ON THE ANTIFEEDANT AND ANTHELMINTIC POTENTIAL OF ANACARDIUM OCCIDENTALE L.
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ABSTRACT: The antifeedant and anthelmintic activities of crude extracts of the shells of Anacardium occidentale were studied by comparing it’s activity with the activity of Azadirachta indica A Juss (Kernel), a commercial standard. The test extracts of A. occidentale in petroleum ether, dichloromethane: methanol showed better activities compared to A. Indica. To Study the anthelmintic activity, the addition of piperine to the formulation significantly enhances the activity. The phytochemical investigation of test extracts showed the presence of phenols namely cordol, cardanol and anacardic acid by TLC studies. The most potent extracts are non toxic to mice.

INTRODUCTION

The genus Anacardium is a tropical American genus mainly composed of shrub and trees, of which one species Anacardium occidentale (Fam: Anacardiaceae) is naturalized in India. Commonly it is known as cashew. It possess high medicinal, confectionary and nutritional values. Its shell oil is indigenously reputed as a mild purgative, pesticide, preservative for wood, anthelmintic and in the treatment of cracks on footsoles, wartz, corns and leprous sores! It is also reported that further the shell oil is used in protection of timber and books from white ants, as a mosquito larvicide, as a molluscicide and as a tyrosinase inhibitor. A few report also document its value in the treatment of hook worm, tapeworm and caecal amoebic infections.

The piperine alkaloids isolated from the fruits of Piper longum have also been reported for its bioavailability enhancing and synergistic properties.

The antifeedant and anthelmintic activities of the shell have yet to be studied systemically and scientifically. This reveals a study of development of biorational plant protection and medicinal agents from natural sources. The preliminary evaluation of these activities were studied in vitro. The preliminary evaluation of these activities were studied in vitro. The synergistic potential of piperine in the anthelmintic formulations were studied. The qualitative analysis of phytoconstituents was carried out. To assess their safety margin, the acute oral toxicity of crude formulations were investigated on mice.

MATERIALS AND METHODS

Mature nuts of A. occidentale were collected in the month of May 1994 from the cashew plantations of pathanamthitta district, Kerala. They were dried and roasted in a sandbath to make the shells brittle and enable easy separation form the kernels. The roasted shells were than dried and powdered (40#). Seed kernels of Azadirachta indica were collected from the plantations in the vicinity of L.M. College of Pharmacy, Ahmedabad, in the month of
May 1994. They were dried in the shade and powdered (40#). The powders were subjected to the following scheme of solvent extraction and their extractive values were determined for two extraction cycles.

**Extraction:**

600g 40# Powder of crude drug

Extract with 1200 ml petroleum ether (60o – 80oC) Defatting (Soxhlet for 6 hrs)

Filter

Filtrate Marc

Concentrate on Water bath Extract with mixture of Dichloromethane: Methanol (1:1) (Soxhlet) Extract with distilled water (boiling)

Filterate Concentrate to Dryness

Residue Determine % extractive value

% Extractive = \( \frac{W_2}{W_1} \times 100 \)

W₁ = before extraction the powder of the crude drug was dried, weighed to constant Wt. W₂ = the extract is evaporated and residue was dried to a constant Wt.

**Formulation**

The crude extracts were formulated as 1%, 2.5% and 5% emulsions, suspensions or solutions in distilled water, depending upon their water solubility and physical nature, Micro – bentonite was used as emulsifying (3%) of suspending (5%) agent.

**Antifeedant Activity**
The third and the fourth instars (most voraciously feeding stages) of the lepidopteran tobacco caterpillar, *Spondoptera litura* Fabr. Were identified, separated and prestarved for three hours prior to treatment. Circular discs of fresh green castor leaves, cut around their petioles to exactly fit the base of a Petri dish were dipped into the formulation of extracts for approximately 10-15 seconds, air dried, moistened with wet cotton plug at the petiolar end (to avoid drying of the leaf disc) and placed into their respective labeled petridish. Crude formulations for *A. indica* and distilled water served as standard and control resp. One pre-weighed larvae of the desired instar stage was released per petridish for feeding on the leaf discs. Observations were made at 24 hrs and 48 hrs for larval weight, faecal matter weight, percentage feeding on leaf discs by insect, percentage protection to leaf discs by treatment and mortality if any.

