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Tumor suppressing ability of myrtenal in DMBA-induced rat mammary cancer: A biochemical and histopathological evaluation

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ABSTRACT

Breast cancer is one of the most common cancers in women and a major cause of deaths. 7,12-Dimethylbenz(a)anthracene (DMBA) is often used to induce breast cancer in rats. This study investigates the potential of myrtenal to prevent breast cancer caused by DMBA in female Sprague-Dawley rats. This study evaluates the dose-dependent chemopreventive effect of myrtenal in female Sprague-Dawley rats with mammary tumors induced by 7,12-dimethylbenz[a]anthracene (DMBA). The subcutaneous injection of DMBA alone in rats resulted in 100% tumor incidence accompanied by an increase in tumor burden, volume, and significant biochemical disruptions. Myrtenal at doses of 100, 200, and 400 mg/kg was orally administered to DMBA-treated rats, and its protective efficacy was evaluated through tumor inhibition potential, histopathological, and biochemical analyses. Myrtenal treatment caused a dose-dependent reduction in tumor incidence and volume and restored altered biochemical parameters (lipid peroxidation byproducts, antioxidants, and phase I and II detoxifying enzymes) toward normal. Histopathological findings further validated the protective effects of myrtenal. This study highlights the potent chemopreventive potential of myrtenal, particularly at 400 mg/kg. Myrtenal's anticancer efficacy is primarily attributed to its strong antioxidant properties and its regulatory influence on the detoxification pathway.

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INTRODUCTION

Breast cancer ranks as the second most frequently occurring malignancy globally, with estimates suggesting that one in eight women may develop it in their lifetime (Karnam *et al.*, 2017). In 2022, breast cancer accounting for approximately 2.3 million new cases and 670,000 deaths globally. In India, breast cancer contributed to 26.6% of all female cancer cases (192,020) and 22.0% of cancer-related deaths (98,337) (Zhang *et al.*, 2025). Breast cancer arises from a combination of factors, including age, genetic predisposition and prolonged exposure to endogenous or exogenous estrogens. Hormonal imbalances, lifestyle factors such as smoking, alcohol consumption and environmental carcinogens also play significant roles (Mathivadhani *et al.*, 2007; Obeagu and Obeagu, 2024). DMBA is commonly used to develop mammary tumors in Sprague-Dawley rats. DMBA is metabolized into its active form, 3,4-diol-1,2-epoxide, which disrupts redox balance and promotes oxidative stress. This leads to increased reactive oxygen species (ROS) generation that damage DNA and proteins, contributing to mutagenesis and carcinogenesis (Wang and Zhang, 2017).

Oxidative stress is implicated in initiation, promotion and progression of cancer by inducing mutations, promoting cell proliferation and supporting tumor growth, invasion and metastasis (Di Carlo and Sorrentino, 2017). Antioxidants help prevent cancer by neutralizing ROS, thus protecting cells from oxidative damage (Jomova *et al.,* 2023). The cellular antioxidant defense system includes Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) [enzymatic components] and reduced glutathione (GSH), vitamin C and vitamin E [Non-enzymatic antioxidants]. These components collectively reduce cellular damage by transforming harmful oxygen radicals into non-toxic molecules (Jena *et al.,* 2023). DMBA metabolism produces large amounts of free radicals that are counteracted by Cytochrome P_{450} and Cytochrome b_5 (Phase I) and Glutathione S-transferase (GST) and Glutathione reductase (GR) [Phase II] detoxification enzymes. These cascade conjugate reactive intermediates with glutathione, thereby reducing oxidative stress and enhancing cellular detoxification (Townsend and Tew, 2003).

Many phytochemicals exhibit anticancer potential through their ability to scavenge ROS and strengthen antioxidant defenses. As dietary agents, these natural compounds offer promising chemopreventive properties (Zhang *et al.,* 2015). Despite advancements in treatment, current breast cancer therapies including surgery, chemotherapy and radiotherapy are often associated with severe side effects and high costs, highlighting the need for safer and more affordable alternatives (Burguin *et al.,* 2021). Myrtenal, a bicyclic monoterpenoid, is found in the essential oils of cumin, mint, cardamom, spearmint, pepper and eucalyptus. It possesses diverse pharmacological properties including bronchodilator, antiaggregant, antihemolytic, hypotensive, antibacterial, anti-inflammatory, antioxidant, antihyperglycemic and neuroprotective effects (Dragomanova *et al.,* 2023). Lokeshkumar *et al.* (2015) demonstrated that myrtenal inhibited colon carcinogenesis through its antioxidant activity. In another study, it was shown to stabilize cellular membranes and maintain homeostasis (Booupathy *et al.,* 2016). Hari Babu *et al.* (2012) revealed its antitumor potential in diethylnitrosamine-induced liver cancer. Although myrtenal has shown efficacy in several experimental cancer models, its role in mammary carcinogenesis remains unexplored. This study thus aims to evaluate the tumor inhibiting potential of myrtenal by investigating its effects on lipid peroxidation and detoxification enzymes in mammary carcinogenesis.

