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# ANTIBACTERIAL ACTIVITY OF VARIOUS LEAF EXTRACT OF *CAJANUS CAJAN L.*

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**ABSTRACT**
The active components of *Cajanus cajan* L. were extracted in various organic solvents such as pet ether, chloroform, methanol, ethanol and aqueous and were tested against *Bacillus subtilis, staphylococcus aureus, Streptococcus pneumonia, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa* using agar well diffusion method. All the extracts inhibited the growth of both Gram-positive and Gram-negative bacteria. However, the chloroform extract did not show any activity against Gram negative bacteria. The ethanolic extract showed the highest activity on all the Gram positive bacteria and Gram negative bacteria *Klebsiella pneumonia* at 100mg/mL, where as *Pseudomonas aeruginosa* was resistant to all the extracts except ethanol. The minimum inhibitory concentration (MIC) of the extracts was in the range of 12.5-75 mg/mL. Preliminary phytochemical analysis of *Cajanus cajan* L. leaves revealed the presence of phenol, tannin, lignins, glycosids, steroids, flavonoids, alkoloids and terpenoids. The results suggest that the ethanolic leaf extract of *Cajanus cajan* L. showed more effective antibacterial activity against both Gram-positive and Gram-negative bacteria. There is a basis for the traditional use of the plant as a local health remedy.

## INTRODUCTION

The search for plants with antibacterial activity has gained increasing importance in recent years due to the development of antimicrobial drug resistance and often the occurrence of undesirable side effects of some antibiotics (Soberon *et al.,* 2007). With the advent of ever increasing resistant bacterial and yeast strains, there has been a corresponding rise in the universal demand for natural antimicrobial therapeutics. About 80% of developing countries use traditional medicine based on plant products. Plants are saffer alternative sources of antimicrobials (Pretorious and Watt, 2001; Sharif and Banik, 2006).

*Cajanus cajan* L is a perennial member of the family Leguminosae, commonly known as 'pigeonpea' or 'redgram' in English and 'arhar' in Hindi. It is one of the most important dietary legume crop predominantly grown in the tropical regions. India contributes about 90% of the world production of *Cajanus cajan* L. compared with other grain legumes, pegionpea ranks only sixth in area and production (Singh *et al.*,1998), but it is used in more diverse ways than others (Chakraborty *et al.*, 2007). The extracts or components of pigeonpea are commonly used all over the world for the treatment of diabetes, dysentery and hepatitis (Grover *et al.,* 2002) Now days, these leaves are used for the treatment of wounds, aphtha, bedsores and malaria as well as diet-induced hypercholesterolemia etc (Aiyeloja and Bello,2006 and Luo *et al.,* 2008). Chemical constituent investigations have indicated that pegionpea leaves are rich in flavonoids, stilbenes which are considered responsible for the beneficiaries of the leaves on human health (Zu *et al.,* 2006 and Zheng *et al.*, 2007). In the present scenario of emergence of multidrug resistance to human pathogenic infections, it has become necessary to search for new antimicrobial substances from other sources such as plants (Doughari *et al.,* 2007). In view of the importance of *Cajanus cajan* L. in ethanobotany as health remedy, the present work has been planned to investigate the antibacterial activity of the leaf extracts against some pathogenic bacteria.

## MATERIALS AND METHODS

The leaves of *Cajanus cajan* L. variety Maruti (ICP-8863) was collected from the field of Gulbarga District in Karnataka. The leaves were dried under shade and made into fine powder using pestle and mortar. Five hundred grams of the powdered plant material was subjected to the soxhlet successive extraction method (60-80ºC ) using 2.5 liter of petroleum ether, chloroform, methanol, ethanol and distilled water (aq) in the order of increasing polarity of solvent for a period of 18hr. The extracts obtained were dried at 40ºC.

**Phytochemical screening:** The extracts of the plant were screened for alkaloids, flavonoids, glycosids, saponins, tannins, phenols, lignins and steroids as described by Harborne (1998).

**Antibacterial activity:** Each extract was tested for the antibacterial activity against five bacteria *Bacillus subtilis, Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Klebsiella pneumoniae, Escherichia coli* and *Pseudomonas aeruginosa*. Antibacterial activity of the leaf extracts of *Cajanus cajan* L. was studied using Agar well diffusion assay (Indian Pharmocopia, 1996). The petroleum ether, chloroform, methanol, ethanol and aqueous extracts

were dissolved in 50% Dimethylsulfoxide (DMSO) to obtain concentration of 12.5, 25, 50 and 100mg/mL. The Petri plates containing 10mL of Muller Hinton Agar medium was inoculated with 200$\mu$L of 18h old bacterial culture of $10^6$ CFU and was evenly spread with a sterile bent glass rod. A sterile cork borer was then used to make five wells (8mm diameter) for different concentrations of the extract, on each of the plates containing cultures of the different test organisms. Wells were loaded with 100$\mu$l of plant extracts and standard solution of streptomycin (0.4mg/mL). For assaying antibacterial activity, plates were incubated at 37ºC for 24hr. The diameter of zone of inhibition (in mm) was recorded.

