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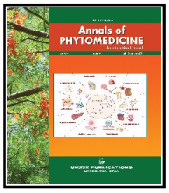

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Exploring the phytochemical composition and medicinal benefits of *Semicarpus anacardium* L. nut extract to combat bacterial infections and reduce pain

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Abstract

This study explores the pharmacological characteristics and phytochemical makeup of a hydroethanolic extract derived from *Semicarpus anacardium* L. nuts. Phytochemical investigation exposed the occurrence of carbohydrates, alkaloids, flavonoids, glycosides, proteins, triterpenoids, phenolic compounds, and amino acids. The antimicrobial activity was assessed by agar well diffusion assay, anti-inflammatory activity by carrageenan-induced foot edema model in rats, anti-nociceptive action by hot plate technique, acetic acid induced writhing test in mice. Antimicrobial activity was assessed against 5 different microbes such as *E. coli*, methicillin-resistant *S. aureus*, vancomycin-resistant *E. faecalis*, MDR-*E.coli*, and MDR-*P. aeruginosa*. The extract showed a significant dosage-dependent antimicrobial activity against all microbes but superior action against methicillin-resistant *S. aureus* with zone of inhibition 20.1 mm. The extract showed significant (p>0.01) anti-inflammatory activity, anti-nociceptive properties at dosages of 200 and 400 mg/kg, with higher dosage showing maximal efficacy. These findings underscore the potential of *S. anacardium* nut extract as a therapeutic agent for managing pain, inflammatory conditions, and bacterial infections.

1. Introduction

Semicarpus anacardium L. (SA), commonly known as the marking nut tree or bhilawa, is a member of the Anacardiaceae family (Jain and Sharma, 2013). This family encompasses a diverse group of flowering plants, including trees, shrubs, and vines, distributed in humid and semitropical provinces globally. SA is notable for its significance in traditional medicine systems, where various parts of the plant are utilized for their therapeutic properties. Additionally, the tree holds cultural and economic importance, with its nuts often used in dyeing, tanning, and even as ink (Patel and Patel, 2023). The botanical characteristics and medicinal attributes of SA render it a subject of interest for botanical, pharmacological, and ethnobotanical studies.

The SA nut extracts encompasses a rich array of phytochemical components, such as alkaloids, tannins, flavonoids, and phenolic compounds (Kupeli *et al*., 2002). These phytoconstituents have been attributed to numerous biological actions, including antibacterial, anti-inflammatory, antioxidant (Barman *et al*., 2013), and analgesic effects (Cunha *et al*., 2024). Understanding the phytochemical composition of SA nut extracts provides insights into their potential therapeutic applications in combating bacterial infections and alleviating pain-related symptoms (Lingaraju *et al*., 2011). Bacterial infections pose a significant public health challenge globally, with the emergence of antibiotic-resistant strains exacerbating the problem. The search for alternative antimicrobial agents has led to renewed interest in natural products, including plant-derived compounds (Sethumathi *et al*., 2021). SA nut extracts have shown promising antibacterial action towards extensive variety of pathogenic bacteria, making them possible applicant intended for the development of novel antimicrobial agents (Prakash *et al*., 2013).

In adding to their antimicrobial properties, SA nut extracts exhibit analgesic effects, offering relief from pain and discomfort associated with various health conditions (Nikam, 2022). Pain management is a critical aspect of healthcare, and the identification of natural remedies with analgesic properties holds significant therapeutic implications (Mishra *et al*., 2024). These research goals to comprehensively explore the phytochemical profile of SA nut extracts and evaluate their therapeutic potential in combating bacterial infections and alleviating pain. By elucidating the phytochemical constituents and biological activities of SA nut extracts, this research contributes significantly to the expanding field of natural remedies for infectious diseases and pain management (Nisa *et al*., 2024).

