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Research Article

Preliminary phytochemical screening of selected Medicinal Plants of Cambodia

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Abstract

Cambodian medicinal plants traditionally have been used to treat various ailments such as backache, burn, cough, diarrhea, sprain, malaria and post-partum. This study was conducted to analyze the preliminary phytochemicals of selected Cambodian plant species including leaves of *Nicotiana tabacum*, whole plant of *Vernonia cinerea*, barks of *Azadirachta indica*, leaves of *Annona muricata*, rhizomes of *Zingiber cassumunar* and rhizomes of *Curcuma longa*. Dried plants of each species were subjected to the tests for alkaloids, phenolic compounds, tannins, flavonoids, cardiac glycosides, steroids, terpenoids, essential oils, resins, saponins, quinones and polypeptides through the phytochemical analysis. These selected Cambodian medicinal plants showed the presences of phenolic compounds, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, resins and saponins. The phytoconstituent profile of these selected plants would be of numerous uses to the alternative medicine practices in Cambodia, and these results are of great importance in performing further isolation research of novel compounds.

Keywords: medicinal plants; Cambodia

Introduction

Traditional practitioners, based on medicinal plant usage, have played an important role in everyday life of Cambodian people for centuries. Cambodian medicinal plants are useful for healing backache, burn, fever, cough, diarrhea, headache, malaria, post-partum, sprain, stomachache and wound (Chassagne et al., 2016). Cambodia-native plants of some species have been reported with antimicrobial activity (Chea et al., 2007), which is in accordance with several studies indicating that medicinal plants originated in Cambodia protect human liver from disease (Lee et al., 2017; Chassagne et al., 2017). For traditional Cambodian medicine, *Nicotiana tabacum*, *Vernonia cinerea*, *Azadirachta indica*, *Annona muricata*, *Zingiber cassumunar* and *Curcuma longa* have been medically applied for a long time ago. The *N. tabacum* has been a plant of remedies against dysentery and virus-caused diseases. *V. cinerea*



has been used traditionally as antipyretic and diaphoretic agents. Moreover, it is used to treat pneumophagia, spasm, dropsy and cancers. *A. indica* has been reported to treat diarrhea, amoebic dysentery, haemorrhoids, abnormal urination and head lice. *A. muricata* has been traditionally prepared as a tonic, aphrodisiac, analgesic and anti-parasitic plant. *Z. cassumunar* has been used to apply to the frontanel of newborn children to prevent them from developing a cold. It is, besides, used for remedies of bruises and rheumatisms. *C. longa* has been applied to fresh wounds, bruises and leech-bites. Additionally, it is administered to treat intermittent fevers, dyspepsia, stomach weakness, skin diseases, sprains, rheumatisms, purulent ophthalmia, conjunctivitis, pains and cancers (Kham, 2004).

Phytochemicals biologically and naturally protect plants from diseases and damages; therefore, it is obviously known that they possess protective roles in human health. Over 4,000 phytochemicals, in which about 150 phytochemicals have been studied in detail, have been identified and classified by protective function, chemical feature and physical characteristics (Saxena et al., 2013). Plant extraction has been widely performed by using some solvents such as water, ethanol, methanol, chloroform, ether and acetone in order to obtain various phytochemicals including alkaloids, phenolic compounds, tannins, flavonoids, coumarins, steroids, terpenoids, cardiac glycosides, saponins and quinones. These classes of phytochemicals are antimicrobial, antidiarrheal, anthelmintic, antiviral and anticancerous (Tiwari et al., 2011). So far there has been scant attention to phytochemicals in Cambodian *N. tabacum* leaves, *V. cinerea* whole plants, *A. indica* barks, *A. muricata* leaves, *Z. cassumunar* rhizomes and *C. longa* rhizomes. Therefore, these medicinal plants were subjected to the study with an aim to analyze their phytochemicals.

Materials and Methods

Collection of plant materials: Leaves of *Nicotiana tabacum*, whole plant of *Vernonia cinerea*, barks of *Azadirachta indica*, leaves of *Annona muricata*, rhizomes of *Zingiber cassumunar* and rhizomes of *Curcuma longa* in dried form were obtained from plant drugstore, Phnom Penh, Cambodia in November 2016. Each plant was authenticated by University of Puthisastra (UP) Herbarium through the comparative examination with the existing voucher specimens. Voucher specimens were lodged as UPFPH-030025 (*N. tabacum*), UPFPH-120011 (*V. cinerea*), UPFPT-210013 (*A. indica*), UPFPT-210041 (*A. muricata*), UPFPH-210033 (*Z. cassumunar*) and UPFPH-210019 (*C. longa*). All the dried plants were well maintained for the phytochemical analysis in Pharmacognosy Laboratory, Faculty of Health Sciences, University of Puthisastra (Table 1).

