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COMPARATIVE STUDY ON ANTI-MICROBIAL AND ANTI-INFLAMMATORY ACTIVITY OF CLERODENDRUM VISCOSUM AND STROBILANTHES ALTERNATA: A COMPREHENSIVE REVIEW

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ABSTRACT :

Clerodendrum viscosum and Strobilanthes alternata are two flowering plants that have gained attention in the field of medicine due to their potential health benefits. Clerodendrum viscosum, which belongs to the Lamiaceae family, has been found to possess anti-inflammatory and anti-microbial properties through various experimental methods. On the other hand, Strobilanthes alternata, a low-creeping herb from the Acanthaceae family, is native to Malaysia and has several common names such as Purple Waffle plant, Aluminium plant, Cemetary plant, Metal leaf, Red flame Ivy, and Java Ivy. This plant has also been discovered to have potent anti-inflammatory and anti-microbial properties, making it a promising candidate for the development of new drugs to treat various diseases. Studies have shown that the roots of Clerodendrum viscosum and the leaves of Strobilanthes alternata contain exceptional properties that can inhibit inflammation and prevent the growth of microbes. These natural extracts exhibit powerful anti-inflammatory and anti-microbial activity, which makes them excellent candidates for developing new therapeutic agents. The research on these plants has shed light on their potential in pharmacology and medicine, and they may provide a natural alternative to synthetic drugs in the future.

INTRODUCTION :

Clerodendrum viscosum belongs to the family Lamiaceae, previously categorized into the Verbenaceae family. This family is represented by herbs, shrubs, and small trees known for heads, spikes, or clusters of small flowers, of which many have aromatic odors1. The main difference between the two families is the ovary. Lamiaceae have a deeply four-lobed ovary with gynobasic style while the Verbenaceae have an unlobed ovary and a terminal style. Clerodendrum, an ethnomedicinally important genus is used for the treatment of various diseases. Several researches have been performed to identify and isolate biologically active compounds from different species of Clerodendrum2. Clerodendrum viscosum is locally known as Gatupata. It comprises 35 genera and 1,200 species found mainly in the tropical and subtropical regions of the world1. The different parts of the plant C. viscosum have been used for the restoration of different physiological hazards such as asthma and other inflammatory diseases3. The roots of the plant have been claimed to be used in dyspepsia, seeds in dropsy, and leaves as a febrifuge and in cephalalgia and ophthalmic4. Because of its copious, widespread availability and folkloric use, the present research was undertaken to enquire into the potentiality of Clerodendrum viscosum root extract as membrane stabilizing, thrombolytic, antidiarrheal activity against important human pathogens. Research reports revealed that steroids, terpenoids, and flavonoids are major among them to use as a potential source for new antimicrobial substances against important pathogens of agricultural and veterinary importance. The plant is used as a bitter tonic, for dyspepsia, diabetes, asthma, malaria, lung disease, rheumatism, inflammatory diseases, febrifuge, fever, swelling, eye disorders, drowsiness, headache, mental disturbances, bronchitis2,5,6. Clerodendrum viscosum vent is traditionally used for antiseptic and expectorant. It is also used in ethnomedicine for its various medicinal properties which include the treatment of scorpion stings, tumors, leprosy, and skin diseases7,8.

The plant Hemigraphis alternata belongs to the Acanthaceae family and is a versatile tropical low-creeping Perennial herb that reaches a height of 15 to 30 cm and is native to tropical Malaysia. It is also known as Purple Waffle plant, Aluminium plant, Cemetary plant, Metal leaf, Red flame Ivy, Java Ivy, etc9. This exotic plant was adapted to Bangladesh as well as India. The notable phytoconstituents that present in Hemigraphis alternata are carbohydrates, alkaloids, phenols, saponins, flavonoids, terpenoids, coumarins, carboxylic acid, xanthoproteins, tannins, proteins, steroids, chlorogenate, cinnamic acids, cinnamate and sterol9,10,11. This plant possesses various medicinal properties. The whole plant or leaves are used to treat fresh wounds, cuts, ulcers, and inflammation, and in folk medicines12. Traditionally the leaves are consumed to mend gallstones, excessive menstruation, and as a contraceptive. It is used internally to cure anemia, gallstones, diuretics, hemorrhoids, and diabetes mellitus9,13,14. Hemigraphis

means “half writing” because the filament of the outer stamen bears brushes. The leaf has a metallic purple lustre on the upper surface and a solid dark purple on the ventral side. It blooms irregularly throughout the year in the tropics. The seeds are small, flat, and white. The leaves are hairy and opposite about 2 to 8 cm long and 4 to 6 cm wide, bearing well-defined veins. The flowers are small (1 to 1.5 cm in diameter), five-lobed, and bell-shaped with imbricate bracts and these flowers are white with faint purple marks within and appear in terminal 2 to 10 cm long spikes15-17. H. alternata is widely used in Indian traditional medicine for its wound healing activity18,19. The antibacterial activity is attributed to the wound-healing effect20. In addition, the methanol and ethyl acetate extracts of Hemigraphis alternata leaves were documented as having effective anti-inflammatory, antinociceptive, antidiarrheal, as well as anticancer activity21,22.

