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Phytochemical Screening of *Leucosyke capitellata* POIR and *Dendrocnide densiflora* C. B. ROB Plant in preference of its utilization in the Municipality of San Miguel, Surigao del Sur, Philippines

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Abstract

The discovery of plant containing secondary metabolites or commonly known as phytochemicals gain interests nowadays because it offers benefit of treating diseases. Most of the indigenous people are advocating the use of plant extracts to treat diseases without knowing its properties but because they have done it for more than a year and for them it was effective. Hence, this paper aims to investigate two plants utilized in the Municipality of San Miguel, Surigao del Sur namely: *Leucosyke capitellata* POIR. and *Dendrocnide densiflora* C.B.ROB commonly known as alagasi and sagay respectively. Roots of each plants were collected and were subjected for ethanolic extraction. Three extracts, (1) *L. capitellata*, (2) *D. densiflora*, and combined formulation with 1:1 ratio was subjected for qualitative phytochemical tests to detect the presence of bioactive compounds. Findings of the study revealed that the following bioactive constituents are present in the three extracts namely: saponins, flavonoids, tannins, and steroids. The presence of this secondary metabolites may be the reason to support its therapeutic value to the local healers to treat inflamed wounds. Richer amount *L. capitellata* bioactive constituents supports the most preferred utilization among local healers. Combined formulations of these plants were found to contain rich amount of its constituents which may be the reason why most of the healers practiced mixing root samples in their traditional medication. Furthermore, there is a need to explore more of its therapeutic value since they possess secondary metabolites that offer more than the present therapeutic utilization.

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Introduction

Medicinal plants and corresponding indigenous preparations have been used for a wide range of purposes. Most local people relied on plants not just in its edible value but also in the healing properties of plants as an alternative remedy against illnesses. For many centuries, people have been trying to treat diseases as well as alleviate symptoms by using different plant extracts and formulations (Cowan, 1999). Such activities could be due to the presence of various complex chemical substances of different composition which occur as secondary metabolites (Balangcod *et al.,* 2012). Plant secondary metabolites provide specific physiological actions on the human body (Sofowora, 1982; Arora and Kaur, 1999; Ahmedulla and Nayar, 1999; Rios, 2005), making it extremely important for human health and well-being (Chiocchio, *et al*., 2021). Because of this, many researchers nowadays are gaining interests in investigating the properties of different medicinal plants and its uses in curing illnesses.

The use of herbal medicines and phytonutrients or nutraceuticals continues to expand rapidly across the world (WHO, 2004), including the Philippines. The Philippines is strategically located in the tropical area of the world, with approximately 13,000 plant species (Banag-Moran *et al.,* 2020), it is a home for abundant floral diversity. Hence, the opportunity for local community to utilized these plant materials for ethno-medicinal tradition is high, usually perceived as a rural practice and slowly traversing the urban areas (Catublas, 2016). With the promising potentials of plants as phytotherapy, and widely used due to its availability (Ekor, 2014), low-cost and effective due to the longevity of utilization (Awuchi, 2019), hence, there is a need for them to be tested and monitored (Ekor, 2014).

In the Province of Surigao del Sur, Philippines, the municipality of San Miguel that faces from the eastern part of the Diwata mountain range is considered as a first-class landlocked municipality in the province characterized by tropical climate conditions and diverse vegetation. This municipality is rich in ethnomedicine, many local people used herbal plants in healing and treating ailments (Montero and Geducos, 2021). Among those plants, are the *Leucosyke capitellata* and *Dendrocnide densiflora*, commonly known as alagasi and sagay plant, respectively. Per interview with the local practitioner, roots of these plants are traditionally utilized for remedy of severely inflamed or swollen part of the body. However, other practitioners combined roots of these plants as they are more effective than using only one plant component. The combined formulations of root plants with water decoction method is a common practice among local herbal users as they are found to be more effective than using one plant component alone. Thus, the present study aims to determine the secondary metabolites present in these plants and relate this to established medicinal properties, to help us understand its therapeutic nature as practiced by the local healers. Furthermore, this study might also unlock opportunities for discovery and development of low-cost plant-based drugs.

Materials and methods

Identification and Collection of Plant Samples

Fresh roots from mature *L. capitellata* and *D. densiflora* plants were collected from the Municipality of San Miguel, Surigao del Sur, Philippines. The collected plants were identified taxonomically with the helped of a botanist from Mindanao State University- Iligan Institute of Technology, Iligan City, Philippines.

