EJPMR, 2026, 13(1), 221-226 **Research Article** **Impact Factor 7.065**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

HPLC–LCMS BASED PROFILING OF *ANNONA MURICATA* PEEL METHANOLIC EXTRACT AND ITS CYTOPROTECTIVE ACTIVITY

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DOI: https://doi.org/10.5281/zenodo.18092933

How to cite this Article: Smitha Grace S. R.1*, Varsha D. S.2, Shringa A. G.2 (2026). Hplc–Lcms Based Profiling Of Annona Muricata Peel Methanolic Extract And Its Cytoprotective Activity. European Journal of Pharmaceutical and Medical Research, 13(1), 221–226.

Article Received on 25/11/2025 **Article Revised on 15/12/2025** **Article Published on 01/01/2026**

ABSTRACT

Oxidative stress, arising from excessive production of reactive oxygen species (ROS), is a major contributor to chronic and degenerative diseases, including cancer. As synthetic drugs often carry significant side effects, natural antioxidants from plant sources are being increasingly explored for their therapeutic value. *Annona muricata*, a tropical fruit known for its diverse medicinal properties, possesses a peel rich in phytochemicals yet commonly discarded as agricultural waste. The present study evaluates the antioxidant and cytoprotective potential of phytoconstituents extracted from *Annona muricata* peel and compares their efficacy with the standard anticancer drug methotrexate using *Saccharomyces cerevisiae* (yeast) as oxidative damage models. Phytochemical screening revealed substantial levels of flavonoids, phenolics, tannins, and alkaloids, compounds well recognized for their strong antioxidant and anti-proliferative activities. Antioxidant assays, including DPPH, H_2O_2, and demonstrated significant free radical scavenging potential of the peel extract. In yeast cells exposed to hydrogen peroxide (H_2O_2)-induced oxidative stress, the extract markedly improved cell viability, reduced ROS accumulation, and maintained membrane integrity when compared with untreated and methotrexate-treated groups. Trypan blue and MTT assays confirmed superior viability and metabolic activity in extract-treated cells, indicating effective cytoprotection with minimal off-target effects. Overall, the findings highlight that *Annona muricata* peel extract possesses strong antioxidant and cytoprotective properties, surpassing methotrexate in both experimental models. This underscores the therapeutic promise of this natural, waste-derived resource in combating oxidative stress and related diseases, particularly cancer.

KEYWORDS: *Annona muricata peel, Antioxidant activity, Cytoprotective effect, Saccharomyces cerevisiae, Methotrexate.*

INTRODUCTION

Health serves as the foundation for human progress, influenced by determinants such as genetics, environment, lifestyle, income, education, and social relationships. Chronic illnesses—including cancer, cardiovascular diseases, diabetes, COPD, stroke, and mental health disorders—persist for long durations and demand continuous medical care. Although pharmaceutical-based allopathic treatments remain essential, they are frequently associated with adverse effects ranging from nausea and insomnia to serious complications like internal bleeding, cardiac abnormalities, allergic reactions, drug interactions, and long-term dependence. As a result, interest has grown in Complementary and Alternative Medicine (CAM), particularly herbal and plant-derived therapies, which have been integral to human healthcare for thousands of years. Medicinal plants contain phytochemicals—such as flavonoids, phenolics, alkaloids, tannins, and essential oils—that exhibit antioxidant, anti-inflammatory, anticancer, antimicrobial, immunomodulatory, neuroprotective, and anti-aging properties. These compounds modulate multiple biological pathways by reducing oxidative stress, inhibiting carcinogen

formation, regulating hormones, and inducing apoptosis in abnormal cells. *Annona muricata* (soursop), belonging to the Annonaceae family, is one such medicinally rich plant traditionally used to treat conditions ranging from fever, diarrhea, and joint pain to infections, skin disorders, anxiety, and tumors. Its leaves, seeds, pulp, and peel contain diverse bioactive constituents, including alkaloids, megastigmanes, flavonol triglycosides, phenolics, cyclopeptides, essential oils, and notably annonaceous acetogenins—recognized for their potent anticancer activity. Since cancer often behaves as a chronic disease requiring long-term control rather than complete cure, the search for effective natural anticancer compounds has intensified. Phytochemicals from *A. muricata* influence multiple molecular pathways involved in carcinogenesis, reinforcing their traditional medicinal relevance. In this study, the peel—an agricultural waste resource comprising 20% of the fruit—was explored for its therapeutic potential through extraction, phytochemical screening, TLC profiling, antioxidant assays, flavonoid quantification, and advanced analyses such as HPLC and LC-MS. Using *Saccharomyces cerevisiae* as a simple eukaryotic model and MCF-7 human breast cancer cells, the research evaluates oxidative stress mitigation, synergy with methotrexate, genotoxic protection, and cytotoxic effects. The significance of this work lies in validating a natural therapeutic alternative with selective cytoprotective properties, transforming waste material into a value-added bioresource, and employing both yeast and cancer cell models for comprehensive biological insight. Overall, the study highlights the promising antioxidant, cytoprotective, and potential anticancer attributes of *Annona muricata* peel phytochemicals and encourages future translational research for nutraceutical and pharmaceutical applications.

