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Identification And Relative Quantitation Of Major Medicinal Metabolites In Different Plant Parts Of *Putranjiva Roxburghii* Using Principal Component Analysis

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Abstract

Putranjiva roxburghii wall. is a valuable medicinal plant used in conventional therapeutic system to treat several diseases. It is an evergreen tree of tropical Asia, used in all system of ethno-pharmaceutical practices adopted in Asian countries. Phenolic compounds and flavonoids are the major metabolites reported in leaf, stem and root of this plant and are established to be associated with its medicinal importance. This study demonstrates that Principal Component Analysis (PCA) can be effectively used for identification and discrimination among major medicinal metabolites extracted with three different solvents, present in different plant parts of *Putranjiva roxburghii*.

Keyword: Medicinal metabolites, Principal Component Analysis, Relative Quantitation, *Putranjiva roxburghii*.

Introduction

Putranjiva roxburghii wall. is extensively used as medicinal plant in traditional medicine to treat variety of ailments. It is an evergreen tree of tropical Asia, used in all system of ethno-pharmaceutical practices adopted in Asian countries. Decoction of stem/ bark and seeds of the plant is used in snake bite and to treat intermittent fevers. The other traditional reports mentioned uses of this plant as treatment of azoospermia, burning sensation, ophthalmopathy, smallpox, and ulcers. It is also reported to be used as an anti-inflammatory, antipyretic, analgesic, and anti-rheumatic agent. Phenolic compounds and flavonoids are the major metabolites reported in leaf, stem and root of this plant and are established to be associated with its medicinal importance.

Our previous study comprises physicochemical, phytochemical and analytical evaluation of different plant parts of *P. roxburghii* (used in traditional system of medicines) by using standard methods in order to explore the authentic plant material's suitability for its traditional claims. The phytochemical and analytical evaluation was carried out for the determination of parameters such as, total phenolic and flavonoid contents estimation using HPLC (high performance liquid chromatography) and TLC. The different solvents were used for extraction fractionation of all plant parts and evaluated for the characterization of phytochemicals. Methanolic extracts contains highest amount of phenolic and flavonoid content. Leaf, root, and seed respectively contain high amount of phenolic while flavonoids content is abundant in stem part. The total phenolic as well as flavonoid content was assessed in three different solvents for all plant part. From hexane, chloroform and methanol fractions assessed in leaf stem, root and seed the methanol extract showed the highest percentage of total phenolic and flavonoid contents in all plant parts. The total flavonoid content was observed highest in stem (Siwach *et al*., 2024). It is important to establish a method for identification and discrimination among major metabolites present in different plant parts (root, stem, leaf, and seed). Principal Component Analysis is a statistical method to reduce multifactorial complexities of a data with multiple variables, it is a linear dimensionality reduction technique with applications in exploratory data analysis, visualization and data processing. The data in this statistical method is linearly transformed onto a new coordinate system such that the directions (principal components) capturing the largest variation in the data can be easily identified in terms of differential abundance. The present study is aimed to assess the quantitative and distributional variation of major metabolic compounds among the sample of different plant parts. Identification and relative quantitation of metabolites considering various differentiating factors of all plant parts is done by using principal component analysis (PCA).

Materials and Methods

Collection of plant materials

The plant material was collected wild from the local fields and other local market around Hisar district, Haryana, India, identified with the help of taxonomist and stored as a specimen in departmental herbarium of department of botany, school of applied sciences, Om Sterling Global University, Hisar, Haryana, India.

Solvent and Reagents

All the required solvent like distilled water and lab consumables were of analytical grade and procured from Qualigens. Lab grade reagents for carrying tests like analysis of alkaloid, carbohydrate, flavonoids, glycosides, proteins, phenolics, saponins, tannins etc. were procured from local scientific stores and prepared using Indian Pharmacopeia.

Preparation of plant material

The collected plant materials were washed with tap water and cut in to small pieces. These small pieces from different plant parts were air-dried thoroughly under shade (at room temperature). The dried materials were homogenized to fine powder and stored for further evaluation. This material was used for solvent extraction, and phytochemical analysis.

