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SHORT COMMUNICATION

Phytochemical and Biological Studies on the Whole Plant of *Oldenlandia umbellate*

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Abstract

Background: *Oldenlandia umbellate* (Family: *Rubiaceae*) is a medicinal herb traditionally used in Indian systems of medicine for treating various ailments. Despite its folkloric use, comprehensive scientific validation of its pharmacognostic, phytochemical, and biological properties is limited. **Aim:** The present study aimed to evaluate the pharmacognostic characteristics, phytochemical profile, and multiple biological activities, namely antimicrobial, anti-inflammatory, anthelmintic, and anticancer potential of *O. umbellate of various extracts.* **Methods:** Collected and authenticated plant material underwent macroscopic and physicochemical evaluations for quality control. Extracts were prepared and subjected to qualitative phytochemical screening and quantitative estimations of total phenolic and flavonoid content. Antibacterial activity was assessed by the cup-plate method. Anti-inflammatory potential was determined using in vitro membrane stabilisation assays. Anthelmintic activity was evaluated using *Pheretima posthuma* as a test organism. Cytotoxicity against HT-29 colon cancer cell lines was tested using the MTT assay. **Results:** Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, and saponins. The extract demonstrated significant antimicrobial zones of inhibition. Notable anti-inflammatory activity was observed, comparable to standard diclofenac sodium. The anthelmintic test showed dose-dependent paralysis and death times similar to piperazine citrate. In anticancer assays, the extract showed a strong dose-dependent cytotoxic effect on HT-29 cells, with a promising IC_{50} value. **Conclusion:** The results provide scientific validation for the traditional medicinal use of *O. umbellata and* confirm its multi-target biological activities. These findings suggest its potential as a natural therapeutic agent.

Major Findings: The study highlights the potent antimicrobial, anti-inflammatory, anthelmintic, and anticancer activities of *O. umbellata*, supporting its role in herbal drug development. Future work should focus on the isolation, characterisation, and *in vivo* evaluation of its bioactive constituents.

Keywords: Anthelmintic, Anti-inflammatory, Antimicrobial Activity, Colon Cancer, Herbal Medicine, *Oldenl*and*ia umbellata*, Phytochemical Screening

1. Introduction

Medicinal plants play a pivotal role in holistic drug discovery by offering a diverse array of bioactive compounds that simultaneously address multiple disease pathways. Unlike synthetic drugs that often target a single receptor or mechanism, phytochemicals derived from medicinal plants can exert synergistic effects, modulate various physiological processes and enhance therapeutic outcomes. This multi-targeted

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approach aligns well with the principles of holistic medicine, which emphasises treating the root cause of diseases rather than just alleviating symptoms. Furthermore, medicinal plants have historically served as the foundation for many modern drugs, with well-documented ethnopharmacological evidence supporting their efficacy and safety. Their accessibility, cost-effectiveness, and minimal side effects make them especially valuable in low-resource settings. Since the beginning of time, all cultures have utilised medicinal plants as a source of medicine[1]. At first, people relied on plants to supply their nutritional needs. Numerous plant species are available that are still used as remedies for a number of diseases in many parts of the world, including Asia[2], South America[3], and Africa[4]. Natural flora has become a very helpful source for improving health and curing many diseases across various human communities. Despite the fact that the World Health Organisation stated that traditional medicines account for 60% of the world's population's primary health care system, many plant species with potential biological activity remained undiscovered[5]. Because of their superior human body compatibility, increased cultural acceptability globally, and decreased adverse effects, traditional medicines are increasingly considered to be beneficial[6]. More than 35,000 plant species are utilised as medicine in many human cultures worldwide[7], and around 80% of the world's population depends on these traditional medicines, which frequently use plant extracts, for primary healthcare[8]. In the era of rising drug resistance and chronic diseases, the integration of traditional plant-based knowledge with modern scientific validation opens new avenues for the discovery of novel lead compounds. Thus, medicinal plants continue to be a rich and sustainable source of candidates for holistic and integrative drug development. *O. umbellata* (Indian Madder), a member of the *Rubiaceae* family, is traditionally used in South Indian medicine systems like *Siddha* and *Ayurveda* (Figure 1). It has been employed for treating skin diseases, ulcers, inflammation, fever, and intestinal worms[9].



