

Original Research Article

<http://dx.doi.org/10.20546/ijcmas.2016.503.057>

Comparative analysis of phytochemical constituents and antibacterial activity of leaf, seed and root extract of *Cajanus cajan* (L.) Mill sp

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A B S T R A C T

Keywords

Cajanus cajan.L,
Phytochemical
constituents,
Antibacterial
activity,
MIC

Article Info

Accepted:
18 February 2016
Available Online:
10, March 2016

Herbal plants are known to be the excellent stimulators of immune system due to antioxidant properties of phytochemical constituent. In the recent consequence, phytochemistry and pharmacognosy are predominately focused areas of research about medicinal plants. The present study is the comparative analysis of phytochemical constituents and natural antibacterial potential of leaf, seed and root extract of *Cajanus cajan* with different solvent such as methanol, ethanol, acetone & aqueous. The phytochemical constituents are analyzed through the standard qualitative analysis and antibacterial potential valued against assured pathogenic species of gram positive and gram negative bacteria (*E.coli*, *Pseudomonas sp*, *Klebsiella sp* & *Streptococcus sp*, *Bacillus sp*) through agar well diffusion technique. From the result of the phytochemical analysis it is indicated the lack of anthraquinone in leaf and lack of reducing sugar, terpenoids, cardiac glycosides, anthraquinone in seed. All bioactive constituents are present in the root. Maximum activity was exhibited by ethanol extracts against *E.coli* and *Pseudomonas sp* and methanol extract against *Streptococcus sp* and *Klebsiella sp* was detected in all the herbal parts of *Cajanus cajan* tested. MIC value of leaf, seed & root was ranging between 0.380 and 0.409 mg/ml for the pathogenic bacteria. It has been known that an MIC 0.380 mg/ml was noted for ethanol leaf extracts and 0.365 mg/ml for methanol leaf extract against pathogenic bacteria respectively. The outcomes of this study support the trifoliolate plant species *Cajanus cajan* had high amount of phytochemicals and antibacterial activity. So that extracts of *Cajanus cajan* used for the pharmacognosy for treat infections initiated by the respective pathogens.

Introduction

Herbal plants have been recently popularized in modern medicine, since many therapeutically important compounds are derived from them (Gosh *et al.*, 2006). Herbs are staging a comeback and herbal

“renaissance” is happening all over the globe. Mankind has been using plants as therapeutic agents for thousands of years and continues to rely on them for healthcare (Ahsan *et al.*, 2009). More than 30% of the

entire plant species, at one time or other was used for medicinal purposes. Herbal plant based drugs have been in use against various infections (Ahmad *et al.*, 2009).

Herbal plants are considered into bio resources of drug. A number of exciting products have been found with the use of a combination of plant extracts to treat diseases. The antimicrobial properties of plants have been studied by a number of investigators worldwide through biological development of plants extracts is vital to ensure their efficacy and safety. These factors are of importance if plant extract are to be accepted as valid medical agents for the treatment of infectious diseases especially for the multi-drug resistance bacteria.

Trifoliolate plants i.e., plants bearing three leaflets at each node produce Flavonoids and isoflavanoid compounds (phytochemicals) (Dogra 2009). Decoctions of the leaves were believed to have chemical compounds which are active against pathogenic microorganism. More people have continued to use these herbs for their treatment of different pathogenic infection in the absence of adequate toxicity data and proper understanding of their medicinal properties. Traditional medicine practitioners consider that these herbs are non-toxic even though there is no scientific backing to support this claim (Oyewole *et al.*, 2010). Quality evolution of plant resources and herbal preparation is a important requirement of industry and other organization dealing with Ayurveda and herbal products (Arsul *et al.*, 2011).

These herbs possesses a good amount of bioactive compounds that make them effective as a potent medicinal plant in this regard, we have chosen *Cajuns cajan*. which is highly grown in Andrapradesh. There is a

lot of information, data and collected works but there is very limited work testified on the present direction.

Pigeon pea (*Cajuns Cajan L.*), a diploid legume crop species is a member of the tribe *phaseoleae*. Pigeon pea (*Cajuns cajan*), which belongs to the legume family is a multipurpose, hardy grain legume crop grown by many developing countries in semi-arid tropics and sub tropics (Zu *et al.*, 2006) pigeon pea occupies an important aspect in human nutrition as a source of dietary protein in several countries (Abdelati *et al.*, 2009).

