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Preparation, sensory evaluation and effectiveness of Philippine Tree Fern (*Cyathea contaminans*) as anti-spasm oil

Mustacisa-Lacaba, M. M. [1*], Dejarme, A. B.[2] and Albina, M. B.[3]

[1]Publications Office, Samar State University, Catbalogan City, Philippines; [2]College of Nursing and Health Sciences, Samar State University, Catbalogan City, Philippines; [3]Center for Engineering Science and Technology Innovation, Samar State University, Catbalogan City, Philippines.

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Abstract *Cyathea contaminans* is an endemic fern which is popularly used as an ornamental plant in the Philippines, its medicinal value is rarely publicized though its chemical components such as ecysdteroids, triterpenoids, and lycodine alkaloid are linked to cure contusions, strains, and swelling with antioxidant, antidepressant, and photo-protectant effects. These facts led to the development of a research-based product, *Cyathea contaminans* as anti-spasm oil. The developed *C. contaminans* anti-spasm oil in three experiment set-ups was voluntarily assessed by 15 respondents for each set-up. The highest frequency count for the speed of efficacy was at set-up A (87.5% *C. contaminans oil;* 6.25% lavandula oil; 5% VCO, and 1.25% surfactant), which means that among the three set-ups, the respondents claimed that prepared product in set-up A had the capacity to produce an effect of less than 5 minutes. The solution prepared in set-up C (90% *C. contaminans oil;* 5% lavandula oil; 4% VCO, and 1% surfactant) had the highest longevity of efficacy with a time span of 5 to 15 minutes. Based on the statistical results, the developed *C. contaminans* anti-spasm oil prepared from three experiment set-ups had no significant differences in color, aroma, and texture. The three products were statistically comparable to each other. However, set-up C which contained 90% by volume of an active ingredients *C. contaminans* is considered to be the most effective based on the results of sensory evaluation. Laboratory tests and screening should be sought prior to commercialization to clinically prove the claim of the product preparation. This study would greatly contribute to agriculture for the economic value of Philippine tree fern, and develop the emerging technology that would cater such progress.

Keywords: Anti-spasm oil, Efficacy, Sensory evaluation, Fern

Introduction

Pteridophytes include the ferns and its allies comprises 305 genera and about twelve thousand species all over the world. They are usually found in tropic countries and occur most in terrestrial habitats and also in some aquatic

[*] **Corresponding Author:** Mustacisa-Lacaba, M. M.; **Email:** marife.mustacisa@ssu.edu.ph

communities, however, they are thought by most people to be useless members of the plant kingdom (Mustacisa, 2016). The harmful effect of its rapid growth is well publicized but their useful aspects are largely ignored (Yatskievych, 2003; Parihar and Parihar, 2006; Srivastava, 2007).

One of the examples is the fern species *Cyathea contaminans*, a member of the tree ferns family well-known throughout the world for their bountiful huge foliage. It is the second largest living fern group among pteridophytes. Common uses of these are for ornamental, horticultural purposes, food and medicinal uses (Singh and Sahu, 2015). The fibrous root-encrusted trunks of tree ferns are a source of fern-fibre. The trunks with larger amounts of fibre are cut down, planted upside down in decorative gardens (mostly in the urban areas) or used as a substrate for certain types of epiphytic ferns and orchids. Often the fibre is cut off in slabs and used for a similar purpose. Crushed fibre is also used as a growing medium, pure or in mixtures with other material. The mass of adventitious roots at the base of the trunk of several species has a pot-like shape and is often used for potting orchids. In some countries a regular industry has been established around the supply of tree fern fibre to horticulturists (Hong, 2016).

In China, it is used for medicinal purpose in which rhizome hairs are considered styptic for coagulating blood, rheumatic problems, old man tonic, topically for wounds and ulcers. In India, it is utilized for wound healing, oft apical portion of caudex is cut into small pieces, crushed in a mortar and made into paste, then applied daily to major cuts and wounds until healed (Shil and Choudhury, 2009; Stuart, 2018). In the Philippines, specifically in Davao, Mindanao where it is known as *pakong buwaya*, its pounded leaves are rubbed in forehead for headaches (Hong, 2016 and Stuart, 2018).

Aside from that, *Cyathea contaminans* exhibit antibacterial and antioxidant agents. It inhibits presence of *E. coli*, *S. aureus* when exposed to methanol and hexane. It has strong antioxidant activity especially in mature fronds. These findings proved that the plant is potential as alternative source of therapeutic agents to encounter the adverse effects of synthetic drugs (Faizal *et al.*, 2020). Moreover, it has some active chemical constituents like alkaloids, glycosides, flavonoids, terpenoids, sterols, phenols, kaempferols, sesquiterpenes, etc., which showed biological activities and having viable potential industrial applications (Singh, 2003).

