RESEARCH PAPER

Antioxidant capacity, antibacterial activity and β sitosterol isolated from chloroform fraction of root extracts of *Parkinsonia aculeata*

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ABSTRACT
Parkinsonia aculeata is alarge spinous shrub or small tree belonging to family *Fabaceae.* Ethyl acetate and n-butanol extract of plant were evaluated for total phenolic and flavonoid content, antibacterial and antioxidant activity by four complementary test systems, namely 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide, reducing power and ferrous ion-chelating assay. The crude chloroform extract of *Parkinsonia aculeata* roots was subjected to column chromatography for separation and isolation of β sitosterol. Phytochemical screening of plant roots extracts revealed the presence of alkaloids, flavonoids, phenolic, amino acids, carbohydrates, steroids and triterpenoids.In DPPH, hydrogen peroxide and ferrous ion-chelating assay, IC_{50} value of ethyl acetate extract was 60.97, 59.51, 444.09 µg/ml and n-butanol extract was236.14, 80.324, 345.24 µg/ml respectively. Further, the extract showed inhibitory activity for gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa)* bacteria at different concentration. The maximum antibacterial activity of chloroform extract was exhibited against *B. subtilis* at concentration 16 µg/ml when compared with amoxicillin. The isolation and purification afforded white crystalline powder, which was subjected to physical and chemical identification by melting point and TLC.The compound was concluded as β-sitosterol described for the first time in the root extract of*Parkinsonia aculeata*.The results clearly indicate that *P. aculeata* roots are effective in scavenging of free radicals and have the potential powerful antioxidant. Thus the result obtained in the present study indicates that *P. aculeata* roots extract could be considered as a potential source of natural antioxidant and that could be used as an effective source against bacterial diseases.

Keywords:*Parkinsonia aculeata*, DPPH assay, *B. subtilis*
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INTRODUCTION

In the recent years, presence of a massive amount of active constituents has been established from the plants or other natural sources. Bioactive compounds/active constituents are remarkable due to their usage in prevention and treatment of various ailments.[1-2] The bioactive compounds are present in the different parts of the plants and are very prone to commotion by various free radial species.[3-7] When free radical formation transpires oxygen and interacts with the active constituents presents in the plants, a number of disruption of the normal synthesis occurs, which is primarily associated with inhibition/inactivation of antioxidant key proteins.[8-9]

Antioxidants are responsible for removal of the free radicals and consist of various exogenous and endogenous compounds which may be classified based on their mechanism of action like, scavenging reactive oxygen species (ROS) or their precursors, inhibiting formation of ROS.[10] An antioxidant, ascorbic acid, possesses beneficial properties and plays a vital role in the activation of immune response along with many other metabolic processes. [11-13]

Parkinsonia aculeata (Fabaceae) is a large spinous shrub or small tree, 4-10 m height with branches spreading near the ground and very thin drooping foliage. Bark is smooth with yellow green color, branches and twigs are often of the same color.[14] Leaf stalk has flattened edge by two rows of 25-30 tiny oval leaflets and fruit are light brown when mature, leathery in appearance.[15] Plantation of this species is raised in arid and semi arid area tract of western Uttar Pradesh, Rajasthan and Gujarat.[16] Decoctions of the

leaf, fruit and stem are given orally to treat fever, malaria and also used as abortifacient.[17]

The active constituents in the *Parkinsonia aculeata*reported are C-glycosyl flavones and C-glycosides.[18-21] Pharmacological investigation of the plant showed various activities such as anti-diabetic, amoebicidal, CNS depressant, antioxidant, analgesic, anti-inflammatory and antipyretic from the total alcoholic and aqueous extract of leaves.[22-24] Though literature revealed *Parkinsonia aculeata* as a useful sedge, so far the phytochemical and pharmacological studies for the roots have not been reported. Therefore, the present paper describes the antioxidant and antibacterial potential of *Parkinsonia aculeata*roots extracts. The root extracts was also subjected for phytochemical screening to determine the presence of various constituents reported earlier in other parts or unknown.

