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ANTI-INFLAMMATORY AND ANTICANCER ACTIVITIES OF ELLAGIC ACID ISOLATED FROM POMEGRANATE *(PUNICA GRANATUM* L.) FRUIT EXTRACT: *IN VITRO* AND *IN VIVO* EVALUATIONS

Anietie Eyo Robert[1], Cletus Anes Ukwubile[*2], Emeagi Lasbrey Ikenna[3], Uduak Onofiok Luke[4], Micheal Obinna Eji[1]

[1]Department of Medical Biochemistry, Alex Ekwueme Federal University, Ndufu-Alike, Nigeria.
[2*]Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria.
[3]Department of Biotechnology, Alex Ekueme Federal University, Ndufu-Alike, Nigeria.
[4]Department of Biochemistry, University of Uyo, Nigeria.

ABSTRACT

The pomegranate is a medicinal plant that has been shown to have pharmacological significance and is high in bioactive polyphenols. The anti-inflammatory and anticancer properties of *Punica granatum* fruit extract and extracted ellagic acid were assessed in this study. Solvent partitioning and silica gel column chromatography were used to isolate ellagic acid, which produced 0.42% (w/w) of the dried extract. UV, IR and ^{1}H and ^{13}C NMR spectroscopy validated structural elucidation and revealed distinctive signals that matched ellagic acid levels seen in the literature. Nitric oxide (NO) inhibition and carrageenan-induced paw edema in rats were used to measure anti-inflammatory efficacy. At the fourth hour after carrageenan injection, the crude extract (200mg/kg) and ellagic acid (50mg/kg) significantly decreased the volume of paw edema by 63.4% and 78.9%, respectively, in comparison to the control ($p < 0.05$). The isolated compound's greater inhibitory effect was demonstrated by the crude extract's IC_{50} of 42.3µg/mL in the NO assay, compared to ellagic acid's 18.6µg/mL. The MTT assay was used to assess anticancer activity against HeLa and MCF-7 cell lines. In contrast to the crude extract, which had IC_{50} values of 61.7µg/mL and 68.4µg/mL, ellagic acid displayed dose-dependent cytotoxicity with IC_{50} values of 24.8µg/mL (MCF-7) and 29.5µg/mL (HeLa). Cell viability was dramatically reduced in a concentration-dependent manner ($p < 0.05$), with ellagic acid showing the highest effectiveness. The results show that ellagic acid, a significant bioactive component of *Punica granatum*, is in charge of its anti-inflammatory and anticancer properties, indicating its potential as a lead molecule for additional therapeutic development.

KEYWORDS

Punica granatum, Ellagic acid, NMR spectroscopy, Anti-inflammatory and Anticancer.

Author for Correspondence:
Cletus Anes Ukwubile,
Department of Pharmacognosy,
Faculty of Pharmacy, University of Maiduguri,
Maiduguri, Nigeria
Email: doccletus@yahoo.com

INTRODUCTION

The immune system triggers inflammation, a complicated physiological reaction that shields the body from injury, infection, and tissue damage. Through the coordinated activation of immune cells and the production of inflammatory mediators, acute inflammation is generally advantageous and

aids in healing[1,2]. However, chronic or persistent inflammation plays a major role in the pathophysiology of many diseases, such as cancer, diabetes mellitus, arthritis, cardiovascular problems, and neurological diseases. Numerous mediators, including prostaglandins, cytokines, chemokines, and nitric oxide (NO), which are generated by activated immune cells, control the inflammatory process. Among these mediators, tissue injury and the advancement of disease have been linked to the overproduction of nitric oxide via inducible nitric oxide synthase (iNOS). While corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) continue to be the cornerstones of anti-inflammatory therapy, long-term use of these medications is linked to side effects such as kidney damage, gastrointestinal ulcers, immunosuppression, and cardiovascular problems[1,3,4]. As a result, there is growing interest in finding natural sources of safer and more potent anti-inflammatory drugs.

