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Original Article

Phytochemical profiling of methanolic extract and petroleum ether soluble fraction of Nami (*Dioscorea hispida* Dennst.) leaves

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Abstract: Medicinal plants are essential in drug development since they serve as raw materials in the pharmaceutical industry. One such plant, Nami (*Dioscorea hispida* Dennst.), which belongs to the family Dioscoreaceae, is well known for its medicinal properties. This study aimed to identify the bioactive metabolites present in the leaves of *D. hispida* The phytochemical screening of methanolic extract and petroleum ether soluble fraction extract of *D. hispida* leaves revealed the presence of tannins, flavonoids, saponins, alkaloids, glycosides, terpenoids, and steroids, all of which possess medicinal properties. Hence, these bioactive metabolites may be employed to develop innovative drugs for several diseases.

Keywords: *Dioscorea hispida*, Phytochemicals, Nami plant, bioactive metabolites

1. INTRODUCTION

Medicinal plants have long been used in various cultures as folklore medicines since ancient times. At least 80% of the population of developing countries use the said plants as a cure. Similarly, around 40% of pharmaceutical products, including landmark products such as aspirin and childhood cancer treatment, have been developed using the said plants (WHO, 2002; WHO, 2024). Studies have shown that their medicinal value may be attributed to the presence of secondary metabolites they contain (Gopalakrishnan K, Udayakumar R, 2014).

One reason why medicinal plants have curative properties is the presence of secondary metabolites, which are classified in three classes. These are phenolics (e.g. tannins and flavonoids), terpenes, and nitrogen-containing compounds, such as alkaloids. These metabolites are known to have properties such as antioxidant, antiparasitic, antimicrobial, anti-inflammatory, and anticancer, among others. Tannins are employed to treat diseases like diarrhea and rhinorrhea among others (Clinton, 2009). Flavonoids studies reported its use as free radical scavengers, antioxidant, anti-inflammatory, and anti-microbial (Saxena et al., 2013; Hartati et al. 2014). Sterols are suggested to bring down cholesterol levels and its

effect is also suggested to overlap with their anti-cancer actions (Grattan, 2013). Alkaloids may also have anthelmintic properties (Pinder, 1957). Indeed, these metabolites have significantly enhanced health systems over the years.

Recent studies on these secondary metabolites have shown that whether in their individual, additive, or synergic state, they may become a significant source of potent drugs not only for the treatment of many diseases but also a potential solution to multidrug resistance (MDR), which is an emerging concern (Gopalakrishnan & Udayakumar, 2014; WHO, 2024). MDR is no longer an imaginary problem since it is already widespread in many livestock production systems (Cabardo & Portugaliza, 2017). Previous studies have already documented instances of MDR when human anthelmintics are applied against soil-transmitted-helminths (Albonico et al., 2003; Albonico et al. 2008; Cabardo & Portugaliza, 2017). Although there is no conclusive data on MDR among humans yet, similar phenomena might occur in the future in the absence of measures to address the declining efficacy of drugs, which may happen approximately within 20 years (Vercruysse et al., 2008; Mehlhorn et al., 2011). Thus, one possible approach that may be used in combatting MDR is the utilization of plants in the development of new drugs (Hammond et al., 1997; Rates, 2001; Sasidharan et al., 2011; Yadav & Singh, 2011).

