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Biological Potential and Phytochemical Evaluation of *Prosopis cineraria*

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Abstract: The objective of the present study was to reports the biological properties (antioxidant, antibacterial and lipoxygenase properties) and phytochemical analysis of 70% ethanolic extract of stem, leaf and bark of *Prosopis cineraria*. The phytochemical analysis of the ethanolic extracts of these plants parts was carried out to explore their phytoconstituents present in this plant. The plant material (stem, leaf and bark) was collected from Cholistan desert of Bahawalpur, Pakistan and extracts was prepared by using 70% ethanol. The ethanolic extracts were subjected for the determination of antioxidant, lipoxygenase and antibacterial activity. The results of DPPH value of stem, leaf and bark ethanolic extracts of *Prosopis cineraria* showed that *Prosopis cineraria* have significant amount of antioxidants which is 82.19, 71.16 and 89.92 while percent Inhibition of lipoxygenase enzyme of stem, leaf and bark extract were 76.82, 88.17 and 83.48 respectively this give us a reason to believe that the plant is active against the lipoxygenase enzyme is It was also illustrated from the results that the plant extracts was also considerably active against *Salmonella typhi (-), E.coli (-), Pseudomonas aeroginosa (-), Bacillis subtilis (+), Staphylococcus aerus (+)*. The phytochemical test confirmed the presence of bioactive secondary metabolites like alkaloid, flavonoids, glycosides, saponins, tannins, steroids and terpenoids. The plant has high value of secondary metabolite which in turn gives antioxidant, antibacterial and lipoxygenase activity to this plant. So, this plant may be considered for potential use as nutraceuticals.

Key words: *Prosopis cineraria* · Antioxidant · Antibacterial · Lipoxygenase · Secondary metabolites · Nutraceuticals

INTRODUCTION

Prosopis cineraria is a flowering tree and contain approximately forty four species and it belongs to Fabaceae family [1]. It is native to Western and South Asia, including Afghanistan, Iran, India and Pakistan. Its common names include Khejri (India), Jand and Kandi (Pakistan). It is the state tree of Rajasthan (India) and provincial tree of the Sindh province of Pakistan [2]. Different parts of the plant are useful for the treatment of many diseases like skin diseases, piles, worms, vertigo and dyspnoea [3], protection from miscarriage, Eye diseases, Snake bite, asthma, bronchitis, dysentery, leucoderma, leprosy, muscle tremors and piles [4].

The basic theme and fundamental need of this plant oriented project was to explore the hidden medicinal potential of the plants. Antioxidants are compounds that can inhibit or reduce the oxidation process of lipids or other molecules by inhibiting the oxidizing chain reactions. The Redox property of phenolic compounds is responsible for their antioxidant activity. It can play an important role in adsorbing and neutralizing free radicals and decomposing peroxides. Normally, there are two basic categories of antioxidants, natural and synthetic. Currently research is going on considerably to find naturally occurring antioxidants for use in therapeutic or food to replace synthetic antioxidants [5]. Phenolic compounds are commonly found in both edible and nonedible plants and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry [6].

In addition these are also results of environmental pollution, UV radiation, chemical, toxins, spicy and deep fried foods and by physical stresses. This leads to

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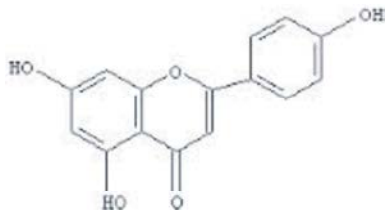
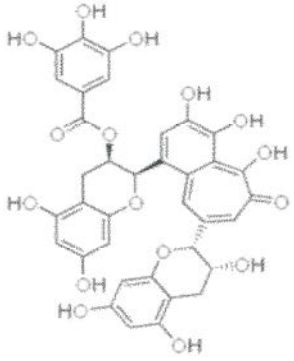
exhaustion of antioxidants which are normally produces in our body and results in anomalous gene expression and anomalous protein synthesis [7]. Oxidation process is a major cause for creation of free radicals in foods drugs and even in alive human body. Catalases and hydro-peroxidases are responsible for normal inactivation of hydrogen peroxide and hydro-peroxide to non free radical form therefore these acts as natural free radical scavengers of human body. But in certain cases body's own free radical scavengers are depleted [8].Therefore it becomes necessary to take free radical scavengers. Currently available synthetic antioxidants are considered to have rather harmful effects on human health. Therefore main focus is on natural resources [9].

