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International Journal on Computational Engineering

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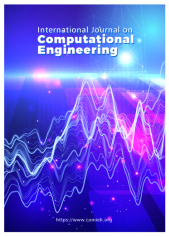

Medicinal Plants as Alternative Anti-Weevil and Antimicrobial Agents: A Study on *Glinus lotoides* and *Croton macrostachyus* Against Selected Test Organisms

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***Abstract*— The rise of antibiotic resistance poses a global public health threat, necessitating the search for alternative anti-weevils and antimicrobial agents. This study investigates the antibacterial and anti-weevil activities of Glinus lotoides (Metere) and Croton macrostachyus (Bissana) against Escherichia coli and Staphylococcus aureus. The antibacterial activity was evaluated using the agar well diffusion method, revealing significant inhibition zones. The highest antimicrobial effect was observed in methanol extracts of Croton macrostachyus. Additionally, anti-weevil properties were assessed, demonstrating promising insecticidal effects. The results support the medicinal use of these plants and indicate their potential as alternative antimicrobial agents.**

***Keywords*— Glinus lotoides; antibacterial activity; agar well diffusion; anti-weevil.**

Manuscript received 15 Oct. 2024; revised 29 Dec. 2024; accepted 2 Feb. 2025. Date of publication 31 Mar. 2025.

I. INTRODUCTION

Medicinal plants have been extensively used for treating diseases, especially in developing countries where access to conventional medicine is limited [1], [2]. With the rise of antibiotic-resistant pathogens, the search for plant-based antimicrobials has gained significant attention [3]. *Glinus lotoides* and *Croton macrostachyus* are widely used in Ethiopian traditional medicine, yet their antimicrobial properties remain underexplored.

In Ethiopia, medicinal plants play a crucial role in healthcare, with approximately 80% of the population relying on plant-based remedies [4]. The country's diverse topography and climatic conditions contribute to the rich biodiversity of medicinal plants. However, scientific validation of traditional knowledge is essential to assess their efficacy and potential use in drug development [5].

The increasing prevalence of antibiotic-resistant bacteria has necessitated the search for alternative antimicrobial agents. Medicinal plants, particularly in developing countries, serve as essential sources for novel bioactive compounds. This study evaluates the antimicrobial properties of *Glinus lotoides* and *Croton macrostachyus*, two traditionally used medicinal plants in Ethiopia, against selected Gram-positive and Gram-negative bacteria. The bioactive compounds present in these plants, including tannins, alkaloids, flavonoids, and terpenoids, may offer effective antimicrobial properties. The findings of this research could contribute to the development of plant-based antimicrobial drugs, addressing the challenges posed by antibiotic resistance [1].

The antimicrobial compounds from plants extract inhibit bacteria than the presently used antibiotics and may have clinical value in treatment of resistant microbial strains [6]. This study evaluates the anti-weevils and antimicrobial activity of *Croton macrostachyus* and *Glinus lotoides* extracts to understand its antimicrobial activity against selected both Gram-negative and Gram-positive bacteria. This contributes part of an effort to identify potential sources of cheap medicinal materials for the synthesis of new medicine to avoid the problem of increasing drug resistance against the selected pathogens.

The emergence of multidrug-resistant (MDR) bacteria has rendered conventional antibiotics ineffective, necessitating the exploration of alternative therapeutic agents [7]. The present study aims to investigate the antimicrobial activity of *Glinus lotoides* and *Croton macrostachyus* extracts against selected bacterial strains. Numerous studies have been carried out on using natural products for screening antimicrobial activity, but no attention has been given to use *Croton macrostachyus* and *Glinuslotoides* for antimicrobial

activity. Therefore, the main objective of this study is to test the antibacterial activity of the two plant extracts against some selected test organism.

II. Materials and Methods

A. Plant Collection and Extraction

Healthy, disease free fresh leaves of *Glinus lotoides* and *Croton macrostachyus* were collected from southern regions near Arba Minch town of Ethiopia and the study was conducted at Arba Minch University, Ethiopia. The plant materials were washed, air-dried, and ground into powder. Extraction was performed using ethanol, methanol, and distilled water following standard protocols [8].

B. Preparation of Plant Extracts

Extracts were prepared differently using methanol, ethanol, and distilled water. The filtrates were concentrated and stored for further analysis.

The plants was grounded using grinder machine and sieved with a mesh to obtain a fine powder. A technical grade solvent (distilled water, ethanol and methanol) was employed for extraction. Plant material (30g) of flour of *Croton macrostachyus* (bissana) and *Glinuslotoides* (Metere) were soaked in 300 ml of each solvents including distilled water both prepared plant powder in differently. The slurry were placed in a shaker for 48 hours and filtered through whatman's filter paper No.1. The filtrate was concentrated by evaporation of solvent in water bath. The collected extract was further separated by Rotary evaporator at 40°C reduced temperature. The yielded extracts were stored in oven up to use for the nest work.