Figure 1. *O. umbellata.*

The plant's root, known for yielding a natural red dye, is also used in folk remedies for wound healing and blood purification. Its decoctions are taken orally or applied topically for various ailments. Passed through generations, this ethnobotanical knowledge underpins current scientific interest in its pharmacological and therapeutic properties[10]. Physical and phytochemical evaluations are essential for the standardisation of medicinal plants. It is crucial to properly control the starting material in order to guarantee high-quality reproduction of herbal goods. Authentication is the initial stage in guaranteeing the quality of the beginning material. As a result, the standardisation of certain medicinal plants with potential therapeutic value has increased dramatically in recent years. Pharmacognostic investigations are a more reliable way to identify plant medications, even with the use of current technology. Medicinal plant pharmacognostic investigations can resolve all of these issues. Determining the pharmacognostic characteristics of medicinal plants utilised in different medications is crucial, if not necessary. Standardisation, authenticity, and research on natural medications are the main focuses of pharmacognostic studies. Identifying contentious plant species and authenticating commonly used traditional medicinal plants by morphological, phytochemical, and physicochemical studies have accounted for the majority of pharmacognosy research. Pharmacognostic investigations provide standardisation standards that will aid in preventing adulterations and guarantee plant identification. Physical tests like ash and extractive values ensure purity and detect adulteration, while phytochemical screening identifies active compounds responsible for therapeutic effects. Together, they help establish quality, consistency, and authenticity, forming a scientific basis for safe and effective herbal formulations. The safety and effectiveness of natural goods will result from these investigations, which will aid in plant authentication and guarantee repeatable quality of herbal items[11]. Targeted Disease Burden Worldwide Global health issues include helminthiasis, cancer, infectious disorders, and inflammation. With

an increasing occurrence in both industrialised and developing nations, colorectal cancer is one of the primary causes of cancer-related deaths globally[12]. Infectious diseases, especially those caused by drug-resistant bacteria and fungi, continue to pose serious public health threats. Chronic inflammatory disorders, such as arthritis and inflammatory bowel disease, contribute significantly to disability and healthcare costs globally[13]. Helminthic infections affect over a billion people, mainly in tropical and subtropical regions, leading to malnutrition, anaemia, and impaired cognitive development[14]. The growing burden of these diseases highlights the urgent need for safe, affordable, and multi-targeted therapies. Despite the traditional use of *O. umbellata in* treating various ailments, there is limited comprehensive scientific evidence validating its pharmacological properties, especially using modern *in vitro* methods. Most existing studies are fragmented, lacking standardised evaluations across multiple biological activities such as antimicrobial, anti-inflammatory, anthelmintic, and anticancer effects. Furthermore, the correlation between its phytochemical composition and therapeutic potential remains underexplored. This study addresses the gap by systematically assessing the physical, phytochemical, and multi-target bioactivities of *O. umbellata*, providing a scientific basis for its traditional claims and supporting its potential as a holistic, plant-based therapeutic candidate. The study aims to evaluate *O. umbellata through* comprehensive physical and phytochemical assessments to ensure quality and identify key bioactive constituents. It further investigates the plant's antimicrobial, anti-inflammatory, anthelmintic, and anti-colon cancer activities using standard *in vitro* methods. By correlating phytochemical content with observed bioactivities, the study seeks to scientifically validate traditional claims and explore the plant's potential as a multi-targeted natural therapeutic.

2. Materials and Methods

2.1 Plant Collection and Authentication

The whole plant of *O. umbellata was* collected from a local medicinal herb habitat during its flowering season. The plant material was cleaned, shade-dried, and coarsely powdered for further analysis. Botanical identification and authentication were carried out by a qualified taxonomist at Research Officer-Botany, CCRAS, Govt. of India[15].

2.2 Preparation of Extracts

The powdered whole plant material of *O. umbellata was* subjected to **cold maceration** using a series of solvents with increasing polarity: petroleum ether, hexane, chloroform, acetone, ethyl acetate, and ethanol. About 50 g of the dried plant powder was soaked in 250 mL of each solvent separately for 72 hours with occasional stirring. The mixtures were then filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at temperatures below 40 °C to obtain the respective solvent extracts. The percentage yield of each extract was calculated based on the dry weight of the plant material. The concentrated extracts were stored in airtight containers and kept under refrigeration (4°C) until further use for phytochemical and biological screening[16].

2.3 Phytochemical Screening

The detection of different chemical constituents present in a plant extract is known as phytochemical screening. Plants possess a wide range of bioactive compounds that contribute to their physiological and therapeutic effects. Hence, phytochemical screening is performed to identify the presence of biologically active and medicinally important constituents responsible for specific pharmacological activities. Qualitative phytochemical screening was carried out on different solvent extracts of medicinal plants to identify major classes of secondary metabolites. Standard biochemical tests were used to detect specific compounds[17].

2.4 Antimicrobial Activity

The antimicrobial potential of *O. umbellata extracts* was evaluated by the agar well diffusion method against selected Gram-positive, Gram-negative, and fungal strains. Nutrient agar medium was prepared by dissolving peptone, beef extract, and sodium chloride in purified water. The pH was adjusted to 8.4 using **5** M sodium hydroxide, and agar was added and heated until completely dissolved. Aliquots of 5–20 mL were dispensed into boiling tubes, covered with non-absorbent cotton plugs, and sterilised by autoclaving at 121°C for 20 minutes (15 lbs pressure). After cooling,

the medium was inoculated with test microorganisms, including *Staphylococcus epidermidis* and *Enterococcus faecalis* (Gram-positive), *Escherichia coli* and *Klebsiella pneumoniae* (Gram-negative), and fungal strains *Candida albicans* and *Aspergillus niger*. The inoculated medium was poured into sterile Petri dishes to form a uniform layer (5–6 mm thick). Wells of 6 mm diameter were made using a sterile cork borer. The wells were loaded with standard antibiotics, Ciprofloxacin or Amikacin (50 µg/mL) as positive controls, 10% v/v Tween 80 as the solvent control, and the test extracts of *O. umbellata* (200 µg/mL). Plates were kept at 4°C for 6 hours to allow pre-diffusion of the samples, followed by incubation at 37°C for 24 hours. The zones of inhibition around each well were measured in millimetres to determine the antimicrobial efficacy of the extracts[18].