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2. Materials and Methods

2.1 Procurement and authentication of SA nuts

The acquisition and authentication process of SA nuts (Figure 1) were procured from local suppliers in Erode, Tamil Nadu, India. The *S. anacardium* nuts was identified by Joint Director, Scientist, C-I/C, Botanical Survey of India, Tamil Nadu Agricultural University Campus, Coimbatore, bears the Reference Number BSI/SRC/5/22/23-24/Tech. 7015. The Voucher Specimen No. 7015 was deposited in the herbarium for future reference.

Figure 1: ***Semicarpus anacardium*** **L. nut.**

2.2 Preparation of extract

The SA nuts were meticulously cleaned and dried to remove any dirt or impurities. Once dried, the nuts were finely ground into a powder using a grinder to increase the surface area of the plant material, facilitating efficient extraction. The SA nut powder was extracted by Soxhlet apparatus, using ethanol and water in a ratio of 70:30. The filtrate containing the extracted compounds was then concentrated to remove the solvent and obtain a more concentrated extract. This concentration process was achieved through evaporation at a temperature of 40-50°C. Finally, the hydroethanolic SA extract (HESA) was dried to remove any residual moisture, resulting in a dry powder or semi-solid extract suitable for pharmacological studies.

2.3 Phytochemical investigation

2.3.1 Test for carbohydrates

5 cc of water and a few drops of HESA extract were combined, and then filtered. After treating the filtrate with a tiny amount of α-napthol (20% in ethyl alcohol), 1 ml of concentrated H_2SO_4 was added laterally along the sides of an inclined test tube. The violet-colored ring in the interface indicated the occurrence of carbohydrates (Kokate, 2002).

2.3.2 Test for glycosides and anthraquinones

Hydrochloric acid was used to hydrolyze the HESA extract in a water bath. Following the hydrolysate's extraction with benzene, the layer that had developed above was treated with diluted ammonia. The development of a reddish-pink tinge suggested the occurrence of anthraquinones and glycosides. Furthermore, the extract was dissolved in pyridine for the Legal test, and freshly made sodium nitroprusside was added after a little amount of NaOH (10%) was added. The blue color indicated the glycosides.

2.3.3 Test for flavonoids

The HESA extract was added to a filter paper strips, ammoniated, and detected for a color change as of white to yellow, indicating the presence of flavonoids (Pavithra *et al*., 2023).

2.3.4 Test for tannins and phenolics

Three portions of the HESA extract were combined with water. One part was treated with 10% Sodium chloride, second part with 1% gelatin and last part with gelatin salt reagent. Precipitate formation in any part indicated the presence of tannins. Positive tests were further confirmed by adding a small amount of 1% ferric chloride, resulting in blue to green-black coloration indicated phenolic compound (Sethumathi *et al*., 2021).

2.3.5 Test for amino acid and proteins

Few drops of the HESA extract has been mixed with water and filtered. Adding few drops of copper sulphate (0.002%) to the ammoniated alkaline filtrate and observing the presence of a red or violet color. Millon's test involved adding 5-6 drops of Millon's reagent to 2 ml of filtrate and observing the formation of red precipitates. The Ninhydrin test and Xanthoprotein test were also performed to detect proteins and amino acids.

2.3.6 Test for triterpenes and sterols

Alcoholic potassium hydroxide served to treat the HESA extract until saponification was achieved. Diethyl ether was utilized to remove the unsaponifiable material. Salkowski's reaction and Libermann-Buchard's reagent were applied to the ether soluble residue in order to identify any sterols or triterpenes.

2.4 Antibacterial study

2.4.1 Selection of bacterial strains

Five multidrug-resistant bacterial strains were preferred for the research due to their clinical significance and resistance profiles. The strains were collected from Microbiology Laboratory at Nandha Medical College and Hospital, *Escherichia coli* (*E. coli*), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), multidrug-resistant (MDR) *Pseudomonas aeruginosa*, and another MDR *E.coli* strain.

