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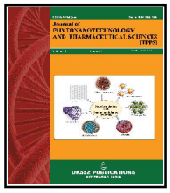

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Microwave-assisted extraction, *in vitro* antioxidant and anticancer activity of *Curcuma longa* L. and *Manilkara zapota* L.

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Abstract

This study is aimed to investigate the synergistic effect of *Manilkara zapota* L. and *Curcuma longa* L. extracts when combined in various combinations (MC-1, MC-2, MC-3, MC-4, and MC-5) for their potential antioxidant activity using the DPPH and ABTS assay as well as anticancer activity. The extracts were prepared using a microwave-assisted extraction method which greatly improved bioactive component extraction from *C. longa* and *M. zapota*. The study demonstrated that both *C. longa* and *M. zapota* extracts individually exhibited significant antioxidant activity and anticancer activity, inhibiting cell proliferation and inducing cell death. However, when combined in different combinations, a synergistic effect was observed, resulting in enhanced anticancer activity compared to the individual extracts alone. The synergistic combinations (MC-1, MC-2, MC-3, MC-4 and MC-5), showed increased cytotoxicity and apoptosis-inducing effects in cancer cells and increased scavenging activity against DPPH and ABTS radicals. These findings suggest the potential of utilizing the synergistic effects of *C. longa* and *M. zapota* extracts for developing novel combination therapies with enhanced anticancer properties. Further research is warranted to elucidate the underlying mechanisms and identify the specific bioactive compounds responsible for this synergistic effect.

1. Introduction

Cancer is a complicated and multidimensional illness characterized by the body's aberrant cells growing and dividing out of control. It is the number one killer in the world and puts a heavy strain on people, families, and healthcare systems (Ferley *et al.,* 2000). Any portion of the body can experience the onset of cancer, which can infect surrounding tissues and spread to distant locations through a process known as metastasis. There are many different varieties of cancer, and each has unique traits, risk factors, and treatment modalities (Hejmadi *et al.,* 2014). Multiple variables contribute to cancer development, including genetic predisposition, carcinogen exposure, lifestyle decisions, and other environmental factors. Early diagnosis techniques, a better understanding of the disease, and various treatment choices, including surgery, chemotherapy, radiation therapy, targeted medicines, immunotherapy, and precision medicine, have all been made possible by cancer research and treatment advancements. Even though, there has been progress, there are still hurdles (Mukavi *et al.,* 2020). In order to improve patient outcomes and quality of life, ongoing efforts are concentrated on prevention, early detection, and the development of more efficient and personalized techniques to diagnose and treat cancer (Dinicola *et al.,* 2014).

The potential healing properties of various medicinal plants have led to a great deal of attention on herbal remedies for preventing and treating cancer. Many herbs have bioactive chemicals that are antioxidants, anti-inflammatory, and anticancer. Some examples are turmeric, green tea, garlic, ginger, and ginseng (Greenwell *et al.,* 2015). The ability of these herbs to limit tumor growth, increase apoptosis, decrease angiogenesis, and alter signalling pathways involved in cancer formation and progression has been examined. While herbal treatments show promise, more research is required to fully understand their mechanisms of action, optimize dosages, and evaluate their efficacy and safety in clinical trials before they can be recommended as standalone treatments or combined with conventional cancer management therapies (Mazumder *et al.,* 2022).

Turmeric (*Curcuma longa* L.), a perennial herb in the ginger family (Zingiberaceae), is used in Indian cookery, skincare, and traditional Indian and Chinese medicine. Turmeric rhizomes contain 75% curcumin, the bioactive component. Turmeric rhizomes also contain demethoxycurcumin (10-20%) and bis-demethoxycurcumin (5%). Curcuminoids are turmeric's three offshoots. Curcumin is antioxidant, antibacterial, anti-inflammatory, hepatoprotective, antitumor, and antiviral. Curcumin prevents and treats digestive tract, breast, lung, skin, head and neck, nervous system, and sarcoma cancers. Turmeric powder's antioxidant potency depends on total curcuminoids. Curcumin, a hydrophobic polyphenol, is turmeric's major component. Curcumin is IUPAC (1E, 6E).1,7-bis (4-hydroxy-3-methoxyphenyl-1, 6-heptadiene-3, 5-dione. Seven-carbon β-unsaturated β-diketone unites two aromatic ring systems with o-methoxy phenolic groups. The diketo group has keto-enol tautomerism in multiple conformers (Desai *et al.,* 2008).

