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# Rotenoids from *Parkinsonia aculeata* L and Their *In Vitro* Amoebicidal Activity

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**Abstract :** Rotenoids the group of naturally occurring insecticidal substances, ketonic in nature were analysed from various plant parts of *Parkinsonia aculeata* L. Three rotenoids viz rotenone, elliptone and deguelin were identified using TLC, GLC, mp, mmp, IR, and UV studies, which were comparable to that of the their respective standard compounds. In different part of the plant the rotenoid recovery has been observed as per the order: root> stem>leaves> pods>seeds, being maximum in the root and minimum in seed. The different doses (1000 ìgmL$^{-1}$ , 500 ìgmL$^{-1}$, 250 ìgmL$^{-1}$ , 125 ìgmL$^{-1}$ , 62 ìgmL$^{-1}$ , 31 ìgmL$^{-1}$) of isolated rotenoids from roots were subjected to *in vitro* antiamoebic activity along with standard rotenone and derris resin for different time intervals (24h 48h 72h). The dose level 500ìg/ml L$^{-1}$ was found most active as compared to 250 ìg/ml L$^{-1}$ of standards. However, the standard antiamoebic drug metroindazole was effective at 8 ìg/ml dose.

**Key words :** Rotenoids, *Parkinsonia aculeata, Entamoeba hystolytica*, amoebicidal

## Introduction :

*Parkinsonia aculeata* L., is a large spinous shrub or small tree, native of America found throughout the drier part of India and commonly known an '*Vilayati Kikar*". Parkinsonia aculeata is a tree from the family Fabaceae; common names include Mexican Palo Verde, Parkinsonia, Jerusalem thorn, or Jellybean tree. It is native to the southwestern United States (western Texas, southern Arizona), Mexico, the Caribbean, South America south to northern Argentina, and the Galapagos Islands.

It grows from 2 to 8 meters high. The leaves and stems are hairless. The flattened leaf stalk is edged by two rows of 25-30 tiny oval leaflets; the leaflets are soon deciduous in dry weather, leaving the green leaf stalks and branches to photosynthesize. The branches grow sharp spines 7-12 mm long. The flowers are yellow and fragrant, 20 mm in diameter, growing from a long slender stalk in groups of eight to ten. The fruit is a pod, leathery in appearance, light brown when mature.

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All the parts of the plant are known as antipyretic, diaphoretic, and abortifacient (Wealth of India, 1948-66). The alcoholic extract of the aerial part possess CNS depressant activity (Rao *et a.l*, 1979). However, there is no report of the presence of insecticidal substances from *P. aculeata.*

Among the waterborne diseases after malaria, amoebiasis is one of the most prevalent and causing health risk to millions every year. The protozoan parasite *Entamoeba hystolytica* infects 10% of the world population mostly in developing countries (WHO,1969). The disease results in considerable morbidity and mortality, and pathogenic strains of the parasites may be responsible for upto 50,000- 100,000 deaths annually. Infection can lead to amoebic dysentery, resulting from trophozoites invading the intestinal wall and amoebic liver abscess and other extraintestinal lesions, resulting from spread of trophozoites from intestinal wall via the blood stream. (Walsh,1986; Benenson,1995; Markell *et al.,* 1999).

WHO (1970) has advocated eradication programme by intermediate vector control strategy. There is ample scope for evaluation of plant origin insecticides against water borne disease under integrated vector management programme (Kamal, 1987; Kamal *et al.,* 1988). There is no report of Amoebicidal activity from *P.aculeata*.

## Materials and Methods :

The different plant parts *P. aculeata* (root, stem, leaves, pods, seeds) were dried first at 100$^{o}$C of 20 minutes to inactivate the enzymes and then at 60$^{o}$C till the constant weight was achieved. The dried plant material was powdered separately and extracted with acetonitrile saturated with n-hexane for 72h at room temperature (Delfel, 1973). Each sample was filtered and concentrated to dryness *in vacuo*, separately. The concentrated extract was dissolved in acetone, filtered and filtrate was mounted on a column of inert alumina to eliminate the impurities. It was continuously eluted with acetone till the last elute gave no positive result on TLC. The various fractions were pooled together, dried and weighed of crude rotenoid content in different plant parts for further analysis.

***Qualitative Analysis***

***Identification*** **:** Thin layer chromatography (TLC) was performed to identify the rotenoids. The various concentrated extracts were dissolved in acetone, spotted on TLC plates with the various reference compounds (*derris* resin, rotenone, deguelin, elliptone) and developed, separately in two solvent systems chloroform, acetone and acetic acid (196:3:1) of Delfel and Tallent (1969) and chloroform and diethyl ether (95:5) of Delfel (1966). The developed chromatograms were dried until they were free of solvent order and thereafter sprayed with hydroiodic reagent (HI; 5N potassium iodide: 45% orthophosphoric acid : 1:30) of Delfel (1965), till the plates appeared slightly damp. A few coloured spots developed immediately after spraying were noted and these plates were then heated in an oven at 120$^{o}$C for 20 min to resolve final development of various coloured spots, which matched with the respective standard reference compounds.

