



Phytochemical analysis and Antimicrobial Activity in *Musa assamica* W.Bull and *Musa balbisiana* Colla

Almina Jabin¹, Prapty Das^{2*}, Syeda Afrin Mumtahina Hussain³

^{1&3}Post Graduate Department of Botany, B. Borooah College, Guwahati, Assam

²Department of Botany, B. Borooah College, Guwahati, Assam

*Corresponding author: das.prapty@gmail.com

Abstract

Musa sp. belonging to the family Musaceae (commonly known as Banana) is a widely distributed plant variety across the world having enormous medicinal and nutritional values. Inflorescence of the plant emerges at top of the plant known as Banana blossom and it is an indigenous food consumed mostly in Asian countries. Banana blossom is protected by thick bracts which are high in fiber, carbohydrates, vitamin E, potassium and minerals. Traditionally flowers have been used to treat ulcer, asthma, heart pain, diabetes, bronchitis etc. This study is aimed at analysing the phytochemical constituents present in the flowers and bract extracts with methanol and ethanol as a solvent. The antimicrobial activities were evaluated against pathogens such as *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma* and bacterial strain *E. coli* using standard agar well diffusion method. Results of the study have shown the presence of phytochemicals such as carbohydrates, tannin, alkaloids, saponin, flavonoids, steroids. Among all the bract and flower extracts highest inhibition zone was observed against *Trichoderma* sp. from ethanol extract of *Musa assamica* flower with 29.6±0.47 mm inhibition at 50mg/μl concentration. Phytochemicals present in the extracts may be attributed to its therapeutic potentials and can be a valuable source of plant-based drugs. This study demonstrated the effectiveness of all the extracts against tested organisms with a conclusion that they can be used as eco-friendly antimicrobial agents rather than synthetic chemicals.

Keywords: Antimicrobial activity, Bract, inflorescence, *Musa assamica*, *Musa balbisiana*, phytochemical analysis.

Introduction

Musa sp. (also known as Banana) is a tall perennial plant belonging to Musaceae family. It is one of the world's most commercially important and widely available tropical fruit grown over more than 100 countries. Every part of the plant (viz. leaf, flower, pseudostem, fruit etc.) except the rhizome is consumed as food with medicinal and nutritional values [1]. The inflorescence of the plant otherwise known as banana blossom or banana heart is an indigenous food. Banana bracts contain fiber, potassium, vitamin E, minerals and unsaturated fatty acids. Crude fibre present in bracts possess health benefits such as reduce type-2 diabetes, blood cholesterol level and inflammation [2]. Banana flowers are rich in carbohydrates, protein and minerals. Traditionally the flowers have been used to treat ulcer, asthma, heart pain, diarrhea, diabetes, bronchitis, etc. Phytochemicals found in banana blossom can show beneficial effect on health. Antioxidant activity present in banana flower may reduce risk of chronic diseases in human. Limited reports have been published about phytochemicals and antimicrobial activity of spathe along with flower extract of *Musa* sp. from North-east India.

Due to its value as a source of many medicinal properties, the present study has been aimed with phytochemical screening and antimicrobial activity of bract and flower extracts of two local varieties of Assam *Musa assamica* W. Bull (malbhogkol) and *Musa balbisiana* Colla (athiyakol) against fungal isolates.

Materials and Methods

Collection and preparation of plant sample for extraction

The plant materials *Musa assamica* (malbhogkol) and *Musa balbisiana* (Athiyakol) were purchased from local market of Kamrup (M) district. The bracts and flowers were separated from the inflorescence and washed to make them free from dust. They were air dried for 1-2 weeks and grinded into uniform powder using grinding machine. The powdered samples were kept in clean bottles at room temperature and used for extraction (Fig. 1).