**Minimum inhibitory concentration (MIC)**

The MIC of the extracts was determined according to the macro broth dilution technique (Baron and Finegold, 1990). Standardized suspensions of the test organisms were inoculated into a series of sterile tubes of nutrient broth containing two-fold dilution of leaf extracts and incubated at 37ºC for 24h. MICs were read as the least concentration that inhibited the growth of the test organisms.

All the experimental results were analyzed and compared by ANOVA using SPSS package version 10.

## RESULTS AND DISCUSSION

The antibacterial activity of various leaf extracts of *Cajanus cajan* L. against Gram-positive and Gram-negative bacteria are shown in Table 1. The ethanolic extracts showed highest

**Table 1: Antibacterial activity of various leaf extract of *Cajanus cajan* L.**

| Extracts Microorganisms | Zone of Inhibition in mm Conc. mg/mL | Pet ether extract | Chloroform extract | Methanol extract | Ethanol extract | Aqueous extract | Streptomy-cin 0.4mg/mL |
|---|---|---|---|---|---|---|---|
| Gram-positive | 12.5 | - | 12.6±0.01 | 14.0±0.01 | 16.0±0.10 | 12.5±0.05 | |
| bacteria | 25 | 11.5±0.12 | 14.5±0.03 | 15.1±0.13 | 17.6±0.02 | 13.0±.0.12 | 25.0±0.1 |
| *Bcillus subtilis* | 50 | 12.5±0.01 | 15.0±0.07 | 16.5±0.02 | 19.1±0.15 | 17.0±0.02 | |
| | 100 | 13.0±0.5 | 18.1±0.05 | 19.0±0.12 | 22.1±0.01 | 18.5±0.1 | |
| *staphylococcus* | 12.5 | 10.0±0.02 | 12.6±0.05 | 15.0±0.03 | 17.1±0.1 | 11.0±0.05 | |
| *aureus* | 25 | 10.5±0.03 | 12.8±0.1 | 16.5±0.02 | 19.4±0.05 | 12.6±0.12 | 20.0±0.0 |
| | 50 | 10.5±0.01 | 14.7±0.02 | 18.1±0.01 | 25.3±0.01 | 16.4±0.0 | |
| | 100 | 11.0±0.01 | 15.0±0.03 | 20.0±0.08 | 30.1±0.01 | 16.3±0.13 | |
| *Streptococcus* | | | | | | | |
| *pneumoniae* | 12.5 | 08.0±0.05 | 11.6±0.08 | 12.0±0.03 | 14.1±0.01 | 10.0±0.05 | |
| | 25 | 08.7±0.07 | 11.8±0.1 | 13.5±0.05 | 15.4±0.02 | 11.8±0.12 | 19.0±05 |
| | 50 | 10.2±0.11 | 12.0±0.05 | 15.1±0.01 | 18.3±0.05 | 13.4±0.0 | |
| | 100 | 11.5±0.01 | 14.0±0.06 | 17.0±0.05 | 22.1±0.11 | 16.0±0.13 | |
| Gram-negative bacteria | | | | | | | |
| *Salmonella typhi* | 12.5 | - | - | 08.0±0.05 | 11.0±0.12 | - | |
| | 25 | - | - | 10.5±0.04 | 12.5±0.05 | - | 21.0±0.2 |
| | 50 | 11.0±0.03 | - | 11.4±0.01 | 16.1±0.06 | 10.0±0.02 | |
| | 100 | 11.5±0.04 | - | 12.0±0.0 | 18.0±0.07 | 15.1±0.01 | |
| *Klebsiella* | | | | | | | |
| *pneumoniae* | 12.5 | 10.0±0.09 | - | 18.4±0.06 | 22.0±0.01 | 16.4±0.06 | |
| | 25 | 13.0±0.01 | - | 20.5±0.01 | 24.4±0.03 | 16.8±0.01 | 22.0±0.5 |
| | 50 | 13.5±0.06 | - | 22.6±0.02 | 25.0±0.12 | 17.0±0.08 | |
| | 100 | 12.0±0.07 | - | 25.7±0.05 | 25.5±0.03 | 17.2±0.09 | |
| *Escherichia coli* | 12.5 | 07.1±0.01 | - | 10.4±0.12 | 12.3±0.05 | 13.6±0.12 | |
| | 25 | 09.0±0.02 | - | 15.6±0.05 | 13.1±0.03 | 16.5±0.05 | 15.0±0.5 |
| | 50 | 11.0±0.05 | - | 15.1±0.04 | 17.0±0.00 | 16.1±0.01 | |
| | 100 | 12.1±0.01 | - | 16.0±0.01 | 17.1±0.05 | 16.6±0.02 | |
| *Pseudomonas* | | | | | | | |
| *aeruginosa* | 12.5 | - | - | - | - | - | |
| | 25 | - | - | - | 10.5±0.12 | - | 27.0±0.2 |
| | 50 | - | - | - | 11.0±0.05 | - | |
| | 100 | - | - | - | 13.4±0.2 | - | |

