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EVALUATION OF ANTIDIARRHEAL, ANTIMICROBIAL, AND ANTIOXIDANT ACTIVITIES OF ETHYL ACETATE EXTRACT FROM ANACARDIUM OCCIDENTALE LEAVES IN EXPERIMENTAL MODELS

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ABSTRACT

Anacardium occidentale, a member of the Anacardiaceae family, is a major source of anacardic acid, primarily obtained from cashew nut shell liquid (CNSL). Interest in this compound has grown due to its unique chemical structure, which combines a salicylic acid ring with an alkyl side chain, giving it important biological and physicochemical properties useful in medicine and industry. Research shows that anacardic acid possesses antibacterial, antioxidant, anti-inflammatory, anticancer, and antidiarrheal activities. Its antioxidant effect is linked to phenolic hydroxyl groups that neutralize free radicals, while its antibacterial action is believed to involve disruption of microbial metabolic pathways and cell membranes. The compound may also help treat diarrheal disorders by reducing inflammation and regulating intestinal secretion and motility. The biological activity of anacardic acid is influenced by factors such as extraction methods, storage conditions, and standardization. Antioxidant potential is commonly evaluated using DPPH, ABTS, and FRAP assays. The medicinal importance of the cashew plant is also supported by its long-standing use in traditional medicine, particularly in Ayurveda, where it is valued for promoting digestive health.

Keywords: *Anacardic acid, Anacardium occidentale, phenolic lipids, plant-derived bioactive molecules, antioxidant activity, antibacterial qualities, and antidiarrheal potential.*

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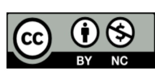

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INTRODUCTION

Anacardic acid is a bioactive compound present in the shells of cashews and other fruit varieties. Its uniqueness lies in its potential to act as both an anti-inflammatory and an antioxidant and is part of the salicylic acid group. One of the compounds that people seek when analysing their food ingredients, health supplements, and biological components is Anacardic acid. Owing to the presence of its long-chain alkyl group, it possesses a unique chemistry, which impacts the metabolism of the body. Modern molecular studies and Ayurvedic diet regimen will be discussed in detail. In this discussion, we will explore the use of foods high in Anacardic acid to promote Dosha balance, prevent Ama formation, and foster Agni [1].

Biological Source

The cashew tree, known scientifically as Anacardium occidentale L., belongs to the family of Anacardiaceae. Its primary objective is the production of cashew nuts. Several valuable parts of the tree, which offer high nutritional value and healing powers, include the seed, shell, fruit, bark, and leaves [2].

Plant Components That Are Useful

Several parts of the cashew tree are important for use in various industries, medical and nutritional purposes. Seeds contain lipids and proteins; therefore, they are consumed as edible nuts. Shell or pericarp provides CNSL (Cashew Nut Shell Liquid), which is required for some industrial purposes. Cashew apple can be found in many fermented drinks. Bark of the tree has always been in use in herbal medicine, while leaves are used for treating ailments due to their healing effects [3].

Anacardic acid is one compound of great interest to science because of its wide range of biological applications. There have been many studies carried out that show its properties as being antibacterial, antioxidative, anti-inflammatory, anticancer, and

antidiarrheal. The antibacterial effect of Anacardic acid is because of its inhibition of bacteria and fungi growth, while its antioxidative properties enable scavenging of free radicals. Therefore, Anacardic acid can be used in the prevention or treatment of some disease conditions involving microbial infection or oxidative stress [4].

In recent years, there has been an increased demand for natural substances extracted from plants that act as safe substitutes for pharmaceutical drugs. One such phytochemical that holds promise in pharmaceutical and biological fields is Anacardic acid. Hence, a detailed study of the biological properties of this compound is essential to evaluate its potential application as a natural drug [5].

The Anacardic acid belongs to the phenolic lipid family of biologically active substances that are characterized by having a hydrophobic hydrocarbon side chain bonded to a phenolic aromatic group. Structurally, it is a salicylic acid derivative and chemically referred to as 2-hydroxy-6-alkylbenzoic acid [6]. The lipophilicity is enhanced through the amphiphilic nature of the structure, which has the nonpolar alkyl end as well as the polar phenolic end. As a result of its hydrophobic nature, Anacardic acid shows poor solubility in water but very high solubility in organic compounds. This property helps in its easy diffusion in plant tissues that contain lipids, especially in structures that protect the plant from pathogens and herbivorous predators [7].

