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PRELIMINARY PHYTOCHEMICAL SCREENING OF ROOTS AND AERIAL PARTS OF *LEPTADENIA PYROTECHNICA*

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Abstract

Leptadenia pyrotechnica (Forssk.) Decne is a medicinal plant that is native to hot deserts of Pakistan. This plant is sporadically known with reference to bioactivity including phytochemical screening especially from Pakistan. The present study was designed to screen out four major groups of phytochemicals such as alkaloids, flavonoids, saponins and tannins from eight solvents based roots and aerial parts extracts viz., hexane, chloroform, acetone, ethyl acetate, butanol, ethanol, methanol and water of the selected plant. The qualitative screening showed the presence of all major groups of phytochemicals in both plant parts extracts in which methanolic ones were the most efficient that extracted all the selected classes of phytochemicals. Quantitative screening revealed various concentrations of selected phytochemicals in both plant parts. The alkaloid contents were 3.267±0.643and 3±0.6 in roots and aerial parts respectively ($p>0.05$). The total flavonoid content was 76.867±2.266 and 139.448±8.677 QE/100g in roots and aerial parts respectively. In the case of total saponin contents, the proportions were 0.34±0.013% and 0.46±0.010% in roots and aerial parts respectively, whereas; total tannin contents were 62.713±4.841 and154.961±5.853 mg of TAE/100g of extract in roots and aerial parts, respectively. This study will serve as a benchmark for further pharmacological studies on the said plant that may be harnessed for drug development in the future.

Key words: *Leptadenia pyrotechnica*, Asclepiadaceae, Flavonoids, Saponins, Alkaloids, Tannins, Pharmacological studies.

Introduction

Phytochemical screening refers to screen a plant or plant parts for the presence of plant constituents. This screening may either be qualitative or quantitative based on the aim of the study. Among all phytochemicals, flavonoids, saponins, tannins and alkaloids are well-known for their medicinal impacts including antioxidant and antimicrobial. People living in rural areas of Pakistan prefer plant based drugs for therapeutic activities which are readily available and inexpensive (Mahmood *et al*., 2012). *Leptadenia pyrotechnica* (Forssk.) Decne (Family Asclepiadaceae) is one of such medicinal plants reported to be used as a source of ethnomedicine in hot deserts of Pakistan. It is an erect, profusely branched, evergreen shrub that attains 3-4 meter height, which is generally leafless or has very minute deciduous leaves. The plant has prolonged and extensive root system mostly distributed in the hot sandy deserts of Sindh, Punjab and Baluchistan. The pods of this shrub are cooked by the residents of deserts as a delicious vegetable (Qureshi *et al*., 2012). As an ethnoveterinary purpose, this plant is boiled in water and given to cattle after delivery for the expulsion of placenta in the Nara Desert (Bhatti *et al.*, 2011). Medicinally, this plant is reported as antispasmodic, anti-inflammatory, purgative, anti-rheumatic, anti-spasmodic, anti-histaminic, antibacterial, diuretic, urolith expulsion and expectorant (Moustafa *et al*., 2009). Previously, there is sporadic information available regarding phytochemical screening of *L. pyrotechnica* (El-Ghani *et al*., 2003; Panwara & Tarafdarb, 2006; Moustafa *et al*., 2007; Alqasoumi *et al*., 2012), especially from Pakistan. Earlier, the authors reported antibacterial and antioxidant activities of this plant (Munazir *et al.*, 2012, 2015). Keeping in view, the present study was carried out for qualitative and quantitative phytochemicals screening of roots and aerial parts for seeking active secondary metabolites.

Materials and Methods

Identification and collection of samples: Plant materials of *Leptadenia pyrotechnica* were collected from Thal desert, Punjab, Pakistan. Flora of Pakistan used for identification and authentication of plant specimens (Ali, 1983). The voucher specimen (RQ-2236) was deposited in the Herbarium of Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan.

Preparation of powdered samples and extracts: The powdered samples of both plant parts and subsequent extractions were carried out in organic solvents ranging from non-polar to polar solvents (i.e. N-hexane, chloroform, acetone, ethylactate, butanol, ethanol, methanol and water) using exhaustive extraction procedure as described by (Munazir *et al*., 2012). Afterwards, the extracts were subjected to a qualitative assessment of different classes of phytochemicals by using slightly modified methods described by (Ekpo & Etim, 2009).

