Phytochemical profile of selected Philippine plants used to treat asthma

Chichioco-Hernandez Christine L.[1]* and Paguigan Noemi D.[2]

[1]Bioorganic and Natural Products Laboratory, Institute of Chemistry, University of the Philippines, Diliman, Quezon City, Philippines 1101
[2]Natural Sciences Research Institute, University of the Philippines, Diliman, Quezon City, Philippines 1101
* Author for correspondence: cchernandez@up.edu.ph

ABSTRACT

Phytochemical analyses were carried out to determine the similarities of the plant metabolites present in the various plants used traditionally for the alleviation of asthma. Plants were collected from the University of the Philippines Diliman campus. The dried samples were homogenized for overnight soaking in methanol at room temperature. The resulting alcoholic extracts were filtered and concentrated *in vacuo* and tested for their various metabolites. Saponins, terpenoids, flavonoids and cardiac glycosides were commonly found in the various plant samples and to some extent, the anti-asthma activity of the plants could be attributed to these types of secondary metabolites.

Keywords: *asthma, plant extracts, phytochemical*.
Editor: Srisailam Kesetti, Phcog.Net

***Author for Correspondence:** cchernandez@up.edu.ph

INTRODUCTION

Asthma is a national health priority because of its increasing prevalence and significant impact on the quality of life.[1, 2] The cost of severe asthma is similar to cardiovascular diseases [3] and has been associated with depression.[4, 5] It is estimated that 300 million people worldwide are suffering from this condition.[6] Around 8 million Filipinos are suffering from asthma according to the International Study of Asthma and Allergies in Childhood and the prevalence of the condition is increasing especially in children. Various medications are available, however, the relief they offer is mainly symptomatic and short-lived, on top of their unwanted side-effects.[7–10] Hence, there is a need for safer and effective medications for asthma. Screening of plants used in traditional medicine is a potential source of compounds that could be developed into more effective asthma drugs.

Thorough literature reviews of plants used in Philippine herbal medicine have shown several plants that are use for the treatment of asthma. Quisumbing [11] has reported several such plants and this includes *Bambusa blumeana* Schultes ('kauayan'), *Pistia stratiotes* Linn ('kiapo'), *Isotoma longiflora* ('estrella'), *Monochoria vaginalis* ('gabing-uak'), *Plumeria rubra* ('kalachuche'), *Euphorbia hirta* ('gatas-gatas'), *Amaranthus viridis* ('kolitis'), *Commelina diffusa* ('alikbangon') and *Eclipta alba* ('tinta-tinta'). *B. blumeana* is also used as a stimulant, astringent, antispasmodic and for coughs. [11] The leaves of *P. stratiotes* is mixed with sugar and used for coughs and asthma.[11] It is used to treat gastroenteritis and fever in Africa.[12] Its interaction with several metals has been studied [13–16] and its possible phytoremediation activity.[17–19] The antiviral, [20] anti-degerative effect, [21] anti-anaphylactic, [22] antimicrobial, [23] anti-allergy, [24] and anti-diarrheal [25] action of *E. hirta* have been investigated. Butanol rhamnosides have also been isolated from its polar and non-polar extracts.[26] *E. alba* has shown hair-growth promoting activity, [27, 28] anti-venom, [29] anti-malarial, [30] anti-aggressive, [31] and anti-hypertensive action.[32] *I. longiflora* is used for its antibacterial properties in Puerto Rico.[33] The leaves of *M. vaginalis* are used for diabetes in India (ext-link-type="uri" xmlns:xlink="http://www.w3.org/1999/xlink" xlink:href="http://www.eseap.cipotato.org">http://www.eseap.cipotato.org). *A viridis* is eaten as a vegetable in India and used as a medicinal herb in Ayuverdic medicine.[34] *C. diffusa* is used as

febrifuge and treatment for diabetes in China.[35] *P. rubra* is a traditional medicinal plant used in Thailand for its molluscicidal and antibacterial activity.[36] It is also one of the five flowers used in China as an herbal beverage for sore throat, halitosis, constipation and irritability.[37]

Different activities have been attributed to these plants. However, no study has been previously reported on the phytochemical constituents of these plants. Therefore, this research aims to determine the phytochemical profile of these plants and establish whether the presence of certain metabolites is responsible for its traditional use.

