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Percentage Extractive Yield and Phytochemical Profile of *Jacquemontia paniculata* (Convolvulaceae)

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ABSTRACT: Leaf and stem of Jacquemontia paniculata (Convolvulaceae**)** were extracted by maceration extraction method using ethanol, acetone and water solvents. The average percentage yield of the leaf part in ethanol (2.86%), acetone (2.37%), and aqueous (1.18%) extracts using gravimetric method were higher compared with stem extracts using the same solvents. Using the t-test there was a significant difference on the percentage extractive yield between the stem and leaf in the aqueous solution. Although aqueous extracts are commonly used traditionally, assays of ethanol extract in the leaf part achieved best yield compared to other solvents. Furthermore, assessment of the phytochemical profile of the leaf and stem extracts (ethanol, acetone and aqueous) of J. paniculata was determined in the study. Qualitative tests for tannins, saponins, flavonoids, phenolic compounds, steroids, alkaloids, cardiac glycosides and reducing sugars were conducted. Overall, tannins, saponins, flavonoids, phenolic compounds, cardiac glycosides and reducing sugars were found to be present in higher abundance in most of the leaf extracts regardless of the solvents used. These are also present in stem but in moderate to trace amount. Flavonoids in leaf extracts were present in the Ferric Chloride and Lead Acetate Tests but absent in the Alkaline Reagent Test. Steroids are present in stem regardless of the solvent used but absent in the leaf part. Both in leaf and stem extracts, alkaloids are absent. The presence of bioactive compounds in the leaf and stem of this plant is an indication of their many possible therapeutic uses. Present findings were preliminary and further investigation is needed to determine the pharmacological applications, antioxidant potential, cytotoxicity test and structural elucidation of functional groups present from the isolate of the plant.

KEYWORDS: Aqueous Extract, Bioactive Compounds, Ethanol Extract, *Jacquemontia Paniculata*, Maceration Extraction, Phytochemical Screening, Therapeutic Potential.

1.0 Introduction

Natural products from plants have shown great potential in the treatment of human diseases such as cancer and other infectious diseases because of its pharmacological or biological activity. Plant materials and extracts are mainly prescribed by traditional healers for the treatment of various diseases. These are usually done by identifying the common symptoms of patients and prescribing the type of plant to use (Hewson, 1998). The practices of using traditional medicine still continue because of its biomedical benefits as well as its cultural beliefs that have made a significant to sustain human health. (http://shodhganga.inflibnet.ac.in/bitstream/10603/15501/7/07_chapter%202.pdf, 2007).

According to the World Health Organization (WHO) 80% or more of the population depends on the traditional medicine for their primary healthcare needs (Sasidharanet et al., 2011). According to Farnsworth et al. (1985) there are 119 identified secondary plant metabolites which are used as drugs. Eleven percent out of 255 drugs considered as basic and important by the World Health Organization (WHO) is obtained from plants (Gupta et al., 2012).

Studies on Philippines indigenous and other local plants phytochemical profile, toxicological property, and antimicrobial potency had developed research interest recently (Penecilla and Magno, 2011; Valle et al., 2015; Uy and Garcia, 2015; Uy and Villazorda, 2015; Latayada and Uy, 2016). The phytochemical studies in the country included: (i) medicinal plant leaves (Peteros and Uy, 2010); (ii) indigenous vegetables leaves and stalks were studied (Baang et al., 2015); (iv) fruit peels (Palmes and Del Rosario, 2012); (v) and herbal vines (Licayan et al., 2016) .The study of Olinan (2009), Rara (2012) as cited by Galarpe and Clemeña (2017) all their studies revealed the phytochemical screening profile of the bark and leaf extracts of the tree species of the indigenous plant of *J.paniculata* (Convolvulacea) using different solvents and extraction procedures. There are only few literatures that comprehensively studied the phytochemical potential of this plant.