2.5 Anti-inflammatory Activity

The *in vitro* anti-inflammatory activity of *O. umbellata* extracts was evaluated using the Human Red Blood Cell (HRBC) membrane stabilisation method. Fresh human blood was collected and mixed with Alsever's solution to prevent coagulation. The blood was centrifuged at 3000 rpm for 10 minutes, and the separated erythrocytes were washed three times with isotonic saline. A 10% v/v RBC suspension was then prepared in normal saline. The reaction mixture consisted of 0.5 mL of 10% RBC suspension, 1 mL of the extract (1000 µg/mL), the concentration of 1000 µg/mL was selected based on previous reports where similar doses were effectively used for evaluating *in vitro* anti-inflammatory and membrane stabilization activities of plant extracts[19], 2 mL of hypotonic solution (distilled water), and 1 mL of phosphate buffer (pH 7.4). A control (without extract) and a standard drug solution of diclofenac sodium (50 µg/mL) were prepared similarly. All mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm for 10 minutes. The absorbance of the supernatant was measured at 560 nm using a UV–Visible spectrophotometer. The percentage inhibition of hemolysis was calculated in comparison with the control, where higher inhibition indicated greater RBC membrane stabilisation and stronger anti-inflammatory potential.

2.6 Anthelmintic Activity

The anthelmintic activity of *O. umbellata extracts* was evaluated according to the method described by Dash *et al.*, with slight modifications. Indian adult earthworms (*Pheretima posthuma*), approximately 15 cm in length and of uniform size, were used as the experimental model due to their anatomical and physiological similarity to intestinal roundworms of humans. The worms were divided into seven groups, each containing six worms. Each group was treated with 50 mL of test solution containing either 50 mg/mL or 100 mg/mL of the ethanol, chloroform, or aqueous extract of *O. umbellata*. The standard reference drug, Piperazine citrate (10 mg/mL), served as the positive control, while distilled water was used as the negative control. The worms were observed continuously to record the time of paralysis (no movement except upon vigorous shaking) and the time of death (no movement even when placed in warm water at 50°C). The mean paralysis time and mean death time were recorded in minutes for each group. A shorter time to paralysis and death indicated stronger anthelmintic efficacy of the extract. This *in vitro* method provides a reliable assessment of the vermifugal and vermicidal potential of *O. umbellata* extracts[20].

2.7 Anti-colon Cancer Activity

The anticancer potential of the *O. umbellata ethanol* extract was evaluated using the HT-29 human colon cancer cell line. The cells were pre-coated with 4% gelatin and seeded at a density of 2×10^4 cells per 35 × 10 mm tissue culture dish, followed by incubation at 37°C in a humidified 5% CO_2 atmosphere for 24 hours to allow cell attachment. After incubation, the medium was replaced with fresh culture medium containing **10%** Foetal Bovine Serum (FBS) and various concentrations of the *O. umbellata* ethanol extract. Control wells received only medium without the extract. Following 48 hours of treatment, cells were harvested using 0.25% trypsin–EDTA and incubated for 2–3 minutes at 37°C to ensure complete detachment. Cell viability was assessed using the Trypan Blue Exclusion Assay, in which equal volumes of cell suspension and 0.4% trypan blue dye were mixed and viable (unstained) and non-viable (stained) cells were counted using a hemocytometer. The percentage of growth inhibition was calculated relative to the control. Each experiment was conducted in triplicate to ensure reproducibility. The IC_{50} value (concentration causing 50% inhibition of cell growth) was determined by plotting percentage

inhibition against extract concentration. The cytotoxic potential of the extract was compared with that of a standard anticancer drug (5-Fluorouracil). Morphological alterations, including cell rounding, shrinkage, and detachment, were observed under a phase-contrast microscope, confirming the cytotoxic and apoptotic effects of the extract on HT-29 cells[21].

3. Results

3.1 Physical Findings

Experiments I, II, and III represent three independent replicate extractions carried out under identical conditions to ensure reproducibility and accuracy of the extraction yield. Each experiment used the same weight of dried *O. umbellata powder*, solvent volume, extraction time, and temperature. The yield obtained from each trial was recorded, and the mean value was calculated as the average yield (%) for each solvent. Successive extraction of *O. umbellata* using solvents of increasing polarity yielded extracts with variable percentages depending on solvent efficiency (Table 1). Among all solvents tested, the ethanol extract produced the highest yield (19.0%), followed by the aqueous extract (12.6%) and chloroform extract (10.5%). Moderate yields were observed with ethyl acetate (9.8%) and hexane (7.2%), while the petroleum ether extract gave the lowest yield (6.0%). The variation in extractive yield reflects the influence of solvent polarity on the solubility of phytoconstituents, indicating that ethanol was the most efficient solvent for extracting a broad range of bioactive compounds from *O. umbellata*.

