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Research Article

Extraction, Qualitative and Quantitative Determination of Secondary Metabolites of *Rumex Nepalensis* Roots

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ABSTRACT

In the Indian ayurvedic system of medicine, *Rumex nepalensis* Spreng. (Polygonaceae) commonly known as Nepal Dock has wide-spectrum therapeutic potencies and is extensively used for centuries in traditional medicine systems. They act as a possible food supplement and are largely used in pharmaceutical industry. Extracts and metabolites from this plant exhibits pharmacological activities including anti-inflammatory, antioxidant, antibacterial, antifungal, antiviral, insecticidal, purgative, analesic, antipyretic, anti-algal, central nervous system depressant, genotoxic, wound healing and skeletal muscle relaxant activity. Due to its remarkable biological activities, it has the potential to act as a rich source of drug against life threatening diseases. The aim of the present study is to examine *Rumex nepalensis* roots for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteu reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The total phenolics content of roots ethanolic extract was (1.658 mg/100mg), followed by flavonoids (1.048mg/100mg). The present study concluded that the crude extract of *Rumex nepalensis* is a potential source of various activates and this justifies its use in folkloric medicine.

Keywords: *Rumex nepalensis*, Qualitative analysis, Quantitative analysis, TPC, TFC, Folins Ciocalteu

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INTRODUCTION

The genus *Rumex* consists of about 250 species of herbs¹. *Rumex nepalensis* Spreng (*R. nepalensis*) belongs to family Polygonaceae and is a perennial, ascending herb², commonly known by the name Nepal dock. Though *R. nepalensis* is an agricultural weed but this wild plant is not really unwanted in the arena of traditional herbal medicines³⁻⁵. Several studies have confirmed striking medicinal benefits of this plant. The juvenile leaves of this plant are cooked as vegetables which gives an acidic-lemon flavor to dishes⁶. The young shoot is also locally eaten as a cooked vegetable⁷. This plant is also used as a colouring agent (dye)⁸. Green colour from the leaves of plants is often used in sweet preparations⁹. Phytochemical screening shows that the *R. nepalensis* (jangli palak) contains various constituents viz., triterpenoids, stilbene glycosides, tannic acid, saponins, resveratrol, sterols, amino acids, quercetin, alkaloid, phenolic components, flavonoids, anthraquinone glycosides, anthraquinones², vitamin C⁶, some cardiac glycoside, naphthalenes, and many more¹⁰. The foundations of modern drugs are based on these

natural compounds. In North Western Himalaya, *R. nepalensis* is a high value medicinal herb due to its high anthraquinone content¹¹. *R. nepalensis* has shown purgative, antioxidant, antifungal, antibacterial¹², antihistaminic, anticholinergic, antibradykinin, antiprostaglandin¹³, antipyretic, antiinflammatory, antialgal, insecticida^{2, 14}, analgesic and CNS depressant properties. The plant is also reported to possess skeletal muscle relaxant activity¹⁵. The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body. These non-nutritive components are called phytochemicals. The qualitative analysis as well as quantification of phytochemicals of a medicinal plant is regarded as vital step in any kind of medicinal plant research. Phytochemical processes have been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals usually employing chromatographic techniques¹⁶. Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available¹⁷. Extensive effort have now been channelled

towards screening of plants for more active and effective new drugs to eliminate diseases which have strains of pathogenic organism that resist the effect of drug in use today¹⁶. Based on the many ethnomedicinal values of this plant, it is becomes imperative to determine the active ingredients present in different parts of the plant as well as their composition.

MATERIALS AND METHODS

Plant materials

The root of plant of *Rumex nepalensis* was collected from from local area of Bhopal (M.P.).

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals used in this study were of analytical grade.

Extraction Procedure

Defatting of plant material

Powdered roots of *Rumex nepalensis* were shade dried at room temperature. The shade dried roots was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place¹⁸.

Extraction

50g. of dried plant material were exhaustively extracted with different solvent using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Qualitative phytochemical analysis of plant extract

The *Rumex nepalensis* roots extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate^{19, 20}. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Quantification of secondary metabolites

Quantitative analysis is an important tool for the determination of quantity of phytoconstituents present in plant extracts. For this TPC and TFC are determined. Extracts obtained from flower of *Rumex nepalensis* plant material of subjected to estimate the presence of TPC and TFC by standard procedure.

Total Phenol Determination

The total phenolic content was determined using the method of Olufunmiso *et al*²¹. A volume of 1 ml of *N. Nouchali* flowers extracts or standard was mixed with 5 ml of Folin Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The

total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total Flavonoids Determination

The total flavonoid content was determined using the method of Olufunmiso *et al*²¹. 1 ml of 2% AlCl₃ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

RESULTS AND DISCUSSIONS

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1.