MATERIALS AND METHODS

Chemicals

DMBA, myrtenal, GSH, NADH and nitroblue tetrazolium were procured from Sigma-Aldrich, India.

Animals

Sprague-Dawley rats (7–8 weeks old female; 130–140 g) were procured from Biogen, Bengaluru and housed

under standard conditions (12 h light/dark cycle, 24 ± 2 °C, 50 ± 10% humidity) at the Central Animal House, Annamalai University. They were given a standard pellet diet and water ad libitum. All experimental procedures followed CCSEA guidelines and were approved by the Institutional Animal Ethics Committee (Approval No.: GMCHC-IAEC/1379/3/24).

Study design

Forty-eight female Sprague-Dawley rats were randomly divided into six groups of eight animals each. Group I acted as the control and was given only the vehicle (1 mL of a sunflower oil-saline emulsion), along with a standard diet and water. Mammary tumors were induced in Groups II to V using a single dose of DMBA (25 mg/kg b.w subcutaneously). Group II served as the DMBA-only group without further treatment. Groups III, IV and V received myrtenal orally at doses of 100, 200 and 400 mg/kg body weight, respectively, starting one week before DMBA injection and continued daily for 16 weeks. Group VI received only myrtenal (400 mg/kg) without DMBA. After 16 weeks, all rats were anesthetized and sacrificed; liver and mammary tissues were collected, homogenized and centrifuged for biochemical analysis. For histopathology, tissues were fixed in 10% neutral buffered formalin, processed, embedded in paraffin, sectioned (2–3 µm) and stained with HandE for microscopic evaluation.

Biochemical estimations

For biochemical analysis, blood was collected in heparinized tubes and plasma was separated by centrifugation at 1000 × g for 15 minutes. Tissues were rinsed with ice-cold saline, blotted, weighed and homogenized using a Teflon-glass homogenizer in appropriate buffer. Protein content was quantified using the protocol described by Lowry *et al.* (1951). Lipid peroxidation products (TBARS) in both plasma and mammary tissues were determined based on the procedures of Yagi (1987) and Ohkawa *et al.* (1979). The activities of antioxidant enzymes, including superoxide dismutase (SOD) and catalase, were assessed according to the methods of Kakkar *et al.* (1984) and Sinha (1972), respectively. Glutathione peroxidase (GPx) activity was evaluated using the approach outlined by Rotruck *et al.* (1973), while reduced glutathione (GSH) levels in plasma, liver and mammary tissues were measured following Beutler and Kelley (1963). Plasma vitamin E concentration was determined by a colorimetric assay as per Desai (1984) and its levels in tissues were analyzed fluorimetrically following the method of Palan *et al.* (1991). Plasma vitamin C content was measured using the technique of Omaye *et al.* (1979).

The activities of glutathione S-transferase (GST) and glutathione reductase (GR) in liver and mammary tissues were estimated using the methods of Habig *et al.* (1974) and Calberg and Mannervik (1985), respectively. DT-diaphorase activity was measured using Ernster (1958) and levels of CYP450 and CYb5 in liver and mammary microsomal fractions were estimated as per Omura and Sato (1964).

Statistical analysis

Data were analyzed using one-way ANOVA and group comparisons were further evaluated with Duncan's multiple range test (DMRT). Results were presented as mean ± standard deviation (S.D.), with significance set at $p < 0.05$. Statistical analysis was carried out using SPSS software (version 12.0, SPSS Inc., Chicago, http://www.spss.com).

RESULTS

Fig. 1 shows the external appearance of control and DMBA-induced rats, with or without myrtenal treatment, after 16 weeks. Tumor incidence, burden and volume are detailed in Table 1.

Rats exposed to DMBA alone exhibited 100% tumor incidence along with a significant rise in tumor volume and burden. However, oral myrtenal treatment at doses of 100, 200 and 400 mg/kg reduced tumor incidence in a dose-dependent manner by 25%, 50% and 75%, respectively, compared to the DMBA alone treated group (Fig. 2). Histopathological evaluation of mammary tissue is presented in Fig. 3.