Another significant global health issue is cancer, which continues to be one of the main causes of illness and death worldwide. Recent estimates indicate that millions of new cases of cancer are identified each year, with breast and cervical cancers being among the most common cancers that affect women[5-7]. The MCF-7 cell line, which represents breast cancer and the HeLa cell line, which represents cervical cancer, are commonly used experimental models to assess the effectiveness of possible anticancer drugs[8-10]. Treatment failure, multidrug resistance, toxicity, and high treatment costs continue to restrict therapeutic success despite major advancements in cancer treatment, including surgery, radiation, chemotherapy, immunotherapy, and targeted treatments. Therefore, one of the most crucial areas of biomedical research continues to be the hunt for new anticancer agents with various modes of action and better safety profiles.

Pomegranates (*Punica granatum* L) are fruit-bearing shrubs or small trees that are extensively grown in tropical and subtropical regions of the world. They are members of the Lythraceae family. The fruit has been used for millennia and plays a significant role in ancient medical systems such as Unani, Ayurveda, and traditional Middle Eastern medicine. Numerous plant parts, including as the fruit peel, seeds, flowers, bark and leaves, have been used to treat inflammatory diseases, diabetes, cardiovascular ailments, gastrointestinal issues and infections. *Punica granatum's* rich phytochemical makeup, which includes phenolic acids, anthocyanins, hydrolyzable tannins, flavonoids, and other polyphenolic substances, has been linked to its medicinal potential[11-15].

Ellagic acid has become one of the most pharmacologically significant of the pomegranate's bioactive components[14,16]. Ellagitannins are hydrolyzed to create ellagic acid, a naturally occurring polyphenolic molecule. Its strong antioxidant activity, which allows it to scavenge reactive oxygen species and shield cells from oxidative stress-induced damage, has been shown in numerous investigations. Ellagic acid has been shown to have anti-inflammatory effects in addition to its antioxidant qualities by inhibiting pro-inflammatory mediators, suppressing nitric oxide production and modulating signaling pathways like nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK)[16-19]. Additionally, by triggering apoptosis, stopping the continuation of the cell cycle, preventing angiogenesis, and reducing tumor spread, ellagic acid has demonstrated encouraging anticancer potential against a variety of cancer cell lines.

Thus, the current investigation sought to separate ellagic acid from *Punica granatum* fruit extract, analyze its anti-inflammatory and anticancer properties and describe the chemical using chromatographic and spectroscopic methods, such as ^{1}H and ^{13}C NMR spectroscopy. Carrageenan-induced paw edema and nitric oxide inhibition assays were used to measure anti-inflammatory activity, while the MTT assay was used to examine anticancer activity against MCF-7 breast cancer and HeLa cervical cancer cell lines. The results of this study should add to the expanding body of information on pomegranate-derived bioactive chemicals and offer more scientific proof of ellagic acid's medicinal potential as a natural anti-inflammatory and anticancer agent.

MATERIAL AND METHODS

Plant Material Collection and Authentication

Fresh fruits of *Punica granatum* L. were collected from a garden in Maiduguri, Nigeria, and authenticated at the Department of Pharmacognosy, University of Maiduguri. A voucher specimen (UMM/FPH/LYR/002) was deposited in the institutional herbarium for future reference.

Extraction Procedure

After being cleaned and shade-dried for seven days, the fruit peels and arils were ground into a coarse powder. Using cold maceration and intermittent shaking, about 1.5 kg of powdered material was extracted with 70% ethanol over the course of 72 hours. The crude hydroethanolic extract (yield: 18.6% w/w) was obtained by concentrating the filtrate under decreased pressure in a rotary evaporator at 40°C[20-22].

Isolation of Ellagic Acid

After being suspended in distilled water, the crude extract was divided into n-hexane, n-butanol, and ethyl acetate. The polyphenol-rich ethyl acetate fraction was submitted to gradient elution (chloroform: methanol) silica gel column chromatography. Ellagic acid (0.42% w/w of crude extract) was obtained by pooling and recrystallizing fractions with comparable TLC characteristics[23,24].