The percentage anti-feedant activity was calculated using the formula

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\text{% Antifeedant activity} = \frac{\text{% protection in treatment} - \text{% protection in control}}{100 - \text{% protection in control}}
\]

Potency of formulation is directly proportional anti-feedant activity.

**Anthelmintic Activity**

Five adult worms of the Indian earthworm, *Pheretima posthuma* (selected due to its anatomical and physiological resemblance with the intestinal roundworm parasites of man) were released into each petridish containing 50ml of the desired formulation of crude extract. Crude formulations of *A. indica* and distilled water served as standard and control respectively. The synergistic potential of piperine (3%) was evaluated for anthelmintic activity with both the test and standard formulations. The parameters recorded were the time taken for paralysis and /or death of more than two worms. Paralysis is recorded when the worms stop their normal agitated wriggling, but revive when transferred into distilled water. Death is recorded when they do not revive even in saline water. Three replications of each treatment was maintained to minimize any sources of error. Potency of formulation is inversely proportional to the time taken for paralysis and / or death of the worms.

**Detection of Phytoconstituents**

The crude extracts were subjected to argentation thin layer chromatography on silica gel G plates impregnated with 10% w/w of silver nitrate as a stationary phase. The mobile phase used was a mixture of diethyl ether, light petroleum, and formic acid (30:70:1). The spraying reagent used was 50% sulphuric acid and subsequently charring the plates. The spots were identified with the help of Rf values and colours and characterized by comparing with the literature.17