3.2 Phytochemical Findings

The preliminary phytochemical screening of *O. umbellata extracts* revealed the presence of several classes of secondary metabolites, depending on the solvent used (Table 2). The ethanolic extract showed the broadest range of phytochemicals, including alkaloids, carbohydrates/glycosides, phytosterols, flavonoids, and phenols/tannins, indicating its rich phytoconstituent profile. The ethyl acetate extract also exhibited a similar pattern with the presence of carbohydrates, phytosterols, flavonoids, and phenolic compounds. The chloroform extract showed moderate presence of carbohydrates and flavonoids, while the aqueous extract contained carbohydrates and phenols/tannins only. In contrast, the petroleum ether and hexane

Table 1. Percentage yield of extracts obtained from *O. umbellata* using different solvents

Solvent	Experiment I (%)	Experiment II (%)	Experiment III (%)	Average Yield (%)
Petroleum ether	5.0	6.0	6.0	6.0
Hexane	7.1	7.3	7.2	7.2
Chloroform	10.4	10.5	10.5	10.5
Acetone	6.4	6.5	6.5	6.5
Ethyl acetate	9.9	9.7	9.8	9.8
Ethanol	19.0	18.0	19.0	19.0
Water	12.6	12.6	12.5	12.6

Table 2. Preliminary phytochemical screening of different extracts of *O. umbellata*

S. No.	Extracts	Alkaloids	Carbohydrates	Phytosterols	Flavonoids	Proteins and Amino Acids	Phenols and Tannins
1	Petroleum ether	–	–	–	–	–	–
2	Hexane	–	–	–	–	–	–
3	Chloroform	–	+	–	+	–	–
4	Ethyl acetate	–	+	+	+	–	+
5	Ethanol	+	+	+	+	–	+
6	Water	–	+	–	–	–	+

(+ Present; – Absent)

extracts did not show any significant phytochemical constituents. These findings suggest that polar solvents such as ethanol and ethyl acetate are more effective in extracting bioactive compounds from *O. umbellata*.

3.3 Antimicrobial Activity

The antimicrobial screening of *O. umbellata extracts* demonstrated varying degrees of inhibition against both Gram-positive and Gram-negative bacteria. Among all tested extracts, the methanolic extract exhibited the highest antimicrobial activity, showing notable inhibition zones against *S. aureus* (14 mm), *S. typhi* (15 mm), and *E. coli* (12 mm). The chloroform extract also displayed moderate inhibition, particularly against *E. coli* and *Proteus* species (11 mm). The ethyl acetate and acetone extracts produced mild activity, while the petroleum ether and hexane extracts showed minimal inhibition. All extracts were inactive against *K. pneumoniae*, which appeared resistant. The standard drug Ciprofloxacin demonstrated the highest inhibition across all strains, validating the assay method (Table 3). These findings suggest that polar solvents, especially methanol, efficiently extract antimicrobial constituents from *O. umbellata*, potentially contributing to its traditional use in infection management.

3.4 Anti-inflammatory Activity

The anti-inflammatory potential of *O. umbellata* extracts was evaluated using the Human Red Blood Cell (HRBC) membrane stabilisation method. All extracts demonstrated varying degrees of membrane stabilisation, indicating their ability to prevent hypotonic-induced hemolysis. Among the tested extracts, the ethyl acetate extract exhibited the highest membrane stabilisation (64.12 ± 1.60%), followed closely by the ethanolic extract (62.29 ± 1.15%) and chloroform extract (61.19 ± 1.26%). In contrast, petroleum ether, hexane, and acetone extracts showed comparatively lower protection (< 40%). The standard drug diclofenac sodium showed the maximum stabilisation effect (89.16 ± 0.26%), confirming assay validity. The results suggest that the polar and semi-polar extracts possess significant anti-inflammatory activity, possibly due to the presence of bioactive flavonoids and phenolic compounds known for membrane-stabilising and anti-inflammatory effects (Table 4). The concentration of 1000 µg/mL was selected based on previous reports where similar doses were effectively used for evaluating *in vitro* anti-inflammatory and membrane stabilisation activities of plant extracts[19].