2.4.2 Storage and maintenance of bacterial strains

The bacterial strains were stored in sterile cryovials containing nutrient broth supplemented with 15 % glycerol at -80°C. Before each experiment, bacterial strains were retrieved from cryostorage and subcultures were prepared on nutrient agar plates using aseptic techniques to avoid contamination. These plates were incubated for 24 h at 37°C, after which single colonies were picked for inoculum preparation.

2.4.3 Preparation of bacterial inoculum

Overnight cultures of the selected bacterial strains were prepared in sterile nutrient broth and calibrated to turbidity comparable to 0.5 McFarland standards.

2.4.4 Agar well diffusion assay

The different strains of bacteria inoculated in a nutrient agar plates. Wells created in the agar with the aid of a borer, and the nut extract at concentrations of 200 and 400 mg was added to the wells (Mohan Kumar *et al*., 2023). The agar plates were incubated for 24 h at 37°C. Following incubation, the plates were examined for the presence of distinct zones, which indicated the inhibition of bacterial growth. A ruler was used to measure the zone of inhibition. After incubation, the plates were detected for the occurrence of clear zones, demonstrating bacterial growth inhibition. The zone of inhibition was measured using a calibrated ruler.

2.5 Pharmacological studies

2.5.1 Animals and husbandry

The Wistar rats (150-200 g) and Swiss albino mice (20-25 g) were taken through an animal husbandry of Nandha College of Pharmacy in Erode, Tamil Nadu, India. Before the study's inquiry began, the laboratory animals were given a seven day acclimatization period. The animals were housed in cages made of polypropylene and were given husk bedding. Housing conditions were kept at 24 ± 2ºC with a comparative moistness range of 30-70%. A 12/12 h lightcycle was maintained throughout the experimental period. The animals were fed commercial pellet chow and were allowed unlimited access to water during the research period.

2.5.2 Ethical approval

The Institutional Animal Ethics Committee (IAEC) of Nandha College of Pharmacy approved the investigative procedure (Approval No: 688/21/C-CPCSEA). Guidelines from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India, were followed in conducting the research.

2.5.3 Anti-inflammatory study (Carrageenan-induced foot edema method)

The carrageenan-induced foot edema model in rats was used to assess the anti-inflammatory properties of HESA extract. Four groups of rats were set up. Saline (10 ml/kg p.o.) was administered to the control group. 10 mg/kg of indomethacin was given orally to Group II. Groups III and IV received dosage of 200 and 400 mg/kg of HESA extract, respectively. After 1 h, rats treated with 0.1 ml of carrageenan (1%) in right foot by subplantar injection. Foot thickness recorded in different time intervals such as 0, 1, 2, 3, and 4 h using digital vernier calipers. The following formula was used to determine the percentage inhibition of inflammation.:

$$\% \text{ Inhibition} = 1 - (dt/dc) \times 100$$

where, "dt" represents a variation in foot thickness in the HESA-treated group and "dc" represents the variation in foot thickness in the control group (Manjuparkavi *et al*., 2022).

2.5.4 Eddy's hot plate technique

Swiss albino mice were used to test the analgesic effect of HESE extract using the hot plate method. There were four groups of mice such as Group I served as control animals, Group II mice treated with pentazocine at a dosage of 3 mg/kg intraperitoneally, Groups III and IV mice ingested HESE extract at a dose of 200 and 400 mg// kg, respectively. The temperature of the hot plate was kept at 55 ± 2ºC. Response duration of mice was noticed at various time intervals after treatment (Gurudeeban *et al*., 2014).

