RESEARCH
www.commprac.com
ISSN 1462 2815

EXPLORING THE SYNERGISTIC POTENTIAL OF METHANOLIC EXTRACTS FROM CARISSA CARANDAS AND COLOCASIA ESCULENTA LEAVES: A COMPREHENSIVE INVESTIGATION INTO ANTIBACTERIAL, ANTIFUNGAL, AND ANTICANCER ACTIVITIES THROUGH IN-VITRO STUDIES

Swati Parcha [1], Mayank Dwivedi [2], Anuj Mittal [3], Seema [4], Dil Prasad Subba [5] and Dr. Satyender Kumar [6]*

[1,2,3,4,6] Department of Pharmaceutical Sciences,
HIMT College of Pharmacy, Greater Noida, Uttar Pradesh.
[5,6] School of Pharmacy, Sharda University, Knowledge Park-3,
Greater Noida, Uttar Pradesh. *Corresponding Author Email: sjinagal@gmail.com

DOI: 10.5281/zenodo.11083701

Abstract

This study investigates the synergistic effects of methanolic extracts from Carissa carandas (MECC) and Colocasia esculenta (MECE) leaves on antibacterial, antifungal, and anticancer activities. The rationale behind this research stems from the long-standing traditional use of Carissa carandas and Colocasia esculenta in treating various diseases, including diabetes, inflammation, bacterial and fungal infections, and cancer. The aim was to assess whether the combination of these plant extracts could enhance their therapeutic properties compared to their individual effects. Methanolic extracts of Carissa carandas and Colocasia esculenta were prepared, and their phytochemical compositions were analyzed using gas chromatography-mass spectrometry (GC-MS). Antibacterial activity was evaluated against Pseudomonas aerginosa and Staphylococcus aureus, while antifungal activity was assessed against Candida albicans and Aspergillus niger. Minimum inhibitory concentrations (MICs) were determined using microdilution assays. Additionally, anticancer activity was examined using the MTT assay on MCF-7 breast cancer cells. The results revealed that the combination of MECC and MECE exhibited enhanced antibacterial, antifungal, and anticancer activities compared to individual extracts. GC-MS analysis showed the presence of similar phytochemicals in both extracts, including hexadecanoic acid methyl ester, stigmasta-3,5-diene, and tris(2,4-di-tert-butylphenyl) phosphate. The combination of MECC and MECE demonstrated higher zones of inhibition against bacterial and fungal strains, albeit lower than standard drugs. However, it exhibited significant anticancer activity with an IC50 value of 76.49 µg/ml (MECC+MECE) against MCF-7 cells. Future studies should further investigate the synergistic effects of MECC and MECE in combating bacterial and fungal infections and explore their potential for cancer therapy.

Keywords: Carissa Carandas, Colocasia Esculenta, Synergistic Activity, Antibacterial, Antifungal, Anticancer, Phytochemicals, Traditional Medicine, Herbal Medicine, Drug Discovery, GC-MS Analysis.

1. INTRODUCTION

The presence of phytochemical compounds in medicinal plant responsible for their pharmacological activity. One phytochemical compound may possess one or more pharmacological properties (Al-Qahtani et al., 2023). Plants are gifted with a range of phytochemical bioactive molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antimicrobial activity and antioxidant activity which is responsible for anticancer activity. Studies have shown that the consumption of natural antioxidants has been associated with decreasing ageing, risks of cancer (Ahirwar & Tembhre, 2021). Two or more drugs that individually produce overtly similar effects will sometimes display greatly enhanced effects when given in combination. When the combined effect is greater than that predicted by their

individual potencies, the combination is said to be synergistic (Tallarida, 2011). Synergistic combinations of two or more agents can overcome toxicity and other side effects associated with high doses of single drugs (Lehár et al., 2009). According to WHO (1993), 80% of the world's population is dependent on the traditional medicines and a major part of the traditional therapies involve the use of plant extracts or their bioactive components (Agarwal et al., 2012).

Carissa carandas is an evergreen thorny shrub belongs to Apocynaceae family, which is commonly known as karonda. It has small berry-shaped fruits, used as additive in many pickles or as a spice in northern India (Agarwal et al., 2012; S. Singh et al., 2020). Ethanolic root extract of C. carandas may produce its anticonvulsant effects (Hegde et al., 2009). Shows clear evidence for the dual effectiveness in constipation, diarrhoea (Mehmood et al., 2014), analgesic, antipyretic, anti-inflammatory agent (Bhaskar & Balakrishnan, 2009), hepatoprotective (Jain et al., 2020). Ripped fruit, leaves shows antibacterial and antioxidant activity, antineoplastic activity (Sudjaroen & Suwannahong, 2017). *Colocasia esculenta* commonly known as Taro, belongs to the family Araceae (Kubde et al., 2010) proven to have antimicrobial, antioxidant and anticancer (Chakraborty et al., 2015), anthelmintic activity (Kubde et al., 2010), anti hyperglycemic (Kumawat et al., 2010).

The current studies aim to find the synergistic relation between methanolic extracts of *Carissa carandas* and *Colocasia esculenta* leaves for Antibacterial, Antifungal and Anticancer activity. Combination use to minimizing their side effects by using lower dose of both plants together and get better outcome of result.

2. MATERIALS AND METHODS

2.1. Materials

The materials utilized in this study included Mueller-Hilton Agar (MHA) plates, bacterial cultures of *Pseudomonas aeruginosa* (MTCC3541) and *Staphylococcus aureus*, Whatman No. 1 filter paper discs (5mm), Dimethyl Sulphoxide (DMSO) as a solvent (SRL Chem 28580), Ciprofloxacin (SRL Chem- 78079) at a concentration of 2mg/ml for antibacterial activity, Sabouraud dextrose agar (SDA) plates, fungal cultures of *Candida albicans* (MTCC 854) and *Aspergillus niger* (MTCC281), Potato Dextrose Agar (PDA) plates, Amphotericin B (Amphocare) at a concentration of 5 mg/ml for antifungal activity, and MCF-7 (Breast cancer cell line) for anticancer activity. All materials were procured from the Akaar Biotechnology Laboratory.

2.2. Sample Collection

Fresh leaves of *Carissa carandas* and *Colocasia Esculenta* were collected from the market near Noida in July 2023. Authentication of the leaves was conducted at the botanical garden of Noida by Dr. Priyanka Ingle.

2.3. Methods

2.3.1. Authentication

Authentication of the plant material was performed by Dr. Priyanka Ingle, Scientist C at the Botanical Garden of India Republic, Gautam Budh Nagar, Uttar Pradesh. A voucher specimen (BSI/BGIR/1/TECH./2023/62) was deposited in the herbarium of the Institute.

