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Research Article

Phytochemical analysis of different extracts of leaves of *Nicotiana tabacum* L. of Cambodia

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Abstract

Nicotiana tabacum L. has been employed in healing of various ailments such as gastrointestinal disorders, abdominal discomfort, constipation, urinary tract obstruction, dental pain and dermatitis. This study was conducted to investigate the phytochemical components of leaf extracts of *Nicotiana tabacum* L. Dried leaves of *Nicotiana tabacum* L. were subjected to the Ultrasound-Assisted Extraction (UAE) with distilled water, methanol, ethanol, ethyl acetate or chloroform, each extract of which was further applied to the phytochemical analysis *via* color tests. The extracting yields of leaves of *Nicotiana tabacum* L. with different solvents gave the values of 13.16% (aqueous extract), 14.20% (methanol extract), 4.88% (ethanol extract), 8.20% (ethyl acetate extract) and 3.63% (chloroform extract). The phytochemical screening revealed the presence of alkaloids, phenolic compounds, tannins, flavonoids, cardiac glycosides, steroids, terpenoids, essential oils, resins, saponins, quinones and polypeptides in different extracts of *Nicotiana tabacum* L. leaves. The current study provides the phytochemical information in term of authentication and standardization of leaves of *Nicotiana tabacum* L. cultivated in Cambodia.

Keywords: *Nicotiana tabacum* L.; ultrasound-assisted extraction; phytochemical

Introduction

Nicotiana tabacum L., which originates from South America has been widely used as a therapeutic plant in Asian countries including China, India, Cambodia, Nepal, Malaysia and Iran (Binorkar & Jani, 2012). Its leaves are beneficial for the treatment of gastrointestinal disorders, abdominal discomfort, constipation, urinary tract obstruction, dental pain and dermatitis (Groark, 2010). The leaves of *Nicotiana tabacum* L. have been reported to activate biological mechanisms such as antibacterial, antinociceptive, antifungal, antimicrobial, anthelmintic and anti-Alzheimer's activities (Rawat & Mali,



2013). The method of UAE or sonication is extensively recognized as the most effective method of plant extraction based on high yield, extraction time (10-30 min) and high selectivity. UAE involves the mechanic effect of acoustic cavitation from the ultrasound increasing the surface contact between solvents and samples; it helps elevate the permeability of plant cell walls by solvents (Azwanida, 2015). Phytochemicals are the chemicals derived from leaves, flowers, seeds, barks, roots and pulps of plants. They play a key role against a number of diseases such as asthma, arthritis, cancers and so on. Currently, phytochemicals become more popular due to their side-effect freeness (Banu & Cathrine, 2015). The main components of plant-derived phytochemicals are alkaloids, flavonoids, glycosides, tannins, saponins, phenolics and terpenoids (Saxena et al., 2013). However, the phytochemical analyses of Cambodian medicinal plants remain of little observation; this study was conducted to investigate the phytochemical constituents of different leaf extracts of *Nicotiana tabacum* L. which is a native plant of Cambodia.

Materials and Methods

Preparation of plant materials: Dried leaves of *Nicotiana tabacum* L. (Khmer name: Tnam Chœk) (Figure 1) were obtained from the local plant drugstore and authenticated by University of Puthisastra (UP)-Herbarium (UPFPH-030025) in October 2016. Elmasonic S100H 50/60 Hz, Germany, was applied to the extraction of dried leaves of *Nicotiana tabacum* L. with various solvents including distilled water, methanol, ethanol, ethyl acetate or chloroform. Each plant was extracted for 30 mins in the room temperature, and extracting yields was estimated and subjected to the phytochemical evaluation (Figure 2)

Phytochemical analysis: The phytochemical evaluation of aqueous, methanol, ethanol, ethyl acetate or chloroform extracts of *Nicotiana tabacum* L. leaves was performed to identify the plant chemicals of 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): 3 ml filtrate of the extract was loaded equally into three test tubes. Each test tube was treated with few drops of Dragendorff's, Mayer's or Wagner's reagents. Orange red precipitate (Dragendorff) indicates the presence of alkaloids; creamy white precipitate (Mayer) points out the presence of alkaloids; and reddish brown precipitate (Wagner) demonstrates the presence of alkaloids (Humayun et al., 2012).

Test for phenolic compounds (ferric chloride test): 1 ml filtrate of the extract was pipetted into the test tube and added with 2 or 3 drops of 5%-FeCl₃. The formation of greenish precipitate indicates the presence of phenolic compounds (Raaman, 2006).