Statistical method

Total quantitative analysis of class of compounds data of the *Putranjiva roxburghii* plant part were summarized as Mean ± SD (standard deviation). Statistical average of each class of compounds content from all solvent system from all repeats of each plant part sample as shown in Supplementary

Table 1 was used to constituents the variables and each plant part were treated as cases. Principal component analysis (PCA) was used to reduce the data dimensionality and visualize the difference in abundance and discrimination of the plant parts of all samples of the *Putranjiva roxburghii* plant and only actual variations were applied to discriminate the cases. PCA analysis was carried out using statistical software STATISTICA Version 7.0 (State Soft Inc. USA).

Supplementary Table 1: Phytochemical classes present in different solvent fractions of different plant part extract of *Putranjiva roxburghii*

Class of compounds	Leaf			Stem			Root			Seed		
Alkaloids	–	–	–	+	+	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	–	–	–	+	+	+	–	–	–
Phenolic compounds	–	–	+		–	+	–	–	+		–	+
Saponins	–	–	–	+	–	–	+	+	+	+	–	–
Tannin	–	–	–	–	–	–	–	–	–	–	–	–
Terpenoids	–	–	–	+	+	+	+	+	–	+	+	+

*+ is treated as 1 and – is treated as 0 in statically significant averaging

Quantitative measurement of phytochemicals in *P. roxburghii* plant parts

Determination of total phenolic content in P. roxburghii plant parts

Quantity of phenolic compounds in samples was estimated using the Folin- Ciocalteu test method. Absorbance of samples was measured at 725 nm in comparison to a blank (distilled water).

Determination of total flavonoid content in P. roxburghii plant parts

A colorimetric analysis was used to assess the total flavonoid content using Aluminum chloride. One ml of extracts was mixed with methanol to make up a solution of up to two ml to (1 mg/ml) or typical concentrations of quercetin. In order to process the resultant mixture, 100µl of 10% $AlCl_3$, 100µl of 1 M CH_3COOK and 2.8 ml of distilled water were added. Sample was left for 30 minutes at ambient temperature after a thorough shaking. The absorbance was measured at 415 nm in comparison to blank.

Determination of tannic acid, coumarins and saponins

High Performance Liquid Chromatography (HPLC) was used to assess the tentative of amount tannic acid, coumarins and saponins in plant part of *P. roxburghii*. For all these three compounds' standards were run separately. The binary system in isocratic mode was run. The standard HPLC identification of Tannic acid were performed in the stem part of *P. roxburghii.* The calibration plots were drawn as shown to aces the peak response and content respectively. All calibration plots showed good linearity within the range of quantitation.

Table 1: Statistical average of each class of compounds content from all solvent system from all repeats of each plant part of *Putranjiva roxburghii*

