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Screening of Biological Activities of Some Leguminosae (Fabaceae) Family Plants

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ABSTRACT

Objective: The objective of this paper was to evaluate some pharmacological activities of 10 indigenous Leguminosae family plants.

Methods: Agar well diffusion assay was used for the estimation of antibacterial activity of the methanolic plant extracts and Broth microdilution assay was used for the determination of minimum inhibitory concentration (MIC). Antibacterial activity was investigated against eight bacteria (both gram positive and negative) that include *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Shigella dysenteriae*, *Helicobacter pylori* and *Proteus vulgaris.* Antioxidant activity was assessed through DPPH inhibition assay. Results indicated that various plants extracts have antibacterial and antioxidant potential.

Result: *Acacia nilotica* showed highest antioxidant potential among tested plant extracts. *Cassia fistula*, *Tephrosia hamiltonii*, *Glycyrrhiza glabra* and *Cassia senna* showed highest antibacterial activity against different strains of bacteria.

Conclusion: It is concluded that tested selected plant extracts have antibacterial and antioxidant activities so can be used in treatment of various ailments. The present study can provide the basis for derivation of synthetic medicines from these tested plants. However, further studies are required to isolate the active constituents from these extracts.

Keywords: Antibacterial, antioxidant, DPPH, leguminosae, plant.

INTRODUCTION

The association of human health and flora is founded 60,000 years ago in fossils history [1]. Among 500,000 species of higher plants on earth, only 6% are in use for biological activities [2]. Leguminosae family is second in importance worldwide as food [3]. It is one of the main plant family that contain ethno botanically important plant species [4]. Leguminosae family has 650 genera and more than 18000 species [5]. Various plants of this family have medicinal properties [6].

Many of the antibiotics have been discovered through random screening. About 75% of drugs for the treatment of infections have been obtained from plants [7]. Moreover, several marketed antibiotics have developed resistance to different pathogens. So, the discovery of new antibiotics is an important public health concern. Moreover, there is an increased trend to find antioxidants from natural sources [8]. Many of

natural plants and foods have antioxidant activity and have the ability to reduce free radicals in body [9].

In current study, methanolic extracts of 10 Leguminosae family plants have undergone for screening of various biological activities. Most of the species used in this study have various traditional medicinal uses. The major reason for choosing methanol as a solvent for extraction is its maximum efficacy to take in the various phyto constituents. Even though methanol is a polar solvent, with polarity index of 5.1 but due to its amphiphillic nature it efficiently absorbs most of the non-polar phyto constituents [10].

The purpose of the study was to evaluate antibacterial, antioxidant, potential of *Acacia nilotica, Caesalpinia bonducella, Cassia fistula, Cassia senna, Cassia tora, Cicer arietinum, Glycyrrhiza glabra, Lens culinaris, Tephrosia hamiltonii, Vigna radiata.*

METHODOLOGY

Plant parts were collected from different areas of Bahawalpur, Pakistan. 10 plants were tested for biological activities: (1) *Caesalpinia bonducella* seeds, (2) *Cassia fistula* leaves, (3) *Tephrosia hamiltonii* leaves, (4) *Cassia tora* seeds, (5) *Glycyrrhiza glabra* bark, (6) *Cassia senna* leaves (7) *Cicer arietinum* seeds, (8) *Lens culinaris* seeds, (9) *Vigna radiata*s eeds, (10) *Acacia nilotica* leaves. The plants were identified by a botanist, Dr. Sarwar, Lecturer The Islamia University, Bahawalpur (Voucher No. 2201/L. S- 2203/L. S, 2206/L. S - 2212/L. S respectively) and were deposited to IUB herbarium.

After authentication of the plants by the botanist, plant parts were dried under shade and then grinded in an electric grinder. The coarsely powdered plants were extracted with methanol. A fine powder (100 g) of each plant was soaked in 400mL methanol separately for two weeks with occasional shaking. The soaked material was strained through the muslin cloth. The process was repeated three times with 200mL methanol to extract maximum contents and material [11].

The solvent was evaporated by using rotary evaporator. The extracts were collected in glass bottles and stored at 4°C until next use. Dried extracts were weighed (5mg each) and dissolved in 1 mL of methanol to get 5mg/mL stock solutions and serial dilutions were prepared from this stock solution.