In the present review, we present piece of updated information on the antimicrobial and anti-inflammatory activity of Clerodendrum viscosum root and Strobilanthes alternata leaves through an intensive literature survey.

PLANT DISTRIBUTION

Clerodendrum paniculatum: Native to South and Southeast Asia, including countries like India, Bangladesh, Sri Lanka, Myanmar, and Thailand.
Strobilanthes alternata: Native to the Western Ghats Mountain range in India.

COMMON NAME

Clerodendrum viscosum**:** Sticky Clerodendrum, wild jasmine
Strobilanthes alternata**:** Murikootti, Murian pacha

Clerodendrum viscosum

Stribilanthes alternata

PLANT PROFILE

Strobilanthes alternata	Clerodendrum viscosum
Kingdom: Plantae Class: Magnoliopsida Order: Lamiales Family: Acanthaceae Superorder: Asteranae Domain: Eukaryota Phylum: Angiosperms Subphylum: Eudicots Genus: Strobilanthes Species: Strobilanthes alternata	Kingdom: Plantae Class: Magnoliopsida Order: Lamiales Family: Lamiaceae Superorder: Asteranae Domain: Eukaryota Phylum: Tracheophyta Subphylum: Angiospermae Genus: Clerodendrum Species: Clerodendrum viscosum

PLANT DESCRIPTION :

- Clerodendrum viscosum[23]:

Plant Type: Shrub or small tree
Height: Up to 6 meters
Leaves: Opposite, simple, ovate, sticky
Flowers: White, tubular, fragrant, clustered
Distribution: India, Bangladesh, Sri Lanka, Myanmar, Thailand
Habitat: Forests, thickets, roadsides
Traditional Uses: Fever, cough, cold, rheumatism, skin diseases
Phytochemicals: Alkaloids, flavonoids, terpenoids, phenolic compounds
Cultural Significance: Sacred in some cultures, used in religious ceremonies
Caution: Some parts may be toxic if consumed in large quantities

- Strobilanthes alternata[24]:

Plant Type: Strobilanthes alternata is a perennial shrub.
Leaves: The leaves are simple, opposite, and ovate to lanceolate in shape. They are around 7-15 cm long, with serrated margins and a pointed tip. The leaves are dark green and hairy on both sides.
Stems: The stems are erect, branching, and covered with fine hairs.
Flowers: The flowers are the most striking feature of Strobilanthes alternata. They are tubular, about 2-3 cm long, and arranged in dense, terminal spikes. The flowers are a vibrant blue-purple color and bloom, creating a stunning visual display.
Fruit: The fruit is a small capsule containing numerous tiny seeds.
Roots: The plant has a fibrous root system.
Habitat: native to the Western Ghats Mountain range in India, where it grows in moist, shady areas, often in forest understories or along stream banks.

PHARMACOLOGICAL ACTIVITY :

- CLERODENDRUM VISCOSUM

1. Anti-inflammatory activity[25]

Prasanth K.G et. al (2012) conducted a study to explore the anti-inflammatory potential of *Clerodendrum viscosum* root (EECVR). The researchers used coarse powders of *Clerodendrum viscosum* root and subjected them to successive maceration processes with petroleum ether, ethyl acetate, chloroform, and ethanol. The extracts were then analyzed for their phytochemical content, and the total alkaloid content was estimated using UV-visible spectroscopy. The phytochemical analysis revealed the presence of alkaloids in EECVR, with a total alkaloid content of 1.4%w/w.
To investigate the anti-inflammatory activity of EECVR, the researchers used the carrageenan-induced paw edema method in Swiss albino mice. The mice were first divided into six groups, with each group receiving either distilled water, standard drug (indomethacin), or different doses of EECVR (100, 200, and 400 mg/kg b.w.). After one hour of treatment, the mice were injected with carrageenan in their right hind paw, and the paw volume was measured at different time intervals. The results showed that the ethanolic extract of *Clerodendrum viscosum* root, at a dose of 200 and 400 mg/kg, exhibited a significant ($p < 0.001$) anti-inflammatory effect compared to the control group. The anti-inflammatory activity of EECVR was found to be dose-dependent, with the highest activity observed at a dose of 400 mg/kg.
In conclusion, the study revealed that EECVR possesses significant anti-inflammatory activity, which can be attributed to the presence of alkaloids. The results suggest that EECVR could be used as a natural anti-inflammatory agent with potential therapeutic applications[25].