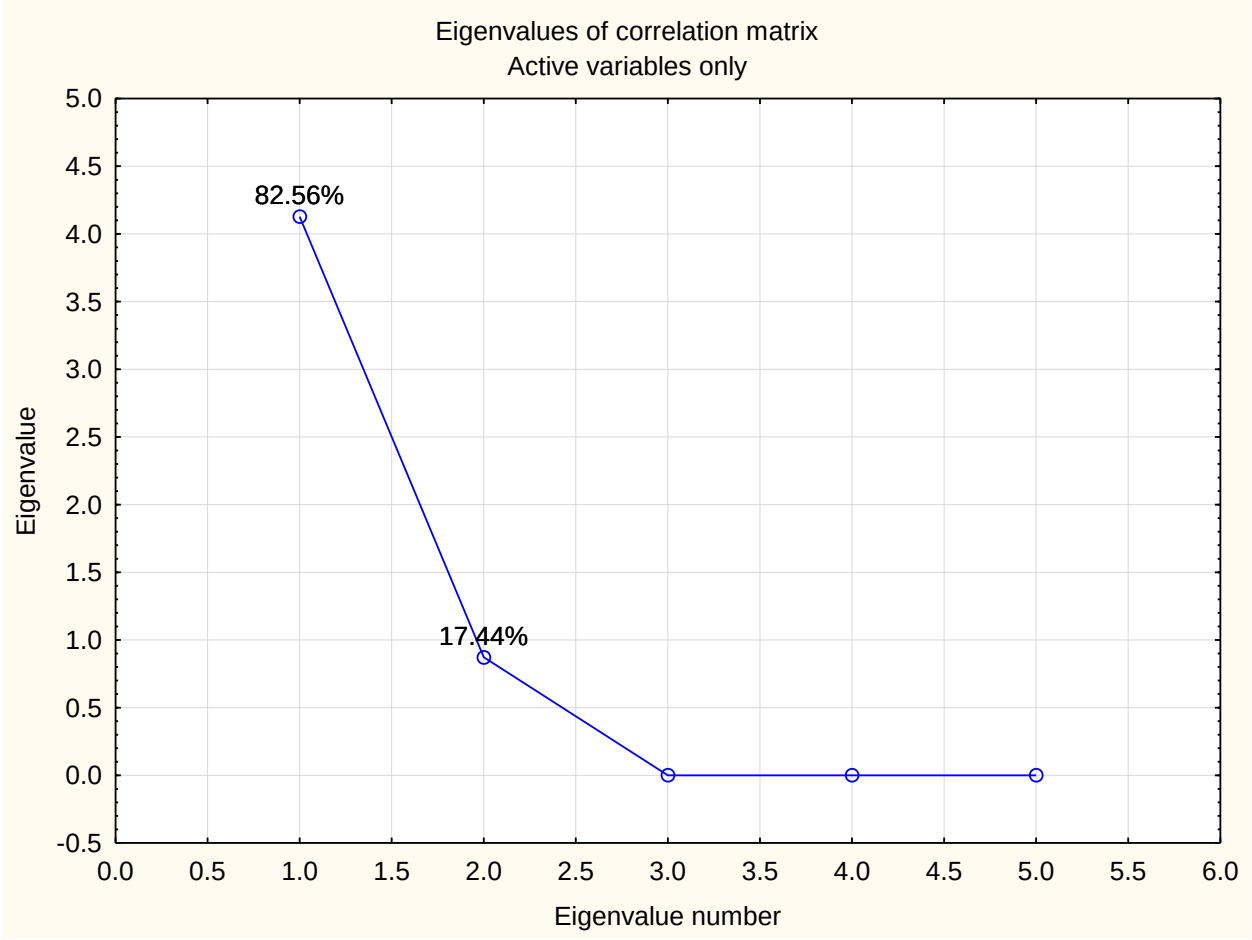
Class of Compounds	Leaf	Stem	Root	Seed
Alkaloids	0.0	1.0	0.5	0.5
Cardiac glycosides	1.0	0.0	0.5	0.5
Phenolic compounds	0.3	0.3	0.3	0.3
Saponins	0.0	0.3	0.2	0.2
Tannin	0.0	0.0	0.0	0.0

Terpenoids	0.0	1.0	0.5	0.5

Results

The presence of all the class of compounds was assessed in three different solvents for all plant part of *Putranjiva roxburghii* is shown in **Supplementary Table 1** were used to identifying the variability in content of all the plant part such as leaves, stem, root and seed of *Putranjiva roxburghii.*

