Preliminary Phytochemical Screening of Different Leaf Extracts of *Juniperus squamata*

Rohaya Ali*[1], Rabia Hamid[1], Durdana Shah[2], Nasreen Rizvi[1] and Showkat Ahamad[3]

[1]Department of Biochemistry, University of Kashmir, Srinagar-190 006, J & K, India
[2]Centre of Research for Development, University of Kashmir, Srinagar-190 006, J & K, India
[3]Department of Clinical Biochemistry, University of Kashmir, Srinagar-190 006, J & K, India
***Corresponding author: rohayaali01@gmail.com**

Abstract

Plants are a source of large number of secondary metabolites comprising to different groups such as alkaloids, terpenoids, flavonoids, steroid, tannins, etc. They can act as antibacterial, antioxidant, antiulcer, anti-inflammatory, antiviral and anticancer agents. It is now believed that nature has given the cure of every disease in one way or another. Therefore, the present study was taken conduct the preliminary phytochemical screening, total flavonoid and phenolic contents assays of various leaf extracts (Hexane, methanolic and aqueous) of *Juniperus squamata*. Total flavonoid content was measured by the aluminium chloride colorimetric assay and total phenolic content was estimated spectrophotometrically by Folin-Ciocalteau method. Preliminary phytochemical screening reveals the presence of phenolics, flavonoids, alkaloids, terpenoids, phylobatanins and cardiac glycosides in methanolic and aqueous extracts.While hexane extract was found to contain steroids mainly. Moreover, in all the three different extracts (hexane ,methanolic, and aqueous) highest content of both phenolics and flavonoids was found in the methanolic extract i.e. 427 mg GAE/g and 255 mg RU/g respectively, and hexane extract with the least i.e. 132 mg GAE/g and 64 mg RU/g.

Keywords: Total phenolic content, folin cioacalteau, phytochemical screening, total flavonoid content.

Introduction

Plants are vital source of natural medicine. A number of modern drugs have been isolated from them. An increasing interest in herbal remedies has been observed in several parts of the world and many of the herbal remedies have been incorporated into orthodox medicinal plant practice (Scalbert *et al.*, 2005). Today in this modern world, even though synthetic drugs are readily available and highly effective in curing various diseases, there are people who still prefer using traditional folk medicines because of their less harmful effects (Shrikumar and Ravi, 2007). Plant products have been part of phytomedicines since time immemorial. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Phytochemical screening of various plants has been reported by many workers. These studies have revealed the presence of numerous chemicals, including alkaloids, flavonoids, steroids, phenols, glycosides and saponins (Rao *et al.*, 2012). The amount of phytochemical substances varies

considerably from species to species and even from plant to plant, depending on the age and various ecological and climatic factors. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. A number of studies have focused on the biological activities of phenolic compounds, which are antioxidants and free radical scavengers. Therefore it is also necessary to have knowledge of chemical constituents of plants before using it as medicine (Audu *et al.,* 2007). Phytochemicals are bio-active chemicals of plant origin. They are regarded as secondary metabolites because the plant that manufactures them may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components (Choudhary *et al*., 2013 and Tiwari *et al.,* 2011). Plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. The secondry metabolites of medicinal plants account for their medicinal value. For example, saponins have hypotensive and cardiodepressant properties. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites that possess an aromatic ring bearing one or more hydroxyl constituents (Edeoga *et al.,* 2005). Phenolic compounds are widely found in the secondary products of medicinal plants, as well as in many edible plants (Khare *et al*., 2007). A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical-scavengers (Rice *et al.,* 1995).

Flavonoids are a broad class of plant phenolics that are known to possess a well established protective ability against membrane lipoperoxidative damages (Gurjar *et al.,* 2012).

Material and Methods

Collection of plant material

Juniperus squamata was collected from higher altitudes of Gulmarg (Apharwat), Jammu and Kashmir state, India, in the months of September and October 2014, identified by the Centre of Plant Taxonomy, Department of Botany, University of Kashmir.A reference specimen has been retained in the herbarium of the Department of Botany at the University of Kashmir under reference number 2211-KASH.