Table 1. Tumor incidence in experimental animals (n=8)

Parameter	Control	DMBA alone	DMBA+ Myrtenal 100mg/kg b.w	DMBA+ Myrtenal 200mg/kg b.w	DMBA+ Myrtenal 400mg/kg b.w	Myrtenal alone 400mg/kg b.w
Tumor incidence	-	100%	75%	50%	25%	-
Number of tumors	-	(8)/8	(6)/8	(4)/8	(2)/8	-
Tumor volume (cm^3)	-	2.89 ± 0.34^a	2.21 ± 0.21^b	1.48 ± 0.11^c	0.70 ± 0.12^d	-
Tumor burden (cm^3)	-	2.89 ± 0.34^a	1.65 ± 0.16^b	0.73 ± 0.04^c	0.17 ± 0.02^d	-

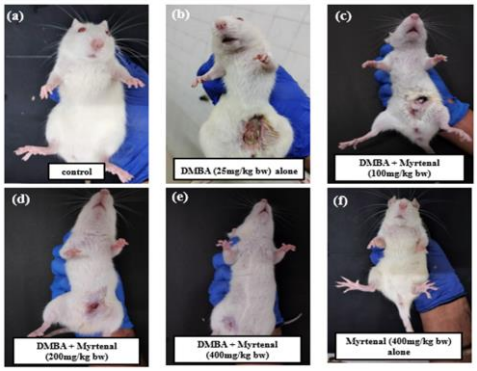

Fig. 1. Morphological appearance of mammary tumors in rats

(a) Control and (f) Myrtenal alone (400mg/kg) group shows no visible lesions. (b) DMBA alone treated rats displayed palpable mammary adenocarcinoma. (c - e) Myrtenal treatment (100-400 mg/kg) reduced DMBA-induced tumor growth in a dose-dependent manner.

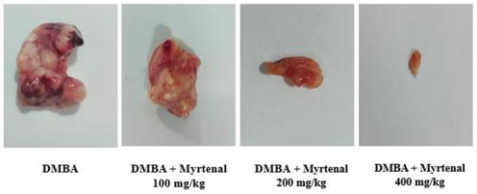

Fig. 2. Images showing mammary tumors excised from various treatment groups

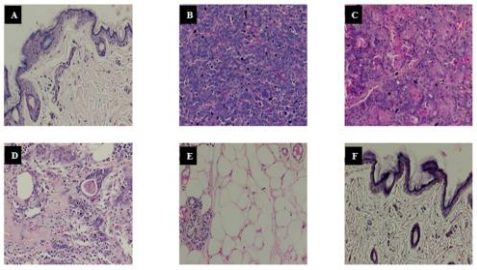

Fig. 3. Microscopic images showing histopathological alterations in mammary tissues of control and treated animals (H & E x40)

(A) Group I (Control) mammary tissue shows normal glandular structures with normal surface epithelium. (B) Group II (Breast cancer bearing rats) shows an invasive neoplasm composed of pleomorphic epithelial cells arranged in solid sheets indicating metaplastic infiltrating ductal carcinoma. (C) Group III (DMBA+ Myrtenal 100mg/kg) shows vast areas of necrosis surrounded by fibroblast proliferation, indicating severe dysplasia. (D) Group IV (DMBA+ Myrtenal 200mg/kg) shows ductal hyperplasia with moderate dysplastic epithelium. (E) Group V (DMBA+ Myrtenal 400mg/kg) shows hyperplastic ducts with mild dysplastic epithelium. (F) Group VI (Myrtenal 400mg/kg alone treated rats) shows normal breast ducts with normal surface epithelium.

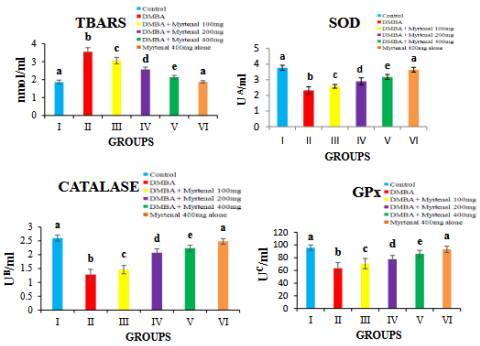

Fig. 4. Plasma TBARS levels and enzymatic antioxidant status in experimental rats (n=8).