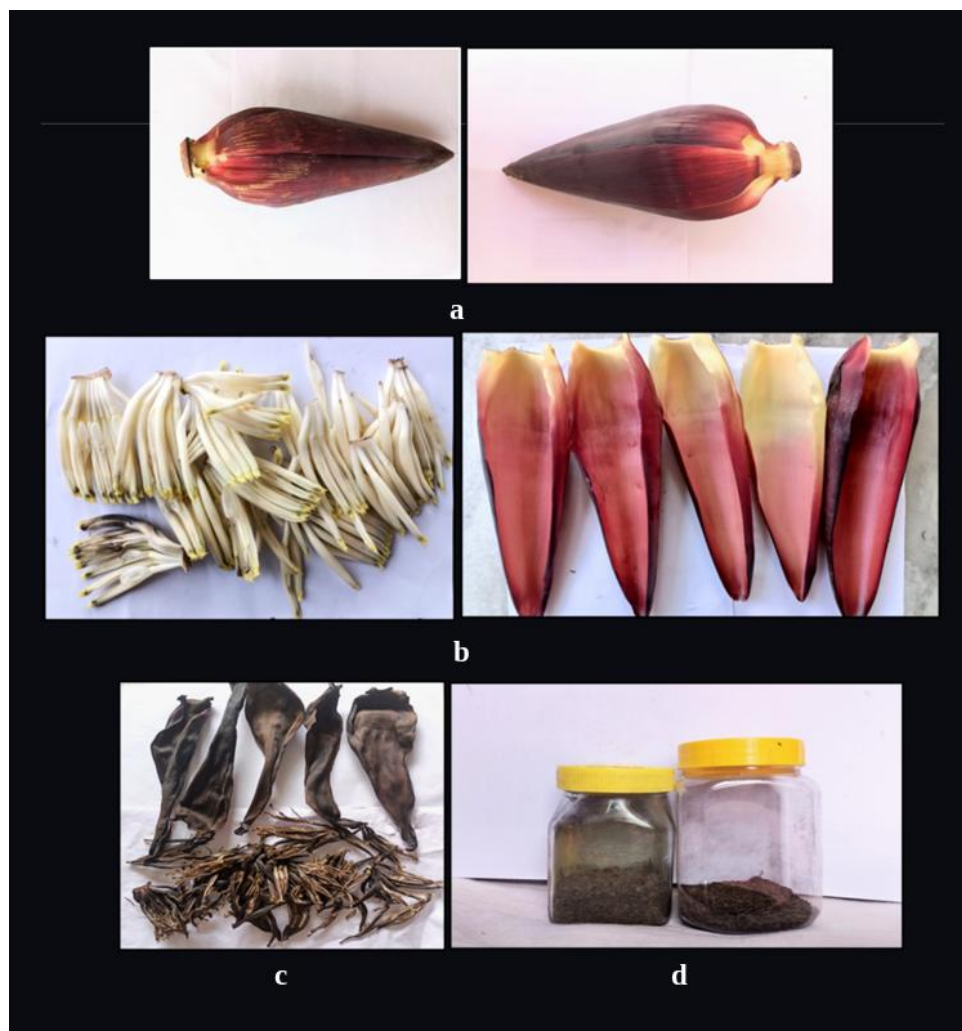


Fig 1. a. Collection of banana blossom of *Musa assamica* (right) and *Musa balbisiana* (left); b. Removal of spathe and flowers; c. Samples are air dried; d. Powder of bracts and flowers.

Preparation of extraction

10gm of each sample i.e, bract and spathe powders of *Musa assamica* W.Bull and *Musa balbisiana* Colla were dissolved in 100 ml of ethanol and methanol separately in small beakers. The mixtures were stirred properly allowed to stand for 48 hrs. Then extracts were filtered through filter papers and supernatants were collected (Fig. 2).

Secondary metabolites screening: The ethanolic and methanolic extracts of *Musa assamica* W. Bull and *Musa balbisiana* Colla were analyzed using standard procedures of [3], [4] for the presence of carbohydrates, alkaloids, saponin, tannin, flavonoids, terpenoids, protein, phenol, quinine and steroids.

