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Antibiofilm Activity of Methanolic Extracts of Ajuga Bracteosa Wall.Ex. Benth: Potential Application in Combating Biofilm Associated Infections

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KEYWORDS

Bacterial, infections, bracteosa, Pneumonia, Gram Positive, Rod shape

ABSTRACT:

Bacterial infection establishes a foremost health concern with an ever rising internationally. Due to the persistent description of the illness and restrictions and dangers of typical therapy, the historical pandemic and endemic has perceived the use of phytomedicines as effective medicinal agents. *Ajuga bracteosa* (*A. bracteosa*) Wall ex Benth. Has been extensively consumed in traditional remedies for bacterial infection and antimicrobial biofilm. The aim of investigation was to development and evaluation of biofilm gel formulation of bioactive extract of *A. bracteosa* and extract obtained from the *in situ* and *in vitro* grown plants of it. Antibacterial action of antibiofilm gel and bioactive extract screened on the *Pseudomonas aeruginosa* and *Bacillus subtilis* bacteria by *in vitro* method. *Pseudomonas aeruginosa* is a Gram-negative, aerobic, non-spore forming rod that is efficient of initiating a variation of contaminations in both immune competent and immune compromised hosts. Its predilection to trigger illnesses amongst immune compromised hosts, life-threatening adaptability, antibiotic confrontation, and an extensive vary of vigorous protections builds it a tremendously intriguing organism to handle in contemporary-day medicine. *B. subtilis* is a Gram-positive, rod-shaped, spore-forming, and facultative anaerobe that is the most isolated Bacillus species from environmental samples. Most *B. subtilis* species are not disease causing; it means not accompanying with microbial infections, but some strains have related to carcinoma disorder viz. fatal pneumonia and septicemia, and contaminations of necrotic axillary tumors in breast cancer.

1. Introduction

For centuries, *Ajuga bracteosa* has been utilized as a therapeutic agent for a myriad of conditions. Its known benefits encompass parasiticidal, anti-Alzheimer[1], antibacterial, anticancer, antileishmanial, antidiabetic, diuretic[2], antioxidant, analgesic, and antidepressant properties[1]. Additionally, it exhibits antimalarial activity[3] and is also effective in addressing intestinal disorders, all while showcasing minimal toxic effects. Further research on *Ajuga bracteosa* has revealed its potential in inhibiting enzymes like lipoxygenase, acetyl cholinesterase, and butyl cholinesterase. Historically, *Ajuga* has been sought as a remedy for a plethora of health concerns, including fevers, diarrhea, dysentery, gastrointestinal complications, pain, glycemic disorders, skin conditions, liver disorders, hypertension, gout, palsy, malaria, and insect bites, among others[4]. A comprehensive approach is vital for its conservation, which includes selecting high quality genotypes and employing both *ex situ* and *in situ* preservation techniques. Biofilm are most commonly observed on implant devices. Their presence severely undermines the

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reliability of health-related devices and poses significant concerns for surgical procedures and public health[5]. In view of this, the aim of study is to Develop and evaluate antibiofilm formulation from bioactive extracts of *Ajuga bracteosa* Wall ex Benth.

Figure 1: Different Species of *Ajuga* distributed worldwide

2. Materials and Method

Antibiofilm Activity of *Ajuga bracteosa* Methanolic Extracts *(AbME):* The anti-biofilm activity of plant extracts were selected on the basis of total phenolic content and total flavonoids content. Previously the comparative study was performed between in vitro grown Plant and in situ grown plant and it was observed that the maximum total phenolic content and total flavonoids content was found with the methanolic extracts of whole plant part of *Ajuga bracteosa* so in current study methanolic extracts was selected for antibiofilm

Table 1: Percentage yield of extracts

S. No	Weight of Raw Materials	Weight of extracts	% Recovery
1	500mg	70mg	14

Table 2: Biofilm inhibition Assay against *Pseudomonas aeruginosa*

S. No.	Sample concentration (µgm/ml)	N1	N2	N3	N4	MEAN	±SD	±SEM
1	0.00	93.43	94.32	110.76	101.47	100	8.02	4.01
2	12.5	88.18	84.81	70.70	68.28	77.98	9.94	4.97
3	62.5	89.012	70.34	88.03	84.02	83.02	8.65	4.32
4	125.00	82.42	77.04	78.38	79.09	79.23	2.27	1.13
5	250.00	84.68	75.74	84.77	78.60	80.95	4.511	2.25
6	500.00	83.38	85.97	84.90	77.75	83.05	3.65	1.82

***Data represents mean± SD; *p<0.05, **P<0.01, ***P<0.001 followed by one way ANOVA**

The selected plant extract of *Ajuga bracteosa* was tested for their anti-biofilm activity at different concentration (12.5, 62.5, 125.00, 250.00 and 500.00 µg/ml) against the bio-film of *Pseudomonas aeruginosa* and *Bacillus subtilis*. On the basis of extractive yield, TPC and TFC, methanol extract was used for the evaluation of anti-biofilm potential of the plant. Methanol is a choice of solvent for most of the extraction of antimicrobial substances. Additionally, the polarity of methanol made it possible to extract polar and moderately polar chemicals from plants, such as terpenoids, tannins, flavones, andpolyphenols that are effective against microorganisms.

