanol is the best solvent to extract antimicrobial properties in Harumanis pruned leaves as it shows the largest inhibition zone, whereas, water is the weakest solvent to extract antimicrobial properties in Harumanis pruned leaves as it shows minimum inhibition zone for all the microbes used. The best extract of Harumanis pruned leaves (i.e. ethanol extract) was used in the formulation of the antibacterial soap. The result shows that formulated soap with Harumanis pruned extract have the ability to inhibit all the five microbes tested and therefore, it has the potential to be commercialized as antibacterial soap in the near future.

Keywords: Natural Product, Personal Care Product, Harumanis, Pruned Leaf Extract, Antibacteria,

1. Introduction

Nowadays, most of the people show consideration about health and environment safety. Various programs and projects have been implemented in order to reduce the disposal of waste. Biological waste such as plant has been attracted many researchers since it is less harmful to environment and consumer. Plant bio-waste ingredients are commonly used in pharmacy, food and cosmetic industries. Moreover, natural based cosmetic has gain worldwide popularity because it is free chemicals and have less side effects. In cosmetic industries, the application of plant and plant extract are widely used for various purposes such as anti-oxidant, anti-bacterial, anti-inflammatory, anti-fungal, anthelmintic, anti- parasitic and many more.

Mango (*Mangifera indica* L.) leaves are rich source of phenolic compounds such as mangiferin and quercetin. Mangiferin is a xanthone which is known as the most potent antioxidant. Some of the researcher refer them as “super antioxidants” due to its high antioxidant content which is higher than vitamin C and vitamin E. Mangiferin act as anti-inflammatory, antioxidant, antibacterial, antidiabetic, immunomodulatory and analgesic properties [1]. The antibacterial activity in this leaves extract is able to inhibit bacterial infections. Harumanis mango leaves have been reported to contain glucoside and mangiferin which is potent antimicrobial agent [2]. Other than that, some other bioactive compounds



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such as quercetin, gallic acid, gallotannins, and iriflophenones have also been identified in mango leaves [3][4]. These antibacterial properties can be used to develop new natural cosmetic product to replace synthetic chemicals, so it can help to minimize the health risk to consumers besides implement green processes that environmentally safe. Therefore, this study will evaluate the potential of Harumanis pruned leaves for its antimicrobial activities and the efficacy of the formulated soap with the Harumanis pruned leaves extract as antimicrobial soap.

2. MATERIALS

2.1. Chemicals

Standard mangiferin and Dragendroff's reagent were purchased from Sigma-Aldrich. Ferric chloride was purchased from Bendosen and sodium hydroxide was purchased from EMSURE. Sulphuric acid was purchased from QReC. Hydrochloric acid was purchased from Fisher Scientific. Glacial acetic acid, methanol and ethanol were purchased from HmBG.

2.2. Plant materials

Harumanis pruned leaves was collected from Institute of Sustainable Agrotechnology (INSAT), Universiti Malaysia Perlis, Perlis. These pruned leaves were washed properly and dried overnight using oven at 50°C. Then, the dried leaves were grinded into fine powder. The powder was kept in an air tight container and stored in freezer at -20°C until further use.

2.3. Bacteria strain

Bacteria used in this study i.e. *E. coli*, *B. subtilis*, *P. putida*, *B. megaterium* and *B. licheniformis* were obtained from School of Bioprocess Engineering, Universiti Malaysia Perlis, Perlis. These bacteria were stored at 4°C on nutrient agar and used for further microbial analyses.

3. PROCEDURES

3.1. Extraction of the Harumanis pruned extract

Hot and cold extraction was carried out by using distilled water, methanol and ethanol as solvent. For each of the extraction process, 10 g of Harumanis leaf powder was soaked in 1L solvent in a sterile conical flask. Cold maceration was carried out in a incubator shaker by shaken the raw materials inside the incubator shaker at 120 rpm, at room temperature for 4 hours. Hot extraction was carried out by soaking the sample in water bath at 50°C for 4 hours. The extracts were then filtered using Whatman no. 1 filter paper and concentrated using rotary vacuum evaporator for further analysis.