Differences between groups were considered significant, as denoted by different superscript letters. A – Enzyme activity required for 50% inhibition of nitroblue tetrazolium reduction, B – µM of H_2O_2 consumed per second, C – µM of glutathione consumed per minute

Control rats and those treated with myrtenal alone exhibited normal tissue architecture. In contrast, DMBA-treated rats (Group II) showed pronounced epithelial cell proliferation, multilayered epithelium, increased cellular density, nuclear atypia and malignant infiltration. Treatment with myrtenal

significantly reduced these pathological features, indicating a protective effect against DMBA-induced cellular proliferation.

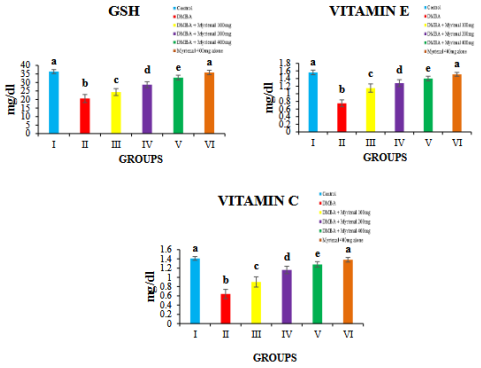

Fig. 5. Plasma non-enzymatic antioxidant status in experimental rats (n=8).
Differences between groups were considered significant, as denoted by different superscript letters.

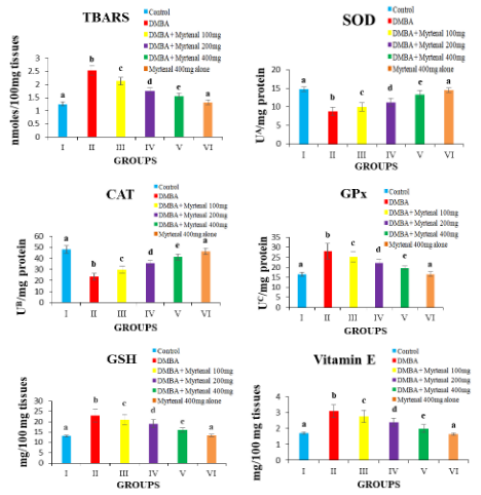

Fig. 6. Mammary tissues TBARS and antioxidants status in experimental rats (n=8)
Differences between groups were considered significant, as denoted by different superscript letters. A – Enzyme activity required for 50% inhibition of nitroblue tetrazolium reduction, B – µM of H_2O_2 consumed per second, C – µM of glutathione consumed per minute.

The myrtenal's efficacy on lipid peroxidation and antioxidant status in plasma are illustrated in Figs. 4 and 5. DMBA alone administered rats exhibited elevated TBARS levels, indicating increased lipid peroxidation. The animals also exhibited reduced antioxidant enzyme activities and lowered concentrations of non-enzymatic antioxidants. Oral treatment with myrtenal decreased TBARS levels and restored antioxidant enzyme and non-enzymatic antioxidant levels in a dose-dependent manner. There was no notable difference between the control group and the group treated with myrtenal alone.

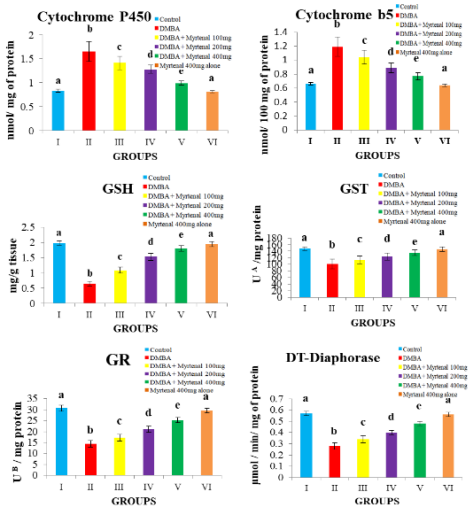

Fig. 7. Liver phase I and II detoxification enzymes in experimental animals (n=8)
Differences between groups were considered significant, as denoted by different superscript letters. A – µM of CDNB-GSH conjugate formed per hour, B – µM of NADPH oxidized per hour, C – µM of 2,6-dichlorophenol indophenol reduced per minute.