Table 1 Result of percentage yield of extract

S. No.	Solvents	% Yield
1.	Chloroform	2.3
2.	Ethyl acetate	1.5
3.	Methanol	3.5
4.	Aqueous	4.8

Preliminary phytochemical screening of *Rumex nepalensis* root extracts revealed the presence of various components such as phenolic compounds, carbohydrates, flavanoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins among which phenols and flavones were the most prominent ones and the results are summarized in Table 2.

Among the secondary metabolites that were quantified, the total phenolic content was the highest with 18.4 mg/100mg of the ethanolic extract followed by the total flavonoids content with 12.4mg/100mg of the ethanolic extract. The results are tabulated in Table 2. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of ethanolic extract of *Rumex nepalensis* root showed the content values of 1.658. But all others extract of *Rumex nepalensis* roots have no phenolic content. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of ethanolic and aqueous extracts of roots of *Rumex nepalensis* showed the content values of 1.048 and 0.987. The above results showed that aqueous extract contain less phenolic and flavonoids content than the alcoholic extract. It may due to the solubility of principle contents presence be higher in case of alcoholic solvent, thus it has been accepted that it is a universal solvent for the extraction of plant constituents. Results are provided in (Table 3 and Fig. 2, 3).

Table 2: Result of phytochemical screening of roots extracts of *Rumex nepalensis*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Alkaloids A) Wagner's Test: B) Hager's Test:	-Ve -Ve	-Ve -Ve	-Ve -Ve	-Ve -Ve
2.	Glycosides A) Legal's Test:	-Ve	-Ve	+Ve	-Ve
3.	Flavonoids A) Lead acetate Test: B) Alkaline Reagent Test:	-Ve -Ve	+Ve -Ve	+Ve +Ve	-Ve +Ve
4.	Saponins A) Froth Test:	-Ve	-Ve	+Ve	+Ve
5.	Phenolics A) Ferric Chloride Test:	-Ve	-Ve	+Ve	-Ve
6.	Proteins and Amino Acids A) Xanthoproteic Test:	+Ve	-Ve	+Ve	+Ve
7.	Carbohydrate A) Fehling's Test:	-Ve	+Ve	+Ve	+Ve
8.	Diterpenes A) Copper acetate Test:	-Ve	+Ve	-Ve	-Ve

Table 3: Estimation of total phenolic and flavonoids content of *Rumex nepalensis*

Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/100 mg of dried extract)
Ethyl Acetate	-	0.675
Ethanol	1.658	1.048
Aqueous	-	0.987

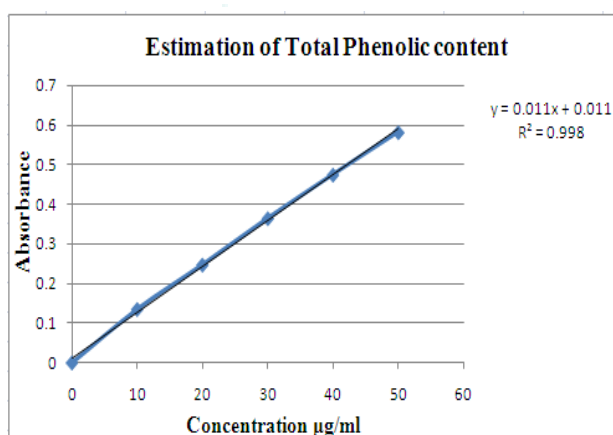


Figure 1: Graph of estimation of total phenolic content

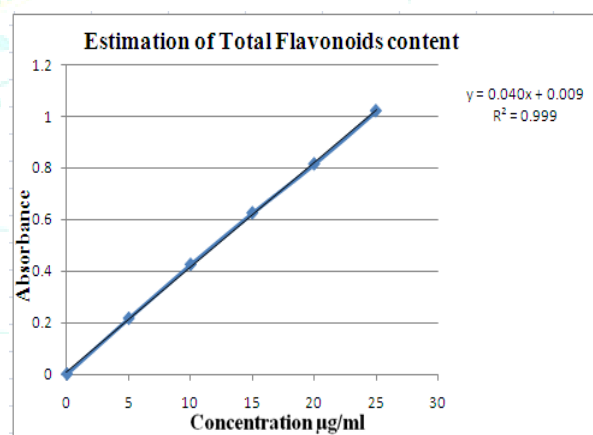


Figure 2: Graph of estimation of total flavonoids content

CONCLUSION

The present study concluded that this medicinal plant viz. *Rumex nepalensis* is a promising source of various activities and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of

the existing data is not enough to suggest a reasonable mode of action for antioxidant effects. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant activity and to explore the existence of synergism if any, among the compounds.

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