2.3.2. Extraction

Leaves of both plants were washed with tap water followed by distilled water and dried at room temperature. The fully dried leaves were ground into a powder. One gram of leaf powder was mixed with 50 ml of absolute methanol solvent. The sample mixture was incubated on a rotary shaker for 24 hours. The extract was then filtered through Whatman filter paper and completely dried in an oven at 40°C. The extracts were collected in microcentrifuge tubes and stored at 4°C.

2.3.3. GCMS Analysis

For GCMS analysis, 10 µl of the sample (50 mg/ml) was taken in a separating funnel and shaken by adding 10 ml of water and ethyl acetate in a 1:4 ratio. The upper layer was collected and concentrated to 1 ml in a rotary evaporator. N,O-Bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA+TMCS) were added, followed by pyridine. The samples were transferred to GC vials, dried using nitrogen gas, and dissolved in methanol before GC-MS analysis. Acquired samples were programmed accordingly.

2.3.4. Antimicrobial Activity

The antibacterial activity was evaluated using the Zone Inhibition Method (Kirby-Bauer method) (Al Laham & Al Fadel, 2014; Pacheco et al., 2013; A. A. Singh et al., 2023). MHA plates were inoculated with bacterial cultures, followed by the placement of discs containing different concentrations of extracts (0, 312.5, 625, 1250, 2500 and 5000) µg/well, positive control Ciprofloxacin (10 µg/disk). The plates were incubated at 37°C for 24 hours, and clear zones around the discs were measured.

2.3.5. Antifungal Activity

The antifungal activity was determined using the Zone Inhibition Method (Kirby-Bauer method) (Al Laham & Al Fadel, 2014; Pacheco et al., 2013; A. A. Singh et al., 2023). SDA plates were inoculated with fungal cultures, and wells containing different concentrations of extracts (0, 312.5, 625, 1250, 2500 and 5000) µg/well were prepared along with along with positive control Amphotericin B (50 µg/disk). The plates were incubated at 37°C for 24 hours, and clear zones around the wells were measured.

2.3.6. Anticancer Activity using MCF-7

The anticancer activity of individual extracts (MECC and MECE) and their combination was evaluated against cancer cell lines such as MCF-7 using MTT assay. Cytotoxicity of the provided samples on MCF-7 cell line (procured from NCCS Pune) was assessed using the MTT Assay. MCF-7 cells (10,000 cells/well) were seeded in a 96-well plate and cultured for 24 hours in DMEM medium supplemented with 10% FBS and 1% antibiotic solution at 37°C with 5% CO2. The following day, cells were treated with varying concentrations (1-1000 µg/mL) of the formulations prepared in incomplete medium. After 24 hours of incubation, MTT Solution (final concentration 250 µg/mL) was added, followed by a further 2-hour incubation. The culture supernatant was then removed, and the cell layer was dissolved in 100 µl Dimethyl Sulfoxide (DMSO). After incubation, MTT Solution was added, and absorbance was measured using an Elisa plate reader. IC_{50} was calculated using Graph Pad Prism-6 software. Images were captured under an inverted microscope using a digital camera. Images were captured under an inverted microscope (Olympus ek2) equipped with a camera (AmSope digital

camera 10 MP Aptima CMOS). This methodology was conducted following established protocols (Kis et al., 2022; Prakash, 2017).

3. RESULTS

3.1 Extraction Yield

After the extraction procedure, the percentage yield was found to be 7.9% for the methanolic extract of *Carissa carandas* leaves (MECC) and 8.8% for methanolic extract of *Colocasia Esculenta* leaves (MECE) as mentioned in table 1.

Table 1: Extraction yield of the methanolic extract of plant leaves

Sample name	*Weight of powder (mg)*	*Weight of extract (mg)*	*% Recovery*
Methanolic extract of Carissa carandas leaves (MECC)	1000	79	7.9
Methanolic extract of Colocasia esculenta leaves (MECE)	1000	88	8.8

3.2 GCMS Analysis

GC-MS analysis (Figure 1) of methanolic extract of leaves of *Carissa carandas* (MECC) indicate the presence of different compounds namely 1-Undecyne (1.24%), 1,2-Benzenedicarboxylic Acid, Diethyl Ester (3.79%), Methyl (3-Oxo-2-Pentylcyclopentyl)Acetate(0.37%), 1-(4-Isopropylphenyl)-2-Methylpropyl Acetate(0.47%), 10(E),12(Z)-Conjugated Linoleic Acid(1.68%), Oleyl Alcohol, Trifluoroacetate(1.74%), 11,14-Eicosadienoic Acid(1.29%), Hexadecanoic Acid, Methyl Ester(6.03%), 9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester(17.96%), 9-Octadecenoic Acid, Methyl Ester, (E)-(20.33%), 8,11-Eicosadienoic Acid, Methyl Ester(1.45%), Methyl Stearate(3.66%), 9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester(1.97%), Glycidyl Palmitate(0.96%), Myristic Acid Glycidyl Ester(0.67%), Linoleyl Acetate(1.22%), 9-Octadecenoic Acid (Z)-, 2,3-Dihydroxypropyl Ester(1.85%), Petroselinic Acid, TMS Derivative(5.72%), Glycidyl (Z)-9-Heptadecenoate(0.70%), Myristic Acid Glycidyl Ester(0.57%), Octadecanoic Acid, Methyl Ester(0.97%), 1,1-Dichloro-2,2,3,3-Tetramethylcyclopropa(1.58%), 5,5-Dimethyl-1,3-Dioxane-2-Ethanol, TBDMS Derivative(0.92%), Glycerol Monostearate, 2TMS Derivative(2.42%), 13-Docosenamide, (Z)-(0.95%), Stigmasta-3,5-Diene(1.27%), Linoleic Acid, Butyl Ester(7.13%), Octanoic Acid, Dodec-9-Ynyl Ester(5.89%), Tris(2,4-Di-Tert-Butylphenyl) Phosphate(3.85%), Linoleyl Palmitate(1.36%).