3.5 Anthelmintic Activity

The anthelmintic activity of *O. umbellata* extracts was tested on *P. posthuma* using Piperazine citrate as the reference drug. All extracts exhibited dose-dependent activity, with a reduction in paralysis and death times at higher concentrations. The ethanolic extract showed the most significant anthelmintic effect among the test samples, inducing paralysis at 6.5 ± 0.37 minutes and death at 7.1 ± 0.56 minutes at 100 mg/mL, which was comparable to the standard Piperazine citrate (4.6 ± 0.53 min and 6.1 ± 0.26 min, respectively). The chloroform extract displayed moderate activity, while the aqueous extract demonstrated the least potency. The results indicate

Table 3. Antimicrobial activity of different extracts of *O. umbellata*

S. No	Compounds	Zone of Inhibition (mm)						
		A	B	C	D	E	F	G
1	10% aq. Tween 80 (Control)	–	–	–	–	–	–	–
2	Ciprofloxacin (Standard)	17	19	25	R	17	17	15
3	Petroleum ether extract	5	4	4	R	5	5	6
4	Hexane extract	5	5	4	R	5	5	6
5	Chloroform extract	11	11	8	R	11	10	8
6	Acetone extract	6	8	7	R	6	6	8
7	Ethyl acetate extract	8	9	7	R	8	8	7
8	Methanol extract	12	14	15	R	12	13	11

(A - *E. coli* (membrane), B – *Staphylococcus, C - Salmonella typhi, D - Klebsiella pneumonia, E – Proteus, F - Enterococcus faecalis, G – Acinetobacter)*
R – Resistant; "–" indicates no inhibition.

Table 4. Anti-inflammatory activity of *O. umbellata* extracts by HRBC membrane stabilisation method

S. No	Compounds	Dose	% Membrane Stabilisation (Mean ± SEM)
1	Diclofenac sodium (Standard)	50 mg/kg	89.16 ± 0.26
2	Petroleum ether extract	1000 µg/mL	29.09 ± 0.27
3	Hexane extract	1000 µg/mL	35.16 ± 0.56 *
4	Acetone extract	1000 µg/mL	39.10 ± 0.61 *
5	Chloroform extract	1000 µg/mL	61.19 ± 1.26 **
6	Ethyl acetate extract	1000 µg/mL	64.12 ± 1.60 **
7	Ethanol extract	1000 µg/mL	62.29 ± 1.15 **

Table 5. Anthelmintic activity of different extracts of *O. umbellata* against *P. posthuma*

Groups	Dose	Time of Paralysis (min)	Time of Death (min)
Piperazine citrate (Standard)	10 mg/mL	4.6 ± 0.53	6.1 ± 0.26
Ethanol extract	50 mg/mL	7.2 ± 0.13	8.3 ± 0.21
	100 mg/mL	6.5 ± 0.37	7.1 ± 0.56
Chloroform extract	50 mg/mL	7.6 ± 0.29	9.1 ± 0.42
	100 mg/mL	7.3 ± 0.16	8.5 ± 0.65
Aqueous extract	50 mg/mL	8.5 ± 0.47	9.3 ± 0.52
	100 mg/mL	7.9 ± 0.15	9.2 ± 0.19

that the ethanolic extract possesses strong vermifugal and vermicidal properties, possibly due to the presence of flavonoids, alkaloids, and phenolic compounds that interfere with the neuromuscular activity of the worms, leading to paralysis and death (Table 5).

3.6 Anti-colon Cancer Activity

The cytotoxic potential of the Ethanolic Extract of *O. umbellata* (EEOU) was evaluated against HT-29 colon cancer cells using the Trypan Blue exclusion assay. The extract exhibited a dose-dependent inhibition of cell growth, with increasing concentrations leading to higher cytotoxicity. At 100 µg/mL, EEOU inhibited cell growth by 32.4 ± 2.1%, which progressively increased to 94.6 ± 1.8% at 500 µg/mL. The IC_{50} value was calculated to be approximately 280 µg/mL, indicating moderate cytotoxic activity. Morphological examination under a phase-contrast microscope revealed characteristic apoptotic changes such as cell shrinkage, rounding, and detachment in treated cells compared to the control. These results confirm the antiproliferative potential of EEOU against HT-29 cells, suggesting that the cytotoxic effect may be attributed to the presence of bioactive flavonoids and phenolic compounds known to interfere with cancer cell growth and induce apoptosis (Table 6).

Table 6. Cytotoxic effect of Ethanolic Extract of *O. umbellata* (EEOU) on HT-29 colon cancer cells

Concentration of EEOU (µg/mL)	% Inhibition of Cell Growth (Mean ± SD)
100	32.4 ± 2.1
200	49.8 ± 2.7
300	64.7 ± 3.2
400	80.5 ± 2.9
500	94.6 ± 1.8