1. MATERIALS AND METHODS

2.1 Chemicals, apparatus and general procedures

All chemicals were of analytical grade. DPPH (2, 2-diphenyl-1-picrylhydrazyl), ferrozine, ascorbic acid, and Folin-&Ciocalteu's reagent were procured from Sigma-Aldrich, USA. All solvents for fractionation, column chromatography and TLC studies were of LR and AR grade, respectively. The extracts were concentrated under vacuum using rotary evaporator (Eylea, Japan). TLC profile was developed using pre-coated silica gel (60-254F) TLC aluminum plates (Merck, Germany). Column of appropriate sizes were used for column chromatography of the fractions. Distilled water was used wherever mentioned. UV-visible double beam spectrophotometer of make Shimadzu, Japan was used for the analysis. Melting points (m.p.) were determined by using glass capillary tubes on a Veego melting point apparatus and are uncorrected.

2.2 Plant collection and Extract preparation

Roots of *Parkinsonia aculeata* were collected from local areas of Vidhyadhar Nagar, Jaipur, Rajasthan and authenticated from the Department of Botany, University of Rajasthan, **(RUBL211332)** Jaipur. The fresh, roots of plant (3 kg) was collected and shade dried to obtain 500 g dry sample which was later coarsely powdered and used for solvent extraction. For sample preparation, 500 g of dried sample were extracted twice (1000 ml for each) with 70 % hydro alcohol at 40-60 °C for 72 h and concentrated using water bath to yield the 70 % hydro alcohol (43.08 %).[25]

2.3 Physiochemical parameters

Physiochemical parameters of the collected material were determined as per WHO guidelines. The alcohol soluble extractive value, water soluble extractive value and moisture content were determined. The following values were determined for the powder drug of the roots of *P. aculeata.*

2.3.1Alcohol/Water soluble extractive value

4.0 g of the accurately weighed coarsely powdered air-dried material was placed in a glass-stopper conical flask. Macerate with 100 ml of alcohol (90 %) / water for 6 hrs, shaking frequently on a rotatory shaker, then placed as such for 18 hrs. Filtered rapidly taking care not to lose any solvent, 25 ml of the filtrate was transferred to a china dish and evaporated to dryness on a water- bath. Dry at 105 °C for 6 hrs, cooled in a desiccator for 30 minutes and weighed without delay. The content of extractable matter in mg per g of air-dried material was calculated.

2.3.2 Determination of moisture content

Accurately weighed 4 gm of powdered plant material was taken in a petridish. It was kept for 30 min in a hot air oven which was adjusted to 105-110 ºC. This procedure was repeated till the constant weight was obtained. The percentage of moisture content was then calculated with reference to the air dried drug. [26-31]

2.4 Antioxidant assays

Each sample was dissolved in 95% methanol to make a concentration of 1 mg/ml and then diluted to prepare the series concentrations for antioxidant assays. Reference chemicals were used for comparison in all assays.

2.4.1 DPPH radical scavenging activity assay

The free radical scavenging activity of the fractions was measured by 2,2' - diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier [32]. The stock solution was prepared by 75 µmol DPPH and stored at 20 °C until required. The working solution was obtained by diluting DPPH solution with methanol . A 2 ml aliquot of this solution was mixed with 2 ml of the sample at various concentrations (50 - 250 µg/ml). The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was taken at 517 nm using the spectrophotometer. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Scavenging effect % = [(control absorbance - sample absorbance) /(control absorbance)]×100

2.4.2 Hydrogen peroxide scavenging activity

Hydrogen peroxide solution (40 mM) was prepared in 0.1M phosphate buffer (pH 7.4). Aliquots (1 ml) of different fractions was transferred into the test tubes and their volumes were made up to 3.4 ml with 0.1M phosphate buffer (pH 7.4) After addition of 0.6 ml hydrogen peroxide solution, tubes were vortexed and absorbance of the hydrogen peroxide at 298 nm was determined after 10 min, against a blank[33]. The

abilities to scavenge the hydrogen peroxide were calculated using the following equation:

H_2O_2 scavenging activity % = (1- absorbance of sample/ absorbance of sample) × 100