A lot of phytoconstituents are present in the medicinal plants like flavonoids [10] phenolics and polyphenols [11], tannins [12], terpenoids [13], sesquiterpenes.



Chemical structure of Flavone



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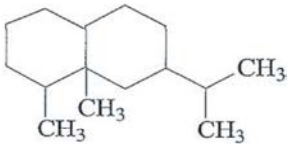

Chemical structure of sesquiterpenes

The medicinal plants play a major role in developing countries to cover their basic health needs and these medicinal plants can be used as a source of antifungal, antiviral and antibacterial constituents which are active against different microorganisms which are notorious to human health. The use of different parts of natural plants as antimicrobial agents and very useful drugs has been employed by many countries from centuries. Many powerful drugs are extracted from different parts of biologically active plants such as flowers, stem, leaf, bark and seeds [14].

Lipoxygenase is an enzyme responsible for catalyzing the process of dioxygenation of polyunsaturated fatty acids in lipids and breakdown arachidonic acid into leukotrienes. Which are used in a variety of diseases such as atherosclerosis, cancer, asthma, osteoporosis and chronic obstructive pulmonary disease [15, 16]. The present study describes the lipoxygenase inhibitory activity of extracts of some plants used in therapeutics.

MATERIALS AND METHODS

Collection of Plant Material: The plant material was collected from the desert of Bahawalpur in the month of March. The identification of *Prosopis cineraria* stem, leaf and bark was done by Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Bahawalpur, Pakistan. The identification document number is Pharm-PC- 00201. Furthermore the stem, leaf and bark part of the *Prosopis Cineraria* were deposited separately in the herbarium in the Department of Pharmacy of The Islamia University of Bahawalpur, Bahawalpur, Pakistan for future reference and this deposited document number for stem, leaf and bark was PC-S-046, PC-L-047 and PC-B-048 respectively.

Preparation of Extract: Each portion of plant was crushed individually until a course powder was obtained by using an electric grinder (National, Japan). Then coarse powder of each part was socked individually in 70 % ethanol in glass beaker and sealed with aluminum foil and kept for three days which was infrequently stirred during this period. After three days socked materials was filtered through two folds of the muslin cloth and then through Whatman filter paper to get a clear solution. This solution was preserved in refrigerator (Dawlance, Pakistan) in airtight glass container. The mark was socked in seventy percent ethanol, again for three days with occasional stirring and was filtered in the similar manner as was done for first time. The two solutions were mixed and solvent was evaporated under reduced pressure and low temperature (35-40°C) by using rotary evaporator (Heidolph Laborota 4000 efficient, Germany). At the end a thick mass was obtained which was preserved in airtight container in freezer at -20°C.

Antioxidant Activity: This method was carried out for the determination of antioxidant activity. Ten µl of test solution was added in 96-wells plate followed by the addition of 90 µl of 100 µM methanolic DPPH solution in a total volume of 100 µl. The contents were mixed and incubated at 37°C for 30 minutes. The reduction in the absorbance was measured at 517 nm using Synergy HT BioTek® USA microplate reader. Quercetin was used as standard antioxidant. All experiments were carried out in triplicates. Data obtained was computed on Ez-fit software. The decrease in absorbance indicates increased radical scavenging activity which was determined by the following formula.

$$\text{Inhibition (\%)} = \frac{(\text{Abs. of control} - \text{Abs. Of test solution})}{\text{Absorbance of control}} \times 100$$

where,

Absorbance of Control = Total enzyme activity without inhibitor

Absorbance of Test = Activity in the presence of test compound

Antibacterial Activity: The antibacterial activity was performed in sterile 96-wells microplates under aseptic environments. The method is based on the principle that microbial cell number increases as the microbial growth proceeds in a log phase of bacterial growth which results in increased absorbance of broth medium. Three gram-negative (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi*) and two gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) were included in the study. The organisms were maintained on stock culture agar medium. The test samples with suitable solvents and dilutions were pipetted into wells (20 µg / well). Overnight maintained fresh bacterial culture after suitable dilution with fresh nutrient broth was poured into wells (180 µL). The initial absorbance of the culture was strictly maintained between 0.12-0.19 at 540 nm. The total volume in each well was kept to 200 µL. The incubation was done at 37°C for 16-24 hours with lid on the microplate. The absorbance was measured at 540 nm using microplate reader, before and after incubation and the difference was noted as an index of bacterial growth. The percent inhibition was calculated using the formula:

Inhibition (%) = 100 *(X – Y) / X

where X is absorbance in control with bacterial culture and Y is absorbance in test sample. Results are mean of triplicate (n=3, ± SEM). Ciprofloxacin was taken as standard.

