Pharmacognostic Evaluation, Phytochemical Screening And Isolation Of Bioactive Compounds Of Different Plant Extracts On Diarrheal Activity

Ashok Kumar[1*], Dr. Israr Ali[2]

[1*]Research scholar, IEC School of Pharmacy, IEC University, Baddi (HP), India
[2]Professor, IEC School of Pharmacy, IEC University, Baddi (HP), India

***Corresponnding Author:** Ashok Kumar
*Research scholar, IEC School of Pharmacy, IEC University, Baddi (HP), India

Abstract
In recent times, there has been a growing interest in exploring the therapeutic and medical potential of natural plant-derived extracts. This study aimed to investigate the impact of different solvents, including methanol, n-hexane, chloroform, ethyl acetate, and water, on the phenolic profile and their associated antioxidant and antidiarrheal properties. As a benchmark antioxidant, ascorbic acid was used to assess the radical-scavenging abilities of leaf extracts from commonly used medicinal herbs. The study employed the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical to evaluate the scavenging activity. Phenolic compounds are known for their bioactive properties, especially their antioxidant effects. Notably, the leaves of *R. ellipticus, Woodfordia fruticosa*, and *Quercus leucotrichophora* exhibited the highest overall antioxidant activity. The primary objectives of this study were to determine phenolic content, reduction power in various leaf extracts, and assess their antioxidant properties using the DPPH and ABTS assays.

Keywords: *R. ellipticus, Woodfordia fruticosa, and Quercus leucotrichophora,* antioxidant

1. INTRODUCTION

Flavonoids and tannins, plant-derived compounds, possess antibacterial, antifungal, antihypertensive, anticancer, and antidiarrheal properties, forming the foundation of modern pharmaceuticals.[1] Phytochemicals, which are found in plants, act as antioxidants to counteract free radicals and may have therapeutic value for illnesses associated with free radical damage.[2] Free radicals are well-established culprits in various chronic and degenerative diseases, encompassing cancer, diabetes, inflammation, coronary heart disease, and stroke.[3]

Antioxidants are substances that protect cells from the cellular damage caused by unstable molecules known as free radicals induced oxidative stress. Antioxidants neutralize free radicals as a natural by-product of normal cell processes. Phenolic compounds, which are widely distributed in many fruits, vegetables, and tea are believed to account mainly for the antioxidant capacity of many plants.[4,5]The context of antioxidant and antimicrobial agents especially those derived from natural sources such as Indian medicinal plants have special attention. An antimicrobial substance which kills or inhibit the growth of microbes especially bacteria, fungi and virus.[6,7]

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods.[8] It has also been used to quantify antioxidants in complex biological systems in recent years. The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. In this work, the antioxidant properties of the different plant extracts were studied by their ability to scavenge free radicals using the 2,2 diphenyl-1- picrylhydrazyl radical (DPPH) reducing power, antidiarrheal activity, phytochemical analysis and hydrogen peroxide scavenging assays.[9] Diarrhea is a gastrointestinal disease which is characterized by an increase in the volume of stool or frequency of defecation, with presence of loose and watery stools. It is one of the most common clinical signs of gastrointestinal disease, but can also reflect primary disorders outside of the digestive system.[10,11]

2.MATERIAL AND METHODS

2.1 Preparation of extracts

*Rubus ellipticus***,** *Woodfordia fruticosa,* and *Quercus leucotrichophora* leaves were separately shade dried. Grinder is use for the pulverization of powder. In Soxhlet apparatus, the roots powder was extracted by ethanol and distilled water. After that the extract was hot filtered. By using distillation process solvents is removed. By reducing the pressure, the solvent is fully removed.

2.2 Phytochemical study

Extract phytochemical screening was carried out using normal methods. Extracts have been preliminarily phytochemically tested to classify different plant substances.

2.2.1 Qualitative chemical tests

2.2.1.1 Tests for carbohydrates

A. Molisch test: To 1ml of test + 2-3 drops of α- naphthol + Conc. sulphuric acid, appearance of purple ring.
B. Fehling's test: To 1 ml of test sample+ Fehling 's solution A and B + heat, brick red precipitate.
C. Benedict's test: To 1 ml of test sample, equal quantity of Benedict 's reagent was added and boiled. Red colour precipitate confirmed the presence of carbohydrates.