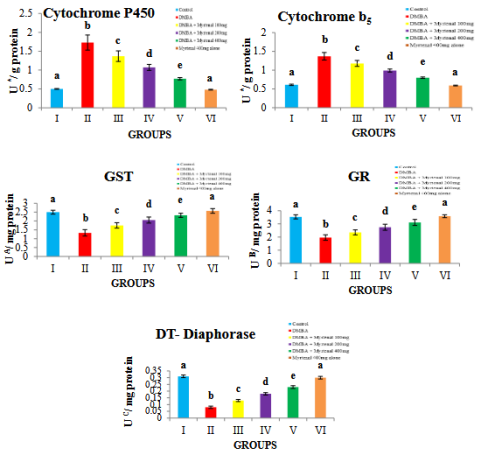

Fig. 8. Mammary tissue phase I and II detoxification enzymes in experimental animals (n=8)
Differences between groups were considered significant, as denoted by different superscript letters. A- µM of CDNB-GSH conjugate formed per hour, B–

µM of NADPH oxidized per hour, C– µM of 2,6-dichlorophenol indophenol reduced per minute.

The myrtenal's efficacy on lipid peroxidation and antioxidant status in breast tumor tissues is shown in Fig. 6. Rats treated with DMBA alone exhibited significantly elevated TBARS levels accompanied by lowered antioxidant activities, indicating increased oxidative stress. Oral administration of myrtenal led to a dose-dependent reduction in TBARS levels and restoration of both enzymatic and non-enzymatic antioxidant levels. There was no notable difference between the control group and the group treated with myrtenal alone.

Figs. 7 and 8 depict the impact of myrtenal on phase I and phase II detoxification enzymes in liver and mammary tissues. DMBA-treated rats showed a notable rise in phase I enzymes (CYP450 and cytochrome b5) and a significant reduction in phase II enzymes (GSH, GST, GR and DT-diaphorase) when compared to the control group. Oral administration of myrtenal at 100, 200 and 400 mg/kg body weight significantly downregulated phase I enzymes and upregulated phase II enzyme activities in a dose-dependent manner in both tissues. These changes were statistically significant when compared to the DMBA only treated group. There was no notable difference between the control group and the group treated with myrtenal alone.

DISCUSSION

Chemoprevention research aims to reduce the burden of cancer by identifying the protective effects of phytochemicals (Wali *et al.,* 2025). Myrtenal, a naturally occurring monoterpene found in herbs such as mint, cumin and black pepper, has demonstrated strong anticancer potential (Kury *et al.,* 2021). This investigation explored the potential of myrtenal to prevent the growth of mammary tumors.

Histological assessment is essential for understanding tumor progression. The DMBA-induced mammary cancer model is well-established for mimicking human breast cancer, particularly due to its origin in ductal epithelial cells, similar to human tumors (Hollern *et al.,* 2018). This model closely resembles the histopathological, biochemical and morphological features of human breast carcinoma (Costa *et al.,* 2002). In our study, 100% tumor incidence with infiltrating duct carcinoma and severe dysplasia was observed in DMBA-only treated rats, confirmed by histopathology. In contrast, rats administered myrtenal at 400 mg/kg exhibited a 75% reduction in tumor occurrence (only 2 out of 8 rats developed tumors) and the remaining 25% showed only minimal tumor burden and mild dysplastic features. These findings highlight myrtenal's ability to preserve tissue architecture and suppress carcinogenic progression, consistent with earlier reports on its protective role against various cancers (Lingaiah *et al.,* 2013; Boopathy *et al.,* 2024).

Lipid peroxidation, driven by reactive oxygen species (ROS), disrupts cellular membranes integrity and metabolic functions. Elevated TBARS levels in DMBA-induced rats confirmed increased oxidative damage, in line with prior observations in mammary tumors (Lakshmi and Subramanian, 2014). Treatment with myrtenal significantly decreased TBARS levels, suggesting its potent free radical scavenging and anti-lipid peroxidation properties. Oxidative stress, resulting from excessive ROS and diminished antioxidant defense, is a well-known contributor to carcinogenesis (Basak *et al.,* 2020). In our study, DMBA administration led to a marked depletion of antioxidant enzyme activity, which was restored dose-dependently by myrtenal, corroborating earlier reports (Kolanjiappan and Manoharan, 2005; Dong *et al.,* 2025).

Interestingly, some studies report elevated GPx and GSH levels in tumors, likely reflecting adaptive responses to high oxidative stress (Adelegan *et al.,* 2024).

Lokeshkumar *et al.* (2015) showed that myrtenal suppressed colon cancer via activation of endogenous antioxidant enzymes. Buddhan *et al.* (2020) reported similar effects in oral carcinogenesis, while Korkmaz

and Tekin (2024) demonstrated that pure myrtenal significantly inhibited MCF-7 breast cancer cell viability in vitro. These studies support myrtenal's role in attenuating cancer progression by modulating oxidative stress and promoting apoptosis, aligning with our results showing antioxidant enhancement in myrtenal-treated DMBA rats.