Lipoxygenase Assay: Lipoxygenase activity was assayed by using a total volume of 200 µl contained 160 µl KH_2PO_4 buffer (100 mM, pH 8.0), 20 µl test compound and 15 µl purified LOX enzyme (127 units per well). The contents were mixed and pre-read at 234 nm and pre-incubated for 10 minutes at 25°C. The reaction was initiated by the addition of 25 µl substrate solution. The change in absorbance was observed after 6-10 min at 234 nm. Quercetin (0.1mg per well) was used as a positive control.

$$\text{Inhibition (\%)} = \frac{(\text{Abs. of control} - \text{Abs. of test solution})}{\text{Absorbance of control}} \times 100$$

where,

Absorbance of Control = Total enzyme activity without inhibitor

Absorbance of Test = Activity in the presence of test compound

RESULTS AND DISCUSSION

Biological Properties of Ethanolic Extracts of *Prosopis cineraria*

Antioxidant Activity: The antioxidant activity was determined by using the DPPH method. It was resulted that ethanolic extract of stem, leaf and bark of *Prosopis cineraria* have antioxidant potential. The percentages of antioxidants are 82.19, 71.16 and 89.92 percent in stem leaf and bark extract respectively against the standard which was ascorbic acid with antioxidant activity of 96.00 percent as shown in the Table 1.

The antioxidants protect us from free radical damage and they are critically needed for good health and for the enhancement of our body defense mechanism. The antioxidant activity of this plant is mainly due to the flavonoids, phenolic compounds which are present in a significant amount and it protects from several inflammatory, pulmonary and heart diseases.

Antibacterial Activity: The result of antimicrobial assay are summarized in Table 2. It was obvious from the results that ethanolic extract of stem leaf and bark of *Prosopis cineraria* have antibacterial properties. The results represents that all the extract dynamically shows percent Inhibition against Salmonella typhi gram negative bacteria, *E. coli* gram negative bacteria, Pseudomonas

Table 1: Antioxidant Activity of ethanolic extract of stem, leaf and bark of *Prosopis Cineraria*:

Sr. No.	Plant Part Extract	Conc.	Antioxidant activity
1	Stem	0.5	82.19 %
2	Leaf	0.5	71.16 %
3	Bark	0.5	89.92 %
4	Ascorbic acid	0.5	96.00

*Sr. no. = Serial Number
Conc. = Concentration

Table 2: Anti Bacterial screening of ethanolic extract of stem, leaf and bark of *Prosopis cineraria*:

			% Inhibition				
Sr. No.	Plant Part Extract	Conc.	Salmonella typhi (-)	*E. coli* (-)	Pseudomonas aeroginosa (-)	Bacillis subtilis (+)	Staphylococcus aerus (+)
1	Stem	0.5	71.95±0.25	75.17±0.61	56.74±1.63	55.00±0.44	51.73±1.20
2	Leaf	0.5	72.40±1.90	73.28±0.94	65.23±0.23	57.56±1.67	56.60±3.27
3	Bark	0.5	69.05±2.15	66.22±4.57	61.51±1.63	52.50±1.06	51.27±4.73
4	Ciprofloxacin	0.5	91.56±1.77	92.04±2.22	92.13±0.55	90.45±1.21	89.99±2.43

*Sr. No = Serial Number
Conc. = concentration
(+) = Gram Positive Bacteria
(-) = Gram Negative Bacteria

Table 3: Lipoxygenase activity of ethanolic extract of stem, leaf and bark of *Prosopis cineraria*

Sr. no	Plant part extract	Conc. (mg)	% Inh
1	Stem	0.5	76.82±0.12
2	Leaf	0.5	88.17±0.75
3	Bark	0.5	83.48±0.32

*Sr. no = Serial number
Conc. = Concentration
% Inh = Percent Inhibition

Table 4: Phytochemical Analysis of the ethanolic extract of stem, leaf and bark of Prosopis cineraria:

Sr. No	Secondary metabolites	Result
1	Alkaloids	++
2	Flavonoids	++++
3	Glycosides	++
4	Tannins	+
5	Saponins	++
6	Steroids	+
7	Terpenoids	+++

*Sr. no= Serial number

aeroginosa gram negative bacteria, Bacillis subtilis gram positive bacteria Staphylococcus aerus gram positive bacteria where Ciprofloxacin taking as standard.

