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Phytochemical analysis, total phenolic content, total flavinoidal content, antioxidant and antimicrobial activity of some extracts of *Caragana gerardiana*

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Abstract

Introduction: Caragana gerardiana is a drought resistant leguminous plant.
Methods: Antimicrobial activity of the of the plant exracts were carried out against against various bacterial and fungal strains like: E. coli (MTCC 407), P.aeruginosa (MTCC 139), S. aureus (MTCC 96), B.subtilis (MTCC 441), K. pneumonia (MTCC 49) ; A. niger (MTCC1344), P. crysogenum (MTCC 947), C. albicans (MTCC), T. rubrum (MTCC 8469), E. floccosum (MTCC 613),M. canis (MTCC 296). Various antioxidant methods were used for evaluation of free radical scavenging activity of the plant extracts , IC_{50} values were found to be lowering with increased polarity.
Results: The plant under study was found to have broad spectrum antimicrobial activity against various bacterial and fungal strains The plant also shows a significant radical scavenging activity with IC50 values of 98.94 124.63, 98.63, 191.88, 100.79 µg/ml in methanolic extract.
Conclusion: The plant can also serve as potential alternative to treat various diseases, as the plant was found to have broad spectrum antimicrobial potential and high antioxidant potential. ***Key Words:*** *DPPH; Phytochemicals*

1. Introduction

Man has been ever desirous of knowledge to explore many new things but still many things remain concealed for many generations to explore them, today even after long period of time with so much scientific and technological advancement, the earths flora and fauna are still remaining the most important source of potential drugs for traditional hakims. Kashmir is a vertical reservoir of plants bearing medicinal value. Kashmir one of the few states of our country where aromatic plants of all kinds can be cultivated because of the suitable climatic

conditions vary from temperate plains to tropical areas,hills,valleys,irrigated soil etc. In Kashmir the use of several plants fragments to cure specific afflictions has been in expedition from ancient times.This system of medicine provides the needs of about 50% people of our population particularly those residing in villages along the hilly areas of our valley not only for its medicinal value but provide means of livelihood to many people involved in their cultivation.Use of plants for curing of diseases is not confined to doctor only but is known to many households as well and our knowledge of medicinal plants has mostly been inherited tradionally. There is a growing need on all over the world to shift to natural based products including medicinal plants (Non-toxic) from synthetic (Toxic) Some of the common medicinally important components isolated from plants are like terpinols, steroids, cortisones, Colchine, limonine, flavoinoids, podophylltoxin etc. Nair,(1994).The components of a plant that aid in curing various diseases are reffered to as 'active principals'.The principal groups of active principals include steroids, terpenoids, essential oils, glycosides,tannins etc.

Free radicals generation is a normal phenomenon in biological system. Sometimes due to over generation, body's defence mechanism is not able to remove them, and as a result a condition called oxidative stress is developed in the body..The free radicals are the chemical species, which have an unpaired electron and are thus vary unstable and reactive. In order to attain stability they react with their neighbouring atoms to gain the electrons resulting the generation of new free radicals,which in turn attack to other nearby molecules causing a web of reactions. Aerobic organism use of oxygen to oxidize food and obtain the energy,a phenomenon essential for their sustenance. But during this oxidation process the oxygen molecule itself get reduced and from an intermediate called as the reactive oxygen species (ROS).The role of free radical reaction in the biology has become an area of intense interest. It is generally accepted that free radicals play an important role in the development of tissue damage and pathological events in living organisms. There is increasing interest in the natural antioxidants contained in the medicinal and the dietary plants, which are the candidates for the prevention of oxidative stress or damage.

Caragana gerardiana is a drought resistant leguminous plant. It is found at the elevation of 3700- 4100 metres.

2. Material and Methods

Caragana gerardiana was collected from its natural source in Kashmir. After identification of the plant from dept. of botany Islamia college, Srinagar, the leaves of the plant were taken and thoroughly washed with tap water, rinsed a few times in distilled water and then dried in shade.The dried leaves were taken

and cut out into small pieces, powdered in a grinder and then extracted with petroleum ether, ethyl acetate and methanol.