2.5.5 Acetic acid-induced writhing response

HESA extract's peripheral pain relief was assessed in mice by inducing writhing reaction *via* acetic acid. There were four groups of mice, Group I control animals, Group II mice were given the conventional medication diclofenac sodium with a dosage of 10 mg/kg orally, Group III and Group IV mice were given HESE extract with a dose of 200 and 400 mg//kg, respectively. Mice were given 0.1 ml of acetic acid (0.7% v/v) intraperitoneally to cause writhing, and a count of writhes was noticed for 10 min after the injection (Srinivasan *et al*., 2003). The following formula was used to determine the percentage of protection:

% Inhibition = [Numbers of writhes (control) – Numbers of writhes (HESE)] /Count (control) × 100 writhes

2.5.6 Statistical analysis

The data were expressed as mean ± SEM of six animals in each group. The statistical analysis was carried out using one-way ANOVA followed by Dunnett's-test. The difference in values at $p<0.05$ was considered as statistically significant.

3. Results

3.1 Phytochemical investigation

Phytochemical investigation revealed HESA extract exposed the occurrence of various constituents, including carbohydrates, phenolic compounds, alkaloids, glycosides, proteins, amino acids, flavonoids, and triterpenoids.

3.2 Antibacterial study

We assessed the HESA's antibacterial efficacy against five bacterial strains that were resistant to multiple drugs. The extract showed dose-dependent inhibition of bacterial development, as proved by the appearance of clear zones of inhibition around the wells in the agar well diffusion assay. The HESA (200 and 400 mg/kg) extract demonstrated varying degrees of inhibition against all tested bacterial strains. Comparison with standard antibiotics, *viz*., ciprofloxacin 5 µg, ampicillin 10 µg, vancomycin 30 µg, and ceftriaxone 30 µg, respectively, revealed a noteworthy zone of inhibition values for the HESA nut extract, indicating its potential as an effective antimicrobial agent against multidrug-resistant bacteria. Detailed results are summarized in Table 1.

Table 1: Zone of inhibition in mm of the HESA alongside standard antibiotics

Bacterial strain	Standard antibiotics	200 mg HESA (mm)	400 mg HESA (mm)	Standard antibiotics (mm)
E. coli	Ciprofloxacin (5 µg)	14.5 ± 0.8	18.2 ± 1.1	14.3 ± 1.0
Methicillin-resistant *S. aureus* (MRSA)	Ampicillin (10 µg)	16.3 ± 1.2	20.1 ± 0.9	No inhibition
Vancomycin-resistant *E. faecalis* (VRE)	Vancomycin (30 µg)	12.8 ± 0.6	15.5 ± 0.7	No inhibition
MDR-*P. aeruginosa*	Ceftriaxone (30 µg)	13.7 ± 0.9	17.6 ± 1.0	No inhibition
MDR-*E. coli* strain	Ciprofloxacin (5 µg)	11.9 ± 0.5	14.8 ± 0.8	No inhibition

3.3 Anti-inflammatory activity

The HESA extract's anti-inflammatory effects on carrageenan-induced foot edema are summarized in Figure 2. HESA extract administered with a low and high dose (200 and 400 mg/kg) demonstrated significant (p<0.01) inhibition against inflammation produced by carrageenan. The HESA extract with low and high dosage revealed inhibition of 30% and 37%, respectively, after 3 h. The standard drug indomethacin showed 44 % of anti-inflammatory activity superior to HESA extract. These findings indicate the HESA extract's showed an anti-inflammatory agent against carrageenan-induced foot edema in rats (Bandigari and Dongamanti, 2023).