2. Anti-microbial activity [26,27]

Sabikunnahar J et.al (2016) conducted a thorough investigation of the methanolic extracts of *Clerodendrum viscosum* roots to determine their in vitro anti-microbial activity. The extraction process involved partitioning the methanolic extract into five different fractions - ethyl acetate soluble fraction (ESF), petroleum ether soluble fraction (PSF), carbon tetrachloride soluble fraction (CTSF), chloroform soluble fraction (CSF), and aqueous soluble fraction (AQSF). Each of these fractions was then subjected to an antimicrobial test to evaluate their antibacterial activity, and the results were compared to the standard drug kanamycin. The study found that the extracts exhibited significant antibacterial activity, which was more pronounced in gram-negative bacteria than in gram-positive bacteria. This suggests that the extracts have the potential to be used as a natural antibiotic against bacterial infections caused by gram-negative bacteria. Furthermore, the study revealed that the presence of flavonoids and tannins in the extracts had a significant and positive correlation with the pharmacological activities of *Clerodendrum viscosum* roots. These findings suggest that the bioactive compounds present in the extracts could be responsible for their antimicrobial properties. Overall, the study provides valuable insights into the potential use of *Clerodendrum viscosum* roots as a natural source of antimicrobial agents[26].

W.T. Oly et. al (2011) conducted experiments to test the crude extracts of *Clerodendrum viscosum* (Verbenaceae) in various solvent systems against six gram-positive bacterial strains (Staphylococcus aureus, Sercinia lutea, Bacillus subtilis, B. megaterium, B. cereus and Streptococcus-β–haemolyticus), nine gram-negative bacterial strains (Salmonellae typhi, Shigella dysenteriae, Escherichia coli, S. Shiga, S. boydii, S. sonnei, Proteus

sp., Klebsiella pneumoniae and Pseudomonas aeruginosa), and seven fungal strains (Aspergillus niger, A. fumigatus, A. flavus, Candida albicans, Vasin factum, Mucor sp., and Fusarium oxysporum) using disc diffusion and micro broth dilution techniques. All the extracts displayed antimicrobial activity with varying potency against a range of microorganisms harmful to humans. Some extracts were bacteriostatic and fungistatic, while others had bactericidal and fungicidal potential. The MIC values (64-128 µg/mL) of ethyl alcohol extract were determined against each gram-positive and gram-negative bacterial strain - S. aureus, B. subtilis, S.-β–haemolyticus, S. typhi, E. coli and Klebsiella sp. In the cytotoxic experiment, the ethyl alcohol root extract was more toxic (LC50 20.845 ppm) than the other extracts analyzed in the Brine shrimp test. The findings on the potential of *C. viscosum* are promising for the development of a broad-spectrum antimicrobial formulation.[27]

- STROBILANTHES ALTERNATA

1. Anti-inflammatory activity[28-31]

In their study, **Divyaa Sreekumar** et. al (2021) investigated the wound-healing effects of *Hemigraphis alternata*, a medicinal herb commonly used in Ayurveda, a traditional Indian system of medicine. The researchers conducted both in vitro cell line and animal model experiments using the *Hemigraphis alternata* leaf extract (HALE) to examine the potential therapeutic properties of this herb. The data showed that HALE treatment facilitated cell survival, cell proliferation, and cell migration leading to wound closure. The researchers examined the marker genes of wound healing, PAI-1 and TGF-β1, using qPCR, which is a technique that quantifies gene expression levels, and determined their concentration and expression fold in 3T3-L1 and L6 cell lines treated with HALE. The results indicated that the concentration of both marker genes of wound healing showed a significant increase in HALE-treated cell line cultures of 3T3-L1 and L6 cells ($P < 0.05$), and the expression fold of both genes was higher in cells treated with HALE than in the control group. These findings suggest that HALE may have therapeutic potential for wound healing. Furthermore, the researchers examined the molecular mechanisms underlying the wound-healing effects of HALE in scratch wounds. Scratch wounds are a widely used in vitro model to study cell migration and wound healing. The researchers found that HALE treatment promoted wound closure in scratch wounds, which was evident from the data on cell proliferation, cell migration, and cell survival ability. The molecular data provide strong evidence to support the use of HALE as an herbal medicine with anti-inflammatory properties for wound healing. This study sheds light on the potential therapeutic applications of *Hemigraphis alternata*, and further research is needed to explore the clinical efficacy and safety of this herb for wound healing[28].