MATERIALS AND METHODS

1. Extraction, Purification, Characterization, and Phytochemical Profiling of Methanolic peel of Annona Muricata using HPLC and LCMS"

The peel of *Annona muricata* was subjected to a systematic extraction and characterization workflow in which dried, powdered peel was methanol-extracted, and the yield was calculated before the extract was purified through silica gel column chromatography using methanol as the mobile phase; fractions collected at 3 mL intervals were scanned at 240–600 nm to select those with maximum absorbance for further analysis. Partial characterization using TLC on activated silica gel plates, developed in an ethyl acetate:methanol:water (5:1:5) system, enabled comparison of the purified fractions with rutin and quercetin standards. Advanced profiling by HPLC (C18 column, PDA detector, water/acetonitrile with 0.1% formic acid gradient) and LC–MS provided retention times and molecular masses to identify major phytochemicals and establish the peel's chemical fingerprint. Complementary phytochemical screening confirmed the presence of diverse secondary metabolites including alkaloids, carbohydrates, glycosides, saponins, steroids, phenols, flavonoids, and tannins through standard colorimetric and precipitation tests, collectively validating the abundance of bioactive compounds in the methanolic peel extract of *Annona muricata*. **Evaluating through antioxidant assays:** The antioxidant potential of methanolic *Annona muricata* peel extract was evaluated using multiple in vitro assays to assess radical-scavenging, reducing power, and total flavonoid content.

2. Evaluating through antioxidant assays

2a. DPPH free radical scavenging activity was measured by incubating varying concentrations of the extract (250–750 µL) with 0.1 mM DPPH solution in methanol under dark conditions for 30 minutes, followed by absorbance measurement at 517 nm using a UV–Vis spectrophotometer, with ascorbic acid (100 µg/mL) as the standard.

2b. Hydrogen peroxide (H_2O_2) scavenging activity was determined by reacting the extract with a 40 mM H_2O_2 solution in phosphate buffer (pH 7.4) for 10 minutes, and absorbance was recorded at 230 nm; the percentage scavenged was calculated relative to a control using % Scavenged $[H_2O_2] = [(A\_c - A\_s)/A\_c] \times 100$, where A_c and A_s denote the absorbance of the control and test sample, respectively.

2c. Total flavonoid content (TFC) was quantified via the aluminum chloride colorimetric assay: the extract reacted sequentially with 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH, and absorbance was measured at 510 nm, using quercetin (100 µg/mL) as the standard. Collectively, these assays provided a comprehensive evaluation of the extract's antioxidant profile, reflecting its potential to scavenge free radicals, reduce oxidized intermediates, and contribute flavonoid-mediated protective effects against oxidative stress.

3. Yeast as a Model Organism for Anticancer Activity Assessment: Saccharomyces cerevisiae was used as a simple eukaryotic model to evaluate the cytoprotective and anticancer potential of methanolic *Annona muricata* peel extract. The yeast cultures were grown in sterilized YEPD medium from 0.1 g of granules and incubated until reaching an OD of 0.3 at 610 nm. Cell viability and treatment responses were then assessed using the Trypan Blue Exclusion method.

