



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading

doi: 10.1016/S2222-1808(14)60654-X

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Phytochemical screening and antioxidant activity of different solvent extracts from *Strychnos minor* Dennst leaves

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PEER REVIEW

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Comments

The study is good where the authors describe about the phytochemical profile, total flavonoids content, total polyphenols content, DPPH radical scavenging activity and chelating effects on ferrous ions in various extracts of a rare south Indian medicinal plant. Thus through this study new drug compounds from plants can be developed for the welfare of the humans to overcome various health problems.

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ABSTRACT

Objective: To study the phytochemical class and its antioxidant activity of various extractions of *Strychnos minor* (*S. minor*), a rare endemic medicinal plant of South India.

Methods: Leaves of *S. minor* were collected from Agasthiyar Malai hills of South India and shade dried. Different solvent extractions were made for the preliminary screening of phytochemicals and their total flavonoids and polyphenols levels were quantified using standard procedures. Finally, the extracts were studied for their antioxidant potential by 1,1-diphenyl-2-picrylhydrazyl and chelating effects on ferrous ions.

Results: Phytochemical screening of the plant shows the presence of various biochemicals like alkaloid, tannins, terpenoids, saponins, flavonoids and resins and absence of phlobatannins. Flavanoids were high with water extracts (6.14 mg/g) and showed high 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (77.25%) whereas total polyphenols was high with methanol extraction (12.12 mg/g). Ethylacetate extract showed poor flavanoid and polyphenol content which reflected their low antioxidant potential when compared to other extracts.

Conclusions: The present study confirms the presence of various phytochemicals which shows good antioxidant potential of *S. minor*. This work will be helpful to explore the biochemical profile and active compound identification in the field of pharmaceutical research.

KEYWORDS

Strychnos minor, Endemic, Antioxidant activity, Medicinal plant, Polyphenols

1. Introduction

Strychnos includes nearly 200 species of trees and lianas distributing around the tropics. The common species of *Strychnos* is strychnine tree (*Strychnos nux-*

vomica), native to tropical Asia, and is the source of highly toxic compound strychnine. Out of the nineteen known species of Indian *Strychnos*, *Strychnos nux-vomica* and *Strychno spotatorum* are the commonly available species in the tropical areas. *Strychnos minor* (*S. minor*) Dennst.

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Foundation Project: Supported by Konkuk University Brain Pool fellowship, Konkuk University, Seoul, South Korea.

Article history:

Received 12 Feb 2014

Received in revised form 2 Mar, 2nd revised form 12 Mar, 3rd revised form 23 Mar 2014

Accepted 22 Jul 2014

Available online 29 Jul 2014

(Synonym: *Strychno scolubrina*) is an endemic plant belonging to the Loganiaceae and is a woody climber (liana) found in Tamil Nadu and Kerala (states of southern India). It is commonly known as snakewood and parts of the plant has a bitter taste. Leaves and roots of the plant are boiled in oil and applied to rheumatic swellings. Kani tribal people in Agasthiyar malai hills of Tamil Nadu use the plant's leaf paste for poisonous bites, particularly snake bites^[1]. The root, wood, stem, bark and seeds of *S. minor* contain β -sitosterol, alkaloids consisting of brucine and strychnine.

S. minor is found in some places of Coromandel Coasts, Western Ghats and Eastern Ghats in Tamil Nadu, India. Eighteen accessions of *S. minor* populations were recorded in the tropical dry evergreen forests in Coromandel Coasts of Tamil Nadu^[2]. We have noticed a prominent number of *S. minor* diversity in Agasthiyamalai hills of Tamil Nadu, India. The plant is also found in Karayar, Papanasam, Kalakad and Manjolai areas of Tirunelveli hills, Tamil Nadu, India^[3]. The plant has been collected for its medicinal purposes and also removed from its natural vegetation for fencing and plantation purposes without knowing its importance and endemic status. The seeds of *Strychnos* species germinate poorly and the seeds which fail to germinate under favorable environmental conditions are considered to be dormant^[4].