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Plants are sources of novel chemical compounds that have potential use in medicine. Many active compounds such as alkaloids, steroids, flavonoids, tannins, glycosides, phenols, terpenoids, anthraquinones, saponins, and reducing sugars are deposited in the specific parts of the plant such as leaves, flowers, barks, roots, etc. Plant-derived products contain a wide range of phytochemicals known to be antioxidants (Gupta et al., 2012), which have a protective role against oxidative stress-related diseases such as cancer and cardiovascular diseases (Anwar et al., 2013). Hence, a diet rich in vegetables and fruits and bioactive compounds found in plants have been associated with natural antioxidants to reduce risk of heart disease, cancer and diabetes (Anwar et al., 2013). Moreover, the bioactive metabolites become the interest of research because of its potential in antimicrobial factors, enzyme inducers and others (Jayachitra et al., 2012).

The Convolvulacea family has approximately 50 genera and 1200 species (Lawrence, 1951) have been known among indigenous communities for its medicinal applications. Specific plant to this family is *J. paniculata* widely distributed species across the tropics including the Philippines. It is commonly known as ‘‘himag” in Panay Bisayan region of the country. The stem is a slender vine not exceeding a stem diameter of 2 cm. The leaf blades are about 4-5 x 3.5-4.5 cm, petioles about 1.2-1.4 cm long. Upper and lower surfaces sparsely clothed in hairs. The flowers are inflorescence a shortly branched panicle on a long peduncle. Outer calyx lobes larger, about 10 x 5 cm. Corolla about 9-10 mm long, apices reddish, tube short or absent.

Parts of this plant especially the bark, is being used as ointment whereas the decoction of stem/bark is used to treat intermittent fever. Moreover, it is locally known to cure coughs and some other illnesses. It has been studied and found to possess analgesic and anti – diabetic properties. The infusion prepared from the bark of the plant has been shown to possess analgesic and anti-inflammatory effect. It has been reported that it is used in folk medicine as poultice in cuts, wounds and all kinds of swellings and for the treatment of ulcer, abscess, hemorrhoids, etc. (Srinivasanet al., 2011).Despite the local abundance and herbal use both pharmacological and phytochemical profiles are limited. This study was therefore conducted to evaluate the possible valuable phytochemical potencies and active metabolites of the ethanol, acetone and aqueous extracts from the leaf and stem of *J. paniculata.* Furthermore, this study aimed to determine the highest percentage yield of leaf and stem extracts soaked in ethanol, acetone and water solvents.

2.0 MATERIALS AND METHOD

2.1 Collection of Samples

Approximately 1 kilogram of fresh leaves and stem of *J. paniculata* commonly known as “himag” plant were freshly collected in Dapitan City and were placed in plastic bags. (see Figure 1**)**. The plant sample was identified by a Biology Instructor in Jose Rizal Memorial State University. The leaves and stem were washed to remove the dirt or any adhering organisms and air–dried to minimize the water content for 2 to 4 days. The dried samples will be cut into small pieces and then stored at an environment of low humidity.

Figure 1. Plant Sample Jacquemontia paniculata known as “Himag”

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2.2 Plant Extraction

A. Gravimetric Method For Percentage Yield

Dried plant parts were then collected and weighed before extraction. A 3×6 factorial design wasemployed on the extracts, giving an output of 18 experiments. Ten (10) g of dried plant part was weighed and soaked in separate containers of 500 mL beaker containing 100 mL of ethanol, acetone and water for 24 hour soaking. The extract solutions were filtered through Whatman filterpaper No. 1. Solid parts were further grinded using mortar and pestle and sieved in a cloth to get all the plant extracts and then filtered again. The filtrates were concentrated by evaporation in a water bath below the boiling point of the solvents used.Crude extract powder was stored at 4^0C until used. The percentage yield of crude extract powder was calculated by the following equation.

$$\text{Percent yield} = \frac{\text{wt of extract}}{\text{wt. of dried sample}} \times 100\%$$

B. For Isolation of the Most Antioxidant Metabolite

Three different samples of 100 g of air–dried leaves and stem parts of the *J. Paniculata* plant was weighed and soaked separately in ethanol, acetone and water for 24 hours and filtered. The different crude extract was concentrated to 50 ml using a water bath procedure in a hot plate with temperature of the water bath below the boiling point of the solvents used.