It also contains triterpenoids compounds, hydrocarbons with molecules belonging to steroidal group of ecydsteroids. Ecydsteroids are very interesting metabolite due to adaptogenic behaviour which offers a lot of potentials for their hepatoprotective, tonic, and anti-depressant effects. It also contains phenolic compounds of which various activities of these compounds are

attributed to mainly antioxidant and photo-protectant. In addition, the presence of lycodine alkaloid is found in the said fern species which is used to cure contusions, strains and swelling (Ho *et al.,* 2010).

The reviews of literature provided a potential use of *Cyathea contaminans* as medicine, readings have pointed out that it is topically applied for headaches, wounds, and rheumatic problems. This information is very useful in order to establish healing effect.

Presently, one of the problems among people such as elderly, pregnant women and those who are working in departments that requires long time of standing is the vulnerability of different kinds of pain including spasm in the different parts of the body due to overuse and muscle fatigue, dehydration, and electrolyte abnormalities. Spasm can affect many different types of muscles in the body, leading to many different symptoms (Newman, 2020).

The need to develop a product that addresses the problems is vital. There are many existing products in the market that claim to reduce pain however, they do not totally eliminate the problem leading to high consumption and tolerance on the use of the existing products. A variety of synthetic antispasmodic drugs have been authorized worldwide by the regulatory agencies, the most important being anticholinergic agents (butylscopolamine), direct smooth muscle relaxants (papaverine), calcium antagonists (pinaverium) or opioid receptor modulators (trimebutine). Despite their clinical efficacy, the use of these molecules is often limited by the development of unpleasant and sometimes severe side effects which may reduce patient compliance and impair treatment efficiency (Chaudhury *et al.,* 2017).

An effective antispasmodic agent should reduce the contraction of smooth and skeletal muscle which is responsible for cramping and discomfort in the different areas of the body. Historically, a long time before the golden age of medicinal chemistry, several aromatic plants were used in traditional medicine for the treatment of different ailments in some parts of the world (Heghes *et al.,* 2019).

Nowadays, antispasmodic botanical remedies are used by a constantly increasing number of patients for symptomatic treatment of functional dyspepsia, intestinal, colonic or ureteral spasms, gallbladder hyperactivity and uterine cramps. In the large category of medicinal plants, aromatic plants rich in essential oils are considered a valuable and easily accessible natural resource for the development of new molecules capable of becoming drug candidates. Essential oils are complex mixtures containing mainly aromatic terpenes classified in monoterpenes and sesquiterpenes according to the number of isoprene units but also phenylpropanoid compounds. The biological effects of essential oils have been extensively researched, as they can easily pass through

cellular membranes and influence a variety of molecular targets from ion channels to intracellular enzymes which is a good candidate for the formulation of antispasmodic oils (Bakkali *et al.*, 2008).

The study aimed to develop an antispasm oil using *Cyathea contaminans* as main component and test its effectiveness through sensory evaluation. The pursuit of this research would be of great benefit to the community to educate them with the formulation of the product which may be potential for commercialization and livelihood, and contribute to agricultural technology.

Materials and methods

Development of anti-spasm oil

The processes of development of antispasm oil out of *Cyathea contaminans* as the main active ingredient is presented in Figure 1. The prior art of the procedure is followed from Rasem *et al.* (2016).

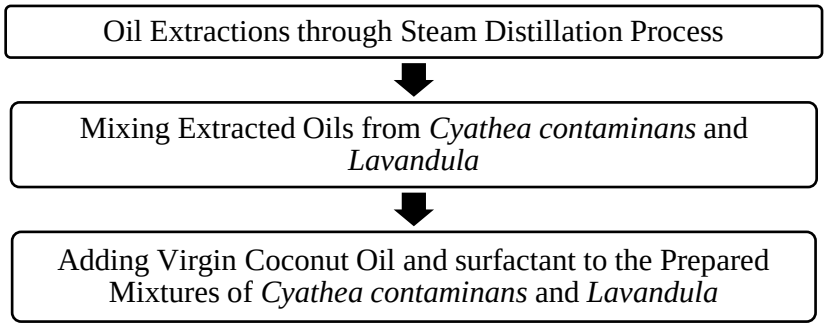

Figure 1. Process of development of anti-spasm oil

For oil extractions through steam distillation process, first, the plant material has been added to a large container called a *Still*, which is usually made of stainless steel. Second, over and done with an inlet, steam is injected through the plant material containing the desired oils, releasing the plant's aromatic molecules and turning them into vapor. Third, the vaporized plant compounds are moved to the condensation flask or the *Condenser*. Two separate pipes made it possible for hot water to exit and for cold water to enter the Condenser. This made the vapor cool back into liquid form. Then, the aromatic liquid by-product is dropped from the Condenser and collected inside a receptacle underneath it, which is called a *Separator*. Because water and oil do not mix, the essential oil floats on top of the water.