Antimicrobial resistance was found to be a serious issue stated by WHO 2013^[5]. Plants are rich in their metabolic content particularly during secondary metabolites. This wide spread medicinal plants and its diversity in their secondary metabolite profile give a constant encouragement for the scientist to extract novel therapeutical compounds. Even though there are some reports about the endemic medicinal plant *S. minor* traditionally using for various diseases^[1], the diversity of its secondary metabolites was unknown. Phytochemical screening of such an important medicinal plant will give prompt advantages in the discovery of novel medicine for the emerging diseases of humans. Because of these reasons the present study was made to identify the diversity of this plant in secondary metabolites by using different extracts along with its antioxidant activity.

2. Materials and methods

2.1. Collection of *S. minor*

Leaves of *S. minor* were collected from the tropical dry evergreen forests in Coromandel coasts of Tamil Nadu,

Southern India (Figure 1). The leaves with flowers and fruits of *S. minor* (Figure 2) were collected, shade dried, and used for the extraction and analysis.

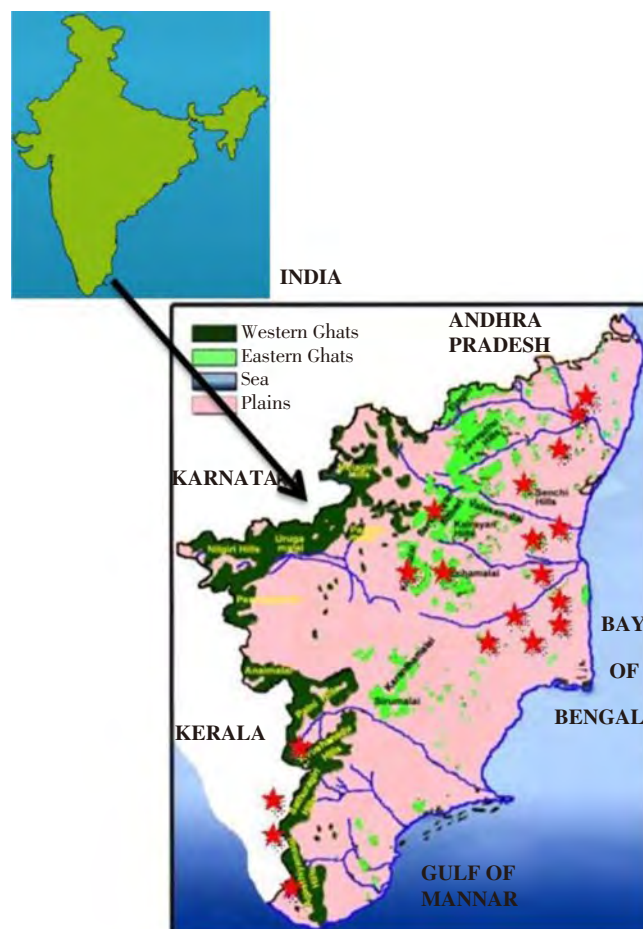


Figure 1. Distribution of *S. minor* in Tamil Nadu, India.



Figure 2. Photographs of *S. minor* collected from tropical dry evergreen forests in Coromandel coasts of Tamil Nadu, Southern India.

A: Leaves and flowers; B: Fruits.

2.2. Plant extracts

Two gram of powdered leaf was extracted with 20 mL of ethanol, methanol and hot water. The extracts were kept under sonication for a period of 10 min followed by centrifuging at 8000 r/min for 10 min. The supernatants were collected separately. The extraction process was repeated three times by using the residue after centrifugation. The collected supernatant was dried using vacuum dryer. Thereafter extracts were taken by

redissolving in 10 mL of methanol and then centrifugation followed by filtration. A separate extraction was made using 2 g samples along with hot water extraction following the above methods. After bringing down the temperature to room condition, equal volume of ethyl acetate was used twice to separate the metabolites from water extracts. The ethyl acetate and water layers were separately dried and served as extracts.

2.3. Phytochemical screening

Since the plant is an endemic and rare medicinal plant, only few leaves were collected and shade dried. The previous method about extracting and analyzing the plant required high quantity of raw materials, therefore, a modified procedure of Jeyaseelan and Jashothan, (2012) was applied for the extraction and analysis of phytochemical screening as well as quantification and antioxidant studies[6].