Test for tannins (ferric chloride test): 10 ml filtrate of the extract was transferred into the test tube and added with 2 or 3 drops of 0.1% of FeCl₃. The brownish green or blue-black coloration interprets the presence of tannins (Karthishwaran et al., 2010).

Test for flavonoids (ammonium test): 10 ml filtrate of the extract was taken into the test tube and added with 1 ml of 1%-ammonium solution. The mixture was shaken vigorously. The formation of yellow color observed in the ammonia layer demonstrates the presence of flavonoids (Sheel et al., 2014)



Figure 1: of *Nicotiana tabacum* L.

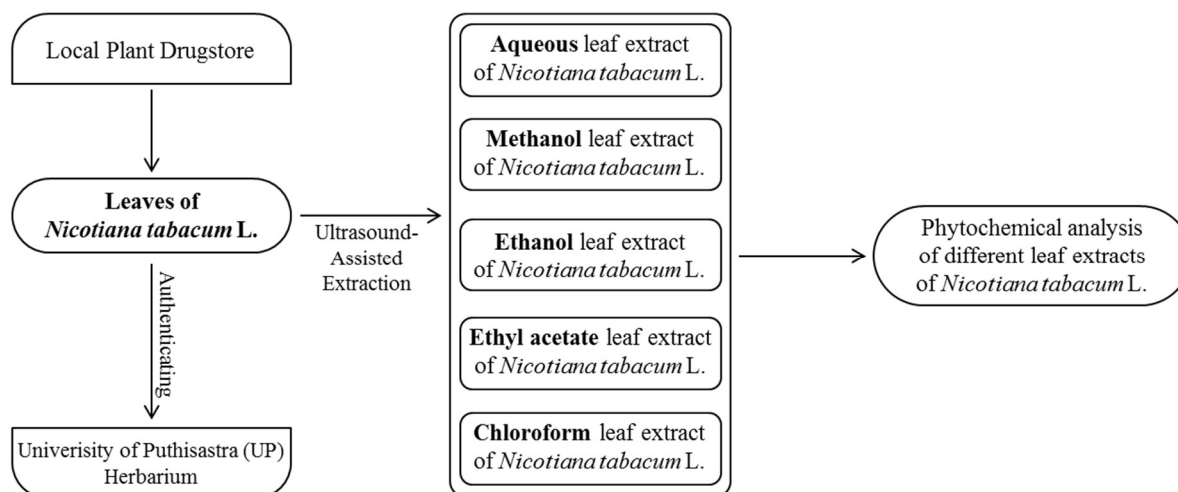


Figure 2: Flowchart of the phytochemical analysis of different leaf extracts of *Nicotiana tabacum* L.

Test for Steroids (Liebermann Burchard Test): 2 ml chloroform was added to the extract of 100 mg and filtered into the test tube. The mixture was added with 1 ml of glacial acetic acid, followed by carefully the addition of 1 ml of H₂SO₄ along the side of the test tube. Greenish color indicates the presence of steroids (Bargah, 2015).

Test for terpenoids (Salkowski test): 5 ml chloroform was added to the extract of 100 mg and filtered into the test tube. The mixture was added carefully with 3 ml of H₂SO₄ along the side of the test tube. Reddish brown color at the interface of the two liquids characterizes the presence of terpenoids (Ajiboye et al., 2013).

Test for Cardiac Glycosides (Keller-Kiliani test): 2 ml of glacial acetic acid with 2 drops of 2%-FeCl₃ were added to 100 mg of the extract in the test tube. The mixture was added with 1 ml of H₂SO₄ along the side of the test tube. Brown ring at the interface indicates the presence of cardiac glycosides (Jaradat et al., 2015; Ajiboye et al., 2013).

Test for essential oils (NaOH-HCl test): In the test tube, 2-ml filtrate of the extract was added with 100 µl of 1M-NaOH, followed by the addition of few drops of 1M-HCl. The mixture was shaken. White precipitate demonstrates the presence of essential oils (Mir et al., 2013).

Test for saponins (froth test): 10 ml of distilled water were added to 200 mg of the extract and filtered into the test tube. The mixture was shaken for 10 min until the formation of stable persistent froth. Formation of stable five-minute-persistent froth indicates the presence of saponins (Djaafar & Ridha, 2014).

Test for Resins (turbidity test): 10 ml of distilled water were added to 200 mg of the extract and filtered into the test tube, and the mixture was observed. Occurrence of turbidity shows the presence of resins (Mir et al., 2013).