2.4.3 Ferrous ion-chelating (FIC) assay

Inthis assay Ferrozine quantitatively form blue color complexes with Fe^{2+}, however, in the presence of chelating agents, the complex formation is prevented resulting red colour of the complex is decreased [34-35]. Various concentrations of the sample (1 ml) were mixed with 2 mM $FeCl_2$ (0.1 ml) and after that 5 mM ferrozine (0.5 ml) was mixed and allowed to stand for 10 min. Absorbance was measured at 562 nm after 10 min. The ability of extracts to chelate ferrous ions was calculated as follows equation:

Chelating activity % = (1–absorbance of sample/ absorbance of sample) × 100

2.4.4 Reducing power

The reducing power was based on Fe (III) to Fe (II) transformation in the presence of the solvent fractions [36-37]. The Fe (II) can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Various concentrations of the sample (1 ml) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (10 mg/ml). The mixtures were incubated at 50 °C for 20 min followed by addition of 2.5 ml of trichloroacetic acid (100 mg/l). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution. A volume of 5 ml from each of the mixture earlier mentioned was mixed with 5 ml of distilled water and 1 ml of 0.1 % (w/v) fresh ferric chloride. After 10 min reaction, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power.

2.5 Estimation of total phenolic content

The total phenolic content was determined by the spectrophotometric method.[38] In brief, a 0.1 ml of sample (1 mg/ml) was mixed with 1.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 4 ml of a 20 % Na_2CO_3 solution was added to the mixture followed by the addition of 4 ml of distilled water and mixed thoroughly. The mixture was kept in the dark for 30 min, after that the absorbance was read at 738 nm using UV-visible spectrophotometer. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

2.6 Estimation of total flavonoid content

Total flavonoid content was determined by aluminiumtrichloride method.[39] In a 10 ml test tube, 0.3 ml of extracts, 3.4 ml of 30 % methanol, 0.15 ml of $NaNO_2$ (0.5 M) and 0.15 ml of $AlCl_3$ (0.3 M) were mixed. After 5 min, 1 ml of NaOH (1 M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 415 nm. The standard curve for total flavonoids was made using quercetin standard solution (0 to 100 mg/l) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of quercetin equivalents(QE) per g of dried fraction.

2.7 Phytochemical screening

Phytochemical screening of different fractions of *Parkinsonia aculeata* roots for the presence of alkaloids, flavonoids, phenolic, amino acids, carbohydrates, steriods and triterpinoids was carried out as per the reported procedures. [40-43]

2.8 Antibacterial activity

Agar well method was used to evaluate the antibacterial activity of the different plant extracts.[44]Antibacterial activities of extracts were tested by zone inhibition method. The different concentrations (1, 2, 4, 8, 16 µg/ml) of extracts and amoxicillin (standard drug) were introduced into the wells of agar plate with the help of micropipettes separately under aseptic condition. The plates were then maintained at room temperature for 1h to allow the diffusion of solution into medium. The plates were later incubated for 18-24 hr at 37 °C. After incubation, the plates were observed for presence of clear zones of growth inhibition as evidence of antibacterial activity.[45]

2.9 Isolation of β sitosterol from chloroform fraction

2.9.1 Fractionation of 70% hydro-alcoholic crude extract

The extract of the plant (22g) was suspended in water (500ml). The suspended extract was fractionated with chloroform (250 ml x 5). All the chloroform fractions were pooled, filtered and concentrated under vacuum, the yield obtained is 43.08%.

2.9.10 TLC of chloroform fraction

After fractionation, 100 mg of chloroform fraction was dissolved in 5 ml of chloroform, and the resultant extract was used for development of TLC profile. Various solvent systems were tried to develop the TLC profile, and best resolution was obtained in toluene : chloroform : methanol (2 : 6.5 : 1.5). The plates were derivatized with anisaldehyde-H_2SO_4 reagent. The sprayed plates were placed in hot air oven at 110° C for 2 min for development of bands.