Identification and Collection of Plant Samples

Root samples of *L. capitellata* (about 1.2 kilograms), *D. densiflora* (1.2 kilograms) and a mixture of the two plants (1.2 kilograms, composed of 0.6 kilograms in each plants) were thoroughly washed under a running tap water to remove unwanted materials, rinsed with distilled water, cut into smaller pieces and were air-dried for 2-3 weeks. The dried roots were powderized with the used of pulverizer/pellet maker machine. Fine, air dried roots were then soaked in a 95% ethanol and distilled water with 80:20 ratios for 72 hours. The resulting mixture was filtered, concentrated in-vacuo using a rotary evaporator to obtain hydro-ethanolic extract. The prepared extracts were used for several phytochemical tests.

Qualitative Analysis for Phytochemical Components

Phytochemical analysis of the three root extracts containing a) *L. capitellata*, b) *D. densiflora*, c) Combined formulation (*L. capitellata* + *D. densiflora* was done using standard qualitative methods of Edeoga *et al.* (2005) and Guevarra *et al. (*2005). The compounds analyzed for phytochemicals were alkaloids, saponins, flavonoids, steroids, tannins, anthraquinone, and cyanogenic glucosides.

Test for Alkaloids

The test of alkaloids follows the methods of Guevarra *et al. (*2005). About 10ml of each root extracts was evaporated to insipient dryness over a steam bath and were cooled at room temperature. The residue was added with 5ml of 2M HCl and were heated for 5 minutes and cooled. After which, a 0.5g of NaCl was added, stirred, filtered and the volume was adjusted to 5ml. A volume of 1ml was taken from the mixture and placed in a test tube. About 2-3 drops of Dragendroff's reagent were added and the color changes were observed. An orange color indicates the presence of alkaloids, however if the intensity of the color is heavier than the extract has a very rich alkaloid content.

Test for Saponins

It is the general characteristic of saponins in a plant to cause persistent foam when the aqueous solution is agitated5. In this manner, froth test was used to test the presence of saponins. A fraction of aqueous filtrate measuring 10ml was mixed with 5ml of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with about three drops of olive oil and shaken vigorously. The formation of a stable or about 2.0cm (or higher) of froth height that can stand for 30 min-1 hour confirmed the presence of saponins.

Flavonoids

For the confirmation of the presence of flavonoids in each extract, methods of Edeoga *et al. (*2005) was followed. About 5ml of diluted ammonia solution were added to an aqueous filtrate of each plant extract. Indication of yellow color shows the presence of flavonoid.

Steroids

For determination of steroid, methods from Guevarra *et al. (*2005) was followed. About 10ml of each extract are evaporated to insipient dryness over a steam bath and cooled to room temperature. Extract was then defatted repeatedly with hexane. The defatted aqueous layer was then warmed over a steam bath to remove the residual hexane. This was then added with 3ml of ferric chloride ($FeCl_3$) reagent and added slowly with 1ml of concentrated sulfuric acid. The formation of reddish-brown coloration signifies the positive result or presence of steroids in the sample.

Tannins

Determination of tannins from the sample was done following the methods of Edeoga *et al. (*2005). Residue was extracted with 20ml of hot distilled water. Presence of tannins was noted by addition of 0.1% ferric chloride ($FeCl_3$) solution resulting to blue, blue-black, green or blue-green color.

Anthraquinone

The presence of anthraquinones was done utilizing Borntrager's test (Fajardo, *et al.,* 2017). About 5 grams of each extract was added with 10mL benzene, filtered and added with ammonia solution. The formation of red or pink color indicates the presence of anthraquinone.

Cyanogenic Glycosides

Guignard's test was used to determine the presence Cyanogenic Glycosides in the plant extract, about 2-5 g of the plant sample was placed in the test tube. It was moistened with water and few drops of chloroform were added to enhance enzyme activity. The tube was covered with cork from which a piece of picrate paper was suspended ensuring that the paper strip must not touch the inner side of the test tube. The tube was warmed at 35-40^{o}C and kept at room temperature for 3 hours. Appearance of various shades of red within 15 minutes indicated positive result.

Results and discussion

Phytochemical analysis results obtained from the three extracts are presented in Table 1. It can be gleaned from the table that the plant extracts possess

only four (4) out of seven (7) secondary metabolites tested. These are the saponins, flavonoids, steroids, and tannins. No alkaloids, anthraquinone and cyanogenic glycosides were detected in all of the extracts. In terms of the degree or intensity of phytochemical contents, results revealed that *L. capitellata* root contains more of these phytochemical constituents than *D. densiflora* plant and when combined it contains rich amount of these secondary metabolites. This only signifies that these plants complement with each other, and this could might be the reason why most of the local community prefer to combine more than one root samples as treatment to a certain disease or illnesses.

Table 1. Phytochemical test results of root extracts of *Leucosyke capitellata* Poir. and *Dendrocnide densiflora* C.B. Rob and combined formulation of these two plants.