The leaves are traditionally used as stringent, diuretic, laxative, anti-inflammatory and oral ulcers. The leaf extracts display defending effects against alcohol induced liver damage and hypo toxic-ischemic brain damage (kundu *et al.*, 2008) there search on chemical elements indicated that the pigeon pea leaves are rich in flavonoids, stilbenes which are considered to be responsible for the beneficiaries of leaves on human health. (zu *et al.*, 2006)

The extracts or components of pigeon pea are mostly used all over the world. For cure the diabetes, dysentery and hepatitis (Grover *et al.*, 2002) currently days, these leaves are used for the treatment of injuries, naphtha, ulcers and malaria as well as diet-induced hypercholesterolemia etc. (Aiyeloja and Bello, 2006, Luo *et al.*, 2008).

Cajanus cajan seeds are now well-thought-out as a non-conventional feed substance in poultry feeding and as a valuable protein feed resource. The seeds of the plant are also claimed to cure gingivitis, stomatitis (Ganeshan, 2008). *Cajanus cajan* roots are considered anthelmintic, expectorant, febrifuge, sedative, vulnerary (Kong, 2010). My research has been planned to examine

the comparative analysis of phytochemical constituents, natural antibacterial potential of the various part of the *Cajanus cajan* plant extract.

Materials and Methods

Collection and Processing of Plant Materials

The fresh leaves, roots and seeds of *Cajanus cajan* L. were simultaneously collected from local area of Theni district. Fresh parts of the plants were Identified and authenticated prior to phytochemical analysis. The leaves, Seed& roots were rinsed thrice with distilled water followed by double distilled water to remove the dust and other contaminants then dried for about 2-3 weeks at room temperature under shade conditions. The dried leaves, seeds, roots were ground separately into powdery form using an electric grinder, then stored and labeled in sterilized containers.

Preparation of Plant Extracts

For phytochemical analysis, the extracts were prepared by taking 2gms of each dried powder into separate 100 ml conical flask and 50ml of each solvent (Aqueous, Methanol, ethanol and Acetone) was added. The conical flasks were plugged with cotton plugs, labeled and allowed to stand for 1-2 hrs. And then filtered using what man No.1 filter paper. Thus, the filtrates obtained were used as test solutions.

Qualitative Analysis of Phytochemical Screening

The plant extracts were tested for the presence of bioactive compounds such as alkaloids, anthocyanin and cardiac glycosides, coumarins, reducing sugars, flavonoids, phenols, quinines saponins,

Steroids, tannins, terpenoids by standard method (Yadav *et al.*,2011).

Test for Flavonoids

Ferric Chloride Test

Few drops of FeCl₃ solution was added to the test solution. Blackish precipitate indicates the presence of flavonoids.

Alkaline Reagent Test

The test solution was treated with sodium hydroxide solution. Yellow to red colour indicates the presence of flavonoids.

Qualitative Analysis of Saponins (Foam Test)

5ml of plant extracts were mixed with equal volume of distilled water and mixed vigorously for 3 to 5min gives intense stable foam development. In addition of 3ml of olive oil mixed vigorously, observed the development of emulsion.

Qualitative Analysis of Glycoside by Keller- Killani Test Method

To 0.5 ml of the plant extract, 2ml of glacial acetic acid and few drops of 5% ferric chloride were added along the side of the test tube. Formation of brown ring at the interface indicates the presence of cardiac glycosides.

Qualitative Analysis of Steroids

Plant extracts mixed in 2ml of chloroform and Conc. H₂SO₄ were added gently, which leads to the development of red colour in the lower chloroform layer indicating the presence of steroid, and was further confirmed with addition of acetic acid which develops the greenish colour formation.

Qualitative Analysis of Terpenoids by Salkowski Test Method

To 0.5 ml of the leaf extract, 2ml of chloroform and 0.5 ml of concentrated sulphuric acid was added carefully. Formation of reddish brown coloration at the interface indicates the presence of terpenoids.

Qualitative Analysis of Alkaloids

About 1ml of plant extract was dissolved in 5% HCl, filtered and tested with Dragendorff's reagent and Mayer's reagent separately. Any precipitate or turbidity with the reagent suggests the presence of alkaloids.

Qualitative Analysis of Anthocyanin and Betacyanin

About 2ml of test extract was added with 1ml of 2N NaOH and heated for 5min at 100^o C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of beta cyanine.

Qualitative Analysis of Coumarins

5ml of the moistened solvent plant extract was taken in a test tube. The mouth of the tube was covered with filter paper treated with 1N NaOH solution. Test tube was placed for few minutes in boiling water and then the filter paper was removed and examined under the UV light for yellow fluorescence indicated the presence of coumarins.

