

EVALUATING THE ANTIMICROBIAL EFFICIENCY OF AQUEOUS EXTRACT OF *Chromolaena Odorata*

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ABSTRACT

Chromolaena odorata (Asteraceae Family) is a conventional herbal remedy known because of its wound-healing qualities. Various portions of this plant have been utilized to treat wounds, burns, and skin diseases. It has also been shown to exhibit anticancer, anti-diabetic, anti-hepatotoxic, anti-inflammatory, antibacterial, and antioxidant properties. Phytochemical constituents include alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids. Other important components of this plant include eupolin, chromomoric acid, quercetagenin, and quercetin, each of which contributes to its healing properties. *Chromolaena odorata* has been shown to have antibacterial action in studies. The purpose of this study is to discover active antibacterial and antifungal compounds from *Chromolaena odorata* eupolin against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Candida tropicalis*, and *Candida albicans*. This investigation indicated that *Chromolaena odorata* contains a greater concentration of phytochemical components and antibacterial agents. These findings showed that with more research, *C.odorata* aqueous extract might be efficiently recognised for therapeutic purposes.

Keywords: *Chromolaena odorata*, Eupolin, wound healing properties, anti-hepatotoxic, tissue engineering

1. INTRODUCTION

Infectious illnesses have afflicted humanity since the start of civilization, and it is believed that they have existed from the dawn of time. Several challenges have developed in the control of infectious illnesses, the most significant being the increase in the number of microorganisms resistant to antimicrobials. As a result, resistance is evident in infections caused by certain bacteria, resulting in failure to react to initial therapy. Resistance to antibiotics is mostly caused by novel bacterial variations, with secondary reasons including antimicrobial medication usage. These problems foster a perfect atmosphere that promotes the dissemination, creation, and tenacity of resistant diseases. Methicillin resistant *Staphylococcus aureus* (MRSA) is a highly contagious bacteria that is resistant to a number of medications, including methicillin, amoxicillin, penicillin, and oxacillin. This resistance complicates therapy and management. Through time, it has developed resistance to beta-lactam medicines. Mec- A is a resistant gene that inhibits -lactam anti-infection drugs from inactivating compound transpeptidases, which are required for cell wall synthesis (Shahkaraniet *al.*, 2014).

Plants have been employed for medicinal purposes for much longer than written history, and the pharmacological properties of plants were noted by ancient Chinese and Egyptians as early as 3,000 years ago (Naidooet *al.*, 2011). The word herbal plant refers to a number of plants that are employed in traditional medicines. It is the use of plants for medical purposes. Folk remedies have historically been utilized alongside mainstream medicine, and it is increasingly becoming one of the key techniques in novel scientific investigations in illness prevention and therapy that are otherwise regarded to be difficult to cure (Bordekeret *al.*, 2002).

An ethnopharmacological investigation in Benin identifies several plants used in traditional medicine to treat intestinal illnesses. The antibacterial activities of *Chromolaena odorata* (previously *Eupatorium odoratum*) were certainly the least explored. Fresh leaves or decoction are used to treat leech bites, soft tissue wounds, scorching wounds, skin infections, and dent alveolitis across Vietnam (Sharmaet *al.*, 1998). *C. odotrata* is also employed in the general practice of postpartum mothers, according to research conducted in northern Thailand. Several people indicate that it helps with indigestion and peptic ulcer wounds, as well as stopping bleeding, accelerating healing, and rejuvenating (Panyaphuet *al.*, 2011).