2.2.1.2 Test for alkaloids

Dil. HCL is pour into the extracts and then filter it.
A. Mayer's test: 1 ml of the filtrate + 1ml of Mayer`s reagent, cream precipitate
B. Hager's test: 1 ml of the filtrate + 1mL of Hager`s reagent, yellow precipitate
C. Wagner's test: 1ml of the filtrate + 1ml of Wagner 's reagent, reddish brown precipitate

2.2.1.3 Tests for terpenoids

A. Salkowski test: Chloroform solution of test sample was treated with equal amount of conc. sulphuric acid. Red colour shows the presence of steroid components.
B. Libermann - burchard test: To 2ml of test sample, chloroform was added before the addition of 2-3 drops of acetic anhydride and conc. sulphuric acid. Bluish colour shows the presence of steroids.

2.2.1.4 Tests for flavonoids

A. Lead acetate test: Lead acetate solution was added to the extract. Flavonoid was confirmed on the basis of formation of yellow precipitate.
B. Alkaline reagent test: 1mL of test sample was dissolved in dilute sodium hydroxide solution that resulted in formation of yellow colour precipitate.

2.2.1.5 Tests for tannins and phenolic compounds

A. 5% $FeCl_3$ solution: In small amount of extract the 5% FeCl3 solution was added. A formation of deep blue-black colour complex.
B. 10% lead acetate solution: In few amount of extract, 10% lead acetate solution were added, white precipitate was formed.
C. Gelatin test: Extract was dissolved in water and add 2ml of gelatin solution it forms a white precipitate which show the presence of phenolic compounds.

2.2.1.6 Tests for saponins

A. Froth test*:* 1 ml of test sample + 20 ml of distilled water + shacked for 15min, Formation of persistent foam.

2.2.1.7 Test for proteins and amino acids

A. Ninhydrin test: 3 ml of the test solution + 3 drops of 5% ninhydrin + heat for 10 min + observe colour change
B. Biuret test: Test sample was treated with same volume of 1% copper sulphate and 4% sodium hydroxide solution and appearance of violet or pink colour was observed.
C. Million's test: extract is mixed with Million 's reagent a brick red colour shows the presence of protein.

2.2.1.8 Tests for glycosides:

A. Borntrager's test: Dil. H_2SO_4 are add to the extract solution and boiled. The solution was filtered and cooled. Same amount of chloroform was added while shaking the mixture. Ammonia was added to the organic solvent layer. Change in color of ammonical layer to pinkish red colour confirmed the anthraquinone type glycosides.
B. Legal test: In a sample sodium nitroprusside and pyridine solution is added. It shows the colour changes.
C. Keller killani test: In a extract solution glacial acetic acid and ferric chloride are added. After that the solution are shift to the beaker which contain con. H_2SO_4.

2.2.1.9 Tests for fats and oils

A. Spot test: On a filter paper a small amount of extract was dropped and it will stand for some minutes to evaporate the solvent. After some minutes a small amount of oils are seen it indicate the presence of fixed oil.

2.3 Pharmacognostic evaluation

2.3.1 Ash Value Determination

a) Ash Value Determination

3gm of powder was weigh and it was tared into silica crucible. Heat is increased gradually. It was cooled and desiccated. The ash value is obtained and percentage was calculated by dried air sample.

b) Determination of acid insoluble ash value
The total ash which was obtained from above in 30ml of HCl for 5 to 6min. after the completion of this filtration was done and collect the insoluble matter on ashless paper. By using hot water wash the paper ignited it in tared crucible, cooled and desiccated. After that it was weigh and calculated the insoluble ash value.

c) Determination of water-soluble ash value
Around 30ml water are boiled for 5minutes. Filter paper is use for the collection of insoluble matter. After that wash it hot water and ignited it for 15minutes. The total ash is substrated by the weight of insoluble matter.

2.3.2 Loss on drying
Weigh the sample and tared the dish and it was completely dried by the use of heating at 105^0C. the loss of amount of sample is calculated by the weight loss of sample.

2.3.3 Extractive values
5gm of dried extract was macerated with 100ml of solvent. It was transfer into a flask for 24hours. In initial 6h the sample was shaken and allowed it for 18h. It was filtered rapidly and the 25ml of filtrate was evaporated to dryness and tared to the bottomed of the cylinder.

2.4 ISOLATION OF BIOACTIVE COMPOUNDS OF EXTRACTS
2.4.1 Determination of Total phenolic content
The total phenolic or tannic acid content of different extracts was determined by the method. The standard tannic acid and plant extract was taken in the range of 0.0 to 1 ml (100µg/ml) and made up to 1 ml with water. Then add 500µl of the foline-ciocateu reagent and 1250µl of the 7% sodium carbonate were added and the same procedure was carried out for different leaf extracts and absorbance was taken at 725 and phenolic content of the extracts was expressed as the tannic acid equivalents.

