

Maceration extraction of oil from tamanu (*Calophyllum inophyllum* L.) seeds: Effect of process parameters and oil characterisations

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ABSTRACT

This study investigated the extraction process of oil from dry seeds of tamanu plant. Various parameters were considered and pairwise examined with respect to final oil yield including moisture content and material size; solvent type and ratio of material/ solvent (w/v); extraction temperature and time. The highest oil extraction yield was obtained under following conditions: material size of 2mm, moisture content of 4%, acetone solvent with a ratio of materials/ solvent (w/v) of 1:30, extraction time of 4 hours, and extraction temperature of 50°C. The obtained oil extraction has density 0.932 g/mL. The acid value, the saponification value, the peroxide value, iodine value and the ester value of tamanu oil was 42.61 (mgKOH/g sample); 190.650 (mgKOH/g sample); 0.338 (meq/kg sample); 93 (gI₂/100g); 148.04 (mgKOH/g sample), respectively. The antimicrobial activity results showed that tamanu oil is resistant to bacteria strains *Staphylococcus aureus* at concentration of 0.65ppt and *Bacillus subtilis* at concentration > 1%. The antioxidant activity, expressed by IC₅₀ value, was 1894 ± 95.13(µg/mL).

Keywords: antimicrobial activity, *Calophyllum inophyllum* L., extraction, tamanu seed oil,

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I. INTRODUCTION

Tamanu (*Calophyllum inophyllum* L.) is a popular plant in Asian countries that is often cultivated for timber. In Vietnam, tamanu is widely cultivated as the shade tree in the Mekong Delta provinces and serves as a vulnerary ingredient in traditional medicine for skin treatment. One important tamanu product that is obtained by pressing is tamanu oil. The oil, in addition to its cultural value, also has several valuable biological properties that have been well documented. Tamanu oil contains terpenic, benzoic, oxygen-benzoic acid, small amounts of vitamin F and phosphor-aminolipids along with glycerides and saturated fatty acids [1]. In terms of fatty acid composition, oleic acid (49%); linoleic acid (21%); palmitic acid (15%); stearic acid (13%), cicosanoic acid (1.7%) and linolenic acid (0.3%) are the major fatty acids found in the oil. Another important ingredient in tamanu oil is complex 4-phenylcoumarin derivatives which are responsible for potent chemopreventive effects [2]. In addition, the tamanu oil also has the unique property of absorbing UV that has the wavelength range from 260 to 400 nm, enabling its application in ophthalmic preparations [3].

Tamanu oil is a common natural substance for cosmetic use. The application of tamanu oil in skin and acne treatment was justified by the study of Léguillier et al. where acne-related bacterial strains such as *Propionibacterium acnes* and *Propionibacterium granulosum* were found to be effectively inhibited by tamanu oil [4]. In addition, Chris (2004) also indicated the oil could also eradicate *Trichophyton schoenleinii* and *T. metagrophyte*, which are fungal strains commonly found in human skin and hairs [5].

Another important use for tamanu oil is for skin treatment and healing [6]. Among tamanu oil constituents, xanthones and coumarins show antioxidant activities and strong inhibition against lipid peroxidation, resulting in cell damage. The antioxidant activity of the oil also confers protective effects to human skin cells [6], promoting the resistance against oxidation and antioxidation mechanisms [7]. In terms of antibacterial activity, friedelin, canophyllo, canophyllic acid and inophynone found in the oil also exhibited antibacterial activities comparable to those of ampicillin and amoxicillin against *Proteus mirabilis*[5]. Other properties of the oil include antiworm [5] and inflammatory activities [8], where

the latter is mostly due to the presence of 4-phenyl coumarin calophyllolide and a group of xanthenes consisting of dehydrocycloguanandin, calophyllin-B, jacareubin, mesuaxanthone-A, mesuaxanthone-B [3].