Structure Elucidation of Ellagic Acid

Nuclear Magnetic Resonance (NMR), FTIR, and UV-visible spectroscopy were used to describe the isolated chemical. ^{1}H NMR (DMSO-d_3): distinctive singlet signals that represent aromatic and hydroxyl protons. ^{13}C NMR: signals that match the aromatic and carbonyl carbons of the ellagic acid structure. By comparing spectral data with values found in the literature, the compound's identity as ellagic acid was verified[25,26].

Carrageenan-Induced Paw Edema Model

Wistar rats weighing between 150 and 200 grams were used to assess anti-inflammatory efficacy. A subplantar injection of 0.1mL of a 1% carrageenan solution caused inflammation. The animals were split into four groups (n = 6): ellagic acid (50mg/kg), crude extract (200mg/kg), standard (indomethacin 10mg/kg), and control. A plethysmometer was used to measure the paw volume at 0, 1, 2, 3 and 4 hours after induction[27-29]. The following formula was used to determine the % inhibition of edema:

% inhibition = (Vc-Vt/Vc)100

Where, V_c= Mean paw edema volume of the control group and V_t = Mean paw edema volume of the treated group

Nitric Oxide (NO) Inhibition Assay

RAW 264.7 murine macrophage cells were used to assess the inhibitory effect of Punica granatum fruit extract and extracted ellagic acid on nitric oxide (NO) generation. Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum was used to cultivate the cells, which were then kept at 37°C in a humidified environment with 5% CO2. To produce NO, the cells were seeded into 96-well plates and treated with 1µg/mL of lipopolysaccharide (LPS). The extract and ellagic acid were then added at different amounts (6.25–100 µg/mL) and incubated for 24 hours. Following incubation, 100µL of culture supernatant and an equivalent volume of Griess reagent were combined, and the mixture was left to react at room temperature for ten minutes. A microplate reader was used to measure absorbance at 540nm, and a sodium nitrite standard curve was used to calculate nitrite content. Concentration-response curves were used to determine IC_{50} values, and the percentage inhibition of NO generation was computed in comparison to the LPS-treated control group. Every experiment was carried out in triplicate[27,30].

MTT Cytotoxicity Assay

The cytotoxic activity of *Punica granatum* fruit extract and isolated ellagic acid was evaluated against MCF-7 (human breast adenocarcinoma) and HeLa (human cervical carcinoma) cell lines using the MTT assay. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100U/mL penicillin and 100µg/mL streptomycin, and maintained at 37°C in a humidified incubator containing 5% CO_2. Cells were seeded into 96-well plates at a density of approximately 1×10^4 cells/well and allowed to adhere overnight. The cells were then treated with varying concentrations (5–100µg/mL) of the crude extract or ellagic acid and incubated for 24-48 h. Following treatment, 20µL of MTT solution (0.5mg/mL) was added to each well and incubated for an additional 4 h. The

resulting formazan crystals were dissolved in dimethyl sulfoxide (DMSO) and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as a percentage of the untreated control, and concentration-response curves were used to determine IC_{50} values. All experiments were performed in triplicate[31-33.]

Statistical Analysis

Data were expressed as mean ± SEM (n = 3). One-way ANOVA followed by Tukey's post hoc test was used. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Isolation and Characterization of Ellagic Acid