Chromolaena odorata (L.) R.M. King and H. Robinson is a flowering plant that is one of the world's top 100 most invasive ecological weeds of wastelands, roadsides, and other outdoor locations (GISD, Chakraborty *et al.*, 2011). This blooming shrub is native to North and Central America and was subsequently imported to portions of Asia, Africa, and Australia. *C. odorata* is also referred to as Armstrong's weed, baby tea, bitter bush, butterfly weed, Christmas bush, devil weed, eupatorium, Jack in the bush, king weed, paraffin bush, paraffin weed, Siam weed, turpentine weed, trifid weed (McFadyen, 2004). It has insecticidal qualities and is used as farmyard manure. It is also used to preserve deceased bodies (Ukwueze *et al.*, 2013). Traditional medicine practitioners have employed the fresh leaves of *C. odorata* or the decoction to treat human burns, soft tissue wounds, ulcerated wounds, burn wounds, postnatal wounds, as well as leech bites, dyspepsia, and skin infection (Panyaphu *et al.*, 2011). The stem decoctions were reported to be efficacious against *Propionibacterium acnes*-caused skin illness (Pandurangan *et al.*, 2015). It is also used to treat amenorrhea, catarrh, cold-associated nasal congestion, diabetes, diarrhoea, fever, pertussis, and rheumatism, as well as a vermifuge (Goodall JM and Erasmus DJ, 1996). This plant also has anthelmintic (Patel *et al.*, 2010), antimalarial (Ongkana, 2003), analgesic, anti-inflammatory, antipyretic, antispasmodic (Oludare *et al.*, 2000), antimycobacterial, insecticidal, antioxidant (Phan *et al.*, 2001), anti-gonorrhoeal (Caceres *et al.*, 1995), fungicidal, diuretic (Gopinath *et al.*, 2009), blood coagulating (Triaratana *et al.*, 1991), and antibacterial activities (Borges *et al.*, 2016). Unfortunately, there is still very minimal information known on the plant's wound-healing qualities (Biswa *et al.*, 1997). One of the most promising fields of biomedical research is the search for effective wound-healing agents (Majumder, 2012 and Kameshwaran *et al.*, 2014).

Phytochemical screens are significant since they are the initial stage in the discovery of potentially helpful medicines (Sivamani *et al.*, 2012). A thorough scientific evaluation is required to fully comprehend such plants and allow them to play their rightful role in contributing to inexpensive healthcare. To analyze the chemical contents of plants and research their bioactivity, several solvent extraction techniques, as well as chromatographic separations and spectroscopic procedures, have been utilized. Much of the research has shown that phenolic acids (protocatechuic, p-hydroxybenzoic, p-coumaric, ferulic, and vanillic acids) extracted from many plants, including *C. odorata*, aid in wound healing in a variety of animal models (Phan *et al.*, 2001 and Paiva *et al.*, 2002). Phan *et al.*, (2001) reported that phenolic compounds from *C. odorata* protected cultured skin cells from oxidative damage. Thang

et al., (2001) suggested that the protective effects of *C. odorata* might be attributable to its strong antioxidant activity which is vital for cutaneous tissue repair and wound healing.

This research includes the investigation of the best solvent for studies through phytochemical analysis, the minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration, antibacterial activity, and antifungal activity. The test organisms were used in this study based on their pathogenicity toward the wounds. The major wound-affecting test organisms are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Candida tropicalis*, *Candida albicans*.

2. MATERIALS AND METHODS

2.1 Collection of herbs and processing

Chromolaena odorata (common pacha-common lantana) leaves were collected from a house garden, in Salem, Tamil Nadu, India. The leaves were pre-washed in deionized water and kept under shade until completely dried for milling.

2.2 Sample preparation – Drying and milling of leaves

The dried leaves were then cut into smaller pieces and arranged separately in aluminium trays and oven dried at 40°C to 50°C for 2h until the moisture content reached below 10%. The dried parts were grounded to powder and sieved using a metal sieve. Sieved particles were stored at room temperature prior to testing.

2.3 Preparation of *Chromolaena Odorata* extract

A total of 5g of fresh *Chromolaena odorata* was weighed and macerated in 100 ml of water. The mixture was left at room temperature for one day in a shaker. The solution was filtered using a muslin cloth and lyophilized the filtrate to get the powdered form of aqueous extract. They were stored at 4°C until use. Other solvent extraction done by soxhlet extraction method.

2.4 Phytochemical analysis

2.4.1 Tests for carbohydrates

Fehling's Test: 1 ml of Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now an equal volume of test solution was added to the above mixture. The solution was heated in a boiling water bath for 5-10 minutes. First, a yellow, brick-red precipitate was observed.

2.4.2 Test for proteins

Biuret Test: To the small quantity of extract 1-2 drops of biuret reagent was added. The formation of violet color precipitate showed the presence of proteins.