Phytochemical screening: The preliminary phytochemical analysis of these dried plant was performed to determine the phytochemicals like alkaloids, phenolic compounds, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, essential oils, resins, saponins, quinones and polypeptides (Harborne, 1984).

Test for alkaloids (Dragendorff's, Mayer's and Wagner's Tests): One gram of dried plant was added with 10 ml of 1M-HCl and ultrasonicated for 15 min at 30 °C. The mixture was filtered, and 3 ml of filtrate was treated with few drops of either Dragendorff's reagent or Mayer's reagent or Wagner's reagent. Orange red, creamy white or reddish brown precipitate indicated the presence of alkaloids (Humayun et al., 2012).

Test for phenolic compounds (Ferric Chloride Test): One gram of dried plant was added with 10 ml of ethanol and ultrasonicated for 15 min at 30 °C. The mixture was filtered, and 2 ml of filtrate was added



with 5 ml of distilled water. The filtrate was treated with few drops of 5%-FeCl₃. Dark green color indicated the presence of phenolic compounds (Raaman, 2006).

Test for tannins (Ferric Chloride Test): One gram of dried plant was added with 10 ml of distilled water and ultrasonicated for 15 min at 80 °C. The mixture was filtered, and the filtrate was cooled down. Two milliliters of filtrate were treated with few drops of 0.1%-FeCl₃. The presence of tannins was indicated by the brownish green or blue-black coloration (Karthishwaran et al., 2010).

Test for flavonoids (Ammonium Test): One gram of dried plant was added with 10 ml of chloroform and ultrasonicated for 15 min at 80 °C. The mixture was filtered, and the filtrate was cooled down. Five milliliters of filtrate were added with 1 ml of 1%-ammonia solution, and the mixture was shaken. Yellow color at the ammonia layer demonstrated the presence of flavonoids (Sheel et al., 2014).

Test for steroids (Liebermann Burchard Test): Ten milliliters of ethanol were added to 1 g of dried plants, and ultrasonicated for 15 min at 30 °C. The mixture was filtered, and the filtrate was evaporated to dryness. Two milliliters of chloroform were added to the crude extract of 100 mg. The mixture was added with 1 ml of glacial acetic acid, followed by careful addition of 1 ml H₂SO₄ along the side of the test tube. Greenish color indicated the presence of steroids (Bargah, 2015).

Test for terpenoids (Salkowski Test): One gram of dried plants was added with 10 ml of chloroform₃ and ultrasonicated for 15 min at 30 °C. The mixture was filtered. Three milliliters of H₂SO₄, along the side of test tube, were added carefully to 5 ml of filtrate. Reddish brown color at the interface of the two liquids characterized the presence of terpenoids (Ajiboye et al., 2013).

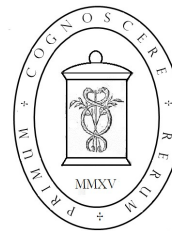
Test for cardiac glycosides (Keller-Killani Test): Ten milliliters of ethanol were added to one gram of dried plants and ultrasonicated for 15 min at 30 °C. The filtrate was evaporated to dryness. Two milliliters of glacial acetic acid with 2 drops of 2%-FeCl₃ were added to 100 mg of the crude extract. The mixture was added with 1 ml H₂SO₄ along the side of the test tube. Brown ring at the interface indicated the presence of cardiac glycosides (Jaradat et al., 2015; Ajiboye et al., 2013).

Test for essential oils (NaOH-HCl Test): Ten milliliters of ethanol were added to 1 g of dried plants, and ultrasonicated for 15 min at 30 °C. Two milliliters of filtrate were added with 100 µl of 1M-NaOH, followed by the addition of few drops of 1M-HCl. The mixture was shaken. White precipitate demonstrated the presence of essential oils (Mir et al., 2013).