The result of antibacterial assay shows that plant have wider antibacterial spectrum against both gram negative and gram positive bacteria. Infections still cause about one-third of all deaths worldwide mainly because of disease in developing countries. One-third of all the deaths in developing countries are due to the infectious diseases which are results from different microorganism [17]. Chemotherapeutic agents, used orally or systemically for the treatment of microbial infections of humans and animals, possess varying degrees of selective toxicity. Although the principle of selective toxicity is used in agriculture, pharmacology and diagnostic microbiology, its most dramatic application is the systemic chemotherapy of infectious disease. The tested plant products appear to be effective against a wide spectrum of microorganisms, both pathogenic and nonpathogenic. Administered orally, these compounds may be able to control a wide range of microbes but there is also the possibility that they may cause an imbalance in the gut microflora, allowing opportunistic pathogenic coliforms to become established in the gastrointestinal tract with resultant deleterious effects. Further studies on therapeutic applications of these plant parts extracts should be undertaken to investigate these issues, especially when considering the substantial number of analytical studies carried out on these natural products.

Enzyme Inhibitory Activity: Lipoxygenases are the enzymes used in the biosynthesis of the leukotrienes. These leukotrienes play a key role in numerous inflammatory diseases. In this study it is demonstrated that the plant extracts have valuable amount of active constituents which inhibit the activity of lipoxygenase enzyme and defend the body against inflammatory and other diseases. Table 3 describes the percent inhibition of lipoxygenase enzyme by plants extracts. It was obvious from the results that 0.5 mg concentration of different parts of plant extracts inhibits the lipoxygenase enzyme up to 83.48 percent.

In lipoxygenase enzyme action inhibition of lipid hydro peroxide formation occurs only because of the fact that Lipoxygenases are very sensitive to antioxidants. So due to this catalytic cycle of lipoxygenase shut down due to unavailability of lipid hydro peroxide. In human tissues Lipoxygenase is expressed in platelet, eosinophils, synovial fluid, neutrophils, colonic tissues, lung tissues, monocytes and bone marrow cells [18]. Neutrophils contain 5-Lipoxygenase which converts the arachiadonic acid to 5-hydroxy-6, 8, 11, 14- eicosatetraenoic acid 5-HPETE. 5-HPETE is converted into a series of leukotrienes and the nature of the final product varies according to the tissue. Leukotrienes are mediators of allergic response and inflammation. Inhibitors of 5-LOX and leukotriene receptor antagonist are used in the treatment of asthma [19].

Phytochemical Screening of *Prosopis cineraria*: Different phytochemical test were performed by using different chemical reagents and ethanolic extract of stem, leaf and bark of *Prosopis cineraria* and it was resulted that a high amount of antioxidants are present in these part of this medicinally active plant. These biologically active constituents originally are secondary metabolites which are present in a significant amount in the ethanolic extract of *Prosopis cineraria* plant. These secondary metabolites comprises of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and different kinds of terpenoids as shown in the Table 4.

The presence of these secondary metabolites in this plant ensure that this plant have medicinal uses in different human diseases [20]. Basic phytoinvestigations of the extracts for their major phytocompounds is vital as the active principles of many drugs are these secondary metabolites found in plants [21]. The selective solubility of bioactive constituents in the present study points to the presence of diverse phytochemical suggesting that the secondary metabolites vary widely which probably is responsible in conferring a wide spectrum of biological activities [22].

CONCLUSION

In view of the importance of plants as cure for diseases and as a source of medicinal lead compounds, there is a need for proper and systematic biological and phytochemical investigations of the plant. Plants are the biggest source of medicine in future. The present study is not only to evaluate the antioxidant, antimicrobial and lipoxygenase inhibition but it is done to relate these activities to each other. Secondary metabolites are not only responsible for free radical scavenging property but also useful in many biological properties such as antibacterial and lipoxygenase enzyme inhibition properties. The medicines which are produce from natural sources are very effective and patient friendly. These natural agents not only diminish the diseases but also minimize reoccurrence. It is cheaper than modern medicine. Thus it is important to characterize the different types of medicinal plants for their biological potentials. Scientific investigations of medicinal plants have been initiated in many countries because of their gifts to human health.

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