Extract preparation

Entire plant material was dried in the shade.Leaves were separated and ground into a powder using grinder.The powder obtained was extracted with different solvents like hexane, ethyl acetate, methanol, ethanol, and water for 48 hrs using a Soxhlet extractor (60–80°C) .The extract was then concentrated with the help of rotary evaporator under reduced pressure and the solid extract was stored in refrigerator for further use.

Phytochemical screening

Preliminary qualitative phytochemical screening

Chemical tests of different plant extracts were carried out qualitatively using standard procedures to identify the major phytochemical constituents. (Harbone, 1998)

Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate

Test for steroids

Two ml of acetic anhydride is added to 0.5 g methanolic extract of each sample with 2 ml H_2S0_4. The colour changed from violet to blue or green in some samples indicating the presence of steroids (Sofowara, 1993).

Test for terpenoids (Salkowski test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids (Sofowora, 1993).

Test for tannins

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added. An intense green, purple, blue or black colour indicated the presence of tannin.

Test for phlobatannin

Extract was boiled with 2 ml of 1% hydrochloric acid. Formation of red precipitate indicated the presence of phlobatannin (Harbrone, 1984)

Test for flavonoids

The presence of flavonoids was estimated by Shinoda test. The alcoholic extract of the crude dry powder of each plant was treated with a few drops of concentrated HCl and magnesium ribbon. The appearance of pink or tomato red colour within a few minutes indicated the presence of flavonoids (Harbrone, 1984).

Test for cardiac glycosides

Keller-kiliani test was performed to assess the presence of cardiac glycosides. The crude dry powder of each plant was treated with 1 ml of FeCl3 reagent (mixture of 1 volume of 5% FeCl3 solution and 99 volumes of glacial acetic acid). To this solution a few drops of concentrated H2SO4 was added. Appearance of greenish blue color within a few minutes indicated the presence of cardiac glycosides (Harbrone, 1984).

Test for Saponins

The presence of saponins was determined by Frothing test. The crude dry powder of each plant was vigorously shaken with distilled water and was allowed to stand for 10 minutes and classified for saponin content as follows:no froth indicate absence of saponins and stable froth more than 1.5 cm indicated the presence of saponins (Harbrone, 1984).

Tests for carbohydrates

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates (Sofowora, 1993).

Molisch's Test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's test

Extracts were dissolved individually in distilled water and filtered. Filtrates were treated with Benedict's reagent and heated gently. Formation of orange red precipitate indicated the presence of reducing sugars.

***Fehling's tes*t**

Filtrates were mixed with equal volume of Fehling's A and Fehling's B solutions and heated. Formation of brick red precipitate of cuprous oxide indicated the presence of reducing sugars.

Test for proteins

Xanthoproteic test: The extracts were treated with a few drops of conc. nitric acid. Formation of yellow colour indicated the presence of proteins (Harbrone, 1984).

Quantitative phytochemical screening

Flavonoid determination:

Aluminium chloride colorimetric method (Chang *et al.,* 2002) with some modifications was used to determine flavonoid content. Plant extract (1ml) in methanol was mixed with 1ml of methanol, 0.5 ml aluminium chloride (1.2 %) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature; then the absorbance was measured at 415 nm.Rutin was used as standard. Flavonoid content is expressed in terms of rutin equivalent (mg/ g of extracted compound).

Determination of Total Phenolic Content

The amount of phenol content of different plant extracts of *Juniperus squamata* were determined with Folin-Ciocalteu reagent (Singleton *et al.,* 1999). 2.5 ml of 10% Folin- Ciocalteu reagent and 2 ml of

Na2CO3 (2% w/v) were added to 0.5 ml of the sample (3 replicates) of each plant extract solution (1mg/ml). The resulting mixture was incubated at 450˚ C for 15 min. The absorbance of each sample was measured at 760 nm using UV Visible Spectrophotometer. Gallic acid (5-30 µg/ml) was used as a standard compound. The gallic acid standard calibration curve was established by plotting concentration (µg/ml) versus absorbance (nm) (y= 0.011x + 0.062; R2= 0.993), where y is absorbance at 760 nm and x is concentration. Total phenolic content in the plant extract was expressed as gallic acid equivalent (mg of gallic acid equivalent/ g of sample) and was calculated by the formula:

T = (C x V)/M
Where,
T = total content of phenolic compounds, mg/g plant extract, in GAE;
C = concentration of gallic acid established from the calibration curve, µg/ml;
V = volume of extract, ml

Results and Discussion

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.

