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In Vitro Anticancer and Antimicrobial Activity Evaluation of Medicinal Plant Extracts

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Abstract- Medicinal plants have long been valued for their therapeutic and pharmacological properties, offering a safer alternative to conventional synthetic drugs, particularly in reducing the toxicity and resistance associated with chemotherapy. The present study was therefore aimed at evaluating the individual and synergistic anticancer and antimicrobial activity of ethanolic extracts of Withania somnifera, Curcuma longa, and Moringa oleifera. The extracts were prepared by the Soxhlet extraction technique using ethanol as solvent, following collection, shade-drying, and botanical authentication of the plant material. Phytochemical screening, in-vitro anticancer evaluation by MTT assay against the HCT116 human colon cancer cell line, and in-vitro antimicrobial evaluation by agar well-diffusion against Lactobacillus bulgaricus were carried out on the individual extracts as well as their combination. Analysis showed that all three extracts possessed good phytoconstituent content and dose-dependent anticancer activity, with turmeric showing the strongest individual effect, and the combined extract producing the lowest IC_{50} value, indicating enhanced, synergistic anticancer and antimicrobial action compared to the individual extracts.

Keywords— Colorectal cancer, Withania somnifera, Curcuma longa, Moringa oleifera, Soxhlet extraction, Synergistic activity, Antimicrobial activity

I. INTRODUCTION

Colorectal cancer (CRC), which affects the colon and rectum, is one of the most common and serious types of cancer globally. It begins in the lower part of the digestive system and usually develops slowly from non-cancerous growths known as adenomatous polyps into cancerous tumors over several years.The World Health Organization (WHO) reports that colorectal cancer is the third most commonly diagnosed cancer worldwide and the second leading cause of cancer-related deaths.In 2022, around 1.9 million new cases were recorded globally, along with over 900,000 deaths.[1,2]The burden of this disease is expected to increase further, with projections indicating more than 3.2 million new cases and over 1.6 million deaths each year by 2040.Several lifestyle factors, such as eating a lot of red meat, being overweight, smoking, drinking alcohol, and not being physically active, can increase the risk of developing this cancer.Moreover, having a family history or genetic conditions that raise cancer risk also contributes to the likelihood of developing this disease.Advances in screening, early diagnosis, and molecular research have led to improved prevention and treatment options, which have helped lower mortality rates in many developed countries.[4,5,6] Colon Cancer Colorectal cancer is the third most common type of cancer worldwide and is a major cause of cancer-related deaths, especially within the gastrointestinal system. Risk factors for this disease include an unhealthy diet, smoking, having inflammatory bowel disease, the presence of polyps, a family history of cancer, and aging.Most individuals diagnosed with this cancer are over the age of 50, with an average age of 64.However, younger patients often face more aggressive forms of the disease.Early detection plays a crucial role in reducing the risk of death, and the

outlook for patients often depends on survival rates. A person may be at higher risk if a close family member was diagnosed with colorectal cancer or polyps, especially if it occurred at a younger age. Screening and detection methods include stool tests such as guaiac, immunochemical, and DNA tests, as well as procedures like sigmoidoscopy, colonoscopy, and barium enema. The stage at which the cancer is diagnosed significantly influences the treatment options, the chances of survival, and the overall prognosis. Colon cancer starts when the cells in the lining of the colon grow out of control and may spread to nearby lymph nodes, the liver, the lungs, or other parts of the body. Risk factors for this disease include being over the age of 50, having a family history of the disease, suffering from inflammatory bowel disease, following a diet high in red or processed meats, smoking, being overweight, and not engaging in regular physical activity.In the early stages, colon cancer may not cause any noticeable symptoms, but as the condition progresses, individuals may experience symptoms such as blood in the stool, abdominal pain, changes in bowel habits, unexplained weight loss, or feelings of fatigue. The World Health Organization (WHO) classifies colon cancer mainly based on the type of cells involved and the characteristics of the tumor. Adenocarcinoma is the most common type, making up between 85% and 95% of all cases, and it develops in the glandular cells that line the colon.Other types of cancer include mucinous adenocarcinoma, signet-ring cell carcinoma, and less common forms.[1,3,4,5]

II. PLANT PROFILE

1. Ashwagandha

Taxonomy [7,8,9]

Table 1: Taxonomy of Ashwagandha

Genus	Withania
Species	Withania Duna
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Angiosperma
Class	Dicotyle
Subclass	Asteridae
Order	Tubiflorae
Family	Solanaceae

Anatomy [7]

Roots

The roots of Withania somnifera are long tap roots, measuring about 15–25 cm in length. They are stout, fleshy, and gradually tapering. The color ranges from brownish-white to light yellow. These roots have a characteristic horse-like odor. They possess a distinctly bitter taste. Roots are the most commonly used medicinal part of the plant. They contain important bioactive compounds like withanolides.

Branches

The branches are round in shape and moderately spread out. They are covered with minute hair-like structures called trichomes. These trichomes provide slight protection to the plant. The surface of branches is somewhat rough due to these structures. Branching helps in supporting leaves, flowers, and fruits. They also aid in proper exposure to sunlight.

Leaves

Leaves are simple, smooth, and slightly shiny in texture. They are about 10–15 cm long and dull green in color. The shape is generally elliptic. Leaves are exstipulate and obliquely petiolate. They are arranged alternately on vegetative shoots. On floral shoots, they appear opposite. Leaves play an important role in photosynthesis.

Flowers

Flowers are small, inconspicuous, and about 4–6 mm in diameter. They are greenish-yellow in color. Flowers are bisexual and pedicellate. They occur in axillary umbellate cymes as inflorescence. The calyx is campanulate and persistent. Corolla shows valvate aestivation. These flowers later develop into fruits.

Fruits and Seeds

The fruit is a small, round berry that turns orange-red when mature. It is fleshy and attractive in appearance. The fruit encloses numerous seeds inside. Seeds are small, light in weight, and smooth. They are either lens-shaped or kidney-shaped. Fruits help in seed dispersal. Seeds are important for propagation of the plant.

Fig 4. Ashwagandha Plant

Chemical constituents [7,9,20]

Table 2: Chemical constituents of Ashwagandha

Plant part	Alkaloids	Withnolides (steroidal lactones)	Glycosides/ Saponins	Flavonoids & Phenolics	Other constituents
Root	Withanine, Somniferine, Anaferine, Tropine, Pseudotropine,	Withaferine A, Withanolides,Withanolide B, Withanone	Sitoindoside VII, VIII	Scopoletin (coumarin)	Starch, Reducing sugars,Iron, Amino acids, β-Sitosterol
Steam (Branches)	Trace alkaloids	Withanolide A (moderate)	Minor glycosides	Flavonoids(antioxidants)	Fibrous material Minor sterols
Leaves	Minor alkaloids	Withaferine A(high), Withanolide D, Withanone	Glycosides	Flavonoids (quercetin), Chlorogenic acid	Proteins, Amino acids
Flowers	Trace alkaloids	Low levels of withanolides	Minor glycosides	Flavonoids, Phenolic acids	Pigments, Nectar compounds
Seeds	Trace alkaloids	Small level of withanolides	-	Minimal phenolics	Fatty oil (fixed oil), Proteins
Fruits (Berries)	Minor alkaloids	Trace withanolides	-	Phenolic compounds	Free amino acids(cysteine), enzyme(protease, esterase), Essential oil

Structure of chemical constituents [20]

Withanoloid B

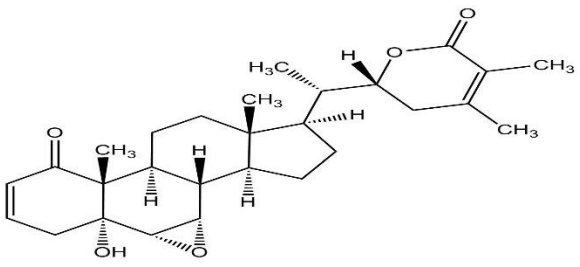


Fig 5. Withanoloid B

Withaferin A

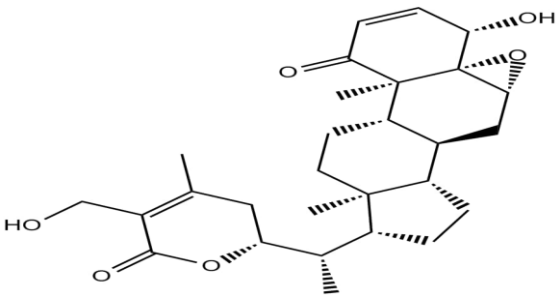


Fig 6. Withaferin A

Uses of plant parts [10]

Root

This is the most frequently consumed portion, utilized as a powerful nervine tonic, aphrodisiac, and adaptogen in the form of powders or extracts to manage stress, anxiety, diabetes, and fatigue. It is a staple in many Ayurvedic formulations, such as Ashwagandharishta, designed to enhance reproductive health and overall stamina.