The study conducted by S**. M. Mushiur Rahman** et.al (2019) aimed to investigate the anti-inflammatory activity of *Hemigraphis alternata* leaf extract in Swiss albino mice. Specifically, the study evaluated the effectiveness of methanol (MHAL) and ethyl acetate (EAHAL) extract in reducing inflammation in mice. Two different anti-inflammatory tests were performed to evaluate the extract's activity. The first test was the xylene-induced-ear edema test, which involved applying xylene to the mice's ears to induce inflammation. The second test was the Cotton pellet-induced granuloma formation test, which involved placing cotton pellets under the mice's skin to induce inflammation. During the anti-inflammatory test, the results showed that both MHAL 400 mg/kg and EAHAL 200 mg/kg & 400 mg/kg significantly reduced ear weight differences and granuloma formation in mice. The highest percentage inhibition was observed in EAHAL 400 mg/kg dose (35.15 ± 11.78% and 34.76 ± 11.30%) in both anti-inflammatory tests, respectively. These results suggest that *Hemigraphis alternata* leaf extract contains plant compounds with anti-inflammatory activities and is non-toxic. The study's findings are significant because they provide evidence that *Hemigraphis alternata* leaf extract could be a potential alternative to traditional anti-inflammatory drugs. The extract's natural properties make it a safer and healthier option for treating inflammatory conditions in mice. Further research could be done to explore the extract's effectiveness in humans and the possibility of using it as a natural treatment for inflammatory conditions[29].

In a scientific study conducted by **Wong Kak Ming** et. al (2014), the anti-protein denaturation activity of the plant *Strobilanthes alternata* was analyzed. The study used the IC50 value, which is the concentration of drug/extract required to prevent 50% protein denaturation. The results showed that the DCM: MeOH leaves extract of *H. alternata* had better activity than the positive control diclofenac sodium. Its IC50 value was 94.3 ug/mL, which was significantly lower than the 311.5ug/mL IC50 value of diclofenac sodium. This indicates that *H. alternata* has strong anti-protein denaturation activity. The researchers then proceeded to extract *H. alternata* sequentially with hexane, dichloromethane, ethyl acetate, methanol, and distilled water. Among the five crude extracts, only ethyl acetate and methanol were found to have anti-protein denaturation properties. Ethyl acetate's IC50 value was 101.0ug/mL, while methanol's IC50 value was 60.4ug/ml. This reveals that methanol crude extract had the greatest anti-protein denaturation activity, making it the most promising candidate for further study. To identify the active fraction and compound of the methanol crude extract, it will undergo fractionation and purification. This process will help the researchers isolate the active compound responsible for the anti-protein denaturation activity in H. alternata. This study provides insights into the potential use of *H. alternata* and its active compound as a natural anti-protein denaturant[30].

A study was conducted by **Boby T. Edwin** (2011) and his team on Hemigraphis alternata to investigate its role in wound healing by examining its effects on fibroblasts and endothelial cells. The study examined the effects of aqueous and ethanolic extract (AEHA and EEHA) on the proliferation and migration of fibroblast by MTT and scratch-wound healing assay. The study also analyzed the expression of angiogenic markers like tyrosine kinase receptors (KDR, FLT 4), Platelet endothelial cell adhesion molecule (CD31), and vascular endothelial cadherin (VE-Cadherin) by endothelial cells when treated with HA by semiquantitative RT PCR. The study showed that AEHA at a concentration of 2.5mg/ml and 3.5mg/ml increased proliferation in serum-free and serum-containing culture conditions respectively, while EEHA was found to be toxic to cells. The proliferation of AEHA was associated with a 60% protein fraction, which revealed a band of 21.8 kD peptide. In a fibroblast scratch wound model, AEHA at a concentration of 180g showed a wound re-epithelialization of 97%. Furthermore, the PCR assay revealed an enhanced expression of angiogenic

markers like KDR, FLT 4, CD31, and VE-Cadherin. These results suggest that Hemigraphis alternata has potential implications in wound healing as it influences fibroblast and endothelial cells, which are the key players during wound healing[31].