2.4.3 Tests for anthroquinone glycosides

Borntrager's Test: To the 3 ml of extracts, dil. H₂SO₄ was added. The solution was then boiled and filtered. The filtrate was cooled and its equal volume of benzene was added. The solution was shaken well and the organic layer was separated. An equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides. (Prashant Tiwari, 2011)

2.4.4 Tests for Cardiac glycosides

Keller-Killiani Test: To the 5ml of extract, 1ml of conc.H₂SO₄, 2 ml of Glacial acetic acid, and 1 drop of FeCl₃ solution were added. The appearance of the brown ring shows the presence of cardiac glycosides.

2.4.5 Test for terpenoids

Salkowski Test: To 2 ml of extract, 2 ml of chloroform, and 2 ml of conc.H₂SO₄ were added. The solution was shaken well. As a result, the chloroform layer turned red and the acid layer showed greenish-yellow fluorescence.

2.4.6 Test for alkaloids

Dragendroff's Test: To the 2-3 ml of filtrate, a few drops of dil. HCl and Dragendroff's reagent was added and shaken well. The formation of an orange-brown precipitate showed the presence of alkaloids

2.4.7 Tests for flavonoids

Lead Acetate Test: To the small quantity of extract lead acetate solution was added. The formation of a yellow precipitate showed the presence of flavonoids. (Prashant Tiwari, 2011)

2.4.8 Test for Tannins and Phenolics compound

Lead Acetate Test: On addition of lead acetate solution to the extract white precipitate appeared.

2.4.9 Test for Saponin

Foam Test: To 1 ml extract 20 ml distilled water was added and shaken well in a measuring cylinder for 15 min. Then 1 cm layer of foam was formed (Prashant Tiwari, 2011).

2.4.10 Test for Xanthoprotein

In 2ml test solution a few drops of conc. nitric acid and 3ml of ammonia were added. The appearance of a red precipitate indicates the presence of xanthoprotein. (Prashant Tiwari, 2011)

2.4.11 Test for Glycosides

The extract was mixed with a little anthrone on a watch glass. One drop of conc. sulphuric acid was added and made into a paste and warmed gently over the water bath. Dark green coloration indicates the presence of glycosides.

2.4.12 Test for Quinone

2ml test solution was treated with a few drops of conc. H₂SO₄ or aq. NaOH solution. Colour formation indicates the presence of the Quinone compound.

2.4.13 Test for fixed oil

A small quantity of powder was pressed between the filter paper. The formation of a grease spot indicates the presence of fixed oil and fats.

2.5 Minimum inhibitory concentration (MIC)

The turbidity test is used to detect the minimum inhibitory concentration. Here *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Candida tropicalis*, and *Candida albicans* were used to detect the MIC.

2.6 Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC)

The Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC) were determined by broth dilution minimum inhibitory concentration tests by subculturing agar plates that do not contain the test agent. Here *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae* were used to detect the MBC and *Candida tropicalis* and *Candida albicans* were used to detect the MFC.

2.7 Antibacterial activity and Antifungal activity

To determine the effective concentration of plant extracts, the antibacterial activity of two different concentrations (S1-500µg/ml and S2-400µg/ml) were tested against the test organisms (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*) using agar well diffusion method. And likewise, the agar well diffusion method was used to detect the antifungal activity of two different concentrations (S1-500µg/ml and S2-400µg/ml) against *Candida tropicalis* and *Candida albicans*. Streptomycin was used as the control.

2.8 Statistical analysis

The antibacterial and antifungal activities were represented as mean $\pm$ SE. After variance analysis, the concentrations of organisms were compared using one-way ANOVA and the Duncan test, with statistical significance established at a P-value of 0.05. SPSS software was used to analyze the data (IBM SPSS STATISTICS version 20).

3.RESULTS

3.1 Phytochemical analysis

The early phytochemical study of *Chromolaena odorata* extracts in water, ethyl acetate, petroleum ether, and hexane demonstrated the existence of certain key phytochemical elements such as alkaloids, flavonoids, saponin, tannins, phenolic compounds, carbohydrates, and proteins. Whenever these extracts were evaluated, the aqueous extract of *Chromolaena odorata* generated the most favorable results. Aqueous extracts contained the majority of the phytoconstituents, including proteins, flavonoids, tannins and phenolic compounds, saponins, fixed oil, carbohydrates, cardiac glycosides, terpenoids, and xanthoproteins. When compared

to hexane and petroleum ether extracts, the ethyl acetate extract included some of the phytochemical components. Only saponins were present in hexane and petroleum ether. Additional phytochemical components were not found in either the hexane or petroleum ether extracts (Table 1).