Leaves

Recognized for their significant anti-inflammatory and antiseptic qualities, the leaves are commonly prepared as topical pastes to soothe skin infections, ulcers, and rheumatic swellings. They are also occasionally used in juice form to treat specific ailments like persistent coughs or ear-related issues.

Flowers

These possess unique astringent, diuretic, and aphrodisiac properties that are frequently tapped to support kidney health and address reproductive fertility concerns.

Seeds

Characterized by their anthelmintic nature, the seeds are employed in traditional medicine to help the body eliminate parasitic worms, and they are sometimes used in preparations to treat localized conditions like corneal white spots.

2. Turmeric

Taxonomy [14]

Table 3: Taxonomy of Turmeric

Kingdom	Plantae
Sub-kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Sub-class	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae
Genus	Curcuma
Species	Longa
Scientific name	Curcuma longa

Anatomy [15]

Root (Rhizome)

The turmeric rhizome is thick, branched, and fleshy, usually measuring about 2–6 cm in diameter and 5–10 cm in length (primary rhizome). It is yellow to orange inside due to curcumin. The surface is rough with ring-like scars. Smaller lateral branches (fingers) are about 3–7 cm long. It stores nutrients and helps in vegetative propagation.

Branches (Pseudostem)

Turmeric does not have true branches but forms a pseudostem made of leaf sheaths. It grows about 60–100 cm in height and 2–4 cm in thickness. The structure is soft and green. It supports leaves and flowers above the ground. It also helps in conduction of water and nutrients.

Leaves

Leaves are large, oblong, and lance-shaped, measuring about 30–60 cm in length and 10–20 cm in width. They have a long petiole (leaf stalk) of about 15–30 cm. The leaves are bright green with a smooth surface. They arise alternately and form a sheath around the pseudostem. They are the main site of photosynthesis.

Flower

The inflorescence (flower spike) is about 10–20 cm long and arises from the center. Individual flowers are small, around 3–5 cm long, and pale yellow or white. Bracts surrounding the flowers may be green or pink and measure about 4–6 cm. The flowering shoot can reach up to 15–25 cm in height. Flowers are bisexual but not commonly used for propagation.

Fruits

Fruits are small capsules, about 1–1.5 cm in diameter. They contain tiny seeds but are rarely seen in cultivated turmeric. The seeds are very small and not used for commercial propagation. The plant mainly reproduces through rhizomes. Therefore, fruits have limited practical importance.

Fig 7. Turmeric plant

Chemical constituents [11,12,13,21]

Table 4: Chemical constituents of Turmeric

Plant parts	Primary Chemical Class	Key Representative Compounds	Typical Concentration/ Occurrence
Rhizome	Curcuminoids, Sesquiterpenes	Curcumin, Demethoxycurcumin, ar-termerone	3-6% Curcuminoids; high sesqueterpene oi content
Roots	Sesquiterpenes	Ar-termerone, zingiberene	Moderate sesquterpene accumulation
Leaves	Monoterpenes, Flavonoids	Terpenolene, Quercetin (trace)	Low-to-moderate; dominant monoterpene profile
Flowers	Monterpenes, Flavonoids	Terpenoline, Linalool	Variable; similar terpenoids profile to leaves

Structure of chemical constituent [21]

Curcumin

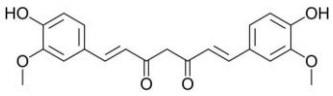
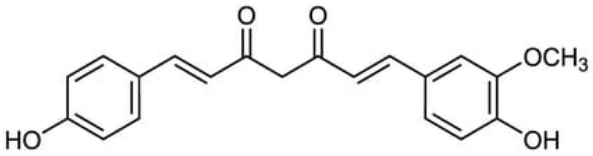


Fig 8. Curcumin

Demethoxycurcumin



Uses of plant parts [11]

Rhizome

Serves as the primary source of bioactive curcuminoids, which provide potent anti-inflammatory, antioxidant, and antimicrobial effects. Widely consumed as a spice, food coloring, and medicinal supplement to manage chronic conditions like arthritis and digestive disorders. Acts as a core ingredient in Ayurvedic and traditional medicines for detoxification and immune support.

Roots (Fibrous structure)

Function primarily as the plant's anchor and nutrient absorption system. While contain similar phytochemicals to the rhizome, they are less concentrated and typically discarded during commercial rhizome processing

Leaves

Used topically as an antiseptic and cooling paste to soothe skin inflammations, cuts, burns, and rheumatic pain. Utilized in culinary practices as natural wrappers during steaming or grilling to infuse food with a unique, earthy aroma. Known to support digestive and respiratory wellness when integrated into traditional herbal preparations.

Flowers

Eaten raw in salads or cooked in various regional dishes for their unique texture and mild flavor. Valued in traditional medicine, particularly for postpartum recovery, to reduce inflammation and promote general healing. Provide concentrated antioxidants that support immune system function, representing a highly versatile but underutilized part of the plant.

3. Moringa

Taxonomy [16,17]

Tables 5: Taxonomy of Moringa

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Dilleniidae
Order	Capparales
Family	Moringaceae
Genus	Moringa
Species	Moringa oleifera

Anatomy [19]

Root

Deep root system; exact size varies by age and soil, but moringa develops a strong taproot for drought tolerance.

Branches

Open crown with fragile, drooping branches; branch length is not fixed, as it depends on tree age and pruning.

Leaves

Usually 7–60 cm long; leaflets are about 1–2 cm long.

Flowers

About 7–14 mm long; flower clusters or panicles are about 10–20 cm long.

Fruit/pod

Usually 10–60 cm long; some sources report 20–45 cm or even up to 1 m in exceptional cases.

Seeds

About 1–1.5 cm in diameter; each seed has three papery wings.

Tree height

Moringa can grow to about 10–12 m tall, with a trunk diameter up to about 46 cm.

Fig 10. Moringa plant

Chemical Constituents [18,19]

Table 6: Chemical Constituent of Moringa

Plant parts	Main constituents	Chemical type	Concentration Range (%Dry weight or extract equiv.)
Leaves	Niazirin, Niazirinin; Quercetin, Kaempferol; Chlorogenic acid	Nitrile glycosides; flavonoid (phenolic); phenolic acid	0.5-2% glycosides; 1-3% flavonoids; 0.2-1% phenolics
Flowers	Kaempferol Quercetin; Ascorbic acid	Flavonoids (phenolics); Vitamin	0.5-1.5% flavonoids; 0.1-0.5% vitamin C
Fruits (pods)	Isothiocyanates; Beta-sitosterol	Glucosinolate derivatives; Sterol	0.3-1% isothiocyanates; 0.1-0.4% sterols
Stem	Vanillin; Beta –sitosterol	Phenolic aldehydeSterol	0.05-0.2% phenolics; 0.1-0.3% sterols
Seeds	Moringine; Niazimicine;Glucosinolates	Alkaloids; Carbamates;Glucosinolates	0.2-0.8% alkaloids; 0.4-1.2% glucosinolates
Roots	Moringine, Moringinine	Alkaloids	0.1-0.5% alkaloids
Bark	Glucosinolates	Glucosinolates	0.2-0.7%

Structure of chemical constituents [22]
Kaempferol

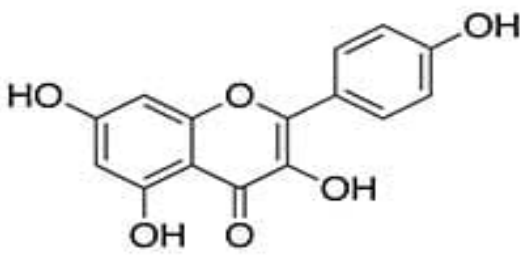


Fig 11. Kaempferol

Quercetin

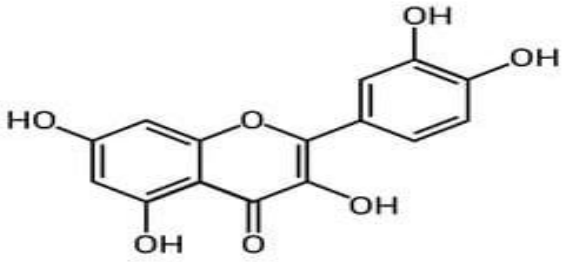


Fig 12. Quercetin

Uses of plant parts [22]
Leaves Uses
Nitrile glycosides like niazirin and niazirinin, along with flavonoids such as quercetin and kaempferol, and phenolic acids like chlorogenic acid in leaves provide potent antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, and antimicrobial effects; they help regulate blood sugar, reduce oxidative stress, and support wound healing.