The plant that can potentially address the need for new anthelmintic medicines is *D. hispida*, which is locally known in the Philippines as Nami. It is a yam indigenous to tropical and subtropical rainforests in Southeast Asia. It is characterized as a robust, herbaceous, and perennial vine that can grow up to several meters. It has trifoliate leaves, with leaflets and small prickles on the underside of its midrib. Flower inflorescence can be either branched (male) or unbranched (female). The fruit is woody, oblong to an ellipsoid capsule, honey-colored, and has three wings. The stem is prickly glabrous, with fine white to brown hairs, and is 6 to 10 cm in diameter. Nami can weigh as much as 15 kg (Stuart, 2013; Lim, 2016). *D. hispida* is used in traditional medicine in Southeast Asian countries such as Thailand, Malaysia, and the Philippines (Neamsuvan et al., 2012; Lim, 2016). In the Philippines, the yam is traditionally used in the treatment of wounds, gonorrhea, dysentery, rheumatism and worm infestation. It is also utilized as an anodyne for tumors and buboes (Kapale & Kumar, 2011; Syazili et al., 2012; Stuart, 2013). Its pounded leaves are used to treat stomach pains, asthma, hernia, and sores (Hanum & Hamzah, 1999). In Malaysia, a decoction of its leaves is used to treat sore feet, along with tubers, which are sometimes mixed with lime or turmeric and benzoin (Burkill, 1966). In Thailand, the decoction of mature leaves is used to treat parasitic diseases (Neamsuvan, 2012). In Bangladesh, its leaves and yams have been utilized in treating whitlow sores, boils, and animal bites. They are also used for female birth control and as anthelmintic drugs. Likewise, they are used to treat myiasis of the scrotum among carabaos (Yusuf et al., 2009; Kapale & Kumar, 2011; Kumar et al., 2012). Apart from medicinal uses, *D. hispida* functions as a deworming medicine and fish poison as in the case of Malaysia (Nashriyah et al., 2012).

The documented uses of Nami plant in traditional medicine prompted the researchers to subject it to phytochemical screening to identify the specific compounds it contains as this first step is essential in drug discovery and development (Gopalakrishnan & Udayakumar, 2014; Starlin et al., 2019). Each plant part contains different bioactive compounds. Hence,

each needs to undergo phytochemical screening to assess the presence of bioactive compounds, which can be used as ingredients in drug development. The recovery of these compounds in plants is not uniform, which implies that each plant or plant part may have a different degree of sensitivity. Thus, appropriate solvents and tests must be applied to accomplish phytochemical screening (Lima et al., 2015). In this study, phytochemical analysis using the solvents methanolic and petroleum ether soluble fraction was conducted to determine the bioactive metabolites present in the *D. hispida* leaf extracts.

2. METHODOLOGY

Plant collection and identification

Fresh leaves of *D. hispida* were collected in Legazpi, Albay, Philippines (coordinates: 13°08′N 123°44′E), from 7:00 a.m. to 8:00 a.m. Sample leaves were identified and authenticated by the curator of the Institute of Biology, Jose Vera Santos Memorial Herbarium (PUH), College of Science, University of the Philippines (UP), Diliman, Quezon City, Philippines.

Preparation of plant material

Leaves of *D. hispida* were washed with distilled water and then dried in a thermostatic oven at 40°C for two days. They were then pulverized into powder using a grinding machine (Dewi et al*.*, 2019). After which, 325 g of powdered material was macerated in 900 mL of 100% methanol for 15 days at room temperature (~28 to 35 ^{0}C). The mixture was filtered using a fresh cotton plug followed by a Whatman No. 1 filter. The filtrate was then concentrated using a rotary evaporator (Heidolph WB, 2000) with a 90/min rotation with temperature 50 ^{0}C for approximately four to five hours. The product obtained from the process was labeled "*D. hispida* methanolic leaf extract" (MLE). Solvent partitioning of methanolic extract was performed using the modified Kupchan method, wherein an aliquot of 5 g concentrated methanolic product was extracted with 300 mL petroleum ether. The product obtained was labeled "petroleum ether leaf extract" (PELE). Both extracts were then lyophilized using the Tabletop Freeze Dyer DC41A/B (VanWagenen et al*.,* 1993; Miah et al*.,* 2018).

Phytochemical screening

Phytochemical screening of the leaf extracts of *D. hispida* (methanolic and petroleum ether soluble fractions) was carried out by the Department of Science and Technology's (DOST) Organic Chemistry Section, which is located in Taguig, Philippines. The sample extracts were tested for the presence of tannins (Ferric chloride test), alkaloids (Mayer's test), flavonoids (Shinoda test), glycosides (Fehling's test), sterols (Lieberman-Burchard test), triterpenes (Lieberman-Burchard test), and saponins (Froth test) (Evans, 2009). The details of this test are found in the official report issued by DOST (see appendix DOST, 2022).