Test for saponins (Froth Test): Eleven milliliters of distilled water were added to 1 g of dried plants, and ultrasonicated for 15 min at 80 °C. The mixture was filtered, and cooled down. Five milliliters of distilled water were added to 10 ml of filtrate. The mixture was shaken about 10 min until the formation of stable persistent froth. Formation of stable five-minute-persistent froth indicated the presence of saponins (Djaafar & Ridha, 2014).

Test for resins (Turbidity Test): Ten milliliters of distilled water were added to 1 g of dried plants, and ultrasonicated for 15 min at 30 °C. The mixture was filtered. Occurrence of turbidity showed the presence of rasins (Mir et al., 2013).

Test for quinones (H₂SO₄ Test): Ten milliliters of ethanol were added to 1 g of dried plants and ultrasonicated for 15 min under the temperature of 30 °C. The mixture was filtered. One milliliter of H₂SO₄ was added to 1 ml of filtrate. Red color indicated the presence of quinones (Rajesh et al., 2014).



Test for polypeptides (Biuret Test): Ten milliliters of distilled water were added to 1 g of dried plants and ultrasonicated for 15 min at 30 °C. The mixture was filtered and added with 1 ml of 40%-NaOH, followed by the addition of 2 drops of 1%-CuSO₄. Violet color indicated the presence of polypeptides (Santhi & Sengottuvel, 2016).

Results

Leaves of *N. tabacum* and rhizomes of *Z. cassumunar* exhibited the positive test of all phytochemicals including alkaloids, phenolic compounds, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, essential oils, resins, saponins, quinones and polypeptides. The rhizomes of *C. longa* showed the presence of all phytochemicals except for the polypeptides which were tested negatively. The alkaloids were present in all plants, except *V. cinerea*. Essential oils and polypeptides were not found in whole plant of *V. cinerea*, barks of *A. indica* and leaves of *A. muricata*. The quinones were tested positively in all plants except for the leaves of *A. muricata*. Moreover, phenolic compounds, tannins, flavonoids, steroids, terpenoids and cardiac glycosides were demonstrated to be present in whole plant of *V. cinerea*, barks of *A. indica* and leaves of *A. muricata*.

Conclusion

The present study was carried out on selected medicinal plants to detect the presence of alkaloids, phenolic compounds, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, essential oils, resins, saponins, quinones and polypeptides. These phytochemicals play an important role in the biological activities of medicinal plants including anti-bacterial, anti-viral, anti-cancerous, anti-oxidant, anti-malarial, anti-neoplastic, anti-inflammatory, anti-diarrheal, anti-fungal, anti-parasitic, anti-allergic, anti-spasmodic, anti-protozoan and anti-tussive activities (Njeru et al., 2013). In this study, the alkaloids were positively tested in all plants except *V. cinerea*; however, a previous report showed the presence of alkaloids (Lakshmi, 2015). Absence of the *V. cinerea* alkaloids in this investigation is probably owed to the change in location and genetic variation because of cross pollination, so their genetic makeup was modified, thereby revealing different outcomes (Wadood et al., 2013); nevertheless, Sangeetha et al. (2011) reported the absence of the alkaloids in this plant. Alkaloids are known to prevent the onset of different degenerative diseases by scavenging free radicals. They also inhibit the growth and development of microorganisms including bacteria, fungi and protozoans (Kuar & Arora, 2015). All the six selected medicinal plants tested positive for phenolic compounds, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, resins and saponins. These phytochemical constituents may account for beneficial roles against cancers, coronary heart disease, diabetes, high blood pressure, inflammation, microbial, viral and parasitic infections, psychotic diseases, spasmodic conditions and ulcers (Dillard & German, 2000). Essential oils were present in *N. tabacum*, rhizomes of *Z. cassumunar* and rhizomes of *C. longa*, but they were not found in *V. cinerea*, *A. indica* and *A. muricata*. Essential oils are used as antispasmodic, antirheumatic, anti-inflammatory, anti-fungal, antioxidant and anti-irritant agents (Rassem et al., 2016). Polypeptides play crucial roles in human physiology, including actions as hormones, neurotransmitters, growth factors, ion channel ligands, or anti-infectives (Fosgerau & Hoffmann, 2015). Quinones were present in all screened plants except *A. muricata*. Quinones regulate the electron transfer reactions resulting in protection against reactive oxygen species (ROS) (Madeo et al., 2013). Sometimes, quinones react as the production of excessive ROS causing the incidence of oxidative damage, which is a common characteristic of anti-cancerous effects (Lu et al., 2013). The phytoconstituent profile of these selected plants is of usefulness to the alternative medicine practices in Cambodia, and these results are of great importance in performing further isolation research of novel compounds.