After the above explained process is the mixing of extracted oils from *C. contaminans* and *Lavandula*. In a small container, the *C. contaminans* oil and *Lavandula* oil (which serve as the aromatic component of prepared product) are mixed together using a spoon.

The last process of the product development is adding virgin Coconut Oil (VCO) and surfactant to the prepared mixtures of *Cyathea contaminans* and *Lavandula*. This phase is started by adding the VCO (which functions as stabilizer component of the prepared product) and blended to the oils. A spoon was used thoroughly to mix in the mineral oil to the oil mixture. Then, the blended oil is carefully poured into bottles or other containers and stored in a cool, dark place. The viscosity and density of the three oils varied from each other, a surfactant was also added.

Experiment set-ups

The experiment set-ups are presented in Table 1. It was done in three replications with varying percentage by volume of the chemical components of *C. contaminans* ant-spasm oil formulation. Set-up A had smaller percentage volume of the main chemical component *C. contaminans* while set-up B conatained greater amount of it but lower in terms of volume for set-up C. It is also observed in the table that the researchers controlled the volumes of other chemical components to prove the effectiveness of the main chemical component.

Table 1. The experiment set-ups used in the study

Components	Set-up A		Set-up B		Set-up C	
	Volume (ml)	**%**	**Volume (ml)**	**%**	**Volume (ml)**	**%**
C. contaminans oil	70	87.50	80	89.00	90	90.00
Lavadula oil	5	6.25	5	5.56	5	5.00
Virgin Coconut Oil (VCO)	4	5.00	4	4.44	4	4.00
Surfactant	1	1.25	1	1	1	1.00
TOTAL	80	100.00	90	100.00	100	100.00

Method for evaluation of effectiveness of the developed product as to speed and longevity of efficacy

For the evaluation of effectiveness of the developed product, it was sampled to three groups of respondents. One group per experimental set-up (A, B, C) to compare the effects of the prepared products with varying amounts of *C. contaminans.* In the identification of research participants, the researchers used convenient and quota sampling due to limited mobility amidst pandemic.

The respondents voluntarily evaluated the prepared product. Since it had a non-probability sampling technique, a total of 45 respondents were asked to rate the product, 15 respondents for each experimental set-up.

Researcher-made questionnaire was utilized with the aid of sensory Hedonic scale evaluation for the collection of data in color, aroma, and texture. The instrument collected data for the speed and longevity of efficacy. The participants checked the corresponding number of minutes when the product took effect upon application, and the corresponding number of hours spasm returned after efficacy.

The researcher-made questionnaire was validated by clinical experts for the face validity (referring to format and alignment of questions to the research objectives). It was validated by an English major and a research expert. Ethical clearance for the conduct of the study was secured, before the product testing, the researchers ensured that the participants were informed that the chemical contents of the formulated product are proven to be edible and nontoxic, thus it is safe to use.

This study developed an antispasm oil using *Cyathea contaminans* as main component and test its effectiveness through sensory evaluation. It specifically designs three experimental set-ups for the development of *C. contaminans* anti-spasm oil. It evaluated the developed products from the three-experimental set-ups as to color, aroma, and texture. Finally, it determined the effectiveness of the developed products from the three-experimental set-ups as to speed of efficacy and longevity of the efficacy.

Results

Sensory evaluation

The average results of *C. contamminans* anti-spasm oil in three-experiment set-ups is presented in Table 2. The product developed by the researchers were in three-experimental set-ups to determine which of these passed the sensory evaluation test of the potential users.

Based on the results of the sensory evaluation, all the set-ups are scored by the respondents between the range of 3.51 – 4.50. However, set-up C which composed of 90% *C. contaminans* oil, 5 % lavender oil, 4% coconut oil, and 1% surfactant had the highest average/overall likeness of the product pegged at 4.31, an indication that the users had Like Very Much the developed products in color, aroma, and texture prepared on the said set-up. The results proved that percentages of the three chemical contents did not matter when it come to the preparation of the product, then respondents tended to have more or less similar responses.

Table 2. Average results of *Cyathea contaminans* oil in three-experiment set-ups

Variable/Experimental Set-ups	Set-up A	Set-up B	Set-up C
Color	4.00 (LVM)	3.67 (LVM)	4.07 (LVM)
Aroma	4.33 (LVM)	3.93 (LVM)	4.47 (LVM)
Texture (*Likeness)*	4.27(LVM)	3.60 (LVM)	4.40 (LVM)
Average/Overall *(Likeness of the product)*	4.20 (LVM)	3.67 (LVM)	4.31 (LVM)

Legend (n=15 per set-ups): 4.51.51 – 5.00 Like Extremely (LE), 3.51 – 4.50 Like Very Much (LVM), 2.51 – 3.50 Moderately (M), 1.51 – 2.50 Like Slightly (LS), and 0.00 – 1.50 Neither Like or Dislike (NLD)

ANOVA single factor analyses for sensory evaluation of the three-experiment set-ups

The analysis of variance of single factor analysis in color, aroma, and texture is summarized in Table 3.