3.2. Phytochemical test

Phytochemical screening was carried out on Harumanis leaves extract using several methods for detecting the presence of alkaloids, tannins, saponins, glycosides and flavonoids [5]. *Detection of mangiferin using HPLC*

The stock solution of mangiferin standard substance was prepared in standard HPLC methanol at a concentration of 0.001 M. The stock solution was kept at 4°C in a refrigerator before use. An isocratic HPLC, C18, Phenomenex column (250 X 4.60mm) have been used in this test. The mobile phase used components methanol-aqueous 0.1% phosphoric acid (31:69, v/v). The analysis was conducted at a flow rate of 1.0 mL/min with ultraviolet (UV) detection at 254 nm [6][7]. The operation was conducted at room temperature. 20 µL of different Mango leaves extracts were injected into the HPLC column [8].

3.3. *Antibacterial test (Kirby-Bauer Disk diffusion assay)*

In this method, the lid of NA plate has been marked into five sections. Two sections for control which is amoxicillin as positive control while water as negative control and three section for three extract. The concentration of organisms was standardized corresponding to 0.5 McFarland standard. 20 μ L of the leaves extract for each extraction solvent have been impregnated into sterile paper disk. The paper disks were immersed in the designated area on the petri dish using sterile forceps. The disks were pressed down gently on the agar surface to ensure full contact with the surface of the agar plate. The plates were incubated at 35°C for 24 hours. Clear zone which is zone of inhibition was observed after incubate 24 hours.

3.4. *Formulation of antibacterial soap*

Three formulation of soap base were prepared before adding Harumanis leaves extract into the soap in order to get the best physical and chemical characteristic of soap. This formulation involves lye solution and three types of oil which are olive oil, palm oil and coconut oil. All the ingredients were mix together using different ratio of formulation based on the characteristic of the oil. Lye solution was mixed with 1:1:2, 1:2:1 and 2:1:1 of olive oil, palm oil and coconut oil. For final evaluation, only the best formulation of soap was chosen to be evaluated.

3.5. *Evaluation of physicochemical properties of the formulated soap*

3.6.1 *Determination of color.* Clarity and color was checked by naked eyes against white background.

3.6.2 *pH.* The pH of all the prepared soap formulations was determined by using Digital pH Meter. The formulations were dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of formulation was done in previously calibrated pH meter.

3.6.3 *Foam Height.* 0.5g sample of soap was taken, dispersed in 25 ml distilled water. Then, it has been transferred into 100 ml measuring cylinder. The volume was made up to 50 ml with water. 25 strokes were given and stand till aqueous volume measured up to 50 ml and measured the foam height, above the aqueous volume.

3.6.4 *Foam Retention.* 25 ml of the 1% soap solution was taken into a 100 ml graduated measuring cylinder. The cylinder was covered with hand and shaken 10 times. The volume of foam at for 5 minutes was recorded.

3.6.5 *High Temperature Stability.* The soap was allowed to stand at 50°C for one week. The stability of soap was observed during this period. The sample which does not melt and stable was indicated as stable and the sample in which the melts at 50°C, then it was said to be as unstable.

3.6.6 *Antimicrobial test of the formulated soap.* The prepared soap was subjected to antimicrobial screening by agar disc diffusion method. Organisms used were *E. coli*, *B. subtilis*, *B. megaterium*, *B. licheniformis* and *P. putida*. One gram of soap was mixed with 5 ml of sterile water and use disc diffusion method to evaluate the antimicrobial activities. The plates were incubated at 37°C for 24 hours and the zones of inhibition were recorded.

4. RESULTS AND DISCUSSION

4.1. *Screening of phytochemical properties of the Harumanis pruned extracts*

Table 1 tabulated the presence or absent of selected phytochemical properties in six Harumanis pruned extracts. Distilled water extract shows positive results for all the phytochemical tested, while ethanol

extract showed the absence of saponin. For Harumanis leaves extracts using methanol, it revealed that only cardiac glycosides, tannin and flavonoids are present but saponin and alkaloid show negative result. This result is supported by [9] which also investigate the phytochemical properties on *M. indica* leaves extract using methanol as solvent. The different existing of phytochemical components in each of different solvents used indicates that these solvents are effective to isolate different active biological compounds due to their chemical properties and polarity. However, different method of extraction does not affect the results of the test because the result for both methods is same.

Harumanis leaves extract was found rich source of phytochemicals as all solvents used for extraction are able to extract phytochemical component that potent to inhibit bacterial growth. Overall analysis on antibacterial activities showed that distilled water and ethanol are the best solvent to extract antibacterial components in Harumanis leaves extract which both were able to extract out tannin, flavonoids and alkaloid. While methanol was the least preferable solvent because it does not able to extract alkaloid.