The cashew tree (Anacardium occidentale) also has a higher content of Anacardic acid in its fruit covering and shell gland oil compared to its edible seed. CNSL (cashew nut shell liquid) is one of the highest sources of Anacardic acid, estimated to have around 10–15% by weight of Anacardic acid. Moreover, traces of cashew have been detected in the peel tissue of mangoes (Mangifera indica), which implies a broader distribution in other members of the Anacardiaceae family. Other members of the Rhus genus (sumac) have traces of Anacardic acid, and their berries are commonly used as food seasonings [7].

Pharmacological Activity

The Anacardic acid possesses biological properties which have gained attention in the field of biomedical sciences recently due to its pharmaceutical value. The acid is capable of disrupting the membranes of microbes as well as inhibiting the activities of important enzymes, thus making it a powerful antibiotic and antifungal agent. It is also capable of acting as an antioxidant due to its phenolic nature [8].

In addition to this, Anacardic acid is said to offer anti-inflammatory properties through modification of inflammatory mediators and enzymes. There have been some promising results regarding its ability to control signalling pathways that are involved with oxidative stress and immune response. Also, based on a preliminary study, the chemical offers anti-cancer properties because it may inhibit uncontrolled growth of cells, leading to apoptosis [9].

Pharmaceutical properties:

In addition, recent studies show that Anacardic acid could possess anti-inflammatory properties through modulation of pro-inflammatory mediators and enzymes. This compound has been shown to be effective in the control of the pathway responsible for oxidative stress and immunity response. In addition, preliminary experiments indicate that the substance could have anti-cancer properties because it could stop uncontrolled cell growth and induce cell apoptosis in certain cells [10].

Taking everything into account, the various applications of Anacardic acid in industries as well as biology indicate that this compound is very important as a versatile organic material. In order to determine how it functions and what its potential medicinal applications are, further investigation is required.(9)

Antibacterial properties:

It was discovered through the experiments done in test tubes that the side chain containing three double bonds is the most effective in combating tooth cavity-causing Streptococcus mutans bacteria. The skin bacteria, cutis bacterium acnes, are insensitive to any amount of unsaturated bonds [11]. As per Eichbaum, the Gram-positive bacteria can be killed in 15 minutes by treating them with a solution of 1:200,000 ratio of Anacardic acid and waters or even a solution having 1:2,000,000 ratio.

The tuberculosis bacteria were eradicated after 30 minutes with somewhat higher ratios. These Anacardic acids change to alcohols (cardanols) when heat treated, and alcohols are less effective than acids. Most of the commercially produced oils are produced via decarboxylation, producing compounds that are least effective. In the Gold Coast area now known as Ghana, the leaves and bark of cashew trees are used to cure toothache by the native population [12].

Traditional Food Practices

The inclusion of Anacardic acid in traditional cooking practices involving the utilization of cashew nuts has been implicit since time immemorial, even though it had not been acknowledged as a separate compound before. Roasted cashew nut kernels are often used in curry dishes, gravy preparations, and chutney recipes along the Indian coast, and these preparations have a slightly astringent taste because of trace amounts of phenolic compounds found in the shells [13]. In Kerala and Odisha, indigenous tribes ferment cashew fruits to prepare vinegar-like beverages or alcoholic drinks. Minor phenolics, including lipid-soluble compounds related to Anacardic acid, may be accumulated or enhanced during fermentation. The same is true for traditional food preservation processes that are incorporated into regional cuisines utilizing the preparation of mango peels, especially those involved in the manufacture of chutneys in parts of western and southern India [14]. Extracts of mango peels possessing phenolic acids, particularly analogues of Anacardic acid, exhibit antibacterial activity that could potentially delay food spoilage, according to recent studies conducted in East Asia.

Therapeutic Effects and Pharmacological Evidence

A number of pharmacological activities mediated by Anacardic acid have been established recently via various in vivo and in vitro experiments. It has shown to possess antibacterial activities against pathogenic bacteria such as Candida albicans and Staphylococcus aureus at low concentrations [15]. Other evidence of its anti-inflammatory properties, such as reduction of edema and inflammatory processes in joints, can be found in the literature in regard to animal experiments. The substance has the potential to trigger programmed cell death in cancer cells, based on cellular studies. Pre-clinical studies using animal

models suggest that the substance may improve insulin resistance under high-fat diets [16].