C. Sources and Preparation of the tested Organisms

A total of 2 strains including gram positive (*Staphylococcus aureus*,) and gram negative (*Escherichia coli)* bacteria were selected to assess the susceptibility test against the prepared extract. The strains were obtained from Ethiopian public health and research institute, Addis Ababa Ethiopia. They were maintained in suspension media (nutrient broth) and kept in incubator at 37^0C. Later on the organism were cultured on Muller Hinton Agar for (*Staphylococcus aurenus*,) and (*Escherichia coli*)), fresh inoculums were taken from the media for test.

D. Preparation of bacterial suspension

Preparation of Suspension for *Staphylococcus S.aureus* and *Escherichia coli:*

The tested microorganisms were separately cultured on Muller Hinton Agar Medium (MHA) at 37^0C for 24 hrs. By spread the inculcating loop containing bacteria at the top end of the agar plate moving in a zig-zag horizontal pattern until 1/3 of the plate was covered.

Then, three to five well isolated overnight cultured colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with sterile bent wire-loop and the growth was transferred into 10ml of broth. The turbidity of the inoculum suspension was adjusted using 0.5 McFarland standards. The turbidity of the actively growing broth culture was adjusted with sterile saline to obtain turbidity optically comparable to that of 0.5 McFarland turbidity standard 1.5×10^8 colony forming units (CFU)/ml. To obtain this, OD value 1.2 will read at 600 wavelength using spectrophotometer.

E. Preparation of culture medium

Muller-Hinton agar was prepared from commercially available dehydrated base according to manufacturer's instructions. Muller Hinton Agar Medium (MHA) was prepared by dissolving 14g of MHA medium in 360ml of distilled water (7.2 PH). The mixture of Muller Hinton Agar Medium powder and sterile distilled water were stirred with a sterilized glass rod and covered with aluminum foil and then autoclaved for 15 min at 121°C. Soon after autoclaving, the agar was allowed to cool in the laminar flow hood prior pouring it into the petri plate. The mixture was heated and stirred until all components fully dissolved.

Then, the dissolved mixture was autoclaved at 121 degrees Celsius for 15 minutes and allowed to cool but not solidify. Then the prepared medium was poured into the prepared petri plates.

F. Antimicrobial Activity Assay

The antimicrobial activity of the extracts was tested against selected Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) using the agar well diffusion method [9]. The minimum inhibitory concentration (MIC) was determined using the broth dilution method [10].

G. Antimicrobial activity determination by agar well diffusion method.

The antimicrobial activity of *Croton macro stachyus* (bissana) and Glinus lotoides (Metere) extract were determined as per method developed by Boyanova *et al*., [11].The antibacterial activities of the different extract were determined by agar well diffusion assay on Muller Hinton Agar Medium. The standardized inoculums of 100µl (1.5×10 CFU/ml, 0.5 McFarland) was added aseptically and streaked with a sterile non-toxic cotton swab on the agar plate surface. 6mm Wells were prepared in the seeded agar plates with sterile cork borer. The test compound or crude extract (100 µl) was carefully dispensed on the wells of agar medium. Extract was allowed diffusing for about 30min before incubation. Standard antibiotics alone use as a negative control. Using aseptic conditions all the selected antibiotic (syploxaxilin 30 µg/disc) and the plant extracts were applied on the MHA and left for 30 minutes to allow the extract to diffuse. The plates were then incubated at 37°C for 18-24 hours in an incubator. All tests were performed in triplicate and zone of inhibition were measured from the edge of each wells/disc after the incubation period by using ruler.

III. Results and Discussion

A. Antimicrobial Activity of Croton macrostachyeus and Glinus Lotoides extract

The antibacterial activity of traditionally used medicinal plant such as *Croton macrostachyeus* and *Glinus Lotoides* has been evaluated against *S. aureus* and *E. coli*. Completely large variation of zone of inhibition of both *Croton Macrostachyeus* and *Glinus Lotoides* has been observed

against test organisms. This shows that both plants have different capability to inhibit the growth of test bacteria. This may also due to the constitutes of compound that they contain and the parts of plants used. *Croton macrostachyeus* extracts have higher range of antibacterial activity against both gram negative and gram-positive bacteria than *Glinus Lotoides* extracts. This showed that the leaf of Bissana is more effective than the leaf of Metere exhibited anti-microbial activity.

Fig. 1 Antimicrobial Activity result of Glinus Lotoides and *Croton macrostachyus respectively*

This result was supported by the previous study reported the extracts of *Glinus lotoides* and *Croton macrostachyus* exhibited significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Ethanolic and methanolic extracts showed the highest inhibition zones, suggesting strong antimicrobial efficacy [12]. The MIC values ranged from 50-200 µg/mL, demonstrating the potential use of these plants as natural antibacterial agents [13].