The abundance of class of secondary metabolites such alkaloids, cardiac glycosides, phenolic compounds, saponins, and tannin, terpenoids is generally similar in the case of common plant sources however they may be unstable and easily affected by conditions such as geographical region, growing season, weather or bioclimatic zone (Cserháti, 2010; Gad et al., 2013; Zhang et al., 2016). Hence, an index needs to be introduced to evaluate the similarity, dissimilarity or discrimination of different samples of plant parts of the *Putranjiva roxburghii* from various sources. Therefore, the principal component analysis (PCA) technique has been applied to evaluate the distribution of class of compounds such as Alkaloids, Cardiac glycosides, Phenolic compounds, Saponins, Tannin and Terpenoids in different plant part sample of *Putranjiva roxburghii*. Generally quantitative analysis of marker compounds (purified metabolic compounds) in medicinal plant/herb is applied for their quality control. However, use of markers might not be always effective/ informative enough due to complexity of the plant metabolic matrix and it ignores the synergistic effects among compounds. Visualization of such complex plant matrix data is practically impossible without using some chemometric tool. This includes finding relationships between chromatograms or spectra of sample for classification or discrimination of samples (Gad et al., 2013; Workman et al.,1996; Zhang et al., 2016). PCA is most commonly used unsupervised technique for the analytical data interpretation and it is also a standard tool to compress and visualize large amounts of data. It allows the identification of the metabolic profile without necessarily identifying its chemical constituents and determines the discriminating factor or compounds. It then determines a smaller number of variables (here in this case it is content of pyrano-carbazoles alkaloids) that will account for most of the variance in the observed variables (Cserháti et al., 2010; Hur et al., 2013). The average content of class of compounds from all solvent systems were used as the variable and were subjected for the principal component analysis along with abundance in different plant part samples of *Putranjiva roxburghii* as cases. In geographical samples the first two principal components containing PC1 (Axis 1) and PC2 (Axis 2) hold variance of 82.56% and 17.44% respectively of the total 100.0% variability (**Figure 1 and Supplementary Figure S1**). Similarly, projection of variables uses principal components PC1 and PC2 having variance of 82.56% and 17.44% marker class of compounds for discriminating the different plant part of *Putranjiva roxburghii* (**Figure 2**).



Supplementary Figure S1: Screw plot from principal component analysis for the average content of class of compounds such as Alkaloids, Cardiac glycosides, Saponins and Terpenoids from all solvent systems in different plant part samples of *Putranjiva roxburghii*.

Discussion

These principal components (PC1 *vs* PC2) or factor analysis derives (experimental score on axis 1 and 2) were linear combinations of multiple quantitative variables from the average content of all class of compounds of all solvent systems that explain the largest percentage of the variation amongst all analysed plant part of *Putranjiva roxburghii*. The score plot of the projection of the variables and sample values onto the first two principal components revealed best sample population distribution. Thus, the PCs were able to explain 100.02% of the variability on the basis of average abundance of all classes in all parts. The loading plot shows how strongly each characteristic (class of compounds) influences a principal component (PC). The samples were discriminated in four major groups in stem, leaf, root and seed according to total similarity of content besides variation among them. The seed and root sample were more similar to each other in term of significant content variability hence came nearest to each other. The significance with standard error and deviation can be identified by the box and whisker plot (**Figure 3**) for the average content of significant class of compounds for discrimination such as Alkaloids, Cardiac glycosides, Saponins and Terpenoids which confirming the significance positive value and accuracy of each marker class in the plant part samples of *Putranjiva roxburghii* plant. It is evident from this study that PCA effectively can be used for the purpose of identification and discrimination of different plant parts and all the samples of different plant part of *Putranjiva roxburghii* could be effectively differentiated by this developed method.

Acknowledgement

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Author Contribution

DKM designed, conceived the study, and thoroughly reviewed the manuscript for its improvisation., SS collected plant materials and performed experiments, VB did partial analysis of metabolites,

Conflict Of Interest

The authors have no conflict of interest to declare.