Test for Quinones (H₂SO₄ Test): 1 ml of H₂SO₄ was added to 1-ml filtrate of the extract. Red color indicates the presence of quinones (Firdouse & Alam, 2011).

Test for polypeptides (Biuret test): 3 ml filtrate of the extract was added with 1 ml of 40%-NaOH, followed by the addition of 2 drops of 1%-CuSO₄. Violet color indicates the presence of polypeptides (Santhi & Sengottuvel, 2016).

Results

The leaf extracting yields of *Nicotiana tabacum* L. with different solvents gave the values of 13.16% (aqueous extract), 14.20% (methanol extract), 4.88% (ethanol extract), 8.20% (ethyl acetate extract) and 3.63% (chloroform extract) (Table 1).

Table 1: Extracting yields of different extracts of leaves of *Nicotiana tabacum* L. of Cambodia

Extracts of leaves of <i>Nicotiana tabacum</i> L.	Extracting Yields (%)
Aqueous	13.16
Methanol	14.20
Ethanol	4.88
Ethyl acetate	8.20
Chloroform	3.63

The phytochemical constituents of leaves of *Nicotiana tabacum* L. extracted by different solvents were analyzed and identified as multiple chemical components. The aqueous extract of *Nicotiana tabacum* L. leaves tested positive for alkaloids, tannins, flavonoids, steroids, cardiac glycosides, essential oils, resins and polypeptides; its methanol extract gave the presence of alkaloids, phenolic compounds, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, essential oils, resins, saponins and quinones; its ethanol extract was positively tested for alkaloids, phenolic compounds, flavonoids, steroids, terpenoids, essential oils, resins, saponins, quinones and polypeptides; its ethyl acetate extract was positively analyzed for tannins, terpenoids, saponins, quinones and polypeptides; and its chloroform extract was positively examined for alkaloids, steroids, terpenoids, cardiac glycosides, essential oils, quinones and polypeptides (Table 2).



Table 2: Phytochemical screening of different leaf extracts of *Nicotiana tabacum* L. of Cambodia

Phytochemicals	Tests	Aqu. Ext.	Met. Ext.	Eth. Ext.	EtOAc Ext.	Chl. Ext.
Alkaloids	Dragendorff	+ve	+ve	+ve	-ve	+ve
	Mayer	+ve	+ve	+ve	-ve	+ve
	Wagner	+ve	+ve	+ve	-ve	+ve
Phenolic compounds	Ferric Chloride	-ve	+ve	+ve	-ve	-ve
Tannins	Ferric Chloride	+ve	+ve	-ve	+ve	-ve
Flavonoids	Ammonium	+ve	+ve	+ve	-ve	-ve
Steroids	Liebermann Burchard	+ve	+ve	+ve	-ve	+ve
Terpenoids	Salkowski	-ve	+ve	+ve	+ve	+ve
Cardiac glycosides	Keller-Kiliani	+ve	+ve	-ve	-ve	+ve
Essential oils	NaOH-HCl	+ve	+ve	+ve	-ve	+ve
Resins	Turbidity	+ve	+ve	+ve	-ve	-ve
Saponins	Froth	-ve	+ve	+ve	+ve	-ve
Quinones	H ₂ SO ₄	-ve	+ve	+ve	+ve	+ve
Polypeptides	Biuret	+ve	-ve	+ve	+ve	+ve

Note: +ve: Positive (present); -ve: Negative (absent); Aqu. Ext.: Aqueous extract; Met. Ext.: Methanol Extract; Eth. Ext.: Ethanol Extract; EtOAc Ext.: Ethyl Acetate Extract; Chl. Ext.: Chloroform Extract

Discussion

Sunil et al. (2011) reported that the petroleum ether, chloroform, ethanol and water extracts of leaves of *Nicotiana tabacum* L. were valued at 0.85%, 0.56%, 11.52% and 12.30% respectively. This is consistent with our observation yielding the extractive values of different solvents. Ethyl acetate and ethanol extracts of *Nicotiana tabacum* L. leaves have been positively tested for triterpenoids, steroids, flavonoids, tannins, phenols and saponins (Sunil et al., 2010; Patil et al., 2015) which is in accordance with our investigation showing the presence of terpenoids, steroids, flavonoids, tannins, phenolic compounds and saponins in the leaves of *Nicotiana tabacum* L. Moreover, Shekins et al. (2016) indicated that the glycoside was positively tested in the leaves of *Nicotiana tabacum* L. which is similar to our result demonstrating the presence of cardiac glycosides in this leaf extract. The basic fraction in the essential oil 2,3'-dipyridyl isolated from Japanese flue cured tobacco leaf has been identified (Ōnishi & Yamasaki, 1957). This is in agreement with our study unveiling the positive test of essential oils in the *Nicotiana tabacum* L. leaves. Quinone formation plays an important role in the callus induction from *Nicotiana tabacum* L.; the activities of quinones are affected by the shaking condition on the tobacco callus growth in suspension culture (Husin et al., 2005). This is comparable with our study observing the presence of quinones in leaves of *Nicotiana tabacum* L. In agreement with our study, Alkhatib et al. (2013) reported the thick section of resins in roots and leaves of *Nicotiana tabacum* L. under the condition of light microscopy. Chen et al. (1975) isolated the polypeptide compositions of fraction I protein from ten cultivars of *Nicotiana tabacum* L. which is compatible with this study indicating the presence of polypeptides in the leaves of *Nicotiana tabacum* L.