Microbial strains: The strains were taken from B. Borooah microbiology laboratory. Strains included *Aspergillus niger*, *Aspergillus flavus*, *Trichoderma* sp. and *E. coli* which were maintained in Potato Dextrose Broth Culture at 4°C for future experiments. The bacterial strain was maintained in Nutrient Agar media for further experimentation.

Preparation of the standard concentration of extracts: Dried bract and spathe extracts were dissolved in 10% DMSO (Dimethyl sulfoxide) to make stock solutions [5]. Stock solutions were diluted to prepare different concentrations of the extracts, the final volume of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml.

Preparation of antimicrobial suspension: Bacterial slants prepared in test-tubes and maintained at 4°C. Active culture was prepared by inoculating Nutrient broth medium with a loopful of bacteria followed by incubation at 37°C for 24 hours. The fungal cultures were grown on Potato Dextrose Agar (PDA) with incubation at 27°C for 48 hrs. and then stored at 4°C. Active cultures were prepared by transferring a loopful of fungi in 50ml PDB media within a conical flask and incubation at 27°C for 48 to 72 hr. [6].

Antifungal activity: To determine the antifungal activity of extract (ethanol and methanol extract), the agar well diffusion technique was used. Fluconazole (100 µl of 10mg/ml) used as positive control. PDA plates were inoculated with 100µl of fungal suspension (10^5 spores /ml), uniformly spreading over agar plates. The extracts of bract and flower concentrations 100µl of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml were placed in the wells punched in inoculated plates. The plates were incubated at 27°C for 24 to 72 hours and the inhibition zones formed surrounding the wells were measured in millimeters (mm).

Antibacterial activity: The technique employed to evaluate antibacterial activity of the ethanol and methanol extracts was Agar Well Diffusion method. Amoxycillin (100 µl of 10mg/ml) used as the positive control. With 100µl of bacterial suspension, Nutrient plates were inoculated by evenly spreading on agar plates. The extracts of bract and flower concentrations 100µl of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml were placed in wells made in inoculated plates. Plates were subsequently incubation at 37°C for 24 hr. and inhibition zones formed surrounding the wells measured and recorded in millimeters (mm) [7].

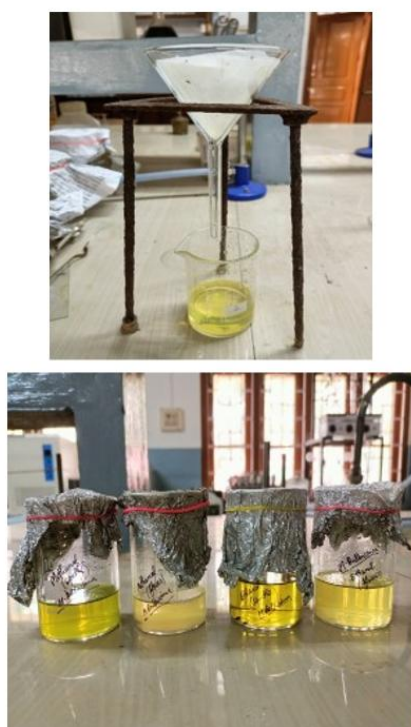


Fig 2. Preparation of Ethanol and Methanol extract of spathe and flowers.