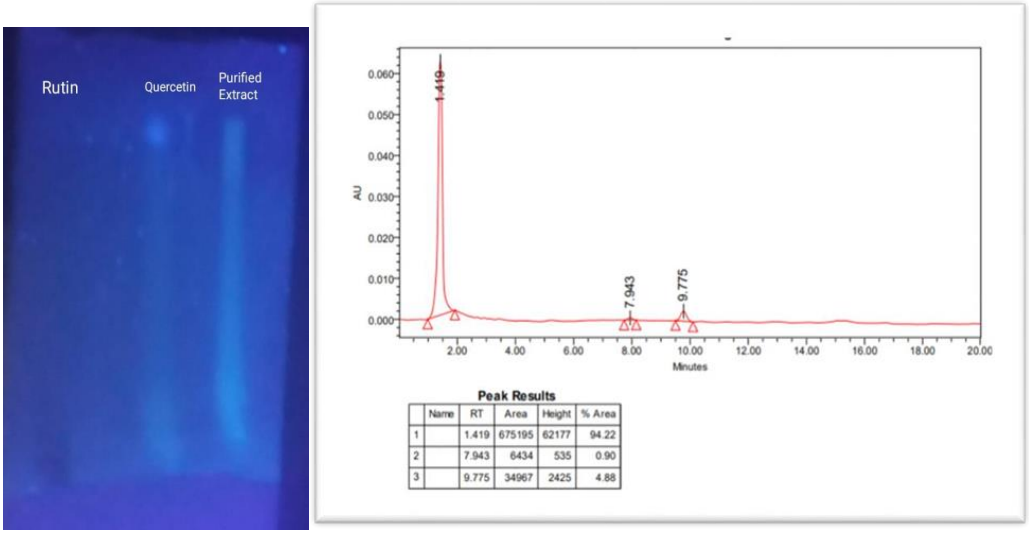
3a. Parallel studies using methotrexate (MTX, 1 mg/mL) were conducted to compare cytotoxic and protective responses, with yeast cultures exposed to MTX (50–250 µL), followed by H_2O_2 and subsequent extract treatment, and absorbance monitored at 595 nm. Lipid peroxidation was quantified via the TBARS assay by resuspending yeast pellets in distilled water, treating with SDS and acetic acid, reacting with thiobarbituric acid at 95°C for 60 minutes, and extracting with n-butanol:pyridine (15:1), with absorbance recorded at 532 nm.

3b. Additionally, cell metabolic activity was evaluated using the MTT assay, where yeast cultures treated with

H_2O_2, methanolic extract, MTX, or their combinations were incubated with MTT reagent (50 µL) at 37°C for 3–4 hours, followed by addition of MTT solvent and OD measurement at 590 nm. Collectively, these assays provided a quantitative assessment of oxidative stress mitigation, cytoprotective effects.

RESULTS AND DISCUSSIONS

1. HPLC, LC-MS, TLC, and Phytochemical Characterization of *Annona muricata* Peel Extract: The chromatographic and phytochemical analyses collectively confirm that the *Annona muricata* peel extract is highly enriched with flavonoid-based antioxidants, predominantly quercetin or closely related analogues.



	Name	RT	Area	Height	% Area
1		1.419	675195	62177	94.22
2		7.943	6434	535	0.90
3		9.775	34967	2425	4.88

Fig 1: TLC & HPLC chromatogram of *Annona muricata* peel extract.

HPLC profiling shows a major peak at 1.419 min contributing 94.22% of the total area—closely matching the quercetin standard at 1.692 min**,** indicating quercetin is the dominant compound in the extract, while minor peaks at 7.943 and 9.775 min suggest additional phenolics or flavonoid derivatives. LC-MS further validates the presence of flavonoids, identifying rutin as a significant component and recommending reinjection for enhanced resolution. TLC fingerprints also align with quercetin and rutin standards, reinforcing the flavonoid-rich nature of the sample. Phytochemical screening supports these findings, showing strong positivity for tannins, phenols, terpenoids, glycosides, alkaloids, and flavonoids**,** all of which contribute to potent antioxidant, cytoprotective, and potential anticancer activities, while the absence of steroids and saponins does not diminish the therapeutic.

2. Evaluating through antioxidant assays

2a. Analysis of DPPH assay: DPPH scavenging assay-. In the present experiment standard ascorbic acid is used in comparison with the peel of *Annona muricata*, the result is indicative of concentration dependent activity Thus, indicating stronger free radical scavenging capabilities with 65 % for the AmP extract and 60% for Std Ascorbic acid.

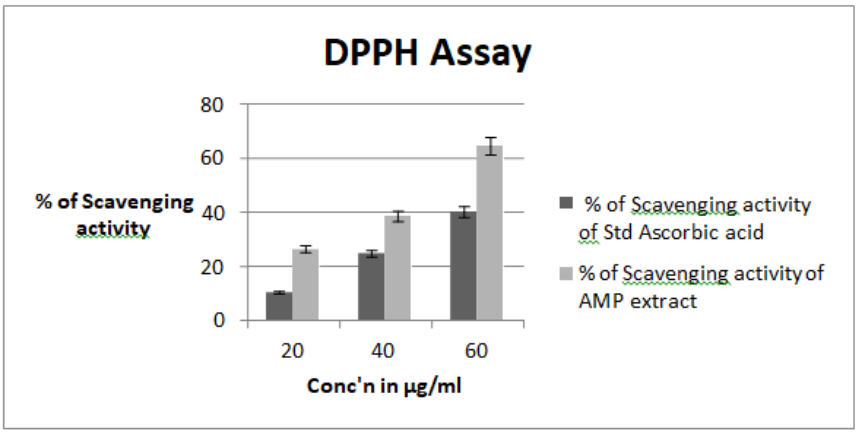


Fig 2: DPPH assay.