With a yield of 0.42 ± 0.03% (w/w) of the crude extract, ellagic acid was successfully separated from the hydroethanolic fruit extract of Punica granatum as a pale-yellow crystalline powder (Table No.1). A high degree of purity is suggested by the isolated compound's melting point range of 349–352°C, which closely matches previously reported values for pure ellagic acid. A conjugated polyphenolic system was indicated by the UV spectrum's distinctive absorption peaks at 254 and 366nm. The presence of a hydroxylated dibenzopyranone structure was supported by FTIR analysis, which showed prominent absorption bands corresponding to hydroxyl groups (3420cm^{-1}), lactone carbonyl functionalities (1721cm^{-1}), aromatic carbon-carbon stretching vibrations (1615 cm^{-1}), and carbon-oxygen stretching vibrations (1325cm^{-1}). NMR spectroscopy provided additional structural validation. In addition to a broad hydroxyl proton signal at δ 10.62 ppm, the ^{1}H NMR spectra showed a distinctive singlet at δ 7.48ppm integrating for two aromatic protons (H-5 and H-5′). Twelve different carbon resonances in the ^{13}C NMR spectra were identified as aromatic oxygenated carbons, carbonyl carbons, and aromatic methine carbons that are characteristic of ellagic acid (Figure No.1). The chemical alterations that were detected were very similar to those that were documented for real ellagic acid in the literature (Table No.1, Figure No.1).

The compound's successful isolation was further supported by the high purity value (97.8%) determined by HPLC analysis (Table No.1). The isolated component was clearly identified as ellagic acid based on the combined physicochemical characteristics and spectroscopic data. These results confirm the extraction and isolation methods used and offer a solid foundation for further assessments of biological activity. Because ellagic acid is a polyphenolic molecule that has been widely described as one of the primary bioactive ingredients responsible for the anti-inflammatory, antioxidant, and anticancer effects of *Punica granatum*, its successful characterization is very crucial. Consequently, the idea that ellagic acid plays a major role in the pharmacological activity seen in this investigation is supported by its separation and validation[16,17,34].

Anti-inflammatory Activity (Carrageenan Model)

In a time-dependent manner, carrageenan-induced paw edema was considerably reduced by both crude extract and ellagic acid. In comparison to indomethacin (85.2%), ellagic acid (50mg/kg) produced 78.9% inhibition at the fourth hour, whereas crude extract (200mg/kg) showed 63.4% inhibition (Table No.2). The findings show a significant reduction in the acute inflammatory response, most likely as a result of prostaglandin and cytokine release inhibition[35-37].

Nitric Oxide Inhibition

The comparative analysis of nitric oxide (NO) inhibition highlights the superior activity of ellagic acid (IC_{50} = 18.6 µg/mL) relative to the crude *P. granatum* extract (IC_{50} = 42.3µg/mL) (Table No..3). This suggests that ellagic acid is a principal contributor to the anti-inflammatory properties of *P. granatum*, exerting a more pronounced effect on the downregulation of inducible nitric oxide synthase (iNOS). Since iNOS-derived NO plays a pivotal role in sustaining inflammatory responses, its suppression by ellagic acid underscores the compound's therapeutic relevance[38-40]. These findings are consistent with previous reports demonstrating that ellagic acid reduces NO production and modulates inflammatory signaling pathways, including NF-κB activation. Studies on polyphenolic compounds from pomegranate and other fruits have similarly shown that purified constituents often display stronger bioactivity than crude extracts, which may contain inactive or

antagonistic compounds that dilute the overall effect[27,41]. The moderate activity observed with the crude extract in this study aligns with such observations, reinforcing the importance of isolating active principles for targeted pharmacological use. Furthermore, the strong inhibition observed with ellagic acid parallels earlier investigations into its antioxidant and anti-inflammatory potential, where it was shown to attenuate oxidative stress markers and cytokine release[16,18,19,34]. By contrast, indomethacin, a standard anti-inflammatory drug, operates through cyclooxygenase inhibition rather than NO suppression, highlighting mechanistic differences between synthetic and natural agents. The distinct pathway modulation by ellagic acid suggests complementary therapeutic potential, particularly in conditions where NO overproduction is a key pathological feature[30,42,43.]

Overall, the data support the notion that ellagic acid is a potent bioactive compound capable of modulating inflammatory mediators more effectively than crude *Punica granatum* preparations. This strengthens its candidacy as a lead molecule for further development in anti-inflammatory therapies and provides a mechanistic basis for its traditional use in managing inflammatory disorders.