Qualitative phytochemical screening

Test for alkaloids: Dilute hydrochloric acid (10 ml) was added to one gram of each solvent-free dried extract and filtered. The filtrate was tested for the presence of alkaloids using different reagents. The first one was Mayer's test in which one ml of filtrate, 2-3 drops of Mayer's reagent were introduced to one side of the test tube and the form of cream or white precipitates was observed at the interface. While the second test was Dragendorff's test in which to every 3 ml of filtrate, 1-2

ml of Dragendorff's reagent was added and observed in the formation of prominent yellowish precipitates.

Test for phenolic compounds: Presence of phenolic compounds in the plant extracts was determined by employing two methods, the first test was a ferric chloride test in which distilled water (5 ml) was added to each solvent extract (50 mg), and constantly shook for a minute to dissolve. Five drops of 5% ferric chloride solution were added and observed for dark green coloration of resultant mixture. The other test was lead acetate test in which three milliliters of distilled water were added to fifty milligrams of each extract and shook for a while. After that, three milliliters of ten percent lead acetate were added and observed in the formation of white precipitates.

Test for flavonoids: Flavonoids in the extracts were determined by two different tests. The first test was an alkaline reagent test in which aqueous solution of each solvent extract was treated with 10% ammonium hydroxide solution and observed for yellow fluorescence in the resultant mixture. The other test involved the addition of few strips of magnesium and concentrated hydrochloric acid to a half gram of each extract of both plant parts and observed for the appearance of faint orange coloration.

Test for tannins: Half gram of extract of each plant part was mixed with 1 ml of distilled water and 2-3 drops of ferric chloride solution and observed in the formation of blue black or blue green precipitates.

Test for saponins: To each solvent extract (50 mg), 20ml of distilled water was added in a graduated cylinder, soaked for 15 minutes and observed for formation of 2 cm layer of foam on the surface.

Quantitative phytochemical screening

Total alkaloid contents: Ethanolic acetic acid (10%, 200 ml) was added to 5g plant powder and incubated for 4 hours. Filtered the mixture and reduced to 1/4th volume and dilute ammonium hydroxide drops were added and precipitates were washed, dried and weighed out.

Total tannin contents: Method of (Polshettiwar *et al*., 2007) was employed to determine total flavonoids content from roots and aerial parts of *L. pyrotechnica*. For this purpose, from 1 g/ml stock solution of tannic acid in distilled water, dilutions of 20, 40, 60, 80 and 100 mg/ml were prepared. Each dilution (0.1 ml) was taken in a flask and half milliliter of Folin-Denis reagent, one milliliter of 0.5% (w/v) sodium carbonate solution was added. Water was added to make the final volume of this mixture to 10 ml and the resultant mixture was observed for its absorption spectrophotometically (755 nm) after twenty minutes against a blank that was devoid of tannic acid. Plant extracts (1 mg/ml) and total tannins in respective extracts were determined from the standard calibration curve and expressed as total tannin content (mg of TAE/100 g of extract) as explained by Sharma *et al*. (2011).

Total flavonoid contents: Aluminium chloride colorimetric method of (Chang *et al*., 2002) was employed to determine total flavonoids contents. 1 ml of deionized water was added to 100 mg of methanolic extract of each plant part. 1.5 ml of 95% ethanol was added to 0.5 ml of this solution and shook well. After that, 10% aluminium chloride hexahydrate (0.1 ml) and 1 Mol potassium acetate was added to the mixture. At the end, 2.8 ml of deionized water was added to the mixture, incubated for 40 minutes at 25^{o}C and the absorbance was recorded spectrophotometrically at 415 nm for reaction mixtures of both plant parts against a blank. Standard (Quercetin) was used to generate standard calibration curve at different concentrations (0, 20, 40, 60, 80, 100 mg/l) and the results were expressed as mg quercetin equivalents per hundred grams of extract (i.e. Mg of QE/100 g). All of the samples were replicated thrice.