2b. Analysis of Hydrogen peroxide (H_2O_2) free radical scavenging assay: The % of H_2O_2 scavenging of *Annona muricata* is compared with known standard 'antioxidant such as ascorbic acid. The result indicates antioxidant activity is increasing with increasing concentration with scavenging activity of 55%v and 51 % respectively.

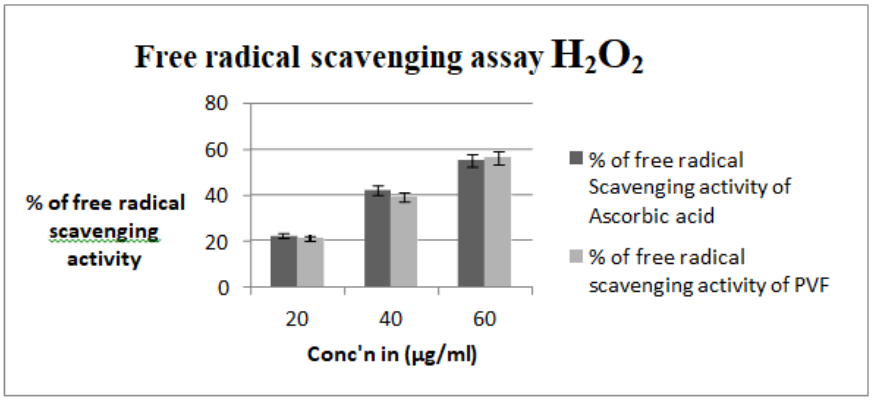


Fig 3: Hydrogen peroxide (H2O2) free radical scavenging assay.

2c. Analysis of Flavonoid: Flavonoid determination, often involving colorimetric assays using aluminum chloride, aims to quantify the total flavonoid content in plant extracts. using a standard flavonoid, like quercetin our assay we have found out the 45.65QE/mg as compared to standard quercetin 48.44QE/mg.

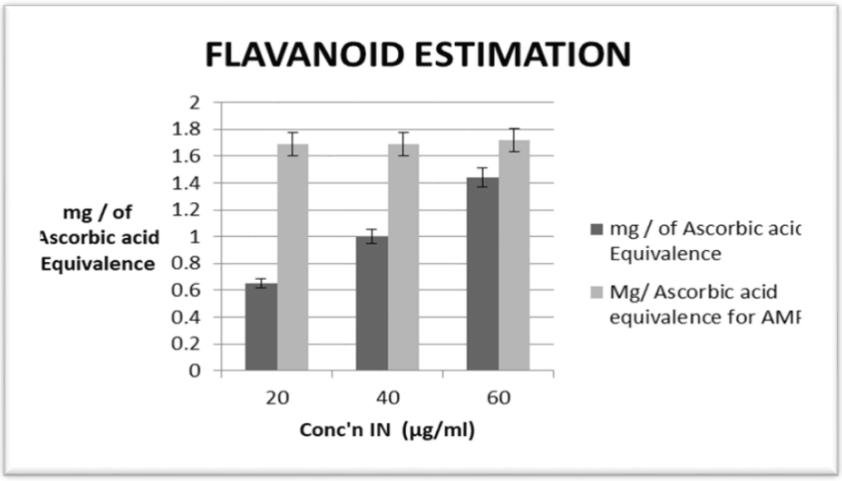


Fig 4: Analysis of Flavonoid.

3 In the present study we used yeast as a model organism because of its conserved cellular pathways sharing with humans making it a suitable model for studying cancer related process. This also proves to be a high-throughput screening of medicinal plant extract and hence we selected this model. The cell analysis using trypan blue exclusion method we found the percentage of viability 56.92%. Further, we treated the yeast cell with oxidant damaging chemicals namely H_2O_2 (30%) and % of cell viability **is** 56.92%.

3a. Analysis of TBAR's assay: When combined with yeast cells and AMP, the TBARS assay has helped us to assess the antioxidant potential of those extracts by measuring their ability to inhibit lipid peroxidation in yeast cells. This synergy in our present study shows protective effects of AMP against oxidative stress in a cellular context.