Preliminary qualitative phytochemical screening

Medicinal plants have potent phytoconstituents which are important source of antibiotic compounds and are responsible for the therapeutic properties (Joselin *et al.*, 2013). These phytoconstituents endow them with medicinal properties. Many plants possess antioxidant properties because of the presence of phenolic compounds (Krings and Berger, 2001).

The phytochemical screening in the present study, has revealed the presence of terpenoids, steroids, glycosides, flavonoids and phylobatanins in metanolic and aqueous extracts.Hexane fraction was found to contain steroids only. Tannins were absent in all the three extracts (**Table 1**). Further the presence of different phytoconstituents in the three different extracts may be responsible for the therapeutic properties of *Juniperus squamata.*

Table 1: Qualitative phytochemical screening of *Juniperus squamata*

Phytochemical screening	Hexane extact	Methanoilic extract	Aqueous extract
Alkaloid	++	++	+
Tannin	-	-	-
Steroid	++	+	+
Saponin	-	+	+
Cardiac glycoside	-	+	+
Phylobatanin	-	++	+
Flavanoid	-	++	++
carbohydrates	-	-	-
Proteins	-	-	-

(++) = strong presence, (+) = moderate presence,(-)=absence

Total phenolic content (TPC)

The evaluation of the phenolic compounds uses the Folin-Ciocalteau reagent, which forms blue complexes in the presence of reducing agents. The TPC of the extracts was determined by extrapolation from the calibration curve (Y = 0.18x + 0.1039; R2 = 0.994) prepared from the gallic acid concentrations and expressed in mg of gallic acid equivalence (GAE) per gram.The amount of phenolic compounds in the various extracts was obtained from regression equation and the values were expressed in gallic acid equivalence. There was no significant difference ($p > 0.05$) in all the three different extracts. The highest phenolic content of 427 GAE/g(TPC) was observed in methanolic extract , while the lowest TPC of 235 GAE/g(TPC) was found in hexane extract.(Figure 1) .High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction (Zheng and Wang, 2001).

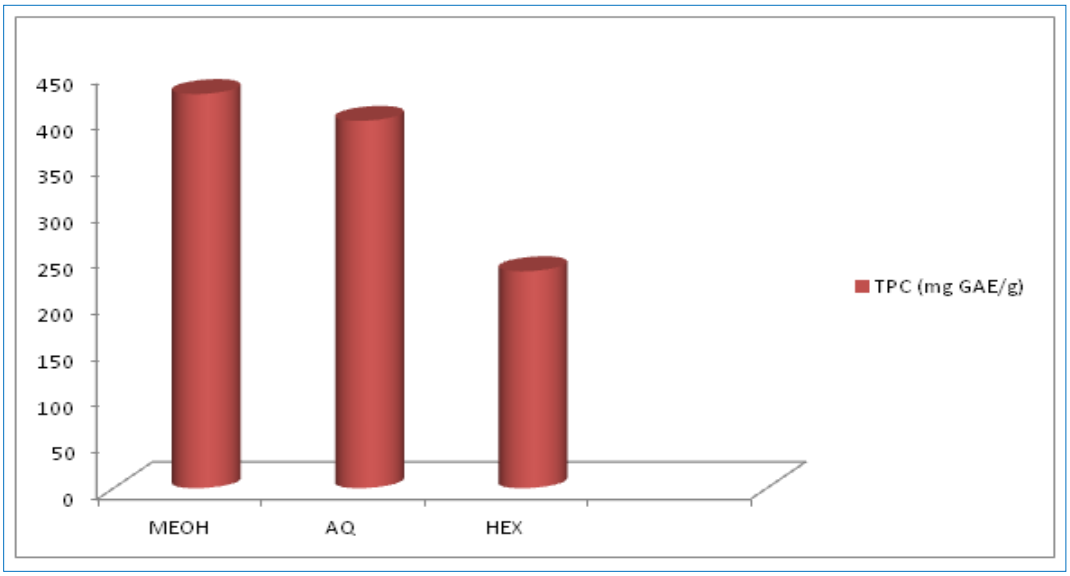

Figure 1. Total phenolic content of various solvent extracts of *Juniperus squamata*