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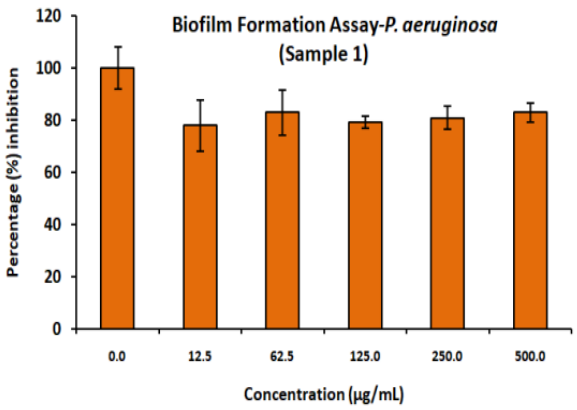

Figure 2: Comparison of percentage inhibition of biofilm of Pseudomonas aeruginosa by various concentrations of the plant extract; (PC) Positive control (ciprofloxacin)

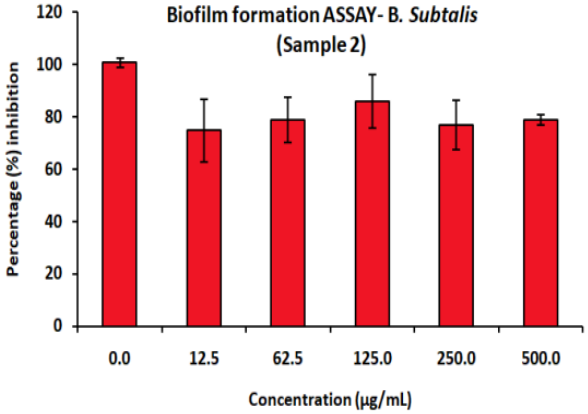

Figure 3: Comparison of percentage inhibition of biofilm of B. Subtalis by various concentrations of the plant extract. PC- Positive control (ciprofloxacin)

Table 1.3: Biofilm inhibition assay against *B.subtalis*

S. No.	Sample concentration (µgm/ml)	N1	N2	N3	N4	MEAN	± SD	±SEM
1	0.00	102.36	99.89	97.63	100.10	100.63	1.93	0.967
2	12.5	93.51	85.48	91.66	67.26	74.79	11.97	5.98
3	62.5	67.98	88.67	73.75	76.22	78.78	8.72	4.31
4	125.00	93.30	88.26	75.19	72.51	85.89	10.05	5.02
5	250.00	88.72	67.00	80.18	72.36	76.78	9.46	4.73
6	500.00	85.89	85.07	81.36	84.04	78.79	1.97	0.98

***Data represents mean± SD; *p<0.05, **P<0.01, ***P<0.001 followed by one way ANOVA**

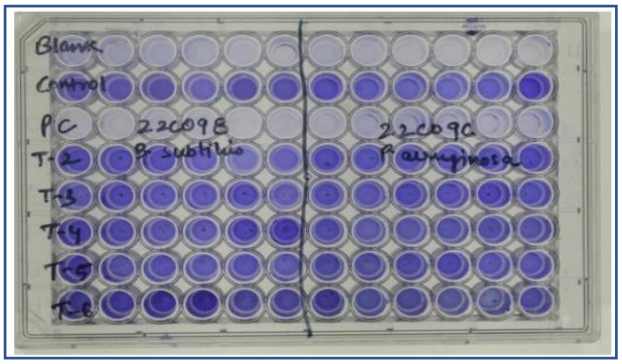

Figure 4: 96 Well Microtitre plates with different concentration of *Pseudomonas Aeruginosa* and *B. Subtalis*

3. Discussion and Conclusion

The biofilm inhibition assay of plant extract (AbME) against Pseudomonas aeruginosa, the extract displayed notable inhibitory effects on biofilm formation. Specifically, at a concentration of 12.5, a significant reduction in biofilm formation was observed. While data remained consistent across different extract concentrations, it's essential to note that the mere presence of inhibitory effects does not guarantee therapeutic potential. Similarly, when examining biofilm inhibition against B. subtilis, the inhibitor showed a significant decrease in biofilm formation, especially at the 12.5 concentration. However, the results varied across concentrations, lacking a distinct dose-dependent trend. The data also revealed some inconsistencies,

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particularly at the 12.5 concentration, indicating that further studies with more replicates may be beneficial.

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