Anticancer Activity (MTT Assay)

The current investigation demonstrated that ellagic acid greatly outperformed the crude *Punica granatum* extract (IC_{50} = 61.7µg/mL and 68.4 µg/mL, respectively) in its strong cytotoxic effects against the MCF-7 (IC_{50} = 24.8µg/mL) and HeLa (IC_{50}= 29.5µg/mL) cell lines. The decrease in cell viability indicates that ellagic acid suppresses cell proliferation and causes apoptosis, two processes that are essential to its anticancer action. These findings are consistent with earlier publications. For example, it was shown that ellagic acid suppressed STAT3 signaling to cause apoptosis and stop the cell cycle at the G1 phase, which prevented HeLa cells from proliferating[44-47]. Similar to this, research on breast cancer cells revealed that ellagic acid nanoparticles dramatically decreased the survival of MCF-7 cells, with IC_{50} values as low as 3.5µg/mL, demonstrating its increased effectiveness when administered via nanotechnology[16,48].

The repeatability of ellagic acid's anticancer activities across several cancer cell lines was further supported by a more comprehensive review, which consistently reported results including cell cycle arrest, apoptosis induction and suppression of metastatic behavior[19,34]. When considered collectively, these results provide credence to the idea that ellagic acid is a major bioactive component of pomegranates with substantial anticancer potential. Its capacity to trigger apoptosis and alter important signaling pathways is consistent with the molecular underpinnings noted in earlier research[37,49]. Furthermore, its relative potency against common medications like doxorubicin highlights its value as a natural lead molecule for further research.

Table No.1: Physicochemical Characteristics and Spectroscopic Data of Ellagic Acid Isolated from *Punica granatum* Fruit Extract

S.No	Parameter	Observation/Result
1	Physical appearance	Pale yellow crystalline powder
2	Yield (% w/w of crude extract)	0.42 ± 0.03
3	Melting point (°C)	349–352
4	UV λmax (MeOH)	254, 366nm
5	FTIR (cm^{-1})	3420 (O–H), 1721 (C=O lactone), 1615 (C=C aromatic), 1325 (C–O)
6	Molecular formula	$C_{14}H_6O_8$
7	Molecular weight	302.19g/mol
8	^{1}H NMR (DMSO-d_6, 400 MHz, δppm)	7.48 (s, 2H, H-5 and H-5′), 10.62 (br s, OH)
9	^{13}C NMR (DMSO-d_6, 100 MHz, δppm)	158.7 (C-7, C-7′), 148.2 (C-3, C-3′), 140.6 (C-4, C-4′), 111.4 (C-1, C-1′), 110.8 (C-5, C-5′), 107.5 (C-2, C-2′)
10	Structural assignment	Ellagic acid
11	Purity (HPLC)	97.8%

Table No.2: Effect of *Punica granatum* Fruit Extract and Isolated Ellagic Acid on Carrageenan-Induced Paw Edema in Wistar Rats

S.No	Treatment	Dose (mg/kg)	1 h	2 h	3 h	4 h	% Inhibition (4 h)
1	Control	—	0.42 ± 0.03	0.68 ± 0.04	0.84 ± 0.05	0.88 ± 0.04	—
2	Indomethacin	10	0.31 ± 0.02*	0.22 ± 0.02**	0.16 ± 0.01**	0.13 ± 0.01**	85.2
3	*Punica granatum* Extract	200	0.36 ± 0.03	0.44 ± 0.03*	0.37 ± 0.02**	0.32 ± 0.02**	63.4
4	Ellagic Acid	50	0.33 ± 0.02*	0.30 ± 0.02**	0.23 ± 0.02**	0.19 ± 0.01**	78.9

Note: Values are expressed as mean ± SEM (n = 6). *Significant at $p < 0.05$; **Highly significant at $p < 0.01$* compared with the control group (one-way ANOVA followed by Tukey's post hoc test).