Clearly, biological activities of the tamanu oil largely depend on its ingredients. However, it has been pointed out that compositional variations of tamanu oil may emerge due to differences in extraction method [9], weather and growing habitat [10, 11] in which the plant is cultivated. To date, most studies involving *Calophyllum inophyllum* materials utilized supercritical fluid extraction [9] or crushing [11] and did not thoroughly characterize tamanu oil obtained via maceration technique, which may hold certain economic advantages. In addition, data on seed oil extracted from tamanu seeds cultivated in Vietnam, a potential area for large scale production of tamanu products, have not been well documented. Therefore, the aim of this study is to further investigate the maceration process of tamanu oil and find out optimal parameters for maximal oil production. In addition, chemical composition and several pharmacological activities of the obtained oil were also examined.

II. MATERIALS AND METHODS

2.1. MATERIALS

Dried fruits of tamanu were harvested from Chau Thanh district, Ben Tre province, Vietnam and sundried for two days. Following that, the seeds were separated from the shell, dried at 40 °C to the appropriate moisture content and crushed into particles with the size of 1-3mm before being measured for fat, moisture and ash content. After the measurements, dried seeds were stored for further extraction and analysis.

2.2. MACERATION OF TAMANU SEEDS

Maceration extraction commenced using various solvents including hexane, acetone and mixture of hexane and acetone (3:2, v/v). To be specific, seed particles with an appropriate size were submerged in solvent in a 250 mL glass beaker. The mixture was heated in a water bath at specific temperature and for a duration depending on experimental specifications. The extract was then filtered using filter paper and washed using the solvent that was previously used for extraction. After removing residues, the obtained mixture consisting of tamanu oil and solvent was subjected to rotary evaporation to afford tamanu oil.

In order to determine effects of technical factors on oil content, a series of five investigations were successively performed. Obtained results will be used for subsequent investigations. Considered

parameters from the first to the fifth experiment included stirring, material size and moisture content, solvent type and solvent ratio, temperature and extraction time, and ultrasonication respectively. In the first experiment, the solvent mixture of acetone and hexane was used. In addition, the moisture content and size of the materials was 4% and 2mm respectively and the beaker was heated at 50 °C in 4 hours under either no stirring or stirring to find out the effect of stirring on oil yield.

2.3. PROXIMATE ANALYSIS

After selecting an appropriate solvent and obtaining optimal conditions, the oil product was determined for physicochemical characteristics, fatty acid composition via GC-MS, antibacterial activity and antioxidant activity.

Fat content was determined using ISO 734-1:2006. Physicochemical indicators of the obtained oil including acid value, saponification, peroxide and iodine were determined according the method of ISO 660:2009, ISO 3657:2013, Vietnamese Pharmacopoeia Annex 7.6, and ISO 3961:2013 respectively. Fatty acid composition was determined via GC-MS.

2.4. ANTIBACTERIAL ASSAY

Antibacterial activity was assayed using the agar diffusion method. The bacteria were first cultured in the growth media of Tryptic Soy Agar before being inoculated into assay agar plate. Turbidity in the growth plate was adjusted to around 1.5×10^8 CFU/mL which is equivalent to 0.5 McFarland. The agar plate for testing was prepared with Mueller – Hinton agar media with the thickness of 3-4mm.

Tamanu oil sample for testing was diluted in Dimethyl sulfoxide (DMSO) supplemented with 0.05% Tween 80 to reach the concentration of 40%. The control sample was prepared identically without the addition of tamanu oil. Following that, 30µL of the mixture was dropped on the paper disk (diameter = 6mm), which was then placed on the agar surface. Lastly, the plates were incubated at 35 – 37°C for 18 – 24 h.

2.5. ANTIOXIDANT ASSAY

Antioxidant activity was determined by DPPH method. First, the buffer solution was prepared by mixing 10mL DPPH with ethanol to create a solution with the absorbance of 1.1 ± 0.02 , measured at 517 nm. Then 0.5 mL of tamanu oil was added into 1.5 mL of buffer solution and allowed to stand in the dark for 30 min. The absorbance of the mixture was measured using a UV-Vis spectrometer. DPPH radical scavenging activity was calculated as in equation (1).

$$DPPH(\%) = \frac{A_{CT} - A_{SP}}{A_{CT}} \times 100 \quad (1)$$

Where A_{ct} and A_{sp} denotes optical absorbance of the sample with and without the tamanu oil respectively. The half maximal inhibitory concentration (IC_{50}) of the tamanu oil was the required concentration to reach the 50% reduction of the DPPH radical. IC_{50} (%) calculated by establishing the standard curve of tamanu concentration against DPPH.

