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Antibacterial Activity And Phytochemical Screening Of Crude Leaves Extract Of *Parkinsonia aculeata Linn.*

S. Parveen Qureshi
P. G. Department of Botany, J. M. Patel College, Bhandara. (M.S.)
drsdprvnqureshi17@gmail.com

ABSTRACT
Plants are the source of very potent and powerful drugs with antimicrobial properties.
The Indian flora offers great possibilities for the discovery of new compounds with important medicinal applications. The antimicrobial compounds found in plants may prevent bacterial infections by different mechanism than the commercial antibiotics and therefore may have clinical value in treating resistance microorganism strains.The medicinal plants offers a new source of antibacterial agents. This is indeed very important because some common pathogens like E.coli, S.aureus, Pseudomonas aeruginosa etc. that have develop resistance to antimicrobials. the present studies states the phytochemiccal investigation and the therapeutic important of Parkinsonia aculeata Linn. The phytochemiccal screening shows the presence of flavonoids, alkaloids, c-glycosides and saponins etc.
Keywords : Antimicrobial, Compounds, Pathogens, Resistance.

INTRODUCTION:

India has a rich culture of Medicinal herbs, plants and species, which include about more than 2000 species has a vast geographical areas with high potential abilities for Ayurvedic, Unani and Siddha traditional Medicines but only few has been studies chemically and pharmacologically for their potential medicinal value. *Parkinsonia aculeata* is a species of perninal flowering tree in the pea family, Fabaceae. All the parts of plant are known as antipyretic, diaphoretic and aberifacient. Plant constituents may be isolated and used directly as therapeutic agents or a starting material for drug synthesis or they may serve as models for pharmacological active compounds in drug synthesis.The antimicrobial compounds which may be present in the plants are:

1.Alkaloids Alkaloids are naturally occurring chemical compound containing basic nitrogen atoms and are produced by a large variety of organisms including bacteria, fungi, plants, and animals. Many alkaloids are toxic and often have a pharmacological effect, which makes them to be used as medications and recreational drugs.

2.Flavonoids Flavonoids are derived from 2-phenylchromen-4-one (2-phenyl-1-4-benzopyrone) and are commonly known for their antioxidant activities. Flavonoids, which are widely distributed in plants, fulfill many functions including producing yellow, red or blue pigmentation in flowers and protection from attacks by microbes and insects. Compared to toher active plant compounds, they are low in toxicity. Flavonoids are referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anto-inflammatory, antimicrobial and anticancer activity.

3. Saponins Saponins are the glycosides of 27 carbon atom steroids, or 30 carbon atom triterpenes in plants. They are found in various plant parts; leaves, stems, roots, bulb, flower and fruits. Saponins are believed to be useul in the human diet for controlling cholesterol. Digitalis-type saponins strengthen the heart muscle causing the heart to pump more efficiently. Saponins aso inhibit cancer tumor growth in animals, particularly, lung and blood cancers, without killing normal cells.

4.Cardiac Glycosides These glycosides are found as secondary metabolites in several plants Cardiac glycosides are drugs used in the treatment of congestive heart failure and cardiac arrhythmia.

MATERIALS AND METHODS

A) Sample collection and Preparation:

The fresh leaves of *P. aculeata* were collected from Salori village, 10 km away from Warora tehsil Dist. Chandrapur (Maharashtra).The plant material was thoroughlly washed with water and kept for drying in shade at room temperature for 20 days. The thoroughly air dried plant material was then grinded to make powder. This powder is then stored in a large plastic container.The pathogenic organism were collected from the microbiology lab of J.M. Patel College, Bhandara. The organism are as follows:-

i) *E.coli*
ii) *S. aureus*

B)Extraction and Preparation of Materials:

1) *Preparation of Aqueous Extract:*

Leaves sample 50gm of thoroughly washed with water and macerated with 100ml of distill water in a warning blender for 10 minutes.

The macerated was first filtered through double layer muslin cloth and then centrifugated at 4000g for 30 minutes.

The supernatant was filtered through Whatmann Filter paper no. 1 and sterilized at 121^{0}c for 30 minutes. The extract was allowed to cool at room temperature and their pH was determined just before subjecting to antibacterial activity assay.

2) Preparation of solvent extracts:

The mature leaves were thoroughly washed shade dried and then powdered with the help of warning blender.2ml of powder was filled in the thimble and extracted successively with ethanol, methanol using soxhlet extractor for 48 hrAll the extract were concentrated using rotary flash evaporator and preserved in airtight bottle prior to the commencement of the analysis. All the extract were subjected to antibacterial activity assay and phytochemical screening.

C)<u>Phytochemical and Antibacterial screening:</u>

1) Phytochemical Screening:

Phytochemical Screening were performed to assess the qualitative chemical composition of different crude extracts using commonly employed precipitation and coloration reactions to identify the maor secondary metabolites like alkaloids, terpenes, flavonoids,saponins, steroids, phenolic compounds, tannins and aminoacids. The phytochemical analyses were carried out using standard procedure.(Singh P. et al., 2011;Vol 3(6)(6,7)

- **Test for flavonoids (Shinoda test):**

To the extract, add 5 ml 95% ethanol, few drops of conc. HCL and 0.5 g magnesium turnings. Pink coloration indicates the presence of flavonoids.

- **Test for Alkaloids (Wagner's test):**

Evaporate the aqueous alcoholic, CHCL3 or ethyl acetate extracts. To residue add dil. HCl. Shake well and filter. 2-3 ml filtrate add few drops of Wagner's reagent. Reddish brown ppt. indicates the presence of alkaloids.