3.2 Minimum inhibitory concentration (MIC)

The broth was employed as a control, and five different concentrations were utilized to identify the minimum inhibitory concentrations (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml). The minimal turbidity for *Streptococcus pyogenes*, *Escherichia coli*, *Candida albicans*, and *Candida tropicalis* was 300 µg/ml. As a result, the minimum inhibitory concentration of the aforementioned test organisms was 300 µg/ml. *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Proteus mirabilis* had the maximum turbidity at 200 µg/ml. As a result, the minimum inhibitory concentration of the aforementioned test organisms was 200 µg/ml.

3.3 Minimum bactericidal concentration (MBC) and Minimum fungicidal concentration (MFC)

The minimum bactericidal concentration of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Staphylococcus aureus* was determined using two concentrations (200 µg/ml and 300 µg/ml) based on the minimum inhibitory concentration. The fewest colonies were formed at 200 µg/ml and no colonies were obtained at 300 µg/ml. As a result, the minimum bactericidal concentration of the above test organisms was 200 µg/ml. The lowest bactericidal concentrations of *Streptococcus pyogenes* and *Escherichia coli* were determined using two distinct concentrations (300 µg/ml and 400 µg/ml). The minimum bactericidal concentration of the aforementioned test organisms was 300 µg/ml. The minimum fungicidal concentration of *Candida albicans* and *Candida tropicalis* were observed at 300 µg/ml. Here also two different concentrations were used (300 µg/ml and 400 µg/ml). There were no colonies found in 400 µg/ml plates.

3.4 Antibacterial and Antifungal activity

The highest zone of inhibition was shown by both *Pseudomonas aeruginosa* (27.66 ± 0.33) and *Escherichia coli* (27.66 ± 0.33) in 500mcg concentration. The least zone of inhibition was shown by *Klebsiella pneumoniae* (24.33 ± 0.33). Other bacterial organisms have shown an average zone of inhibition. When compared with *Candida tropicalis* and *Candida*

albicans, *Candida tropicalis* were shown the highest zone of inhibition (28.33 ± 0.33 in 500 mcg and 25.33 ± 0.33 in 400 mcg). The *Candida albicans* have moderate antifungal activity.

4. DISCUSSION

Wound management encompasses hospitalization for a definite period, ridiculously priced pharmacotherapy, and different surgical methodologies accompanied by an extended recovery process. (Thomas et al. 2009). Most dermatologic antibacterial and disinfectants are successful in avoiding infectious diseases, but the existence of skin problems and allergies induced by these agents lowers the rate of tissue regeneration and extends the time needed to achieve full healing process (Burks 1998). Besides that, the most overpriced tissue-engineered wound dressings are out of reach for patient populations in nations that are underdeveloped or developing. Several therapeutic plants and products manufactured from herbal plants have been proven to have satisfactory wound-healing potential with no or minimal cytotoxicity and to be less affordable than synthetic medications. A number of the herbal medicines investigated for wound healing properties have historically been utilized in folk medicine for the treatment of wounds (Phan et al. 2001b; Upadhyay et al. 2009; Hashemi and Davoodi 2011; Vaisakh and Pandey 2012).

The existence of phytoconstituents in *Chromolaena odorata* leaves indicates they are able to be utilized to control bacteria. This is corroborated by (Edeoga et al., 2005), who claimed that the therapeutic properties of plants are found in their constituent phytochemicals such as alkaloids, tannins, flavonoids, and other phenolic compounds, each of which has a diverse physiological impact on the human body. The appearance of alkaloids in the leaves could indicate their efficacy in preventing pathogens, which is consistent with the findings of (Tsuchiya et al., 2006), who claim that alkaloids are major plant components responsible for antimicrobial activity because they cleave with DNA and interrupt cell division.