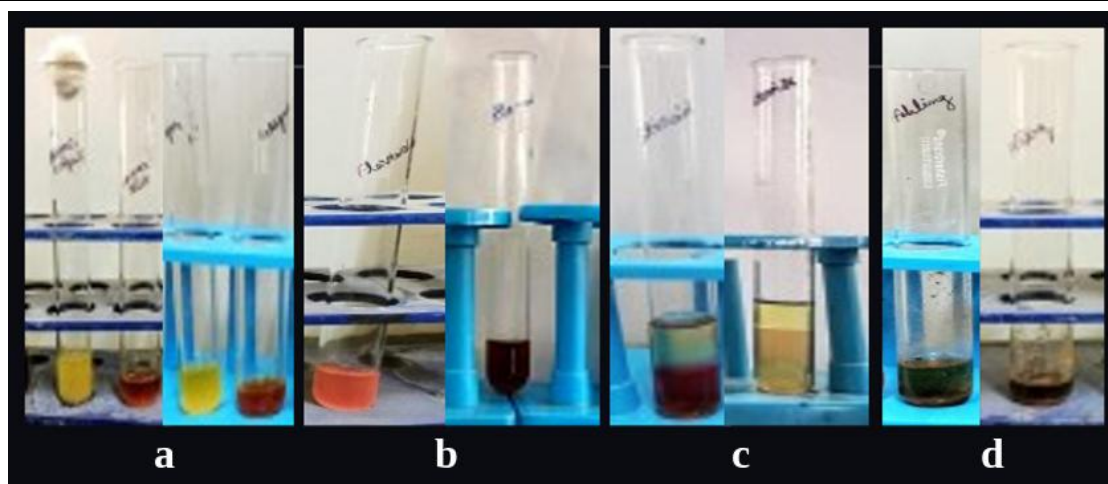
Results and Discussion

Secondary metabolites screening of Musa assamica W.Bull spathe and flower extract:

The present study on the spathe and flower extract of *Musa assamica* revealed presence of carbohydrates, alkaloids, saponin, tannin, flavanoids, and steroids in ethanol and methanol extracts which corroborates with the study of Begashaw *et al.*, 2023 [8]. But terpenoids, phenol and quinone were absent in both the extracts (Table 1).

Table 1. Qualitative analysis of secondary metabolites in spathe and flower extract of *Musa assamica* W. Bull

Phytochemical Test	Spathe extract		Flower extract	
	Ethanol	Methanol	Ethanol	Methanol
Test for Carbohydrates				
i) Benedict's Test	+	+	+	+
ii) Fehling Test	+	+	+	+
Test for alkaloid				
i) Mayer's Test	+	+	+	+
ii) Wagner's Test	+	+	+	+
Test for Saponin	+	+	+	+
Test for tannin	+	+	+	+
Test for flavonoids	+	+	+	+
Test for terpenoids	—	—	—	—
Test for phenol	—	—	—	—
Test for quinone	—	—	—	—
Test for steroids	+	+	+	+

**Fig. 3.** Analysis of phytochemical test for ethanol and methanol spathe extract (*Musa assamica*). a) Alkaloid (Mayer's test, Wager's test) b) Flavonoids, c) Steroids, d) Flavonoids**Secondary metabolites screening of *Musa balbisiana* spathe and flower extract:**

In the present study on the *Musa balbisiana* spathe extracts shows presence of carbohydrates, alkaloids, saponin, tannin, flavanoids, and steroids in both ethanol and methanol extract. But terpenoids, phenol, quinone absent in both extracts.

In *Musa balbisiana* flower carbohydrates, alkaloids, tannin, flavanoids and steroid was present in both ethanol and methanol extract. But saponin was absent in ethanol extract and present in methanol extract. Terpenoids, phenol, quinine was absent in both extracts as shown in Table 2.

Table 2. Qualitative analysis of secondary metabolites in spathe and flower extract of *Musa balbisiana*

Phytochemical Test	Spathe extract		Flower extract	
	Ethanol	Methanol	Ethanol	Methanol
Test for Carbohydrates				
i) Benedict's Test	+	+	+	+
ii) Fehling Test	+	+	-	+
Test for alkaloid				
i) Mayer's Test	+	+	+	+
ii) Wagner's Test	+	+	+	+
Test for Saponin	+	+	+	+
Test for tannin	+	+	+	+
Test for flavonoids	+	+	+	+

Phytochemical Test	Spathe extract		Flower extract	
	Ethanol	Methanol	Ethanol	Methanol
Test for terpenoids	—	—	—	—
Test for phenol	—	—	—	—
Test for quinone	—	—	—	—
Test for steroids	+	+	+	+

Antimicrobial activity of *M. assamica* and *M. balbasiana* spathe extract

In the study, Antifungal activity of *M. assamica* and *M. balbasiana* spathe extract were tested against the fungal strains *Aspergillus niger*, *Aspergillus flavus* and *Trichoderma* sp.. Agar Well Diffusion method was used to assess antimicrobial activity against *E. coli*.

