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# PHYTOCHEMICAL INVESTIGATION OF ANACARDIC ACID FROM ETHYL ACETATE EXTRACT FROM CASHEW LEAVES AND ITS BIOLOGICAL ACTIVITIES

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## ABSTRACT

The present study investigates the biological potential of Anacardium occidentale leaf extract. The main objective was to evaluate its antidiarrheal,antimicrobial, and antioxidant activities. The leaf extract was prepared using appropriate extraction techniques and assessed through standard in-vitro experimental methods. The findings revealed noticeable antimicrobial effects against selected microbial strains along with considerable antioxidant activity. In addition, the extract demonstrated promising antidiabetic potential. These results indicate that Anacardium occidentale leaf extract could be a valuable natural source of bioactive compounds with possible therapeutic applications.

**KEYWORDS:** Anacardic acid, Anacardium occidentale leaf extract, Plant-derived bioactive compounds, Antimicrobial potential, Antioxidant properties, Antidiabetic activity, Phytochemical constituents, Natural therapeutic agents.

## AIM AND OBJECTIVES

The present study aims to investigate the biological activities of Anacardium occidentale leaf extract by evaluating its antimicrobial, antioxidant, and antidiarrheal properties. The objectives include assessing its therapeutic potential, identifying its bioactive effects through experimental analysis, and exploring its possible application as a natural plant-based medicinal agent.

## INTRODUCTION

Anacardic acid is a naturally occurring phenolic compound mainly obtained from the cashew plant, Anacardium occidentale (family Anacardiaceae). It is abundantly present in cashew nut shell liquid and possesses a salicylic acid structure with a long alkyl side chain. Traditionally, different parts of the plant have been used in herbal medicine to treat various disorders such as infections, inflammation, and diarrhea. Recent studies have reported that anacardic acid exhibits several biological activities including antimicrobial, antioxidant, anti-inflammatory, and anticancer effects. These properties highlight its potential importance in pharmaceutical and biomedical research.

## MATERALS AND METHODS

### Plant material collection and extraction

Fresh leaves of Anacardium occidentale were collected, shade-dried for 15 days, and powdered. About 50 g of the powdered material was extracted using Leishman's reagent and filtered to obtain a clear extract. The filtrate was sequentially partitioned using n-hexane, ethyl acetate, and chloroform in a separating funnel to isolate different solvent fractions. Each fraction was concentrated by evaporation and dried in a desiccator. The final residue was recrystallized using acetone and Leishman's reagent, dissolved in methanol, and completely dried prior to further analytical studies.

**Antidiarrheal activity**

**Castor Oil–Induced Diarrhoea Model**

Young cheeks (30-60 g) were used to evaluate antidiarrhoeal activity using the castor oil–induced diarrhoea model. Diarrhoea was induced by oral administration of castor oil (0.75 mL per animal). Test drugs or vehicle were administered orally 1 h prior to castor oil treatment. Cheeks were observed for 4 h following induction, and the frequency of diarrhoeal episodes was recorded. All faecal matter excreted during the observation period was collected, and fecal water content was determined to assess the severity of diarrhoea and the therapeutic effect of the treatments.

Experimental animals were randomly divided into six groups, with five rats in each group.

**Group I – Vehicle Control**

Animals received the vehicle only.

**Group II – Standard Treatment**

Animals were administered loperamide (10 mg/kg, p.o.) as the reference antidiarrhoeal drug.

**Group III – Low Dose Treatment**

Animals received ethyl acetate leaf extract of anacardic acid at a dose of 150 mg/kg.

**Group IV – High Dose Treatment**

Animals received ethyl acetate leaf extract of anacardic acid at a dose of 300 mg/kg.