2.9.11 Column chromatography

The crude chloroform fraction of *Parkinsonia aculeata* roots (4.0 g) was subjected to column chromatography for separation and isolation of different components. Silica gel (60-120 mesh) was used as a stationary phase. During the preparation of column, the lower part of the glass column was stocked with glass wool with the aid of glass rod. The slurry, prepared by mixing 120 g of silica gel and 300 ml of hexane, was poured down carefully into the

column. The tap of the glass column was left open to allow free flow of solvent into a conical flask below. The wet packing method was used in preparing the silica gel column. The column was allowed 24 h to stabilize, after which, the clear solvent on top of the silica gel was allowed to drain down to the silica gel meniscus. The sample was prepared by adsorbing 4 g of the extract to 10 g of silica gel. The dry sample was gently layered on top of the column. Elution of the extract was done with the following ratios of solvent combinations; Hexane: chloroform 100:0, 75:25, 50:50, 25:75. The eluted fractions (250 ml each) were collected and concentrated under vacuum. The TLC profile of different fractions were carried out TLC plates by using suitable solvent system and were derivatized by anisaldehyde-H_2SO_4 reagent. fractions with similar TLC profile were pooled to get final four fractions (F1-F4).

2.9.12 Column chromatography of fraction F-3

Fraction F-3 was further column chromatographed using column grade silica gel (60-120). Column was packed in hexane: chloroform (50:50) solvent system and was eluted in chloroform in increasing order of polarity. Various fractions (100 ml each) were obtained and pooled on the basis of similar TLC profile to obtain final five fraction (F-3.1-F-3.5). After that white needle shaped crystals were obtained from fraction which was subjected to physical and chemical identification by melting point and TLC.

2.9.13 Confirmation of isolated β sitosterol using TLC and melting point

Melting points (m.p.) were determined by using glass capillary tubes on a Veego melting point apparatus and the needle shaped crystals R_f was compaired with the standardβ sitosteroland found to be same.

RESULTS AND DISCUSSION

3.1 Physiochemical parameters

Physiochemical values like extractive values and moisture content are tabulated in Table 1. The Physiochemical parameters precious source of information that gives suitable standard to establish plant quality in future study or application. Extractive values are useful for evaluation of crude drugs and give an idea about the nature of chemical constituents present in them.Extracts obtained by exhausting crude drugs are indicative of approximate measures of certain chemical compounds they contain, the diversity in chemical nature and properties of contents of drug. Moisture contain of drug could be at minimal level to avoid microbial growth during storage.

Table 1. Physiochemical parameters

Parameters	**Determined value % (w/w)**
Moisture content	14
Extractive values	
Water soluble extractive	7
Alcohol soluble extractive	4

3.2 Phytochemical screening

Phytochemical screening of *Parkinsonia aculeata* roots fractions demonstrated the presence of alkaloids, flavonoids, phenolic, carbohydrates, steriods and triterpenoids.

3.3 Antioxidant activity

Antioxidant capacity of *Parkinsonia aculeata* rootsfractions was examined using four different assays.

3.3.1 DPPH radical scavenging activity

Figure 1A & B shows that the scavenging effects of samples on DPPH radical and were in the following order: ethyl acetate >*n*-butanol fraction. The IC_{50} value of *P. aculeata* roots ethyl acetate and n-butanol extracts were found to be 60.97 and 236.14 µg/ml respectively(Table-2). Standard drug ascorbic acid IC_{50} value was 6.78 µg/ml. Though the antioxidant potential of fractions was found to be low than those of ascorbic acid, the study revealed that ethyl acetate fraction and n-butanol fraction have prominent antioxidant activity for the presence of phenolic compounds. As we have earlier mentioned that antioxidants are the agents which remove free radicals, scavenging of reactive oxygen speciesor their precursors. ROS intermingle with the biological molecules and interrupt the normal synthesis and renovation of DNA. To evaluate antioxidant activity, DPPH is widely used method in a relatively short time as compared to other methods. DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The effects of n-butanol and ethyl acetate extract on DPPH scavenging are thought to be due to their hydrogen donating ability. The decrease in absorbance of DPPH caused by antioxidant is due to the reaction between antioxidant molecules and radical which results in scavenging of radical by hydrogen donation and visualized by discoloration from purple to yellow.