Manilkara zapota L. belongs to the family Sapotaceae. The sapotaceae family is a diverse family of flowering plants, commonly known as the Sapodilla family. It includes various tropical and subtropical tree

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species that are distributed across different regions of the world. *M. zapota*, medium-sized sapodilla trees feature glossy, dark green leaves. Small bell-shaped flowers produce round or oval fruits. As fruits ripen, their rough, brownish skin relaxes and darkens. When fully matured, the fruits' soft, brown pulp tastes like caramel, pear, and brown sugar. Vitamins, minerals, antioxidants, and dietary fiber make *M. zapota* fruit healthy.Traditional medicine uses the delectable *M. zapota* fruit for its health benefits. Bark, leaves, and fruit are antibacterial, anti-inflammatory, antidiabetic, and antioxidant. Antioxidants shield cells from cancer-causing free radicals (Mohanapriya *et al.,* 2019). *M. zapota* antioxidants reduce oxidative stress and inflammation, protecting cells and preventing cancer. *M. zapota* extracts destroy cancer cells in several studies. These extracts reduce cancer cell proliferation and promote apoptosis (Angelica *et al.,* 2022).

Using a solvent, "extraction" separates a plant's medicinally active components. Marc is produced by solubilizing plant material in a solvent while leaving inert materials undissolved. The solubility gradient between the solute, other molecules, and stabilizing solvent drives extraction (Ingle *et al.,* 2017). Soxhlet extraction, solvent extraction, and hydrodistillation are standard extraction procedures. These methods are simple but rarely selective, require a long time, and can damage heat-sensitive components. It encompasses solvent extraction, hydrodistillation, infusion, digesting, decoction, and continuous hot extraction (Soxhlet) (Altemimi *et al.,* 2017).

Ultrasound, microwave, enzyme, and supercritical liquid extraction are new methods. These novel extraction methods are ecologically friendly and technologically advanced.

Microwave-assisted extraction (MAE), the latest extraction method, consumes less solvent, is faster, and extracts more. Effective MAE extraction of phenolics, flavonoids, essential oils, and glycosides is done. Microwaves enhance solvent diffusion and bioactive component extraction. Microwaves generate heat. Solvent polarity impacts conversion. MAE heats compounds faster, lowers temperature gradients, yields more, and requires less equipment. This ecological approach for extracting organic and organometallic compounds uses less organic solvent (Hamuel *et al.,* 2012). Variable microwave extraction times helped plant metabolite research generate quality results. Nature of the solvent, type of plant material used, contributes towards determination of extraction time (Wang *et al.,* 2007). Most favorable combination of microwave power and extraction time maximizes yield. Most extractions require low power and lengthy irradiation time (Javad *et al.,* 2014). But in various experiments, high power and short irradiation duration also yield high extraction. And high power and longer extraction time destroy active plant compounds, so the selection of suitable microwave power and extraction is essential for extraction. Closed-container extraction at increased temperatures enhances target compound mass transfer from the sample matrix. The extraction requires 15-30 min and 10-30 ml solvent. MAE has extracted plant extract under various conditions. Researchers explore microwave power, irradiation time, cooling time, temperature, solvent type, plant material size, and extraction. MAE uses high-dielectric constant solvents (Hamid *et al.,* 2021).

In this research, the *C. longa* dry powder and the *M. zapota* leaves were extracted using MAE, and their antioxidant and anticancer activities were tested *in vitro*.

2. Materials and Methods

2.1 Collection and preparation of plant material

Fresh plant leaves of *Manilkara zapota* L. (Sapotaceae) were collected from Tirumala Hills, Tirupati, Chittoor District, Andhra Pradesh, India, in January (Voucher specimen No. 4527). The collected leaves were cleaned, dried, and then blended. After the leaves were ground into a powder, they were placed in an airtight container and kept at room temperature. The powder of *Curcuma longa* L. was taken from the local market.