Flowers Uses
Flavonoids including kaempferol and quercetin, plus ascorbic acid in flowers exhibit anti-inflammatory and antioxidant properties, aiding in reducing inflammation and boosting immunity through vitamin C's role in collagen synthesis and free radical scavenging.

Fruit (Pods) Uses
Isothiocyanates and beta-sitosterol in pods contribute to antimicrobial, antiulcer, and cholesterol-lowering activities, promoting digestive health and cardiovascular protection by inhibiting bacterial growth and modulating lipid profiles.

Stem Uses
Phenolics like vanillin and sterols such as beta-sitosterol in stems support anti-inflammatory and analgesic effects, potentially easing pain and inflammation in traditional remedies.

Seeds Uses
Alkaloids like moringine, carbamates such as niazimicin, and glucosinolates in seeds deliver antimicrobial, anti-cancer, and water-purifying benefits, with antitumor activity against various cancers and cholesterol reduction.

Root Uses
Alkaloids including moringine and moringinine in roots possess anti-inflammatory, analgesic, and circulatory stimulant properties, used traditionally for rheumatism, hypertension, and as cardiac tonic.

Bark Uses
Glucosinolates in bark exhibit anti-inflammatory and antimicrobial actions, supporting skin health and infection control in folk medicine.

III. LITERATURE REVIEW

Zubaidah et al. (2025) reported that Javanese turmeric kombucha exhibits enhanced physicochemical and anti-cancer properties compared to its unfermented form. Fermentation significantly increased phenolic content and antioxidant activity, contributing to stronger cytotoxic effects on T47D breast cancer cells. The study demonstrated reduced cell viability and induced apoptosis in more than 22% of the cancer cell population, supported by morphological and flow cytometry analyses. These findings indicate that fermentation improves the bioactive potential of Javanese turmeric, suggesting its promise as a complementary approach in breast cancer therapy.

Bharat B. Aggarwal et al. (2013 (2013) emphasises that turmeric has several bioactive substances in addition to curcumin, such as turmerones, elemene, and other components with notable anti-inflammatory and anticancer qualities. The abstract emphasizes that curcumin-free turmeric (CFT) also demonstrates strong biological activities, suggesting

synergistic or independent effects of non-curcumin components (Aggarwal et al., 2013). Various studies cited in the review indicate that these compounds act through mechanisms such as apoptosis induction, inhibition of inflammatory mediators, and modulation of signalling pathways. The conclusion underscores that several non-curcumin compounds may be equally or more potent than curcumin and even comparable to standard anti-inflammatory drugs. However, despite promising preclinical evidence, clinical studies on these components remain limited (Aggarwal et al., 2013). Therefore, future research should focus on validating their therapeutic potential through clinical trials and exploring their synergistic effects.

Singh et al. (2019) described Moringa oleifera as a highly nutritious and medicinal plant rich in proteins, vitamins, minerals, and bioactive compounds. The review highlights its significant antioxidant, antibacterial, and anticancer activities, mainly attributed to phytochemicals like flavonoids and phenolics. It also emphasizes its wide pharmacological potential, including anti-inflammatory, hepatoprotective, and neuroprotective effects. Overall, the study concludes that Moringa oleifera is a cost-effective, multifunctional plant with promising therapeutic applications, although further research is needed to confirm its clinical efficacy and expand its use.

Mazurkiewicz et al. (2024) conducted a comprehensive review on Withania somnifera highlighting its significant antibacterial and anticancer potential. The study reported that ashwagandha exhibits inhibitory activity against both Gram-positive and Gram-negative bacteria and shows promising effects in cancer treatment by inducing apoptosis and enhancing sensitivity to radiation. Furthermore, various phytochemicals such as withanolides and alkaloids were identified as key contributors to its bioactivity. Despite these encouraging findings, the authors emphasized the need for more extensive in vivo and clinical studies to validate its long-term efficacy and safety. Overall, the review suggests that ashwagandha could serve as a potential natural therapeutic agent, but further research is required to establish its clinical applications.

Dahot et al. (1998) investigated the antimicrobial potential of small proteins isolated from Moringa oleifera leaves using chromatographic techniques. The study demonstrated that purified fractions (P1, P2, P3) exhibited significant antibacterial activity against E. coli, Staphylococcus aureus, and Bacillus subtilis, with moderate antifungal effects. The findings suggest that these bioactive peptides contribute to the plant's natural defense mechanism against pathogens. Furthermore, variation in activity among fractions indicates selective antimicrobial specificity. In conclusion, the study highlights the potential of Moringa oleifera as a source of natural antimicrobial agents and supports further research for pharmaceutical applications.

Peixoto et al. (2011) investigated the antibacterial activity of aqueous and ethanolic extracts of Moringa oleifera leaves against various Gram-positive and Gram-negative bacteria. The findings showed that higher concentrations of extracts exhibited significant inhibitory effects, particularly against Staphylococcus aureus, Enterococcus faecalis, Vibrio parahaemolyticus, and Aeromonas caviae. However, some strains like Escherichia coli, Pseudomonas aeruginosa, and Salmonella enteritidis showed resistance. The study concluded that Moringa oleifera possesses promising antibacterial properties due to its phytochemical constituents. Overall, the results suggest its potential as a natural alternative for treating certain bacterial infections (Peixoto et al., 2011).

Singh et al. (2014) investigated the antibacterial activity of Moringa oleifera leaf extracts against common pathogenic bacteria using the agar well diffusion method. The results showed that aqueous, ethanol, and methanol extracts exhibited inhibitory effects, with ethanol and methanol extracts demonstrating significantly higher activity, especially at increased concentrations. Escherichia coli was found to be more sensitive compared to Staphylococcus aureus and Pseudomonas aeruginosa. The study concluded that Moringa oleifera leaves possess strong antibacterial potential

against both Gram-positive and Gram-negative bacteria. Furthermore, the extracts were suggested to be more effective than some conventional antibiotics, indicating their possible use as natural therapeutic agents. Overall, the findings support the potential of Moringa oleifera as a cost-effective and safe alternative in treating bacterial infections.

Sobota et al. (2024) investigated the anticancer potential of Withania somnifera (ashwagandha) and reported its significant role in modulating multiple cancer-related pathways. The study highlights that ashwagandha exhibits strong anti-inflammatory and antioxidant properties by regulating pathways such as NF-κB, MAPK, and JAK-STAT, thereby reducing oxidative stress and tumor progression. It also induces apoptosis and inhibits angiogenesis, which helps in controlling cancer cell proliferation and metastasis. Furthermore, the herb enhances immune response by activating T-cells and NK cells, contributing to improved tumor defense. The conclusion suggests that ashwagandha may serve as a promising adjunct in cancer therapy due to its multi-targeted mechanisms. However, Sobota et al. (2024) emphasize the need for further clinical studies to validate its safety and therapeutic efficacy in humans.

Motavallihaghi et al. (2023) reported that hydatid cyst fluid showed significant in vitro anticancer activity against C26 colon cancer cells. The study demonstrated reduced cell viability and induced apoptosis after treatment with the extract. Increased BAX gene expression and decreased BCL2 expression confirmed activation of apoptotic pathways. Cell cycle arrest was observed mainly in G0/G1 and G2/M phases, limiting cancer cell proliferation. These findings suggest that parasite-derived bioactive compounds may have potential as novel anticancer agents.

Nelson et al. (2020) investigated the in vitro anticancer activity of methanolic extract of Eclipta alba against various cancer cell lines. The study reported significant cytotoxicity against HCT-116 human colon cancer cells with minimal toxicity toward normal WI-38 cells. The extract also inhibited colony formation and cell migration in a dose-dependent manner. Phytoconstituents such as wedelolactone and β-sitosterol were suggested to contribute to the anticancer effect. These findings indicate the potential of Eclipta alba as a promising source for colon cancer therapy.

Singirala et al. (2025) reviewed the extraction of bioactive compounds from Withania somnifera and highlighted its importance as a functional medicinal plant. The study reported that the plant is rich in withanolides, alkaloids, flavonoids, and phenolic compounds responsible for various therapeutic effects. Advanced extraction methods such as microwave-assisted, ultrasound-assisted, and supercritical fluid extraction were found effective for obtaining higher yields of active constituents. The review also emphasized antioxidant, anti-inflammatory, anticancer, and immunomodulatory activities of the plant. Furthermore, the authors discussed its potential incorporation into food products to improve nutritional and health benefits.