**Acute Oral Toxicity**

The most potent crude extracts were screened for acute oral toxicity to a group of five mice by an initial high single dose limit test. The mice were administered 5ml of the formulation containing 2000mg. per kg body wt. of the extract by oral intubation. Determination of the LD 50 value becomes necessary only if death or any other toxic manifestations are observed in the animals with this high single dose limit test.
| Crude Drug     | Extraction Cycle | Petroleum Ether Extraction (%) | Dichloromethane: Methanol Extraction (1:1) | Distilled Water Extract (%) |
|---------------|------------------|---------------------------------|-------------------------------------------|-----------------------------|
| Anacardium    | 1                | 36.66%                          | 10.00%                                    | 32.66%                      |
| Occidentale Linn | 2                | 3.33%                           | 3.11%                                     | 5.83%                       |
| Azadirachta   | 1                | 46.00%                          | 12.41%                                    | 8.62%                       |
| Indica A. Juss | 2                | 6.00%                           | 3.17%                                     | 3.11%                       |
| Extract Stage | Parameter | Percentage Larval Weight | Percentage Faecal Matter Weight | Percentage Feeding | Percentage Protection | Percentage Antifeedant Activity |
|---------------|-----------|--------------------------|--------------------------------|-------------------|----------------------|---------------------------------|
| Plant Conc. (%) | Conc. (%) | Time 0hrs 24hrs 48hrs 24hrs 48hrs 24hrs 48hrs 24hrs 48hrs 24hrs 48hrs |
| Anacardium Occidentale Linn. | 1 | 146.10 -13.35 -15.42 6.10 -68.85 15.11 9.69 84.89 90.31 61.86 80.56 |
| | 2.5 | 305.90 -2.84 -4.71 4.30 -44.19 18.12 6.83 81.88 93.17 54.27 86.31 |
| | 5 | 305.60 -4.58 -30.46 2.20 -90.91 7.89 7.38 92.11 92.62 80.08 85.91 |
| Standard (Azadirachta indica A. Juss.) | 1 | 314.00 -0.06 -4.20 11.60 -38.79 33.73 25.22 66.27 74.78 14.86 49.41 |
| | 2.5 | 182.70 -0.32 -19.98 2.80 -75.00 8.36 6.86 91.64 93.14 78.89 86.24 |
| | 5 | 253.20 -4.54 -28.32 6.40 -46.87 21.92 14.38 78.08 85.62 44.67 71.15 |
| Control | | 264.80 +10.05 +23.15 11.90 +4.20 39.62 49.85 60.38 50.15 |
| Anacardium Occidentale Linn. | 1 | 335.40 -33.54 -38.25 3.80 -2.63 7.47 5.38 92.53 94.62 86.24 90.78 |
| | 2.5 | 379.60 -43.07 -49.53 20.80 -4.80 8.04 6.94 91.96 93.06 85.18 88.12 |
| | 5 | 412.50 -47.54 -49.96 11.40 -87.72 3.98 2.41 96.02 97.59 92.67 95.87 |
| Standard (Azadirachta indica A. Juss.) | 1 | 399.80 -10.21 -12.68 7.80 -21.79 29.00 24.57 71.00 75.43 46.56 57.93 |
| | 2.5 | 379.20 -58.83 -61.74 0.40 -25.00 11.77 6.82 88.23 93.18 78.31 88.32 |
| | 5 | 426.70 -13.41 -20.99 5.60 -16.07 9.16 8.86 90.84 91.14 83.12 84.83 |
| Control | | 367.80 +42.31 +57.73 52.70 +5.31 54.27 58.41 45.73 41.59 |
| Extract | Instar Stage | Parameter | Percentage | Percentage | Percentage | Percentage | Percentage | Percentage |
|---------|--------------|-----------|------------|------------|------------|------------|------------|------------|
|         |              | Plant     | Conc. (%)  | Larval Weight | Faecal Matter Weight | Feeding | Protection | antifeedant Activity |
|         |              | Anacardium Occidentale Linn. | 1 | 236.30 | -23.02 | -47.27 | 0.90 | -77.77 | 2.63 | 1.92 | 97.37 | 98.08 | 94.99 | 96.16 |
|         |              |            | 2.5 | 235.70 | -14.86 | -34.62 | 1.20 | -75.00 | 2.64 | 1.05 | 97.36 | 98.95 | 94.97 | 97.90 |
|         |              |            | 5 | 282.30 | -22.95 | -53.10 | 0.80 | -50.00 | 4.10 | 1.45 | 95.90 | 98.55 | 92.19 | 97.10 |
|         |              | Anacardium Occidentale Linn. | 1 | 257.00 | -10.86 | -29.22 | 1.00 | -60.00 | 8.69 | 7.11 | 91.31 | 92.89 | 83.45 | 85.79 |
|         |              |            | 2.5 | 199.30 | -17.61 | -36.23 | 1.30 | -53.85 | 6.77 | 6.33 | 93.23 | 93.67 | 87.10 | 87.35 |
|         |              |            | 5 | 261.10 | -25.39 | -52.85 | 1.60 | -87.50 | 11.67 | 9.02 | 88.33 | 90.98 | 77.77 | 81.96 |
|         |              | Standard (Azadirachta indica A. Juss.) | 1 | 209.30 | +41.61 | +62.02 | 9.40 | +126.59 | 52.50 | 50.02 | 47.50 | 49.98 | 38.75 | 40.50 |
|         |              |            | 2.5 | 191.60 | -35.13 | -62.31 | 0.40 | -117.30 | 2.90 | 2.50 | 99.66 | 99.30 | 97.80 | 97.20 |
|         |              |            | 5 | 249.70 | -31.76 | -52.85 | 0.30 | -62.85 | 0.80 | 0.40 | 99.92 | 99.92 | 99.70 | 99.70 |
|         |              | Control | | | | | | | | | | | | |
|         |              | Anacardium Occidentale Linn. | 1 | 211.40 | -37.63 | -62.02 | 0.80 | -126.59 | 52.50 | 50.02 | 47.50 | 49.98 | 38.75 | 40.50 |
|         |              |            | 2.5 | 191.60 | -35.13 | -62.31 | 0.40 | -117.30 | 2.90 | 2.50 | 99.66 | 99.30 | 97.80 | 97.20 |
|         |              |            | 5 | 249.70 | -31.76 | -52.85 | 0.30 | -62.85 | 0.80 | 0.40 | 99.92 | 99.92 | 99.70 | 99.70 |
|         |              | Standard (Azadirachta indica A. Juss.) | 1 | 271.20 | -52.88 | -62.02 | 0.10 | -126.59 | 52.50 | 50.02 | 47.50 | 49.98 | 38.75 | 40.50 |
|         |              |            | 2.5 | 259.40 | -50.69 | -62.02 | 1.50 | -126.59 | 52.50 | 50.02 | 47.50 | 49.98 | 38.75 | 40.50 |
|         |              |            | 5 | 228.70 | -61.08 | -62.02 | 0.40 | -126.59 | 52.50 | 50.02 | 47.50 | 49.98 | 38.75 | 40.50 |
Table – IV

