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Research Journal of Agricultural Sciences  
An International Journal

P- ISSN: 0976-1675

E- ISSN: 2249-4538

Volume: 13

Issue: 05

*Res. Jr. of Agril. Sci.* (2022) 13: 1492–1497



# Phytochemical Screening of Different Extracts of *Musa acuminata* Flower: Its Antimicrobial Activity and Potentiality of Synthesizing Silver Nanoparticles

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Received: 15 Jul 2022 | Revised accepted: 04 Sep 2022 | Published online: 26 Sep 2022  
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## ABSTRACT

Traditionally, banana has been a staple food in India, especially in the southern and eastern parts of India. Furthermore, it is for its diverse usage in food, medicine, religious rituals, and festivals. In this study, we focused on investigating the medicinal potentiality of the *Musa acuminata* flower (MAF). First, the preliminary screening of MAF phytochemical constituents using the different extracts such as ethanol, acetone, butanol, and ethyl acetate. Gas chromatography-Mass spectroscopy (GC-MS) was used to screen the bioactive compounds of MAF. Secondary, investigating the ability of MAF extracts in antibacterial activity and in the synthesis of silver nanoparticles (SNPs). From the results obtained, it shows ethanol and acetone gave the best results in extracting the MAF phytochemicals and showed that it has the capability of reducing silver nitrate to silver nanoparticles. While comparing the MAF ethanol and acetone extract with MAF-SNPs ethanol and acetone extract gave promising antibacterial activity results. This portrays the good potential of developing a medicinal drug for various applications.

**Key words:** *Musa acuminata* flower, Gas chromatography-Mass spectroscopy, Silver nanoparticles, Banana, Antibacterial

Infectious diseases are rising and the complication related to it is skyrocketing. Exclusively, bacterial infections are common in the worldwide scenario and the prevalence of the same is a global concern [1]. However, there are a plethora of antibacterial medications available in the market but the side effects are huge leading to different health-related issues and even mortality [2-3].

Consequently, herbal medicines come to the rescue, and the phytochemical constituents and antioxidants present in the plant possess various medicinal properties namely antimicrobial, antipyretic, antiviral, anticancer, antifungal, and in the treatment of various ailments. Perhaps, numerous researches are going on in the field of bioactive compounds [4-6].

*Musa acuminata* is one of the cash crops grown by many farmers in India due to its towering market value. It is a perennial herbaceous plant with a hard fibrous pseudo stem composed of the overlapping compound leaf and grows up to 6-7 feet. Inflorescence emerges at top of the stem; bracts of Inflorescence are red to dark purple with male and female flowers. Fruits originate from flowers that are green in colour and slowly change the colour to yellow when it fully ripens [1], [7]. The whole of the banana plant is edible not even a single part is wasted, and moreover is renowned for its nutritional and medicinal values. Overall, banana is rich in potassium, calcium, iron, vitamins A, B, C, D and other bioactive components such as norepinephrine, dopamine, and serotonin. According to the parts of the *Musa acuminata* the medicinal properties differs; for instance, the stem core is used in the treatment of stomach upset, diabetes, reducing weight and dissolving stones in the kidney and urinary tract, the flower is used to treat ulcers, dysentery, bronchitis and cooked flower reduces diabetes, the sap is used to treat leprosy and insect bite, the peel is good

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source of fibre and the fruit is used in the treatment of reducing high blood pressure, heartburn, prevention of stroke and constipation. Antibacterial and antifungal properties are found in peel, fruit and flowers [8-9].

Each day, nanotechnology is expanding its application in different fields such as electrical, optical, bio sensing, imaging, drug delivery, Nano-device fabrication and medicine. The size of particles is a unique feature nanoparticle thus opening the most interesting area of research [10-13]. Therefore, in our study, we have selected silver nanoparticles due to their antibacterial, antifungal and antiviral ability as such SNPs are used in various fields. Whereas, we here have used *Musa acuminata* flower for the synthesis of silver nanoparticles. Thus, reducing the toxic level of silver nanoparticles synthesized from physical and chemical methods.

## MATERIALS AND METHODS

### Collection of samples and materials used

Banana flower (*Musa acuminata*) was harvested from the farm and rinsed with water to remove any dust particles. Silver nitrate was purchased from Hi Media, Mumbai and all other chemicals from Sigma-Aldrich, Mumbai.

### Preparation of solvent extract

The fresh flower (MAF) was harvested from the farm and rinsed with water to remove atmospheric dust particles. The flower was air dried under shade for 3 weeks days and reduced to coarse powder using grinded to a fine powder. The powder was stored in an airtight bottle until needed for use. Sample (10 gm) of the shade-dried powder of flower was extracted in a Soxhlet extractor successively with 200 ml ethanol, acetone, ethyl acetate and butanol respectively until colourless extract was obtained on the top of the extractor. Each of the extracts was concentrated separately under reduced pressure; each of these extracts was weighed and subjected to further tests. Extracts were maintained at a temperature between 2 - 8°C for further studies [14].