## RESULTS

**Table 1. Effect of Treatment on Castor Oil–Induced Diarrhea in young cheeks.**

| Group | Treatment | Dose (mg/kg) | Castor Oil Administered (ml) | Onset of Diarrhea (min) (Mean ± SEM) | Number of Wet Feces (Mean ± SEM) | Severity of Diarrhea | Inhibition of Diarrhea (%) |
|---|---|---|---|---|---|---|---|
| Group I | Vehicle Control | — | 2 ml | 28.4 ± 1.2 | 12.6 ± 0.8 | Severe | — |
| Group II | Standard Drug (Loperamide) | 10 | 2 ml | 64.2 ± 2.1 | 3.2 ± 0.4 | Mild | 74.6 |
| Group III | Extract (Low Dose) | 150 | 2 ml | 41.5 ± 1.8 | 7.4 ± 0.6 | Moderate | 41.3 |
| Group IV | Extract (High Dose) | 300 | 2 ml | 55.7 ± 2.0 | 4.9 ± 0.5 | Mild–Moderate | 61.1 |

**Conclusion**

The study demonstrated that the leaf extract exhibited significant antidiarrheal activity by delaying the onset of diarrhea and reducing fecal output in a dose-dependent manner. The higher dose showed greater effectiveness, comparable to the standard drug, indicating the potential of the extract as a promising natural agent for diarrhea management.

**Antimicrobial activity**

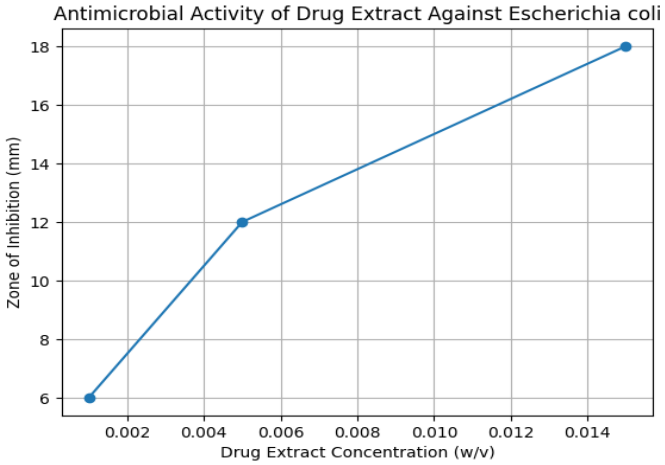
**Preparation of agar plates and sample solution**

Sterile nutrient agar plates were prepared under aseptic conditions and allowed to solidify before wells were created using a sterile cork borer. The n-hexane extract was dissolved in analytical-grade solvent to obtain a stock solution, from which different concentrations were prepared by serial dilution. A standardized culture of Escherichia coli was uniformly spread on the agar surface to form a bacterial lawn. The prepared extract solutions were introduced into designated wells, while solvent alone served as the negative control. The plates were incubated at 37 °C for 24–48 h, after which antibacterial activity was determined by measuring the diameter of inhibition zones around each well using the agar well diffusion method.

**Preparation of Drug Extract and Microbial Culture**

The drug extract was prepared by dissolving the crude sample in analytical-grade chloroform to obtain a stock solution, followed by serial dilution to achieve concentrations of 0.001, 0.005, and 0.015 (w/v), each adjusted to a final volume of 5 mL under aseptic conditions. For antimicrobial evaluation, a pure culture of Escherichia coli was maintained on nutrient agar and subcultured prior to experimentation to ensure active bacterial growth.

## RESULTS




**Graph-1: Antimicrobial activity of extract against e. coli.**

**Conclusion**

The prepared drug extract demonstrated antibacterial activity against Escherichia coli as observed in the agar well diffusion assay. The inhibition zones increased with rising extract concentration, indicating a dose-dependent response. These results suggest the presence of active phytoconstituents capable of suppressing bacterial growth.