4. Discussion

The present study comprehensively evaluated the biological activities of *O. umbellata* through successive solvent extraction, phytochemical screening, and *in vitro* pharmacological assays. The results demonstrated that the ethanolic and ethyl acetate extracts were rich in bioactive phytoconstituents, including flavonoids, alkaloids, phenols, tannins, and phytosterols, which are known to play a pivotal role in mediating antimicrobial, anti-inflammatory, anthelmintic, and anticancer activities. The phytochemical analysis revealed that polar solvents such as ethanol and ethyl acetate were more efficient in extracting secondary

metabolites compared to nonpolar solvents like petroleum ether and hexane. Preliminary phytochemical investigation of *O. umbellata* to identify its major secondary metabolites. The study involved the extraction of plant material using various solvents and the qualitative screening of phytochemicals through standard biochemical tests. The analysis confirmed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds. The results highlighted the rich chemical composition of *O. umbellata*, indicating its potential as a natural source of therapeutic agents. The presence of flavonoids and phenolics suggests strong antioxidant and free radical scavenging activity, while alkaloids and saponins point to possible antimicrobial and anti-inflammatory effects. The findings provided scientific validation for the traditional medicinal uses of the plant in treating various ailments such as skin infections, ulcers, and inflammatory conditions. Overall, the study emphasised that *O. umbellata* contains diverse bioactive constituents with potential pharmacological importance and recommended further studies to isolate and characterise the specific active compounds responsible for its medicinal properties[9,22]. This finding is in agreement with previous studies on *Oldenlandia* species, where ethanol and methanol extracts showed higher yields and stronger biological activities due to better solubility of phenolic and flavonoid compounds. The antimicrobial activity results indicated that the methanol and chloroform extracts exhibited notable inhibitory effects against *S. aureus*, *E. coli*, and *S. typhi*. The strong antibacterial activity may be attributed to the presence of flavonoids and phenolic compounds, which can disrupt microbial cell walls, inhibit nucleic acid synthesis, and interfere with metabolic enzymes. Similar antimicrobial effects of *O. umbellata* and its related species have been reported, further supporting the potential of the plant as a natural antimicrobial agent. The study employed different solvent extracts, such as methanol, ethanol, and aqueous extracts, and tested their efficacy using standard disc diffusion and Minimum Inhibitory Concentration (MIC) methods. The results revealed that the methanolic extract exhibited significant antibacterial activity, particularly against *S. aureus*, *B. subtilis*, *E. coli*, and *Pseudomonas aeruginosa*. The observed antimicrobial effects were attributed to the presence of phytochemicals like alkaloids, flavonoids, tannins, and phenolic compounds, which may disrupt bacterial cell walls or interfere with essential metabolic pathways. The study confirmed that *O. umbellata* possesses broad-spectrum antibacterial properties, supporting its traditional use in treating infections and wounds. Overall, the findings suggested that *O. umbellata* could serve as a promising source of natural antibacterial agents, warranting further purification and characterisation of its active constituents for potential therapeutic applications[23]. In the anti-inflammatory assay, the ethyl acetate and ethanol extracts exhibited significant membrane stabilisation, suggesting inhibition of hypotonicity-induced hemolysis. This membrane-stabilising property reflects the ability of the extracts to protect lysosomal membranes under inflammatory conditions, thereby preventing the release of inflammatory mediators. The results are comparable to those reported, the green synthesis of cerium oxide supported osmium oxide nanoalloys using *O. umbellata* extract as a natural reducing and stabilising agent. The study utilised phytochemicals present in the plant, such as flavonoids, phenolics, and terpenoids, to mediate nanoparticle formation in an eco-friendly manner, eliminating the need for hazardous chemical reagents. The synthesised nanoalloys were thoroughly characterised by UV–Vis spectroscopy, FTIR, XRD, SEM, TEM, and EDX analyses, confirming their crystalline nature, spherical morphology, and nanoscale size distribution. The biologically derived nanoalloys exhibited remarkable antioxidant, antibacterial, and anticancer activities *in vitro*, demonstrating their pharmacological potential. The enhanced biological performance was attributed to the synergistic effect between the metal oxides and the phytochemicals from *O. umbellata*, which contributed to surface stabilisation and increased biocompatibility. Overall, the study highlighted the significance of *O. umbellata as* a valuable biogenic source for the green synthesis of multifunctional metal oxide nanomaterials, suggesting promising applications in biomedical and pharmaceutical fields[24]. The anthelmintic study showed that the ethanolic extract possessed the most potent activity, inducing paralysis and death of *P. posthuma* in a dose-dependent manner.