Table 1: Information on selected medicinal plants of Cambodia for phytochemical analysis (Kham, 2004)

Plant Species	Voucher Specimens	English Names	Khmer Names	Plant Families	Plant Parts	Plant Types
<i>Nicotiana tabacum</i>	UPFPH-030025	Tobacco	Tnam chœk	Solanaceae	Leaves	Herb
<i>Vernonia cinerea</i>	UPFPH-120011	Vernonia	Smäv ruy	Compositae	Whole plant	Herb
<i>Azadirachta indica</i>	UPFPT-210013	Margosa	Sdav	Meliaceae	Barks	Tree
<i>Annona muricata</i>	UPFPT-210041	Soursop	Tieb bârañ	Annonaceae	Leaves	Tree
<i>Zingiber cassumunar</i>	UPFPH-210033	Wild ginger	P'œn-ley	Zingiberaceae	Rhizomes	Herb
<i>Curcuma longa</i>	UPFPH-210019	Tumeric	Mom rôomit	Zingiberaceae	Rhizomes	Herb

Table 2: Preliminary phytochemical screening of selected medicinal plants of Cambodia

Phytochemicals	Tests	Leaves of <i>N. tabacum</i>	Whole Plant of <i>V. cinerea</i>	Barks of <i>A. indica</i>	Leaves of <i>A. muricata</i>	Rhizomes of <i>Z. cassumunar</i>	Rhizomes of <i>C. longa</i>
Alkaloids	Dragendorff	+ve	-ve	+ve	+ve	+ve	+ve
	Mayer	+ve	-ve	+ve	+ve	+ve	+ve
	Wagner	+ve	-ve	+ve	+ve	+ve	+ve
Phenolic compounds	Ferric Chloride	+ve	+ve	+ve	+ve	+ve	+ve
Tannins	Ferric Chloride	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids	Ammonium	+ve	+ve	+ve	+ve	+ve	+ve
Steroids	Liebermann Burchard	+ve	+ve	+ve	+ve	+ve	+ve
Terpenoids	Salkowski	+ve	+ve	+ve	+ve	+ve	+ve
Cardiac glycosides	Keller-Killani	+ve	+ve	+ve	+ve	+ve	+ve
Essential oils	NaOH-HCl	+ve	-ve	-ve	-ve	+ve	+ve
Resins	Turbidity	+ve	+ve	+ve	+ve	+ve	+ve
Saponins	Froth	+ve	+ve	+ve	+ve	+ve	+ve
Quinones	H ₂ SO ₄	+ve	+ve	+ve	-ve	+ve	+ve
Polypeptides	Biuret	+ve	-ve	-ve	-ve	+ve	-ve

Note: +ve: Positive (present); -ve: Negative (absent)

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Declaration of Conflict of Interest

No conflict of interest associated with this work.