2.1 Preparation of Plant Extracts

A standardized solvent extraction protocol was used. 50 g of plant powder was fed to a Soxhlet extractor fitted with a 0.5 L round-bottom flask and a condenser. Extraction was carried out serially with different solvents using petroleum ether, ethyl acetate and methanol.. The extraction was executed on a water bath for 12 hrs with 0.4 L of each solvent. After completion of extraction, the solvent was distilled off in a rotary evaporator at 35- 45 °C. The dried extracts were weighed to determine the yield of soluble constituents. All the extracts of the plant were stored under refrigerator (4 °C), until used for further analyses.

2.2 Phytochemical screening

The phytochemical screening tests of the different plant extracts were performed by using standard procedures (Sofowora 1993; Ayoola *et al*. 2008)

2.3 Determination of Total of phenolic content

The total content of soluble phenolic compounds in plant extracts was determined with Folin Ciocalteau reagent (FCR) according to the method described in (Chang et al. 2002; Roy et al, 2010). Gallic acid was used as a standard. 0.5 ml of each extract with a concentration of 5 mg /ml was separately mixed with Folin–Ciocalteu reagent (0.2 N, 2.5 ml) and aqueous Na_2CO_3 (1 M, 2 ml) solution. The reaction mixture was allowed to stand at room temperature for 15 min. The absorbance was measured at 765 nm using a UV–visible spectrophotometer. The calibration curve (Slope = 57.44 ± 1.10; R2 = 0.997) was prepared using solutions of gallic acid (standard) in methanol: water mixture (50:50, v/v) with concentrations ranging from 0–30 μg ml-1. The total polyphenol content was expressed in terms of milligram of gallic acid equivalent per gram of dry mass (mg GAE g-1). Three replicates were performed for each sample concentra concentration to check the reproducibility of the experimental result and to get more accurate results.

2.4 Determination of Total Flavonoid Content

The Aluminium chloride colorimetric method was used for flavonoid content determination of each extract (Roy et al, 2010;Mcdonald et al 2001). 0.5 ml of each extract (5 mg ml-1) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The reaction mixture was kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm with a UV–visible spectrophotometer. The calibration curve (Slope = 65.68 ± 0.76; R2 = 0.999) was recorded by using quercetin (standard) solutions in methanol with concentrations

ranging from 0 to 20 μgml-1. The total flavonoid content was expressed in terms of milligram of quercetin equivalent per gram of dry mass (mg QE g^{-1})

2.5 Evaluation of Antioxidant Activity

The antioxidant activities were performed using assays viz. DPPH radical scavenging assay, Ferric ion reducing assay, FRAP assay, Hydrogen peroxide scavenging assay and Lipid per-oxidation assay. The methods of these assays are described below:

Modified DPPH (Shameem *et al.,*2015), Ferric (Fe^{3+}) reducing power assay(Yildirim *et al*.,2000), FRAP assay (Ruch *et al*. 1989), hydrogen peroxide scavenging assay (Feerreira *et al*, 2010) and) and Lipid per-oxidation Method (Padmaja *et al*., 2011) were followed for determining antioxidant activity.

2.6 Antimicrobial Activity

The purified lectin was screened for their antimicrobial activity by using agar ditch diffusion method (Barry., 1980) by measuring the diameter of the inhibitory zones in mm using different concentration of purified lectin in methanol. The diameters of the zones of inhibitions of the samples were than compared with the diameter of the zone of inhibition produced by the standard antibiotic such as ciprofloxacin (antibacterial) and fluconozol (antifingal). Nutrients agar medium and and potato dextrose agar were used for determining antibacterial and antifungal activities respectively.

3. Results and Discussion

The yields of various extracts of the plants under study are given in **Table 1**.All the extracts were obtained as dark-green semi-solid material.

Table 1: Yields of various extracts of plant material (in grams).

