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Qualitative and Quantitative Screening for Different Active Phytochemicals of Medicinal Plant- Himalayan Alder species, *Alnus nepalensis*

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ABSTRACT

Phytochemicals are the plant derived metabolites. Phytochemical are functionally different from plant to plants. The study includes qualitative and quantitative screening of secondary metabolites of Alder nepalensis plant. Screening reveled flavonoids and tannin are present thus plant have potential to act as a drug. These potential compounds further can be examine, screened on experimental animals for particular disease.

Keyword*:Flavonoids, Tannin, Secondary Metabolites, Phytochemicals.*

INTRODUCTION

Plants possess different type of chemicals these are technically known as phytochemicals[1]. Medicinal plants possess different kinds of phytochemicals and these all have potential to cure of various disease[2]. The use of medicinal plants have been used since ancient times. People, scientist, researcher have been exploring the nature mainly medicinal plants in search of new novel drugs. Near about 80% of world's population used the medicinal plants for their basic health concern[3]. India is the birth place of traditional uses of plant derived medicine as "Ayurveda". A scripture about the herbal or medicinal plants and their utilization in different kinds of illness practiced in this literature. The Himalayan region of India are enriched place for different kinds of highly potential medicinal plants. The Himalayans region of India are one of the most hot spot area's in the worlds and highly specialized area for medicinal plants that's are endemic to Himalayan region[4].

MATERIAL AND METHOD

Collection of plants

Plant sample were collected personally from Himalayans region of India. Plant leaves were identified, and fresh and healthy leaves of plants were collected from its branch.

Preparation of plant extraction

First of all better quality leaves were washed under tap water to remove the surface pollutants and adhere materials. Then leaves dried under shade. The powder of leaf were obtained. Fine quality powder subjected to successive extraction with methanol, hexane, butanol, ethyl acetate and aqueous using Rotavapor®[5]. The extract thus obtained was used for various analyses of plant material.

Preliminary Phytochemical screening of extracts

Methanol, hexane, butanol, ethyl acetate and aqueous extracts were used for preliminary phytochemical investigation with using standard procedures[6]. The following qualitative tests for both the secondary metabolites were conducted as follows.

A. Test for Flavonoid's

Shinoda Test[7]: first of all 10 mg of sample was added to pieces of magnesium, then hydrochloric acid is dripped on the sample (1-2 drops of concentrated).

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Soon after Pink-red color formed that's indicates the presence of Flavonoids.

Lead acetate test[8]: 10 mg of sample extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow, creamy color precipitate indicates the presence of flavonoids.

B. Test for Tannin

Ferric chloride test (Fecl3)[9]: 5 mg of extract of plant sample was taken and allowed to mix with 0.5 ml of $FeCl_3$. Dark brown color indicates the presence of Tannins in sample.

C. Test for Phenol

Sodium hydroxide test (NaOH)[10]: 5 mg of extract was dissolved in 0.5 ml of H_2So_4 solution. Followed by addition of few drops of aqueous sodium hydroxide solution, sudden blue color appeared that's indicates the presence of phenols.

Quantitative determination of secondary Metabolites

Determination of Total Phenolic and Tannins

Total phenolic contents was determined by following standard method described in[11]. Ten microliter aliquots of the extract (2 mg/ 2 ml) was taken and made upto the volume of 3 ml with adding distilled water. 0.5 ml of folin-ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each tube of sample. Then vortexing the reaction mixture, followed by incubated for 1 min in boiling water bath. Absorbance was measured at 650 nm against a reagent blank. TPC was expressed as mg of Catechol equivalent per gram of sample.

The Tannin content in samples was estimated by the method [Price and Butler]. Different aliquots of sample was taken and final volume to 3 ml was obtained by distilled water. The solution after vortexing was mixed with 1ml of 0.016 M $K_3Fe\ (CN)_6$, followed by 1 ml of 0.02 M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. 5 ml of stabilizer (3:1:1 ratio of water, H_3PO_4 and 1% gum arabic) was added followed by revortexing. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001 M Gallic acid.

