Phytochemical Screening And Isolation Of Bioactive Compounds Of Plant Extracts On Diarrheal Activity

Damit 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

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

Abstract
Natural products from medicinal plants, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Due to an increasing demand for chemical diversity in screening programs, seeking therapeutic drugs from natural products, interest particularly in edible plants has grown throughout the world. Botanicals and herbal preparations for medicinal usage contain various types of bioactive compounds. 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. 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: Bioactive compound, Plant Extraction, Isolation

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] Diarrhoea is a disease in which there is decrease in the form of stool or an increase in the frequency of bowel movements. It is one among the foremost common clinical signs of gastrointestinal disease, can also reflect primary disorders outside of the digestive system. [6,7] It may be defined as a situation in which an adult daily stool exceeds 200 g and contains 60-95% water. Diarrhoea is a neglected tropical disease despite being a global scourge and international health challenge.[8] Diarrhoea exacts large tolls of morbidity and mortality among all age groups and is particularly endemic in developing countries. It causes about 1.7 billion deaths worldwide.[9,10] The global burden of incidence and severity for diarrhoea is highest in Southeast Asia and Africa. According to WHO report released in May 2017, Diarrhoeal diseases account for 1 in 9 child deaths worldwide, in children under the age of 5; it is the second leading cause of death.[11] Diarrhoea is also associated with other infections such as malaria and measles.[12]

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 DPPH assay for determination of Free Radical Scavenging Activity:
0.1mM DPPH solution in methanol (4.44mg in 200ml) was freshly prepared. Plant extract solution of 1mg/ml was prepared in methanol and varying concentration (1-500 µg/ml) was achieved by serial dilution method. 750µl plant extract solution of varying concentrations were mixed with 750µl of DPPH solution in 1.5ml disposable cuvette and kept for 30 minutes in dark at room temperature (28°C). The mixture of methanol and extract solution of corresponding concentration was used as blank sample. Mixer of 750µl methanol and 750µl DPPH solution was used as control. Ascorbic acid at various concentrations (1-100µg/ml) was used as reference standard. All test samples and reference were assayed in triplicate and the absorbance *(A)* was measured at 517 nm using UV-Vis spectrophotometer.

2.4.2 Determination of Total Phenolic Content (TPC):
TPC of the plant extracts were determined by the Folin Ciocalteu's method given by Singleton and Rossi 10 with some modifications. 0.2 ml plant extract (1mg/ml) was mixed with 2 ml distilled water. To this 0.3 ml of Folin Ciocalteu's phenol reagent was added and mixed well. After 5 minutes 0.8 ml of 20% $NaCO_3$ in water was added and final volume was made to 5ml. The mixtures were kept for 2 hrs at room temperature (28°C) and the absorbance were measured at 765 nm using UV-Vis spectrophotometer (SPECORD-250, Analytic Jena, AG, Germany). Reaction was carried out in triplicate.

2.4.3 Measurement of total flavonoids
The total flavonoid content of extracts was estimated using Aluminium chloride ($AlCl_3$) colourimetric assay. About 0.3 mL of 5% NaNO2 was added to 1 mL extract. After 5 min, 0.3 mL of 10% AlCl3.6H2O was added, and incubated for 5 min. About 2 mL NaOH (1M) was added, and the final volume of the solution was adjusted to 5 mL with distilled water. After 15 min of incubation, the mixture turned to pink and the absorbance was measured at 510 nm.

2.4.4 Measurement of FRAP reducing power
The reducing power of the extracts was assessed as per the method. About 100 mL of fruit extracts were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). This mixture was incubated at 50^0C for 20 min, to which 2.5 mL aliquots of trichloroacetic acid (10%) was added. The content was centrifuged at 3,000 rpm for 10 min. The upper layer of the solution (2.5 mL) was extracted and mixed with 2.5 mL of distilled water and 0.5 mL of freshly prepared ferric chloride solution (0.1%). Then the measurement of absorbance was recorded at 700 nm (UV-visible spectrophotometer, Model: UV 3200) and the reducing power was expressed in terms of ascorbic acid equivalent (AAE) in milligram per gram of extract (mg AAE/g).