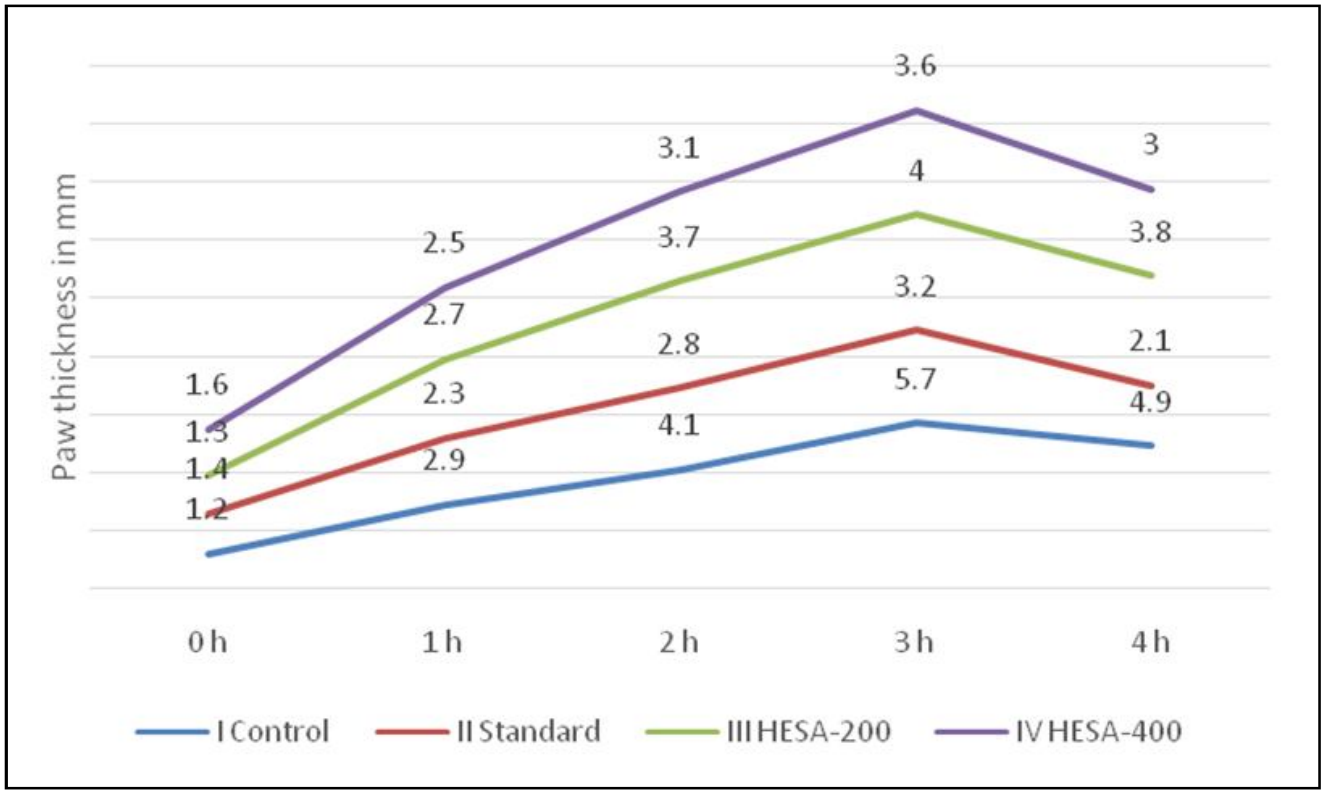

Figure 2: Anti-inflammatory activity of HESA on carrageenan induced paw edema method in Wistar rats.

3.4 Analgesic activity hot plate technique

Swiss albino mice were used to test the antinociceptive effect of the HESA using the hot plate technique and the results are presented in Table 2. When HESA extract was given orally at 200 and 400 mg/kg, there was a noticeable analgesic effect as compared to the control group. The highest analgesic effect was found at 400 mg/kg, and the reaction time at 90 min was 5.4 ± 0.35, which was less than the reference treatment pentazocine (6.8 ± 0.54).

Table 2: Analgesic effect of HESA on hot plate test in Swiss albino mice

Groups	Paw licking or jumping in seconds			
	After 30 min	After 60 min	After 90 min	After 120 min
Group I Control	2.4 ± 0.24	2.2 ± 0.18	2.5 ± 0.16	2.6 ± 0.12
Group II Pentazocine (3 mg/kg)	2.9 ± 0.18	5.9 ± 0.42**	6.8 ± 0.54**	7.9 ± 0.28**
Group III (200 mg/kg)	2.5 ± 0.26	3.4 ± 0.14*	4.2 ± 0.28**	4.1 ± 0.34**
Group IV (400 mg/kg)	2.8 ± 0.14	4.8 ± 0.36**	5.4 ± 0.35**	5.2 ± 0.42**

Values were mean ± SEM, (n=6), *p<0.05 **p<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnet's test.