Determination of Total flavonoid content

77 mg/ml, AE: 44 mg/ml were prepared with methanol to a suitable concentration for analysis. With slightly modifications using standard curve generated with rutin Aliquots of each extract (ME & AE) were pipetted out in series of test tubes and volume was made up to 0.5 ml with adding distilled water; Sodium nitrate (5% : 0.3 ml) was added to each tube. Then incubated for 5 min at room temperature. Aluminum chloride solution (10%; 0.06 ml) was added and incubated again for 5 min at room temperature then Sodium hydroxide (1M; 0.25 ml) was added and total volume was made to 1 ml with distilled water. Then Absorbance was measured at 510 nm against a reagent blank using Schimadza model 150 – 02 double beam spectrophotometer. Concentration of flavonoids in the test sample was determined and expressed as mg of Rutin equivalent per gram of sample. The analysis was performed in triplicate and the results were expressed as rutin equivalent.

RESULT AND DISCUSSION

Phytochemical analysis is the techniques to identify the chemical constituent of the medicinal plants. This technique employed to determine the various chemical constituents of plants. These regards as secondary metabolites. In this phytochemical screening we find the potential of phytochemicals act as an effective drug. In above analysis Valuable compounds of plants have been chemically investigated. Above analysis revealed different secondary metabolites that's were qualitative and quantitative analyzed using of *Alder nepalensis* plant species. Qualitative result summarized in Table 1 while Table 2 revealed the amount of flavonoids and standard curves of Quercetin shown on figure 1. Table 3 revealed the amount of tannin and standard curves of tannic acid shown on figure 2. Table 4 revealed the amount of phenolic compound. And standard curves of Catechol Shown on figure 3. above study of Alder nepalensis leaf extracts in different solvent such as, chloroform, methanol, Butanol were used.

Different phytochemicals have been identified as they found in different plant. They possess a wide range of medicinal properties, which may help in protection against various diseases. For example, alkaloids protect against chronic diseases; saponins protect against hypercholesterolemia and steroids and triterpenoids show the analgesic properties[12]. While flavonoids act as antioxidants[13].

Table 1: Preliminary phytochemical screening of plant sample

Plant constituents: Phytochemical	Plant Extracts				
	Chloroform Extract	Methanol Extract	Butanol Extract	Aqueous Extract	Name of the Test
Flavonoids	+	+	+	-	Shinoda Test,Lead Acetate Test
Tannin	+	+	+	-	Sodium hydroxide Test
Phenolic compound	+	+	+	-	Ferric Chloride Test

Fig. 1: Standard calibration curve of Quercetin

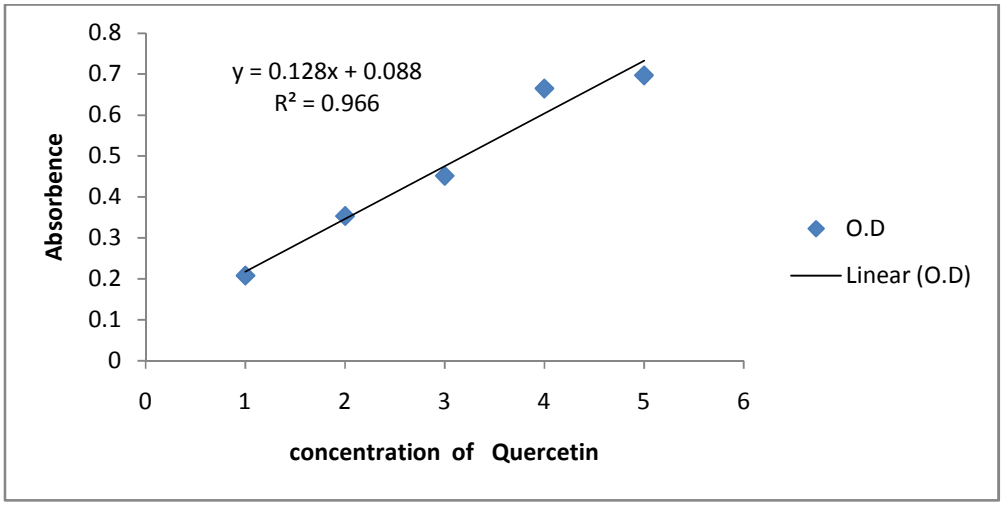

Table 2:Revealed the amount of flavonoids in plant sample

S. No.	Extracts	Absorbance	Mg of Quercetin Equivalent
1	Chloroform	0.24 Å	1.88 mg/gm.
2	Methanol	0.23 Å	1.77 mg/gm.
3	Butanol	0.22 Å	1.64 mg/gm.