MATERIAL AND METHOD

Plant material

Leaves were collected from the University of the Philippines, Diliman Campus, Quezon City. A voucher specimen for each plant was submitted to the Dr. Jose

Table 1: Phytochemical tests

Test	Procedure	Expected Result
Tannins	Dissolve around 2 mg of sample in 5 mL distilled water. Add 15° $FeCl_3$ solution drop wise.	The formation of a brownish-green precipitate indicates condensed tannins while the appearance of a blue-black precipitate indicates the presence of hydrolysable tannins.
Saponins	Boil approximately 5 mg of sample in 5 ml distilled water. Allow the mixture to cool then vigorously shake.	Frothing suggests the presence of saponins.
	Another test is to layer the solution with corn oil then shake vigorously.	Presence of emulsion at the froth-water interface.
Terpenoids	Prepare a TLC chromatogram of the samples using Silica Gel 60 F_{254}. Develop the chromatogram in $CHCl_3$ then spray with vanillin-H_2SO_4 solution.	The formation of red to purple spots upon heating of the TLC plate.
	Confirmatory analysis is done using the Salkowski Test. Treat 2 mg of the sample with 2 ml $CHCl_3$ then layer with H_2SO_4	Formation of reddish-brown color at the interface.
Flavonoids	Dissolve around 2 mg of the sample in 2 ml of 1M NaOH followed by the addition of several drops of 0.6M HCl	A yellow to orange solution with NaOH that turns colorless upon addition of HCl.
	Confirmatory test is done by dissolving the sample in 70° EtOH then spot on a Silica Gel 60 TLC plate. Develop the chromatogram in Forestal solution (30:3:10 glacial acetic acid: concentrated HCl: water), allow to dry, then view under UV light, and then fume with NH_4OH.	Note for fluorescence or change in color.
Cardiac glycosides	Killiani-Keller test — add one drop of 15° $FeCl_3$ to 2 mg of sample dissolved in 2 ml distilled water. Then layer the solution in 1 ml concentrated H_2SO_4.	Formation of a brown ring at the interface indicates the presence of cardiac glycosides.
Phenolic compounds	Add a few drops of 1° $FeCl_3$ to two mg of sample dissolved in 2 ml distilled water.	Formation of a green, purple, blue, or black solution.
Steroids	Add 2 ml of diluted H_2SO_4 to 2 mg of sample dissolved in 2 ml acetic anhydride.	Formation of a blue or green solution.
Alkaloids	Dissolve 5 mg of sample in 2 mL distilled water then add 3 drops of Wagner's reagent. This reagent is prepared from 2 g iodine and 6 g potassium iodide dissolved in 100 mL water.	Formation of a blue black precipitate.

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Plant extraction

Dried leaves were air-dried, homogenized, weighed then soaked in methanol (MeOH). The resulting MeOH extracts were concentrated *in vacuo* at 40°C using a rotary evaporator. The MeOH used was technical grade and distilled before use.

Phytochemical analysis

Phytochemical screening of the crude extracts was based on several procedures with slight modifications [38–40] as shown in Table 1.

RESULTS AND DISCUSSION

The phytochemical profiles of nine Philippine plants that are being used in traditional medicine have been determined. Phytochemical screening of the different plants revealed that they contain several types of metabolites as shown in Table 2. It is worth noting that most of the extracts were positive for saponins, terpenoids, flavonoids and cardiac glycosides. It is probable that such compounds that were found to be present in the plants are responsible for their anti-asthma activity.

Several studies have shown that compounds from these classes inhibit asthma. For instance, astragaloside IV, a new cycloartane-type triterpene glycoside extract of *Astragalus membranaceus* Bunge, has been shown to inhibit ovalbumin-induced chronic experimental asthma.[41] Triterpenoid saponins have been identified from the stem bark of *Pteleopsis suberosa*, a traditional remedy for asthma.[42] The seeds of *Allium tuberosum* which is also used for asthma have yielded steroid saponins.[43] A triterpenoid glycoside isolated from *Bupleurum falcatum* has shown inhibitory action against allergic asthma in rats.[44] Flavonoids found in saboku-to, a herbal medicine for bronchial asthma, have shown inhibitory action against the release of leukotrienes from human leukocytes.[45[Licorice flavonoids inhibit airway eonosiphilic inflammation which is a major feature of allergic asthma.[46]

CONCLUSIONS

To some extent, the observed inhibition of asthma could be attributed to the various plant secondary metabolites detected in the plant materials. Further studies are currently being done to determine which plant exhibits the highest activity against asthma.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR'S CONTRIBUTION

CCH designed the study and was involved in the preparation of the manuscript while NDP was involved in the experiments and collection of data.

ACKNOWLEDGEMENTS

This research was funded by the Natural Sciences Research Institute, University of the Philippines, Diliman, Quezon City, Philippines.

Table 2: Phytochemical profile of the nine Philippine plants

Sample	Tannin	Saponin	Terpenoid	Flavonoid	Cardiac Glycoside	Phenolic Compounds	Steroids	Alkaloid
1. B. spinosa	–	–	+	–	–	–	–	–
2.P.stratiotes	–	+	+	+	–	–	–	–
3. I. longiflora	–	+	–	–	+	–	–	–
4. M. vaginalis	–	+	+	+	–	–	–	–
5. P. rubra	–	+	+	–	+	–	–	–
6. E. hirta	+ blue black ppt.	+	+	+	+	+	+	–
7.A. viridis	–	+	–	–	+	–	–	–
8. C. diffusa	–	+	+	+	+	+	–	–
9. E. alba	+ brownish green ppt.	–	+	+	+	+	–	–

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