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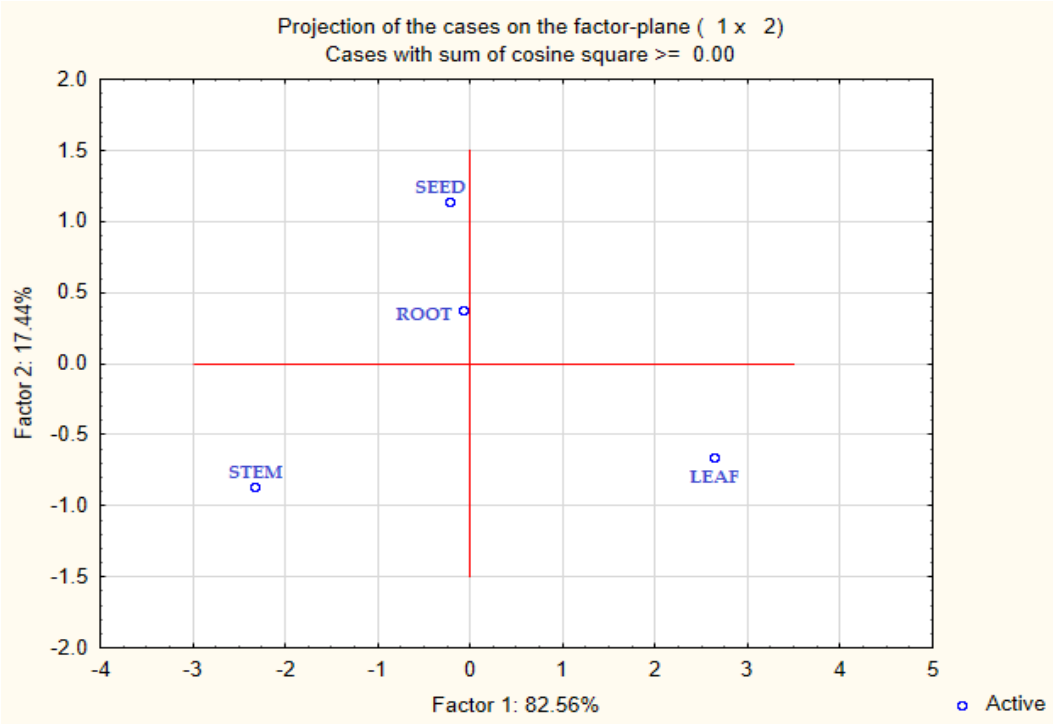

Figure 1: Principal component analysis for the average content of class of compounds such as Alkaloids, Cardiac glycosides, Phenolic compounds, Saponins, Tannin and Terpenoids from all solvent systems in different plant part samples of *Putranjiva roxburghii*. Projection of the contents of all classes onto the first two principal axes (+ and – indicate positive and negative correlations with the axes) and score plot to shows a distinctive discrimination between the different plant parts on the basis of only four statistically significant class alkaloids, cardiac glycosides, Saponins and Terpenoids.