3.5 Acetic acid-induced writhing response in mice

The results of the analgesic activity of HESA were shown in Table 3. The intraperitoneal administration of acetic acid into control mice formed 47.4 ± 3.8 writhes. Pre-treatment with HESA at the dose of 200 and 400 mg/kg decreased the number of writhes to 38.1 ± 2.8 (19.62% protection) and 32.4 ± 1.6 (31.64% protection), respectively. Among the two doses, 200 and 400 mg/kg showed slightly lower analgesic activity compared to the standard drug diclofenac sodium, which exhibited 23.5 ± 2.4 writhes (50.42% protection). Additionally, pre-treatment with the HESA extract delayed the onset of writhing and shortened the duration of writhing. These findings suggest the potential of HESA as an analgesic agent against acetic acid-induced pain in mice.

Table 3: Analgesic effects of HESA on acetic acid writhing test in Swiss albino mice

Groups	Number of writhes	% Inhibition
Group I Control	47.4 ± 3.8	-
Group II Diclofenac Sodium (10 mg/kg)	23.5 ± 2.4**	50.42
Group III (200 mg/kg)	38.1 ± 2.8**	19.62
Group IV (400 mg/kg)	32.4 ± 1.6**	31.64

Values were mean ± SEM, (n=6), **p<0.01 Vs control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

4. Discussion

In the current research, HESA extracts were evaluated for their antimicrobial, anti-inflammatory, central and peripheral analgesic activity by using suitable animal models. Phytochemical screening of the extract showed the presence of carbohydrates, phenolic compounds, alkaloids, glycosides, proteins, amino acids, flavonoids, and triterpenoids. The HESA extract showed *in vitro* bactericidal activity against three Gram-negative strains (*E. coli,* MDR-*E.coli,* MDR-*P. aeruginosa)* and two Gram-positive strains (*S. aureus,* vancomycin-resistant *E. faecalis),*which commonly implicated in clinical infections.

Plant extracts inhibit microbial enzymes, blocking critical processes like DNA replication, protein synthesis, and energy metabolism. Phenolic compounds like tannins and flavonoids can denature microbial proteins or inhibit key enzymes involved in energy production (*e.g.*, ATP synthase), leading to the loss of metabolic activity and cell death (Farhadi *et al*., 2019). Previous studies suggested that the herbal extract can disrupt microbial DNA and RNA synthesis, either by directly interacting with nucleic acids or inhibiting enzymes involved in replication and transcription (Yan *et al*., 2021). Alkaloids are important group of compounds possess antibacterial properties and many research have reported that they can play a significant role in treating infectious diseases. Their mechanism of action might be due to the enzymatic alterations affecting physiological processes, including inhibition of DNA synthesis and repair mechanisms by intercalating nucleic acids (Mithofer and Boland, 2012).

The current study correlates with the previous study that the alkaloids like berberine present in the HESA extract bind to microbial DNA, preventing its replication and transcription, while flavonoids in the extract can inhibit DNA gyrase, an enzyme required for DNA supercoiling in bacteria. Efflux pumps are used by microbes to expel toxic substances (including antibiotics) from their cells. Some plant compounds inhibit these pumps, enhancing the susceptibility of microbes to antimicrobial agents (Khameneh *et al*., 2021).