- **Test for C-glycosides (Modified Borntrager's test) :**

To 5 ml of extract add ml of 5% $FeCl_3$, and 5ml dil. HCl. Heat for 5 min. in boiling waterbath. Cool and add benzene or any organic solvent. Shake well. Separate the organic layer and add equal volume of dil. Ammonia. Ammonical Layer shows pinkish red color.

- **Test for Terpenoids (Salkowski test) :**

To 0.5 gm of the extract 2ml of CHCl3 was added. 3ml of conc. H2SO4 was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

- **Test for Saponin glycosides (Foam test):**

To 0.5g of extract 5ml of distilled water was added. The solution was shaken vigorously. Persistent foam indicates the presence of saponins.

- **Test for Phenolic compounds** :

To 2-3ml of aq.Or. alc. Extract few drops of 10% aq. ferric chloride solution was added. Formation of blue or green colour indicates the presence of phenolic compounds.

- **Test for gums and mucilages:**

10 ml of extract was slowly added to 25 ml of absolute alcohol under constant stirring. Precipitation indicates the presence of gums and mucilages.

- **Test for reducing sugars (Fehling's test):**

The aqueous ethanol extract (0.5g in 5ml of water) was added to boiling fehling's solution (A+B). brick red ppt. at the bottom of the test tube indicates the presence of reducing sugars.

- **Test for tannins:**

5ml of extract was added with few drops of 1% lead acetate. Formation of yellow or white ppt. indicates the presence of tannins.

2) Antibacterial Assay:-

The four different concentrations of leaf extract were tested for antibacterial activity using agar disc diffusion assay. The bacterial culture were maintained nutrient broth. The nutrient agar was prepared and poured in autoclaved petri plates well were made with the help of sterile cork borer (6-7 mm) and inoculating bacterium were spread on solidified plates with the help of coon swab. Then the wells were filed with 50ml of crude extract. The four different concentration (50%; 25%;75100%/ml) of leaves extracted were prepared and were tested for antibacterial activity. The experiement were done three times and the mean value were presented. Distilled water were used as standard.

.OBSERVATION & RESULT:

A) Pytochemical screening:

1) The phytochemical screening of *P. aculeata Linn* leaves ethanolic extract shows the presence of Steroids, Terpenoids, Tannins, Flavonoids, Alkaloids.

1) The phytochemical screening of *Parkinsonia aculeata Linn shows* the presence of Flavonoids, Terpenoids, Alkaloids, Saponins, Tannins, Phenolic Compound, C-glycosides and Reducing sugars. (Table-1)

B) Antibacterial assay:

Antibacterial activity of leaves extracts of *Parkinsonia aculeata* was assayed by well diffusion method against some bacteria. Table-2 summarizes the microbial growth inhibition of leaves extracts.

The methanolic leaves extracts shows maximum antibacterial response against *S.aureus* with maximum zone of inhibition 30.8 mm at conc. of 75% of 50mg/ml. The extract also shows antibacterial response against *E.coli* maximum with zone of inhibition i.e., 30mm at conc. of 75% of 50mg/ml. Both the strains do not show any inhibition against Aqueous extract.

Table-1: The phytochemical screening of *Parkinsonia aculeata Linn* Leaves extract.

Compounds	Aqueous extract	Ethanolic extract
Terpens	+	+
Flavonooids	+	+
Alkaloids	+	+
C- glycosides	+	-
Saponins	-	-
Gums mucilages	+	-
Reducing sugar	-	+
Tannins	+	-
Phenolic compounds	-	-

Table-2: Antibacterial activity of *Parkinsonia aculeate Linn.* Leaves extract.

Sr. No.	Bacterial strains	Zone of inhibition (in mm)									
		Std.	Aqueous extract			Ethanolic extract			Methanolic extract		
		(d/w)	25%	50%	75%	25%	50%	75%	25%	50%	75%
1.	*S.aures*	-	-	-	-	20.5	20.7	30.2	30.00	30.3	30.8
2.	*E.coli*	-	-	-	-	20.4	20.7	20.9	20.3	20.7	30.00

DISCUSSION AND CONCLUSION

Plants are the rich source for discovering new antimicrobial compounds. The ultimate goal of this work is to screen plant for the antibacterial activity and phyochemical investigatioThe phytochemical investigation shows the different-different chemicals are present in the plants which are considered as primary and secondary metabolites. The antibacterial activity of plants extract is due to different chemical agent in the extract which are classified as active antimicrobial compound (Singh P et al)10. The study therefore provided basis to the folklonic use of this plant as a remedy for Urinary Tract Infection and skin diseases and other infection caused by pathogen.

The present study shows the *Parkinsonia aculeata* leaves has maximum antibacterial activity against *S.aureus* , so this plant can be used to made natural products for the treatment of skin infections generally caused by *S.aureus*. it also has antibacterial activity against *E.coli* which uses generally causes Urinary Tract Infection. It may serve as leads for development of new pharmaceuticals.On the other side it is observed that aqueous leaves extract do not show any activity on *S.aureus* and *E.coli*.The result of present investigation clearly indicates that the antibacterial activity vary with the species of plant and the plant material used.Thus the study indicates that . the value of plant at the time of ayurveda, could be of great interest to the development of new drugs.leaves of*parkinsonia aculeate* has great antimicrobial property of ethanolic and methanolic solvent shows the maximum antibacterial activity against *S.aureus* and *E.coli* the conc.of 50mg/ml. It is observed that the minimum inhibitory conc. of the extract at which its shows its antibacterial property is 25% of 50mg/ml i.e. 12.5mg/ml.

Suggestion for further studies, more screening is needed to identify bioactive component responsible for antibacterial activity and its use in the treatment of various diseases.

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