Nevertheless, clinical evidence in humans is still not available. Although mild skin irritation was observed in some subjects, an experimental pilot study conducted in Brazil evaluated the effect of the substance applied topically in a formulation derived from cashew shell on the treatment of warts and demonstrated limited efficacy. The substance should be considered as an interesting experimental substance and not a medication since no large-scale clinical evidence of its systemic efficacy has been provided yet [17].

Anticancer and Enzyme-Modulating Activities

Anacardic acid is a biologically active molecule with anti-proliferative activity and is the focus of many studies. It holds promise as a lead molecule for medicinal purposes because it possesses the ability to interfere with the enzyme system responsible for inflammatory and tumour development processes [18]. Being structurally flexible, physiologically active analogues can be prepared.

Anti-Inflammatory and Antioxidant Properties

There has been considerable evidence that Anacardic acid possesses substantial antioxidant and anti-inflammatory activity in experiments on animals. According to studies, inflammation is lowered, pain sensation decreases, and inflammation mediators are decreased in animal models. The phenolic nature of the compound helps to reduce free radicals and manage oxidative stress. Moreover, antioxidants have been found in CNSL compounds in foods as well as in biological systems [17, 19].

METHODOLOGY

Collection

Fresh and healthy young leaves of Anacardium occidentale (cashew) were collected from confirmed plant sources and thoroughly cleaned to remove dust, soil particles, and other extraneous factors.

Drying of leaves:

These leaves were allowed to dry in shade at room temperature for approximately 15 days. Shade drying was particularly selected to minimize the destruction or alterations in the structure of phytoconstituents that can easily be thermally degraded either due to sunlight exposure or at high temperatures. This process was continued till there was no change in the weight, indicating that sufficient moisture had been removed.

Figure 01: Dried cashew leaf powder

Maceration

Leishman's reagent, about 130 ml in quantity, served as the extraction solvent for the initial extraction process. In order to ensure constant interaction between the plant material and the extraction agent, the plant powder was thoroughly mixed with the extraction solvent. The solution was allowed to stand for a while and was occasionally stirred so as to facilitate dissolution of the active compounds into the solvent layer. Coarse plant material was separated from the mixture using sterile muslin fabric immediately after the extraction process. Using Whatman filter paper, the filtrate was further purified to produce a clear extract.

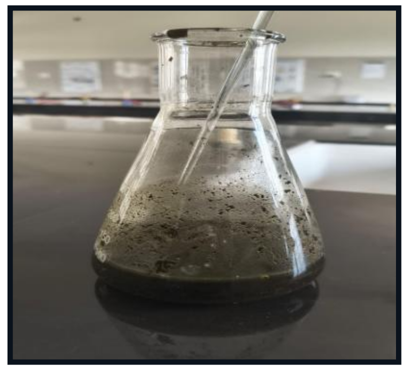


Figure 02: Maceration of leaf powder

Figure 03: Filtration of leaf extract

Extraction of Leaves:

For aiding in the phase separation during solvent partitioning, 25 mL of the extract that has been treated earlier was introduced to a separating funnel, and 50 mL of distilled water was added. To ensure uniformity of the solution, the contents of the funnel were agitated. Then, 25 mL of n-hexane was added to the contents of the funnel, which was then vigorously shaken and vented off from time to time for releasing any built-up pressure. Until the formation of two different layers, no intervention was made. After this, the top n-hexane layer was removed.

The aqueous layer was further processed with the use of other 25 mL portions of n-hexane until the solvent used up amounted to 100 mL in total volume.

Aqueous layer was continually subjected to further extraction by additional 25 mL of n-hexane up to the usage of a total amount of 100 mL of n-hexane to ensure maximum recovery of nonpolar components. The resulting n-hexane fractions were pooled together and kept for further processing.