Piecuch et al. (2024) reviewed the anticancer potential of Withania somnifera (Ashwagandha) and reported its multi-targeted therapeutic effects. The study highlighted that Ashwagandha exhibits anti-inflammatory and antioxidant activities, which help reduce oxidative stress and cancer progression. It was also found to induce apoptosis and inhibit angiogenesis in various cancer cell lines. Additionally, the herb enhances immune responses by stimulating T-cells and NK cells. The authors concluded that Ashwagandha may serve as a promising adjunct in cancer therapy, though further clinical studies are required to confirm its safety and efficacy.

Need

High burden of colorectal cancer [23,25]

Colorectal cancer (CRC) is among the most frequent cancers worldwide and HCT116 is well-established human colon adenocarcinoma cell line widely used to model CRC biology and drug-response studies. Conventional chemotherapy agents often cause severe toxicity and drug resistance, so there is a strong need to explore safer, natural alternatives such as plant-derived extracts.

2. Potential of medicinal plant as anticancer agents [24]

Many traditional medicinal plants have shown promising in-vitro cytotoxicity and pro apoptotic effects on HCT116 and other cancer cell lines, indicating their potential as anticancer lead. The majority of research, however, concentrates on single plant extracts; systematic assessment of the individual and combinational (synergistic) effects of several plant extracts on HCT116 is yet comparatively unexplored.

Rationale for evaluating synergy [25,26]

Combination of plant extracts may produce synergistic, additive or antagonistic interactions, which can either enhance efficacy or reduce required doses and side effects. In vitro synergy testing allows selection of the most potent combinations before moving to expensive in vivo or clinical studies.

Aim and Objective

Aim

To evaluate in vitro individual and synergestic anticancer activity of ashwagandha, turmeric and moringa plant extract against human colon cancer cell line (HCT 116)

Objectives

Primary objectives

- To evaluate the individual cytotoxic activity of three selected medicinal plants extracts on HCT116 human colon cancer cells using standard assays (e.g., MTT/XTT, SRB, or similar). [26]
- To determine the IC50 values, dose response curve and time-dependant effects of each plant extracts on HCT116 proliferation and viability.

Synergistic interaction objective

- To assess the combinational (two-way and three-way) effects of the selected plants extacts on HCT116 cells and to quantify synergy using models such as combination index (CI), Fractional Inhibitory Concentration (FIC) or isobologram analysis.
- To identify the most synergistic combination that show significantly lower IC50 and/or reduce cytotoxicity on normal cells compared with individual extracts

IV. MATERIALS AND EQUIPMENT

Weighing balance, Soxhlet apparatus, Beaker, Round bottom flask, Test tubes, Test tube stand, Stirrer, Butter paper, Cotton bags, Metallic cage, Aluminium foil, Distilled water, Conical flask, Petri plates, Sterile spreader, Sterile loop, Cork, Glass containers, etc.

1. Plants

Table 7: List of Plants

Plant Name	Biological source	Family	Locality
Turmeric	Dried rhizomes of *Curcuma longa*.	Zingiberaceae Martinov	Palus, Dist. Sangli
Ashwagandha	Dried leaves of *Withania somnifera (L)*.	Solanaceae Juss.	Vita, Tal. Khanapur, Dist. Sangli
Moringa	Dried leaves of *Moringa oleifera Lam*.	Moringaceae Martinov	Vita, Tal. Khanapur, Dist. Sangli

2. List of Standard drugs

Table 8: List of Standard Drug

Standard drug	Manufactured by
Erythromycin	Medibios LaboratoriesPvt.Ltd.
5-Flurouracil	Celon Laboratories

3. List of Chemicals

Table 9: List of Chemicals

SN	Name of chemicals	Name of supplier
1.	Ethanol	MARINEX LABORATORY
2.	Dragendorff's reagent	LOBA CHEMIE PVT.LTD.
3.	Ferric chloride	LOBA CHEMIE PVT.LTD.
4.	Mayer's reagent	LOBA CHEMIE PVT.LTD.

5.	Lead acetate Solution	LOBA CHEMIE PVT.LTD.
6.	Sulphuric acid	LOBA CHEMIE PVT.LTD.
7.	Hager's reagent	LOBA CHEMIE PVT.LTD.
8.	Wagner's reagent	LOBA CHEMIE PVT.LTD.
9.	Hydrochloric acid	LOBA CHEMIE PVT.LTD.
10.	Acetic acid	LOBA CHEMIE PVT.LTD.
11.	Sodium hydroxide	LOBA CHEMIE PVT.LTD.
12.	Chloroform	LOBA CHEMIE PVT.LTD.
13.	Sodium Chloride	LOBA CHEMIE PVT.LTD.
14.	Nutrient agar media	LOBA CHEMIE PVT.LTD.
15.	Methylene blue	LOBA CHEMIE PVT.LTD.
16.	Crystal violet	LOBA CHEMIE PVT.LTD.
17	Carbapol Fuschin	LOBA CHEMIE PVT.LTD.
18	Gram iodine solution	LOBA CHEMIE PVT.LTD.
19	Cidarwood oil	LOBA CHEMIE PVT.LTD.
20	Dulbecco's Modified Eagle Medium	VIJAY CHEMICAL
21	Fetal Bovine Serum	A.J. CORPORATION
22	Dimethyl Sulfoxide	A.J. CORPORATION
23	Phosphate Buffered Saline	VIJAY CHEMICAL
24	MTT reagent	A.J. CORPORATION
25	5-Fluorouracil	VIJAY CHEMICAL

4. List of Instruments

Table 10: List of Instruments

SN	Instrument	Model	Company
1.	96-well plate	96-well microplate	HiMedia Laboratories Pvt Ltd, Mumbai
2.	CO_2 Incubator	BB150 CO_2 Incubator	Saksham Technologies Pvt Ltd
3.	Biosafety cabinet		Hally Instruments
4.	ELISA Plate Reader	E21 ELISA Microplate Reader	Transasia Biomedicals Ltd, Mumbai

V. EXPERIMENTAL WORK

1. Collection of plants

The plants like Curcuma longa (L.) cultivated in the field of islampur, Moringa oleifera Lam collected from farm of vita, Withania somnifera (L.) Dunal collected from rood side of vita, sangli, Maharashtra India.

2. Drying of plants

After collection plants were washed and kept for shade drying. After shade drying of 1week plants get ready for the authentication.

3. Authentication of plants

Plants were submitted in the museum of Department of Botany, Balwant college, vita Dist. Sangli (Specimen No. AKS001, AKS002, AKS003) for the botanical authentication.

4. Preparation of extracts [27,28,29]

Extraction in pharmacognosy refers to the process of separating active pharmaceutical constituents from plant sources. Coarse powder of Turmaric, Ashwagandha and Moringa were collected from ayurvedic shop of Islampur. The powder was kept at room temperature.

In the present study, the powder of Moringa oleifera Lam, Withania somnifera (L.) Dunal and Curcuma longa (L.) were subjected to extraction using ethanol as a solvent. Approximately 250-300 mL of ethanol were used for the extraction process.

The extraction was carried out using a Soxhlet apparatus (Shri Krishna Surgicals). The process was continued until 10 extraction cycles were completed to ensure efficient extraction of Phytoconstituents from the plant material.

After completion of the extraction cycles of each plant material, the ethanolic extract of Moringa oleifera Lam, Withania somnifera (L.) Dunal and Curcuma longa (L.) were obtained. The extract was then collected and stored in a properly labeled container, protected from sunlight to prevent degradation of active constituents.

Soxhlet extraction procedure [30]

Extraction is the primary step in the analysis of plant material, involving the separation of chemical constituents from plant tissues. The choice of extraction method depends on several factors such as the nature of phytochemicals, type of plant materials and the intended purpose of extraction.

Soxhlet extraction is a continuous extraction technique that utilizes a heated organic solvent. It is particularly suitable when the desired compounds have limited solubility in the solvent and impurities are insoluble. This method allows efficient and repeated extraction without constant supervision.

The Soxhlet apparatus, developed by Franz von Soxhlet in 1879, consists of the three main components:

- Percolator (Boiler and Reflux): Facilitates continuous circulation of the solvent.
- Thimble: Made of cellulose or filter paper, it holds the plant material.
- Siphon Mechanism: Periodically transfers the solvent back to the flask, completing the extraction cycle.