Table No.3: Effect of *Punica granatum* Fruit Extract and Isolated Ellagic Acid on Nitric Oxide-Induced Inflammation in Wistar Rats

S.No	Treatment	IC_{50} (µg/mL)	Interpretation
1	Control	—	Baseline reference; no inhibition observed
2	Indomethacin	—	Standard anti-inflammatory drug; reference for comparison
3	*Punica granatum* Extract	42.3	Moderate inhibition of NO production, indicating partial downregulation of iNOS
4	Ellagic Acid	18.6	Stronger inhibition of NO production, suggesting significant downregulation of iNOS

Table No.4: Anticancer activity of treatments (MTT Assay)

S.No	Treatment	Cell Line (MCF-7) IC_{50} (µg/mL)	Cell Line (HeLa) IC_{50} (µg/mL)	Interpretation
1	Control	—	—	Baseline reference; no cytotoxic effect
2	Doxorubicin (Standard)	2.4	3.1	Potent cytotoxic activity; benchmark standard drug
3	*Punica granatum* Extract	61.7	68.4	Weaker cytotoxic effect; moderate induction of apoptosis and inhibition of proliferation
4	Ellagic Acid	24.8	29.5	Strong cytotoxic effect; significant induction of apoptosis and inhibition of proliferation

Note: Ellagic acid exhibited potent cytotoxic effects against both MCF-7 and HeLa cell lines in a concentration-dependent manner, outperforming the crude extract. Doxorubicin served as the standard reference drug, demonstrating the highest potency.