Staphylococcus aureus (S.A) ATCC-6538, *Pseudomonas aeruginosa* (P.A) ATCC-9027, purchased from Microbiologics Inc. *Bacillus subtilis* (B.S), *Helicobacter pylori* (H.P), *Escherichia coli* (E.C), *Klebsiella pneumonia* (K.P), *Proteus vulgaris* (P.V), *Shigella dysenteriae* (S.D) purchased from first fungal culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of The Punjab, Lahore, Pakistan. Accession numbers were 12, 14, 72, 147, 74 and 174 respectively. All the bacteria used were kept at 37°C for 24 h before the experiment. Before use, the bacterial solution was diluted to a final concentration of 10^5 McFarland Standard and set aside.

Chemicals used were DPPH (2, 2 diphenyl 1-picryl hydrazyl), Muller Hinton agar and Muller Hinton Broth (Sigma Aldrich, Germany), Dimethyl sulfoxide (Merck, Germany), nutrient agar, nutrient broth (Merck, Germany), Ciprofloxacin (Sami Pharmaceuticals (Pvt) Ltd)

Agar Well Diffusion Assay

The experiment was performed by the method of [12, 13] with slight modifications. Petri dishes were sterilized in hot air oven at 150°C and then placed in laminar flow hood for the aseptic environment. 20mL of Muller Hinton agar was placed in petri dishes and allowed to solidify. A suspension of the microorganism of 60µL was evenly spread on the surface of agar with sterile cotton- tipped swab. Wells of 6 mm in diameter were made on solid agar surface with the help of Cork borer in each petri dish. 20µL of extract solution was added to each well. Place the petri dishes in the incubator at 37°C for 24 hours. After 24 hours, the zones of inhibition were measured to estimate the antibacterial activity. The experiment was done in triplicate and results were taken as an average of the three experiments.

Broth Microdilution Method

The antibacterial activity assay was explained by Kaspady *et al*. and Mondal [14, 15]. The method explains, the antibacterial activity that was performed in sterile 96-wells micro plates in laminar flow hood to attain aseptic environment. Total mixture volume in a well was 200µL, contained 20µL of methanolic extract solution and 180µL suspension of bacterial culture in Muller Hington broth. At 540 nm absorbance was measured this was taken as pre-read. Then for 16-24 hours the plates were incubated at 37°C. After-read was taken at 540 nm and the difference between pre-read and after the read was taken as an index of

bacterial growth. All readings were taken as triplicate. Results are mean of triplicate (n=3, ± S.E.M). The positive inhibitor was ciprofloxacin and instead of test sample methanol was added in assay as negative control.

The % inhibition was calculated by following formula

Inhibition (%) = 100 *(X – Y) / X

Where

X= absorbance in negative control with bacterial culture

Y= absorbance in test sample with bacteria

Serial dilutions of the test samples were made to calculate the minimum inhibitory concentration (MIC) was measured.

DPPH Radical Scavenging Activity

The antioxidant activity of different serial dilutions of crude plant extracts was checked with 2, 2 - diphenyl 1picrylhydazyl (DPPH). The experiment was performed by the method of [16,17] with slight modifications. 90µL of DPPH solution (0.1mM) and 10µL of test plant extract was used in this micro assay. The reaction mixture was incubated for 30 minutes at 37°C. Absorbance was taken at 517 nm by using BioTek® USA ELISA micro plate reader. All the assays were done in triplicate. Radical scavenging capacity of sample was calculated by the following formula:

% radical scavenging activity= 100- [$A_{sample}/A_{control}$] * 100

Where,

$A_{control}$= Absorbance of negative control

A_{sample}= Absorbance of sample

Statistical Analysis

SPSS version 20.0 was used for statistical analysis. One-way ANOVA followed by Tukey post Hoc test was applied for checking significance. A P-value of ≤0.05 was considered significant.

RESULT

The growth inhibition value of methanolic extracts of selected plant extracts on different bacterial strains was shown in Table **1** & **2**.

Table 1. MIC determination results of Leguminosae family plant methanolic extracts.

Tested material	S.A	K.P	B.S	S.D	P.V	E.C	H.P	P.A
	Minimum inhibitory concentration (MIC) (mg/mL)							
Ciprofloxacin (P.C)	0.001[a]	0.001[a]	0.005[a]	0.002[a]	0.005[a]	0.000015[a]	0.008[a]	0.001[a]
C.bonducella	----	3[d]	3[d]	----	3[d]	2.1[c]	2.5[c]	4[e]
Cassia fistula	2[c]	2.7[d]	3[d]	4[e]	2.5[c]	2.5[c]	0.5[b]	4[e]
Tephrosiahamiltonii	----	2.3[c]	2.5[c]	3[d]	2.5[c]	2.3[c]	0.5[b]	3[d]
Cassia tora	----	1[b]	5[f]	----	3[d]	4[e]	2.5[c]	4[e]
Glycyrrhizaglabra	5[f]	0.5[b]	2[c]	2.5[c]	3[d]	3.7[e]	1.5[b]	1[b]
Cassia senna	----	0.5[b]	4[e]	4[e]	2.5[c]	3.9[e]	1[b]	4[e]
Cicerarietinum	----	2[c]	4[e]	4[e]	4[e]	4.2[e]	3[d]	3[d]
Lens culinaris	----	2.5[c]	5[f]	----	5[f]	4[e]	4[e]	----
Vignaradiata	----	2.7[c]	4[e]	----	5[f]	3.5[d]	4[e]	----
Acacia nilotica	----	2.9[d]	3.5[d]	5[f]	4[e]	4.7[f]	1[b]	5[f]