3. RESULTS AND DISCUSSION

Table 1. Phytochemical constituents of methanolic and petroleum ether soluble fraction of *D. hispida* leaf:

Compounds	Phytochemical Test	Methanolic Leaf Extract	Petroleum Ether Soluble Fraction Leaf Extracts
Tannins	Ferric chloride test	+	+
Saponins	Froth test	+	+
Triterpenes	Lieberman-Burchard test	+	-
Alkaloids	Mayer's test	+	+
Flavonoids	Shinoda test	+	+
Sterols	Lieberman-Burchard test	+	+
Glycosides	Fehling's test	+	+

(+) presence of the compound
(-) absence of the compound

The phytochemical screening of the methanolic leaf extracts of *D. hispida* revealed the presence of tannins, flavonoids, saponins, alkaloids, glycosides, terpenoids, and steroids. The phytochemical screening of the petroleum ether soluble fraction leaf extracts revealed the presence of tannins, flavonoids, saponins, alkaloids, glycosides, and steroids with the exception of triterpenes (see Table 1).

The preliminary phytochemical screening investigation of plants is very important because it will determine if the plant has a pharmacological potential as indicated by secondary metabolites, which are essential in drug discovery and development. In addition, these tests may be helpful in determining the plants' qualitative and quantitative estimation of their secondary metabolites (Palanisamy et al., 2012).

Plant extracts contain various secondary metabolites that may have therapeutic properties. The present study revealed the presence of tannins, flavonoids, saponins, alkaloids, glycosides, terpenoids, and steroids, while petroleum ether soluble fraction leaf extracts showed the presence of tannins, flavonoids, saponins, alkaloids, glycosides, and steroids with the exception of triterpenes. The results concur with the previous study of Suresh et al. (2011) where the phytochemical screening of the *D. hispida* methanolic leaf extracts revealed the presence of secondary metabolites such as tannins, saponins, glycosides, and alkaloids. These results demonstrate the presence of various secondary metabolites which are important in phytomedicine. Most of the essential bioactive metabolites, also known for their medicinal value, have also been detected in just one plant, thereby potentially optimizing the drug production process (Gopalakrishnan & Udayakumar, 2014; Starlin et al., 2019). For instance, phenolic tannins have been reported to be responsible for inducing a stress response in a manner different from known anthelmintics and decreasing glucose uptake, growth rate, and protein digestibility, all of which may cause weight gain (Chung et al., 1998; Kumarashingha et al., 2014). The phenolic flavonoids of the extract can also potentiate tannin and affect calcium pump and ATPase, which may cause the death of parasites (Hrckova & Velebny, 2010;

Klongsiriwet et al., 2015). Flavonoids have also been shown to have an anti-oxidative effect through their ability to scavenge most oxidizing molecules, such as singlet oxygen, which are implicated in several diseases, such as coronary heart diseases, inflammation, and cancer (Saeed et al., 2012). Saponins and tannins, with their potential to activate the mucous membrane's protective factors and reduce chemical irritation, hold significant promise for gastrointestinal health. They also influence mitochondrial action, which can combat pathogens like parasitic worms (Melzig et al., 2001; Santos et al., 2018). This combined action of saponins and tannins reduces inflammation and provides a protective shield for the stomach mucosa, potentially alleviating excessive acidity. Other bioactive metabolites, such as terpenoid and alkaloid compounds, have demonstrated their efficacy in treating gastric ulcers (Sreeja et al., 2018). Moreover, a study on terpenes has unveiled their promising potential in controlling anthelmintic resistance (AR), particularly in the case of albendazole-resistant worms (Mirza et al., 2020). The exact mechanisms, however, that make particular terpenes effective against AR are still unknown. A study by Ndjonka et al. (2014) has documented how phenolic acids from axle wood tree (*Anogeissus leiocarpus*to) effectively inhibit *Caenorhabditis elegans* and the AR filarial *Onchocerca ochengi*. Similarly, a study utilizing the aqueous extracts of guava (*Psidium guajava)* and African marigold (*Tagetes erecta)* also recorded vigorous activity against the AR *C. elegans* CB193 (Piña-Vázquez et al., 2017). This research indicated the effectiveness of the bioactive metabolites from plants against the AR strains.