Qualitative Analysis of Phenols and Tannins

Plant extract was mixed with 2ml of 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.

Qualitative Analysis of Reducing Sugars

1ml of the extract was added with 2ml of Fehling's reagent and 3ml of water. It was then boiled for 2minutes.

Antibacterial Assay

Test Organisms

Gram negative bacterial strain *Escherichia coli*, *Pseudomonas sp*, *Klebsiella sp* and gram positive strain *Streptococcus sp*, *Bacillus sp* were used as a present study. They were obtained from laboratory of Department of Microbiology, Nadar Saraswathi College. They were maintained at 4°C on the slants of nutrient agar medium for further use. The isolates were identified by standard biochemical method.

Well Diffusion Method

Antimicrobial activity was performed using the well-diffusion method (NCCLS 1993). The plant extracts (leaf, seed root) were tested on Mullen Hinton agar plates to detect the presence of antibacterial activity.

Bacterial suspensions for each of the tested organism were prepared in 9ml sterile nutrient broth and were incubated at 37^oc for 18hrs to obtain a turbidity of 0.5 MacFarland. Each bacterial suspension corresponding were spread on the surface of Mullen Hinton agar plates with sterile cotton swab and kept to dry. The antimicrobial assay was achieved with agar diffusion technique, consequently five equally distant 6mm wells were made on the inoculated Mullen Hinton agar plates with the help of a sterile cork borer. Each well was loaded with 50µl of different solvent plant extract respectively using a micropipette, the extract was allowed to diffuse for 30minutes at room temperature and the loaded plates were

then incubated at 37°C for 18 to 24hrs. Standard streptomycin used as a control. Appearance of a zone indicated the presence of antibacterial of the plant extract being tested against the bacterial isolates.

Determination Minimum Inhibitory Concentration

The MIC of the extracts was determined according to the macro broth dilution technique (NCCLS 2000). From the prepared plant extracts 25µl, 50 µl, 75 µl, and 100 µl, was taken. It is added to the series of test tubes containing 10 ml of nutrient broth. About 0.1 ml of microbial culture is also added to each and every tube. The tubes were incubated at 37°C. After overnight or 48 hours the turbidity is measured using spectrophotometer in terms of optical density at 540nm.

Results and Discussion

The phytochemical components & antibacterial activity *Cajuns Cajan* (leaf, seed, and root) extracts was examined in the present study. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to humans and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their important, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with a hope of safety and security.

From the result of my study show that predominant phytochemical constituent's present in the *Cajanus cajan* leaf, seed & root extract of different solvent. Comparative screening indicated that lack of anthraquinone in leaf and lack of reducing sugar, terpenoids, cardiac glycosides,

anthraquinone in seed. Almost all phytochemical constituents are present in the root extract.

The three parts of the *Cajuns Cajan L.* extracts efficacy were screened against gram negative *E.coli*, *pseudomonas sp*, *Klebsiella sp*, and gram positive *Streptococcus sp*, *Bacillus sp* isolates. To measure the antibacterial activities through well diffusion assay. And zone of inhibition was measured in millimetre. Maximum activity was exhibited by ethanol extracts against *E.coli* and *pseudomonas sp* and methanol extract against *Streptococcus sp* and *Klebsiella sp* was observed in all the plant parts of *Cajanus cajan* tested. Comparative antibacterial potential indicated that leaf extract exhibit the maximum activity against pathogenic bacteria. Compare to different solvent, acetone extract showed least activity. Along with the three tested seed extract showed the least activity against the bacteria (table2-6).

Minimum inhibitory concentration of leaf, seed & root was observed between the 0.383 and 0.409mg/ml for the pathogenic bacteria. It has been identified that an MIC 0.383mg/ml was recorded for ethanol leaf extracts and 0.365mg/ml for methanol leaf extract against pathogenic bacteria respectively (table7).

From my study high amount of phytochemicals obtained from *Cajanus cajan* (leaf, seed and root). So, *Cajanus cajan* contains good source of phytochemicals (table-1).