2.6. STATISTICAL ANALYSIS

Results from triplicate experiments were inputted in Microsoft Excel. ANOVA & LSD analysis were carried out using Statgraphics Centurion XVI.

III. RESULTS AND DISCUSSIONS

3.1. PROXIMATE ANALYSIS OF DRIED TAMANU SEED

Proximate analysis showed the moisture and ash content of the dried seeds were 26.367 ± 2.58 % and 2.283 ± 0.08 %, respectively. The moisture of this level is a favorable environment for development of microorganisms. Therefore, for long-term preservation purposes, tamanu seeds

should be dried in a longer period to achieve a lower moisture content. The fat content was 65% and 78.3%, respectively based on wet weight and dry weight. This fat content is higher than the oil extraction efficiency previously reported (67.2%) by Crane et al. [12]. The difference may originate from harvesting seasons and the growing habitat of the plant.

3.2. EFFECT OF STRIRRING ON TAMANU OIL CONTENT

Fig.1 displayed results of the first experiment in which the oil content extracted under no stirring showed a minor reduction in comparison to that under stirring. However, the difference in oil contents resulted from the two conditions was statistically insignificant at the 95% confidence interval ($P=0.9452$). This could possibly due to the high fat content of the seeds, in turn leading to fast and facilitated diffusion during maceration. As a result, the agitated extraction was unable to improve the oil yield any further. Therefore, to simplify the maceration process, no stirring was selected as the condition for subsequent experiments.

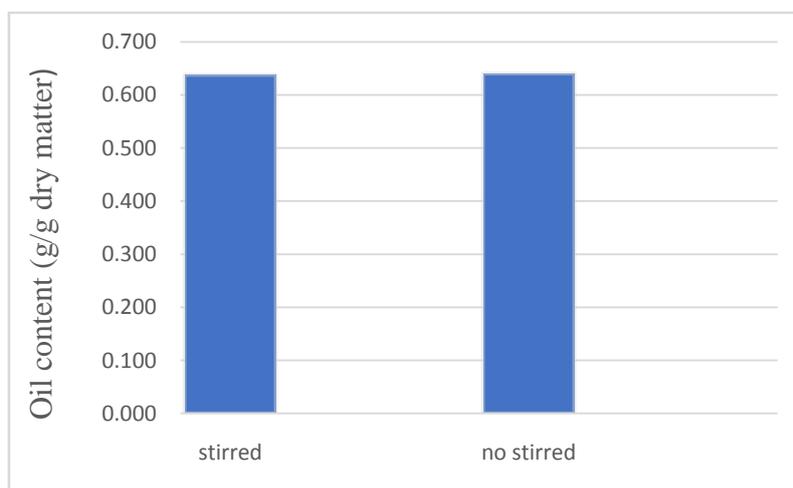


Fig. 1: Oil content extracted with stirring and without stirring

3.3. EFFECT OF MOISTURE CONTENT AND MATERIAL SIZE ON TAMANU OIL CONTENT

Oil content in relation to various moisture contents and material sizes was displayed in Fig. 2. Clearly, the peak oil recovery, at around 0.515 (g/g d.w.), was attained at the material size of 2mm and moisture content of 4%. This oil yield accounts for nearly 65% of total fat content of the materials (0.783 g/g d.w.) and is higher than the oil content achieved under the larger material size of 6mm (0.336 g/g d.w.).

Regarding the effect of moisture, ANOVA results indicated that the influence of moisture on oil content was statistically significant at 95% confidence interval and that the peak oil content is achieved at 4% moisture. Furthermore, multiple range analysis also confirmed these results by pointing out that oil yield difference achieved between moisture contents was significant that the oil yield obtained from the fresh materials was lower than materials of lower moisture. This inverse relationship could be explained by the eased penetration of solvent into the material, which is

possibly caused by low moisture and promotes diffusion and expulsion of oil out of the seeds.