2.4.2 Free Radical Scavenging Assay by DPPH Method
The DPPH radical scavenging activity of *R. ellipticus, W. fruticose* and *Q. leucotrichophora* leaves extracts and ascorbic acid (Standard) were determined according to the method. 0.1mM DPPH reagent was prepared by using ethanol and standardize to 1.9 OD at 517nm. The standard ascorbic acid and Plant extract (concentration range between 0 to 50µg) was taken separately in different aliquots in the range of 0.0 to 1 ml and made up to 1ml using ethanol. To this 2ml of the 0.1mM DPPH was added and incubated in dark for 30 minutes at room temperature. Absorbance was read at 517nm and percentage of the inhibition was calculated using formula.
Radical scavenging Assay = A DPPH - A Sample/A DPPH X 100
A DPPH is the absorbance of DPPH radical + ethanol and A sample is the absorbance DPPH radical + sample or standard.

2.4.3 Free radical scavenging activity by ABTS method
Determination of antioxidant activity by ABTS assay, the procedure followed the method of 11 with some modification. The reagent solutions included 7.5mM ABTS and 3mM potassium persulfate. The working solution was prepared by mixing equal volume of two reagents and allowing them to react for 12-16 hrs at room temperature in dark. The reagent was then
diluted by mixing 1ml ABTS solution with 60ml of ethanol to obtain an absorbance of 1.1±0.02 at 734nm. Fresh ABTS was preferred for each assay. The standard ascorbic acid and Plant extract (concentration range between 0 to 50µg) was taken separately in different aliquots in the range of 0.0 to 1 ml and made up to 1ml using ethanol. 1ml of freshly prepared ABTS was added and incubated in dark for 2h. Then the absorbance was read at 734nm using a spectrophotometer. The % of inhibition was calculated.

2.4.4 Total reducing power
The reducing power of *R. ellipticus, W. fruticose* and *Q. leucotrichophora* was determined according to the method. The aliquots of standard ascorbic acid in range of 0.0 to 1ml (10mgµg/ml) and plant extract in the range of 0.0 to 1ml(10mg/ml) were taken and volume was made up to 1ml by ethanol, this was followed by addition of 2ml phosphate buffer, 2.5ml 1% potassium ferric cyanide and incubated at 50˚C for 20 minutes. After incubation 2.5ml 10% TCA was added and mixed well. Then 2.5ml of distilled water and 0.5ml of 0.1% ferric chloride was added. Mixed well and absorbance was taken at 700nm and total reducing power of the extract was expressed in ascorbic acid equivalents.

3. RESULT
3.1 Phytochemical test for *Rubus ellipticus, Woodfordia fruticose and Quercus leucotrichophora*

Test for phytoconstituents	*Rubus ellipticus*		*Woodfordia fruticosa*		*Quercus leucotrichophora*	
	Water	Ethanol extract	Water	Ethanol extract	Water	Ethanol Extract
Test for Starch	+	+	-	-	+	+

Test for Terpenoids	+	+	-	-	+	+
Test for Proteins	+	+	-	-	+	-
Test for Amino acid	+	+	-	-	+	+
Test for Mucilage	+	+	+	+	+	+
Test for Alkaloids	+	+	+	+	+	+
Test for Anthraquinone glycoside	+	+	+	-	-	+
Test for Cardiac glycoside	+	+	-	-	+	+
Test for Saponin	+	-	+	+	+	+
Test for Tannins	+	+	-	-	+	+
Test for Steroids	-	-	-	-	-	-
Test for Flavonoids	-	-	+	+	+	+

Table 1. Phytochemical screening

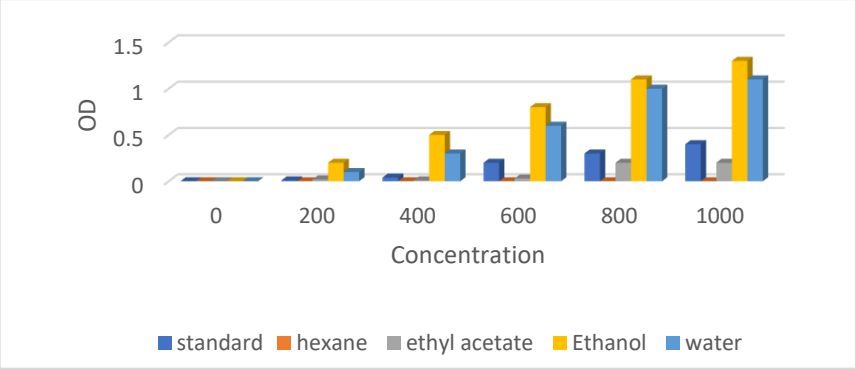
3.2 Physicognostic evaluation of *Rubus ellipticus, Woodfordia fruticose and Quercus leucotrichophora*