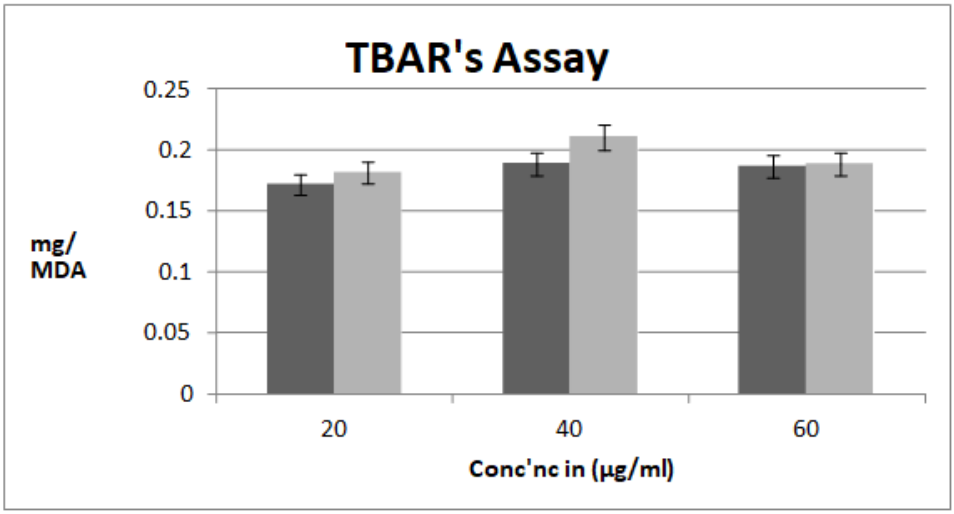


Fig 5: TBAR's assay.

Analysis of MTT assay: The cell viability is supressed and as we treat it with combination of AMP and MTX, the extracts are not found to be cytotoxic and the viability is retained and hence these compounds can be used as therapeutic agents.

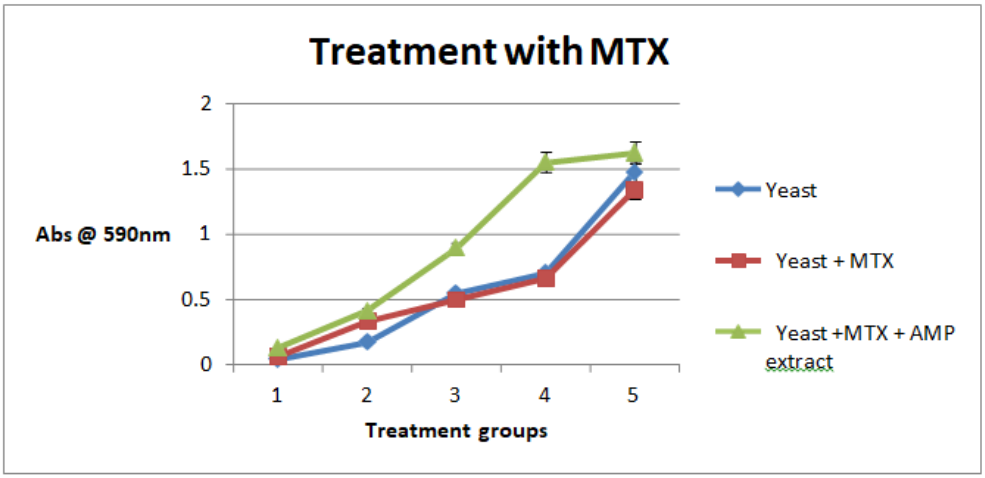


Fig 6: MTT assay.

CONCLUSION

The present study demonstrates that the methanolic peel extract of *Annona muricata* possesses significant antioxidant and cytoprotective properties, strongly supported by phytochemical richness and validated through TLC, HPLC, LC–MS, DPPH, H_2O_2, TBARS, and MTT assays. The extract not only scavenged free radicals efficiently but also protected yeast cells from oxidative and methotrexate-induced stress, indicating its potential as a natural therapeutic agent with minimal cytotoxicity. In line with the growing recognition of plant-derived compounds, this study reinforces the idea that **"**nature remains the world's greatest chemist, offering remedies hidden in its simplest forms." Overall, the findings highlight that *A. muricata* peel—often considered agricultural waste—can be transformed into a valuable bioresource with promising applications in antioxidant therapy, cytoprotection, and future anticancer research.

Conflict of interest statement: The authors declare that there is no conflict of interest concerning this study.

ACKNOWLEDGEMENT: The authors thank the Director of Pooja Bhagavat Memorial Mahajana Education Centre, PG Wing of SBRR Mahajana First Grade College & Mahajana Research Foundation(MES) –for Seed money (Ref No : MES /535A/2023 -24) and DST -FIST (Ref : No SR/FST/College-329/2016) for providing the infrastructure for conducting the experiments, invaluable support and encouragement.

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