Some findings suggest that *C. odorata* leaf extract exhibits antimicrobial potential against *Pseudomonas aeruginosa*, *Streptococcus faecalis* [Irobi, 1992], *Neisseria gonorrhoeae* [Caceres et al., 1995], and antifungal properties against *Cryptococcus neoformans*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* [Ngono Ngane et al., 2006].

Plant phenolics, one of the biggest families of specialised metabolites in the plant kingdom, continue to be of significant interest to researchers due to their vast variety of activities in plants as well as their linkages to a number of health-promoting features in humans. Phenolics, namely flavonoids, have been identified as one of the key taxonomic markers for the genus *Chromolaena* (Oliveira et al., 2017). *C. odorata* includes a variety of phenolic chemicals, which may be further classified as flavonoids and basic phenolic acids and derivatives. Plant terpenoids are significant sources of bioactive metabolites with a wide variety of pharmacologic potentials, including antidiabetic, wound healing, and antibacterial properties (Castellano et al., 2013). A previous study has revealed that *C. odorata* contains a large quantity of saponin. For example, Vijayaraghavan et al. (2018) previously discovered that the concentration of *C. odorata* saponins was greater in water leaf extracts than in ethanol leaf extracts. Omokhua et al. (2017) observed a modest amount of saponin in leaf extracts of *C. odorata* produced from dried (55 °C, 72 h) powder from both the Asian/West African biotype and southern African biotype implementing a solvent for extraction equivalent to the one used in the current research (70% ethanol).

Several investigations have emphasized the therapeutic value of *C. odorata* compounds. Although some prior research revealed that *C. odorata* extracts were cytoprotective towards oxidative damage (Hung et al., 2011), other investigations indicated that the extracts might be cytotoxic (Anyanwu et al., 2017). For example, Pakdeepak et al., 2020 discovered that 5,6,7,4'-tetramethoxyflavone, a polyphenol derived from *C. odorata*, protects mice from dexamethasone-induced degeneration by inhibiting amyloidogenesis. Ikewuchi et al., 2021 recently demonstrated that aqueous extract of *C. odorata* defended against doxorubicin-induced cardiotoxicity in Wistar rats by increasing endogenous cardiac antioxidants, enhancing cardiac electrolyte balance, and decreasing cardiac oxidative stress, among other things. Omotuyi et al., 2020 previously established that *C. odorata* flavonoid extract reduced streptozotocin-induced neuropathy in Wistar rats by inhibiting reactive oxygen species production and cytokine upregulation.

The anti-microbial potential of *Chromolaena odorata* extracts is corroborated by the findings of (Ugwoke et al., 2007), who discovered *Chromolaena odorata* leaves to be effective against *S. aureus*, *S. typhii*, *E. coli*, *Candida albicans*, and *Aspergillus niger*. The comparison between both the control (ciprofloxacin) and the ethanol extract of *Chromolaena odorata* leaves was statistically significant at $p < 0.05$. The ethanolic extracts of *Chromolaena odorata* leaves performed well in comparison to ciprofloxacin (control). The MIC of the plant extracts

was also evaluated against the test organism. The MIC of *C.odorata* leaf extracts in ethanol and chloroform was reported to be 6.25mg/ml. The MIC findings indicate that *C. odorata* extracts were effective against the test organism even at low concentrations.

Aqueous extracts were shown to have a stronger inhibitory action on the test organism than the other extracts in this investigation. This might be because aqueous extracts more essential oils and secondary plant compounds, which are thought to have an antibacterial effect on the test organism. On the other hand, this investigation validates the scientific usage of these herbs in folk medicine to treat illnesses produced by the test organisms.

5. CONCLUSION

The aqueous extract showed a high level of phytochemical constituents in this study. It was also observed that the aqueous extract exhibited higher inhibitory activity on test organisms like *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Candida tropicalis*, and *Candida albicans*. So, it has wound-healing properties by preventing wound-affecting microorganisms. In the future, it can be used for tissue engineering for wound healing and it can be also used for diabetic wound healing through tissue engineering by averting puss and enhancement of keratinocytes and fibroblasts. It is significant because of the possibility of the components present in *Chromolaena odorata* and its use against multi-resistant organisms.

6. CONFLICT OF INTEREST

The authors have declared that they have no conflict of interest.

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