Parameters	***Rubus ellipticus***	***Woodfordia fruticosa***	***Quercus leucotrichophora***
Total ash	3.05	5.05	4.05
Acid insoluble ash	1.0	0.05	0.05
Loss on drying at 110°C	13.35	14.35	13.35
Foaming index	3.6	4.6	5.6
Swelling index	0.0	0.0	0.0
Extractive values			
Ethyl alcohol	21.94	52.94	43.94
Petroleum ether	3.52	2.52	3.52
Chloroform	6.2	5.2	4.2
Water	16	15	17

Table 2. Physicognostic evaluation

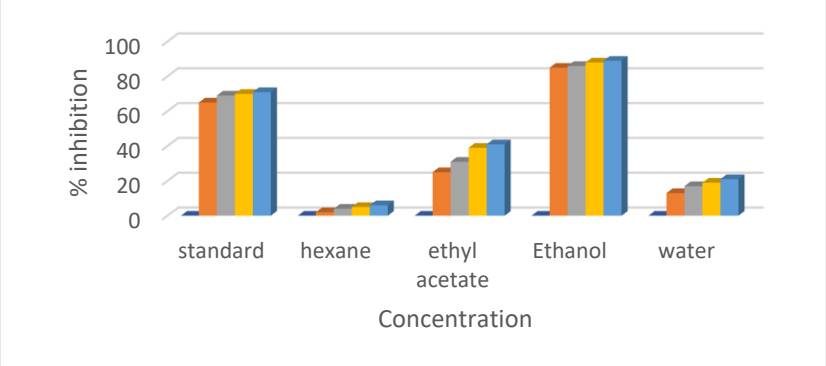
3.3 ISOLATION OF BIOACTIVE COMPOUNDS

3.3.1 Determination of Total Phenolic Content: All the plant extracts had Phenolic content except Cold water, among all ethanol extract had High phenolic content and Hexane had very less.



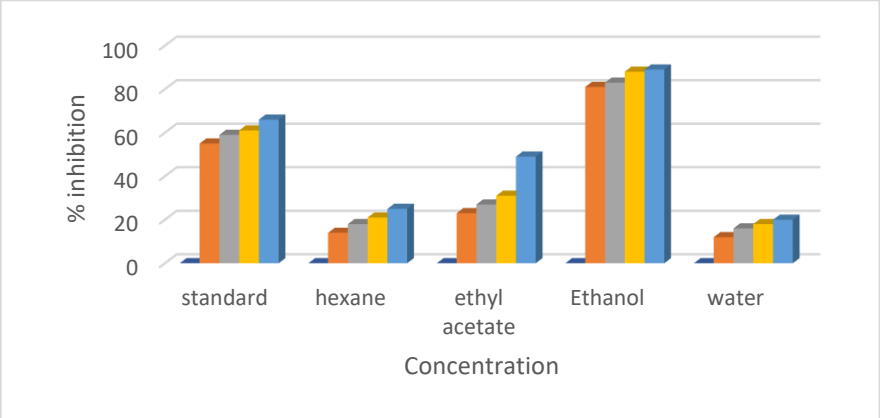
Total phenolic contents

3.3.2 Free Radical Scavenging Assay by DPPH Method: The ethanol and Ethyl acetate are showing the free radical scavenging activity more. However, among this Methanol extract had higher activity.