Two-dimensional TLC was also carried out to achieve better separation by using solvent system of chloroform and diethyl

ether in first direction and chloroform, acetone and acetic acid in second direction (Kamal and Mangla,1987). The concentrated test extracts as well as the reference mixture of rotenoids were applied at one corner of TLC plants (30x30 cm), separately; both the plates were developed in same manner for co-comparison. These two dimensionally developed chromatograms were air dried and sprayed with HI spray reagent. Spots coinciding with the standard reference compounds in various experimental test samples were noted and their Rf values were compared.

***Preparative thin layer chromatography*** **(PTLC) :** The known amount of crude rotenoid extracts so obtained were subjected PTLC for quantification. The spots of different extracts along with their respective standard reference rotenoids were applied on activated TLC plates and developed unidirectionally as described in qualitative analysis. The spots were identified on TLC plates by spraying with HI reagent, to one of the column in each plate and the spots coinciding with their standard were marked, scrapped separately from 200 developed unsprayed plates. Each of the scrapped spots were extracted in acetone and subjected to crystallization using carbon tetra-chloride as per AOAC (Kamal and Mangla, 1987). The crystals were subjected to mp, mmp, IR and UV spectral studies.

***Gas Liquid Chromatography (GLC)***

The following conditions were maintained for the GLC analysis of the sample.

Instrument – Variance 600

Temperature – 150-180$^{o}$C

Carrier gas – Nitrogen at 40-PSI column OV 17.3%

Chart speed – 0.5 cm/min

Mixture of standard rotenoids, as well as extract of the test plants were run on above condition.

***Quantitative Analysis***

Preparative TLC of all known amount of crude extracts of rotenoids were carried for quantitation using the method described above. The scrapped spots from identical bands were bulked together and extracted in acetone. The extracts so obtained were dried and weighed for total rotenoids percent recovery separately.

***In vitro testing of antiamoebic activity against Entamoeba histolytica***

The trophozoites of 48 to 72 h old cultures growing in modified Diamond's medium (1968) were collected by centrifugation following the method of Das and Prasad (1973).

***The test material***

Rotenoid extract from the seeds of *P. aculeata*, standard rotenone, and *Derris* resin were used for screening their Amoebicidal activity.

**Procedure of antiamoebic activity**

Various samples were weighed and 2-3 drops of dimethy1 sulphoxide was added. After keeping for 1-2 h distilled water was added to make the concentration 2 mg/ml. In 1 ml of the test solutions 1 ml of culture medium was added. Various concentrations of these test solutions were then prepared by serial dilutions in screw capped test tubes. The tubes were incubated for overnight. Inoculum 0.2 ml, containing about 2,000 amoebae, as determined by

**Table 1.** ***In vitro*** **amoebicidal of activity standard and isolated rotenoids against** ***E. histolytica***

*Standard antiamoebic drug metroindazole (8µgmL$^{-1}$ )*
*+ amoebae living*
*- amoebae dead*
*± mixed population of living and dead*

| Test materials | *Treatment doses* | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1000 µgmL$^{-1}$ | | | 500 µgmL$^{-1}$ | | | 250 µgmL$^{-1}$ | | | 125 µgmL$^{-1}$ | | | 62 µgmL$^{-1}$ | | | 31 µgmL$^{-1}$ | | | | |
| | (*Observations after hours*) | | | | | | | | | | | | | | | | | | | | |
| | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h |
| *P. aculeata* | - | - | - | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Rotenone (standard) | - | - | - | + | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| *Derris* resin | - | - | - | ± | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

haemocytometer count, was put into cavity slide along with 0.2 ml of the various test samples dilutions (Das, 1975). The cavity was covered with cover slips and the edges of cover slips were sealed with paraffin wax. Slides were kept in petridishes and incubated at 37°C. Petridishes which served as moist chamber, were sealed from outside by adhesive tape to avoid contamination. Duplicate sets were run for each drug dilutions. In the case of control, no drug was added. Observations were taken after 24, 48 and 72h under the microscope to find out whether the amoebae were dead or alive. The dead were rounded and appeared vacuolated, while living amoebae appeared hyaline and refractile.

## Results and Discussion :

The qualitative and quantitative estimations of rotenoids from different plant part of *P. aculeata* were made using TLC, PTLC, and GLC analysis.