2.3.1. Test for tannins

The tannins in the extracts were tested by using 250 μ L of extract solution with 500 μ L of distilled water and two drops of ferric chloride solution. Appearance of blue black coloration in the test confirms the presence of tannins.

2.3.2. Test for terpenoids

Terpenoids were tested by using 500 μ L of extract mixed with 200 μ L of chloroform in a test tube. Concentrated sulfuric acid (300 μ L) was added to the mixture carefully and observed interface with a reddish brown coloration.

2.3.3. Test for saponins

Saponins in the extracts were tested by adding 2 mL of extract in a test tube and were shaken vigorously to obtain a stable persistent froth. Mixing of two drops of olive oil in the froth allowed for the formation of an emulsion, which indicated the presence of saponins.

2.3.4. Test for flavonoids

Yellow color appearance when the extract (1 mL) was added with three drops of 1% ammonium solution, which indicates the presence of flavonoids.

2.3.5. Test for cardiac glycosides

The extract (500 μ L) was mixed with glacial acetic acid (200 μ L) containing one drop of ferric chloride and the mixture was added to 100 μ L of concentrated sulfuric acid. Formation of a brown ring indicates the presence of cardiac glycosides.

2.3.6. Test for phlobatannins

Extract (1 mL) was boiled with 1% hydrochloric acid (1 mL) and the appearance of red precipitate indicated the presence of phlobatannins.

2.3.7. Test for alkaloids

Hydrochloric acid (1%, 500 μ L) along with 1.5 mL of extract in a test tube was treated with three drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids.

2.3.8. Test for resins

Copper solution (1 mL) was added to the extract (1 mL) and shaken vigorously. Green precipitate was observed as the presence of resin.

2.4. Total flavonoids content

Total flavonoids content was estimated by using 96-well microplate reader by adding 20 μ L of each extracts along with 180 μ L of 90% diethylene glycol and 20 μ L of 1 mol/L NaOH. After 15 min the absorbance was measured at 515 nm, and flavonoids content was expressed as milligram of naringin equivalents per gram of tissue[7].

2.5. Total polyphenols content

The total phenolics content of the different extracts were measured by adapting the modified procedure of Isabelle *et al.* (2008) using a 96-well microplate reader[8]. Twenty microliters of extracts taken in different wells were added with 100 μ L of 0.2 mol/L Folin-Ciocalteu's phenol reagent. Saturated sodium carbonate (80 μ L) was added to the mixture after 3 min and incubated at room temperature for 1 h. The absorbance was measured at 750 nm, and the results were expressed in milligram gallic acid equivalent per gram of tissue. All the data was analyzed statistically ($P < 0.05$) and based on that the error bar was plotted and compared.

2.6. DPPH radical scavenging activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the different plant extracts were compared by adapting the procedure of Kim *et al.*, 2013[8]. Extract (20 μ L) was added in 96-well microplate and 180 μ L of DPPH was added to the wells. Methanol (20 μ L) served as blank and after 20 min incubation the optical density were measured at 515 nm. Results were analyzed statistically and the percentage of antioxidant potential was calculated based

on the difference between the blank and treated samples, using the following calculation:

$$\text{Scavenging(\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where A is absorbance, the control contained only methanol and samples contained the soybean extract.

2.7. Chelating effects on ferrous ions

The extracts were measured for its metal chelating activity by using modified procedure of Dinis *et al*[9]. In brief, 20 μL of extracts were placed under 96-well plates and 10 μL of 1 mmol/L FeCl_2 was added and mixed. After 1 min 170 μL of 5 mmol/L ferrozine was added to the mixture, and after 30 min the optical density was measured at 562 nm. The inhibition percentage of ferrozine- Fe^{2+} formation was calculated by using the same formula of DPPH radical scavenging activity.

3. Results

The plant extracts were tested for its various phytochemicals. The results are presented in Table 1. The terpenoids were observed with methanol, ethyl acetate, hot water and water layers; whereas tannins, saponins and flavonoids were observed with hot water and water layers extracts. Cardiac glycosides only observed with methanol extracts suggested that further confirmation for this phytochemical was required since other extracts didn't contain this compound. In all the extracts phloba tannins were not observed which indicated either the non-existence of the compound in the plant or the need of an extraction with other solvents for confirming this phytochemicals. Alkaloids and resins were observed with ethanol, methanol and hot water extracts, confirming the presence of alkaloid bioavailability in the plant.