| Extract           | Instar Stage | Parameter       | Percentage Larval Weight | Percentage Faecal Matter Weight | Percentage Feeding | Percentage Protection | Percentage antifeedant Activity |
|-------------------|--------------|----------------|--------------------------|-------------------------------|-------------------|-----------------------|---------------------------------|
|                   |              | Plant          | Time 0hrs 24hrs 48hrs 24hrs 48hrs 24hrs 48hrs 24hrs 48hrs 24hrs 48hrs |                                 |                   |                       |                                 |
| Distilled Water   | 3            | Anacardium Occidentale Linn. | 1  187.60 -22.60 -28.78 10.20 -69.61 32.55 29.00 67.45 71.00 15.76 49.75 |                                 |                   |                       |                                 |
|                   |              |                | 2.5  124.80 -4.16 -9.38 8.10 -70.37 29.38 25.47 70.62 74.53 23.96 55.87 |                                 |                   |                       |                                 |
|                   |              |                | 5  279.10 -3.37 -13.15 9.10 -32.67 36.33 29.36 63.67 70.61 5.97 49.08 |                                 |                   |                       |                                 |
|                   | 4            | Standard (Azadirachta indica A. Juss.) | 1  266.60 -7.69 -14.02 10.70 -14.90 37.22 26.41 62.78 73.59 3.60 54.24 |                                 |                   |                       |                                 |
|                   |              |                | 2.5  150.60 -0.99 -1.79 11.90 -48.74 35.85 29.77 64.15 70.23 7.22 48.42 |                                 |                   |                       |                                 |
|                   |              |                | 5  283.40 -1.69 -3.95 24.70 -21.05 38.22 39.35 61.78 60.65 1.08 31.38 |                                 |                   |                       |                                 |
|                   | 5            | Control        | 124.50 +48.46 +130.84 9.90 +80.81 38.64 57.72 61.36 42.28 |                                 |                   |                       |                                 |
|                   |              | Anacardium Occidentale Linn. | 1  397.90 -45.76 -49.68 6.60 -3.30 13.58 12.66 86.42 87.34 75.27 77.77 |                                 |                   |                       |                                 |
|                   |              |                | 2.5  429.70 -17.10 18.75 8.10 -20.86 13.76 79.14 86.42 62.02 75.84 |                                 |                   |                       |                                 |
|                   |              |                | 5  401.90 -7.02 -8.53 10.30 -5.82 16.75 12.22 83.25 87.78 69.50 78.55 |                                 |                   |                       |                                 |
|                   |              | Standard (Azadirachta indica A. Juss.) | 1  649.10 -5.05 -7.26 18.70 -10.16 29.02 30.35 70.98 69.65 47.15 46.72 |                                 |                   |                       |                                 |
|                   |              |                | 2.5  339.60 -35.60 -39.01 2.90 -10.34 28.05 27.51 71.95 72.49 48.92 51.71 |                                 |                   |                       |                                 |
|                   |              |                | 5  410.90 -2.48 -3.91 3.80 -5.26 25.27 13.75 74.73 86.25 53.98 75.86 |                                 |                   |                       |                                 |
|                   |              | Control        | 351.20 +48.46 +60.59 33.40 +73.65 54.92 56.97 45.08 43.03 |                                 |                   |                       |                                 |
Table – V
Anthelmintic Activity