Name of Plant	Pet ether Extract	Ethyl acetate Extract	Methanol Extract
Caragana gerardiana	1.346	2.051	3.106

3.1 Phytochemical screening

Different methods were followed to determine qualitatively the presence of phytochemical constituents present in the plant methanol extract. The amount of

crude extracts varied among the solvents used. Under the present study, the methanol extract (3.106 g) showed higher yield. The qualitative phytochemical screening of crude extracts of *Caragana gerardiana* revealed that alkaloids, phenols, anthraquinones, and flavonoids were present. Saponins, glycosides, and +tannins were absent in both the RP and AP extracts while terpenoids were present in only the RP extract (Table 2).

Table 2: Phytochemical analysis of different secondary metabolites present in the aerial extracts of *Caragana gerardiana.*

Phytochemical constituents	**Pt. ether**	**Ethyl acetate**
Methanol. Extract		
Alkaloids +	+	+
Glycosides +	+	+
Anthraquinones +	+	+
Saponins –	+	+
Tannins –	–	-
Terpenoids –	+	+
Flavonoids +	+	+
Phenolic compounds +	+	+
Yield (%) 1.3	2.05	3.1

(+) = present, (–) = absent

The results obtained in the present study revealed that the level of the total phenolic (TP) content and total flavonoid content in different extracts of the selected plant was considerable. The TP content was measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent. The TP content of different extracts in terms of GAE µg/ml is shown in **Table 3**. Among the extracts methanol extract shows highest phenolic content.

The TF content was measured in terms of quercetin equivalent . The TF content of different extracts in terms of QE µg/ml is shown in **Table 4.** Among the extracts methanol extract shows highest flavinoidal content.

Table 3: Total Phenolic content of the plant under study, expressed as gallic acid equivalents in µg/ml for 100 g of extract.

Plant name	Total Phenolic Content Gallic Acid Equivalent) (µg/ml for 100 g of extract)		
	Pet. ether Extract	Ethylacetate Extract	Methanol Extract
Caragana gerardiana	23.83	665.08	844.56

Table 4: Total flavonoid content of the plants under study, expressed as Quercetin equivalents in µg/ml for 100 g of extract.

Plant name	Total FlavonoidContent Quercetin Equivalent) (µg/ml for 100 g of extract)		
	Pet. ether Extract	Ethylacetate Extract	Methanol Extract
Caragana gerardiana	8.87	664.24	1004.84

3.2 Antioxidant Activity

3.2.1 DPPH radical scavenging assay

DPPH assay is one of the most widely used method for screening antioxidant potential. DPPH being a stable free radical at room temperature and accepts an electron or hydrogen radical to become stable diamagnetic molecule. The reduction capability of DPPH radical was determined by decrease in its absorbance at 517 nm, which is induced by different antioxidants. The activities obtained as a function of various concentrations of different extracts are presented (**Figure 1**).It is clear from the figure that the methanolic extract shows higher DPPH radical scavenging activity. The IC_{50} values of the extracts for this activity were determined from the graph and values are given in the **Table 5** for all extracts.

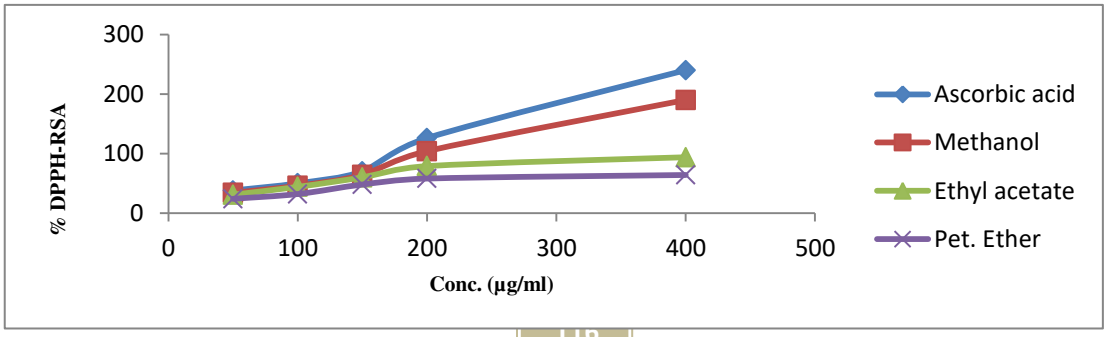

Figure 1: % DPPH Radical Scavenging Activity of various extracts of under study plants and standard (Ascorbic acid).