**Antioxidant Activity**

**DPPH Free Radical Scavenging Assay**

The antioxidant activity of the ethyl acetate extract was evaluated using the DPPH assay, which measures the reduction of purple DPPH radicals to a yellow-colored compound in the presence of antioxidants. The extract was dissolved in distilled water, sonicated, filtered, and subjected to serial dilutions. A DPPH solution (0.01 g in 100 mL methanol) was prepared and kept in the dark for 30 minutes. Each sample dilution was mixed with DPPH solution, and absorbance was measured at 370 nm using a UV–visible spectrophotometer. The absorbance values obtained were 0.108, 0.021, and 0.011 for the first, second, and third dilutions, respectively, while the DPPH control showed an absorbance of 0.592.

The antioxidant activity was expressed as percentage inhibition of DPPH free radicals and calculated using the following equation:

Percentage Inhibition (%)=A0A0 /A1×100

where:

$\mathbf{A_0}$ represents the absorbance of the DPPH control solution (0.592).

$\mathbf{A_1}$ represents the absorbance of the test sample at the respective concentration.

## RESULTS

**Table 2: DPPH Antioxidant Activity.**

| Sample | Absorbance ($A_1$) | Control Absorbance ($A_0$) | % Inhibition (DPPH Scavenging Activity) |
|---|---|---|---|
| DPPH Control | 0.592 | 0.592 | — |
| Methanol (Blank) | 0.004 | — | — |
| 1st Dilution | 0.108 | 0.592 | 81.75% |
| 2nd Dilution | 0.021 | 0.592 | 96.45% |
| 3rd Dilution | 0.011 | 0.592 | 98.14% |

**Conclusion**

The DPPH assay results confirm that the extract exhibits strong antioxidant activity, as indicated by high radical scavenging percentages. The decrease in absorbance values demonstrates effective free radical neutralization, suggesting the presence of active antioxidant compounds**.**

## DISCUSSION

The present investigation examined the biological activities of Anacardium occidentale leaf extract, including antidiarrheal, antimicrobial, and antioxidant properties, to evaluate its potential therapeutic value. The overall findings indicate that the extract possesses notable pharmacological activity, which may be associated with naturally occurring phytochemicals such as phenolic compounds and anacardic acid derivatives.

In the antidiarrheal study, the extract significantly reduced the severity of castor oil–induced diarrhoea. Castor oil is known to stimulate intestinal secretion and motility, resulting in increased fecal output. Administration of the leaf extract delayed the onset of diarrhoea and decreased the number of wet feces compared with the control group. The effect was dose-dependent, with the higher dose showing greater protection and results comparable to the standard drug loperamide. This suggests that the extract may help regulate intestinal movement and fluid secretion, thereby contributing to its antidiarrheal activity.

The antimicrobial assessment demonstrated that the extract inhibited the growth of Escherichia coli, confirming its antibacterial potential. The increase in inhibitory response with higher extract concentrations indicates a concentration-dependent effect. The antimicrobial activity may be attributed to bioactive plant constituents capable of interfering with microbial cell integrity and metabolic functions. These observations support the traditional medicinal use of A. occidentale in managing infections.

The antioxidant activity evaluated by the DPPH assay revealed strong free radical scavenging ability of the ethyl acetate extract. A marked reduction in absorbance values and high percentage inhibition indicated efficient neutralization of DPPH radicals. The antioxidant effect is likely related to phenolic compounds that donate electrons or hydrogen atoms to stabilize reactive species and reduce oxidative stress.

Taken together, the results suggest that Anacardium occidentale leaf extract exhibits multiple biological activities through complementary mechanisms, including antioxidant protection, antibacterial action, and control of intestinal disturbances. These findings highlight the plant as a promising natural source of therapeutic agents and provide scientific support for further phytochemical and pharmacological investigations.

## CONCLUSION

The present study confirms that Anacardium occidentale leaf extract exhibits notable antidiarrheal, antimicrobial, and antioxidant activities. The observed biological effects suggest the presence of active phytoconstituents with therapeutic potential. These findings support the plant as a promising natural source for the development of safe and effective plant-based pharmaceutical agents, warranting further detailed investigations.

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