Additional components include:

- Round Bottom Flask (RBF): Contains the extraction solvent.
- Heating Mantle: Provides controlled heating for solvent evaporation and reflux.
- Reflux condenser: Positioned above the extractor, the condenser facilitates the cooling and condensation of solvent vapours.
- Water inlet & outlet: Connected to the condenser, these allow for the continuous flow of water to regulate the condenser's temperature.

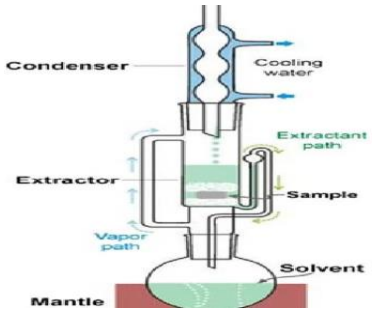


Fig 13. Soxhlet Extract

VI. METHODOLOGY [27,28,29]

The powder of turmeric, ashwagadha and moringa was placed into each thimble made of cotton cloth (cotton bag). This thimble was then positioned inside the Soxhlet extractor.

A round-bottom flask containing approximately 200–250 ml of ethanol was attached below the extractor, and a condenser was fixed at the top.

The apparatus was heated so that the ethanol started boiling and producing vapors. These vapors moved through the distillation arm into the condenser, where they cooled and converted back into liquid form. The condensed solvent then dripped into the chamber containing the cotton thimble with the powder.

As the solvent accumulated, it extracted the active compounds from the powder. When the liquid reached the siphon level, it automatically drained back into the flask along with the dissolved constituents.

This cycle of evaporation, condensation, and siphoning was repeated for about 10 cycles to achieve effective extraction. After completion, the ethanolic extract of each turmeric, ashwagandha, moringa powder was collected in the flask for further use.

After about 10 cycles, or nearly 8 hours, the desired compound becomes concentrated in the distillation flask.

Once the extraction process is complete, the solvent is removed to obtain the extract, commonly by allowing it to evaporate in the shade. The remaining insoluble material stays inside the thimble and is usually discarded.

Fg14. Turmeric Extract

Fg15. Ashwagandha Extract

Fg16. Moringa Extract

Evaporation of Extract [33]

After completion of the Soxhlet extraction, the ethanolic extract was transferred into a porcelain dish and kept in a dry place in the laboratory for about 3-4 days to allow complete evaporation of the solvent.

Fig 17. Evaporation of Turmeric, Ashwagandha, Moringa

Phytochemical Screening

The Phytochemical screening of ethanolic extract was performed according to standard procedures.

Turmeric [31]

Flavanoid test: Lead Acetate test

To 2 ml of turmeric extract add a few drops of 10% lead acetate solution resulted in the formation of a yellow precipitate confirms the presence of flavonoids.

Tannins test: Ferric Chloride Test

To check for tannins, boil 1 ml of turmeric extract in 20 ml of water, then filter the mixture. The test is examined for a brownish green or a blueblack colour when a few drops of 0.1% are added.

Alkaloids test

Mayer's test

To 1ml of turmeric extract was added two drops of the Mayer's reagent along the side of the test tube. A white or creamy precipitate confirmed the test as positive.

Dragendorffs test

To 2 ml of the filtrate was added 1 ml of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish-brown precipitate indicated the test as positive.

Curcumin test

a) Boric acid test-

Add 2 ml of liquid sample of boric acid with few drops of HCL in a test tube then add 1ml of turmeric extract, gently heat the test tube on a water bath. A red or red-brown colour confirmed the test as positive.

Sulphuric acid test

Add concentrated sulphuric acid to the extract. The formation of a crimson-red colour confirms the presence of curcumin.

Fig 18. Chemical test of Turmeric

Ashwagandha [32]

Withanolides: Terpenoids test-

About 0.2 g of each extract was mixed with 2 ml of chloroform and conc. H_2SO_4 (3 ml) was carefully

added to form a layer. The formation of reddish-brown coloration at the interface indicates positive results for presence of terpenoids.

Flavanoid test: Lead Acetate test

To 2 ml of turmeric extract add a few drops of 10% lead acetate solution resulted in the formation of orange-yellow precipitate confirms the presence of flavonoids.

Alkaloids test

Hager's test

To 1 ml of test solution or filtrate, a drop or two of Hager's reagent was added. The formation of yellow precipitate indicated the test as positive.

Dragendroffs test

To 2 ml of the filtrate was added 1 ml of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the test as positive.

Fig 19. Chemical test of Ashwagandha

Moringa

Flavanoid test: Lead Acetate test

To 2 ml of turmeric extract add a few drops of 10% lead acetate solution resulted in the formation of a greenish-yellow precipitate confirms the presence of flavonoids.

Alkaloids test-

Hager's Test-

To 1 ml of test solution or filtrate, a drop or two of Hager's reagent was added. The formation of yellow or green precipitate indicated the test as positive.

Wagner Test-

Two drops of Wagner's reagent were added to 1ml of the test solution along the the side of the test tube. The formation of yellow or brown precipitate confirmed the test as positive alkaloids.

Mayer's Test-

To 1 ml of test solution or filtrate was added a drop or two of the Mayer's reagent along the sides of the test tube. A creamy or white precipitate indicated a successful test.

Dragendorff's Test-

To 2 ml of the filtrate was added 1 ml of Dragendorff's reagent along the side of the test tube. Formation of orange or orange reddish brown precipitate indicated the test as positive.

Fig 20. Chemical test of Moringa

Anticancer Activity:

Sample- Turmeric extract, Ashwagandha extract, Moringa extract and Combination mixture.

Sample form- solid

Activity- In Vitro Cell Viability Assay

Cell line- HCT 116 (Human Colon Cell line)

Media

- McCoy's Medium with high glucose (CatNo-11965-092)
- FBS (Gibco, Invitrogen) CatNo-10270106
- Antibiotic- Antimycotic100Xsolution (Thermo fisher Scientific) CatNo-15240062
- 5-Flurouracil

Materials Required

- 96 well plate
- CO2 incubator (Thermo fisher Scientific)

- Biosafety cabinet (SAS filtration technologies Pv.t. Ltd. Pune.)
- Elisa plate reader (BenespheraE21)

Principle of Assay: [34,35,36]

This Colorimetric assay is based on the capacity of Mitochondria succinate dehydrogenase enzymes in living cell store duce the yellow watersoluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) into an insoluble, purple colored formazan product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

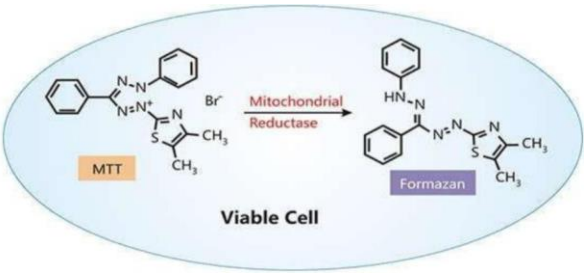


Fig 21. Colorimetric Assay

Procedure: [34,35,36]

- HCT 116 (Human Colon Cell line) was procured from National center for cell sciences (NCCS), Pune maintained in DMEM Medium supplemented with10%fetalbovineserum.
- Cells were incubated at a concentration of 1 × 104 cells/ml in culture medium for 24 h at 37°C and5%CO2.
- Cells were seeded at a concentration(70µl)104cells/wellin100µlculture mediumand100µl i. Samples (10-100µg/ml) into microplates respectively (tissue culture grade, and 96 wells).
- Control wells were incubated with DMSO (0.2% in PBS) and cell line. All samples were incubated in triplicate. Controls were maintained to determine the control cell survival and the percentage of live cells after culture.
- Cell cultures were incubated for 24 h at 37°C and 5% CO2 in CO2 incubator (Thermo scientificBB150)
- After incubation, the medium was completely removed and Added 20µl of MTT reagent (5mg/min PBS).
- After addition of MTT, cells incubated for 4hrs at 37oC in CO2 incubator.
- Observed the wells for formazan crystal formation under microscope. The Yellowish MTT was reduced to dark colored formazan by viable cells only.
- 8. After removing the medium completely. 200 microlitres of DMSO were added, left for ten minutes, and then incubated at 370 degrees Celsius while covered with aluminium foil.
- Triplicate samples were analyzed by measuring the absorbance of each sample by a Elisa microplate reader (BenespheraE21) at a wavelength of 570nm.

Antimicrobial Activity: [37,38,39]

Medicinal plants are widely used in traditional medicine due to their therapeutic and antimicrobial properties. Plant extracts contain bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and curcumin which help inhibit microbial growth. Antibiotic resistance has increased the need for alternative natural antimicrobial agents. Ashwagandha (Withania somnifera), Turmeric (Curcuma longa), and Moringa (Moringa oleifera) are known for their antimicrobial and antioxidant activities.

