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Phytochemical analysis and antioxidant activity evaluation of ethanolic extract of pitcher plant pitchers *(Nepenthes bellii)*

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Abstract

Plants provide a wealth of medicinal benefits even for severe illnesses. Medicinal herbs are commonly used to treat and prevent a variety of illnesses. They often contain phytochemicals that contribute to developing novel medications to combat the threat of resistant strains. The current study investigated a medicinal plant commonly used in the northern part of Mindanao. The pitcher plant (*Nephentes bellii*) is a carnivorous plant that is endemic to the area. *Nephentes bellii*, ethanolic pitchers extract was evaluated for its medicinal property through phytochemical analysis and antioxidant capacity utilizing 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Results revealed that *Nephentes bellii* pitchers contain bioactive compounds that are essential components in a medical plant, such as; Alkaloids, Anthraquinones, Flavonoids, Saponins, Steroids, and Tannins. Moreover, the *Nephentes bellii* ethanolic pitcher extracts possess a potent free radical scavenging activity.

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Introduction

Medicinal plants serve as a rich source of novel drugs that forms the ingredients in customary systems of medicine, current medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles, and lead compounds in synthetic drugs (Parveen *et al.*, 2010). Different medicinal plants are typically extracted or decocted of their various parts in cultural traditions to prepare for treatment (Hosseinzadeh *et al.*, 2015). As a result, screening these plants to verify their usage in traditional medicine and discover the active principle by extracting and identifying their constituents is of great interest.

The world is currently dealing with multiple drug resistance pathogens (MDR) as a result of the indiscriminate use of commercial antimicrobial drugs. According to Yala*et al.* (2001), because bacteria have the genetic ability to acquire and transmit resistance to therapeutic agents, the widespread use of antibiotics in human therapy has resulted in the development of several resistance mechanisms. This resistance issue necessitates the development of alternative antimicrobial drugs for the treatment of infectious diseases. Screening local medicinal plants for potential medicinal properties is one approach.

There have been numerous published reports on the effectiveness of traditional plants against harmful organisms, and as a result, plants are still recognized as the foundation for modern medicine in the treatment of infectious diseases (Evans, 2002). Cunha (2001) claims that medicinal plants have incredibly large clinical efficacy. They are beneficial for treating infectious diseases while significantly reducing many of the side effects commonly caused by synthetic antimicrobials or antibiotics.

Natural sources of medicinal plants are gaining popularity worldwide due to their safety, efficacy, cultural acceptance, and lower side effects compared to manufactured medications (Aktar & Foyzun, 2017). On the other hand, herbal medicine must be assessed for efficacy using the standard trial technique, even though some individual herbal extracts are effective for specific ailments (Firenzuoli & Gori, 2007). Due to the vast spectrum of pharmacological relevance of medicinal plants generated from natural products, numerous studies have been done on them, adding that in order to prevent side effects, which are primarily caused by overdosage and a lack of understanding of some plants' constituents, scientific screening and testing such as phytochemical analysis, antioxidant evaluation, and on other medicinal plants have generally been examined (Shukla *et al.*, 2010; Olowa and Nuñeza, 2013). This will also advance our understanding of plant metabolites, or chemical molecules, which have a variety of biochemical and bioactivity properties and are used in the pharmaceutical industry to develop potential cures for specific ailments (Yamane *et al.*, 2010).

Herbal cures are essential to humankind's oneness with nature and are said to be one of the ways Mother Earth cares for humanity. Many plants have been created as herbal medicines in the Philippines, although only a few numbers have passed traditional trials for pharmacological study. *Nepenthes bellii*, also known as the pitcher plant, is one of those medicinal plants. The pitcher plant Nepenthes bellii also referred to as "Hara-Hara" locally, is an endangered species native to the Philippines.

The municipalities of Hayanggabon and Carassal in the islands of Mindanao are where *Nepenthes bellii* was first discovered (Kondo, 1969). Carnivorous plants are found in nutrient-poor soil, whereas typical plants take nutrients from the soil. They "capture" insects that they then transform into forms they can digest or absorb to obtain sustenance (Rogers, 2018). This particular pitcher plant trait may play a significant role in conventional medicine and is noteworthy in pharmacological research. One of the plants used as an alternative medicine to treat conditions like fluid retention, constipation, urinary tract issues, and digestion issues is *Nepenthes bellii*, along with the other known species of the Nepenthes genus. In this sense, documentation of indigenous knowledge through ethnobotanical studies is essential in conserving and utilizing a plant's biological resources (Cakilcioglu & Turkoglu, 2010).