Fig. 2: Standard Calibration curve of Tannic acid

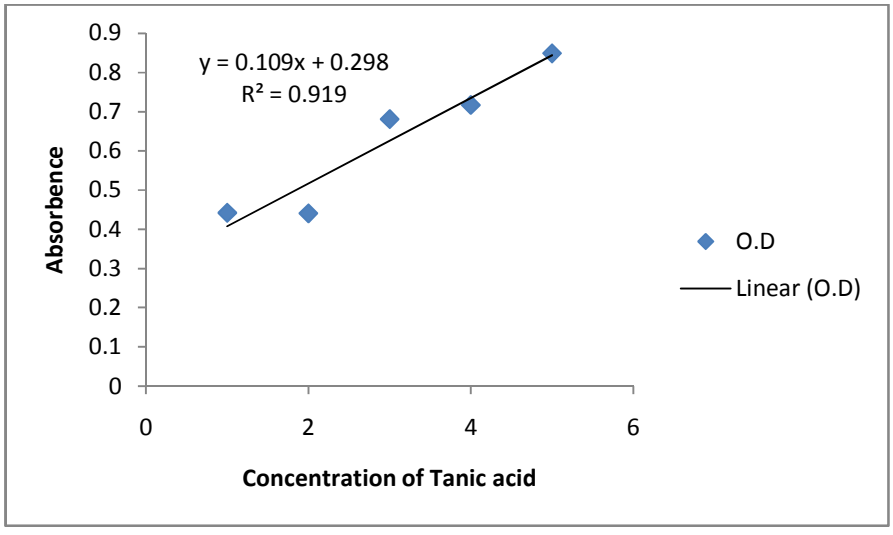

Table 3: Revealed the amount of Tannin in plant sample

S. No.	Extracts	Absorbance	Mg of Tannic acid Equivalent
1	Chloroform	0.46 Å	98.6 mg/gm.
2	Methanol	0.41 Å	68.1 mg/gm.
3	Butanol	0.48 Å	110.66mg/gm.

Fig. 3: Standard calibration curve of Catechol

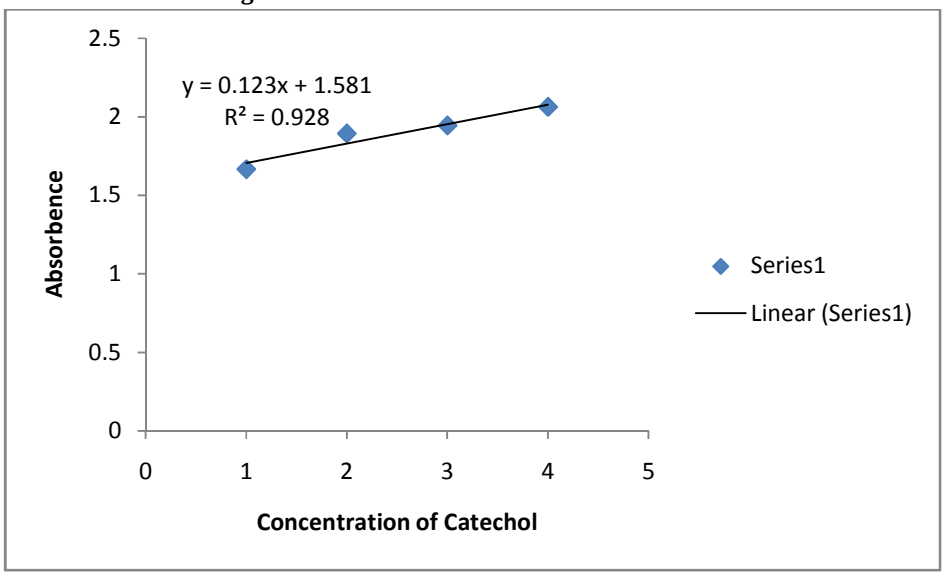

Table 4: Revealed the amount of phenolic compound in plant sample

S. No.	Extracts	Absorbance	Mg of Catechol Equivalent
1	Chloroform	1.837 Å	104 mg/gm.
2	Methanol	1.62 Å	15.85 mg/gm.
3	Butanol	1.61 Å	11.75mg/gm.

CONCLUSION

The results obtained in the present study indicates leaves of this plants have the potential to act as a source of useful drugs. Because as results revealed the presence of various phytochemical components like phenols, flavonoids and tannin. Further experiment of these compound can be conducted on animal models for predict the toxicity and efficacy. Before formulation of drug, pure sample or chemical compound should be isolated from extract that's must be free from contamination as well as other harmful impurities[14]. Further scientific experts committee put forward it and clinical trial approval is must and required before trail on humans in particular disease condition[15].

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