The ethyl acetate layer was collected once well-separating layers became evident. Extraction was repeated by adding fresh 25 mL of ethyl acetate solution to extractors until the volume of the solvent reached 100 mL. The semi-polar fraction containing phytoconstituents of interest was obtained after pooling all the ethyl acetate layers. For complete removal of organic solvents from samples, all solvent fractions (including those obtained in the previous step), as well as all extracts, were evaporated to dryness under proper conditions. For dehydration purposes, dried residues were placed in a desiccator. Following solvent partitioning, the remaining mother liquor was further extracted using 25 millilitres of

chloroform to isolate other components that had intermediate polarity. A concentrated solution was prepared by carefully evaporating the extract to dryness and then redissolving the residue in 2.5 mL of Leishman's reagent. After the removal of contaminants by filtration, the mixture was gradually heated. Following solvent partitioning, the remaining mother liquor was further extracted with 25 mL of chloroform to isolate components of intermediate polarity. Then, the residue was diluted using two millilitres of methanol before placing it inside a desiccator packed with anhydrous desiccating agent, which consisted of a carbohydrate-based drying agent. This ensured that the extract was thoroughly dried.

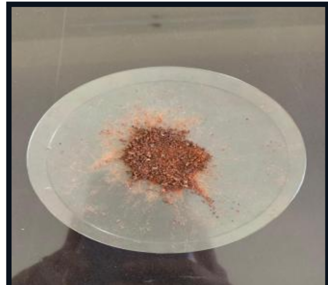


Figure 04: Anacardic acid drug leaf extract with ethyl acetate

Figure 05: Weighing of leaf extract

Antidiarrheal Activity

Castor Oil–Induced Diarrhoea Model

The potential use of the antidiarrheal properties of ethyl acetate leaf extract containing Anacardic acid was assessed through the castor oil-induced diarrhoea test, a common experimental technique for screening drugs with anti-diarrhoeal activity. Chicks weighing 30 to 60 g and in good health condition were used for the experiment. They were kept in a conventional laboratory setting wherein light, temperature, and humidity were controlled. Prior to the experiment, they were allowed access to water and were deprived of food to adapt them to the environment.

The anti-diarrhoeal activity of the ethyl acetate leaf extract of the Anacardic acid was determined through the castor oil-induced diarrhoea test, which is commonly used in testing the effectiveness of antidiarrheal drugs. Chicks aged three days with body weight ranging from 30 to 60 grams were used for the study. They were kept in a standard laboratory setting that maintained the normal environmental conditions, including temperature, light-dark cycle, and humidity. Prior to the experiments, they were allowed to have access to water and were deprived of food for an appropriate amount of time to acclimate to the laboratory setting.

To induce the diarrhoea in each subject, 0.75 millilitres of castor oil was given orally to each animal. Since the castor oil is known to cause diarrhoea through the increase in intestinal motility and secretion brought about by the formation of ricinolein acid in the intestine, it serves as a reliable model in determining antidiarrheal properties.

The number of instances of diarrhoea and when they began were monitored during the entire experiment. The faeces of each animal were analysed to see if there was any occurrence of diarrhoea and also the consistency of their stool. To estimate the amount of fluid that was being produced in the intestines and the degree of diarrhoea, the faeces moisture content was determined.

Figure 06: Young chicks

To achieve uniform distribution and reduce experimental bias, the animals were randomly assigned to six groups of five animals each for experimental evaluation.

- **Group I:** Vehicle Control: In order to establish a baseline diarrheal reaction, the animals in this group were given just the vehicle.
- **Group II:** Standard Treatment: Loperamide, the standard antidiarrheal medication because of its well-known inhibitory impact on intestinal motility and secretion, was administered to the animals at a dose of 10 mg/kg body weight (p.o.).
- **Group III:** Low-Dose Treatment: Animals were given an oral dose of 150 mg/kg body weight of ethyl acetate leaf extract containing anacardic acid**.**
- **Group IV:** High-dosage Treatment: To assess dosage-dependent antidiarrheal activity, animals were given the ethyl acetate leaf extract containing anacardic acid at a higher dose of 300 mg/kg body weight.

Figure 07: Groups of young chicks

Figure 08: Castor oil injected into chicks

The effectiveness of the treatments was determined by comparing the frequency of diarrheal stools, onset time, and faecal water content between the experimental groups. By comparing the percentage inhibition of diarrhoea to the vehicle control group, the preventive effect of the extract was assessed.