Studies have shown that *Nepenthes bellii* leaves extracts can inhibit the growth of gram positive and gram negative bacteria (Buniel, 2023). Yet, other parts of the plant have still to be revealed as to its medicinal potential. Hence, this study is prompted to evaluate *Nepenthes bellii* pitchers' phytochemical components, which helps researchers understand the nature of how it can cure and heals (Wadood *et al.*, 2013) and antioxidant property to determine if the substances present possess free radical chain reaction breaking properties (Veeru *et al.*, 2009). Further, through this investigation, scientific proofs can be added and recorded that *Nepenthes bellii* pitchers has medicinal property as what can be seen in its phytochemical evaluation and antioxidant assay results.

Materials and methods

Collection of Plant Material

Permits were obtained first from Cantilan municipal government before collecting the samples. Fresh pitchers of *Nepenthes bellii* were gathered and placed into a glass container ready for processing.

Preparation of Plant Extract

The pitcher samples of *Nepenthes bellii* were processed according to the protocol described by Nair *et al.*, 2005: pitcher samples were air-dried under the shade for three weeks at room temperature until crispy, powdered using a blender, and stored inside an airtight plastic container prior to extraction. One hundred grams of powdered pitcher sample were steeped in 200mL of pure ethanol for seven days to make the ethanolic extract. The mixtures were placed in a container covered with black fabric and foil and stored at room temperature in a locker. Filtered residues were re-extracted with ethanol. Whatman No. 1 filter paper was used to separate the ethanol (solvent). The solvent was evaporated in a vacuum using a rotary evaporator after filtering. It was possible to get a concentrated leaf ethanolic crude extract of *Nepenthes bellii* pitcher. The crude extracts were chilled at seven °C in a sealed glass container.

Phytochemical screening

The ethanolic pitcher extracts of *Nepenthes bellii* were subjected to phytochemical screening in accordance with the procedure Oloya *et al.*, 2022, with modifications. To identify the presence of different bioactive chemical constituents like alkaloids, anthraquinones, cyanogenic glycosides, flavonoids, saponins, steroids, and tannins, ethanolic pitcher extracts of *Nepenthes bellii* were subjected to qualitative phytochemical screening.

DPPH radical scavenging activity

High-grade chemicals were employed, and appropriate chemical preparation was monitored. Following the protocol developed by Saed *et al.*, 2012; Pereira *et al.*, 2015 with slight modifications, the antioxidant capacity of *Nephentes bellii* pitcher extracts was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) on the ability of free radicals to decolorize in the presence of antioxidants. 24mg of DPPH was dissolved in 100mL of ethanol to create the stock solution, which was then stored in the refrigerator at -10°C until needed. 2.5mL of 6.5x10-5 M DPPH solution and 0.5mL of sample extracts diluted in ethanol—as the control—were used to create the reaction mixture. Leaf extracts were evaluated in DPPH solution for 12 hours in a dark, room-temperature environment.

The concentrations used were 1 g/mL, 2 g/mL, 3 g/mL, 5 g/mL, 10 g/mL, 20 g/mL, 30 g/mL, and 50 g/mL. A UV-Vis spectrophotometer measured absorbance at 515 nm (SHIMADZU UV mini 1240). The percentage of DPPH radical scavenging activity was calculated using the equation listed below. For each test solution, the IC50 value—the sample concentration necessary for 50% inhibition—was calculated. % DPPH scavenging activity = (A control – A sample/A control) x 100. A control and A sample are the test samples and the blank sample's respective absorbance values.

For purple coloring to be observable, the DPPH radical must have odd electrons that operate on absorption in a 517 nm range. The reduction in absorption was used to gauge how much radical scavenging had occurred. Three trials were used to make all determinations.

Result and discussion

Phytochemical Screening

The phytochemical screening in this study showed that Nepenthes bellii ethanolic pitcher extract has the presence of bioactive compounds such as alkaloids, anthraquinones, flavonoids, saponins, steroids, and tannins and the absence of cyanogenic glycosides.

As shown in Table 1, *N. bellii* ethanolic pitcher extract is rich in anthraquinones, flavonoids, steroids, and tannins. Anthraquinones are an important group of bioactive components that are found not only in N. bellii but also in many other species of medicinal herbs, such as rhubarb, aloe, senna, and purslane (Huang *et al.*, 2006). It has anti-fungal, antibacterial effects, which also shows potential protective properties for gastrointestinal and renal systems and even a potential treatment for cancer (Schorkhuber *et al.*, 1998; Wojcikowski *et al.*, 2004; 2006; Zhang X *et al.*, 2005; Huang *et al.*, 2006).

Table 1. Phytochemical constituents of *N. bellii* ethanolic pitcher extracts.

Alkaloids	Anthraquinones	Cyanogenic glycosides	Flavonoids	Saponins	Steroids	Tannins
+	+++	_	+++	+++	+++	+++

Flavonoids are a group of natural substances with variable phenolic structures found in a plant's leaf, fruit, vegetable, grain, bark, root, stem, and flower parts. They are now considered indispensable in various nutraceutical, pharmaceutical, medicinal and cosmetic applications (Panche *et al.*, 2016). Moreover, Flavonoids are well-known as antibacterial agents against many pathogenic microorganisms.