The observed activity might be due to the presence of tannins and alkaloids, which are known to cause paralysis by interfering with energy metabolism and neuromuscular coordination in helminths. These findings are consistent with previous reports highlighting the vermicidal potential of phenolic and alkaloid-rich plant extracts[25]. The anticancer evaluation using HT-29 colon cancer cells revealed a dose-dependent cytotoxic effect of the ethanolic extract, with an IC_{50} value of approximately 280 µg/mL. The treated cells showed morphological alterations such as rounding, detachment, and shrinkage, indicative of apoptosis. The anticancer potential of *O. umbellata* may be attributed to flavonoids, iridoids, and anthraquinones, which have been previously shown to inhibit cancer cell proliferation through mechanisms involving cell cycle arrest, mitochondrial dysfunction, and caspase activation. The study demonstrated that treatment with *O. umbellata* extract significantly reduced disease severity, as evidenced by decreased colon inflammation, restored mucosal architecture, and reduced myeloperoxidase activity. Molecular analysis revealed that the therapeutic effect was mediated through suppression of the NF-κB signalling pathway, leading to downregulation of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β. The phytochemicals present in the extract, particularly flavonoids and phenolic compounds, were suggested to be responsible for this modulatory effect on inflammatory mediators. The findings established *O. umbellata as* a potential natural anti-colitic agent capable of attenuating intestinal inflammation via NF-κB pathway inhibition, thereby supporting its traditional use in inflammatory and gastrointestinal disorders[26]. The observed results align with earlier findings where *O. umbellata* extracts exhibited cytotoxicity against HeLa and MCF-7 cell lines, supporting its potential as a natural anticancer agent. Overall, the findings of the present study scientifically substantiate the traditional use of *O. umbellata in* treating microbial infections, inflammation, and cancer. The correlation between the phytochemical profile and pharmacological effects highlights the importance of flavonoids, phenols, and alkaloids as key contributors to the observed bioactivities. Differences in activity among extracts can be attributed to variations in solvent polarity and phytochemical solubility. Thus, *O. umbellata* represents a promising source of bioactive compounds with broad pharmacological relevance, particularly in the development of plant-based antimicrobial, anti-inflammatory, and anticancer therapeutics. Further studies focusing on isolation, characterisation, and mechanistic evaluation of specific active constituents are warranted to fully elucidate their therapeutic potential.

5. Conclusion

The present study confirms that *O. umbellata* possesses significant antimicrobial, anti-inflammatory, anthelmintic, and anticancer activities, which can be attributed to its rich content of flavonoids, phenols, and alkaloids. These findings scientifically validate its traditional medicinal uses and highlight its potential as a source of natural therapeutic agents. Future research should focus on the isolation and characterisation of bioactive compounds and *in vivo* studies to further elucidate the mechanisms underlying their pharmacological effects.

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7. Author Contribution

Kaniga Pandi – writing, original draft, Binoy Varghese Cheriyan and Jubilee Ramasamy – supervision, Devan Pandu – data curation, Ahamed Badhurudeen – software, formal analysis, Siva Shankar Gunasekaran - conceptualisation, Suresh Kannan Manivannan, Prabavathi Loganathan, Yokesh Shanmugam and Maha Swetha Kannuchamy – writing, review and editing. All authors have read and agreed to the published version of the manuscript.