References

- Ajiboye BO, Ibukun EO, Edobor G, Ojo AO, Onikanni SA (2013) Qualitative and quantitative analysis of phytochemicals in *Senecio bialfræ* leaf. *Int. J. Invent. Pharm. Sci.* 1(5):428-432.
- Bargah RK (2015) Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma* Gaertn. *J. Pharmacogn. Phytochem.* 4(1):07-09.
- Chassagne F, Deharo E, Punley H, Bourdy G (2017) Treatment and management of liver diseases by Khmer traditional healers practicing in Phnom Penh area, Cambodia. *J. Ethnopharmacol.* 202:38-53.
- Chassagne F, Hul S, Deharo E, Bourdy G (2016) Natural remedies used by Bunong people in Monduliri province (Northeast Cambodia) with special reference to the treatment of 11 most common ailments. *J. Ethnopharmacol.* 191:41-70.
- Chea A, Jonville MC, Bun SS, Laget M, Elias R, Duménil G, Balansard G (2007) In vitro antimicrobial activity of plants used in Cambodian traditional medicine. *Am. J. Chin. Med.* 35(5):867-873.
- Dillard CJ, German JB (2000) Phytochemicals: nutraceuticals and human health. *J. Sci. Food Agric.* 80:1744-1756.
- Djaafar Z, Ridha OM (2014) Phytochemical study of selected medicinal plant, *Solanum nigrum*, the Algerian Dessert. *Int. Let. Chem. Phys. Astron.* 1:25-30.
- Fosgerau K, Hoffmann T (2015) Peptide therapeutics: current status and future directions. *Drug Disc. Today.* 20(1):122-128.
- Harborne JB (1984) *Phytochemical methods: a guide to modern techniques of plant analysis.* Chapman and Hall Ltd, London.
- Humayun S, Ibrar M, Barkatullah, Ahmad I (2012) Comparison of three extracts of *Fumeria indica* for the evaluation of cytotoxic and phytotoxic activities. *Int. J. Biosci.* 2(12):112-119.
- Jaradat N, Hussen F, Ali AA (2015) Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* Decne. *J. Mater. Environ. Sci.* 6(6):1771-1778.
- Karthishwaran K, Mirunalini S, Dhamodharan G, Krishnaveni M and Arulmozhi V (2010) Phytochemical investigation of methanolic extract of the leaves of *Pergularia daemia*. *J. Biol. Sci.* 10(3):242-246.
- Kaur R, Arora S (2015) Alkaloids-important therapeutic secondary metabolites of plant origin. *J. Crit. Rev.* 2
- Kham L (2004) *Medicinal plants of Cambodia: habitat, chemical constituents and ethnobotanical uses.* Bendigo Scientific Press, Australia.
- Lakshmi PJ (2015) Therapeutic uses of *Vernonia cinerea*—a short review. *Int. J. Pharm. Clin. Res.* 7(4):323-325.
- Lee DS, Keo S, Cheng SK, Oh H, Kim YC (2017) Protective effects of Cambodian medicinal plants on tert-butyl hydroperoxid-induced hepatotoxicity via Nrf2-mediated heme oxygenase-1. *Mol. Med. Rep.* 15(1):451-459.
- Lu JJ, Bao JL, Wu GS, Xu WS, Huang MQ, Chen XP, Wang YT (2013) Quinones derived from plant secondary metabolites as anti-cancer agents. *Anti-Can. Agents Med. Chem.* 13:456-463.
- Madeo J, Zubair A, Marianne F (2013) A review on the role of quinones in renal disorders. *SpringerPlus.* 2(139): 1-8.
- Mir SA, Mishra AK, Reshi ZA, Sharma MP (2013) Preliminary phytochemical screening of some pteridophytes from District Shopian (J & K). *Int. J. Pharm. Pharm. Sci.* 5(4):632-637.
- Njeru SN, Matasyoh J, Mwaniki CG, Mwendia CM, Kobia GK (2013) A review of some phytochemicals commonly found in medicinal plants. *Int. J. Med. Plants.* 105:135-140.



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Raaman N (2006) Phytochemical techniques. New India Publishing, New Delhi.

Rajesh H, Rao SN, Rani MN, Shetty PK, Rejeesh EP, Chandrashekar R (2013) Phytochemical analysis of methanolic extract of *Curcuma longa* Linn rhizome. Int. J. Uni. Pharm. Bio Sci. 2(2):39-45.

Rassem HHA, Nour AH, Yunus M (2016) Techniques for extraction of essential oils from plants: a review. Aus. J. Basic Applied Sci. 10(16):117-127.

Sangeetha T, Venkatarathinakumar T, Sankari G (2011) Preliminary phytochemical investigation including HPTLC profile on aerial parts of *Vernonia cinerea* (L). Int. J. Pharm. Sci. Rev. Res. 11(2): 65-68.

Santhi K, Sengottuvel R (2016) Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Int. J. Curr. Microbiol. App. Sci. 5(1):633-640.

Saxena M, Saxena J, Nema R, Singh D, Gupta A (2013) Phytochemistry of medicinal plants. J. Pharmacogn. Phytochem. 1(6):168-182.

Sheel R, Nisha K, Kumar J (2014) Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. IOSR J. Appl. Chem. 7(1):10-13.

Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H (2011) Phytochemical screening and extraction: a review. Int. Pharm. Sci. 1(1):98-106.

Wadood A, Ghufuran M, Jamal SB, Naeem M, Khan A, Ghaffar R, Asnad (2013) Phytochemical analysis of medicinal plants occurring in local area of Mardan. Biochem. Anal. Biochem. 2(4):1-4.



Nicotiana tabacum

Vernonia cinerea



Azadirachta indica

Annona muricata



Zingiber cassumunar

Curcuma longa

Figure 1: Selected medicinal plants of Cambodia used for phytochemical analysis