Environmental carcinogens like DMBA are bioactivated by phase I enzymes (e.g., CYP450 and CYb5) into electrophilic intermediates that bind DNA. Phase II enzymes (e.g., GST, GR, DT-diaphorase) facilitate detoxification through conjugation with nucleophiles like GSH, enhancing excretion and reducing carcinogen-induced damage (Iacopetta *et al.,* 2023; Wen *et al.,* 2013).

Our study revealed elevated phase I enzyme activity and reduced phase II enzyme levels in DMBA-only rats. Myrtenal administration by the oral route markedly downregulated phase I enzymes while upregulating key phase II enzymes activities in both hepatic and mammary tissues, thereby re-establishing the cellular detoxification equilibrium. These findings are consistent with Mathivadhani *et al.* (2007) and Babu *et al.* (2012) who also observed that myrtenal mitigated DEN-PB-induced liver cancer by normalizing the activities of detoxifying enzymes. Collectively, these findings indicate that myrtenal may reduce the carcinogenic burden by inhibiting metabolic activation of procarcinogens and promoting their detoxification and elimination.

CONCLUSION

This investigation offers compelling support for the protective efficacy of myrtenal in preventing DMBA-triggered mammary tumor development. Oral administration of myrtenal effectively mitigated oxidative stress by modulating oxidative stress biomarkers and phase I and phase II detoxifying enzymes activities. These biochemical investigations were further supported by histopathological observations, which revealed preservation of normal mammary tissue architecture in myrtenal-treated animals. Collectively, these findings suggest that myrtenal may serve as a promising natural compound for breast cancer prevention. Additional molecular studies are necessary to unravel the precise pathways and targets through which myrtenal exerts its chemopreventive effects.

REFERENCES

Adelegan AA, Talabi AA, Dokunmu TM, Iweala EEJ. 2024. Anticancer activity of ethyl acetate fraction and ethanol leaf extract of *Olax subscorpioidea* against DMBA-induced female rats. Tropical Journal Natural Product Research **8**(1), 6039-6044. https://doi.org/10.26538/tjnpr/v8i1.47.

Al Kury LT, Abdoh A, Ikbariah K, Sadek B, Mahgoub M. 2021. In Vitro and In Vivo Antidiabetic Potential of Monoterpenoids: An Update. Molecules **27**(1), 182. https://doi.org/10.3390/molecules27010182

Basak D, Uddin MN, Hancock J. 2020. The Role of Oxidative Stress and Its Counteractive Utility in Colorectal Cancer (CRC). Cancers (Basel) **12**(11), 3336. https://doi.org/10.3390/cancers12113336

Beutler E, Kelly BM. 1963. The effect of sodium nitrite on red cell GSH. Experientia **19**, 96-97. https://doi.org/10.1007/BF02148042

Boopathy LK, Roy A, Gopal T, Kandy RRK, Arumugam MK. 2024. Potential molecular mechanisms of myrtenal against colon cancer: A systematic review. Journal of Biochemical and Molecular Toxicology **38**(1), e23525. https://doi.org/10.1002/jbt.23525.

Booupathy LK, Venkatachalam S, Natarajan N, Thamaraiselvan R, Arumugam M, Periyasamy BM. 2016. Chemopreventive effect of myrtenal on bacterial enzyme activity and the development of 1,2-dimethyl hydrazine-induced aberrant crypt foci in Wistar Rats. Journal of Food and Drug Analysis **1**, 206-213. https://doi.org/10.1016/j.jfda.2015.07.003.

Buddhan R, Manoharan S, Naidu RM, Karthik M, Neelakandan M. 2020. Chemopreventive potential of myrtenal in 7,12-dimethylbenz(a)anthracene induced experimental oral carcinogenesis in golden Syrian hamsters. Journal of Clinical and Diagnosis Research **14**(4), XC01-XC06.
https://doi.org/10.7860/JCDR/2020/43399.13644

Burguin A, Diorio C, Durocher F. 2021. Breast Cancer Treatments: Updates and New Challenges. Journal of Personalized Medicine **11**(8), 808.
https://doi.org/10.3390/jpm11080808.

Carlberg I, Mannervik B. 1985. Glutathione reductase. Methods in Enzymology **113**, 484-90.
https://doi.org/10.1016/s0076-6879(85)13062-4.