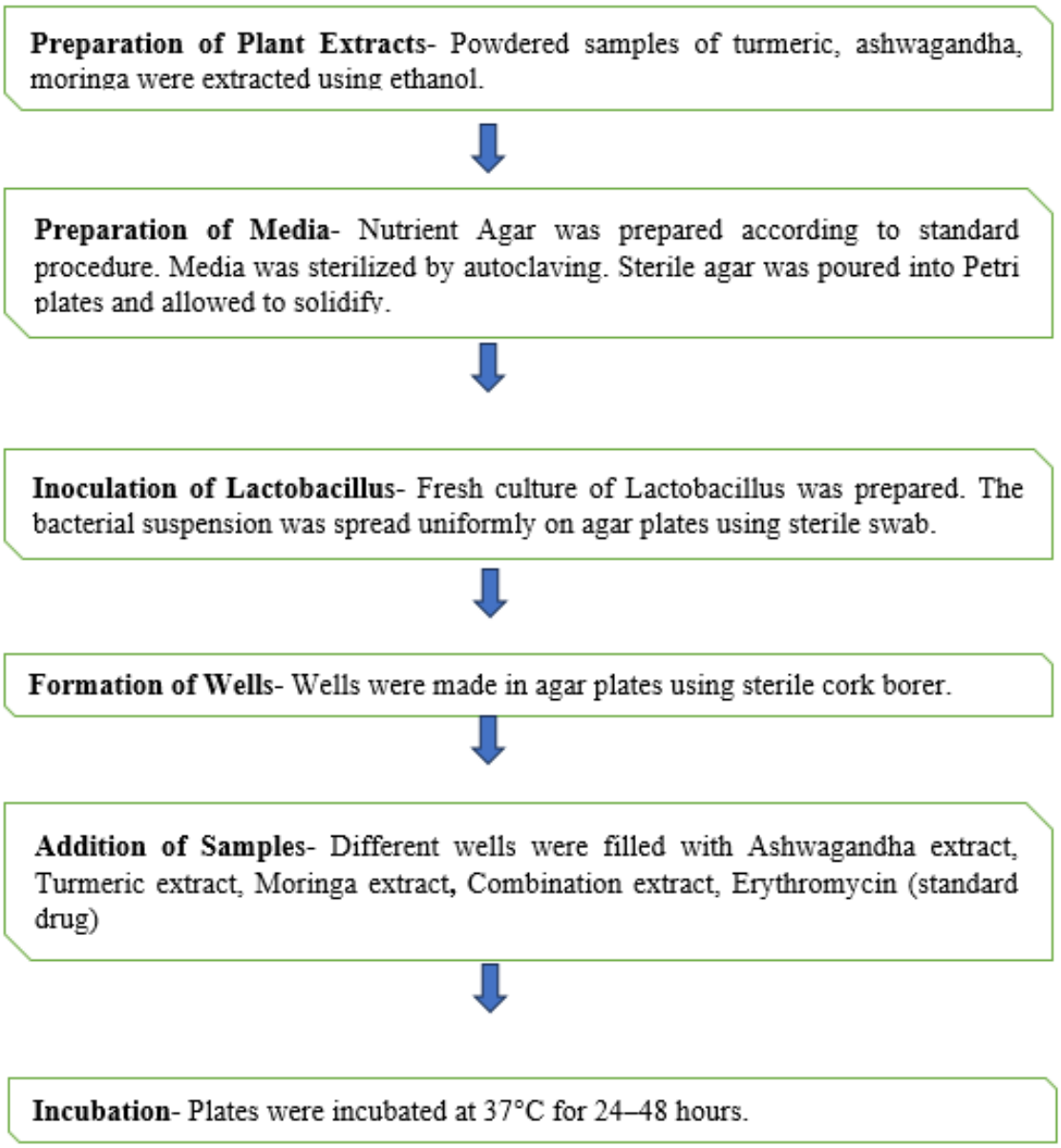
Erythromycin is used as a standard antibiotic for comparison of antimicrobial effectiveness. Lactobacillus species are important microorganisms commonly used in microbiological and pharmaceutical studies. The present study evaluates the antimicrobial activity of individual and combined plant extracts against Lactobacillus using the zone of inhibition method.

Method Used- Zone of Inhibition

Principle- Antimicrobial compounds diffuse through the agar medium and inhibit microbial growth around the well/disc. The clear circular area formed around the sample is called the Zone of Inhibition. Larger zones indicate stronger antimicrobial activity.

Procedure- [37,38,39]

Observation- The diameter of clear zones around wells was measured in millimeters (mm). Results were compared with standard drug Erythromycin.



VI. RESULT AND DISCUSSION

1. Collection of Plant

The plants like Curcuma longa (L.) cultivated in the field of islampur, Moringa oleifera Lam collected from farm of vita, Withania somnifera (L.) Dunal collected from rood side of vita, sangli, Maharashtra India.

2. Authentication of Plant

Plants were submitted in the museum of Department of Botany, Balwant college, vita Dist. Sangli (Specimen No. AKS001, AKS002, AKS003) for the botanical authentication.

3. Plant extracts

The photograph of plant extracts is given in Figure. According to the literature survey, ethanol was used as the solvent and the ma ceration process was used for extraction. The percentage practical yield of ethanolic extract of turmeric 5% w/w, ashwagandha is 2 %w/w and moringa is 13% w/w

4. Phytochemical screening

Table 11: Phytochemical screening of Turmeric, Ashwagandha, Moringa

SN	Test	Specification	Observations	Results
1	Turmeric			
	a) Lead acetate test	Formation of yellow precipitate	Formation of yellow precipitate	Present
	b) Ferric chloride test	Bluish green colour	Bluish green colour	Present
	c) Mayer's test	White or Creamy precipitate	Creamy precipitate	Present
	d) Dragendorff's test	Orange or orange brown precipitate	orange brown precipitate	Present
2.	Ashwagandha			
	a) Terpenoids test	Formation of reddish or brown colour	Formation of brown colour	Present
	b) Flavonoid test	Formation of yellow orange precipitate	Formation of orange precipitate	Present
	c) Hager's test	Formation of yellow precipitate	Formation of yellow precipitate	Present
	d) Dragendorffs test	Formation of orange or orange reddish-brown precipitate	Formation of orange reddish-brown precipitate	Present
3.	Moringa			
	a) Lead acetate test	Formation of greenish yellow precipitate	Formation of greenish yellow precipitate	Present
	b) Hager's test	Formation of yellow or greenish precipitate	Formation of greenish precipitate	Present
	c) Wagner's test	Formation of yellow or brown precipitate	Formation of brown precipitate	Present
	d) Dragendorff's test	Formation of orange or orange reddish-brown precipitate	Formation of oranorange reddish-brown precipitate	Present

Fig 22. Phytochemical screening of turmeric

Fig 23. Phytochemical screening of Ashwagandha

Fig 24. Phytochemical screening of Moringa

Anticancer activity (MTT Assay)

Anticancer Activity Result Analysis (HCT 116 Cell Line)- The anticancer activity of Moringa, Turmeric, Ashwagandha, and their combined mixture was evaluated against the HCT 116 human colon cancer cell line using the MTT assay. The assay measures mitochondrial activity in viable cells. A lower cell viability percentage and lower IC50 value indicate stronger anticancer activity.