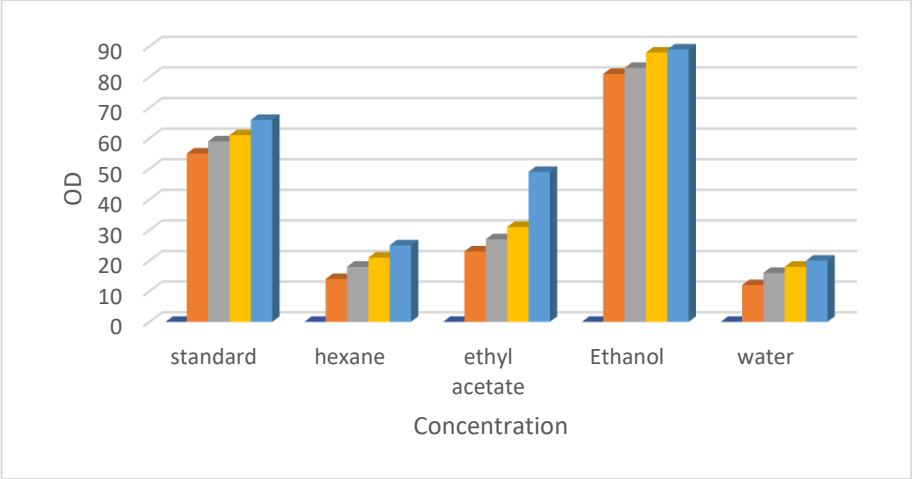
Percentage inhibition

3.3.3 Free radical scavenging activity by ABTS method: ethanol extract was given more activity compared to the other five extract showed better ABTS scavenging activity than the cold water and hot water extract.



Free radical scavenging activity

3.3.4 Total reducing power: Methanol extract had higher reducing power, followed by ethyl acetate extract. Cold water extract showed least reducing power.



Total reducing power

4. CONCLUSION

This study establishes the antioxidant and antidiarrheal properties of *Rubus ellipticus, Woodfordia fruticose and Quercus leucotrichophora.* All fractions including the crude extract (ethanol Extract) showed free radical scavenging activity in a variety of antioxidant assays. In all of the tested configurations, the ethyl acetate fractions outperformed the crude extract and all other fractions when it came to scavenging free radicals. The ethyl acetate fraction is very effective at scavenging free radicals because its polyphenols (Phenolic and flavonoid content) have been concentrated through fractionation. It's well established that free radicals have a role in multiple diseases. In addition, *Rubus ellipticus, Woodfordia fruticose and Quercus leucotrichophora* showed significant antidiarrheal activity. This new evidence lends credence to the idea that the plant's historical use as a curative agent is due to its free radical scavenging properties.

5. references

1. M. Taib, Y. Rezzak, L. Bouyazza, B. Lyoussi, Activities of Quercus species, evidencebased complement, Alternative Med. 2020 (2020).
2. P. Semwal, S. Painuli, Antioxidant, antimicrobial, and GC-MS profiling of saussurea obvallata (brahma kamal) from Uttarakhand himalaya, Clin. Phytosci. 5 (2019).
3. A.F. Vinha, J.C.M. Barreira, A.S.G. Costa, M.B.P.P. Oliveira, A new age for Quercus spp. fruits: review on nutritional and phytochemical composition and related biological activities of acorns, Compr. Rev. Food Sci. Food Saf. 15 (2016) 947–981.
4. G.P. Singh, J. S., & Singh, S. P, Nainital, India, Forests of Himalaya: Structure, Functioning, and Impact of Man., (n.d.).
5. P. Semwal, S. Painuli, H. Badoni, R.K. Bacheti, Screening of phytoconstituents and antidiarrheal activity of leaves and bark of plant. Camus from Uttarakhand Himalaya, Clin. Phytosci. 4 (2018).
6. W. Al-Rousan, R. Ajo, K. Al-Ismail, A. Attlee, R. Shaker, T. Osaili, Characterization of acorn fruit oils extracted from selected mediterranean Quercus species, Grasas Aceites 64 (2013) 554–560.

7. P.L. Soni, H. Sharma, D. Dun, M.M. Gharia, Physicochemical properties of Quercus leucotrichophora (oak) starch, Starch-Starke 45 (1993) 127–130.
8. C. of S, I.R. Chopra, N. R, S. Nayar, I. Chopra, New Delhi, Glossary of Indian Medicinal Plants, 1956.
9. E. Burlacu, A. Nisca, C. Tanase, A comprehensive review of phytochemistry and biological activities of Quercus species, Forests 11 (2020) 1–24.
10. S.C. Sati, N. Sati, O.P. Sati, Chem. Investigat. Screen.Antimicrob. ACTIV. Stem Bark Querc.Leucotrichop. OH 3 (2011) 4–6.
11. S. Dhiman, S. Singla, I. Kumar, P. Palia, P. Kumar, S. Goyal, Protection of Violaodorata L. against neurodegenerative diseases: potential of the extract and major phytoconstituents, Clin. Complement. Med. Pharmacol. (2023).