Among the tested samples, the highest zone of inhibition was found against *Trichoderma* sp. from methanol extract of *Musa assamica* with 28.6 ± 0.386 mm inhibition at a concentration of 50mg/μl as shown in (Table 3). For comparison positive control (fluconazole) the inhibition zones was measured 29.5 ± 0.33 mm against *Trichoderma*. With rise in extract concentrations, there was also an rise in antimicrobial efficacy against the tested varieties of microorganism. Similar type of findings was attained by Nhon *et al.*, 2025 and Sirajudin *et al.*, 2014. [9], [10].

Table 3. Zone of Inhibition Test (mm) of Methanol and Ethanol extracts of *M. assamica* and *M. balbasiana* spathe

Extract	Concentration (mg/ml)	Zone of Inhibition (mm)		
		Fungal strains		
		<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Trichoderma</i> sp.
Methanol extract (<i>Musa assamica</i>)	6.25	15.4±0.434	18.4±0.411	20±0.25
	12.5	15.6±0.35	22.9±0.258	23.7±0.275
	25	17.3±0.25	23.5±0.427	27.6±0.336
	50	17.5±0.42	24.4±0.465	28.6±0.386
Fluconazole	10	17.8±0.268	24.6±0.948	28.5±0.377
Ethanol extract (<i>Musa assamica</i>)	6.25	14.5±0.416	21.5±0.341	20.2±0.386
	12.5	15.4±0.411	23.6±0.336	22.9±0.619
	25	18.6±0.365	24.4±0.443	24.1±0.478
	50	19.6±0.263	26.4±0.434	25.8±0.341
Fluconazole	10	18.5±0.5	29.5±0.5	29.5±0.33
Methanol extract (<i>Musa balbisiana</i>)	6.25	14.4±0.365	21.5±0.34	17.4±0.411
	12.5	15.4±0.341	22.6±0.336	21.8±0.443
	25	16.3±0.258	25.5±0.443	24.7±0.457
	50	17.3±0.275	28.5±0.33	28.5±0.341
Fluconazole	10	16.7±0.545	27.5±0.411	28.8±0.268
Ethanol extract (<i>Musa balbisiana</i>)	6.25	13.7±0.221	16.2±0.365	22.7±0.822
	12.5	14.5±0.416	20.1±0.416	23.3±0.35
	25	15.7±0.25	22.1±0.341	25.5±0.233
	50	16.7±0.316	23.5±0.35	26.4±0.427
Fluconazole	10	16.5±0.341	23.1±0.298	28.7±0.386

Table 4. Zone of Inhibition Test (mm) of Methanol and Ethanol extract of *M. assamica* and *M. balbasiana* spathe

Extract	Concentration (mg/ml)	Zone of inhibition (mm)
		Bacterial strain
		<i>E. coli</i>
Methanol extract (<i>Musa assamica</i>)	6.25	20.5±0.416
	12.5	21.9±0.258
	25	22.6±0.263

Extract	Concentration (mg/ml)	Zone of inhibition (mm)
		Bacterial strain
		<i>E. coli</i>
Antibiotic	50	23.6±0.341
	10	24.1±0.33
Ethanol extract (<i>Musa assamica</i>)	6.25	22.4±0.411
	12.5	23.5±0.42
	25	24.7±0.258
	50	25.5±0.377
Antibiotic	10	24.7±0.275
Methanol extract (<i>Musa balbasiana</i>)	6.25	22.7±0.17
	12.5	23.5±0.341
	25	24.8±0.17
	50	25.7±0.258
Antibiotic	10	25.60.298
Ethanol extract (<i>Musa balbasiana</i>)	6.25	18.7±0.221
	12.5	19.6±0.294
	25	21.6±0.25
	50	25.8±0.17
Antibiotic	10	23.9±0.464