2.2 Microwave-assisted extraction

In order to extract the active constituent of *C. longa*, 0.5 g of turmeric powder must be dissolved in 10 ml of acetone and then placed in a microwave chamber. The extraction is carried out at various microwave operating powers between 100 and 450 W and irradiation periods between 0.5 and 3 min for optimal yield. The samples were treated to three minutes of 300W power microwave irradiation, starting with a 30-second microwave exposure, then 30-second cooling, and another 30-second microwave exposure. This intermittent method of irradiation-cooling-irradiation is used only because longer exposure times and greater radiation powers cause the solvent to boil and the active ingredients in turmeric powder to degrade. Following extraction, the solvent is evaporated, the residue is weighed, and it is dissolved in a 95:5 solution of chloroform and methanol for column chromatography. The extraction process of *M. Zapota* was done by taking 1 g of powder dissolved in a methanol and acetone mixture (40:60) and then placed in a microwave chamber and performed the same experiment as for *C. longa*.

Table 1: Different combinations of extracts of *C. longa* (CL) and *M. Zapota* (MZ)

Code	Combination of plant extracts (CL:MZ)
MC-1	1:4
MC-2	2:3
MC-3	3:2
MC-4	4:1
MC-5	2:2

2.3 Determination of antioxidant activity by DPPH (1, 1dip heny l2 picrylhydrazyl) assay

For evaluating radical scavenging activity, the previously established DPPH test technique was adopted (Brand *et al.,* 1995).The results regarding % inhibition per 100 µg dry sample weight in methanol were expressed using a calibrated ascorbic acid reference curve. The statistical results acquired are expressed as the mean of three independent replications.

2.4 ABTS (2, 22 azinobis (3 ethylbenzothiazoline 6 sulfonic acid)) free radical assay

In brief, this study used 100 µg of methanol extracts from each plant. Spectrophotometric analysis of a decolorization experiment based on 2, 22 -azinobis 3-ethylbenzothiazoline-6-sulfonic acid (Re *et al.,* 1999) was used to assess the free radical scavenging activity.

2.5 *In vitro* cytotoxicity (MTT assay)

As an indicator of cell viability, proliferation, and cytotoxicity, the MTT ((3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium

bromide) assay quantifies cellular metabolic activity. This colorimetric assay relies on the ability of metabolically active cells to convert yellow tetrazolium salt into purple formazan crystals (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT). The antiproliferative effects of combination of extracts on Skmel-23 cells were evaluated using the MTT assay. The skmel-23 human melanoma cell line was obtained from the National Centre for Cell Sciences (NCCS) in Pune, India, and then sub-cultured at PGP Life Sciences in Hyderabad.The rationale for selecting Skmel-23 cell line lies in the evaluation of curcumin on the melanoma cell. As curcumin is traditionally reported to have many benefits on the skin, its effect in treating melanoma is evaluated. Similarly, as *M. Zapota* was reported to possess anticancer properties, the combination of *M. Zapota* and *C. longa* was evaluated for their anti-proliferative effect against Skmel-23 cells. The original source of the cell line was American culture collections (ATCC number: HTB-71) (Divya *et al.,* 2023).

3. Results

3.1 Antioxidant activities

This study investigates the IC_{50} values of MC-1, MC-2, MC-3,MC-4 and MC-5 using the DPPH and ABTS assays. Our results inferred that the combination of extracts showed potential antioxidant activity as compared to the standard drugs.

In DPPH radial scavenging activity, the combination of extracts elicited prominent antioxidant activity. The IC_{50}values indicate that both the extracts possessed antioxidant activity and the results are comparable with that of the standard ascorbic acid. Both the extracts when employed in equal concentration (2:2 ratio) exhibited synergistic effect and the IC_{50}value was found to be 4.16 µg/ml which is very much nearer to the standard (Table 2).

The results of ABTS assay were found to be similar to that of DPPH assay. All the combination of extracts showed good antioxidant activity, and the activity was enhanced when combined in equivalent ratio with an IC_{50} value of 3.32 µg/ml (Table 2).