Table 12: Effect of 5-Flurouracilagainst HCT 116 MTT Assay

Conc (ug/ml)	Absorbance (OD)				Cell Viability (%)	IC50 (ug/ml)
	1	2	3	Average		
Control	2.316	2.315	2.319	2.16±		
Standard 5FU 100	0.546	0.542	0.541	0.543±0.002	23.3448	3(ug/ml)
50	0.653	0.652	0.654	0.653±0.001	28.07395	
25	0.754	0.752	0.756	0.754±0.002	32.41617	
12.5	0.859	0.851	0.856	0.855±0.004	36.77271	
6.25	1.563	1.562	1.564	1.563±0.001	67.1969	

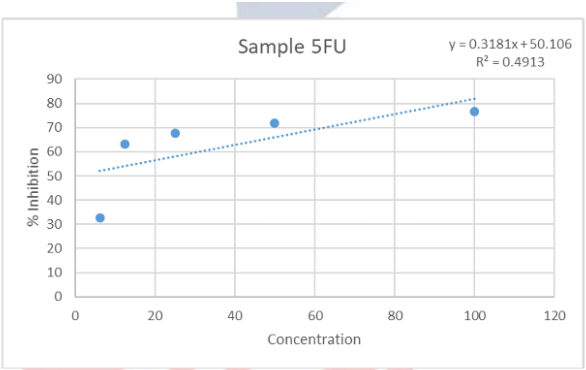
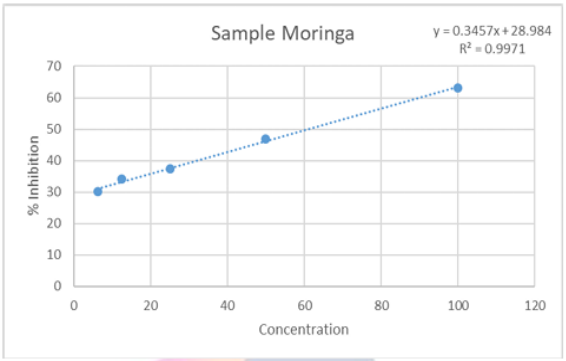
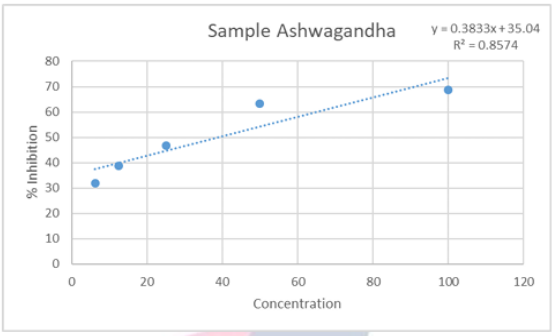


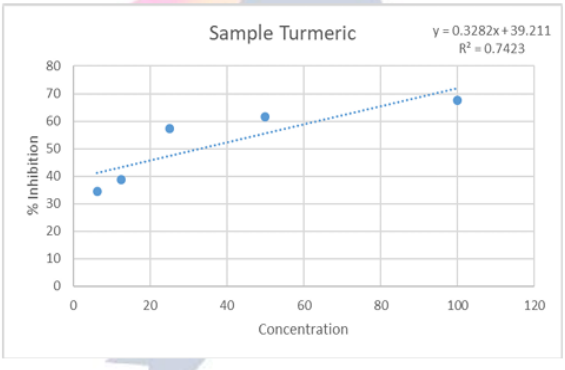
Table 13 Effects of Sample against HCT 116 (Human Colon Cell line) by MTT assay

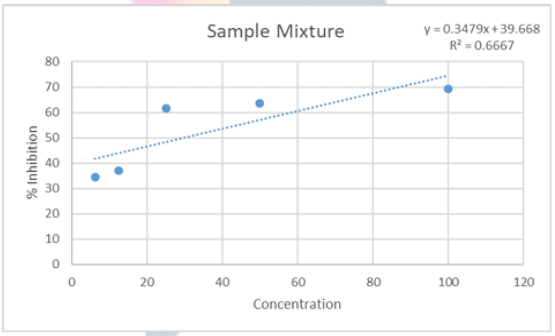
Conc.(ug/ml)	Absorbance (OD)				Cell Viability (%)	IC50 (ug/ml)
	1	2	3	Average		
Control	2.326	2.325	2.328	2.326±0.002		
Sample Moringa 100	0.854	0.852	0.853	0.853±0.001	36.6724	60.79
50	1.233	1.235	1.236	1.234±0.001528	53.08111	
25	1.452	1.456	1.453	1.453±0.002082	62.49642	
12.5	1.552	1.526	1.523	1.533±0.15948	65.9358	
6.25	1.622	1.625	1.623	1.623±0.001525	69.79077	

Sample Turmeric 100	0.752	0.751	0.756	0.753±0.002646	32.37317	
50	0.896	0.896	0.894	0.895±0.001155	38.4924	32.87
25	0.998	0.991	0.993	0.994±0.003606	42.73431	
12.5	1.423	1.4269	1.423	1.424±0.002252	61.23388	
6.25	1.521	1.526	1.523	1.523±0.002517	65.49154	
Sample Ashwagandha 100	0.725	0.726	0.729	0.726±002082	31.24104	
50	0.852	0.851	0.856	0.853±0.002646	36.6724	39.02
25	1.236	1.235	1.239	1.236±0.002082	53.1671	
12.5	1.425	1.426	1.423	1.424±0.001528	61.24694	
6.25	1.589	1.582	1.586	1.585±0.003512	68.1714	
Sample Mixture 100	0.715	0.716	0.718	0.716±0.001528	30.79679	
50	0.845	0.845	0.843	0.844±0.001528	36.31413	29.69
25	0.896	0.835	0.891	0.894±0.002646	38.43508	
12.5	1.463	1.468	1.469	1.46694±0.003215	63.05532	
6.25	1.523	1.528	1.529	1.52694±0.003215	65.63485	









Antimicrobial Activity

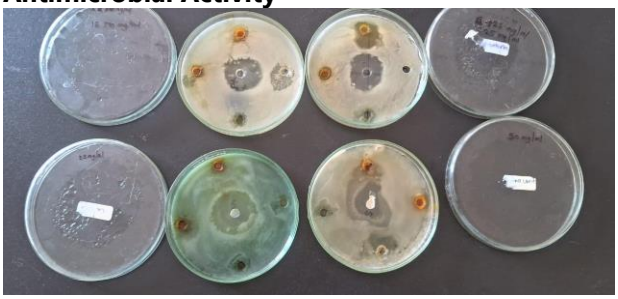


Fig 25 Antimicrobial Activity of Standard drug, Turmeric, Ashwagandha, Moringa extract with combination of three extracts

VII. DISCUSSION

The present study evaluated the individual and synergistic antimicrobial and anticancer activities of Withania somnifera, Curcuma longa, and Moringa oleifera extracts against Lactobacillus bulgaricus and Colorectal Cancer using HCT116 cell lines. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, and terpenoids, which contribute to significant biological activities. The extracts showed dose-dependent anticancer and antimicrobial effects, while the synergistic combination demonstrated enhanced activity compared to individual extracts. The improved effect may be due to the combined action of bioactive compounds that induce apoptosis, inhibit cell proliferation, reduce oxidative stress, and interfere with microbial metabolic pathways. Overall, the findings suggest that these medicinal plant extracts possess promising therapeutic potential as safer natural alternatives for the management of microbial infections and colon cancer, although further in-vivo and mechanistic studies are required for clinical validation.

VIII. CONCLUSION

Anticancer Activity

The cytotoxic potential of different samples against the HCT 116 (human colon cancer cell line) was evaluated using the MTT assay. All tested samples exhibited a dose-dependent decrease in cell viability, indicating significant anticancer activity. Among the individual extracts, Turmeric showed the highest cytotoxic effect with the lowest IC_{50} value (32.87 µg/ml), followed by Ashwagandha (39.02 µg/ml) and Moringa (60.79 µg/ml). This suggests that Turmeric possesses stronger antiproliferative activity against HCT 116 cells compared to the other individual plant extracts. Notably, the mixture of all extracts demonstrated the most potent cytotoxic activity, with the lowest IC_{50} value (29.69 µg/ml), indicating a possible synergistic effect among the bioactive compounds present in the combined formulation. At the highest concentration (100 µg/ml), all samples significantly reduced cell viability, with the mixture showing the maximum inhibition (~30.79% cell viability), further confirming its enhanced anticancer potential. Overall, the results suggest that while individual extracts possess moderate to strong anticancer activity, the combined formulation exhibits superior efficacy, making it a promising candidate for further investigation in colon cancer therapy.

Antimicrobial Activity

The present study evaluated the antimicrobial activity of Turmeric, Ashwagandha, and Moringa extracts individually and in combination against Lactobacillus species using different concentrations of 50 mg/ml, 25 mg/ml, 12.50 mg/ml, 6.25 mg/ml, and 3.125 mg/ml. The antimicrobial activity was compared with the standard drug Erythromycin (250 mg). The results indicated that all three plant extracts, as well as their polyherbal combination, exhibited antimicrobial activity against the tested microorganism. The activity was found to vary with concentration, with higher concentrations showing greater antimicrobial effect. The synergistic combination of the extracts demonstrated comparatively enhanced activity, suggesting possible synergism among the phytoconstituents present in the plants. Therefore, the study concludes that these medicinal plant extracts possess significant antimicrobial potential and may serve as a natural source for the development of antimicrobial agents.