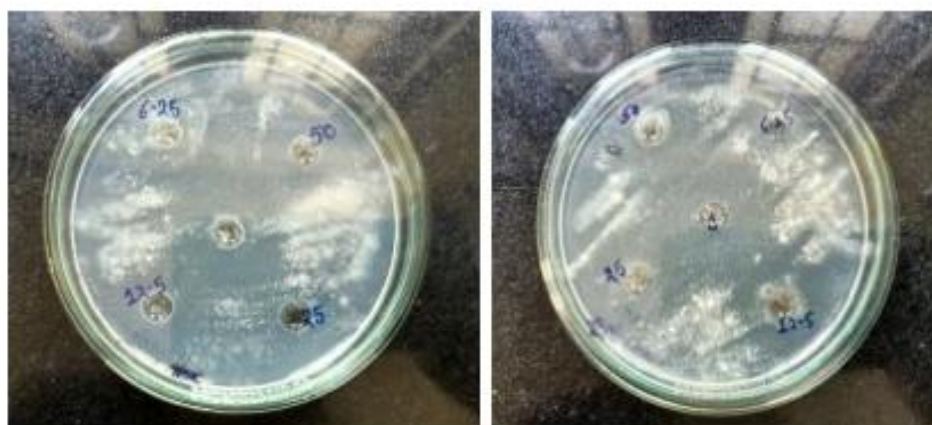


Fig 4. Zone of Inhibition for methanol and ethanol extract of *Musa assamica* spathe against *Aspergillus flavus*.

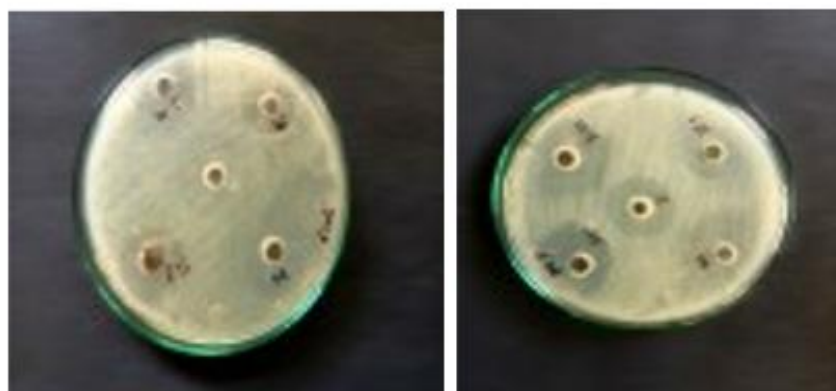


Fig 5. Zone of inhibition for methanol and ethanol extract of *Musa assamica* spathe against *E. coli*.

Antimicrobial activity of *Musa assamica* and *Musa balbasiana* flower extract

Among all the extract, zone of inhibition was observed the maximum against *Trichoderma* sp. from ethanol extract *Musa assamica* with **29.6±0.47 mm** inhibition at concentration of 50mg/μl (Table 5). In the positive control (fluconazole), the inhibition zone was found to be 29.8±0.80 mm against *Trichoderma* sp.

Table 5. Zone of Inhibition Test (mm) of Methanol and Ethanol extract of *M. assamica* and *M. balbasiana* flower.