C.Phytochemical Screening

The crude extracts from the leaf and stem of*J. paniculata* that were obtained from soaking in different solvents were separately screened for the presence of certain phytochemicals. The following phytochemical tests were carried out using their respective procedures as presented in Appendix 1.

2.3 Data Analysis

All the analyses were done in triplicate. The results were expressed as means. Statistical analyses were conducted using Minitab 17.One – way analysis of variance (ANOVA) was used for comparison of more than two means. The difference was considered statistically significant when $p \leq 0.05$.The confident limits used in this study were based on 95% ($p < 0.05$).

3.0 RESULTS AND DISCUSSION

3.1 Extraction Yield

Samples of ethanol, acetone and aqueous extracts were obtained from leaf and stem plant parts in triplicates, as shown in Table 1. Leaf extracts gave significantly higher percentage yields than the stem part in the three solvents used. The average percentage yield of ethanol (2.82%), acetone (2.36%), and aqueous (1.18%) extracts from leaf part were significantly higher compared with stem extracts using the same solvents.Although aqueous extracts are commonly used traditionally, results in the study revealed that it has the lowest percentage yield both in leaf (1.18%) and stem extracts (0.66%).Ethanol extract (2.82%) from the leaf part achieved best yield compared to acetone and aqueous extracts. While in the stem part it is the acetone extract (2.11%) among the three solvents used. The ethanol and acetone solvents showed significantly higher yields compared with the other extraction solvents of the plant leaf and stem respectively as shown in figure 1.

Table 1. Extraction yields of leaf and stem of *J. paniculata* in different solvents.

Plant parts (10 g)	Extraction solvents	Extractive Value				Percent Yield	% CV
		Trial 1 (g)	Trial 2 (g)	Trial 3 (g)	Average (g)		
J. paniculata Leaf	Ethanol	0.3428	0.2275	0.2894	0.2866	2.82	20.13
	Acetone	0.1745	0.2989	0.2367	0.2367	2.36	26.28
	Aqueous	0.1183	0.1152	0.1214	0.1183	1.18	2.62
J. paniculata Stem	Ethanol	0.1646	0.2525	0.1209	0.1793	1.75	37.38
	Acetone	0.2180	0.2100	0.2141	0.2140	2.11	1.87
	Aqueous	0.0589	0.0687	0.0697	0.0658	0.66	8.94

Note. %CV = percent coefficient of variation.

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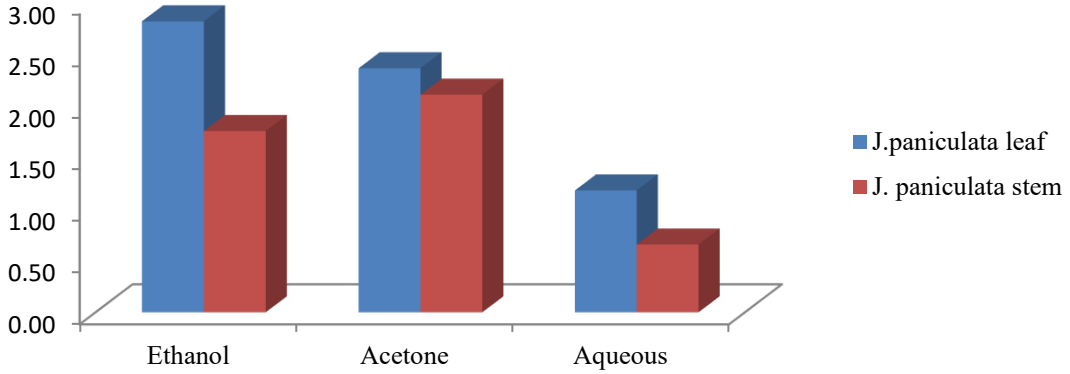
Figure 1. Percentage Yield of Leaf and Stem of *J.paniculata* extracts



3.2. Effect of Solvents and Plant Parts on Yields and Compounds of Extracts J. paniculata extracts

The results indicated that ethanol in leaf extract showed the highest percentage yield followed by acetone and aqueous extracts. While in stem extract it is in acetone solvent that showed the highest percentage yield followed by ethanol and aqueous solvents, when using a 1:10 (w/v) ratio of dried plant material and solvent. The effects on antibacterial activity were associated with the plant part and extraction solvent. The leaf was absorbed by polar organic solvents more than the other plant parts. These results were similar to the report of Stanley et al. (2014) but different from Mondal et al. (2012).