In addition, the present study also highlighted the importance of noting the sensitivity of using the appropriate solvent in the extraction of plants' metabolites. It is crucial to understand that the recovery of bioactive compounds varies in sensitivity, which necessitates the use of specific solvents and tests (Lima et al., 2015). This study demonstrated that both solvents used in extraction of leaves are effective in terms of the number of bioactive metabolites it revealed (except triterpenes). Theoretically, nonpolar solvents such as methanol and petroleum ether can easily degrade the nonpolar cell walls of the plants, thereby releasing more varieties and a large number of secondary metabolites (Brunet & Hoste, 2006; Wang et al., 2010; D'addabbo et al., 2011; Novobilský et al., 2013; Spiegler et al., 2017). Although triterpenes were absent in the petroleum ether soluble fraction leaf extracts (Table 1), other kinds of triterpenes that might be present in the extract may be more potent compared to the kind of triterpene present in the methanolic extract. As mentioned, the recovery of different bioactive metabolites such as triterpene acids from plant tissues requires specific kinds of solvents and tests (Lima et al., 2015). These findings underscore the practical relevance of this research in the field of drug development.

4. CONCLUSIONS AND RECOMMENDATIONS

In the present study, *D. hispida* methanolic and petroleum ether soluble fraction leaf extracts demonstrated the presence of various bioactive metabolites that have pharmacological properties as antioxidant and anthelmintic, among others. Hence, the therapeutic effects of the plant are attributed to the presence of bioactive metabolites they contain. These findings underscore the potential of *D. hispida* as a source for novel drugs, thus warranting further investigation.

While the *D. hispida* leaf extracts have yielded promising results, and a variety of essential bioactive metabolites were detected, it is crucial to conduct a comprehensive

study to uncover the biochemical characteristics of the plant extracts, thereby elucidating the precise mechanisms that could transform the plant into a potential source of groundbreaking drugs for specific diseases. Additionally, a detailed analysis of the quantity or concentrations of bioactive metabolites in the extract could provide further clarity on the potential mechanism of action of the plant extracts. Furthermore, future studies for the efficient extraction of triterpenes that focus on the polarity and solubility should be explored. Understanding the mechanism and interaction between solvents and structures of triterpenes could optimize the yield and purity of the substance, thereby, enhancing its scalability and application in various industries.

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Competing interest

The authors declare no competing interest.

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Appendix

Excerpt from the DOST Test Report

9.2 Test Procedure:

9.2.1 Test for Saponins:

Froth Test - Dissolve the alcoholic extract in hot water, and then filter. The aqueous filtrate extract when shaken vigorously should become frothy; honeycomb in nature should persist for at least 30 minutes.

9.2.2 Test for Tannins:

Ferric Chloride Test - Dissolve the dried extract in hot water, filter .Add 1-2 drops of Ferric Chloride T.S. Production of dark coloration that may either be black, dark blue, blue-black, indicate the presence of tannins

9.2.3 Test for Glycosides:

Fehling's Test - Dissolve the alcoholic extract in hot water, and then filter. The filtrate is used for the test. Get 2 test tubes. Place 2 mL of sample in each test tube. To the tube 1, add 1.0 ml dilute HCl; to the tube 2, add nothing (control tube). Place the two test tubes in a boiling waterbath for 5 minutes, then allow them to cool. The samples are neutralized with anhydrous sodium carbonate until no effervescence is produced. Then add 1.0 mL Fehling's solution (mix together 3 mL each of Fehling's A & B). Heat the sample tubes in a water bath for 2 minutes.
Observe the amount of brick red precipitate formed. An increase in the amount of brick red precipitate in the hydrolyzed sample (the sample to which dilute acid was added) as compared to the control tube indicates the presence of glycosides.