(A) **(**

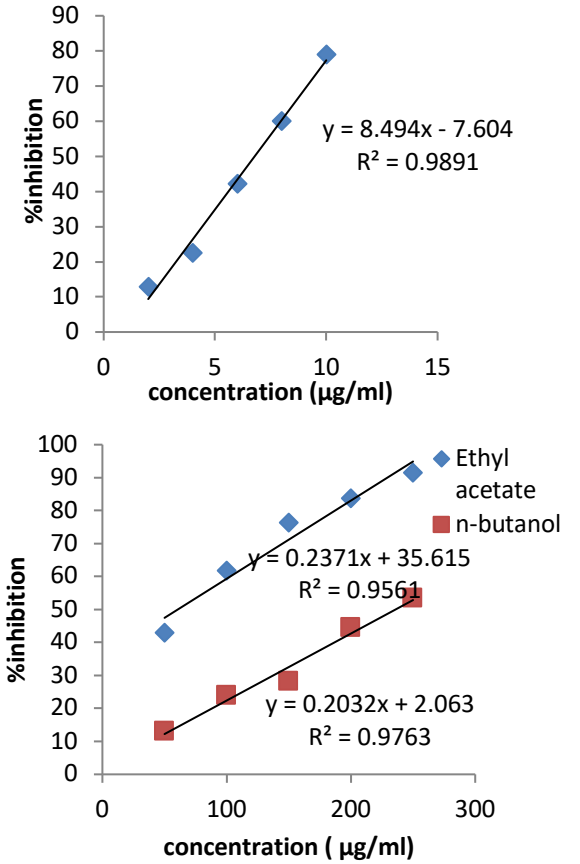


Figure 1 Antioxidant activities of different extracts from the 70 % hydro alcoholic extract of *Parkinsonia aculeata* roots by different solvents at various concentration. (A) DPPH radical scavenging activity of standard ascorbic acid , (B) DPPH radical scavenging activity of different extracts(**C**) Hydrogen peroxide radical scavenging activity, (**D**)Ferrous ion-chelating (FIC) assay.

Table 2. Radical scavenging activities of *Parkinsonia aculeata* roots fractions at different concentrations

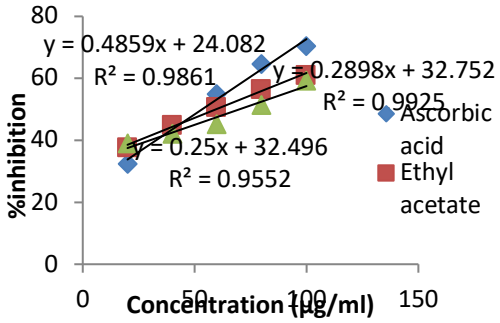
IC_{50} values (µg/ml) of radical scavenging			
Plant extracts/Chemical	**DPPH radical**	**Hydrogen peroxide**	**Ferrous ion-chelating**
Ethyl acetate fraction	60.97	59.51	444.09
n-Butanol fraction	236.14	80.324	345.24
Ascorbic acid	6.78	6.78	-
BHT	-	-	192.53

3.3.2 Hydrogen peroxide radical scavenging activity

The scavenging effect of ethyl acetate and *n*-butanol fractions of *Parkinsonia aculeata* roots on hydrogen peroxide was concentration-dependent (20 - 100 µg/ml) as shown in Figure 1C. Ethyl acetate fraction displayed strong H_2O_2 scavenging activity (IC_{50}59.51 µg/ml) whereas that of the standard, ascorbic acid exhibited 6.78µg/ml. The scavenging activities of*n*-butanol fraction was 80.324 µg/ml (Table-2).IC_{50} values of the fractions in scavenging hydrogen peroxide were significantly different ($P < 0.05$) from the IC_{50} values obtained for ascorbic acid. The scavenging activity for hydrogen peroxide of various solvent extracts from *Parkinsonia aculeata* roots was in the order of ethyl acetate extract >*n*-butanol fraction respectively.

Hydrogen peroxide is a non-radical form of ROS that is formed by superoxide dismutase in living organisms and generates hydroxyl radicals. These hydroxyls radical are very toxic to cells and can damage the numbers of active constituents. Thus for the protection of living organisms removing of hydroxyl radical is very important.