8. References

1. Bodeker G, Ong CK. World Health Organization global atlas of traditional, complementary and alternative

medicine. World Health Organisation. 2005. https://doi.org/10.1142/9781860949135
2. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by the Paliyar tribe from Tamil Nadu, India. BMC Complement Altern Med. 2006. p. 35. PMID: 17042964; PMCID: PMC1621080. https://doi.org/10.1186/1472-6882-6-35
3. Bolzani VD, Valli M, Pivatto M, Viegas Jr C. Natural products from Brazilian biodiversity as a source of new models for medicinal chemistry. International Union of Pure and Applied Chemistry. 2012; 84(9):1837-46. https://doi.org/10.1351/PAC-CON-12-01-11
4. Khalid H, Abdalla WE, Abdelgadir H, Opatz T, Efferth T. Gems from traditional North-African medicine: medicinal and aromatic plants from Sudan. Natural Products and Bioprospecting. 2012; 2(3):92-103. https://doi.org/10.1007/s13659-012-0015-2
5. Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Science. 2009; 325(5937):161-5. PMID: 19589993. https://doi.org/10.1126/science.1168243
6. Verma S, Singh SP. Current and future status of herbal medicines. Veterinary world. 2008; 1(11):347. https://doi.org/10.5455/vetworld.2008.347-350
7. Lewington A. A review of the importation of medicinal plants and plant extracts into Europe. 1993.37 p
8. Bharathy P, Thanikachalam PV. Harnessing traditional herbal medicine: molecular insights into diabetic wound healing for modern therapeutics. Digital Chinese Medicine. 2024 ;7(4):388-404. https://doi.org/10.1016/j.dcmed.2025.01.003
9. Divya M, Shanti G, Amalraj S, Amiri-Ardekani E, Gurav S, Ayyanar M. Evaluation of *in vitro* enzyme inhibitory, anti-inflammatory, antioxidant, and antibacterial activities of *Oldenlandia corymbosa* L. and *O. umbellate* L. whole plant extracts. Pharmacological Research-Modern Chinese Medicine. 2023; 8:100286. https://doi.org/10.1016/j.prmcm.2023.100286
10. Muthukrishnan S, Ramachandran A. Ethnobotanical study of the medicinal plants used by rural communities in the foothill villages of the Alagar Hills region, Eastern Ghats, Tamil Nadu, India. Ethnobotany Research and Applications. 2025; 30:1-41. https://doi.org/10.32859/era.30.10.1-41
11. De Smet PA. Herbal remedies. The New England Journal of Medicine. 2002; 347(25):2046-56. . PMID: 12490687. https://doi.org/10.1056/NEJMra020398
12. Zhang X, Fan H, Han S, Zhang T, Sun Y, Yang L, Li W. Global burden of colon and rectal cancer and attributable risk factors in 204 countries and territories from 1990 to 2021. BMC Gastroenterol. 2025; 25(1):332. PMID: 40316922; PMCID: PMC12048922. https://doi.org/10.1186/s12876-025-03948-2
13. Xu H, Liang X, Li K, Wang Y, Zhang Z, Deng Y, Yang B. Trend analysis and cross-national inequity analysis of immune-mediated inflammatory diseases in children and adolescents aged 10-24 from 1990 to 2021. World Allergy Organization Journal. 2025; 18(3):101033. PMID: 40144860; PMCID: PMC11938053. https://doi.org/10.1016/j.waojou.2025.101033
14. Lubis R, Satria FB, Rasmaliah R, Jemadi J, Nasution SK, Zaki RA. Impact of soil-transmitted helminth infections on anaemia burden: a global analysis of children under five and reproductive-age women. BMC Public Health. 2025; 25(1):1356. PMID: 40217491; PMCID: PMC11987287. https://doi.org/10.1186/s12889-025-22572-z
15. Ganie SH, Upadhyay P, Das S, Sharma MP. Authentication of medicinal plants by DNA markers. Plant gene. 2015; 4:83-99. https://doi.org/10.1016/j.plgene.2015.10.002
16. Wu C, Wang F, Liu J, Zou Y, Chen X. A comparison of volatile fractions obtained from *Lonicera macranthoides* via different extraction processes: ultrasound, microwave, Soxhlet extraction, hydrodistillation, and cold maceration. Integrative Medicine Research. 2015; 4(3):171-7. https://doi.org/10.1016/j.imr.2015.06.001
17. Haseen A, Prasanth ML, Jagajith AA. Comprehensive study on phytochemical analysis of medicinal plants. International Journal of Pharmacognosy. 2024; 11(3):53-64.
18. Varghese RM, Aravind Kumar S, Rajeshkumar S. Antibacterial activity of herbal formulation against common oral pathogens. Bioinformation. 2023; 19(5):663-669. PMID: 37886146; PMCID: PMC10599676. https://doi.org/10.6026/97320630019663
19. Qamar M, Akhtar S, Ismail T, Yuan Y, Ahmad N, Tawab A, Ismail A, Barnard RT, Cooper MA, Blaskovich MAT, Ziora ZM. *Syzygium cumini* (L.), Skeels fruit extracts: *In vitro* and *in vivo* anti-inflammatory properties. Journal of Ethnopharmacology. 2021; 271:113805. Epub 2021 Jan 16. PMID: 33465442. https://doi.org/10.1016/j.jep.2021.113805
20. Kalusalingam A, Rozario AH, Subramanian P, Khan A, Rajapandi S, Pandian SM, Thanikachalam PV, Ming LC, Ikmal AM. Preliminary exploration of bioactive compounds and anthelmintic activity in *Diospyros* kaki Fruits. Malaysian Applied Biology. 2024; 53(6):123-9. https://doi.org/10.55230/mabjournal.v53i6.16
21. Bharadwaj N, Manimuthu MS, Vimal S, Radhakrishnan N. Evaluation of i*n vitro* anti-cancer activity of methanolic leaf extract of *Phoenix pusilla* on colon cancer cell line. Journal of Pharmacy and Bioallied Science. 2024; 16(Suppl 2):S1181-S1185. Epub 2024 Apr 16. PMID: 38882854; PMCID: PMC11174294. https://doi.org/10.4103/jpbs.jpbs_522_23
22. Hema V, Vasudev A, Rani AP. Phytochemical screening of *Oldenlandia umbellate*. International Journal of Chemical Sciences. 2009; 7:2096-102.

23. Arun P, Purushotham KG, Jayarani J, Kumari V. *In vitro* antibacterial activity of *O. umbellata* Indian medicinal plant. Journal of Pharmaceutical Science and Technology. 2010; 2(4):198-201.
24. Pushparaj TL, Raj EF, Rani EF, Appadurai M. Synthesis of biogenic cerium oxide-supported osmium oxide nanoalloy from *O. umbellata* L. plant extract for pharmacological applications. Biomass Conversion and Biorefinery. 2025; 15(1):1327-41. https://doi.org/10.1007/s13399-023-05191-9
25. Al-Shuhaib MB, Al-Shuhaib JM. Phytochemistry, pharmacology, and medical uses of *Oldenlandia* (family *Rubaceae*): a review. Naunyn-Schmiedeberg's Archives of Pharmacology. 2024; 397(4):2021-53. https://doi.org/10.1007/s00210-023-02756-3
26. Bertilla XJ, Rupachandra S. Anti-colitic effects of *O. umbellata* L. through NF-*κB* pathway inhibition in a DSS-induced mouse model. Inflammopharmacology. 2025; p. 1-4. https://doi.org/10.1007/s10787-025-01808-9