Table 2: The IC_{50} values of various combinations of extracts using DPPH and ABTS assays

Sample	DPPH assay IC_{50} (µg/ml)	ABTS assay IC_{50} (µg/ml)
MC-1	5.45	5.98
MC-2	6.12	4.75
MC-3	6.32	4.35
MC-4	5.39	5.68
MC-5	4.16	3.32
Standard*	3.98	2.98

The values are represented as average mean ± SEM *For DPPH assay, Ascorbic acid (Vit-C) is used as standard. For ABTS assay, Trolox (Vit-E) is used as standard.

3.2 Anticancer activity

The anticancer potential of the combination of extracts assessed using MTT assay revealed potential anticancer effects of both the extracts when used in combination. The IC_{50} value of the combination of *C. longa* and *M. zapota* when used in the ratio of 2:2 elicited highest efficacy to inhibit Skmel-23 cells (Table 3).

Table 3: The comparison of IC_{50} values of MC-1 to MC-5 on Skmel-23 cells

Extract	IC_{50} (µg/ml)
MC-1	40.11 ± 3.96
MC-2	59.32 ± 6.54
MC-3	60.45 ± 5.34
MC-4	52.22 ± 4.22
MC-5	25.32 ± 2.88
Doxorubicin	15.75 ± 1.12

4. Discussion

Herbal medicine uses plants and plant extracts for therapeutic purposes. It is a centuries-old healing practice in many cultures. These plant-derived chemicals comprise various active components that may have pharmacological effects. They promote natural therapies to boost the body's healing capacities and restore balance. Despite continuous modern research, traditional herbal medicines have shown promise in treating many health ailments.

MC is a various combination of extracts (Table 1) that consists of *M. Zapota* and *C. longa*. The extract is prepared by the microwave-assisted method. These plants contain a variety of phytochemicals with potential health benefits such as antioxidant and anti-inflammatory and have been used traditionally to treat various ailments. The study reveals the individual extracts of *C. longa* and *M. Zapota* as significant anticancer activity, inhibiting cell proliferation and inducing cell death in the tested cancer cell lines.The study aimed to see whether these natural extracts could work together to suppress cancer cell growth and encourage cell death. However, our study has demonstrated encouraging results, when these extracts were combined in different combinations (MC-1, MC-2, MC-3, MC-4 and MC-5), a synergistic effect was observed, leading to enhanced anticancer activity.The extract MC-5 showed the maximum synergistic effect within the group. The specific bioactive compounds responsible for this synergistic effect need further elucidation through advanced analytical techniques and identification methods. Antioxidants neutralize reactive oxygen species and hydroxyl radicals. Free radicals accumulate and harm cells and are thought to be at the root of many diseases. In this investigation, we tested the efficacy of a *C. longa*/ *M. Zapota* extract combo on antioxidant activity. The findings of the antioxidative analytical methods demonstrated that MC-5 has significant antioxidant potential. The test extracts' effect on scavenging DPPH and ABTS radicals has been attributed to their ability to donate hydrogen. Based on these findings, all four extracts (MC-1, MC-2, MC-3, MC-4 and MC-5) possessed promising antioxidant and anticancer properties. Further more, our study indicated that MC-5 exhibited good cytotoxic and antiproliferative effects against Skmel-23 cancer cells.

5. Conclusion

In conclusion, the combination of *C. longa* and *M. Zapota* extracts has demonstrated a synergistic antioxidant and anticancer effect, showing greater efficacy in inhibiting cancer cell growth and promoting

cell death than individual extracts alone. For extraction, green synthetic method, microwave-assisted extraction method was used. The 300W power microwave irradiation of three minutes reduces longer exposure time and it is most suitable radiation powers to maximize the extraction yield of plants. These findings highlight the potential of utilizing natural extracts in combination therapies for enhanced anticancer strategies.

However, the results of this study imply that MC-5, which contains equal concentration of both the plant extracts, could be a useful adjuvant biological in cancer treatment. It can be the significant bioactive molecule responsible for synergistic therapeutic potential. It shows promise as an adjuvant chemical for the treatment of cancer.

Conflict of interest

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

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