RESULTS

Table 01: Effect of Treatment on Castor Oil–Induced Diarrhoea in Young Chicks

Group	Treatment	Dose (mg/kg)	Castor Oil Administered (ml)	Onset of Diarrhoea (min) (Mean ± SEM)	Number of Wet Faeces (Mean ± SEM)	Severity of Diarrhoea	Inhibition of Diarrhoea (%)
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

With a high number of wet faeces (12.6 ± 0.8) and onset time of 28.4 ± 1.2 min, the results indicated that castor oil induced diarrhoea significantly in the vehicle control group. Delaying the onset of diarrhoea and decreased number of faecal output by administration of Loperamide drug, there was a percentage inhibition of 74.6%. There were dose dependent antidiarrheal effects shown by ethyl acetate leaf extract. Though the low dose (150 mg/kg) offered moderate protection with a percentage inhibition of 41.3%, the high dose (300 mg/kg) offered high effectiveness with a percentage inhibition of 61.1%.

Antimicrobial Activity

Agar plate preparation

The nutrient agar plates were prepared according to established microbiology protocol under aseptic conditions. The melted nutrient agar medium was poured into sterile petri dishes and left to cool down to room temperature. Once the nutrient agar cooled down, uniform holes were made using a sterile cork borer, which would enable inoculating of samples and controls.

Test Extract Preparation

The stock solution of the n-hexane extract was prepared by dissolving it in analytical grade n-hexane. The required concentration levels were obtained by serial dilution of this stock solution. The working concentrations of 0.001, 0.010, and 0.1 (w/v) were prepared in a total volume of 5 mL using n-hexane. In a sterile environment, standard bacterial slurry of Escherichia coli was applied uniformly to the agar medium to create an even bacterial lawn. There were four wells on each plate; three wells were filled with varying amounts of the extract, while the fourth well was used as the negative control with the solvent only.

Assay for Agar Well Diffusion:

For assessing the antibacterial effect, the agar well diffusion technique was used. The culture of E. coli was inoculated evenly onto the agar plates by applying the culture onto them with a sterile brush, which allowed for uniform distribution of bacteria on the medium. Following that, holes were created in the medium by using the sterile cork borer. Wells were filled with different concentrations of the drug extract (0.001, 0.005, and 0.015 ml). Chloroform was used as the only solvent in one well.

Antibacterial Activity Assessment and Incubation:

The plates containing the inoculums were incubated for 24-48 hours at a temperature of 37 degrees centigrade under laboratory conditions that are ideal. The activity of the antibiotic was evaluated based on the measurement of the inhibition zone formed around the wells upon incubation. The inhibition zone measured was used as an index of the effectiveness of the bacterial activity of the extract.

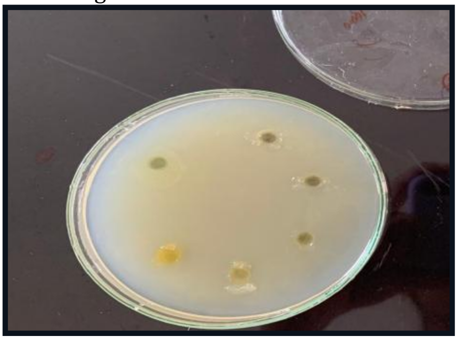
Drug Extract Preparation:

The chemical extract was extracted using chloroform as the extraction solvent. The crude material was dissolved in pure chloroform, resulting in a stock solution. The stock solution was further diluted into three different solutions by serial dilution technique. The

concentrations obtained were 0.001, 0.005, and 0.015 (w/v), with chloroform as the solvent, to obtain a total volume of 5 mL. All the procedures undertaken were carried out under aseptic and sterile conditions to avoid any microbial contamination.

Preparing Microbial Cultures:

The organism chosen for testing the antibacterial activity was a pure culture of E. coli. The bacteria were grown on nutrient agar medium to produce an active culture before the experiment. Nutrient agar plates were made using standard microbiological procedures and left to solidify inside the laboratory before being inoculated. The new cultures obtained were utilized to guarantee reliable bacterial growth during the test.