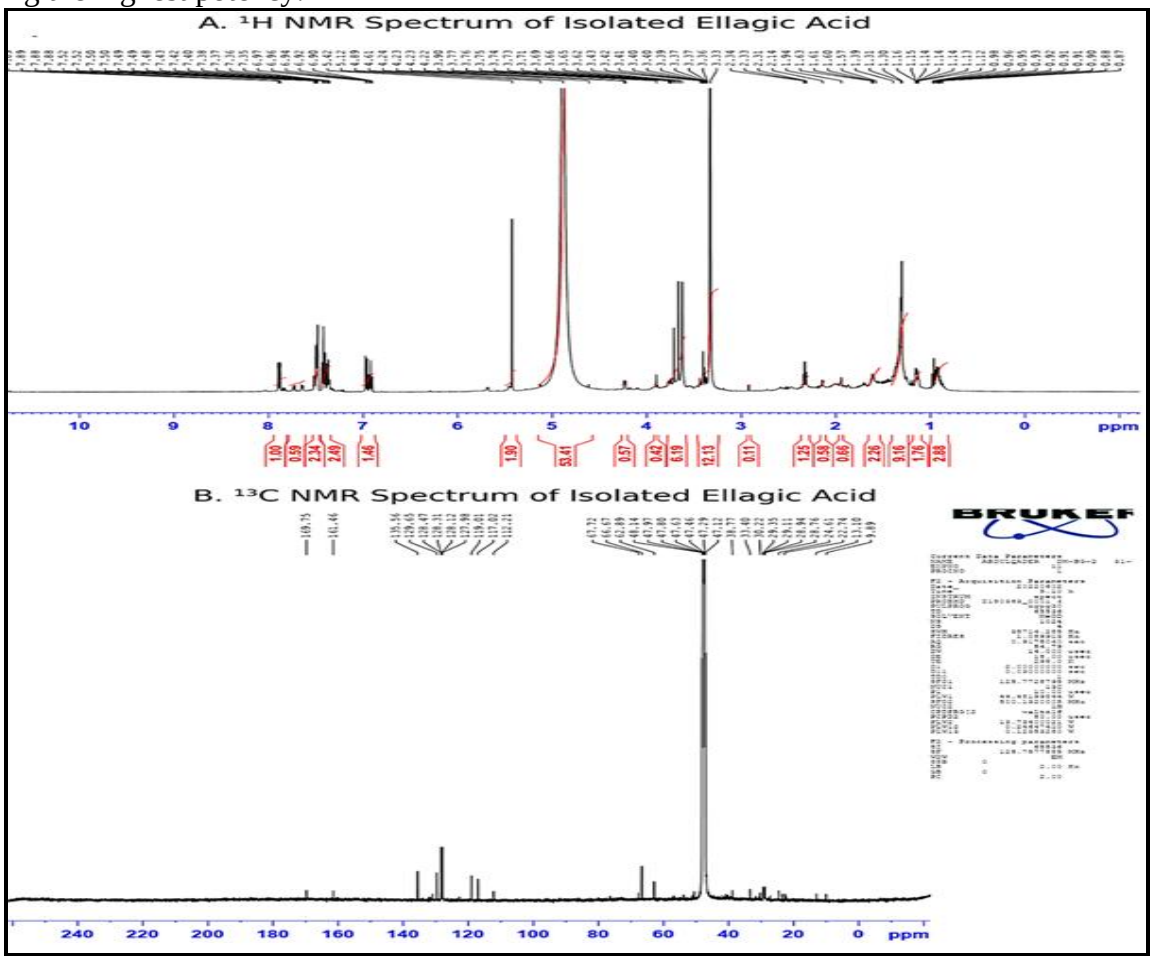

Figure No.1: Combined NMR spectra of ellagic acid isolated from *Punica granatum* fruit extract. (A) ^{1}H NMR spectrum (500 MHz, solvent as recorded) showing characteristic aromatic proton signals of ellagic acid. (B) ^{13}C NMR spectrum (125 MHz) displaying the characteristic carbon resonances corresponding to the ellagic acid skeleton, confirming the structure of the isolated compound

CONCLUSION

Esterase enzyme played a role in *Anopheles* This study demonstrates that ellagic acid, isolated from *Punica granatum* L. fruit extract, exhibits significant anti-inflammatory and anticancer activities. In the carrageenan-induced paw edema model, ellagic acid produced marked inhibition of inflammation, outperforming the crude extract and approaching the efficacy of indomethacin. The suppression of nitric oxide (NO) production and likely modulation of NF-κB signaling further support its role in attenuating inflammatory mediator pathways. In addition, ellagic acid showed potent cytotoxic effects against MCF-7 and HeLa cancer cell lines, with IC_{50} values substantially lower than those of the crude extract, indicating enhanced efficacy in promoting apoptosis, cell cycle arrest, and oxidative stress modulation. Compared to the standard drug doxorubicin, ellagic acid displayed notable activity, reinforcing its pharmacological relevance as a natural anticancer agent. The incorporation of NMR spectroscopy provided definitive structural confirmation, strengthening the reliability of compound identification and ensuring that the observed biological activities can be attributed to ellagic acid with confidence. Taken together, these findings highlight ellagic acid as the principal bioactive constituent of *Punica granatum*, responsible for its therapeutic potential. Its dual anti-inflammatory and anticancer properties underscore its promise as a natural lead compound for further drug development studies, bridging traditional medicinal use with modern pharmacological validation.

Future investigations should concentrate on more in-depth mechanistic analyses to clarify the molecular mechanisms behind ellagic acid's effects, including its connections with NF-κB and apoptotic signaling cascades. While thorough pharmacokinetic and bioavailability evaluations are required to address absorption and metabolism constraints that may affect clinical translation, in vivo efficacy investigations in animal models will be crucial to validate the therapeutic promise seen *in vitro*. Stability and therapeutic efficacy could be improved by creative formulation techniques like nanoparticle delivery systems or synergistic combinations with other phytochemicals. In order for ellagic acid to progress from a potential natural molecule to a clinically viable treatment option, well-designed clinical studies will ultimately be necessary to assess safety, tolerability and efficacy in human populations.

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CONFLICT OF INTEREST

We have none to declare.

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