3. RESULT
3.1 Phytochemical test for *Pyrus pashia, Prunus avium and Cedrus deodara*

Test for phytoconstituents	*Pyrus pashia*		*Prunus avium*		*Cedrus deodara*	
	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 *Pyrus pashia, Prunus avium and Cedrus deodara*

Parameters	***Pyrus pashia***	***Prunus avium***	***Cedrus deodara***
Total ash	9.50 ± 0.50	6.09	5.99
Acid insoluble ash	4.55 ± 0.20	0.04	0.08
Loss on drying at 110°C	3.83 ± 1.2	13.98	14.95
Foaming index	Less than 100	3.9	4.7
Swelling index	4.5 ± 0.81	2.8	2.9
Extractive values			
Ethyl alcohol	25.98	62.45	49.55
Petroleum ether	4.99	3.68	2.34
Chloroform	5.1	4.8	3.9
Water	19	19	19

Table 2. Physicognostic evaluation

3.3 ISOLATION OF BIOACTIVE COMPOUNDS OF EXTRACTS

3.3.1 Free Radical Scavenging Activity

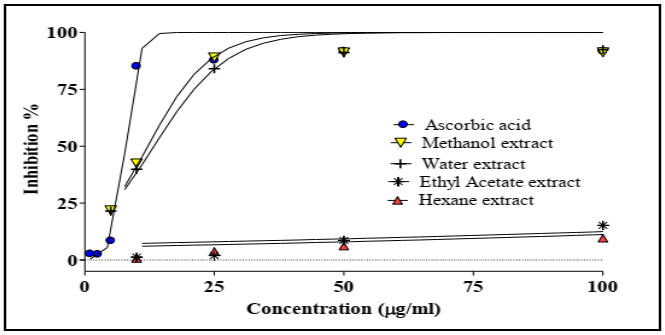


Figure 1. Free Radical Scavenging Activity

3.3.2 Total Phenolic Content (TPC):

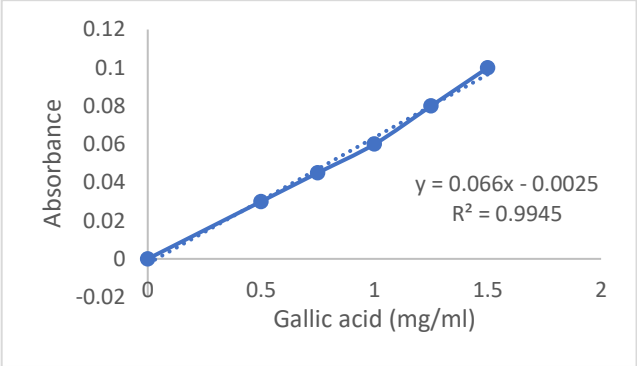
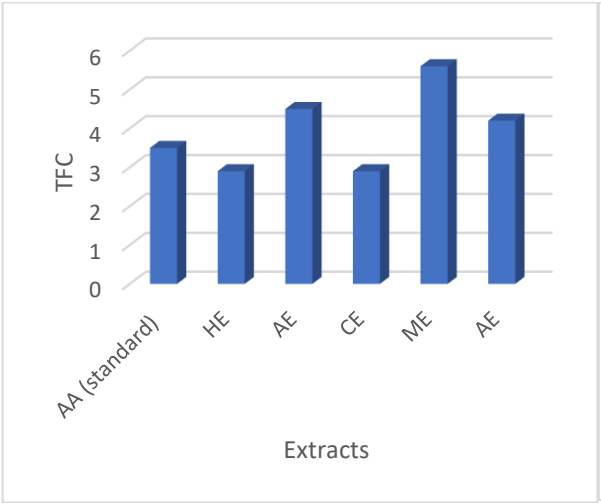
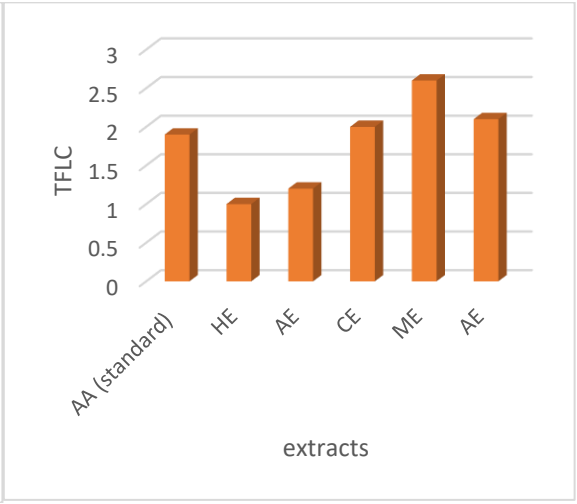


Figure 2. Gallic Acid Standard Curve

3.3.3 Total flavonoids and flavonols



Total flavonoids content (TFC, mg QE /g)