Each value is expressed as mean ± S.D (n = 3) and statistically significant at $p < 0.05$; '-'No zone of inhibition

**Table 2: Minimum inhibitory concentration of various leaf extract of *Cajanus cajan* L.**

| ExtractsMicrorganisms | Gradient crude extract mg/mL Petether extract | Chloroformextract | Methanol extract | Ethanol extract | Aqueous extract |
|---|---|---|---|---|---|
| *Bcillus subtilis* | 6.25±0.12 | 6.25±.02 | 6.25±0.05 | 6.25±0.01 | 6.52±0.06 |
| *staphylococcus aureus* | 60±0.12 | 75±0.15 | 6.25±0.01 | 3.125±0.07 | 6.25±0.06 |
| *Salmonella typhi* | 70±0.01 | - | 6.25±0.02 | 6.25±0.01 | 6.25±0.11 |
| *Klebsiella pneumoniae* | 65±0.05 | - | 3.125±0.01 | 70±0.17 | 6.25±0.01 |
| *Esherichia coli* | 12.5±0.06 | - | 12.5±0.15 | 6.25±0.11 | 6.25±0.05 |
| *Pseudomonas aeruginosa* | - | - | | 50±0.06 | - |

Each value is expressed as mean ± S.D (n = 3) and statistically significant at $p < 0.05$; '-'No zone of inhibition

**Table 3: Phytochemical screening of various leaf extract of *Cajanus cajan* L.**

| Photochemicals | Pet ether extract | Chloroform extract | Ethanol extract | Aqueous extract |
|---|---|---|---|---|
| Alkaloids | - | - | + | + |
| Flavonoids | - | - | + | + |
| Terpenoids | - | - | + | + |
| Steroids | + | + | + | + |
| Tannins | + | + | + | + |
| Phenols | + | + | + | + |
| Glycosides | + | + | + | + |
| Lignin's | + | + | + | + |
| Saponins | - | - | - | - |

+ = Present; - = Absent

activity on both Gram- positive and Gram-negative bacteria compared to the methanol, aqueous, chloroform and petether extracts. While chloroform extract did not show any antibacterial activity against Gram negative bacteria in all concentrations. The highest inhibition zone was observed against *Staphylococcus aureus* and *Bacillus subtilis* and *Klebsiella pneumoniae* at 100mg/mL of ethanol extract. However, the *Pseudomonas aeruginosa* was resistant to all the extracts except the ethanolic extract. The difference in the observed activities of the various extracts may be due to varying degrees of solubility of the active constituents in the solvents used. It has been documented that different solvents have diverse solubility capacities for different phytoconstituents (Majorie, 1999). Ethanolic, chloroform, petether and distilled water extracts of several plants have shown antimicrobial activities in *Madhula butyracea, striga densiflora* and *striga orobanchioods* (Hiremath *et al.,* 1996). Of all the bacteria tested the Gram-positive bacteria were slightly more susceptible to the extracts than the Gram-negative bacteria, therefore the present results are in agreement with earlier reports (Jigna and Sumitra, 2006; Dougharia and Manzara, 2008).

The MIC method was applied on extracts has proved their high efficacy against microorganisms by the disc diffusion method. The MIC of petether, chloroform, methanol, ethanol and aqueous extract as shown in Table 2. The ethanolic extracts of *Cajanus cajan* L. showed highest sensitivity at 3.125mg/mL against *Klebsiella pneumoniae* and at 6.25mg/mL against *Staphylococcus aureus*. The results support the use of *Cajanus cajan* L. in the treatment of gastroenteritis, pneumonia and urinary tract (Kilani, 2006; Juanid *et al*., 2006).

The phytochemicals detected in *Cajanus cajan* L. leaf extracts are listed in table-3. Test for alkaloids, steroids, tannins, phenols, glycosides and lignins were positive in all extracts, where as flavonoids and terpenoids were detected only in methanol and ethanol extracts. The saponine is absent in all extracts. Results show that *Cajanus cajan* L. are rich in phytochemicals. Biologically important specific compounds have been identified in extracts of the plant by many workers (Ducker-Eshun *et al.,* 2004; Zu *et al.,* 2006; Zheng *et al.,* 2007). The presence of phytoconstituents in the leaf extracts may be responsible for the antibacterial activity of the plant (Majorie, 1999).

It is concluded that antibacterial activity of *Cajanus cajan* L. and its active constituents would be helpful in treating various kinds of diseases. It also revealed that ethanolic extract have a broad spectrum of activity against both gram-positive and gram-negative bacteria. Further investigation of its activity against a wider range of bacteria, identification and purification of its active chemical constituents and toxicological investigations of the plant extracts should be carried out with a view to developing novel drugs for human consumption.

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