Material size exhibits similar effects on oil content. To be specific, the material size of 2mm gave consistently higher oil content in comparison with 6mm at all moisture levels. In addition, the difference was also confirmed as statistically significant by multiple range test. As reduced material size could enlarge contact area between

materials and the solvent, solvent penetration could therefore be promoted, leading to expedited extraction of components. However, the use of very small particles may hamper the separation process after maceration, which, in many cases, results in higher operation cost and reduced yield due to oil loss. Therefore, the moisture of 4% and material size of 2mm were selected for subsequent experiments.

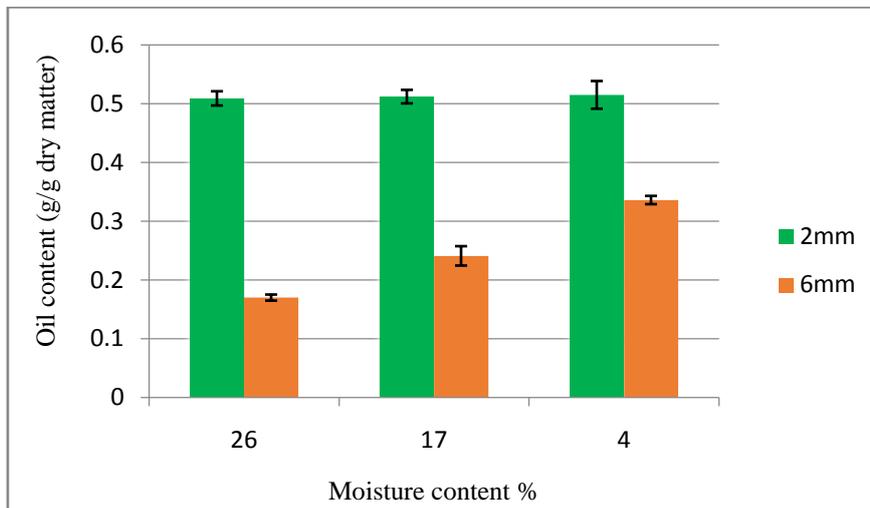


Fig. 2: Effect of moisture content and size of material on oil content

3.4. EFFECT OF SOLVENT AND MATERIAL: SOLVENT RATIO ON TAMANU OIL CONTENT

Fig. 3 showed results of oil content when performing maceration using different types of solvent and at various material:solvent ratios. When utilizing the acetone solvent with the material:solvent ratio of 1:30 (w/v), the oil content peaked at 0.644 (g/g d.w.). This result accounts for approximately 82.4% of the initial fat content of the fresh material and is higher than that obtained when using hexane and hexane:acetone solvent.

ANOVA results also confirms the influence of material:solvent ratio on the oil yield with 95% confidence. In addition, the interaction between the ratio and the solvent was also indicated in the statistical results. Multiple range analysis which compared influences exerted by three ratios also pointed out that the 1:30 (w/v) ratio showed

the largest LSD and that its differences with other LSD were statistically significant. Similar results were obtained when examining multiple range analysis results regarding effects of solvent type, confirming that acetone solvent gave the highest oil content.

The positive relationship between material:solvent ratio and oil content could be explained by the enhanced contact between solvent and oil sacs, promoting diffusion of oil into the solvent and causing greater concentration difference between solubles and solvent [13]. In addition, symmetric structure of acetone also causes the solvent to be less polar, permitting better dissolubility against compounds with low polarity existing in the oil. Therefore, the material:solvent ratio of 1:30 (w/v) would be selected for further investigations.