3.2.2 Ferric (Fe^{3+}) reducing power assay

The reducing ability to convert Fe^{3+} to Fe^{2+} is also an indirect evidence for the antioxidant activity of an extract or a compound (Matsushige *et al.*, 1996). In the ferric reducing antioxidant power assay, the antioxidants i.e. the reducing species present in the extract causes the reduction of the Fe^{3+} ferricyanide complex to form Fe^{2+} ions; this reaction was monitored spectrophotometrically by recording the absorbance of the reaction mixture at 700 nm . The reducing power characteristics of different extracts is shown in (**Figure 2).** From the figure it is clear that, out of all three extracts of the plants studied, methanolic extract has highest ferric ion reducing activity at different concentration, followed by ethyl acetate extract and least for pet. ether extract. The reducing power increases with increasing the concentration of extracts in the solutions. IC_{50} value is shown in **Table 5.**

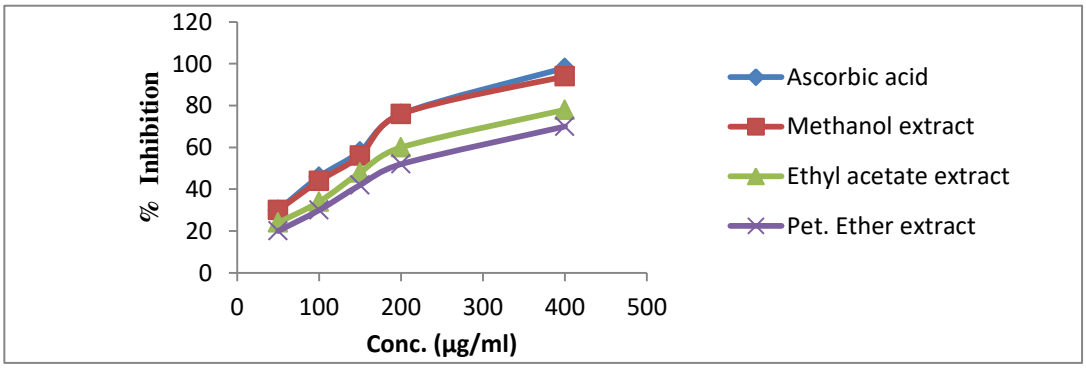

Figure 2: Ferric reducing activity of different extracts of under study plant and standard Ascorbic acid

3.2.3 FRAP

The ferric reducing antioxidant power (FRAP) assay measures the reducing ability of antioxidants against the oxidative effects of reactive oxygen species. This assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of tripyridyltriazine (TPTZ), whereby an intense blue Fe^{2+}–TPTZ complex with an absorbance maximum at 593 nm is formed. Increasing absorbance indicates an increase in reductive ability. Among the different samples isolated from the studied plant at various concentrations (50, 100, 150, 200, 400 µg/ml) were examined. From **Figure 3** it is clear that there was a concentration dependent

increase in reducing activity in all extracts and methanol extracts possesses highest activity among all the extracts. IC_{50} value is presented in **Table 5.**

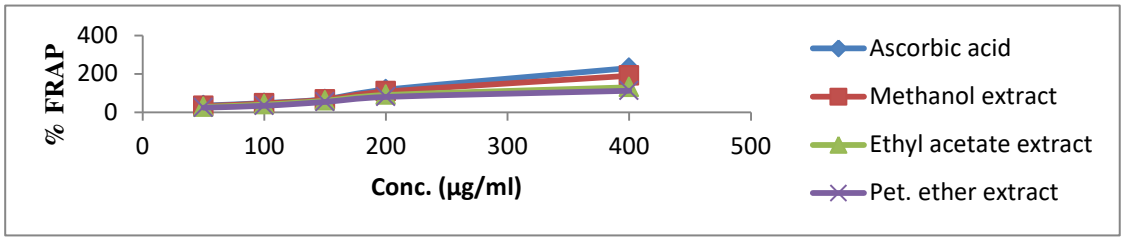

Figure 3: FRAP activity of different extracts of under study plant and standard Ascorbic acid