2. Anti-microbial activity[32, 33]

Hemigraphis alternata is a species of medicinal plant that has been traditionally used in Indian medicine due to its potential antimicrobial properties. To investigate this claim, **Sushmita Roy** et. al (2020) conducted a study that tested the methanol extracts of the leaves of *H. alternata* for antibacterial activity. The testing was performed using the disk diffusion method and involved six bacterial species, including four Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella typhimurium) and two Gram-positive bacteria (Micrococcus luteus and Bacillus subtilis). Unfortunately, the results of the study showed that *H. alternata* did not exhibit any inhibitory activity against any of the bacterial species tested. Although this is disappointing, it is still important to continue exploring the potential medicinal properties of *H. alternata* and other natural remedies[32].

Santhiya Sasidharan et. al (2020) conducted a study on the leaf extract of Hemigraphis alternata or Murikuti, which is a traditionally used medicinal plant. The leaves were found to contain bioactive reducing agents as per phytochemical screening. The study aimed to prepare gold nanoparticles using an aqueous extract of HA leaves and optimize the concentration variation of gold nanoparticles under room temperature conditions. Various conventional methods, such as refluxing and homogenization, were used to prepare reduced graphene oxide (HAarGO) and nanocomposite (HAaNC), respectively. The synthesized nanoparticles were characterized using spectroscopic techniques, including UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Raman spectroscopy, Thermal analysis (TGA/ DTG), and Field Emission Scanning Electron Microscopy (FE SEM). The average size of the gold nanoparticles was calculated as 22.02 nm using XRD, with desired peaks at 38.4° and 44.6° attributed to 111 and 222 planes, respectively. Spherical-shaped gold nanoparticles of size 34.80 nm were observed under FE SEM. The study found that the synthesized nanoparticles had effective antibacterial properties against Gram-positive and Gram-negative bacteria. Additionally, the nanoparticles exhibited less cell viability (40-20%) on skin cancer cell lines A431. The study also used the Acridine Orange (AO)/ Ethidium bromide (EtBr) dual staining and the Propidium Iodide (PI) staining method to reveal apoptotic cell death for HAaNP-treated cancer cell lines. The findings of this study portray the potential application of HA nanoparticles as an alternative antibiotic for infectious diseases and as a therapeutic agent for skin cancer. The detailed analysis of the synthesized nanoparticles using various spectroscopic techniques highlights their physical and chemical properties, which can aid in their application in various fields, including healthcare and biotechnology[33].

CONCLUSION :

Clerodendrum viscosum, a flowering plant, is classified under the Lamiaceae family, which was formerly grouped under the Verbenaceae family. The Lamiaceae family includes 35 genera and about 1,200 species that are mainly distributed across tropical and subtropical regions around the world. Numerous research studies have suggested that the plant possesses potent anti-inflammatory and anti-microbial properties, supported by various experimental methods. The anti-inflammatory activity of the plant has been confirmed through several methods, including the carrageenan-induced paw edema method. Additionally, the anti-microbial activity of the plant has been studied in various solvent extracts, along with other supportive research studies. These findings have opened up new avenues for further research on the potential health benefits of *Clerodendrum viscosum* and its potential use in various domains, including medicine and pharmacology.

The *Hemigraphis alternata* is an interesting plant that belongs to the Acanthaceae family. It is a low-creeping perennial herb that grows up to a height of 15 to 30 cm. This tropical plant is native to Malaysia and has many common names such as Purple Waffle plant, Aluminium plant, Cemetary plant, Metal leaf, Red flame Ivy, and Java Ivy, among others. This versatile plant has been the subject of numerous studies, which have revealed its potent anti-inflammatory and anti-microbial properties. Researchers have employed various methods to confirm the anti-inflammatory activity of the plant, including the MTT and scratch-wound healing assay, xylene-induced-ear edema test, and Cotton pellet-induced granuloma formation test. Additionally, the anti-microbial activity of the plant has also been studied using the disc diffusion method in various solvent extracts, along with other supporting studies. The *Hemigraphis alternata* is a fascinating plant that has captured the attention of researchers due to its medicinal properties. Its anti-inflammatory and anti-microbial activities make it a promising candidate for the development of new drugs to treat various diseases.

It has been observed that the roots of *Clerodendrum viscosum* and the leaves of *Strobilanthes alternata* possess remarkable properties of inhibiting inflammation and microbe growth. Results from various studies indicate that both these natural extracts exhibit potent anti-inflammatory and anti-microbial activity, making them promising candidates for use in the development of novel therapeutic agents.

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