9.2.4 Test for Flavonoids:

Mg+ Turning Test - one mL or a small amount of the dried alcoholic extract is treated with 1.0 mL 10% HCl and magnesium turnings.
A red coloration is observed for positive result:

9.2.5 Test for Alkaloids:
Mayer's Test - The dried alcoholic extract is extracted with 1% HCl. Filter. To the filtrate add 2 drops of the Mayer's reagent.
A cream colored precipitate is observed.

Wagner's Test - To the small amount of dried extract dissolve it with 1.0 mL of dilute acetic
acid.
A white of cream colored precipitate is formed.

Note : For false positive reactions: To remove impurities capable of giving false positive reactions (i.e. proteins) from an initial aqueous acidic extract-salt out these materials by adding powdered NaCl

9.2.6 Test for Sterols and Triterpenes:
Liebermann-Burchard Test - Dissolve a small amount of dried extract in acetic anhydride. Decant the soluble portion. To this add 1-2 drops of concentrated sulfuric acid.
A green color, either immediately or slowly going to red or blue tones, will form. A pink to red color is indicative of triterpenoids, while a blue color indicates the presence of steroids.

Salkowski's Test - Add concentrated sulfuric acid to several mg of the substance, and 2 drops of acetic anhydride to its solution in chloroform. Production of red color indicates of triterpenoids, blue for steroids.

REPUBLIC OF THE PHILIPPINES
DEPARTMENT OF SCIENCE AND TECHNOLOGY
INDUSTRIAL TECHNOLOGY DEVELOPMENT INSTITUTE
STANDARDS AND TESTING DIVISION

A Member of DOST OneLab

TEST REPORT

ITDI-112022-OCS-0395

Customer's Name	:	Adjale G. Wyson
Address	:	Unit 201 B Fidel Apartment, Fidel St. T. S. Cruz Subd., Novaliches, Quezon City
Contact Details	:	CP# 0925.367.4025
Date Received	:	November 03, 2022
Sample	:	***Dioscorea hispida Dennst.*** (2 Samples)
Description and Identification	:	OCS-2022-0760: About nine (9) mL dark-colored liquid extract in a graduated glass tube with screw cap marked as *Nami MeOH* OCS-2022-0761: About 1 g crude extract in a graduated glass tube marked as *PETSF Nami*
Date(s) Tested	:	November 28, 2022

Phytochemical test for plant constituents:

Test Parameters	OCS-2022-0760 *Dioscorea hispida Dennst. / Nami Methanolic Extract*	OCS-2022-0761 *Dioscorea hispida Dennst. / Nami Pet ether sol frac*	**Test Method**
Sterols	+	+	Lieberman-Burchard Test
Triterpenes	+	-	
Flavonoids	+	+	Shinoda Test
Alkaloids	+	+	Mayer's Test
Saponins	+	+	Froth Test
Glycosides	+	+	Fehling's Test
Tannins	+	+	Ferric Chloride Test

Note:
(+) Presence of Constituents
(-) Absence of Constituents

Reference: Evans, W.C (2009). *Trease and Evans' Pharmacognosy* (16th ed). Saunders Ltd.

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December 07, 2022
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CERTIFICATE OF IDENTIFICATION

This is to certify that the accepted scientific name of the specimens by **Adjale G. Wyson** from the **Ateneo de Manila University - Main Campus** is:

Family Name	**Scientific Name**
Dioscoreaceae	*Dioscorea hispida* Dennst.

This specimen was identified using materials at the Institute of Biology, Jose Vera Santos Memorial Herbarium (PUH), College of Science, University of the Philippines, Diliman, Quezon City.

This certificate is issued as requested by the researchers and may be used for whatever legal matter pertaining and related to this authentication.

Edwino S. Fernando, Ph. D.
Curator
Jose Vera Santos Memorial Herbarium (PUH)

Date: April 21, 2022