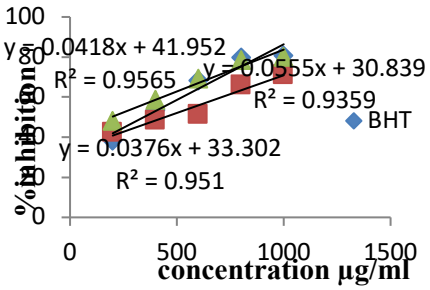
(**C**)



3.3.3 Ferrous ion-chelating (FIC) assay

Maximum scavenging activity (80.17%) was observed at 1000 µg/ml concentration in n-butanol extract. The IC_{50} value of *P. aculeata* roots ethyl acetate and n-butanol extracts were found to be 444.09 and 345.24 µg/ml respectively (Table-2). Standard drug BHT IC_{50} value was 192.53 µg/ml as shown in Figure 1D. IC_{50} values of the fractions in scavenging hydrogen peroxide were significantly different ($P < 0.05$) from the IC_{50} values obtained for BHT. The scavenging activity for Ferrous ion-chelating of various solvent extracts from *Parkinsonia aculeata* roots was in the order of *n*-butanol > ethyl acetate extract fraction respectively.

(**D**)



3.3.4 Reducing power activity

Figure 2A and 2B shows the dose response curves for the reducing powers of standard ascorbic acid(100-500 µg/ml) and all extracts (200 - 1000 µg/ml) from *Parkinsonia aculeata* roots . It was found that the reducing power increased with concentration of each sample. The ranking order for reducing power was ethyl acetate >*n*-butanol extract. Reducing power assay measures the electron-donating capacity of an antioxidant. Presence of reducers causes the conversion of the Fe^{3+} /ferricyanide complex to the ferrous form which serves as a significant indicator of its antioxidant capacity.

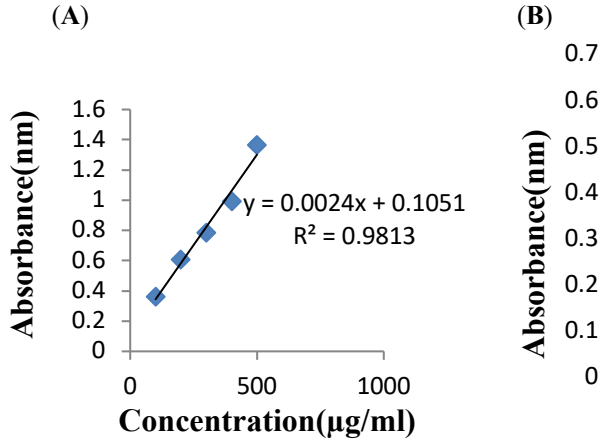


Figure 2 Reducing power activity:(**A**) standard ascorbic acid, (**B**) Different extracts of *Parkinsonia aculeata* roots.

3.4 Antibacterial activity

The antibacterial activities of chloroform, ethyl acetate and n-butanol extracts of the roots of *Parkinsonia aculeata* against various strains of bacterial microorganisms (2 Gram positive and 2- Gram negative) in different concentrations (**Figure-3**). The chloroform extract of the plant was found to be more effective against *B. subtilis* in comparison to amoxicillin as standard.