REFERENCES

1. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. Global Cancer Observatory:

Cancer Today. Lyon: International Agency for Research on Cancer; 2020 (https://gco.iarc.fr/today, accessed February 2021).
2. Mármol, I., Sánchez-de-Diego, C., Pradilla Dieste, A., Cerrada, E., & Rodriguez Yoldi, M. J. (2017). Colorectal carcinoma: a general overview and future perspectives in colorectal cancer. International journal of molecular sciences, 18(1), 197.
3. Granados-Romero, J. J., Valderrama-Treviño, A. I., Contreras-Flores, E. H., Barrera-Mera, B., Herrera Enríquez, M., Uriarte-Ruíz, K., ... & Arauz-Peña, G. (2017). Colorectal cancer: a review. Int J Res Med Sci, 5(11), 4667.
4. Center, M. M., Jemal, A., Smith, R. A., & Ward, E. (2009). Worldwide variations in colorectal cancer. CA: a cancer journal for clinicians, 59(6), 366-378.
5. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. Nat Med. 2003;9:669-76.
6. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature. 1993;362:841-4.
7. Garkal, A. G., Bakal, R. L., & Hatwar, P. R. Ashwagandha (Withania somnifera): A comprehensive review of its taxonomy, phytochemistry, pharmacological effects and traditional uses.
8. Meena LK, Gupta AK, Patel J, Khan MY, Kumar S. Ashwagandha (Withania somnifera L.). In: Medicinal Plants in India: Importance and Cultivation. 2020:81-94.
9. Srivastav, Y., Bind, S. B., & Srivastav, A. Ashwagandha (Withania somnifera), Taxonomy, Pharmacological Properties and Its Role in COVID-19 and Extraction Process: A Comprehensive Expansion.
10. Khanchandani N, Shah P, Kalwani T, Ardeshna A, Dharajiya D, et al. Antibacterial and antifungal activity of Ashwagandha (Withania somnifera L.): a review. Journal of Drug Delivery & Therapeutics. 2019;9(5- s):154-161. DOI: 10.22270/jddt.v9i5-s.3573.
11. Krup, V., Prakash, L. H., & Harini, A. (2013). Pharmacological activities of turmeric (Curcuma longa Linn): a review. J Homeop Ayurv Med, 2(133), 2167-1206.
12. Naz S. Antibacterial activity of Curcuma longa varieties against strains of bacteria. Pakistan Journal of Botany, v. 2010; 42:455-462.
13. Negi PS. Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. Journal of Agricultural and Food Chemistry. 1999; 47:4297-4300. PMid:10552805.
14. Chanda, S., & Ramachandra, T. V. (2019). Phytochemical and pharmacological importance of turmeric (Curcuma longa): A review. Research & Reviews: A Journal of Pharmacology, 9(1), 16-23.
15. Nair, K. P. (2013). The agronomy and economy of turmeric and ginger: the invaluable medicinal spice crops. Newnes.
16. Mallenakuppe, R., Homabalegowda, H., Gouri, M. D., Basavaraju, P. S., & Chandrashekharaiah, U. B. (2015). History, taxonomy and propagation of Moringa oleifera–a review. crops, 3(3.28), 3-15.
17. Sharmin, F., Sarker, M. S. K., Sarker, N. R., Huque, K. S., Hossain, S. M. J., & Basher, M. K. (2019). Taxonomical identification, biomass production and nutrient composition of Moringa sp. as a fodder crop. Bangladesh Journal of Livestock Research, 26(1-2), 61-72.
18. Dubey, D. K., Dora, J., Kumar, A., & Gulsan, R. K. (2013). A multipurpose tree-Moringa oleifera. International journal of pharmaceutical and chemical sciences, 2(1), 415-423.
19. Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera: a food plant with multiple medicinal uses. Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, 21(1), 17-25.
20. Shinde, S., Balasubramaniam, A. K., Mulay, V., Saste, G., Girme, A., & Hingorani, L. (2023). Recent advancements in extraction techniques of Ashwagandha (Withania somnifera) with insights on phytochemicals, structural significance, pharmacology, and current trends in food applications. ACS omega, 8(44), 40982-41003
21. Li, S., Yuan, W., Deng, G., Wang, P., Yang, P., & Aggarwal, B. (2011). Chemical composition and

product quality control of turmeric (Curcuma longa L.).
22. Shervington, L. A., Li, B. S., Shervington, A. A., Alpan, N., Patel, R., Muttakin, U., & Mulla, E. (2018). A comparative HPLC analysis of myricetin, quercetin and kaempferol flavonoids isolated from Gambian and Indian Moringa oleifera leaves. International Journal of Chemistry, 10(4), 28.
23. Yeung, T. M., Gandhi, S. C., Wilding, J. L., Muschel, R., & Bodmer, W. F. (2010). Cancer stem cells from colorectal cancer-derived cell lines. Proceedings of the National Academy of Sciences, 107(8), 3722-3727.
24. Howells, L. M., Sale, S., Sriramareddy, S. N., Irving, G. R., Jones, D. J., Ottley, C. J., ... & Brown, K. (2011). Curcumin ameliorates oxaliplatin-induced chemoresistance in HCT116 colorectal cancer cells in vitro and in vivo. International journal of cancer, 129(2), 476-486.
25. Aiello, P., Sharghi, M., Mansourkhani, S. M., Ardekan, A. P., Jouybari, L., Daraei, N., ... & Kooti, W. (2019). Medicinal plants in the prevention and treatment of colon cancer. Oxidative Medicine and Cellular Longevity, 2019(1), 2075614.
26. Cen, L., Hutzen, B., Ball, S., DeAngelis, S., Chen, C. L., Fuchs, J. R., ... & Lin, J. (2009). New structural analogues of curcumin exhibit potent growth suppressive activity in human colorectal carcinoma cells. BMC cancer, 9(1), 99.
27. Popuri, A. K., & Pagala, B. (2013). Extraction of curcumin from turmeric roots. Int J Innovative Res Stud, 2, 289-299.
28. Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., Wongkrajang, Y., & Gritsanapan, W. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Industrial crops and products, 44, 566-571.
29. Nile, S. H., Nile, A., Gansukh, E., Baskar, V., & Kai, G. (2019). Subcritical water extraction of withanosides and withanolides from ashwagandha (Withania somnifera L) and their biological activities. Food and Chemical Toxicology, 132, 110659.
30. De Castro, M. L., & Ayuso, L. E. (2000). Soxhlet extraction. Environmental Applications, 2701-2705.
31. Oghenejobo, M., & Bethel, O. (2017). Antibacterial evaluation, phytochemical screening and ascorbic acid assay of turmeric (Curcuma longa). MOJ Bioequiv Availab, 4(2), 00063.
32. Baskaran, C., & Velu, S. (2012). Phytochemical analysis and in-vitro antimicrobial activity of Withania somnifera (Ashwagandha). J Nat Prod Plant Resour, 2(6), 711-6.
33. Zulmardi, Z., Mitayani, M., Febriyanti, F., & Soo, K. M. (2022). Phytochemical Screening of Turmeric (Curcuma longa Linn.) Extract With 97% Ethanol Solution. Jundishapur Journal of Microbiology, 15(2), 926-931.
34. Santos, P. A. S. R., Avanço, G. B., Nerilo, S. B., Marcelino, R. I. A., Janeiro, V., Valadares, M. C., & Machinski, M. (2016). Assessment of cytotoxic activity of rosemary (Rosmarinus officinalis L.), turmeric (Curcuma longa L.), and ginger (Zingiber officinale R.) essential oils in cervical cancer cells (HeLa). The Scientific World Journal, 2016(1), 9273078.
35. Ahmed, W., Mofed, D., Zekri, A. R., El-Sayed, N., Rahouma, M., & Sabet, S. (2018). Antioxidant activity and apoptotic induction as mechanisms of action of Withania somnifera (Ashwagandha) against a hepatocellular carcinoma cell line. Journal of International Medical Research, 46(4), 1358-1369.
36. Charoensin, S. (2014). Antioxidant and anticancer activities of Moringa oleifera leaves. J. Med. Plants Res, 8(7), 318-325.
37. Gul, P., & Bakht, J. (2015). Antimicrobial activity of turmeric extract and its potential use in food industry. Journal of food science and technology, 52(4), 2272-2279.
38. Moyo, B., Masika, P. J., & Muchenje, V. (2012). Antimicrobial activities of Moringa oleifera Lam leaf extracts. African Journal of Biotechnology, 11(11), 2797-2802.
39. Baskaran, C., & Velu, S. (2012). Phytochemical analysis and in-vitro antimicrobial activity of Withania somnifera (Ashwagandha). J Nat Prod Plant Resour, 2(6), 711-6.