Costa I, Solanas M, Escrich E. 2002. Histopathologic characterization of mammary neoplastic lesions induced with 7,12 dimethylbenz(alpha)anthracene in the rat: a comparative analysis with human breast tumors. Archives of Pathology & Laboratory Medicine **126**(8), 915-927.
https://doi.org/10.5858/2002-126-0915-HCOMNL

Desai ID. Vitamin E analysis methods for animal tissues. 1984. Methods in Enzymology **105**, 138-147.
https://doi.org/10.1016/s0076-6879(84)05019-9.

Di Carlo E, Sorrentino C. 2024. Oxidative Stress and Age-Related Tumors. Antioxidants (Basel) **13**(9), 1109.
https://doi.org/10.3390/antiox13091109

Dong R, Wang J, Guan R, Sun J, Jin P, Shen J. 2025. Role of Oxidative Stress in the Occurrence, Development, and Treatment of Breast Cancer. Antioxidants (Basel) **14**(1), 104.
https://doi.org/10.3390/antiox14010104.

Dragomanova S, Andonova V, Volcho K, Salakhutdinov N, Kalfin R, Tancheva L. 2023. Therapeutic Potential of Myrtenal and Its Derivatives-A Review. Life (Basel) **13**(10), 2086.
https://doi.org/10.3390/life13102086.

Ernster L. 1958. Diaphorase activities in liver cytoplasmic fractions. Federation Proceedings **17**(1), 9650.

Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. Journal of Biological Chemistry **249**(22), 7130-7139.

Hari Babu L, Perumal S, Balasubramanian MP. 2012. Myrtenal attenuates diethylnitrosamine-induced hepatocellular carcinoma in rats by stabilizing intrinsic antioxidants and modulating apoptotic and anti-apoptotic cascades. Cellular Oncology (Dordrecht) **35**(4), 269-283.
https://doi.org/10.1007/s13402-012-0086-4.

Hollern DP, Swiatnicki MR, Andrechek ER. 2018. Histological subtypes of mouse mammary tumors reveal conserved relationships to human cancers. PLoS Genetics **14**(1), e1007135.
https://doi.org/10.1371/journal.pgen.1007135

Iacopetta D, Ceramella J, Catalano A, Scali E, Scumaci D, Pellegrino M, Aquaro S, Saturnino C, Sinicropi MS. 2023. Impact of cytochrome P450 enzymes on the phase I metabolism of drugs. Applied Sciences **13**(10), 6045.
https://doi.org/10.3390/app13106045.

Jena AB, Samal RR, Bhol NK, Duttaroy AK. 2023. Cellular Red-Ox system in health and disease: The latest update. Biomedicne and Pharmacotherapy **162**, 114606.
https://doi.org/10.1016/j.biopha.2023.114606.

Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. 2023. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Archives of Toxicology **97**(10), 2499-2574.
https://doi.org/10.1007/s00204-023-03562-9.

Kakkar P, Das B, Viswanathan PN. 1984. A modified spectrophotometric assay of superoxide dismutase. Indian Journal of Biochemistry & Biophysics **21**(2), 130-132.

Karnam KC, Ellutla M, Bodduluru LN, Kasala ER, Uppulapu SK, Kalyankumarraju M, Lahkar M. 2017. Preventive effect of berberine against DMBA-induced breast cancer in female Sprague Dawley rats. Biomedicine & Pharmacotherapy **92**, 207-214. https://doi.org/10.1016/j.biopha.2017.05.069.

Kolanjiappan K, Manoharan S. 2005. Chemopreventive efficacy and anti-lipid peroxidative potential of *Jasminum grandiflorum Linn*. on 7,12-dimethylbenz(a)anthracene-induced rat mammary carcinogenesis. Fundamental & Clinical Pharmacology **19**(6), 687-693. https://doi.org/10.1111/j.1472-8206.2005.00376.x.

Korkmaz E, Tekin S. 2024. Cytotoxicity of myrtenal on different human cancer cell lines. Annals of Medical Research **31**(5), 404-408. https://doi.org/10.5455/annalsmedres.2024.04.065

Lakshmi A, Subramanian S. 2014. Chemotherapeutic effect of tangeretin, a polymethoxylated flavone studied in 7, 12-dimethylbenz(a)anthracene induced mammary carcinoma in experimental rats. Biochimie **99**, 96-109. https://doi.org/10.1016/j.biochi.2013.11.017.