| Crude Drug | Conc | Petroleum Ether Extract | Dichloromethane: Methanol Extract | Distilled Water Extract |
|------------|------|--------------------------|-----------------------------------|------------------------|
|            |      | Time taken for Paralysis | Time taken for death              | Time taken for Paralysis | Time taken for death |
| Anacardium occidentale L. | 1%  | 13.33 | 20.00 | 9.33 | 16.00 | 20.02 | 28.02 |
|            | 2.5% | 13.06 | 20.00 | 7.33 | 11.33 | 14.02 | 24.33 |
|            | 5%  | -     | 6.00  | -    | 6.00  | 6.33  | 12.00 |
| Anacardium occidentale + Piperine | 1%  | 13.06 | 19.06 | 6.02 | 10.00 | 13.02 | 17.02 |
|            | 2.5% | 12.56 | 15.06 | 3.02 | 6.33  | 7.33  | 16.00 |
|            | 5%  | -     | 4.06  | -    | 2.02  | -     | 5.33  |
| Azadirachta indica A.Juss | 1%  | 17.33 | 28.00 | 18.02 | 26.33 | 23.33 | 42.02 |
|            | 2.5% | 15.00 | 22.06 | 12.02 | 20.02 | 18.33 | 29.33 |
|            | 5%  | -     | 8.00  | -    | 7.00  | 9.02  | 13.33 |
| Azadirachta indica A.Juss + Piperine | 1%  | 11.33 | 22.06 | 10.00 | 15.00 | 16.33 | 29.06 |
|            | 2.5% | 10.06 | 15.06 | 7.06  | 9.33  | 10.00 | 20.06 |
|            | 5%  | -     | 6.00  | -    | 5.06  | -     | 7.06  |

Table – VI
Phytochemical Analysis

| Spots       | Petroleum Ether Extract | Dichloromethane: Methanol Extract | Distilled Water Extract |
|-------------|-------------------------|-----------------------------------|------------------------|
| A           | Rf=0.2 Cardol           | Rf=0.2 Cardanol                   | Rf=0.2 Cardol          |
| B           | Rf=0.58 Cardanol        | Rf=0.58 Cardanol                  | Rf=0.58 Cardanol       |
| C           | Rf=0.76 Anacardic Acid  | Rf=0.76 Anacardic Acid            | Rf=0.76 Anacardic Acid |

*Fluorescent

RESULT AND DISCUSSION

Table 1 shows the extractive values of the crude drugs in the respective solvents for two extraction. The high yield of both drugs in petroleum either extract indicated their salty nature.

The results of the parameters evaluated for antifeedant activity are shown in Table II, III and IV. There was a decrease in larval weight, faecal matter weight and percentage feeding in the treatments, in contrast to that in control. This indicates the feeding
deterrent activity of the treatments, which was confirmed by the calculated values of percentage antifeedant activity. A. occidentale showed better antifeedant activity that A. Indica on both third and fourth instars of the larvae, suggesting its superiority over the latter. Interestingly the dichloromethane: methanol extract of both the drugs showed insecticidal activity too, killing the larvae within 48hrs. The (-) mark in the table indicate that the larvae were dead and thus reading could not be taken. No dose relationship was observed. The most potent crude extracts were the 2.5% and 5% dichloromethane: methanol extracts of A. occidentale on the third and fourth instar larvae respectively.

Table V enlists the values of the parameters evaluated for anthelmintic three replications. All test extracts showed better anthelmintic activity than the std No paralysis or death were recorded in the control. All extracts showed a proportionate dose response relationship.

The combination of the crude extracts with piperine showed a statistically significant (student “t” test at P<0.05) improvement in activity. Here too a proportionate dose response relationship was observed with all extracts. The combination of piperine with the 5% dichloromethane: methanol extract of A. occidentale was most potent.

The individual spots obtained by TLC of the crude extracts were characterized and identified in Table VI.

Acute oral toxicity screening of the most potent crude extracts i.e the 2.5% dichloromethane: methanol extract of A. occidentale the 5% dichloromethane: methanol extract of A. occidentale and the combination of piperine (3%) with the 5% dichloromethane: methanol extract of A. occidentale showed neither mortality nor any other toxic manifestations even at 2000mg/kg. body wt. in mice. No gross behavioral changes were observed. Thus categorizing the formulations as non toxic and safe.

**CONCLUSION**

The study revealed that A. occidentale deterred or interfered with the feeding mechanism of S. litura larvae, the activity being even better than a commercialized std A. indica, thus its potential should be exploited in various pest management schemes. The anthelmintic activity of A. occidentale and its combination with piperine suggest that it can be a useful tool in the development of biorational plant protection and medicinal gents from natural sources.

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