3.2.4 Hydrogen peroxide scavenging activity

Hydrogen peroxide is a weak oxidizing agents and can inactivate a few enzymes directly by the oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly, once inside the cell, H_2O_2 can probably react with every possible molecule in living organism especially with DNA, Proteins and Lipids and this may be the origin of many of toxic effects [27]. Hydrogen peroxide scavenging activity percentage of various extracs is presented in graph (**Figure 4**) and IC_{50} value is presented in (**Table 5**). The extracts showed the concentration dependent scavenging as compared with standard ascorbic acid.

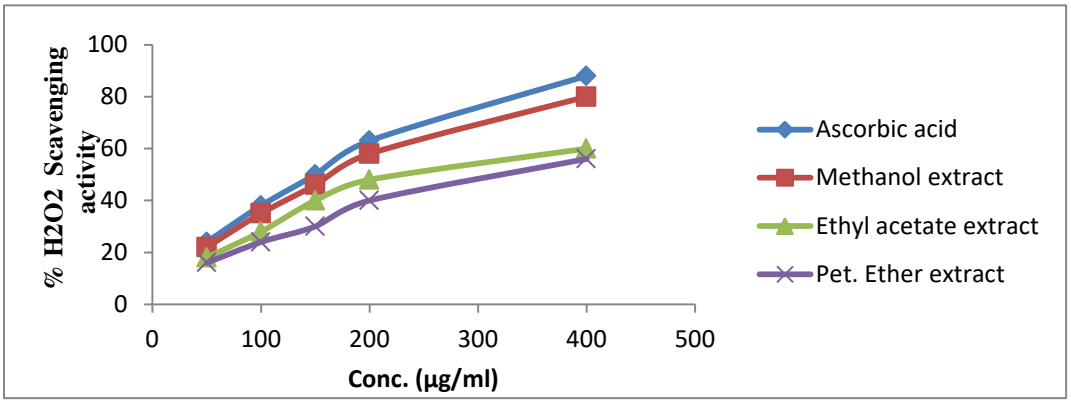

Figure 4: Hydrogen peroxide scavenging activity of various plant extracts and standard Ascorbic acid.

3.2.5 Lipid per-oxidation Method

Lipids Peroxidation has been a major problem for the shelf stability of foods. Due to oxidation of lipids, lipid hydroperoxides formed in the food systems are cleaved or polymerized to form various secondary products. These products are

responsible for the inferiority of the food quality such as deterioration of taste and flavor (Spanier *et al*.,1992; Jensen *et al*., 2001) and decreased nutritional value (Ames, 1983). Lipids Peroxidation activity percentage of various extracs is presented in graph (**Figure 5**) and IC_{50} value is presented in (**Table 5**). The extracts showed the concentration dependent activity as compared with standard ascorbic acid.