Secondary Metabolites	Composition of Plant Root Extract		
	L. capitellata	*D. densiflora*	Combined Formulation (*L. capitellata* + *D. densiflora*)
Alkaloids	-	-	-
Saponins	++	++	+
Flavonoids	+++	+	+++
Steroids	+++	+++	+++
Tannins	+++	++	+++
Anthraquinone	-	-	-
Cyanogenic Glycosides	-	-	-

Legend: (-) absence or not visible; (+) light amount; (++) moderate amount; (+++) rich amount

The present study is in consonance with the work of Lagunay and Uy (2015), which shows *L. capitellata* Poir. possess tannins, steroids, cardiac glycosides and flavonoids except saponins which were not detected in their work. The presence of flavonoids would entail anti-fungal properties (Trease and Evans 1983), and with saponins, it is potential for anti-inflammatory activities (Chawla *et al.*, 1987; Requena and Kenner, 1996). In addition, the presence of tannins has been found to have antibacterial and anti-inflammatory property (Kolodziej and Kiderlen, 2005). Steroids, on the other hand, also have therapeutic value as anti-inflammatory agents (Pajarillaga *et al.,* 2018). This might be the possible reason why *L. capitellata* are utilized by local healers to treat wounds with severe infections.

Respective to *D. densiflora*, results revealed that it contains rich amount of steroids, while saponins, flavonoids and tannins, are in light and moderate amount compared to *L. capitellata*. The present result, provides support on the current practices of the local healers in San Miguel, Surigao del Sur. For them, this plant is used to treat wounds that are basically inflamed. The presence of steroids, as potential anti-inflammatory agents (Pajarillaga *et al.,* 2018) may account to this therapeutic value of this plant. As of this writing, there is a limited literature recorded relative to the ethnomedicinal uses *D. densiflora*, in particular.

Respective to the preference of utilization between these two plants, personal interview with the local healers in the Municipality of San Miguel, Surigao del Sur, preferred *L. capitellata* in treating severely infected wounds. The richer amount of the present secondary metabolites in *L. capitellata*, as documented in this study, may support the local healer's therapeutic preference. Often, this *D. densiflora* are used to treat wounds only if *L. capitellata*, is unavailable in the area. The unavailability of *L. capitellata* can be due to its rareness in the lowland areas and the risk of extinction in mountainous areas due to the observed indiscriminate collection, overexploitation and habitat destruction that poses threat (Chen *et al.*, 2016) to this medicinal plant.

On the other hand, although *L. capitellata* is the most preferred remedy to treat infected wounds, *D. densiflora*, are often used by the local healers, if the wounds are severely inflamed due to infections. For them, a combined formulation of *L. capitellata* and *D. densiflora,* are more effective because they find that both plants complement with each other, where *L. capitellata* acts against wound infections while *D. densiflora* acts on reducing inflammations. Such preference of utilization can be supported by the presence of secondary metabolites in richer amounts (Table 1). The bioactivity of natural extracts can be represented by synergism between several compounds (Gorlenko *et al.*, 2020). The possibility of these secondary metabolites as antibacterial or

inflammatory agents against important human pathogens could act independently or enhance its action. Therefore, the key, and often the most challenging aspect of research, is not only to quantitatively measure the bioactivity of any plant extract, but also to connect a particular chemical structure(s) with a particular clinical effect (Li *et al*., 2020).

Although, the primary therapeutic nature of the plant as used by the local healers in San Miguel, Surigao del Sur are being supported by this study, examining the other phytochemical present in the plants revealed other therapeutic potentials can be furthered explored. The presence of tannins and flavonoids for example, has important roles as potent anti-oxidants (Trease and Evans, 1983; Chawla *et al*., 1987; Stauth, 2007; Zain *et al*., 2011). In addition, the presence of tannins, terpenoids, cardiac glycosides and flavonoids are secondary metabolites that have been reported to have anticancer as well as antioxidant properties (Zain *et al*., 2011). Accordingly, quantitative analyses of these secondary metabolites may be done to guide the researchers on which particular bioactive class of compounds may be subjected to subsequent target isolation.

Conclusion

The present study showed that *Leucosyke capitellata* Poir. and *Dendrocnide densiflora* C.B. Rob) plants be it prepared alone or in combined formulation, contains secondary metabolites namely saponins, flavonoids, steroids, and tannins. The richer amount of secondary metabolites *L. capitellata* supports the local healer's preference of utilization than *D. densiflora* in treating infected wounds. Moreso, their preference of combining these plants to treat severely inflamed and infected wounds due to infections may be supported by the rich amount of secondary metabolites namely steroids, flavonoids and tannins.

Recommendation

Though, the primary therapeutic value of the plant in preference to the utilization of the local healers in San Miguel, Surigao del Sur is being supported by this study. But still there is a need to explore more, by quantifying and isolating these secondary metabolites and testing them to further support the claim of the local healers. Furthermore, molecular confirmation of species using integrative molecular approach will also help in understanding the true identity of medicinal plants in the treatment of health-related problems.

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