Table 1. Phytochemical properties in Harumanis leaves extract

Method of extraction	Type of Solvent	Saponin	Glycoside	Tannin	Flavonoid	Alkaloid
Hot Maceration	Methanol	-	+	+	+	-
	Ethanol	-	+	+	+	+
	Distilled water	+	+	+	+	+
Cold Maceration	Methanol	-	+	+	+	-
	Ethanol	-	+	+	+	+
	Distilled water	+	+	+	+	+

Key: (+) Presence; (-) Absent

4.2. Detection of mangiferin from Harumanis Leaves Extract

Mangiferin was detected by high performance liquid chromatography (HPLC) with UV detection at 254 nm. A total of 20 μ L of standard working solution with concentration 0.001 M was injected into the HPLC system to determine the peak areas of mangiferin. All the obtained results had been summarized in Table 2. Due to limited mangiferin standard available, quantification of the mangiferin in each of the extracts cannot be done. However, based on the area under the peak, it can be concluded that water and methanol extracts contains high amount of mangiferin compared to ethanol extract.

Table 2. Mangiferin content in Harum Manis pruned leaves extract from HPLC.

Samples	Retention time (min)	Area under the peak	Height of the peak
Standard Mangiferin	3.851	25109029	119496
Methanol extract	3.871	66908289	1435183
Ethanol extract	3.612	29759388	603026
Water extract	3.871	71938784	1870415

4.3. Antibacterial test of Harumanis Leaves Extract

There are five bacteria tested for antibacterial test consist of three Gram-positive bacteria which are *B. subtilis*, *B. megaterium* and *B. licheniformis* and two Gram-negative bacteria which are *E. coli* and *P. putida*. Three different solvent extract of Harumanis pruned leaves extract demonstrated different zone of inhibition. Amoxycillin was used as positive control while water as negative control. From Table 3, it showed that hot maceration technique is better than cold maceration technique. It is because most of the inhibition zones for hot maceration are nearer value to the positive control than cold maceration. For bacterial inhibition, the results revealed that ethanol extract of Harumanis leaves using hot maceration showed no significantly different with amoxycillin against *B. subtilis* since the inhibition zone were quite close each other which is 15.11mm and 15.12 mm . It means that this extract falls in strong inhibitory activity range followed by methanol and ethanol extraction for cold maceration which shows slight significant different. While ethanol and methanol extracts showed moderate activity against strains of *E. coli*, *P. putida* and *B. megaterium* which slightly significantly different with amoxycillin. The value of inhibition zone for *B. licheniformis* shows ethanol is the highest followed by methanol and water. These results are supported by the study of [10] [11] in which he reported that ethanol, methanol and water extracts can be effective against some of tested bacteria.

Table 3. Diameter of inhibition zone at optimum condition at different extraction methods

Method	Type of solvent	Total inhibition (mm)				
		<i>B. subtilis</i>	<i>E. coli</i>	<i>P. Putida</i>	<i>B. megaterium</i>	<i>B. licheniformis</i>
Hot	Methanol	14.32±0.08 <i>b</i>	15.18±0.03 ^c	14.89±0.07 <i>bc</i>	12.18±0.08 ^b <i>c</i>	14.48±0.60 ^c
	Ethanol	15.11±0.03 <i>a</i>	16.14±0.05 <i>b</i>	16.01±0.38 <i>b</i>	12.31±0.19 ^b	15.97±0.57 ^b
	Water	6.92±0.07 <i>d</i>	10.15±0.13 <i>f</i>	9.87±0.15 ^d	9.66±0.05 ^c	9.67±0.10 ^f
	Amoxycillin	15.12±0.10 <i>a</i>	17.11±0.10 <i>a</i>	21.00±1.32 <i>a</i>	31.60±0.96 ^a	17.25±0.28 ^a
	Distilled water	na	na	na	na	na
Cold Maceration	Methanol	12.37±0.38 <i>c</i>	14.18±0.08 <i>d</i>	13.48±0.1 <i>3^c</i>	11.28±0.1 <i>0^b</i>	11.38±0.3 <i>4^e</i>
	Ethanol	12.34±0.30 <i>c</i>	15.11±0.10 <i>c</i>	14.77±0.2 <i>1^b</i>	11.93±0.3 <i>8^b</i>	12.67±0.7 <i>6^d</i>
	Water	7.18±0.07 <i>d</i>	13.63±0.32 <i>e</i>	9.58±0.08 <i>d</i>	9.77±0.25 ^c	9.53±0.11 <i>f</i>
	Amoxycillin	15.12±0.10 <i>a</i>	17.11±0.10 <i>a</i>	21.00±1.3 <i>2^a</i>	31.60±0.9 <i>6^a</i>	17.25±0.2 <i>8^a</i>
	Distilled water	na	na	na	na	na