The extraction efficiency from plants is related to solvent type, plant material to solvent ratio, time of extraction, and particle sizes of the plant (Tiwari, Kumar, M. Kaur, G. Kaur, & H. Kaur, 2011). The rate of extraction can be increased by increasing the surface area and using a sample to solvent ratio of 1:10 (w/v)(Tiwari et al., 2011).Although aqueous extracts are used traditionally, ethanol extracts in stem part used in the study achieved better activity in the assays as corroborated in the study of Qader et al., 2011.

3.3. Statistical note on the active metabolites content

The results shown in Table 2 can be the basis to claim the potential pharmacological properties of the leaves and stem of *J. paniculata.*To empirically evaluate the active metabolites in the studied extracts a One-way ANOVA were employed. Summary of result is presented in Table 2. It can be extrapolated from the statistical analysis that there was a significant difference between the stem and leaf extracts (p>.05). This was evidenced by the higher concentrations of metabolites in the leaf extracts compared to the stem extracts in the different solvents used. Results can be ranked in the order of ethanol > acetone > aqueous.

Table 2. Significant Difference on the Percentage Yield between the Leaf and Stem Parts of *J. paniculata*

Solvents	Leaf Part Mean Percentage Yield	Stem Part Mean Percentage Yield	t-value	p- value
Ethanol	2.86	1.79	2.082	0.106
Acetone	2.37	2.14	0.630	0.563
Aqueous	1.18	0.66	13.531	0.000

3.4. *Phytochemical Profile*

The ethanol, acetone and aqueous extracts of *J. paniculata*leaf and stem were screened for the presence of phytochemicals. These were carried employing qualitative tannins, saponins, flavonoids, phenolic compounds, steroids, alkaloids, cardiac glycosides and reducing sugarstests respectively.

Regardless of solvent used and *J. paniculata* parts (e.g.leaves and stem) tannins, saponins, flavonoids, phenolic compounds, cardiac glycosides and reducing sugars were found to be present (refer to Table 3 on the next page).The presence of bioactive compounds in the leaf and stem of this plant is an indication of their many possible therapeutic uses.

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Alkaloids were absent in all plant extracts. The absence of alkaloids in the leaf extract and in the stem extract may be due to variations of alkaloid distribution in the different plant parts. These metabolites have only about 9%-10% distribution in vascular plants and are specific to a few related plants as reported by Harborne (1998). Alkaloid detection in plants is dependent on factors such as age, climate, plant part, habitat, season, time of harvest, chemical races of plants and sensitivity of alkaloid (Nduagu et al., 2008).

Table 3. Summary of result for the phytochemical profile

Phytochemicals	**Specific Test**	**J. paniculata**					
		Leaf extract			**Stem extract**		
		water	**Ethanol**	**Acetone**	**water**	**Ethanol**	**Acetone**
Tannins	Ferric Chloride Test	++	+++	++	+	++	+++
Saponins	Froth Test	+++	+++	+	++	++	+
Flavonoids	Ferric Chloride Test	++	+++	+	++	+	+
	Lead Acetate Solution Test	++	+++	+	+	+	+
	Alkaline Reagent Test	-	-	-	+	+	-
Phenolic Compounds	Ferric Chloride Test	++	+++	+	++	+	++
Steroids	Acetic Anhydride in acid Test	-	-	-	+	+	+
Alkaloids	Wagner's Test	-	-	-	-	-	-
Cardiac Glycosides	Keller-Killiani Test	++	+++	+	++	+++	++
Reducing Sugars	Fehling's test	++	+++	+	++	++	++

Legend: absent (-); trace (+); moderate (++); abundant (+++).