With the increasing prevalence of untreatable infections induced by antibiotic resistance bacteria, flavonoids have attracted much interest because of the potential to be substituted for antibiotics (Xie *et al.*, 2015). Another bioactive compound, steroids had immense pharmacological activity like any other compound; however, steroids have several restrictions (Dewick, 2011). Further, another rich bioactive compound in *Nepenthes bellii* pitchers is tannins. Tannins are produced by plants in adverse environmental conditions, being responsible for their protection against herbivores and pathogenic diseases, and are essential for the growth and reproduction of plants (Sartori, 2012). Medicinally, tannins are employed in antidiarrheal, hemostatic, and anti-hemorrhoidal compounds and have significant properties as anti-viral (Lin *et al.*, 2004), antibacterial (Funatogawa *et al.*, 2004) and anti-parasitic effects (Kolodziej *et al.*, 2005). Henceforth, the presence of these bioactive compounds that had been identified using phytochemical screening presumes Nepenthes bellii pitchers' is a potential plant with pharmacological properties in searching for cures and treating several target diseases, respectively.

Table 2. DPPH radical scavenging assay of the *Nepenthes bellii* ethanolic pitchers extracts.

% inhibition at different concentration	
control	0 ± 0.3
1 µg/mL	27.92 ±0.2
2 µg/mL	31.85 ± 0.2
3 µg/mL	36.62 ± 0.1
5 µg/mL	46.26 ± 0. 02
10 µg/mL	73.89 ± 0.01
20 µg/mL	95.75 ± 0.01
30 µg/mL	96.07 ± 0.01
50 µg/mL	96.50 ± 0.01
IC_{50} µg/mL	5.47

*Mean ± SD

α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method is an approach to evaluate the antioxidant potential of a compound, an extract, or other biological sources. A process wherein the prospective compound or extract is mixed with DPPH solution and absorbance is recorded after a defined period (Kedare & Singh, 2011). The odd electron of a nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Contreras-Guzman & Srong, 1982) or by evaluating antioxidants by spectrophotometry (Huang *et al.*, 2005). The result shows that rapid assessment for an antioxidant property using a DPPH assay demonstrated that the ethanolic pitchers extracts of *N. bellii* possess

vigorous free radical scavenging activity with an LC50 value of 5.47 µg/mL. As shown in the table and Fig. 1, the free radical scavenging activity had stabilized at concentrations of 10 µg/mL to 50 µg/mL; thus, the optimum activity of the Nepenthes was at these concentrations bellii pitchers' ethanolic extract was reached. In confirmation to the study of Vargas *et al.*in 2016, samples only exhibiting LC50 < 10 µg/mL are considered very active antioxidants as they have the potential comparable to the antioxidant standards of quercetin, β-carotene, ascorbic acid, gallic acid, and Trolox® and the plant extracts exhibiting the most significant antioxidant potential were those with the highest levels of total polyphenols. Screening of phytochemicals in *Nepenthes bellii* is a basis for the antioxidant activity of the pitcher's ethanolic extract.

Polyphenolic compounds such as flavonoids and tannins that are abundant in *Nepenthes bellii* pitchers' are contributors to the high free-radical scavenging activity since the chemical activities of polyphenols in terms of their reducing properties as hydrogen or electron-donating agents predict their potential for action as free-radical scavengers (Rice-Evans *et al.*, 1997). Further, alkaloids, flavonoids, and other phytochemicals are considered antioxidants and have a beneficial effect against inflammation, microbial activity, and certain cancers (Kevat, 2013).

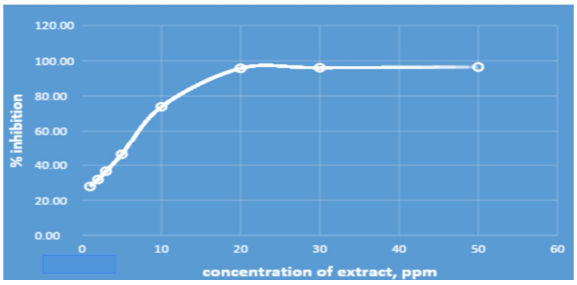

Fig. 1. Plot of the percent inhibition vs concentration of *Nepenthes bellii* ethanolic pitchers' extract.

Conclusion

Nepenthes bellii ethanolic pitcher extracts have bioactive compounds such as alkaloids, anthraquinones, flavonoids, saponins, steroids, and tannins. These bioactive compounds contribute to the plant part's high activity of antioxidant properties. These bioactive compounds present in the pitchers indicate the therapeutic potential the locals can utilize for their folkloric medicine by proper administration.

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