***** Results were expressed as mean ± S.E.M (n =3). Superscripts [a-f] in a specific column were statistically different from each other (P ≤ 0.05). PC- Positive control

---- There was no inhibition at a dosage of 5mg/mL.

Table 2. Screening of Leguminosae family plants for antibacterial activity determined by agar well diffusion.

Tested material		S.A	K.P	B.S	S.D	P.V	E.C	H.P	P.A
	Dose (mg/mL)	Diameters of Zone of Inhibition (mm)							
Ciprofloxacin	200/100	32±0.1	30±0.01	35±0.01	30±0.01	31±0.01	35±0.01	26±0.01	32±0.1
C. bonducella	5	----	13±0.5	13±0.5	---	13±0.5	15.5±1	15±0.5	12±1
	1	----	09±0.5	09±0.5	---	09±0.5	11±0.5	10 ±1	8±0.5
Cassia fistula	5	16±0.5	14±0.5	13±0.5	12±1	15±0.5	16±0.5	20±1	17±0.5
	1	12±0.5	10 ±1	09±0.5	8±0.5	10 ±1	11±0.5	15±1	12±0.5
T. hamiltonii	5	8±0.5	17±1	14±1	13±0.5	15±0.5	17±1	20±1	13±0.5
	1	----	12±0.5	10 ±1	09±1	10 ±0.5	11±0.5	15±1	09±0.5
Cassia tora	5	----	18±0.5	11±1	8±0.5	13±0.5	12±1	13±0.5	12±1
	1	----	16±0.5	8±0.5	---	09±0.5	8±0.5	09±0.5	8±0.5
Glycyrrhiza glabra	5	12±1	20±1	16±1	15±0.5	13±0.5	11.5±1	17±0.5	18±1
	1	8±0.5	15±1	12±0.5	10 ±0.5	09±0.5	8±0.5	14±0.5	16±0.5
Cassia senna	5	8±0.5	20±1	12±1	12±1	13±0.5	11.5±1	18±1	12±1
	1	----	15±1	8±0.5	8±0.5	09±0.5	8±0.5	16±0.5	8±0.5
Cicer arietinum	5	---	16±0.5	12±1	12±1	12±1	11.5±1	13±0.5	13±0.5
	1	----	12±0.5	8±0.5	8±0.5	8±0.5	8.5±1	09±0.5	09±0.5
Lens culinaris	5	----	13±0.5	12±1	----	12±1	12±1	12±1	8±1
	1		09±0.5	8±0.5		8±0.5	8±0.5	8±0.5	---
Vigna radiata	5	----	14±0.5	12±1	----	12±1	11.5±1	12±1	----
	1	----	10 ±1	8±0.5	----	8±0.5	8±0.5	8±0.5	---
Acacia nilotica	5	8±0.5	13±0.5	12±1	13±1	12±1	18±1	18±0.5	12±1
	1	----	09±0.5	8±0.5	9±0.5	8±0.5	16±0.5	16±0.5	8±0.5

***** Results were expressed as mean ± S.E.M (n =3). Superscripts $^{a-f}$ in a specific column were statistically different from each other (P ≤ 0.05) in each column

---- There was no inhibition at a dosage of 5mg/mL.

Many of the tested plant extracts have relatively good antibacterial activity. *Cassia fistula* and *Tephrosia hamiltonii* showed highest antibacterial activity against *Helicobacter pylori*. *Glycyrrhiza glabra* and *Cassia senna* showed highest antibacterial activity against *Klebsiella pneumonia.* All the tested plants showed minimum antibacterial activity against *Staphylococcus aureus.* The least active antibacterial tested plant extracts include *Lens culinaris* and *Vigna radiata.* The diameters of growth of inhibitions of different extracts were 0-20 mm. Ciprofloxacin showed diameters of inhibitions in a range of 26-35mm against different bacterial strains. There was significant difference between the zone of inhibition values of ciprofloxacin and extract (P ≤ 0.05).