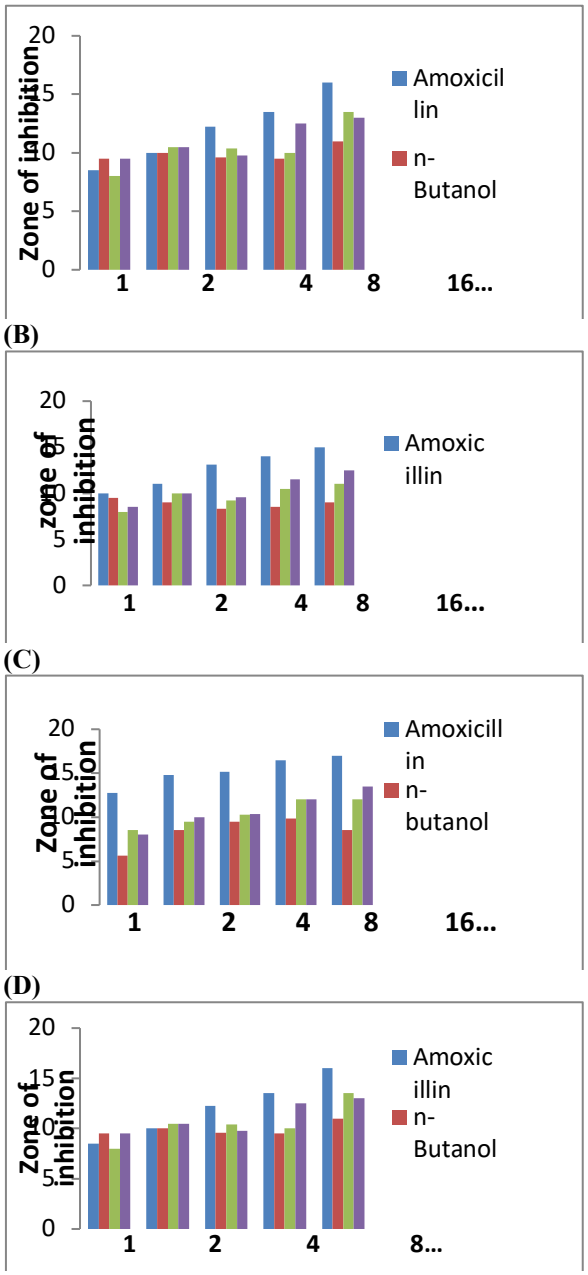


Figure 3 Antibacterial activity of different extracts of *P.aculeata* rootagainst: (A) *E.coli*, (B) *S.aureus*, (C) *B.subtilis*, (D) *P.aerogenosa*.

3.5 Extraction yield, total phenolic and flavonoid contents

The extraction yield of different fractions of *Parkinsonia aculeata*roots varied from 1.5 % to 22.08 % with a descending order of 70 % hydro alcohol > chloroform > *n*-hexane >*n*-butanol > ethyl acetate extracts (Table 3). So the extraction with 70 % hydro alcohol resulted in the highest amount of total extractable compounds whereas the extraction yield with ethyl acetate was only small in comparison with that of the other solvents. Total phenolic content was estimated by using Folin- Ciocalteu reagent. Total

phenolic content of the different fractions of *Parkinsonia aculeata*roots was solvent dependent and expressed as milligrams of gallic acid equivalents (GAE) equivalent. Table 3 summarizes that total phenolic compounds in fractions varied widely, ranging from 13.33 and 27.83 mg/g expressed as gallic acid equivalents (GAE). Ethyl acetate extract exhibited the highest total phenolic content. The content of flavonoid expressed as quercetin equivalents, varied from 18.58 and 30.03 mg quercetin equivalent/g extract (Table 3). The ethyl acetate extract showed the highest amount of flavonoid contents.

Phenolic compounds of plants are also very significant because their hydroxyl groups bestow the scavenging ability. It has also been familiar that flavonoids shows activity through scavenging or chelating process which effects on human nutrition and health are also considerable. Studies on the flavonoid derivativ Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals implicated in several diseases. So comparable with the findings in the literature for other extracts of plants our results suggested that phenolic acids and flavonoids may be the major contributors for the antioxidant activity as the IC_{50} values of radical scavenging activity of various soluble fractions of*Parkinsonia aculeata*roots and the contents of phenolic or flavonoids exhibited significant correlation.Studies have shown a wide range of antibacterial, antiviral,anti-inflammatory, anti-cancer, and anti-allergic activities.