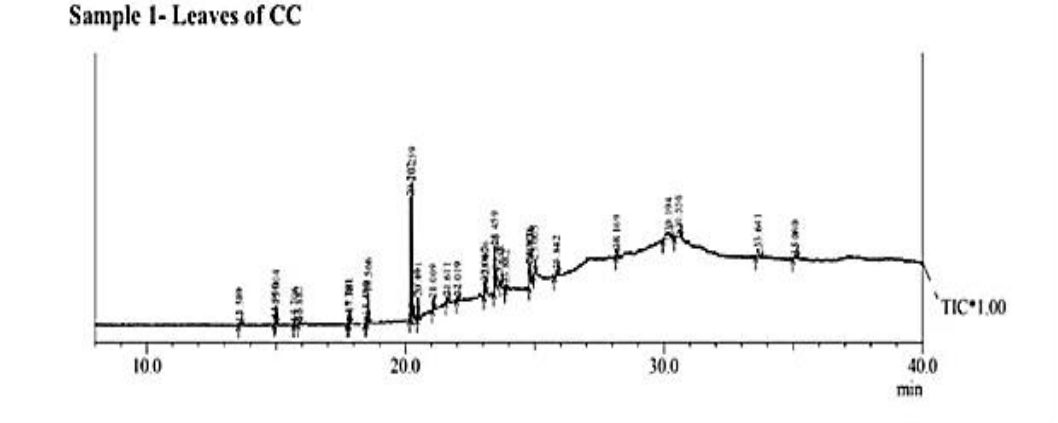

Figure 1: GC-MS analysis of MECC

GC-MS analysis (Figure 2) of methanolic extract of leaves of *Colocasia Esculenta* (MECE) indicate the presence of different compounds namely 1,9-Nonanediol(1.08%), 1,2-Benzenedicarboxylic Acid, Diethyl Ester(2.61%), 1-(4-Isopropylphenyl)-2-Methylpropyl Aceta(0.35%), 10(E),12(Z)-Conjugated Linoleic Aci(1.52%), Oleyl Alcohol, Trifluoroacetate(1.32%), 11-Octadecynenitrile(1.41%), Hexadecanoic Acid, Methyl Ester(5.75%), 9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester(17.37%), 9-Octadecenoic Acid, Methyl Ester, (E)-(18.41%), 10,13-Eicosadienoic Acid, Methyl Ester(1.18%), Methyl Stearate(3.23%), 9,12-Octadecadienoic Acid (Z,Z)-, Methyl Ester(1.71%), Hexadecanoic Acid, 2-Hydroxy-1-(Hydroxym(1.17%), Myristic Acid Glycidyl Ester(0.83%), 2,3-Dihydroxypropyl Elaidate(1.68%), Hexadecanoic Acid, 2-[(Trimethylsilyl)Oxy](6.61%), Glycidyl Palmitate(3.65%), Hexacosanoic Acid, Methyl Ester(0.54%), 1-Cyclohexyldimethylsilyloxybutane(8.45%), 1h-Indole-3-Ethanamine(2.56%), 2-Iodoethyl Linoleate(0.39%), Stigmasta-3,5-Diene(1.94%), Linoleic Acid, Butyl Ester(12.24%), Tris(2,4-Di-Tert-Butylphenyl) Phosphate(4.01%).

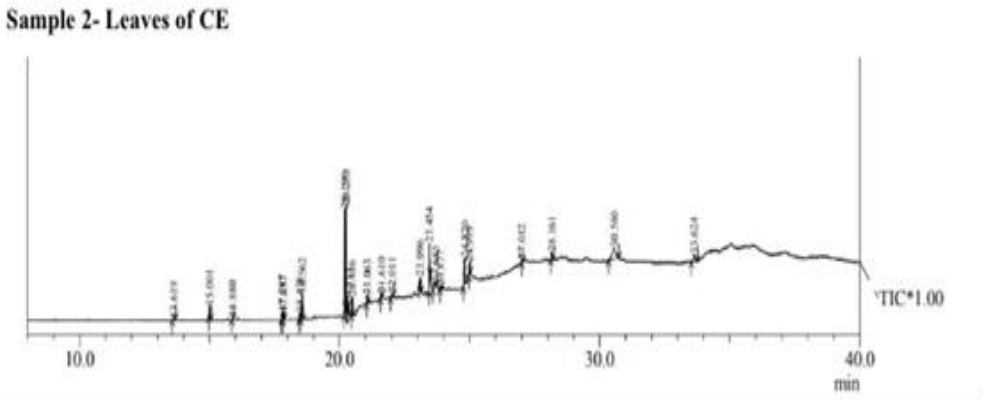

Figure 2: GC-MS analysis of MECE

3.3 Antimicrobial Activity

The percentage of inhibition for the antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* for various concentrations (0, 312.5, 625, 1250, 2500 and 5000) µg/well. of methanolic extracts (MECC and MECE) and their combination (MECC+MECE) was measured.

Percentage of inhibition for *Staphylococcus aureus*: MECC: (0, 4.9, 6.2, 7.3, 7.9, 7.9)%, Positive control (Ciprofloxacin) (28%) (Figure 3), MECE: (0, 5.1, 5.8, 6.4, 7.2, 7.7)%, Positive control (Ciprofloxacin) (27%) (Figure 4), MECC+MECE (1:1): (0, 4.8, 6.4, 7.4, 7.7, 8.3)%, Positive control (Ciprofloxacin) (29%) (Figure 5).

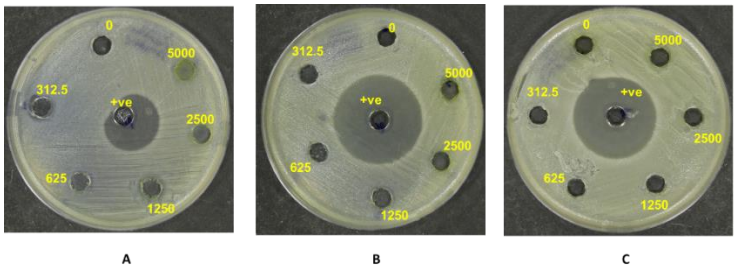

Figure 3: Inhibition zone at different concentrations of MECC against Staphylococcus aureus

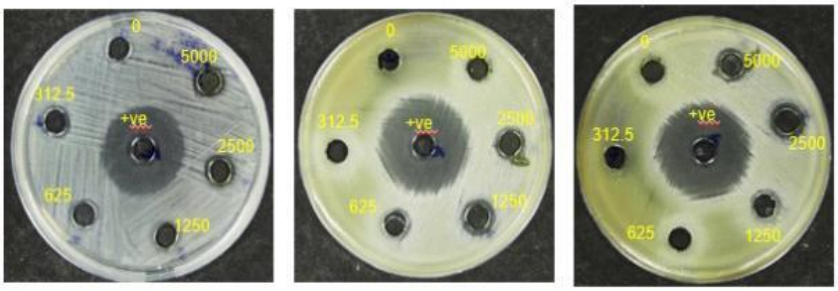

Figure 4: Inhibition zone at different concentrations of MECE against Staphylococcus aureus

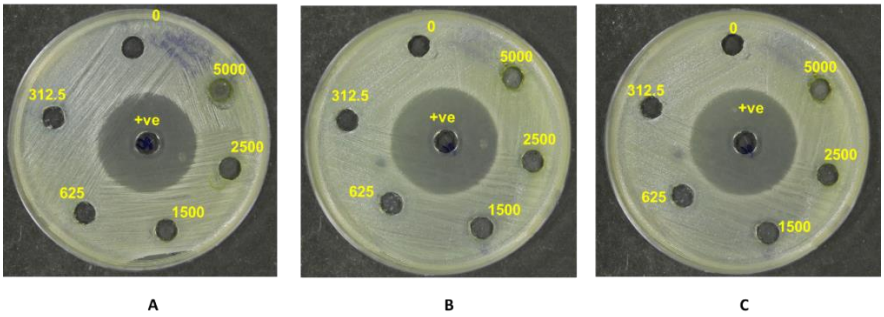

Figure 5: Inhibition zone at different concentrations of MECC+MECE against Staphylococcus aureus

Percentage of inhibition against *Pseudomonas aeruginosa*: MECC: (0, 7.1, 8.3, 8.5, 9.4, 9.5), Positive control (Ciprofloxacin) (28%) (Figure 6), MECE: (0, 6.9, 8.2, 9.2, 9.4, 11.2)%, Positive control (Ciprofloxacin) (27%) (Figure 7); MECC+MECE (1:1): (0, 6.2, 7.2, 8.4, 10.3, 12.9)% and Positive control (Ciprofloxacin) (29%) (Figure 8).