Compared to all other solvent extracts, ethanolic leaf and stem extracts had higher number of secondary metabolites in terms of tannins, saponins, flavonoids, phenolic compounds and cardiac glycosides and reducing sugars with high degree of precipitation (+++) and color change. Steroids are present only in stem extract in trace(+) amount and absent in leaf extracts. Alkaloids were absent (-) in all extracts regardless of plant parts.

Tannins were detected in all studied extract and very much abundant in ethanolic leaf extract and acetonic stem extract. This phytochemical was detected since it is innate to be found in vascular plants with woody tissues like *J. paniculata*. Tannins are used chiefly in tanning leather, dyeing fabric, making ink, and in various medical applications (Encyclopaedia Britannica,2018).

Presence of saponins was also confirmed through the formation of persistent honeycomb froth upon shaking an ethanol and water solutions dominantly of *J. paniculata* extracts. This was further verified by layering the solution with corn oil followed by vigorous shaking, then observing for the presence of emulsion at the froth-water interface. Plants rich in saponins have immune boosting and anti-inflammatory properties (Olinan, 2009). Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics include formation of foams in aqueous solution, haemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004).Saponins have the beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system.
(http://www.phytochemicals.info/phytochemicals/saponins.php).

Phytochemical screening of flavonoids was done with three different tests namely: Ferric chloride test, Lead Acetate Test and Alkaline Reagent Test. Both extracts (leaf and stem) showed positive result in Ferric chloride test and Lead Acetate tests in all solvents but absent in the leaf extract using the Alkaline Reagent Test. Flavonoid**s** found dominantly in leaf sample extracted using ethanol solvent.

Flavonoids have antioxidant activity and some of the activities attributed includes; anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral (http://www.phytochemicals.info/phytochemicals/flavonoids.php). The presence of flavonoids in the extract can be associated to the blood glucose lowering property. The free radical scavenging ability of many flavonoid-containing extracts has been postulated as the mechanism that sustains relief in many distressful diseases including diabetes.

This result indicated high antioxidant activity of *J. paniculata* leaf. Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution

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pattern of hydroxyl groups (Sharififar et al., 2009). The concentration of flavonoids in plant extracts depends on the polarity of solvents used in preparation, consequently the reason for the negative result using acetone as the solvent.

Phenols were detected in all studied extract and dominantly found in ethanolic leaf extract. Its presence was detected by forming a bluish black solution. Phenols have wide applications in different fields like medicines. Like exachlorophene which is a phenolic compound is antiseptic in nature and acts as a main constituent of mouthwashes, deodorant and medicinal skin cleansers.

Steroids were also detected through Acetic Anhydride Test. The stem extract with different solvents showed a positive result but the leaf extract gave a negative result. Steroids have beneficial effects on human body by increasing lean muscle mass, strength, and body endurance (Olinan, 2009).

Cardiac glycosides through Keller – Killiani test were detected in all studied extract and very much abundant in leaf and stem extracts using ethanol. Cardiac glycosides comprise the most drug-like molecules subjected to several investigations and they were proved to be fruitful in developing potential drugs. They are chemical compounds responsible for the poisoning of livestock and the treatment of congestive heart failure.

Reducing sugars were also detected through Fehling's test after the samples were shaken and immersed in boiling water until a brick – red precipitate forms as an indicative result. Reducing sugars act as a reducing agent and can donate electrons to another molecule. Specifically, a reducing sugar is a type of carbohydrate or natural sugar that contains a free aldehyde or ketone group. Reducing sugars can react with other parts of the food, like amino acids, to change the color or taste of the food. (https://sciencing.com/common-reducing-sugars-6193580.html.)

Overall, the present phytochemical study corroborated with the previous findings of Olinan (2009), Rara (2012) and Clemeña and Galarpe (2017) except for steroids in their studies that showed a positive result using the Keller-Killiani test from the bark and leaf of the indigenous tree specie of *J. paniculata.* Present findings in the study were preliminary and further test may be necessary on the medicinal applications of the plant such as antioxidant potential, cytotoxicity test and structural elucidation of functional groups present from the isolate of the plant.

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