Table 3. Total phenolics, flavonoid and extraction yield of 70% hydro alcohol extract and soluble fractions of *Parkinsonia aculeata* roots

Plant extracts	**Total phenolics (mg gallic acid equivalent/g)**	**Total flavonoid (mg quercetin Equivalent/g)**	**Extraction yield (%)**
70% hydro alcohol	-	-	22.08
Chloroform	-	-	4.9
n-hexane	-	-	3.88
Ethyl acetate	27.83	30.03	1.50
n-butanol	13.33	18.58	2.45

3.6 Identification of isolated compoundβ sitosterol from chloroform fraction

3.6.1 TLC of chloroform fraction

Chloroform fraction (100 mg) was dissolved in 5 ml of chloroform, and the resultant extract was used for development of TLC profile. Various mobile phase were tried to develop the TLC profile, and best resolution was obtained in toluene : chloroform : methanol (2 : 6.5 : 1.5). The plates were derivatized with anisaldehyde-H_2SO_4 reagent. A total of 10 prominent spots were observed (Figure 4).

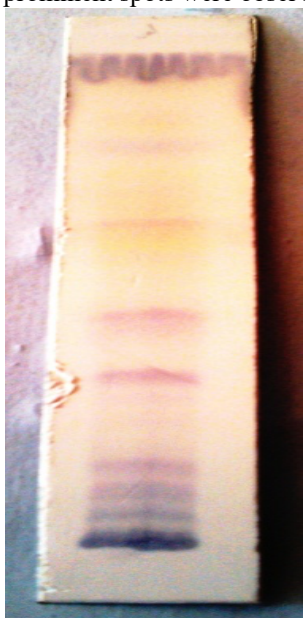


Figure 4 TLC of chloroform fraction

2.6.2 Column chromatography of chloroform fraction

column chromatography of chloroform fraction was carried out using solvent systems hexane, hexane : chloroform and chloroform in increasing order of polarity. A total of 35 fractions each of 250 ml were collected and fractions with similar TLC profile were pooled to get final four fractions (Table 4).

Table 4. Fractions quantity after column chromatography

Fractions	**Quantity (g/mg)**	**Solvent ratio (Hexane:Chloroform)**
F1	0 .30	100 : 0
F2	0 .10	75:25
F3	1.77	50:50
F4	0.44	25:75

3.6.3 Column chromatography of fraction F-3

Fraction F-3 (1.5 g/mg) was further column chromatographed using solvent system hexane:

chloroform (50:50) to chloroform (100%) in increasing order of polarity. A total of 30 fractions of 100 ml each were collected and those with similar TLC profile were pooled to get final five fractions (Table 5).

Table 5. Quantity of fractions (F-3.1 to 3.5) in column chromatography

Fractions	Quantity (g)	Solvent ratio (Hexane:$CHCl_3$)
F-3.1	0.20	50 : 50
F-3.2	0.40	50:50
F-3.3	0.05	50:50
F-3.4	0.20	25:75
F-3.5	0.55	25.75

Fraction F-3.2 gave fine needle shaped white crystals (7 mg). These were further purified by washing with hexane to get 5 mg of the pure crystals. The crystals were identified as β-sitosterol, when compared on TLC with standard β-sitosterol (Figure 5).

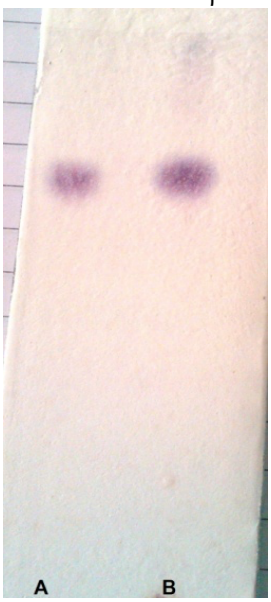


Figure 5: TLC of the compound isolated A: Standard β-sitosterol; B: Crystals isolated

3.7 Melting point

Melting points (m.p.) were 135-140 ˚C determined by using glass capillary tubes.

4. Conclusions:

In the present paper, analysis for free radical scavenging activity of the extract (ethyl acetate and *n*-butanol) of roots of *P. aculeata*was carried out and showed that it can be source of natural antioxidants. The *in vitro* antioxidant tests of *P. aculeata* roots extract has confirmed the presence of reducing agents showing good antibacterial activity against the test organisms. The antibacterial effect of chloroform extract against these microorganisms may be due to presence of some bioactive compound. The study reveals that there is enough potential in this plant and further isolation of the active constituents can give a lead to some potential candidate.

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