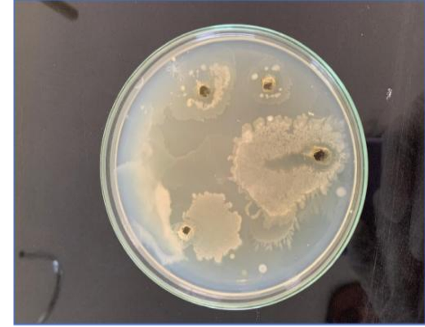


Figure 09: Sample and E. coli inoculation in agar broth wells

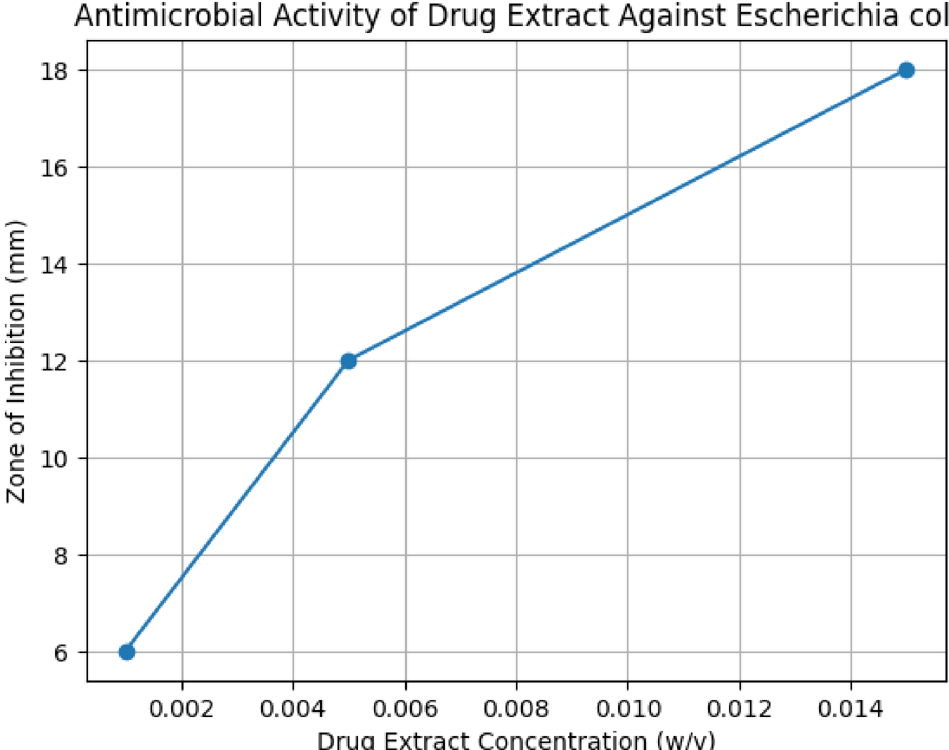


Figure 10: Inhibition of bacterial growth

The antibacterial effect of the extract of the drug on Escherichia coli showed a very concentration-dependent nature, as shown by the graph. With increasing concentrations of the extract, the inhibition zone steadily grew larger, indicating increasing antibacterial efficacy. At the minimum concentration (0.001 w/v), an inhibition zone of roughly 6mm was formed, implying moderate antibacterial action. An increase in the concentration to 0.005 w/v saw the formation of a much bigger inhibition zone of about 12 mm, implying better suppression of bacterial growth. An even larger inhibition zone exceeding 18 mm was formed with maximum concentration (0.015 w/v), implying the greatest antibacterial effect.

Antioxidant Activity

Assay for DPPH Radical Scavenging:

The antioxidant capacity of the ethyl acetate fraction was examined through the DPPH free radical scavenging assay. This particular experiment takes advantage of the reduction process in the stable DPPH free radical, which possesses a deep purplish hue when dissolved in solution. With the presence of antioxidants that can potentially donate hydrogen or electron components, there will be reduction in DPPH to diphenyl picrylhydrazine, resulting in a colour change from purple to yellow.

Materials

The assay's components included DPPH (molecular weight 394.32 g/mol), methanol, ethyl acetate extract, distilled water, and a UV-visible spectrophotometer for absorbance measurement.

Extract Solution Preparation:

The volume of ethyl acetate extract needed should then be diluted in thirty millilitres of distilled water to form the extract solution. The mixture was sonicated for about ten minutes at a temperature of fifty degrees centigrade to ensure complete dissolution of the phytochemicals. The supernatant fluid was collected after filtration of the insoluble components from the solution.