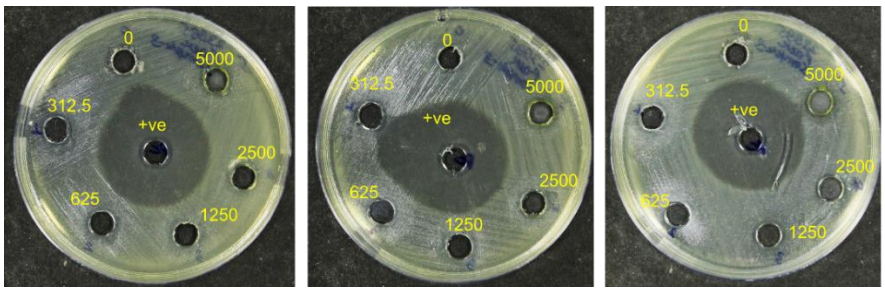

Figure 6: Inhibition zone at different concentrations of MECC against Pesudomonas aeruginosa

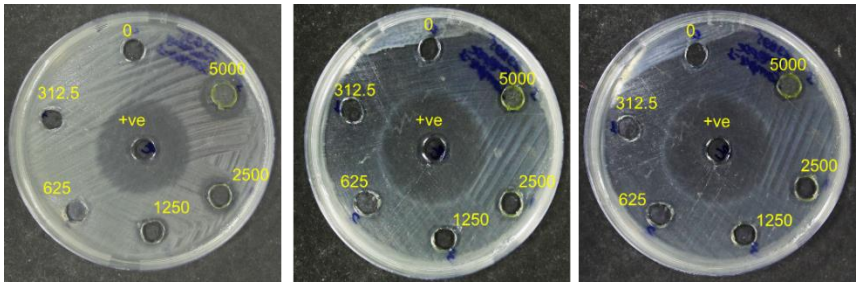

Figure 7: Inhibition zone at different concentrations of MECE against Pesudomonas aeruginosa

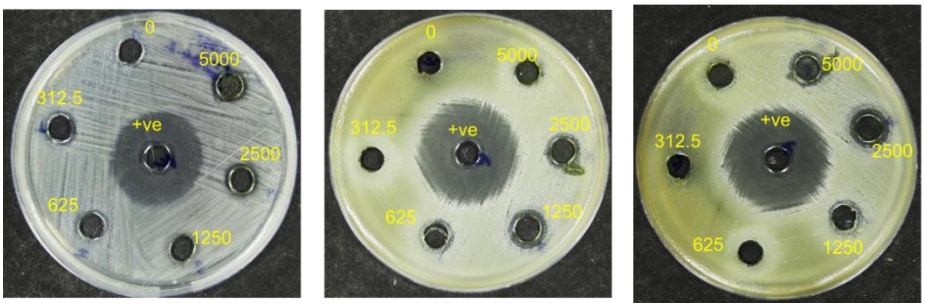

Figure 8: Inhibition zone at different concentrations of MECC+MECE against Pesudomonas aeruginosa

In Antibacterial activities, **Ciprofloxacin** was used as a positive control and denoted as **PC.**

3.4 Antifungal Activity

The percentage inhibition for the antifungal activity against *Aspergillus niger* and *Candida albicans* for various concentrations of methanolic extracts (MECC and MECE) and their combination (MECC+MECE) at different concentrations (0, 312.5, 625, 1250, 2500 and 5000) µg/well was measured.

Percentage of inhibition against *Aspergillus niger*: MECC: (0, 14.1, 15.2, 18.1, 18.6, 20.2)%, Positive control (Amphotericin B) (28%) (Figure 9); MECE: (0, 15.2, 16, 17.3, 18.7, 19.8)%, Positive control (Amphotericin B) (27%) (Figure 10), MECC+MECE (1:1): (0, 15.3, 16.8, 18, 19.6, 22.5)%, Positive control (Amphotericin B) (28%) (Figure 11).

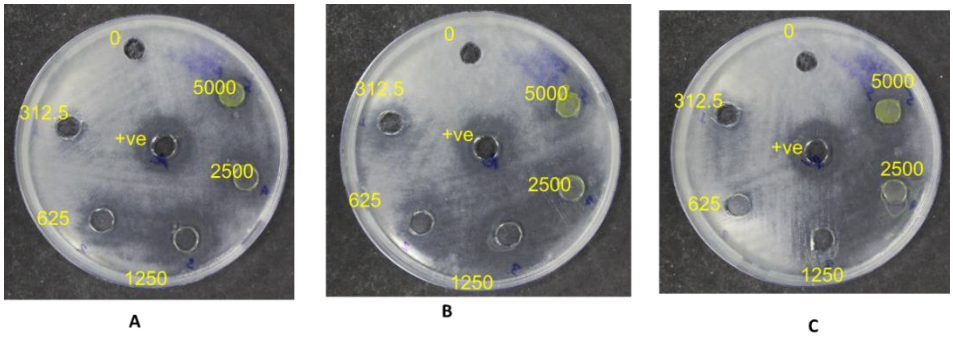

Figure 9: Inhibition zone at different concentrations of MECC against Aspergillus niger

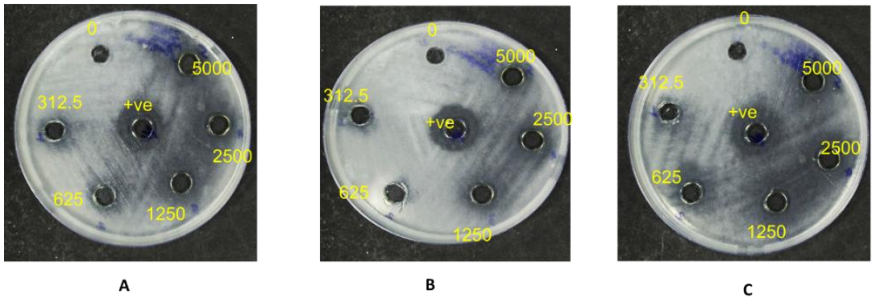

Figure 10: Inhibition zone at different concentrations of MECE against Aspergillus niger