Table 3. ANOVA single factor results for sensory evaluation

Indicators	Total SS	Total df	F	p-value	F crit
Color	61.64	44	0.48	0.62	3.22
Aroma	44.78	44	0.57	0.64	3.22
Texture (*Likeness)*	43.64	44	3.03	0.06	3.22

*Level of significance at 0.05, two-tailed

For the color, the result was represented by F with a computed value of 0.48 and a p-value of 0. 62; in terms of aroma the computed F value was 0.57 and the computed p-value was 0.64, and for the texture the computed F value was 3.03 and p-value was 0.06. All the computed F values were much lower than the F critical value of 3.22, and all the computed p-values tended to be higher than the level of significance at 0.05, two-tailed. These results indicated that there were no significant differences on three prepared experimental set-ups in color, aroma, and texture. These three-experimental set-ups were comparable to each other and proved that any of the set-ups was preferably accepted.

Effectiveness of the developed product prepared in three-experiment set-ups

Speed of efficacy

The survey result for the effectiveness of the developed product in three-experimental set-ups as to speed of efficacy is provided in Table 4. The modal speed of efficacy for set-ups A and B was less than 5 minutes, and for set-up C, it pegged at 5 to 15 minutes.

Table 4. Speed of efficacy of the developed product in three-experiment set-ups

Speed of Efficacy/Experimental	**Set-up A**	**Set-up B**	**Set-up C**
Less than 5 minutes	**10**	**9**	5
5 minutes to 15 minutes	3	5	**9**
16 minutes to 30 minutes	2	-	-
31 minutes to 1 hour	-	1	-
More than an hour	-	-	1
Modal Speed of Efficacy *(n=15 per set-up)*	*Less than 5 minutes*	*Less than 5 minutes*	*5 minutes to 15 minutes*

Longevity of efficacy

The survey result for the effectiveness of the developed product in three-experimental set-ups to longevity of efficacy is depicted in Table 5. The modal longevity of efficacy for set-ups A and B was less than 5 minutes, and for set-up C was 5 to 15 minutes.

Table 5. Effectiveness of the developed product as to Longevity of efficacy

Speed of Efficacy/Experimental	**Set-up A**	**Set-up B**	**Set-up C**
Less than 5 minutes	**9**	**7**	4
5 minutes to 15 minutes	2	3	**7**
16 minutes to 30 minutes	3	4	2
31 minutes to 1 hour	-	1	1
More than an hour	1	-	1
Modal Speed of Efficacy *(n=15 per set-up)*	*Less than 5 minutes*	*Less than 5 minutes*	*5 minutes to 15 minutes*

Discussion

The highest frequency counts for the speed of efficacy was in set-up A, which means that among the three set-ups, the respondents claimed that the prepared product in set-up A (87.5% *C. contaminans* oil*;* 6.25% lavandula oil; 5% VCO, and 1.25% surfactant) had the capacity to produce an effect in less than 5 minutes, this definition of efficacy is anchored to the work of Lynch (2019). A solution with higher percent by volume of main chemical component provided the faster rate of reaction (Soult, 2020). Set-up A had lower speed of efficacy compared to set-up B, then it had lower main chemical component concentration. Although set-up C had higher percent by volume as compared to the two set-ups, there was a difference on the rating of the participants from

each set-up. Human perceptional evaluation would be considered as extraneous factor on this regard (Danziger *et al.*, 2011).

Corollary, the respondents claimed that solution prepared in Set-up C (90% *C. contaminans oil*; 5% lavandula oil; 4% VCO, and 1% surfactant) had the highest longevity of efficacy with a time span of 5 to 15 minutes. Longevity of efficacy was the maximum response that could be achieved in a medicinal drug (Mandal, 2019). Set-up C which had higher concentrations of the active ingredient in *C. contaminans*, Oakley (2016) and Wiechers (2005) confirmed that higher concentrations penetrated the skin as compared to the lower concentrations. With this result, set-up C proved to have a longest efficacy.

The developed *C. contaminans* anti-spasm oil prepared from three experimental set-ups had no significant differences in color, aroma, and texture. The three products were statistically compared to each other.

With the literature cited and the results of the study, it is suggested to use set-up with higher concentrations of the active ingredient to developed speed and longevity of efficacy. It was the preparation of anti-spasm oil with 90% *C. contaminans* content.

The development of the product in three-experiment set-ups was conducted amidst pandemic where limited mobility was considered and phytochemical screening was not pursued. Thus, it is a recommendation to resort to lab test and screen the suggested product preparation to clinically prove the speed and longevity of efficacy.

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