Note: na -No antibacterial activity

Values are expressed as mean ± standard deviation (n=3). Means within a column followed by different small letters (a, b, c,d,e,f) are significantly different at the level of p<0.05. The differences between values are analyzed with General Linear Model ANOVA followed by Turkey's test. Standard disc diameter is 6.0

4.4. Physicochemical test of formulated soap

All the olive oil, palm oil and coconut oil were blended in different ratio. Based on Table 4, the best formulated soap is formulation 2 with 1:1:2 ratio of olive oil, palm oil and coconut oil. This is because the appearance was good which is has the average hardness. Combination of these three types of oil had stabilized the hardness of formulation 2 of this soap. While formulation 1 was very soft which contain more olive oil than other oils which were 2:1:1 and formulation 3 was very hard which contain more palm oil than other oils which were 1:2:1. For cold process soap, the formulations, the normal pH is 9 to 10 which is in alkaline condition. Although it is alkaline pH, it will not give side effect for normal skin since the usage is for wash off soap. All the soap shows the range of pH in between 9 to 10 including opaque soap. For the durability of the soap, formulation 3 shows the longest foam retention time which is 9.8 min followed by formulation 2, opaque and formulation 1. The foam retention is directly proportional to the foam height which also indicate the durability of the soap. The other test was on the stability of the soap against high temperature. This test shows that all the formulated soap can withstand high temperature than opaque soap.

Table 4: Physicochemical properties of soap formulations

Formulation	Color	Hardness	Foam retention (min)	pH	Foam height (cm)	High temperature stability at 50°C
1	White	Soft	6.0	9.49	10.4	Stable
2	Cream	Hard	8.5	9.58	12.3	Stable
3	Cream	Very Hard	9.8	9.67	14.5	Stable
Opaque	Yellow	Hard	8.0	9.20	12.3	Melts above 60°C

4.5. Antibacterial test of Harumanis Leaves Extract Soap

The concentration of Harumanis leaves extract varied by 0.20g/ml, 0.40g/ml, 0.60g/ml, 0.80g/ml and 1.00g/ml. of leaf extract. All the observation data for in vitro antimicrobial activity evaluation of Harumanis leaves extract soap is presented in Table 5. The zone of inhibition was directly proportional to the concentration of Harumanis leaves extract. Formulated soap with concentration 1.0 g/ml showed the strongest antibacterial activity towards all the bacterial tested while the formulated soap with concentration 0.2 g/ml showed the weakest antibacterial activity towards all the bacterial tested. However, overall analysis of inhibition zone showed that all the concentration applied can inhibit bacterial growth. Its means that Harumanis leaves extract soap formulation proved to be beneficial with excellent activity against all the tested microorganisms.

Table 5: Diameter of Zone of Inhibition (mm) on bacteria by various concentration of soap

Concentration of extract (g/ml)	Total Inhibition (mm)				
	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. Putida</i>	<i>B. megaterium</i>	<i>B. licheniformis</i>
0.2	1.05±0.05 ^c	1.12±0.02 ^b	1.10±0.08 ^c	0.82±0.04 ^d	1.08±0.68 ^b
0.4	1.15±0.01 ^b	1.25±0.03 ^b	1.15±0.30 ^b	0.96±0.11 ^c	1.29±0.01 ^b
0.6	1.26±0.04 ^b	1.34±0.07 ^b	1.31±0.07 ^b	1.12±1.63 ^b	1.48±0.04 ^a
0.8	1.42±0.04 ^a	1.50±0.05 ^a	1.46±0.11 ^a	1.32±0.53 ^a	1.52±0.04 ^a
1.0	1.50±0.05 ^a	1.61±0.05 ^a	1.52±0.11 ^a	1.42±0.53 ^a	1.54±0.05 ^a

Values are expressed as mean ± standard deviation (n=3). Means within a column followed by different small letters (a, b, c,d) are significantly different at the level of p<0.05. The differences between values are analyzed with One Way ANOVA followed by Turkey's test. Standard disc diameter is 6.0 mm.