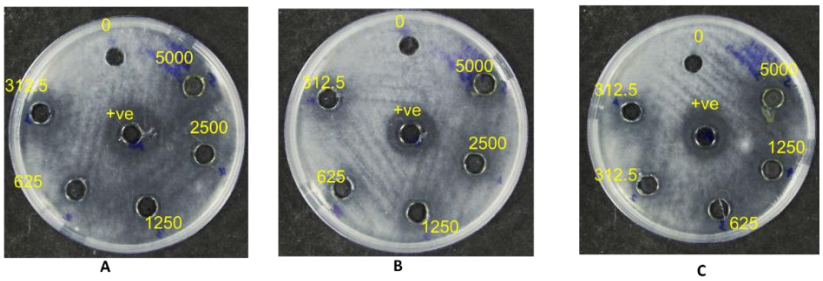

Figure 11: Inhibition zone at different concentrations of MECC+MECE against Aspergillus niger

Percentage of inhibition against *Candida albicans*, the antifungal activity showed concentration-dependent effects: MECC: (0, 4.6, 5.7, 6.6, 8.9, 10.7)%, (Figure 12), Positive control (Amphotericin B) (28%), MECE: (0, 5.7, 6.4, 8.6, 9.4, 12.1)%, (Figure 13), Positive control (Amphotericin B) (27%) and MECC+MECE: (1:1) (0, 6.3, 7.7, 9.4, 11.3, 14.1)%, (Figure 14), Positive control (Amphotericin B) (28%).

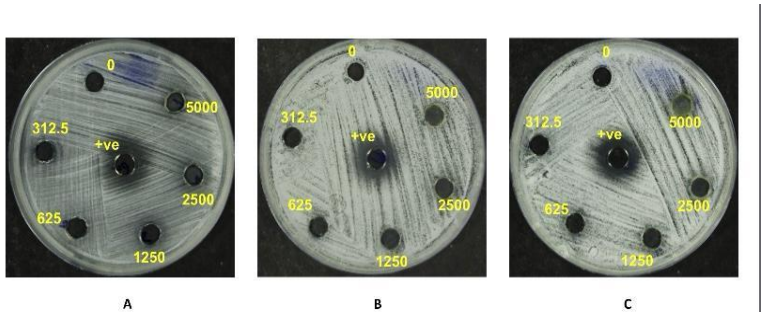

Figure 12: Inhibition zone at different concentrations of MECC gainst Candida Albicans

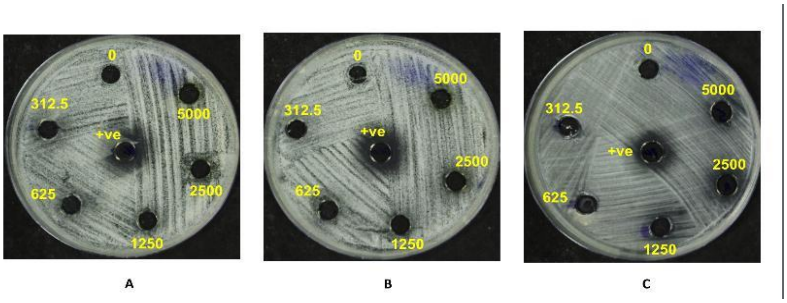

Figure 13: Inhibition zone at different concentrations of MECE against Candida Albicans

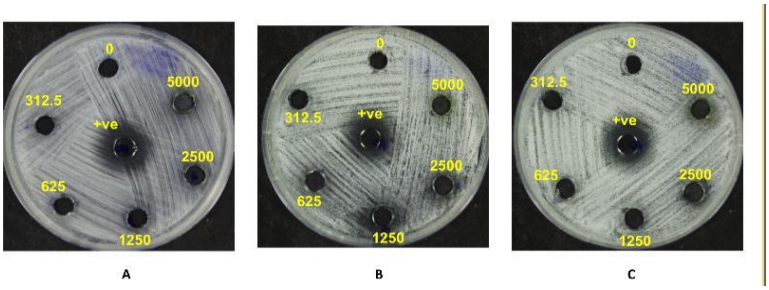

Figure 14: Inhibition zone at different concentrations of MECC+MECE against Candida Albicans

3.5 Anticancer Activity

The cell viability assay was conducted on MCF-7 breast cancer cells using methanolic extracts (MECC and MECE) individually and their combination (MECC+MECE) at various concentrations (0, 1, 10, 50, 100, 250, 500, 1000 µg/ml). The MTT assay revealed percentage cell viability inhibition for MECC: *(98.72%, 94.89%, 82.41%, 75.00%, 65.63%, 56.74%, 47.67%, 25.33%), (Figure 15), MECE: (99.00%, 91.69%, 86.21%, 78.45%, 67.73%, 55.31%, 35.38%, 28.08%), (Figure 16) and MECC + MECE (1:1): (99.99%, 92.98%, 66.12%, 54.33%, 46.16%, 41.12%, 27.81%, 14.91%), (Figure 17),* respectively. The IC50 values obtained were 183.40µg/ml for Carissa Carandas, 182.69µg/ml for Colocasia Esculenta, and 76.49µg/ml for the combination of Carissa Carandas and Colocasia Esculenta extracts.