Table 1

Phytochemical screening of an endemic medicinal plant of south India (*S. minor*).

Phytochemicals	Et ^a	Me ^b	Wa ^c	EA ^d	RW ^e
Tannins	–	–	+	–	+
Terpenoids	–	+	+	+	+
Saponins	–	–	+	–	+
Flavonoids	–	–	+	–	+
Cardiac glycosides	–	+	–	–	–
Alkaloids	+	+	+	–	–
Resins	+	+	+	–	+
Phlobatannins	–	–	–	–	–

^a: Ethanol; ^b: Methanol; ^c: Hot distilled water; ^d: Ethyl acetate; ^e: Water collected after ethyl acetate partitioning; +: Presence of the phytochemicals; -: Absence of the phytochemicals.

The total flavonoids and polyphenols contents were

measured with different extracts and presented in Figure 3. Results revealed that in all the extracts the amount of flavonoids was less than 6 mg/g. This compound was comparatively less with ethanol (4.00 mg/g) and methanol (1.72 mg/g) when compared to hot water (6.14 mg/g) extract. Since this plant has other metabolites, it affects the dissolving of this compound to be low in the analysis levels. ethyl acetate registered low level of flavanoids (0.97 mg/g) and this result coincided with that of Sathishkumar *et al.*, 2013[10], whereas water extracts showed high yield in flavanoid extraction. However, total polyphenols content was high with methanol (12.12 mg/g) followed by ethanol (10.21 mg/g) extracts, which suggests that the plants are rich in total polyphenols.

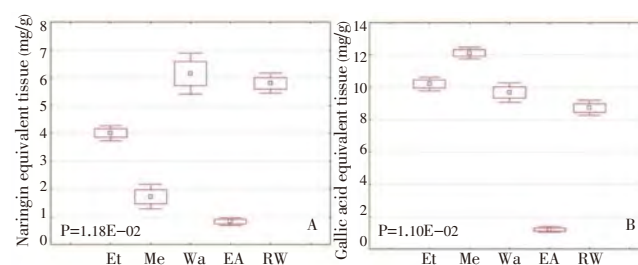


Figure 3. Total flavonoids and polyphenols content of an endemic medicinal plant of south India (*S. minor*) extracts.

A: Flavonoids; B: Polyphenol; Et: Ethanol; Me: Methanol; Wa: Hot distilled water; EA: Ethyl acetate; RW: Water collected after ethyl acetate partitioning.

Antioxidant activity of the different extracts were compared and presented in Figure 4. The DPPH radical scavenging potential of the ethanol (44.32%) and methanol (62.98%) extracts registered lower than hot water (77.25%) extracts. Based on the total flavonoids analysis it was clear that the levels of flavonoids were low in ethanol and methanol extracts, and are high in hot water extracts, which reflects their DPPH scavenging potential high. Here the ethyl acetate extracts registered low (16.94%) and these results directly correlated with flavonoid content of the extracts. Based on the preliminary screening flavonoids were low in the extracts resulting variation in their antioxidant potential. In contrast, the metal chelating activity was high in ethanol (23.34%) followed by hot water (21.91%) extracts.

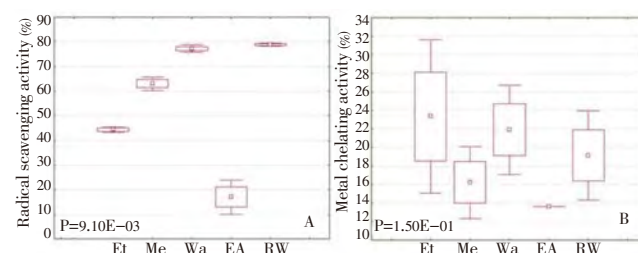


Figure 4. Free radical scavenging potentials of various extracts from an endemic medicinal plant of south India (*S. minor*).