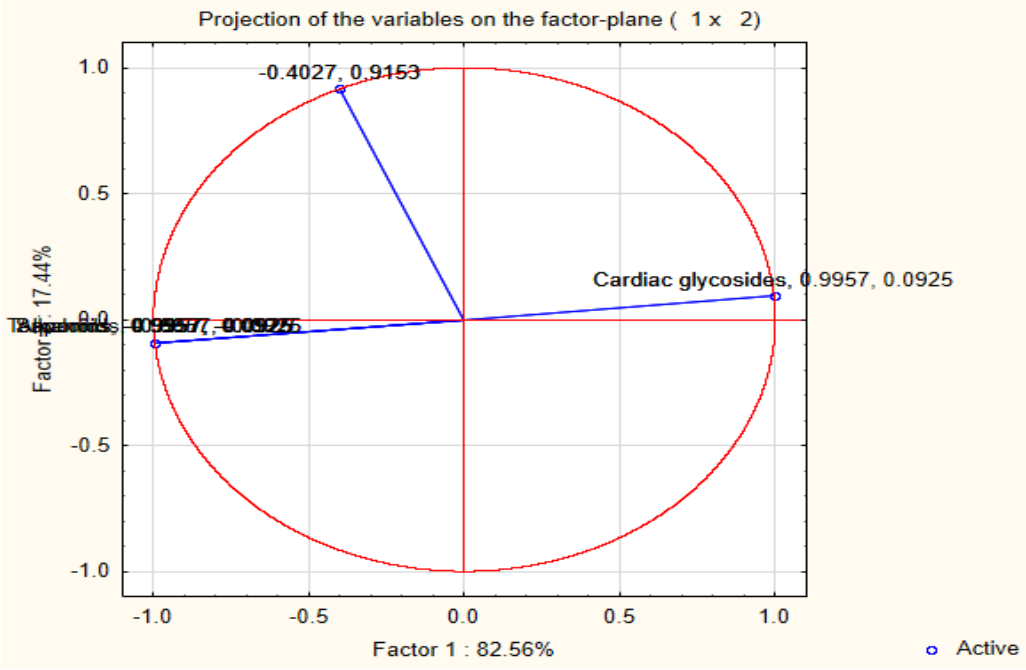

Figure 2: Projection of variables using principal component analysis for the average content of class of compounds such as Alkaloids, Cardiac glycosides, Saponins and Terpenoids in different plant part samples of *Putranjiva roxburghii* showing loading plot depicting marker classes for discrimination of the samples.

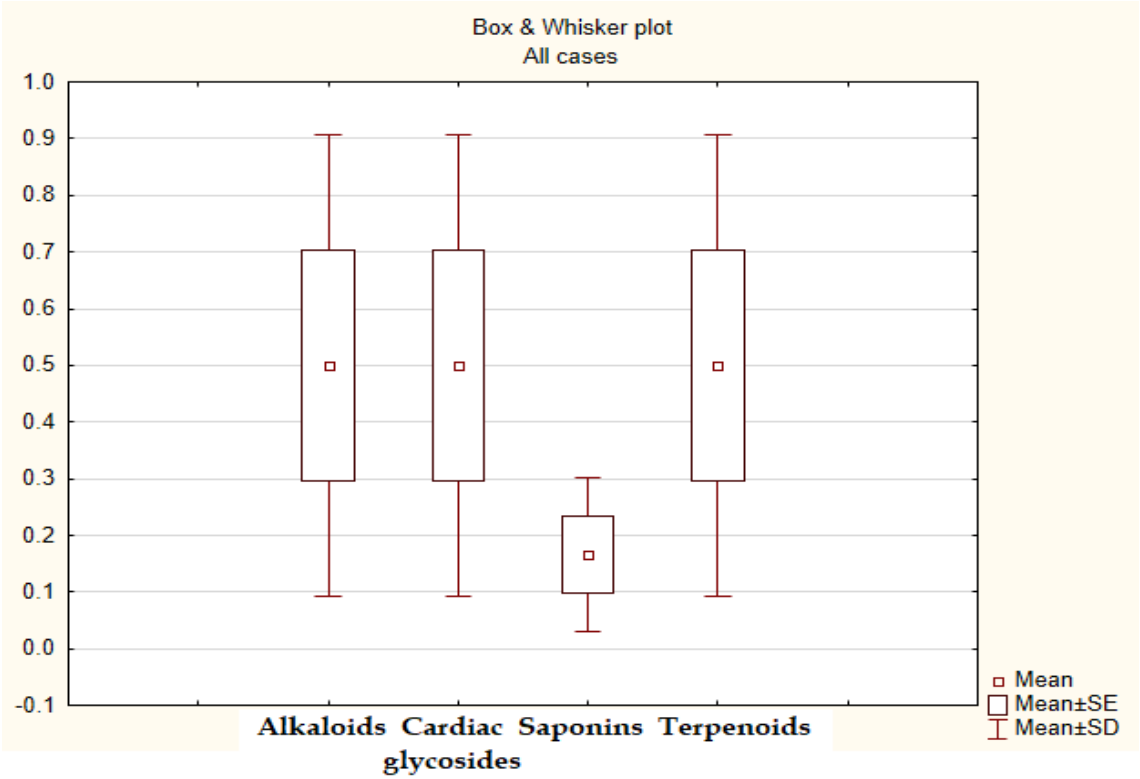

Figure 3: The box and whisker plot for the average content of significant class of compounds for discrimination such as Alkaloids, Cardiac glycosides, Saponins and Terpenoids showing significance positive value of each marker class in the plant part samples of *Putranjiva roxburghii*.