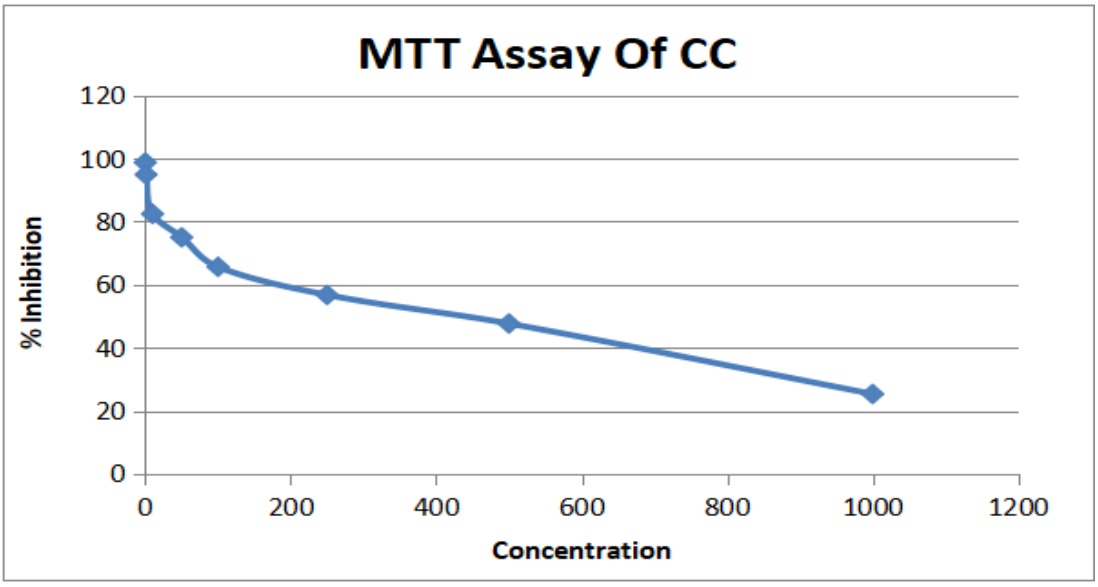

Figure 15: Cell viability assay of MECC in MCF-7 cell line

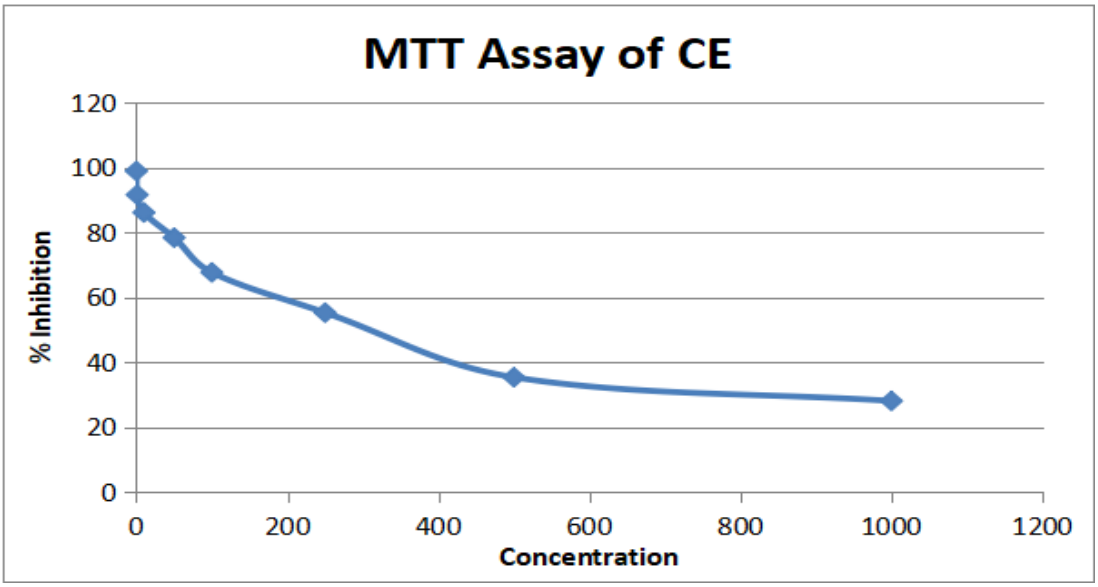

Figure 16: Cell viability assay of MECE in MCF-7 cell line

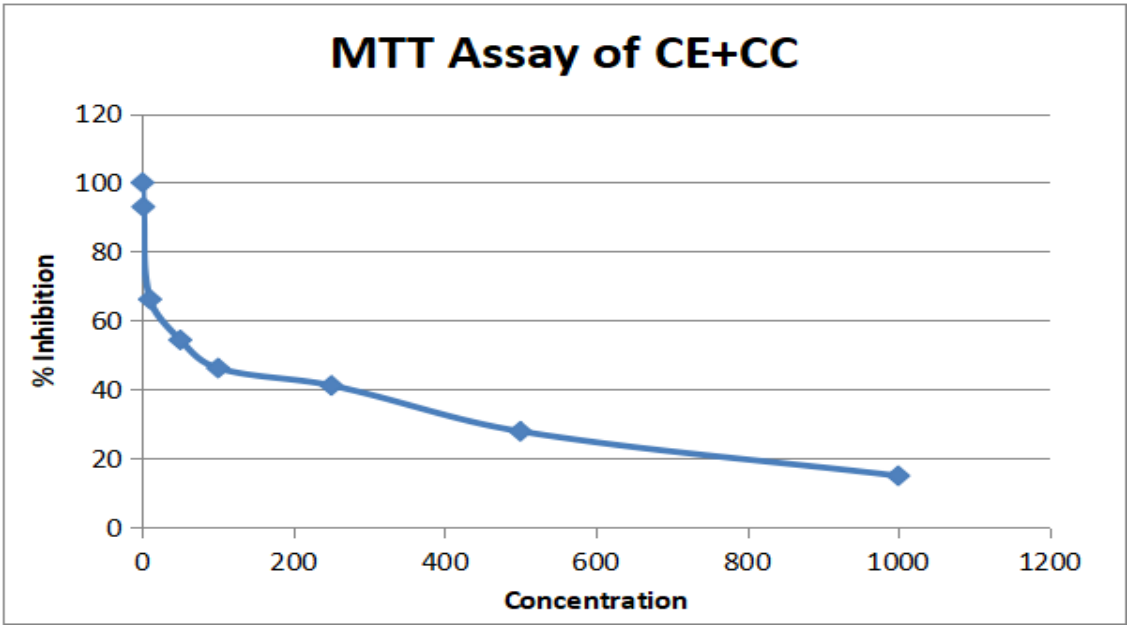

Figure 17: Cell viability assay of MECC + MECE in MCF-7 cell line

4. DISCUSSION

The research presents an importance exploration into the synergistic potential of methanolic extracts from *Carissa carandas* (MECC) and *Colocasia esculenta* (MECE) leaves for antibacterial, antifungal, and anticancer activities. This study was planned on the basis of traditional medicinal uses of *Carissa carandas* and *Colocasia esculenta* in traditional medicine systems for treating various diseases, including microbial infections and cancer (Agarwal et al., 2012; Sudjaroen & Suwannahong, 2017). This report investigated whether the combination of these plant extracts could amplify their therapeutic effects compared to their individual actions. Phytochemical analysis of the methanolic extracts using gas chromatography-mass spectrometry (GC-MS) revealed the presence of several bioactive compounds known for their pharmacological properties. Among these compounds, notable ones include hexadecanoic acid methyl ester, stigmasta-3,5-diene, and tris(2,4-di-tert-butylphenyl) phosphate (Kis et al., 2022). These compounds have been previously associated with antibacterial, antifungal, and anticancer activities. The compound 9-Octadecenoic Acid, Methyl Ester, (E)-(20.33%), commonly known as oleic acid, has been extensively studied for its diverse biological activities. It has shown promising antibacterial effects against various pathogens, including *Staphylococcus aureus* and *Escherichia coli* (Chakraborty et al., 2015). Additionally, oleic acid exhibits potential antifungal activity against Candida albicans (Prakash, 2017). Furthermore, several studies have reported its anticancer properties, including inhibition of cancer cell proliferation and induction of apoptosis in various cancer types. Linoleic Acid, Butyl Ester (7.13%): Linoleic acid, a polyunsaturated omega-6 fatty acid, has demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria. It has also shown promising antifungal effects against dermatophytes and *Candida* species (Kubde et al., 2010). Moreover, linoleic acid exhibits potential anticancer activity by inhibiting tumor cell growth and promoting apoptosis. 10(E),12(Z)-Conjugated Linoleic Acid (1.68%): Conjugated linoleic acid (CLA) is a group of isomers of linoleic acid with various biological activities. Studies have reported its antibacterial effects against