Total saponin contents: Method of Obadoni and Ochuko (2001) was used to determine total saponin content from roots and aerial parts of *L. pyrotechnica*. For this purpose, 100 cm^3 of 20% aqueous ethanol were added to twenty grams of each of powdered samples and heated over a water bath at 55^{o}C for four hours while constantly stirring. Filtered the extract and the residue was washed with 200 ml of 20% ethanol, the resulting extracts were combined and reduced each sample to forty milliliters in a water bath adjusted at 90^{o}C and the resultant concentrate was transferred into a separating funnel. Afterwards, 20 ml of diethyl ether was added and the mixture was vigorously shaken for two to three minutes to recover the aqueous layer and the ether layer was discarded. The same procedure was repeated for purification of aqueous layer and 60 ml of n-butanol was added and combined extracts were washed with 10 ml of 5% aqueous sodium chloride. This final mixture was concentrated over a water bath in pre-weighed falcon tubes until complete dryness of the extracts. The saponin contents were left at the bottom, which were weighed out and percent saponin contents were determined.

Statistical analysis: Regression models (standard calibration curve) were built for quercetin and tannic acid to determine total flavonoids contents and total tannin contents respectively. Total alkaloids and saponin contents of each sample were determined by following formulae:

$$\text{Total alkaloids content} = \frac{\text{Weight of alkaloids}}{\text{Weight of plant powder}} \text{ X } 100$$

$$\text{Percent saponins} = \frac{\text{Weight of saponins}}{\text{Weight of plant powder}} \text{ X } 100$$

Results and Discussion

Qualitative phytochemical screening: Detection of five phytochemical classes of compounds viz., alkaloids, flavonoids, saponins, tannins and terpenoids was carried out for extracts prepared in eight different solvents. These selected compounds have been reported to have dramatic physiological antibacterial, antifungal and antioxidant

activities. Results of phytochemical screening of both plant parts are provided in Tables 1 and 2. In the case of aerial parts, methanol showed presence of all five phytochemical groups of compounds followed by ethanol (4 groups), acetone (3 groups), ethyl acetate, butanol (2 groups) while it was interesting to note that n-hexane extracts gave negative results for all phytochemical classes. Same was the case with the findings of Daud *et al*. (2011), where all other phytochemicals were missing except tannins in the said extract.

In the case of roots, alkaloids were detected in all solvent based extracts except in n-hexane and water. Flavonoids were detected in ethanol and methanol extracts only. Saponins were found in methanol and water, tannins in acetone, ethanol and methanol and terpenoids were expressed by all solvents except n-hexane, chloroform and water extracts. Roots extracts also showed the presence of all classes of compounds. Alkaloids were found in all solvent extracts except n-hexane, chloroform and water, flavonoids were detected in ethanol, methanol and water extracts, saponins in chloroform extracts, tannins in methanolic extracts and terpenoids in all extracts except n-hexane and butanol extracts. Methanolic extracts showed the highest richness in phytochemicals in roots as well, with the presence of four groups of compounds, followed by ethanol (3 groups), ethanol (3 groups), chloroform, acetone, ethyl acetate (2 groups), butanol (1 group) while n-hexane extract gave negative results for all phytochemical classes. Similar results were determined by Ashwani & Ashish (2012). A similar study was by Vijayalakshmi & Ravindhran (2012) for two selected medicinal plants *Diospyrus ferrea* and *Aerva lanata*. They observed presence of tannins, flavonoids, phlobatanins, alkaloids, reducing sugars, terpenoids and quinines in the aforementioned plants.

Quantitative phytochemical screening

Total alkaloid contents: Total alkaloid contents (TAC) were 0.15±0.03g (3±0.6%) and 0.163±0.032g (3.267±0.643%) in aerial parts and roots of *L. pyrotechnica* respectively, with non-significant difference ($p>0.05$) in TAC of both parts (Table 3). Difference in TAC of different plant organs was observed by Schneider & Wolfling (2004) for three selected plant species. Such differences may rightly be attributed to differences in genetic make-up, environmental impacts (including exposure to sunlight and growing conditions) and physiological functions of plant organs. The TAC determined in *L. pyrotechnica* is higher than many other plant species.