In this study, there is highly statistical significant antibacterial effect *Croton macrostachyeus* against *S.aures* (17.3±1.52) for methanol leaf extract, by ethanol leaf extract with an inhibition zone of (15.3±3.05). The extract also shows (13.3±2.08 mm) by distilled water respectively. The highest inhibition zone was observed for methanol. For the second plant *Glinus Lotoides*, ethanol shows highest zone of inhibition (12±2.51) mm against *s.aures* and all the rest methanol and distilled water shows (11±2.08) mm and (9±0.35) mm zone of inhibition on *s.aures* respectively.

TABLE I
THE MEAN INHIBITION ZONE (MEAN ±SD (MM)) OF THE LEAVES CROTON MACROSTACHYEUS OF AGAINST THE TEST ORGANISM

Inhibition zone (mm)			
Test organisms	**ethanol**	**methanol**	**Distilled water**
S. aureus	16±0.42	17.3±1.52	13.3±2.08
E.coli	15.6±3.05	13±2.64	11.3±1.05

TABLE II
THE MEAN INHIBITION ZONE (MEAN ±SD (MM)) OF THE LEAVES OF GLINUS LOTOIDES AGAINST THE TEST ORGANISM

Inhibition zone of the extracts (mm)			
Test organisms	**ethanol**	**methanol**	**Distilled water**
S. aureus	12±2.51	11±2.08	9±0.35
E.coli	10±1.5	9±1.5	7±1.75

The anti-microbial test on the selected bacterial strains, the leaf of *Croton macrostachyeus* extract showed a promising effect than leaf of *Glinus Lotoides* extract. The *Croton macrostachyeus* extract also had a good effect when extracted by ethanol (95%) when compared with the methanol and distilled water extracts. The effect was compared based on the diameter formed as a clear zone. This implies that Bissana leaves are a very good antimicrobial agent. The findings of this study contribute to the growing body of evidence supporting the medicinal potential of Ethiopian plants.

This finding was supported by previous reported research which explained the increasing antibiotic resistance, plant-derived antimicrobial compounds could serve as alternatives or adjuvants to existing antibiotics [14], [15].

B. Anti-weevil activity

Methanol, Ethanol and aqueous extract of *Croton macrostachyeus* and *Glinus Lotoides* plants have shown variation in killing curculionoidea. The highest anti weevils result was recorded for ethanol extract of *Glinus Lotoides* (four death in one minute).The lowest yield was observed for distilled water extract of *Croton macrostachyeus*.(one death in 5 minute).

The result of anti-weevils activity showed that the seed of Metere (*Glinus Lotoides*) are more effective than leaf of Bissana (*Croton macrostachyeus*).

As it indicated below ethanol extract of *glinus lotoieds* (Metere) has highest yield and antiweevils activity than that of *Croton macrostachyeus* extracted. Also when it compared with other extracted yield from *glinus lotoides* (methanol extract of *glinus lotoiedes* and distilled water extract of *glinus lotoides*) methanol is more effective. This implies that Metere has a high anti larval activity and has a promising effect to act as insecticidal for many other pests.

Fig. 2 Ethanol extracted *Croton macrostachyeus*

Fig. 3 Distilled water extracted *Croton macrostachyeus*

Fig. 4 Methanol *extracted* Glinus lotoides test

Fig. 5 Methanol extracted Croton *macrostachyeus* test.

TABLE III
PLANT EXTRACT EFFECT ON ANTI-WEEVIL ACTIVITY

Extracts	<1min	1-2min	2-3min	Above 3min
Methanol *extracted* Glinus lotoides	3 deaths (50 sec)	1 death (2:12min) 1 death (2:40min)		
Methanol *extracted Croton macrostachyeus*		2 deaths (2:05min) 2 deaths (2:52min)	1 death (3:55min)	
Ethanol extracted Glinus lotoides	2 death (55sec) 2 deaths (58sec)	1 death (2min)		
Distilled water extracted *Croton macrostachyeus*				death (5min)

IV. CONCLUSION

The study demonstrates that *Glinus lotoides* and *Croton macrostachyus* possess promising antimicrobial properties against drug-resistant bacteria. These plants could serve as potential sources of new antimicrobial agents. Further research is required to isolate and characterize the active compounds responsible for the observed antibacterial activity. On the basis of the present findings extracts of *Croton macrostachyeus* and *Glinus Lotoides* possess the capability of being a good candidate in the search for natural antimicrobial agents against bacterial diseases or infections which are caused by the pathogenic bacterial strains. As per the investigation of this study, it can be concluded that the traditional medicinal plant has a certain degree of efficacy against the test microorganisms.

ACKNOWLEDGMENT

We would like to thank Causal Productions for permits to use and revise the template provided by Causal Productions. Original version of this template was provided by courtesy of Causal Productions (www.causalproductions.com).

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