foodborne pathogens such as *Listeria monocytogenes* and *Salmonella enterica*. CLA also possesses antifungal activity against *Candida* species. Additionally, CLA has shown promising anticancer properties by inhibiting cancer cell proliferation and inducing apoptosis *in vitro* and *in vivo.* Octanoic Acid, Dodec-9-Ynyl Ester (5.89%): Octanoic acid, also known as caprylic acid, has demonstrated significant antibacterial activity against various pathogens, including Helicobacter pylori and Streptococcus mutans. It also exhibits antifungal effects against Candida species. Furthermore, caprylic acid has shown potential anticancer activity by inducing apoptosis and inhibiting cell proliferation in cancer cells. Tris(2,4-Di-Tert-Butylphenyl) Phosphate (3.85%): Tris(2,4-di-tert-butylphenyl) phosphate, a flame retardant compound, has been reported for its antibacterial properties against both Gram-positive and Gram-negative bacteria. Additionally, it exhibits antifungal activity against Aspergillus flavus and other fungal pathogens. Although limited, some studies suggest its potential anticancer activity by inhibiting cancer cell proliferation (Al-Qahtani et al., 2023). The antibacterial activity of the individual and combined extracts was evaluated against clinically significant bacterial strains, including *Staphylococcus aureus,* and *Pseudomonas aeruginosa*. The results demonstrated that the combination of MECC and MECE exhibited enhanced antibacterial activity compared to their individual counterparts. This synergistic effect was particularly pronounced against Pseudomonas aeruginosa. Similarly, the antifungal activity of the extracts was assessed against common fungal pathogens, including Candida albicans and Aspergillus niger. The combination of MECC and MECE displayed superior antifungal activity compared to the individual extracts, with significant inhibition observed against both fungal strains. The most striking finding of the study pertains to the anticancer activity of the combined extracts. The MTT assay conducted on MCF-7 breast cancer cells revealed a notable IC50 value of 183.40 µg/ml (MECC), 182.69 µg/ml (MECE) and 76.49 µg/ml (MECC+MECE) indicating both plant have moderate cytotoxic activity, in combination active cytotoxic activity. It shows their synergistic effect and significant cytotoxicity against cancer cells. This result underscores the potential of the MECC and MECE combination as a promising candidate for cancer therapy.

Overall, the findings of this study provide compelling evidence for the synergistic effects of methanolic extracts from *Carissa carandas* and *Colocasia esculenta* leaves in combating bacterial and fungal infections, as well as their promising anticancer activity. However, further in-depth studies are warranted to elucidate the underlying mechanisms of action and to evaluate the safety and efficacy of these extracts in preclinical and clinical practices.

5. CONCLUSION

In conclusion, the research uncovers promising synergistic potential in the combination of methanolic extracts from *Carissa carandas and Colocasia esculenta* leaves, showcasing enhanced antibacterial, antifungal, and anticancer activities. Notable bioactive compounds identified through phytochemical analysis, such as oleic acid, linoleic acid, conjugated linoleic acid, caprylic acid, and tris(2,4-di-tert-butylphenyl) phosphate, contribute to these therapeutic effects. The study highlights significant improvements in antibacterial and antifungal efficacy against key pathogens, along with notable cytotoxicity against MCF-7 breast cancer cells. While promising, further research is essential to elucidate mechanisms and validate clinical applications. The research contributes valuable insights into the therapeutic potential

of plant-based synergies in addressing multifaceted health challenges, paving the way for the development of novel botanical therapies with enhanced efficacy and reduced side effects.

6. Acknowledgement

Authors want to acknowledge to Director of HIMT College of Pharmacy for his endless support throughout the study.

References

1) Agarwal, T., Singh, R., Shukla, A. D., & Waris, I. (2012). In vitro study of antibacterial activity of Carissa carandas leaf extracts. *Asian Journal of Plant Science & Research*, *2*(1), 36–40. https://www.imedpub.com/abstract/in-vitro-study-of-antibacterial-activity-of-carissa-carandas-leaf-extracts-13203.html

2) Ahirwar, P., & Tembhre, M. (2021). Preliminary Phytochemical Analysis, Antioxidant Activity, Phenolic and Flavonoid Contents of Annona reticulata Leaf Extract. *Asian Journal of Experimental Sciences*, *35*(2), 19–25. www.ajesjournal.com,

3) Al Laham, S. A., & Al Fadel, F. M. (2014). Antibacterial Activity of Various Plants Extracts Against Antibiotic-resistant Aeromonas hydrophila. *Jundishapur Journal of Microbiology*, *7*(7), 11370. https://doi.org/10.5812/JJM.11370

4) Al-Qahtani, J., Abbasi, A., Aati, H. Y., Al-Taweel, A., Al-Abdali, A., Aati, S., Yanbawi, A. N., Abbas Khan, M., Ahmad Ghalloo, B., Anwar, M., & Khan, K. ur R. (2023). Phytochemical, Antimicrobial, Antidiabetic, Thrombolytic, anticancer Activities, and in silico studies of Ficus palmata Forssk. *Arabian Journal of Chemistry*, *16*(2), 104455. https://doi.org/10.1016/J.ARABJC.2022.104455

5) Bhaskar, V., & Balakrishnan, N. (2009). Analgesic, anti-inflammatory and antipyretic activities of Pergularia daemia and Carissa carandas. *DARU*.