5. CONCLUSION

Harumanis pruned extract contains various types of phytochemicals such as saponin, glycoside, tannin, alkaloid and flavonoids and acceptable amount of mangiferin. All these phytochemicals are proven to be potent as antimicrobial agent. Results shows that soap enriched with Harumanis pruned extract have the potential to inhibit bacterial growth namely *B. subtilis*, *B. megaterium*, *B. licheniformis*, *E. coli* and *P. putida*. The enriched soaps also show a very good appearance in term of hardness, pH, foam retention, foam height and stable at high temperature compared to opaque soap. The Harumanis pruned extract have a great potential to be utilize as antimicrobial agent and may be further exploit for commercialization.

REFERENCES

- [1] Mohan, C. G., Deepak, M., Viswanatha G.L., Savinay G., Hanumantharaju V., Rajendra C.E., & Praveen D. H. (2013). Anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica*. *Asian Pacific Journal of Tropical Medicine*, 311-314.
- [2] Hannan, A., Asghar, S., Naeem, T., Ullah, M. I., Ahmed, I., Aneela, S., & Hussain, S. (2013). Antibacterial effect of mango (*Mangifera indica* Linn.) leaf extract against antibiotic sensitive and multi-drug resistant *Salmonella typhi*. *Pak. J. Pharm. Sci.*, 4(26), 715-719.
- [3] Zhang, Y., Qian, Q., Ge, D., Li Y., Wang, X., & Chen, Q. (2011). Identification of benzophenone C- glucosides from mango tree leaves and their inhibitory effect on triglyceride accumulation in 3T3-L1 adipocytes. *J. Agric. Food Chem*, (59), 11526– 11533.
- [4] Fernández-Ponce , M. T., Casas, L., Mantell, C., & Martínez E. (2015). Use of high pressure techniques to produce *Mangifera indica* L. leaf extracts enriched in potent antioxidant phenolic compounds, Innovative. *Food Sci. Emerg. Technol.*, (29) , 94– 106.
- [5] Prashant. T., Bimlesh. K.,Mandeep. K., Gurpreet. K., Harleen. K. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Scientia*, (1), 98- 106.
- [6] Deng J. G., Feng X., Wang Q., Qin J.P., Ye Y., & Chen F. (2006). Comparison research on content of mangiferin between mango leave and mango branch, Guangxi. *Journal of Traditional Chinese Medicine*, (292), 53-55.
- [7] Nagaraj, G., Pradeep, K., Surender, P., & Mashra R. (2010). ICH guidance in practice: validated reversed-phase HPLC method for the determination of active mangiferin from extracts of *Mangifera indica* Linn. *Journal of Chromatographic Science*, (48), 156-160.
- [8] Javier, E., Ramirez, R. Z., Beatriz, S., & Mario, J. (2014). Simirgiotis. Antioxidant Properties and Hyphenated HPLC-PDA-MS Profiling of Chilean PicaMango Fruits (*Mangifera indica* L. Cv. piqueño). *J. Molecular.*, (19), 438-458.
- [9] Joon, K., Sowmia, C., Kp, D., & Mj, D. (2013). Preliminary Phytochemical Investigation of *Mangifera indica* leaves and screening of Antioxidant and Anticancer activity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 4(1), 1112–1118.
- [10] Islam, M. R., Mannan, M. A. , Kabir, M. H. B. , Islam, A. & Olival, K. J (2010). Analgesic, anti- inflammatory and antimicrobial effects of ethanol extracts of mango leaves. *J. Bangladesh Agril. Univ.*, 8(2), 239–244.
- [11] Vega-Vega, V., Silva-Espinoza, B. A., Cruz-Valenzuela, M. R., Bernal-Mercado, A. T., Gonzalez- Aguilar, G. A., & Ruiz-Cruz, S. (2013). Antimicrobial and antioxidant properties of byproduct extracts of mango fruit. *Journal of Applied Botany and Food Quality*, (86), 205-211.