The result of the study is in support with the reports of K.K Harris *et al.*, (2014). They have stated that high amount of phytochemicals present in leaf, seed, and shoot of *Cajanus cajan L.*

Table.1 Phytochemical Constituents of *Cajanus Cajan*

Compounds	Methanol extract			Ethanol extract			Acetone extract			Aqueous extract		
	1*	2°	3*	1*	2°	3*	1*	2°	3*	1*	2°	3*
Leaf, seed and root	1*	2°	3*	1*	2°	3*	1*	2°	3*	1*	2°	3*
Alkaloids	P	P	P	P	P	P	P	P	P	P	P	P
Flavonoids	P	P	P	P	P	P	P	P	P	P	P	P
Tannins	P	P	P	P	P	P	P	P	P	P	P	P
Terpenoids	A	A	P	P	A	P	A	A	P	A	A	p
Phenol	P	P	P	P	P	P	P	P	P	P	P	P
Steroid	P	P	P	P	P	P	P	P	P	P	P	P
Cardiac glycosides	P	P	P	P	A	P	P	P	P	P	A	P
Anthraquinone	A	A	P	A	A	P	A	A	P	P	P	A
Coumarins	P	A	P	P	A	P	P	A	P	P	A	P
Reducing sugar	P	A	P	P	A	P	P	A	P	P	A	A
Saponins	P	P	P	P	P	P	P	P	P	P	P	P

P = PRESENCE, A= ABSENCE, 1*=LEAF, 2°=SEED, 3* =ROOT

Table.2 Antibacterial Activity Against *E.Coli*

Zone of inhibition(mm)			
SOLVENT	LEAF	SEED	ROOT
Methanol	14.2	10.8	11.1
Ethanol	17.2	12.2	13.4
Acetone	7.1	6.0	6.1
Aqueous	6.6	5.2	5.4
Standard	19.6	20.6	19.2

Table.3 Antibacterial Activity Against *Pseudomonas Sp*

Zone of inhibition(mm)			
SOLVENT	LEAF	SEED	ROOT
Methanol	13.2	8.3	9.9
Ethanol	17.1	11.1	12.2
Acetone	4.2	2.9	4.9
Aqueous	5.9	4.4	6.6
Standard	20.2	18.2	19.3

Table.4 Antibacterial Activities Against *Streptococcus Sp*

Zone of inhibition(mm)			
SOLVENT	LEAF	SEED	ROOT
Methanol	18.9	13.6	14.2
Ethanol	13.7	9.3	11.4
Acetone	6.2	4.0	4.2
Aqueous	6.7	3.2	7.5
Standard	21.9	20.5	19.0

Table.5 Antibacterial Activities Against *Klebsiella Sp*

Zone of inhibition(mm)			
SOLVENT	LEAF	SEED	ROOT
Methanol	17.9	11.1	12.2
Ethanol	13.6	6.9	9.2
Acetone	4.0	3.4	4.9
Aqueous	12.6	9.7	11.2
Standard	20.3	19.8	19.9

Table.6 Antibacterial Activities Against *Bacillus Sp*

Zone of inhibition(mm)			
SOLVENT	LEAF	SEED	ROOT
Methanol	6.6	4.9	5.7
Ethanol	7.9	5.2	6.5
Acetone	5.2	3.4	3.3
Aqueous	4.5	2.9	3.3
Standard	19.3	19.8	21.4

Table.7 Minimum Inhibitory Concentration of *Cajanus cajan L*

S.NO	<i>E.coli</i>	<i>Pseudomonas sp</i>	<i>Streptococcus sp</i>	<i>Klebsiella sp</i>	<i>Bacillus sp</i>
Leaf	0.385	0.392	0.387	0.33	0.435
Seed	0.391	0.365	0.389	0.401	0.395
Root	0.434	0.408	0.401	0.398	0.402

