



RESEARCH PAPER

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Phytochemical profile of bark and leaf extracts of *Jacquemontia paniculata* (Convolvulaceae)

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Abstract

This study evaluated the phytochemical profile of the bark and leaf extracts (aqueous, ethanol, and hexane) of *Jacquemontia paniculata* (Convolvulaceae). Qualitative tests for alkaloids, saponins, tannins/polyphenols, steroids, tannins, anthraquinones, cyanogenic glycosides, and flavonoids were conducted. Further quantification of flavonoids using the Quercetin acid equivalence was employed in all extracts. Overall, alkaloids, flavonoids, saponins, steroids, tannins, and anthraquinones were found to be present in most of the extracts. The total flavonoids varied from 12.81 to 15.51mg/g in extracts. The maximum flavonoid content was found in the ethanolic bark extract (15.51mg/g) while the lowest flavonoid content was found in hexane leaf extract (12.81mg/g). Present findings were preliminary and further investigation is needed to determine the pharmacological applications of the plant.

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Introduction

In developing countries the use of traditional medicine by utilizing endemic and indigenous plants as sources is widely practiced because of its presumed affordability, availability and cultural importance (Mander, 1998). 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 fundamental basis for plants therapeutic efficacies are the phytochemicals it contains and plants anti-oxidative potentials.

Studies on Philippines indigenous and other local plants phytochemical profile, toxicological property, and antimicrobial potency had grown 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 region 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).

None of these literature investigated phytochemical potential of another indigenous plant *Jacquemontia paniculata* (Convolvulaceae).

Convolvulaceae is a family of 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 'ching cham' in Thai, 'arog pondolandak' in Sundanese, 'lawatan' in Javanese, 'siembukan' in Madurese, and 'himag' in Panay Bisayan - Philippines. 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. Despite the local abundance and herbal use both pharmacological and phytochemical profiles are limited. This study was therefore conducted to evaluate the possible beneficial phytochemical potencies of the aqueous, ethanol, and hexane extracts from the barks and leaves of *J. paniculata*.

Materials and Methods

Sample Preparation

Freshly collected *J. paniculata* bark and leaf samples were placed in plastic bags (see Fig. 1). The bark and leaf samples were washed and air-dried for seven days and then grounded using a blender prior to extraction. The prepared samples were stored in a clean amber bottle.



Fig. 1. Bark and leaf of *J. paniculata* .

Preparation of Extracts and phytochemical tests

Upon collection, the samples were cleaned and subjected to air-drying for about 3 to 7 days. Thereafter, the samples both bark and leaves were cut

into strips. The dried bark strips was grounded using a mechanical grinder while the leaves was cut into rectangular or square shapes. The samples were subjected to aqueous, ethanol, and hexane extracts.

For the aqueous extract, the samples were mixed with distilled water and then boiled up until the mixture was concentrated. On the other hand, for the ethanol extract, the samples were milled using a grinder.

The dried powder was soaked to 500mL to 700mL of ethanol for about 3 to 5 days. The solution was then filtered using a whatman filter paper no. 42 followed by concentrating the extracts using a rotary evaporator. On the other hand, another set on 300g of dried and powdered samples were soaked in enough volume of hexane for 44h for hexane extract. The mixture was then filtered and concentrated in a rotary evaporator at a temperature below 50°C (see Fig. 2). The concentrated hexane extract was evaporated over a steam bath to a syrupy consistency.

The plants extracts were then stored in clean and closed-capped containers. For the phytochemical screening, observable results were determined by physical changes such as color change, and formation of precipitate upon addition of chemical reagents in each specific test (see Table 1).



Fig. 2. Concentrating the extracts using the rotary evaporator.

Table 1. Phytochemical qualitative test methods.

Phytochemicals	Specific test
Alkaloids	Mayer's reagent
	Wagner's reagent
Flavonoids	Bate-Smith and Metcalf method
Saponins	Froth test
Tannins and polyphenols	Ferric chloride test
Anthraquinones	Borntrager's Test
Steroids	Keller-Killiani test
Cyanogenic glycosides	Cyanogenic glycosides

Total flavonoid

Total flavonoid in the plant extract was estimated using aluminum chloride method according to Sahu and Saxena (2013) and Sultana et.al. (2012). Extract samples were evaluated at a final concentrations of 0.1mg/mL and 1mg/mL. While quercetin. Concentrations of 20, 40, 60, 80, and 100mg/mL was used to obtain the calibration curve. A 1mL of aliquot of extract was placed in a 10 mL volumetric flask containing 4 mL distilled water.