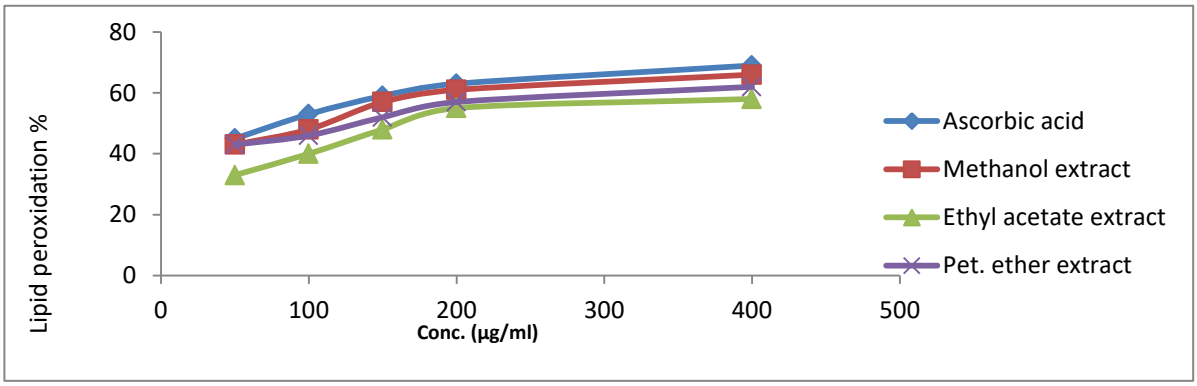

Figure 5: Lipid peroxidation activity of various plant extracts and standard Ascorbic acid.

3.3 Antimicrobial activity

3.3.1 Antibacterial Activity:

Investigation is being carried out of plant material as alternative source of antimicrobial agent. It has become more common over the past few years, due to the increased rate of development of antibiotic resistance organism. The inhibition of bacterial growth *in-vitro* by the extracts of plants could be due to the presence of some active compounds in the extracts. These active compounds may act alone or in combination to inhibit bacterial growth. It may be due to crude plant extracts containing multiple organic components including flavonoids, tannins, alkaloids, triterpenoids, all of which are known to have antibacterial affects (Santhi et al, 2006; Rabe and Staden, 1997).

In our study all the three extracts showed significant antibacterial property. The methonolic extract was found to provide more consistent antibacterial activity compare to other extracts like petroleum ether and ethyl acetate. The results of our studies are shown in **Table 6**.

3.3.2 Antifungal Activity:

In vitro antifungal of different extracts of the plant under study was determined against various fungal strains *Aspergillus niger, Penecillium crysogenum, Candida*

albicans, Cryptococcus neoformis, Rhizopus microsporus with fluconozole as positive control.Results are shown in **Table 6.**

Table 6: Antimicrobial activity of some extract of the the plant under study against pathogenic bacterial and fungal strains.

Strains	**Inhibition zone diameter (mm)**			
	Methanol extract	Ethyl acetate extract	Pet. ether extract	Control
Bacterial Strains				
E. coli (MTCC 407)	15	13	11	24
P.aeruginosa (MTCC 139)	16	13	10	24
S. aureus (MTCC 96)	15	12	09	20
B.subtilis (MTCC 441)_	15	13	10	22
K. pneumonia (MTCC 49)	13	12	08	22
Fungal Strains				
A. niger (MTCC1344)	12	11	10	20
P. crysogenum (MTCC 947)	12	10	09	22
C. albicans (MTCC	11	11	10	22
T. rubrum (MTCC 8469)	12	10	08	24
E. floccosum (MTCC 613)	10	08	07	22

Values are represented as mean ± SD (n = 3); control- Ciprofloxcin and Fluconozole

Table 5: IC_{50} values of various extracts under different assays of plant under study.

Plant extract	IC_{50} (µg/ml of extract)				
	DPPH	**Ferric reducing assay**	**FRAP**	**H_2O_2**	**Lipid peroxidation assay**
Petroleum ether extract	**223.69**	**226.66**	**138.17**	**330.98**	**229.55**
Ethyl acetate extract	**114.65**	**188.94**	**118.49**	**279.56**	**143.33**
Methanol extract	**98.94**	**124.63**	**98.63**	**191.88**	**100.79**
Ascorbic acid	**89.73**	**112.94**	**77.20**	**165.88**	**56.45**

4. Conclusion

Caragana gerardiana is belongs to family leguminosaea, before this work no such study has been conducted on this plant. The plant can serve as potential alternative to treat various diseases, as the plant was found to have broad spectrum antimicrobial potential and high antioxidant potential of plant under study, suggest its medicinal value point to an easily accessible source of natural antioxidants that could be used in the pharmaceutical industry as precursors of therapeutic drugs that can be implemented as antithesis against oxidative stress and consequent toxicity to cellular biomolecules.

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