The outcomes of the present study corroborate those reported in previous research that flavonoids like quercetin inhibit bacterial efflux pumps, increasing the retention of toxic compounds within the microbial cell (Purushothaman *et al*., 2017; Salehi *et al*., 2020). This suggests the therapeutic potential of *S. anacardium* as an alternative medicine for combating antibiotic-resistant infections, thereby addressing the growing concern of antimicrobial resistance. Furthermore, the anti-inflammatory and anti-nociceptive activities of the HESA extract were assessed using established animal models. The observed reduction in paw edema and pain response substantiates the traditional use of *S. anacardium* in alleviating inflammatory conditions and pain (Ramprasath *et al*., 2006; Bandigari *et al*., 2023). These results showed the pharmacological significance of *S. anacardium* in managing inflammatory disorders and pain syndromes. Plant extracts often contain bioactive molecules such as flavonoids, terpenoids, alkaloids, and phenolic acids, which contribute to their anti-inflammatory effects. Many plant compounds contains flavonoids like quercetin which inhibit enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), which are involved in the synthesis of pro-inflammatory mediators such as prostaglandins and leukotrienes (Malhotra *et al*., 2001; Upreti *et al*., 2016).

The findings of the study demonstrate that the polyphenols in herbal extracts can downregulate the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). Alkaloids like berberine in the extracts can modulate immune cell activity, including macrophages, T-cells, and neutrophils, which play a role in the inflammatory response (Costa *et al*., 2016).

Tannins in plant compounds stabilize lysosomal membranes, preventing the release of pro-inflammatory enzymes from lysosomes, thus reducing inflammation.Voltage-gated sodium (Na^+) and calcium (Ca^{2+}) channels play a key role in the transmission of pain signals along nerves. Some plant extracts inhibit these channels, reducing the excitability of pain pathways. Alkaloids like berberine can block Na^+ and Ca^{2+} channels, decreasing the transmission of pain signals (Khan *et al.*, 2022).

Nitric oxide is a signaling molecule that contributes to pain sensation and inflammation. Some plant extracts inhibit the enzyme nitric oxide synthase (NOS), particularly inducible NOS (iNOS), reducing nitric oxide production and thereby relieving pain. Quercetin inhibit iNOS activity, reducing nitric oxide levels and pain. Inflammation often involves oxidative stress due to the overproduction of reactive oxygen species (ROS) (Tungmunnithum *et al*., 2018), antioxidant compounds such as flavonoids and phenolic acids in plant extracts scavenge ROS, reducing oxidative stress and inflammation.

Comparison with contemporary literature reveals consistent findings regarding the pharmacological potential of *S. anacardium*. However, advancements in extraction techniques, phytochemical analysis, and mechanistic studies have provided deeper insights into its therapeutic properties. For instance, recent studies have identified specific bioactive compounds, such as β-sitosterol and flavonoids, responsible for the observed pharmacological activities (Mahajan and Mehta, 2011; Semalty *et al*., 2010). Moreover, innovative approaches utilizing nanotechnology and formulation strategies have enhance the bioavailability and efficacy of *S. anacardium*-based therapeutics (Singh *et al*., 2017).

5. Conclusion

In research, herbal extracts have gained significant attention due to their diverse therapeutic potentials and long-standing use in traditional medicine. Their natural origins and wide range of biological activities, such as anti-inflammatory, antimicrobial, and antioxidant properties, make them promising candidates for developing new drugs and therapies. The present study revealed that the HESA extract have a broad spectrum antimicrobial activity and active against MDR- *E.coli,* MDR-*P. aeruginosa,* and vancomycin-resistant *E. faecalis*. The HESA extract showed significant antinociceptive effect by acting centrally as well as peripherally. It showed strong anti-inflammatory activity evaluated by carageenan-induced paw edema method. These actions are presumably attributed to the drug's flavonoid, tannins and alkaloid content. The pharmacological potential of *S. anacardium* nuts as a valuable tool of bioactive components with diverse therapeutic properties. The growing interest in herbal extracts underlines the importance of understanding their mechanisms of action, bioavailability, and therapeutic potential in modern medical research. The herbal extracts represent a vital link between traditional healing practices and modern pharmacology, providing valuable insights for the development of future therapeutic agents.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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