Serial Dilution Preparation:

- Concentration-dependent antioxidant activity was determined through serial dilution of the extract.
- FIRST DILUTION – Distilled water was added into a volumetric flask measuring 100 mL with ten mL of the extract solution inside it for dilution.
- SECOND DILUTION – One mL of the first dilution was mixed with ten mL of distilled water.
- THIRD DILUTION – One mL of the second dilution was mixed with twenty mL of distilled water.

DPPH Solution Preparation:

Preparation of DPPH Solution

DPPH was dissolved in 100 mL of methanol at a concentration of 0.01 g/100 mL. The solution was shielded from light for 30 minutes to allow for full decomposition and stabilization of the radical.

Reaction Mixture Preparation:

Each dilution was combined using reaction mixtures consisting of 1.6 mL of DPPH and 2.6 mL of the corresponding sample dilution. The reactions were appropriately mixed and reacted under dark conditions before analysis.

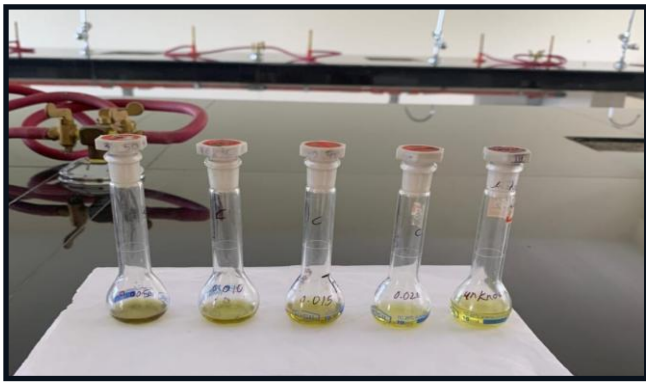


Figure 11: Dilutions of leaf extract

Measurement of Absorbance

For every dilution, a reaction mixture was made by mixing 1.6 mL of DPPH solution with 2.6 mL of the corresponding sample dilution. The mixes were properly mixed and allowed to react in the dark prior to examination.

Observations:

The absorbance values obtained for different sample dilutions are presented below:

Table 02: Observations of dil. samples

Sample	Absorbance
First dilution	0.108
Second dilution	0.021
Third dilution	0.011
Methanol (blank)	0.004
DPPH control	0.592

DPPH Activity Calculation

- The percentage suppression of DPPH radicals was calculated using the procedure below.

  ($A_0/A_1 \times 100$ = Percentage Inhibition (%), where A_0 (0.592) represents the absorbance of the DPPH control solution.
- A_1 represents the absorbance of the test sample at the relevant concentration.

The calculated percentage inhibition values were as follows: First dilution inhibition was 81.75%; second dilution inhibition was 96.45%; and third dilution inhibition was 98.14%. The results demonstrated the extract's strong antioxidant efficacy by showing a consistent increase in radical scavenging activity with dilution.

Table 03: DPPH Antioxidant Activity

Sample	Absorbance (A_1)	Control Absorbance (A_0)	% Inhibition (DPPH Scavenging Activity)
DPPH Control	0.592	0.592	—

Sample	Absorbance (A_1)	Control Absorbance (A_0)	% Inhibition (DPPH Scavenging Activity)
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%

The extract's antioxidant activity was evaluated using the DPPH radical scavenging assay; the results are shown in Table X. The DPPH control's high absorbance value (0.592) demonstrated the maximum radical intensity in the absence of antioxidant compounds, while the blank, methanol, showed very low absorbance. The investigated extract demonstrated strong free radical scavenging action at all dosages. The first dilution showed a significant drop in absorbance (0.108), suggesting an 81.75% suppression of DPPH radicals. Further dilution increased the antioxidant effect; the second dilution resulted in 96.45% inhibition and a much lower absorbance value (0.021). The third dilution had the highest antioxidant effectiveness, with a minimal absorbance of 0.011 and an inhibition of 98.14%. The progressive decrease in absorbance values and corresponding rise in % inhibition show the extract's considerable radical scavenging activity overall, suggesting the presence of potent antioxidant components that may neutralize free radicals.