The TAC of *Coccinia cordifolia* (0.1117g/5g, 2.23%) as reported by Ganga *et al*. (2011) was lower than both plant parts of *L. pyrotechnica*. Similarly, TAC of powdered samples of *Ipomoea batata*, as determined by Ameyaw & Eshun (2009) was lower than both parts of *L. pyrotechnica* (345.65mg/100g i.e. 1.83%). Similarly, TAC of various parts of *Sophora flavescens* determined by Sandanov & Pankrushina (2011) ranged between 0.27 and 2.48%, which is lower than that of *L. pyrotechnica*. In some instances, TAC of some plants was higher than that of *L. pyrotechnica*. One such example is that of *Vitex negundo* (10% TAC) studied by Brindha *et al*. (2012). These differences in TAC between different plant species may be related to differences in genetic makeup, environmental impacts, stage of plant growth, method of analysis and others. Edeoga & Enata (2001) reported that alkaloids possessed analgesic and anti-inflammatory properties and their therapeutic values can be attributed to the presence of these phytochemicals in plants.

Table 1. Qualitative analysis of various solvent extracts of aerial parts of *Leptadenia pyrotechnica*.

Extractants	Alkaloids	Flavonoids	Saponins	Tannins	Terpenoides
S1	-	-	-	-	-
S2	+	-	-	-	-
S3	+	-	-	+	+
S4	+	-	-	-	+
S5	+	-	-	-	+
S6	+	+	-	+	+
S7	+	+	+	+	+
S8	-	-	+	-	-

Legend: S1= Stands for n-hexane, S2= Chloroform, S3= Acetone, S4= Ethylacetate, S5= Butanol, S6= Ethanol, S7= Methanol, S8= Water, where (+) stands for Presence and (-) for Absence

Table 2. Qualitative analysis of various solvent extracts of roots of *L. pyrotechnica*.

Extractants	Alkaloids	Flavonoids	Saponins	Tannins	Terpenoides
S1	-	-	-	-	-
S2	-	-	+	-	+
S3	+	-	-	-	+
S4	+	-	-	-	+
S5	+	-	-	-	-
S6	+	+	-	-	+
S7	+	+	-	+	+
S8	-	+	-	-	+

Legend: S1= Stands for n-hexane, S2= Chloroform, S3= Acetone, S4= Ethylacetate, S5= Butanol, S6= Ethanol, S7= Methanol, S8= Water, where (+) stands for Presence and (-) for A

Table 3. Total alkaloid content of aerial parts and roots of *L. pyrotechnica*.

Plant part(s)	W_1 (g) = Weight of Filter paper	W_2 (g) = Weight of filter paper + precipitates	Weight of alkaloids (g)	Percent alkaloids
Aerial parts	1.177 ± 0.021	1.32 ± 0.01	0.15 ± 0.03	3 ± 0.6
Roots	1.173 ± 0.032	1.34 ± 0.05	0.163 ± 0.032	3.267 ± 0.643

Table 4. Total saponin contents of aerial parts and roots of *L. pyrotechnica*.

Plant part(s)	W_1 (g) = Weight of Falcon tubes	W_2 (g) = Weight of falcon tubes + ppt	Weight of saponins (g)	Percent saponins
Aerial parts	10.63 ± 0.036	10.723 ± 0.035	0.093 ± 0.0021	0.46 ± 0.010
Roots	10.61 ± 0.02	10.678 ± 0.018	0.068 ± 0.0026	0.34 ± 0.013

Table 5. Total flavonoid contents of aerial parts and roots of *L. pyrotechnica*.

Plant part(s)	Absorbance at 415nm	R.E. (y=ax+b)	Total flavonoid contents (Mg of QE/100g of extract)
Aerial parts	1.351 ± 0.081	Y = 0.0093x + 0.0538	139.448 ± 8.677
Roots	0.769 ± 0.021	Y = 0.0093x + 0.0538	76.867 ± 2.266

Table 6. Total tannin contents of aerial parts and roots of *L. pyrotechnica*.