Extract	Concentration (mg/ml)	Zone of Inhibition (mm)		
		Fungal strains		
		<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Trichoderma</i>
Methanol extract (<i>Musa assamica</i>)	6.25	12.5±0.416	20.9±0.861	23.7±0.208
	12.5	13.6±0.275	23.3±0.258	24.6 ±0.275
	25	15.5±0.341	24.4±0.668	26.7±0.531
	50	16.6±0.34	26.5±0.341	27.7±0.476
Fluconazole	10	16.2±0.66	25.8±0.244	29.8±0.80
Ethanol extract (<i>Musa assamica</i>)	6.25	14.5±0.416	20.1± 0.264	20.1±0.35
	12.5	17.6±0.25	22.9± 0.454	26.6±0.336
	25	18.5±0.41	23.9± 0.416	28.9±0.33
	50	21.7±0.258	26.8± 0.365	29.6±0.47
Fluconazole	10	21.5±0.33	25.7±0.607	25.9±0.258
Methanol extract (<i>Musa balbasiana</i>)	6.25	12.7±0.258	21.9±0.25	15.2±0.531
	12.5	13.7±0.221	23.1±0.416	18.9±0.341
	25	14.4±0.432	23.9±0.25	20±0.386
	50	17.7±0.22	25.9±0.5	23.8±0.5
Fluconazole	10	12.8±0.258	25.9±0.42	21.9±0.33
Ethanol extract (<i>Musa balbasiana</i>)	6.25	12.6±0.34	20.5±0.718	18.8±0.369
	12.5	13.5±0.3	21.9±0.464	20.5±0.5
	25	14.7±0.29	25.4±0.434	22.2±0.424
	50	15.5±0.416	27.6±.262	23.9±0.404
Fluconazole	10	14.5±0.33	28.4±0.42	19.8±0.298

Table 6. Zone of Inhibition Test (mm) of Methanol and Ethanol extract of *M. assamica* and *M. balbasiana* flower.

Extract	Concentration (mg/ml)	Zone of inhibition (mm)
		Bacterial strain
		<i>E. coli</i>
Methanol extract (<i>Musa assamica</i>)	6.25	11.7±0.208
	12.5	11.8±0.478
	25	13.6±0.330
	50	15.5±0.419
Antibiotic	10	17.7±0.585
Ethanol extract (<i>Musa assamica</i>)	6.25	14.4±0.432
	12.5	15.4±0.434
	25	17.5±0.330
	50	18.6±0.294
Antibiotic	10	20.7±0.369
Methanol extract (<i>Musa balbasiana</i>)	6.25	20.2±0.14
	12.5	22.5±0.33
	25	25.4±0.434
	50	28.6±0.298
Antibiotic	10	27±0.613
Ethanol extract	6.25	19.8±0.365

Extract	Concentration (mg/ml)	Zone of inhibition (mm)
		Bacterial strain
		<i>E. coli</i>
<i>(Musa balbasiana)</i>	12.5	22.9±0.258
	25	24.6±0.330
	50	25.6±0.341
Antibiotic	10	24.7±0.476



Fig 6. Zone of inhibition for methanol and ethanol extract of *Musa balbisiana* spathe against *Aspergillus niger*.



Fig 7. Zone of inhibition for methanol and ethanol extract of *Musa balbisiana* spathe against *Aspergillus flavus*.

Various phytochemicals found in both the spathe and flower extracts of *Musa assamica* and *Musa balbasiana* may contribute to its therapeutic potentials. They have shown antimicrobial activity against many pathogens hence it can offer as a potential eco friendly alternative to antibacterial and antifungal agents rather than synthetic chemicals. Banana bracts usually discarded during banana harvesting can be repurposed, reducing agricultural waste and thus promoting sustainability.

Conclusion

In this present study, phytochemical analysis and the antimicrobial activity of two parts of banana blossom (spathe and flower) showed significant antibacterial and antifungal activity along with presence of some phytochemicals. These phytochemicals can be produced in large scale which are crucial raw ingredient for food and pharmaceutical industries. Further studies on the phytochemical compounds and other medicinal benefits of banana blossom can be done to provide new approaches that can help to improve various health problems.

From this present investigation as well as earlier reports on spathe and flower of *Musa* sp. it can be said that the presence of antimicrobial activity of them opens up new avenues of researches to be done on clinical

isolates with different *Musa* sp. and its extracts. The extracts of spathe and flower of *Musa* sp. can serve as a eco-friendly, low-cost source of natural antimicrobial agent.

Declarations

The authors have no conflicts of interest.

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