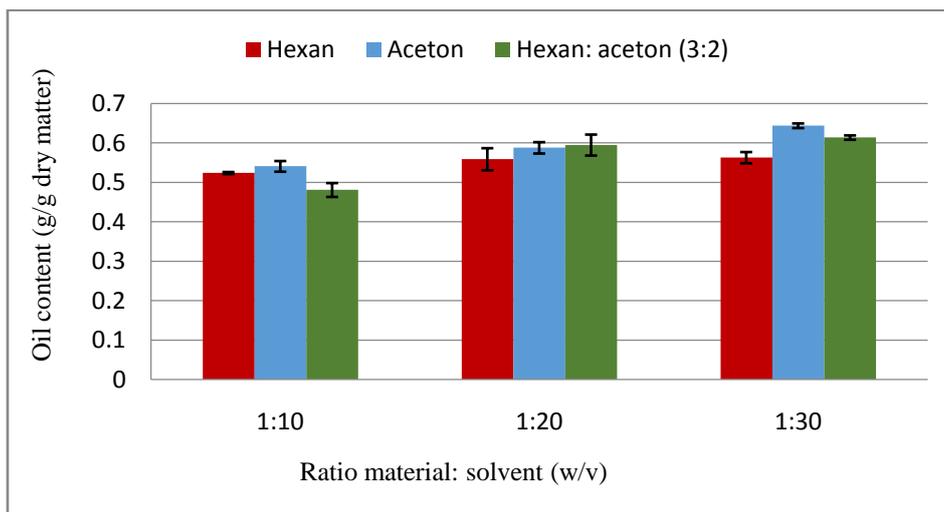


Fig. 3: Effect of solvent type and ratio material: solvent (w/v) on oil content

3.5. EFFECT OF TEMPERATURE AND EXTRACTION TIME ON TAMANU OIL CONTENT

Results experiments that were performed under different combinations of temperature and time conditions were illustrated in Fig. 4. Visually, the peak oil content (0.645 g/g d.w.) was observed at the experiment performed at 50°C and 4 hours of extraction time. This figure is 1.6 times higher than the lowest oil content which were obtained under the condition of 6 hours and 60 °C. In addition, prolonging the time from 4 hours to 6 hours under temperature of 40 °C seemed to reduce oil content. The optimal oil content is higher than result of a previous study [14] and accounts for 82.4% of the initial fat content.

Statistical analysis (ANOVA and multiple range test) also indicated that both factors are influential to the oil yield and have mutual interactions. In addition, multiple range test results indicated that the temperature of 50 °C and the extraction time of 4 hours both gave largest LSD and resulted in oil yield that are statistically different from other conditions.

At 4 hours of extraction time, by elevating the temperature from 40 to 50 °C, the oil content

was also accordingly increased. According to a previous study, thermal treatment in oil extraction confers manifold benefits including reducing solvent viscosity, improving diffusivity of compounds, and enhancing mass transfer of solvents into materials, which leads to better contact and thereby oil yields [15]. However, at the extraction temperature of 60 °C, oil content was reduced in comparison with those of lower temperatures. This is due to the insufficient amount of solvent presenting in the mixture occurring as a result of the evaporation of acetone solvent, which has the boiling point of 56-57 °C.

Extraction time also affects oil content following a similar trend. To be specific, the peak oil, which was caused by the equilibrium between oil concentration in and outside of the oil cells, was observed at the extraction time of 4 hours. Furthermore, extended maceration period that was longer than 4 hours induced oil yields that was relatively invariant, suggesting that 4 hours is the optimal time. As a result, optimal extraction time and temperature was selected as 4 hours and 50 °C respectively.