DISCUSSION

In an attempt to investigate the biological potential of Anacardium occidentale leaf extract with regard to its antimicrobial, anti-diarrhoea and antioxidant characteristics, the following experiments were conducted. The results have confirmed the presence of a pharmacological effect of Anacardium occidentale leaf extract; this might explain why traditional medicine has relied on it for years. This effect might be due to the existence of some phytochemicals including but not limited to flavonoids, phenols, and Anacardic acid derivatives. In the case of the experiment on the antidiarrheal effect of the extract, a protective effect against castor oil induced diarrhoea was clearly observed. It is noteworthy that castor oil causes diarrhoea as it triggers the production of ricin oleic acid in order to increase motility and secretions from the intestine. In this case, the treatment with Anacardium occidentale leaf extract delayed the onset of diarrhoea in comparison with the control group and reduced the number of wet faeces. This means that the higher the dosage of the extract, the better the effect achieved. Higher dosages seem to result in effects similar to those of loperamide in terms of regulating intestinal motility and secretion. The antibacterial effect was exerted against Escherichia coli.This behaviour may result from the phytochemicals found in the extract and their interference with the metabolism of bacteria by interfering with their cell walls. The antioxidant activity was determined by the DPPH test, and the result was a strong radical scavenging action by the extract. The antioxidant activity of the extract may be attributed to the presence of phenolic acids because of their reducing nature. In all aspects, the extract displayed various bioactivities whose combined effects would yield medical benefits. However, further research is necessary before its use.

CONCLUSION

There is sufficient evidence to prove the high degree of biological activity of the leaf extract of the Anacardium occidentale, which is indicated by its antibacterial, antidiarrheal, and antioxidant characteristics. It can be proven by the fact that the pharmacological activity of the substance along with the possibility to use it as a source of different biological activities can be shown by the results from all experiments. Such activity may be determined by different phytochemicals such as phenolic compounds, flavonoids, and derivatives of Anacardic acid. As to the antidiarrheal experiment, it is apparent that the reduction of the incidence rate and symptoms of diarrhoea suggests the ability of the substance to regulate motility and fluid secretion in the intestine. Thus, the substance has the ability to maintain the gastrointestinal system, preventing the issues related to diarrhoea. The antimicrobial investigation proved the extract's inhibitory activity against pathogenic bacteria and its effectiveness in limiting bacterial growth. The antibacterial properties highlight the usage of A. occidentale for the treatment of diseases based on the possibility that the bioactive agents might affect microbial cellular structures and metabolic functions. With the growing antibiotic resistance, these characteristics underscore the significance of plant-based substances as prospective candidates for the production of novel antimicrobial drugs.

The remarkable antioxidant activity demonstrated through the DPPH test indicates the extract's efficiency in alleviating oxidative stress and scavenging free radicals. The capability of scavenging reactive oxygen species can enhance the therapeutic benefits of the plant while ensuring that there is no cellular damage within the biological system.

Anacardium occidentale leaf extracts have been identified as a promising natural medicine resource that can be used in different medical conditions. The presence of multiple properties, including antibacterial, antidiarrheal, and antioxidant, implies a synergic mechanism, which increases the importance of this natural product in the treatment of various diseases. Therefore, this research justifies the application of Anacardium occidentale leaf extracts as medicine and confirms that this natural product can be considered an alternative to creating pharmaceutical products based on natural resources.

However, more investigations should be carried out to reveal the medical opportunities of Anacardium occidentale leaves, despite the fact that this research provides valuable

information about its properties. Phytochemical analysis, chemical components separation, pharmacological and toxicological tests, and clinical trials should be conducted to verify the effectiveness and safety of this product in clinical settings. In this way, future investigations may facilitate the development of innovative medications created using natural resources, such as Anacardium occidentale leaves.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Informed Consent

Not applicable, as the study did not involve human participants.

ETHICAL STATEMENT

All experimental procedures involving animals were carried out according to standard laboratory ethical guidelines and institutional regulations.

AUTHOR CONTRIBUTION

Narayanamma D. L. Degala contributed to the conceptualization, supervision, and manuscript preparation of the study, while Narendra Devanaboina provided research guidance and was involved in the review and editing of the manuscript. Brunda Devi Kaki, Sravya Gurrani, Navya Sahithi Guttula, and Akshaya Kandukuri participated in the experimental work, data collection, data analysis, and literature review.

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