Conclusion

Collectively, the different extracts of *Nicotiana tabacum* L. leaves contain the phytochemical components of alkaloids, phenolic compounds, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, essential oils, resins, saponins, quinones and polypeptides. This study provides the phytochemical information in term of identification and authentication of different extracts of leaves of *Nicotiana tabacum* L. growing in Cambodia.

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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 bialfrae* leaf. Int. J. Invent. Pharm. Sci. 1(5):428-432.
- Alkhatib R, Bsoul E, Blom DA, Ghoshroy K, Creamer R, Ghoshroy S (2013) Microscopic analysis of lead accumulation in tobacco (*Nicotiana tabacum* var. Turkish) roots and leaves. J. Microsc. Ultrastruct. 1:57-62.
- Azwanida NN (2015) A review on the extraction methods use in medicinal plants, principle, strength and limitation. Med. Aromat. Plants 4(3):1-6.
- Banu KS, Cathrine L (2015) General techniques involved in phytochemical analysis. Int. J. Adv. Res. Chem. Sci. 2(4):25-32.
- 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.
- Binorkar SV, Jani DK (2012) Traditional medicinal usage of tobacco-a review. Spatula DD 2(2):127-134.
- Chen K, Kung SD, Gray JC, Wildman SG (1975) Polypeptide composition of fraction I protein from *Nicotiana glauca* and from cultivars of *Nicotiana tabacum*, including a male sterile line. Biochem. Genet. 13:771-778.



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Djaafar Z, Ridha OM (2014) Phytochemical study of selected medicinal plant, *Solanum nigrum*, the Algerian Dessert. Int. Let. Chem. Phys. Astron. 1:25-30.

Firdouse S, Alam P (2011) Phytochemical investigation of extract of *Amorphophallus campanulatus* tubers. Int. J. Phytomed. 3:32-35.

Groark KP (2010) The angel in the gourd: ritual, therapeutic, and protective uses of tobacco (*Nicotiana tabacum*) among the Tzeltal and Tzotzil Maya of Chiapas, Mexico. J. Ethnobiol. 30(1):5-30.

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.

Husin MAG, Hasan M, Taha RM (2005) Callus induction from tobacco (*Nicotiana tabacum*) leaf explants for the production of quinone. Dev. Chem. Eng. Mineral Process. 13(5/6):563-572.

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.

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.

Ōnishi I, Yamasaki K (1957) Studies on the essential oils of tobacco leaves. Bull. Agr. Chem. Soc. Japan 21(3):177-180.

Patil RS, Desai AB, Wagh SA (2015) Comparative study of antimicrobial compounds extracted from leaves of *Nicotiana tabacum* and cigarette. World J. Pharm. Pharm. Sci. 4(3):1511-1518.

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

Rawat A, Mali RR (2013) Phytochemical properties and pharmacological activities of *Nicotiana tabacum*: a review. Indian J. Pharm. Bio. Res. 1(1):74-82.

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



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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.

Shekins OO, Dorathy EU, Labaran ML, Joel P (2016) Phytochemical screening of tobacco (*Nicotiana tabacum*) and its effects on some haematological parameters and histopathology of liver and brain in male rats. Int. J. Biochem. Res. 14(4):1-9.

Sunil K, Paras S, Amit J, Mukesh SS (2010) Preliminary phytochemical screening and HPTLC fingerprinting of *Nicotiana tabacum* leaf. J. Pharm. Res. 3(5):1144-1145.

Sunil K, Sayeed A, Paras S (2011) Pharmacognostic evaluation and HPTLC fingerprinting of *Nicotiana tabacum* stem collected from different geographical regions of India. Der Pharm. Sin. 2(5):1-11.