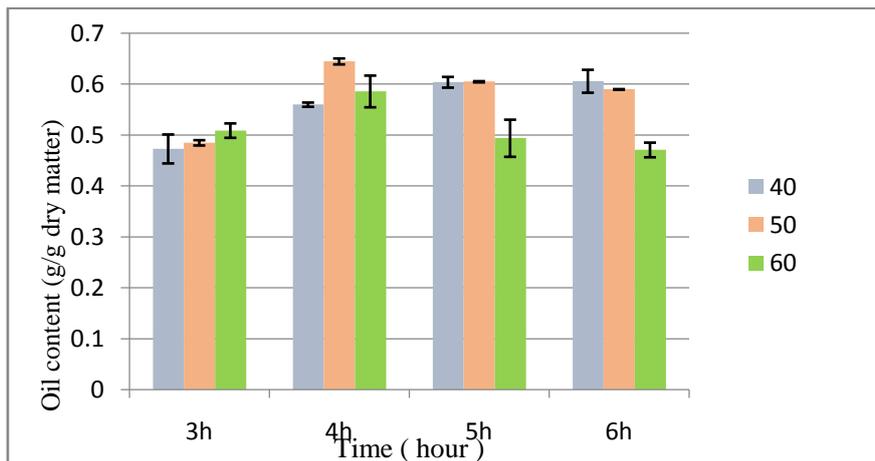


Fig. 4: Effect of temperature and time on oil extraction process

3.6. EFFECT OF ULTRASONICATION ON OIL CONTENT

We performed another comparison to find out whether ultrasonication could affect the oil maceration process. The results were displayed in Fig. 5. With ultrasonication, a marginal improvement in oil yield was found in comparison with results that were performed with no ultrasonication. However, the increase was not statistically

significant at 95% confidence, suggesting that ultrasonication exerted no clear impact on the maceration process. It was expected that ultrasonication may break oil cells and therefore facilitates the extraction of compounds. However, due to the high oil content in the seeds, extraction of oil was already maximal without ultrasonication. As a result, no ultrasonication was required in the extraction process.

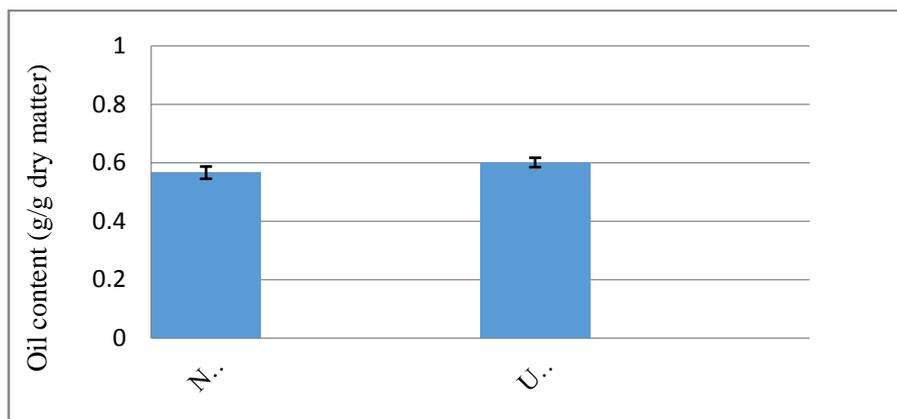


Fig. 5: Effect of ultrasonic condition

3.7. PHYSICOCHEMICAL CHARACTERISATION

The oil sample that was obtained under optimized extraction conditions were subjected to multiple

testing methods to determine several important oil physicochemical indicators. The results were shown as in Table 1.

Table 1: Physicochemical indicator of the obtained tamanu oil

Parameter	Method	Tamanu oil	Unit
Density	Vietnamese Pharmacopoeia	0.932 ± 0,007	g/ml
Acid value	ISO 660:2009	42.61 ± 0,04	mgKOH/g
Saponification value	ISO 3657:2013	190.65 ± 1,39	mgKOH/g
Peroxide value	Method Wijs	0.338 ± 0,04	meq/ kg
Iodine value	ISO 3961:2013	93.00	gI ₂ /100g

The obtained oil was abundant in free fatty acids, as indicated by the high acid value. This suggests that the tamanu oil would be susceptible to denaturation under room conditions. The high saponification value of 190.65 mgKOH/g is possibly due to the high content of short chain triglycerides. This, in conjunction with low molecular weight, implies that the tamanu oil is suitable for manufacture of soaps. The peroxide value of the oil was 0.338 meq/kg sample, which is indicative of unsaturation of fatty acids, or in other word, high rancidification of the oil. The unsaturation could promote oxidation of fatty acids in the oil which, when in contact with air, creates peroxides. Iodine value of 93.0 gI₂/100g suggests the high liquidity and susceptibility to oxidation of the oil. Therefore,

it is recommended to adopt an appropriate storage measure to prevent denaturation of tamanu oil.