Total flavonol content (TFLC, mg QE /g)

3.3.4 Total phenolic content

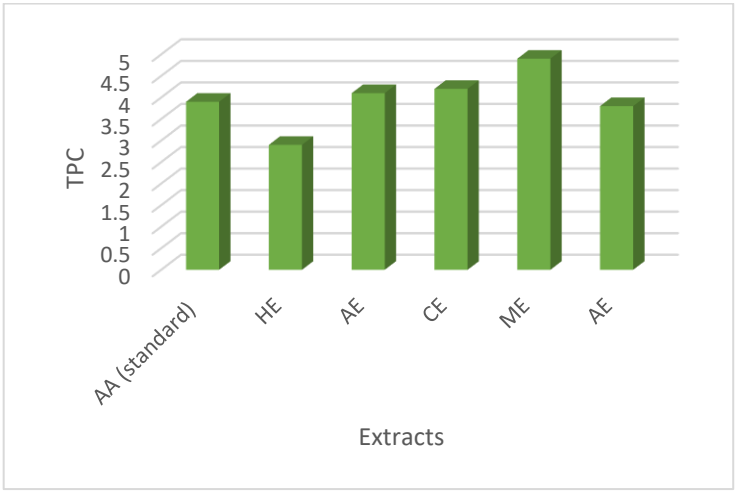


Figure 3. Total phenol content (TPC, mg GAE /g)

3.3.5 DPPH free radical scavenging activity

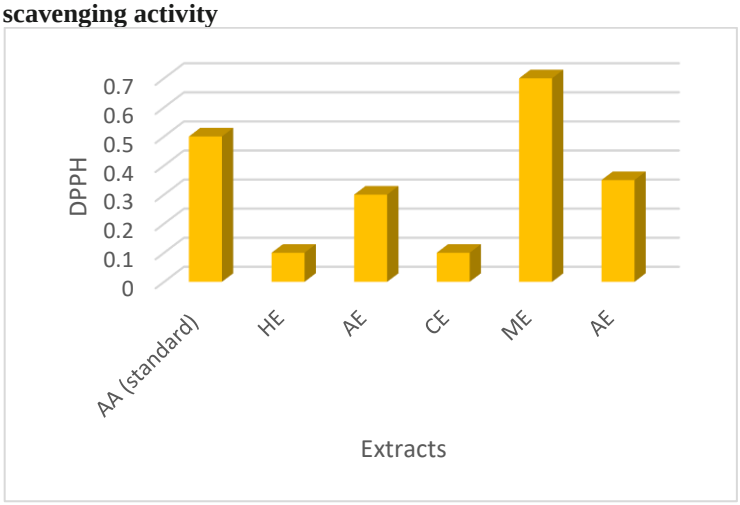


Figure 4. DPPH antioxidants capacity (DDPH, IC50 value mg /mL)

3.3.6 FRAP reducing power

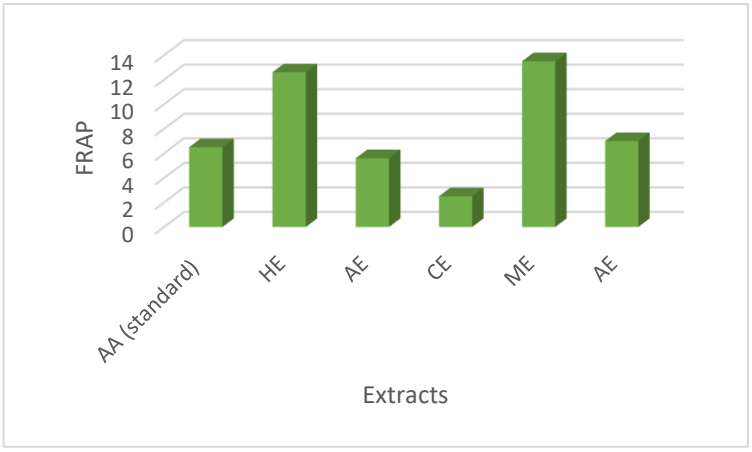


Figure 5. FRAP antioxidants capacity (FRAP, mg AAE/g)

4. CONCLUSION

This study establishes the antioxidant and antidiarrheal properties of *Pyrus pashia, Prunus avium and Cedrus deodara*. 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. In addition, *Pyrus pashia, Prunus avium and Cedrus deodara* showed significant antidiarrheal activity.

5. REFERENCES

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