A: DPPH; B: Metal chelating activity; Et: Ethanol; Me: Methanol; Wa: Hot distilled water; EA: Ethyl acetate; RW: Water collected after ethyl acetate partitioning.

4. Discussion

Water was found to be one of the best sources for the extraction of alkaloids from plants. According to Wang *et al.*, (2012) the water extract showed very high extractability of alkaloids than that of ethanol^[11]. Commonly, flavonoids were extracted by methanol, ethanol, hot water and acetone since these compounds were highly dissolved with these solvents. According to Upadhyay *et al.*, (2013) the total phenolic compounds were highly extracted by acetone whereas total flavonoids with methanol^[12]. But the present study shows poor yield with methanol and ethanol, this is due to solubility of the flavanoids were strongly affected by the nature of the solvent and the flavonoid structure^[13]. On the other hand, the bioavailability of the compounds plays a vital role in the extraction and its dissolving potential against various solvents. Hence, some other compounds like terpenoids and alkaloids dissolved highly with the methanol and ethanol resulting in the variation of the extracts. *Moringa oleifera* was studied for its biochemical classes by various test and confirmation for their antioxidant potentiality^[14]. Flavonoids are rich in antioxidant potential compound produced by plants^[15], and highly dissolved in hot water^[16,17]. Variation in polyphenols and flavonoids content of plants directly influences their antioxidant potential. Since flavanoids and polyphenols are highly dissolved in water and show higher activity in their DPPH radical scavenging potential^[17]. Alkaloids are a good source of free radical scavengers, hence our results were well correlated with Jang *et al.*, 2009^[18], stating the hydroxyl radical scavenging activities of isoquinoline alkaloids. Based on the biochemical screening and quantification of flavonoids, it was confirmed that their levels found to be low within this plant, meanwhile, alkaloids and other terpenoids were highly presented. Since this plant contains high amount of other metabolites like terpenoids and alkaloids reflects high metal chelating activity with their corresponding extractions^[19].

Phytochemicals are important for the plants to protect themselves from various pests and diseases. Knowing its immense medicinal properties they were used from ancient time as a source of medicine^[20]. The research about medicinal plants to screen phytochemical classes is very important since these plants show good activity against chronic diseases like cancer and heart diseases by scavenging free radicals^[21,22]. Studies of biological active compounds in medicinal plants demonstrated the importance of identifying new metabolites in the area of human diseases. The phytochemical screening analysis

of *S. minor* revealed the presence of saponins, cardiac glycosides, tannins, alkaloids, flavonoids and terpenoids but presence of phlobatannins was not confirmed. Among the phytochemicals not only flavonoids but also terpenoids, tannins and alkaloids are good sources of antioxidant and antimicrobial compounds. To explore the phytochemical profiles and to study the properties will be helpful for humans to overcome various health problems. The present study will be advantageous for scientists who engage in new active compound profiling and developing drugs against various diseases.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to Mr. Joshua Thangaraj and Mrs. Joan Thangaraj for their constant help during the experiment. This study was financially supported by Konkuk University Brain Pool fellowship, Konkuk University, Seoul, South Korea.

Comments

Background

Phytochemical screening of such important medicinal plants will give prompt advantages in the discovery of novel medicine for the emerging diseases of humans.

Research frontiers

Studies have been performed on *S. minor*, a rare endemic medicinal plants of South India, about its phytochemical class and its antioxidant activity of its various extracts.

Related reports

The result obtained for flavanoids was similar to that of Thiagarajan *et al* (2013). The results obtained for alkaloids were well correlated with Jang *et al* (2009).

Innovations & breakthroughs

Exploration of phytochemical profiles of *S. minor* in all the extracts phlobatannins were not observed indicating either the non-existence of the compound in the plant or the need of an extraction with other solvent conforming

this phytochemicals.

Applications

The study will be useful to overcome various health problems. The present study will be advantageous for scientists who engage in new active compound profiling and developing drugs against various diseases.

Peer review

The study is good where the author describes about the phytochemical profile, total flavonoids content, total polyphenols content, DPPH radical scavenging activity and chelating effects on ferrous ions in various extracts of rare south Indian medicinal plant. Thus through this study new drug compounds from plants can be developed for the welfare of the humans to overcome various health problems.

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