3.8. CHEMICAL COMPOSITION OF TAMANU OIL

GC-MS results revealed that a total of 13 identified constituents, accounting for approximately 92.935% of the total content, were found in the obtained oil (Table 2). Meanwhile, unidentified compounds accounted for 7.065% total oil content. Among identified constituents, palmitic and arachic acid constitute 8.824% and 1.851% respectively. There were two major esters found in the oil including Glycidyl palmitate (8.465%) and Methyl 2-methoxy-1-(2-[(1E)-3-methoxy-3-oxo-1-propenyl]benzyl)-4-oxo-2,5-cyclohexadiene-1-carboxylate (1.088%).

Table 2: Chemical composition of tamanu oil

No.	Name	Content (%)
1	1,4-Diethylbenzene	1.568
2	Palmitic acid	8.245
3	6,1-Dimethyl-2,6,10-dodecatrien-1-ol	2.267
4	Glycidyl palmitate	8.465
5	Arachic acid	1.851
6	Glycidyl oleate	34.23
7	β-Monostearin	7.846
8	Methyl 8-(7-hexyl-3,7-dihydro-4a(4H)-naphthalenyl	1.061
9	Methyl 2-methoxy-1-(2-[(1E)-3-methoxy-3-oxo-1-propenyl] benzyl)-4-oxo-2,5-cyclohexadiene-1-carboxylate	1.088
10	Oleamide	2.094
11	Pregnan-12-one	7.258
12	7-Hydroxy-4-methylcoumarin, tert-butyldimethylsilyl ether	15.65
13	3a, 10a-Dihydroxy-5-(hydroxymethyl)-2,10-dimethyl-3a,4,6a,7,9,10,10a,10b-octahydrobenzo[e]azulen e-3,8-dione	2.880

3.9. ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF TAMANU OIL

Results from the disk-diffusion agar assay of the obtained oil against *Pseudomonas aeruginosa*,

Staphylococcus aureus and *Bacillus subtilis* were summarized as in **Table 3**.

Table 3. Diameters of inhibition zone against three bacterial strains

	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>
Tamanu oil	ND*	12	24
Control sample	ND	ND	ND

(*): ND= not detected

The antibacterial activity of the tamanu oil against *Bacillus subtilis* was two-fold higher than that against *Staphylococcus aureus*. On the other hand, the oil seemed to be unable to inhibit the growth of *Pseudomonas aeruginosa*. Further experimenting against three bacterial strains revealed that MIC against *Staphylococcus aureus*

and *Bacillus subtilis* was 0.65 ppt and >1% respectively.

Antioxidant activity of the oil was determined using DPPH method and the DPPH radical scavenging activity was summarized as in **Table 4**.

Table 4. DPPH radical scavenging activity of the tamanu oil

Concentration (µg/ml)	Rate (%)
12800	85,539 ^a ± 0,435
6400	76,090 ^b ± 6,847
3200	51,946 ^c ± 7,343
1600	48,411 ^d ± 0,545
800	34,421 ^e ± 2,126
400	21,586 ^f ± 5,112
200	11,702 ^g ± 4,500
100	3,518 ^h ± 2,451
50	2,301 ⁱ ± 2,484
0	0

Note: Figures having different letters are statistically different (p<0.05)

Results from the **Table 4** indicated that the scavenging capability of tamanu oil was increasing with oil concentration. The peak scavenging rate, at 85.539%, was achieved at 12800µg/ml concentration. The differences between all reported numbers were also statistically significant. Regarding IC₅₀ value, further calculation from antioxidant data resulted in IC₅₀ value of 1894 ± 95.13(µg/ml) and 5.514 ± 0.231(µg/ml), respectively for tamanu oil and vitamin C. This indicates the antioxidant activity of tamanu oil is considerably lower than that of vitamin C.

IV. CONCLUSION

This study has attempted to isolate the tamanu oil from seeds of *Calophyllum inophyllum* L. via maceration extraction. The extraction process was investigated with respect to different experimental parameters. In order to achieve maximal oil yield, maceration should be performed under following conditions: material size of 2mm, material moisture of 4%, solvent of acetone with the material:solvent ratio of 1:30 (w/v), extraction time of 4 hours and extraction temperature of 50 °C. Physicochemical indicators of the obtained oil suggest that the oil is suitable as additive in manufacture of soap. Furthermore, the tamanu oil exhibit antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*.

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