Figure.1 *In vitro* Antibacterial activity of Leaf

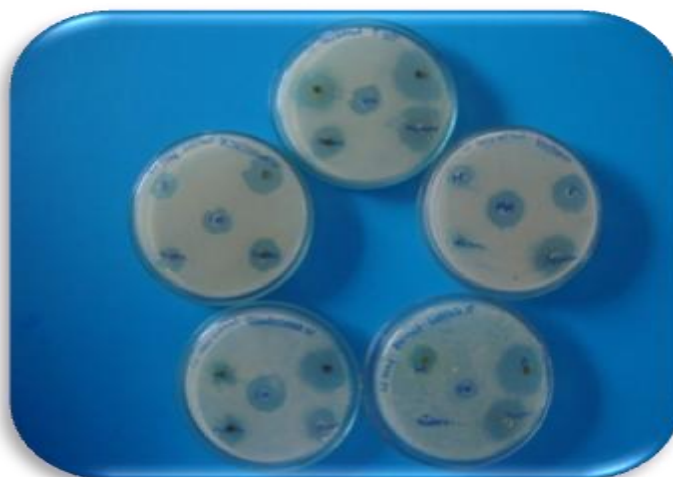


Figure.2 *In vitro* Antibacterial Activity of Seed

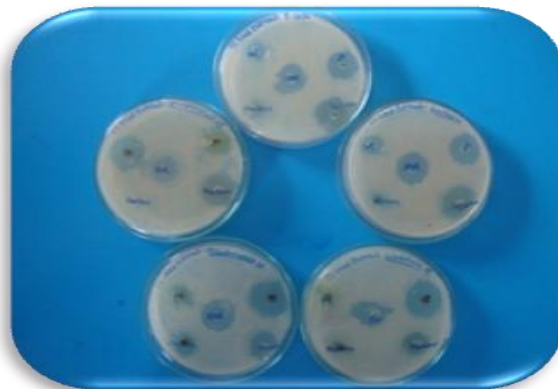
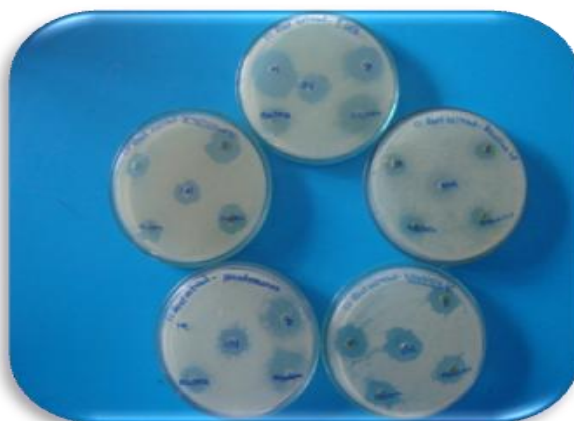


Figure.3 *In vitro* Antibacterial Activity of Root



In all plant parts of *Cajanus cajan*. Maximum activity was exhibited by methanol extract against *Streptococcus sp* and *Klebsiella sp* and ethanol extract against *E.coli* and *pseudomonas sp* tested respectively. The result of our study are in conformity with the reports of Ramaswamynanna *et al.*, (2014). They have reported that *ethanol* extract of *Cajanus cajan* showed higher inhibitory activity against gram negative bacteria & methanol extract showed higher inhibitory activity against gram positive bacteria.

The current study supported that the organic solvent extracts were more effective than the aqueous extracts. It has been described that the varieties of solvents have diverse

solubility capacities for different phytoconstituents [Marjorie *et al.*, 2000)].

The effect of aqueous and various organic solvent extracts of different plant parts of *Cajanus cajan* for antibacterial activity was determined to be compared among the tested organisms.

Based on the dosage of various solvent and aqueous extracts of *Cajanus cajan* inhibited the growth gram positive and gram negative bacteria tested respectively. The methanol and ethanol extracts of all the plant parts of *Cajanus cajan* showed more inhibitory effects in comparison to the other extracts tested. So that active ingredients are better extracted in methanol and ethanol compare

to other extracts. It is indicated to facilitate presence of broad spectrum nature of the antimicrobial compounds present in the extracts of the methanol and ethanol. Related interpretation were also made by Prathima and Prathima Mathad [2011] who described that the ethanol extracts exhibited maximum activity on both gram positive and gram negative bacteria in *Cajanus cajan*.

Consequently, methanol extracts against *Streptococcus* sp & *Klebsiella* sp and ethanol extracts against *E.coli* and *Pseudomonas* sp of *Cajanus cajan* was studied to determine minimum inhibitory concentration. High activity indicated by low MIC value. vice versa

It can be concluded that different extracts of leaf, seed & root of the *Cajanus cajan* are a great potential source of antibacterial compounds. The proximate analysis reveals high amount of phytochemical constituents. It is concluded from the result that *Cajanus cajan* (leaf, seed & root) could be used in formulation of new antimicrobial drugs of natural basis.

Acknowledgement

The project was supported by laboratory of Department of Microbiology & Biochemistry in Nadar Saraswathi College of Arts & Science, Theni. I thank the Management, Principal, Head of the Department, Faculty members for their guidance and support.

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How to cite this article:

Raveena Devi, R., R.Premalatha and Saranya, A. 2016. Comparative analysis of phytochemical constituents and antibacterial activity of leaf, seed and root extract of *Cajanus cajan* (L.) Mill sp *Int.J.Curr.Microbiol.App.Sci*. 5(3): 485-494. doi: <http://dx.doi.org/10.20546/ijcmas.2016.503.057>