The mixture was added with 0.3mL of 5% NaNO₂. After 5 min., a 0.3mL of 10% AlCl₃ and 2mL of 1M NaOH were added to the above mixture and diluted to mark with distilled water. The solution was mixed/shaken thoroughly and were read against the blank at 510 nm. All determinations were done in triplicates. Total flavonoids content was expressed as quercetin equivalents (mg/g) using the following equation based on the calibration curve: $y = ax + b$, where x was the absorbance and y was the quercetin equivalent (mg/g).

Data Analysis

To summarize the gathered data, each were subjected into different types of statistical means. Descriptive statistics was used in the analysis and interpretation of phytochemical screening. Two-way ANOVA was used to determine if there was interaction between the different parts and solvents for flavonoid contents.

Results and discussion

Phytochemical profile

The aqueous, ethanol, and hexane extracts of *J. paniculata* bark and leaf were screened for the presence of phytochemicals. These were carried employing qualitative (alkaloids, flavonoids, saponins, tannins, anthraquinones, steroids, and cyanogenic glycosides) and quantitative (total flavonoids) tests respectively.

Regardless of solvent used and *J. paniculata* parts (e.g. bark and leaves) flavonoids, alkaloids, saponins, anthraquinones, and steroids were found to be present (refer to Table 2).

Tannins on the other hand were detected in aqueous and ethanolic extracts. The results shown in Table 2 can

be the basis to claim the potential pharmacological properties of the bark and leaves of *J. paniculata*.

Table 2. Summary of result for the phytochemical profile.

Phytochemicals	Specific test	<i>J. paniculata</i>					
		Bark extract			Leaf extract		
		Aqueous	Ethanol	Hexane	Aqueous	Ethanol	Hexane
Alkaloids	Mayer's reagent	+	+++	+++	+	+++	+++
	Wagner's reagent	+	+++	+++	+	+++	+++
Flavonoids	Bate-Smith and Metcalf method	++	+++	+	++	+++	+
Saponins	Froth test	+++	++	+	+++	++	+
Tannins and polyphenols	Ferric chloride test	++	+++	-	+	+++	-
Anthraquinones	Borntrager's Test	+	+	+	-	+	+
Steroids	Keller-Killiani test	+++	+++	++	+++	+	++
Cyanogenic glycosides	Cyanogenic glycosides	-	-	-	-	-	-

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

The presence of alkaloids in the leaf extract and in the bark extract may be due to variations of alkaloid distribution in the different plant parts. Harborne (1998) reported that alkaloids have about 9%-10% distribution in vascular plants and are specific to a few related plants. 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). Pronounced result was detected in ethanol extract due to affinity of its water soluble salt to ethanol.

Presence of saponins was also confirmed through the formation of persistent honeycomb froth upon shaking an aqueous solution dominantly of *J. paniculata* extracts. 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). Steroids were also detected through Keller-Killiani Test. Steroids have beneficial effects on human body by increasing lean muscle mass, strength, and body endurance (Olinan, 2009).

Tannins were also detected in all studied extract. This phytochemical was detected since it is innate to be found in vascular plants with woody tissues like *J. paniculata*.

Its presence was detected by forming water insoluble copolymers in the qualitative test. At the latter was salted out as protein-tannin complex upon action of NaCl (Talib and Mahasneh, 2010). Tannins are known to possess general antimicrobial and antioxidant activities (Riviere *et al.*, 2009).

Anthraquinones of known laxative property (Talib and Mahasneh, 2010) were also detected. The folkloric use of *J. paniculata* bark and leaves for treating herpes simplex and skin diseases may be confirmed by the presence of anthraquinones.

This was screened through Borntrager's test developing a red color in the lower ammoniacal layer. Both bark and leaf extracts (ethanol and hexane) of *J. paniculata* showed positive result. Cyanogenic glycoside however was absent in all extracts of *J. paniculata*. Picrate method was used to supposedly detect its presence.