Lingaiah HB, Natarajan N, Thamaraiselvan R, Srinivasan P, Periyasamy BM. 2013. Myrtenal ameliorates diethylnitrosamine-induced hepatocarcinogenesis through the activation of tumor suppressor protein p53 and regulation of lysosomal and mitochondrial enzymes. Fundamental & Clinical Pharmacology **27**(4), 443-54. https://doi.org/10.1111/j.1472-8206.2012.01039.x.

Lokeshkumar B, Sathishkumar V, Nandakumar N, Rengarajan T, Madankumar A, Balasubramanian MP. 2015. Anti-Oxidative Effect of Myrtenal in Prevention and Treatment of Colon Cancer Induced by 1, 2-Dimethyl Hydrazine (DMH) in Experimental Animals. Biomolecules & Therapeutics **5**, 471-478. https://doi.org/10.4062/biomolther.2015.039

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry **193**(1), 265-275.

Mathivadhani P, Shanthi P, Sachdanandam P. 2007. Effect of *Semecarpus anacardium* Linn. nut extract on mammary and hepatic expression of xenobiotic enzymes in DMBA-induced mammary carcinoma. Environmental Toxicology and Pharmacology **23**(3), 328-34. https://doi.org/10.1016/j.etap.2006.12.004.

Obeagu EI, Obeagu GU. 2024. Breast cancer: A review of risk factors and diagnosis. Medicine **103**(3), e36905. https://doi.org/10.1097/MD.0000000000036905.

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry **95**(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Omaye ST, Turnbull JD, Sauberlich HE. 1979. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods in Enzymology **62**, 3-11. https://doi.org/10.1016/0076-6879(79)62181-x.

Omura T, Sato R. 1964. The Carbon Monoxide-Binding Pigment Of Liver Microsomes. I. Evidence For Its Hemoprotein Nature. Journal of Biological Chemistry **239**, 2370-2378.

Palan PR, Mikhail MS, Basu J, Romney SL. 1991. Plasma levels of antioxidantβ-carotene and α-tocopherol in uterine cervix dysplasias and cancer. Nutrition and Cancer **15**(1), 13–20. https://doi.org/10.1080/01635589109514106.

Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. 1973. Selenium: biochemical role as a component of glutathione peroxidase. Science **179**(4073), 588-590. https://doi.org/10.1126/science.179.4073.588.

Sinha AK. 1972. Colorimetric assay of catalase. Analytical Biochemistry **47**(2), 389-394. https://doi.org/10.1016/0003-2697(72)90132-7.

Townsend DM, Tew KD. 2003. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene **22**(47), 7369-7375. https://doi.org/10.1038/sj.onc.1206940

Wali AF, Pillai JR, Talath S, Shivappa P, Sridhar SB, El-Tanani M, Rangraze IR, Mohamed OI, Al Ani NN. 2025. Phytochemicals in Breast Cancer Prevention and Treatment: A Comprehensive Review. Current Issues in Molecular Biology **47**(1), 30. https://doi.org/10.3390/cimb47010030.

Wang Z, Zhang X. 2017. Chemopreventive Activity of Honokiol against 7, 12 - Dimethylbenz[a]anthracene-Induced Mammary Cancer in Female Sprague Dawley Rats. Frontiers in Pharmacology **8**, 320. https://doi.org/10.3389/fphar.2017.00320.

Wen H, Yang HJ, An YJ, Kim JM, Lee DH, Jin X, Park SW, Min KJ, Park S. 2013. Enhanced phase II detoxification contributes to beneficial effects of dietary restriction as revealed by multi-platform metabolomics studies. Molecular & Cellular Proteomics **12**(3), 575-586. https://doi.org/10.1074/mcp.M112.021352.

Yagi K. 1987. Lipid peroxides and human diseases. Chemistry and Physics of Lipids **45**(2-4), 337-351. https://doi.org/10.1016/0009-3084(87)90071-5

Zhang Y, Ji Y, Liu S, Li J, Wu J, Jin Q, Liu X, Duan H, Feng Z, Liu Y, Zhang Y, Lyu Z, Song F, Song F, Yang L, Liu H, Huang Y. 2025. Global burden of female breast cancer: new estimates in 2022, temporal trend and future projections up to 2050 based on the latest release from GLOBOCAN. Journal of the National Cancer Center **1**, 287-296. https://doi.org/10.1016/j.jncc.2025.02.002.

Zhang YJ, Gan RY, Li S, Zhou Y, Li AN, Xu DP, Li HB. 2015. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. Molecules **20**(12), 21138-21156. https://doi.org/10.3390/molecules201219753.