Total flavonoid content (TFC)

The concentration of flavonoids in various plant extracts of the species *Juniperus squamata* was determined using spectrophotometric method with aluminum chloride.The content of flavonoids was expressed in terms of rutin equivalent (the standard curve equation: y = 17.231x – 0.0591, r2 = 0.999), mg of RU/g of extrac.Methanolic extract contains the highest flavonoid concentration. The concentration of flavonoids in methanol extract was 132 mg RU/g.The lowest flavonoid concentration was measured in hexane extract. (**Figure 2**)

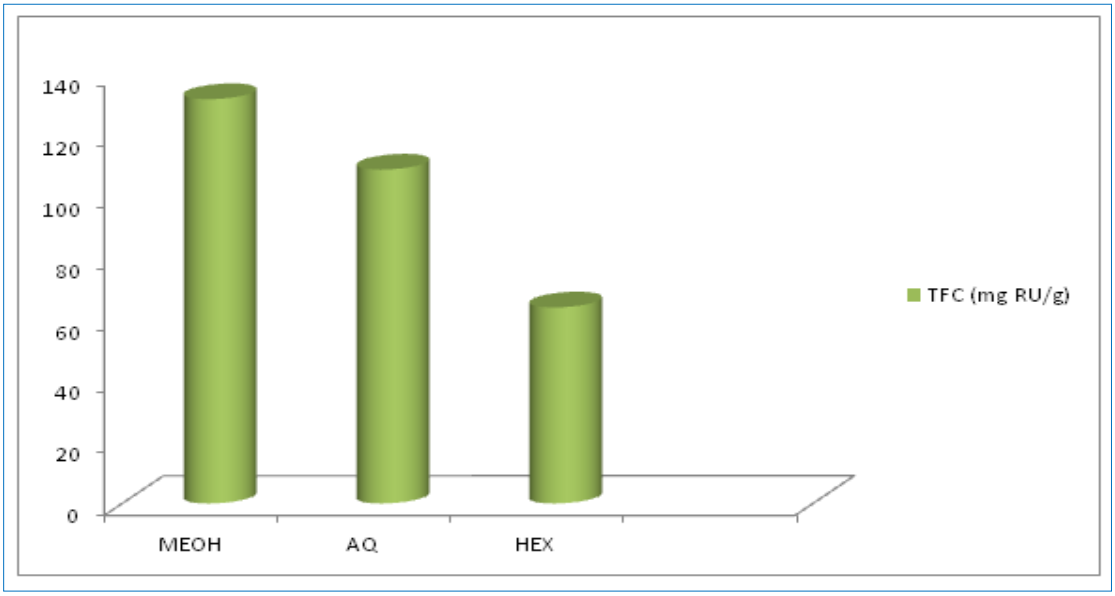

Figure 2. Total flavanoid content of various solvent extracts of *Juniperus squamata*

Conclusion

The presence of phytoconstituents make the plant useful for treating different ailments and have a potential of providing useful drugs of human use. In the present study, we have found that most of the biologically active phytochemicals were present in the methanolic, and aqueous extracts.Since the methanolic extract contains more constituents it can be considered beneficial for further investigation.It has also been found that the aqueous extract of root of *Juniperus squamata* also contains a good quantity of phenolic compounds. Thus these extracts can be studied further to know their biological effects which could be a beneficial in the treatment and controlling of various diseases. The phytochemical characterization of the extracts, the identification of responsible bioactive compounds and quality standards are necessary for future study.

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