The results obtained from ciprofloxacin showed resistance to all selected bacterial strains representing MIC ranged from 0.015- 8µg/mL There was significant difference between MIC values of ciprofloxacin and extract (P ≤0.05).

Results revealed that Acacia nilotica showed highest inhibition of DPPH and Lens culinaris showed minimum inhibition of DPPH among the tested species of plants (Table **3**). The percent inhibition of DPPH by selected leguminosae family plants were 1.5- 90% at a dosage of 5mg/mL

Table 3. Screening of Leguminosae family plants for DPPH inhibition assay.

S. No.	Plants of Leguminosae Family	%age Inhibition	IC $_{50}$(µg/mL)
1.	*Caesalpinia bonducella*	19 ± 0.5	----
2.	*Cassia fistula*	83 ± 0.1^{b}	201
3.	*Tephrosia hamiltonii*	27 ± 1.0	----
4.	*Cassia tora*	24 ± 0.5	----
5.	*Glycyrrhiza glabra*	36 ± 0.5	----
6.	*Cassia senna*	90 ± 1.5 ^{a}	181
7.	*Cicer arietinum*	57 ± 0.1 ^{c}	395
8.	*Lens culinaris*	1.5 ± 0.5	----
9.	*Vigna radiata*	3.9 ± 0.5	----
10.	*Acacia nilotica*	92 ± 0.5 ^{a}	173
11.	Ascorbic acid (Standard)	93.74±0.12 ^{a}	0.01

***** Results were expressed as mean ± S.E.M (n =3). Superscripts $^{a-f}$ in a specific column were statistically different from each other (P ≤ 0.05)

DISCUSSION

Test performed for flavonoids exhibited yellow color which revealed the presence of flavonoids. Persistence of frothing was observed for more than half hour when the test was performed for saponins which indicated the presence of high contents of saponins. Different tests are performed to identify the presence of cardiac glycosides. By Keller Killani method brown ring was not appeared at the interface, similarly kedde's and baljet test also gave negative results which confirmed the absence of cardiac glycoside but positive result with Lieberman-burchard test exhibited that cardiac glycosides are absent but phytosterols are present. Leibermanburchard test indicated positive result for all sterols double bond containing triterpenes, cholesterol and sitosterol.

Erdmmann's test performed for phenolics showed reddish color which confirmed the presence of phenolics. Color of sodium picrate paper remains same in guignard's test indicated the absence of cyanogenic glycosides. Tests performed for alkaloids produce precipitates of different colors which assured the presence of alkaloids.

Wagner test produced reddish brown precipitate. Mayer test presented cream color precipitate. Hager test gave yellow precipitate and Dragendorffs test gave orange precipitate. All these precipitates assured the presence of alkaloids.

Six Gram negative (*Shigella dysenteriae*, *Helicobacter pylori*, *Proteus vulgaris Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) and two Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) were used for this the study. These bacterial strains are the commonly infection causing bacteria in humans as *Bacillus subtilis* involve in the various allergic conditions of respiratory track, food poisoning and eye infections [18]. *Staphylococcus aureus* causes variety of skin diseases including (boils, itch), soft tissue, bone, joint, food poisoning, cardiovascular, wound infections, and other respiratory problems. *Pseudomonas aeruginosa* can cause urinary tract infections, ear & eye infections, pneumonia, and traumatic wound infections. *Klebsiella pneumoniae* is responsible for urinary tract infections, endocarditis, wound infections, cholecystitis, and Meningitis. *Escherichia coli* is an opportunistic organism which can cause pneumonia and even sepsis in immunocompromised person [19]. The plants of Cassia genus proved to be highly active in this study and this matched to previously published literature [20-21]. Moreover, *Acacia nilotica* also showed highest activity among tested plants of Leguminosae family.

CONCLUSION

In this experiment, Leguminosae plants extracts were used to conduct in vitro antibacterial test against *Staphylococcus aureus* & *Bacillus subtilis* (Gram positive bacteria) *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella pneumonia, Shigella dysenteriae, Helicobacter pylori, Proteus vulgaris* (Gram negative bacteria). MIC was determined by broth microdilution method, the study found that Leguminosae plants extracts have certain antibacterial effects. The present study can provide the suggestion for presence of antibacterial active principle in certain plants of Leguminosae family. Various plants extracts as *Cassia fistula* have both antioxidant and antibacterial effects thus can prevent and cure the diseases. However, further study is required to isolate constituents responsible for antimicrobial and antioxidant activities of the extracts.

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