Plant Parts	Absorbance at 415nm	R.E. (y=ax+b)	Total tannin contents (Mg of TAE/100g of extract)
Aerial parts	0.129 ± 0.003	Y = 0.0043x + 0.0627	154.961 ± 5.853
Roots	0.089 ± 0.0021	Y = 0.0043x + 0.0627	62.713 ± 4.841

Total saponin contents: Total saponin contents (TSC) of aerial parts and roots of *L. pyrotechnica* were 0.46±0.010% (0.093±0.0021g) and 0.34±0.013% (0.068±0.0026g) with a significant difference ($p<0.05$) as shown in Table 4. These contents were lower than that of many plant species. For example, TSC of the stem, roots and leaves of *Ipomoea obscura* determined by Mungole *et al*. (2012) ranged between 120 and 149mg/g that are much higher than that from both parts of *L. pyrotechnica.* This difference may be attributed to differences in plant genera that are totally unrelated. Saponin contents of fruits of *Donax grandis* determined by[17] were 2.39%, which are quite higher than both parts of *L. pyrotechnica.* This difference may be regarded to differences in the plant parts. In addition to differences in plant genera and plant organs/parts, geographical conditions, nature of the soil (Ashwani & Ashish, 2012) and other factors also take part in changing the scenario of phytochemical contents of plants and the same may be the case with a difference in saponin contents of both parts of the selected plant. The study of TSC is of paramount significance in realizing the therapeutic potential of a plant. It has been mentioned by[17] that the saponins might be involved in free radicals scavenging activity of plant extracts. In addition, saponins are known to play role toward Na^{+}-Ca^{+2}influx and efflux thus maintaining a balance of these ions in the cardiac muscles (Ashwani & Ashish, 2012). In a recent study it has been highlighted that the saponins play cancer, lowering or preventive roles, especially in the case of dietary supplements (Mungole *et al*., 2012).

Total flavonoid contents: The TFC was found to be 139.448±8.677 and 76.867±2.266mg QE/g from aerial parts and roots of *L. pyrotechnica* respectively (Table 5). There was a significant difference ($p<0.05$) in TFC of both plant parts. Comparing with other plants, TFC of the under investigated plant was higher than *Acacia catechu*, *A. sinuata*, *A. nilotica*, *Albizia lebbeck*, *Caesalpinia sappan*, *Senna tora*, *Cassia fistula*, *Saraca asoca*, *Emblicaribes*, *Aervalanata*, *Biophytum reinwardtii*, *Jasminum gradndiforum*, *Holoptelea integrifolia*, *Gmelina arborea*, *Plumbago indica*, *Justicia adhatoda*, *Oroxylum indicum*, *Pseudarthria schulli*, *Hygrophila schulli*, *Ficus racemosa*, *F. microcarpa* having 8.54, 3.65, 7.86, 6.87, 7.58, 21.58, 9.56, 11.36, 1.35, 6.99, 1.86, 1.23, 1.08, 2.65, 0.65, 2.65, 0.08, 0.25, 0.35, 0.86 and 1.65 mg QE/100g TFC respectively (Sulaiman & Balachandran, 2012).

On the other hand, some studies reported higher TFC than the undertaken plant such as *Ipomoea batatas* (662.02mg/100g) studied by Ameyaw & Eshun (2009) and *Satureja hortensis*, *Majorana hortensis* and *Thymus vulgaris* with TFC ranging about 0.36 to 4.10% (Vábková & Neugebauerová, 201) and *Marrubium peregrinum* (18.72 to 54.77 mg/g rutin equivalents) reported by Stankovic (2011). Based on significant TFC values present in both plant parts, it is hypothesized that the undertaken plant possesses free radical scavenging activity that may be investigated further in detail.

Total tannin content: There was a significant difference in both parts ($p<0.05$) for yielding total tannins content (TTC) as shown in Table 6. The TTC of aerial parts of *L. pyrotechnica* was 154.961±5.853 (0.055%) and 62.713±4.841mg/100g (0.063%) respectively. Some studies reported various concentrations of TTC from many plants. For instance, *Indigofera aspalathoides* (34.59±1.788 mg/g Gallic acid, 0.035%) by Tamilselvi *et al*. (2012) was lower than present findings. On the other hand, the TTC of *Vitex negundo* was 15%, which is higher than present study (1.55 and 6.2% in aerial parts and roots) as reported by Brindha *et al*. (2012).

Conclusion

From these results, it can be concluded that all solvent-based extracts except n-hexane expressed the presence of all selected phytochemicals. Furthermore, root and aerial parts possessed various quantities of these phytoconstituents, so their presence may serve as antimicrobial and antioxidant activities. This study will serve as a benchmark for further pharmacological studies on the said plant that may be harnessed for drug development in the future.

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