6) Chakraborty, P., Deb, P., Chakraborty, S., Chatterjee, B., & Abraham, J. (2015). Cytotoxicity and antimicrobial activity of Colocasia esculenta. *Journal of Chemical and Pharmaceutical Research*, *7*(12), 627–635. www.jocpr.com

7) Hegde, K., Thakker, S. P., Joshi, A. B., Shastry, C. S., & Chandrashekhar, K. S. (2009). Anticonvulsant Activity of Carissa carandas Linn. Root Extract in Experimental Mice. *Tropical Journal of Pharmaceutical Research*, *8*(2), 117–125. https://doi.org/10.4314/TJPR.V8I2.44519

8) Jain, D., Chaudhary, P., Kotnala Azoth Biotech LLP, A., Buddha Nagar, G., Pradesh, U., Rajib Hossain, I., Bisht, K., Nabil Hossain, M., Author, C., Kotnala, A., & Hossain, R. (2020). Hepatoprotective activity of medicinal plants: A mini review. *Journal of Medicinal Plants Studies*, *8*(5), 183–188. https://doi.org/10.22271/PLANTS.2020.V8.I5C.1212

9) Kis, B., Pavel, I. Z., Avram, S., Moaca, E. A., Herrero San Juan, M., Schwiebs, A., Radeke, H. H., Muntean, D., Diaconeasa, Z., Minda, D., Oprean, C., Bojin, F., Dehelean, C. A., Soica, C., & Danciu, C. (2022). Antimicrobial activity, in vitro anticancer effect (MCF-7 breast cancer cell line), antiangiogenic and immunomodulatory potentials of Populus nigra L. buds extract. *BMC Complementary Medicine and Therapies*, *22*(1). https://doi.org/10.1186/S12906-022-03526-Z

10) Kubde, M. S., Khadabadi, S. S., Farooqui, I. A., & Deore, S. L. (2010). In-vitro anthelmintic activity of Colocasia esculenta. *Pharmacia Lettre*, *2*(2), 82–85. www.scholarsresearchlibrary.com

11) Kumawat, N., Chaudhari, S., Wani, N., Deshmukh, T., & Patil, V. (2010). Antidiabetic activity of ethanol extract of Colocasia esculenta leaves in alloxan induced diabetic rats. *International Journal of PharmTech Research CODEN*, *2*(2), 1246–1249. www.graphpad.com.

12) Lehár, J., Krueger, A. S., Avery, W., Heilbut, A. M., Johansen, L. M., Price, E. R., Rickles, R. J., Short, G. F., Staunton, J. E., Jin, X., Lee, M. S., Zimmermann, G. R., & Borisy, A. A. (2009). Synergistic drug combinations improve therapeutic selectivity. *Nature Biotechnology*, *27*(7), 659. https://doi.org/10.1038/NBT.1549

13) Mandal S, Vishvakarma P. Nanoemulgel: A Smarter Topical Lipidic Emulsion-based Nanocarrier. Indian J of Pharmaceutical Education and Research. 2023;57(3s):s481-s498.

14) Mandal S, Jaiswal DV, Shiva K. A review on marketed Carica papaya leaf extract (CPLE) supplements for the treatment of dengue fever with thrombocytopenia and its drawback. International Journal of Pharmaceutical Research. 2020 Jul;12(3).

15) Mandal S, Bhumika K, Kumar M, Hak J, Vishvakarma P, Sharma UK. A Novel Approach on Micro Sponges Drug Delivery System: Method of Preparations, Application, and its Future Prospective. Indian J of Pharmaceutical Education and Research. 2024;58(1):45-63.

16) Mishra, N., Alagusundaram, M., Sinha, A., Jain, A. V., Kenia, H., Mandal, S., & Sharma, M. (2024). Analytical Method, Development and Validation for Evaluating Repaglinide Efficacy in Type Ii Diabetes Mellitus Management: a Pharmaceutical Perspective. Community Practitioner, 21(2), 29–37. https://doi.org/10.5281/zenodo.10642768

17) Singh, M., Aparna, T. N., Vasanthi, S., Mandal, S., Nemade, L. S., Bali, S., & Kar, N. R. (2024). Enhancement and Evaluation of Soursop (Annona Muricata L.) Leaf Extract in Nanoemulgel: a Comprehensive Study Investigating Its Optimized Formulation and Anti-Acne Potential Against Propionibacterium Acnes, Staphylococcus Aureus, and Staphylococcus Epidermidis Bacteria. Community Practitioner, 21(1), 102–115. https://doi.org/10.5281/zenodo.10570746

18) Khalilullah, H., Balan, P., Jain, A. V., & Mandal, S. (n.d.). Eupatorium Rebaudianum Bertoni (Stevia): Investigating Its Anti-Inflammatory Potential Via Cyclooxygenase And Lipooxygenase Enzyme Inhibition - A Comprehensive Molecular Docking And Admet. Community Practitioner, 21(03), 118–128. https://doi.org/10.5281/zenodo.10811642

19) Mandal, S. (n.d.). Gentamicin Sulphate Based Ophthalmic Nanoemulgel : Formulation And Evaluation , Unravelling A Paradigm Shift In Novel Pharmaceutical Delivery Systems. Community Practitioner, 21(03). https://doi.org/10.5281/zenodo.10811540

20) Mandal, S., Tyagi, P., Jain, A. V., & Yadav, P. (n.d.). Advanced Formulation and Comprehensive Pharmacological Evaluation of a Novel Topical Drug Delivery System for the Management and Therapeutic Intervention of Tinea Cruris (Jock Itch). Journal of Nursing, 71(03). https://doi.org/10.5281/zenodo.10811676

21) Mehmood, M. H., Anila, N., Begum, S., Syed, S. A., Siddiqui, B. S., & Gilani, A. H. (2014). Pharmacological basis for the medicinal use of Carissa carandas in constipation and diarrhea. *Journal of Ethnopharmacology*, *153*(2), 359–367. https://doi.org/10.1016/J.JEP.2014.02.024

22) Pacheco, A. O., Morán, J. M., González Giro, Z., Hidalgo Rodríguez, A., Mujawimana, R. J., Tamayo González, K., & Frómeta, S. S. (2013). In vitro antimicrobial activity of total extracts of the leaves of Petiveria alliacea L. (Anamu). *Article Brazilian Journal of Pharmaceutical Sciences*, *49*(2).

23) Prakash, R. (2017). Anti-cancer activity of Trachyspermum ammi against MCF-7 cell lines mediates by p53 and Bcl-2 mRNA levels. *The Journal of Phytopharmacology*, *6*(2), 78–83. www.phytopharmajournal.com

24) Singh, A. A., Naaz, Z. T., Rakaseta, E., Perera, M., Singh, V., Cheung, W., Mani, F., & Nath, S. (2023). Antimicrobial activity of selected plant extracts against common food borne pathogenic bacteria. *Food and Humanity*, *1*, 64–70. https://doi.org/10.1016/J.FOOHUM.2023.04.002

25) Singh, S., Bajpai, M., & Mishra, P. (2020). Carissa carandas L. – phyto-pharmacological review. *Journal of Pharmacy and Pharmacology*, *72*(12), 1694–1714. https://doi.org/10.1111/JPHP.13328

26) Sudjaroen, Y., & Suwannahong, K. (2017). In vitro antioxidant, antibacterial, and cytotoxicity activities from Karanda (Carissa carandas L.) fruit extracts. *International Journal of Green Pharmacy (IJGP)*, *11*(01), 189. https://doi.org/10.22377/IJGP.V11I01.893

27) Tallarida, R. J. (2011). Quantitative Methods for Assessing Drug Synergism. *Genes & Cancer*, *2*(11), 1003. https://doi.org/10.1177/1947601912440575