Flavonoid content

Other studied phytochemical was flavonoid which was present evidenced by the development of red color upon qualitative test. This was further screened quantitatively using Quercetin acid equivalence (refer to Table 3).

Table 3. Total flavonoid content in different extracts of *J. paniculata* (n =3).

Extracts	Total flavonoid (mg/g); mean \pm SD
<i>Bark</i>	
Aqueous	14.19 \pm 0.51
Ethanol	15.51 \pm 1.26
Hexane	12.87 \pm 0.26
<i>Leaves</i>	
Aqueous	13.07 \pm 0.46
Ethanol	13.89 \pm 1.56
Hexane	12.81 \pm 0.15

Table 3 shows the contents of total flavonoid quantitatively measured by AlCl₃ reagent in terms of Quercetin acid equivalent. The concentration of flavonoids in plant extracts from *J. paniculata* ranged from 12.81 to 15.51mg/g. The maximum flavonoid content was found in the ethanol bark extract (15.51 \pm 1.26mg/g). This result indicated high antioxidant activity of *J. paniculata* bark. Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties.

Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Shariffar *et al.*, 2009). In contrast lowest flavonoid concentration was found in hexane extract of both bark and leaf of *J. paniculata*. The concentration of flavonoids in plant extracts depends on the polarity of solvents used in preparation, consequently the reason for the negative result using hexane as the solvent.

Statistical note on flavonoid content

To empirically evaluate flavonoids in studied extracts a Two-way ANOVA was employed. Summary of result is presented in Table 4. It can be extrapolated from the statistical analysis that there was a significant difference between the bark and leaf extracts ($p = 0.017$). This was evidenced by the higher concentrations of flavonoids in the bark extracts.

Further, a significant difference ($p = 0.0018$) among studied extracts (aqueous, ethanol, and hexane) was similarly determined. This can be associated to the dominance of flavonoid in ethanol extract regardless of *J. paniculata* parts. Results can be ranked in the order of ethanol > aqueous > hexane.

Comparison from past findings

To tabulate all the results of phytochemical screening, Table 5 shows all the bioactive components present at different extracts of *J. paniculata* bark and leaf with its corresponding author from previous study to present. Overall, present phytochemical study corroborated with previous findings.

Table 4. Two-way ANOVA of total flavonoid in bark and leaf extracts of *J. paniculata*.

Source	SS	df	MS	F	p-value	F critical
Sample	5.261	1	5.264	6.826	0.017	4.413
Extract	13.97	2	6.987	9.061	0.0018	3.554
Interaction	2.503	2	1.251	1.623	0.224	3.554
Within	13.88	18	0.771			
Total	35.62	23				

Table 5. Phytochemicals of the different extracts from *J. panicula* bark and leaves.

Extract	Phytochemicals						Reference
	Alkaloids	Flavonoids	Saponins	Anthraquinones	Steroids	Cyanogenic glycosides	
<i>Bark</i>							Olinan (2009)
Decoction	+	+	+	+	+	-	
Ethanol	+	+	+	+	+	-	
<i>Leaf</i>							Rara (2012)
Decoction	-	+	+	-	+	-	
Methanolic	-	+	+	+	-	-	
<i>Bark</i>							This study
Aqueous	+	+	+	+	+	-	
Ethanol	+	+	+	+	+	-	
Hexane	+	+	+	+	+	-	
<i>Leaf</i>							
Aqueous	+	+	+	-	+	-	
Ethanol	+	+	+	+	+	-	
Hexane	+	+	+	+	+	-	

Legend: present (+); absent (-).

Conclusion

This study covered phytochemical screening of the bark and leaf extracts (aqueous, ethanol, and hexane) of *J. paniculata*. Alkaloids, flavonoids, saponins, steroids, tannins, and anthraquinones were found to be present in most of the extracts. The total flavonoids varied from 12.81 to 15.51mg/g in extracts. The maximum flavonoid content was found in the ethanolic bark extract (15.51mg/g) while the lowest flavonoid content was found in hexane leaf extract (12.81mg/g). Present findings were preliminary and further test may be necessary on the medicinal applications of the plant.

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