The TLC of different plant samples revealed presence of three spots in solvent system of chloroform, acetone, and acetic acid were coinciding with Standard rotenoids–rotenone, deguelin and elliptone in the Rf values 0.58, 0.72 and 0.87, respectively. The hydroiodic sprayed chromatograms developed characteristic colours of rotenone (blue) and elliptone (violet) before heating which were comparable to the colours developed in the standard. After heating the chromatograms colour changes to purplish-blue (elliptone), pink (deguelin) and blue (rotenone), which also corresponded with that of standard run parallel. Two-dimensional chromatography also resolved three distinct spots of rotenone, deguelin and elliptone, which coincided with authentic marker of rotenoids.

Crystals of rotenone and elliptone were subjected to melting point determination, were 163°C and 181°C, respectively; these were comparable to that of the respective standards. However, deguelin could not be crystallized. The mixed melting point was undepressed. The IR spectral studies of each of the isolated compounds showed characteristic super imposable absorption peaks. The UV max (MeOH): 223 nm of rotenone and others between 222-225 nm also corresponded with standard. The GLC studies showed that the retention times and peaks of individual ketonic fractions of isolates were similar to standard.

In different plant parts the common trend of rotenoid recovery was observed as below, where roots gave maximum and seeds gave minimum rotenoid recovery (Fig 1):

***Root > stem > leaves > pods > seeds***

Rotenoid isolated from *P. aculeata* showed antiamoebic activity at the concentration of 500 ìg/ml as compared to 250 ìg/ml activity of rotenone and derris resin, which may be due to pure crystals of the standard compounds. However, the metronidazole gave antiamoebic activity at 8 ìg/ml (Table 1). In western medicine there is no drug that can be considered as ideal for the treatment for amoebiasis, particularly for treatment of severe infection. Currently, metronidazole and emetin are regarded as drug of choice in the treatment of amoebiasis but have serious side effects such as bad taste and tumor formation (Anon,1983).

Misra *et al*. (1981) observed that *Pistacia integerrima* extract gave 80% recovery with a dose of 30mg/day, in clinical manifestation of intestinal amoebiasis. Van

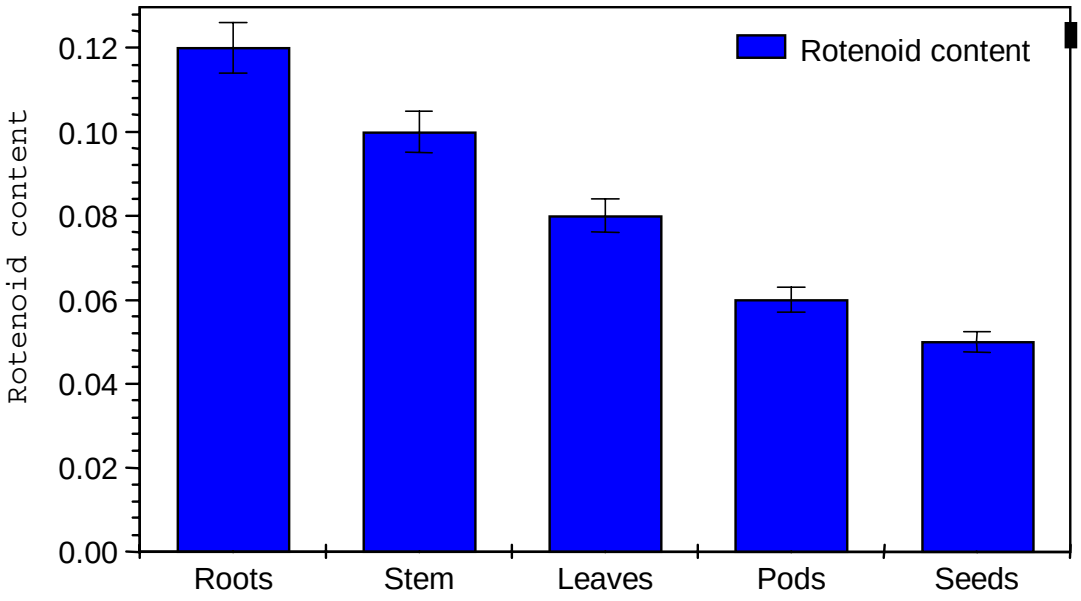


**Fig. 1 : Rotenoid content in different plant parts of *P. aculeata***

Beek *et al.* (1984) reported significant and antiamoebic activity at 1.5mg/ml of three *Tabernaemontana* sps as compared to extract of *Cepahalis ipecacuanha* containing 2% emetine (0.45mg/ml). There is no report of antiamoebic action of rotenoids. The results of the present study indicate that *P.aculeata* aerial part rotenoid extract can be useful as an amoebicidal agent.

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