### Qualitative phytochemicals study

The MAF samples were tested for phytochemical constituents such as alkaloids, saponins, flavonoids, phenols, tannins, terpenoids, quinones, steroids, carbohydrates, proteins and the presence and absence are determined by the colour change of the solution as mentioned [15-17].

### Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of MAF acetone extract was performed using GCMS-TQ8030 SHIMADZU. The obtained constituents were identified by using the mass spectral library of NIST 0.8L.

### Determination of antibacterial activity

The antibacterial activity is carried out by agar well diffusion method. Test pathogens such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *Salmonella* sp. and *P. mirabilis* ( $10^8$  CFU/ml) were spread on MHA medium. Then the 5mm wells were punctured with borer on the agar plates and 100µl of different concentrations of MAF extract were added to respective wells along with control samples ampicillin (5µg/ml) and respective solvent (ethanol, acetone, ethyl acetate and butanol) in another well. Incubate the plate at 37°C for 24hrs, and then observe for the zone of inhibition [18].

### Synthesis of silver nanoparticles

Among the various solvent extracts, acetone extract showed better activity against all bacterial isolates. Therefore, MAF acetone extract subjected to nanoparticle synthesis. The 5 mL of silver nitrate ( $\text{AgNO}_3$ ) 1 mM was prepared and in that 0.5ml of extract was added, note for colour change of the solution from ruby red to brown, due to the reaction of silver ions and formation of SNPs [19]. Then synthesized nanoparticles were subjected to antimicrobial activity with agar well diffusion method and silver nitrate was used as a control agent.

### Minimum inhibitory concentration assays

This minimum inhibitory concentration analysis was done with MAF-SNPs following the procedure. Overnight nutrient broth culture was prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of the McFarland 0.5 standard. The inoculums thus prepared to expect to obtain  $10^6$  CFU/ml [20].

## RESULTS AND DISCUSSION

For the past few decades, researchers have given a lot of importance in the investigation of phytochemical compounds, which has been extracted, from different biological sources such as plants (bark, leaf, latex, flower, bract, stem), mushroom, fungus, algae etcetera due to its cost, environmental friendly nature, availability and affordability. Therefore, medicinal plants play a momentous role in the treatment of various ailments in rural areas. In India, Ayurveda has an immense role in the maintenance of primary health and indigenous plants are widely consumed by locals for medicinal values. In that, the banana plant is one among it and has greater value among the people not only because of the edibility but also due to medicinal usage and festival rituals.

### Qualitative phytochemicals and GC-MS analysis

The qualitative phytochemical screening showed most of the respective compounds are present in acetone extract of MAF. Acetone is the effective solvent compared to other solvents (ethanol, ethyl acetate, and butanol) used in this study. Alkaloids, Carbohydrates, Flavonoids, Phenols, Saponins, Terpenoids, and Proteins are present in acetone extract, Alkaloids, Flavonoids, Terpenoids, Tannins, and Quinines are present in ethanol extract, Carbohydrates are present in butanol extract and Alkaloids, and Terpenoids are present in ethyl acetate (Table 2).

The study result correlates with the result of the *Musa acuminata* flower [21] and revealed the existence of glycosides, tannins, saponins, phenols, steroids, and flavonoids in the *M. acuminata* flower methanolic extract. A similar type of result obtained using *M. acuminata* peel and shows the presence of alkaloids, terpenoids, saponins, sugar and proteins [22].

Table 1 Phytochemical analysis of *Musa acuminata* flower

Phytochemical test	Acetone extract	Ethanol extract	Butanal extract	Ethyl acetate
Alkaloids	+	+	-	-
Carbohydrates	+	-	+	+
Flavonoids	+	+	-	-
Phenols	+	-	-	-
Sterols	-	-	-	-
Saponins	+	-	-	-
Terpenoids	+	+	-	+
Tannins	-	+	-	-
Quinines	-	+	-	-
Proteins	+	-	-	-

GC-MS analysis of MAF acetone extract was done to explore the bioactive fatty acids and volatile compounds present in them. A total number of 32 compounds were identified using the GC-MS NIST data library (Table 1) and the peaks obtained are displayed in (Fig 1). All the compounds identified has unique feature and usage, n-Hexadecanoic acid found to be the major compound and can be used as Lubricant, antiandrogenic, flavor, hypocholesterolemic, hemolytic, antioxidant, nematocide, pesticide, anti-inflammatory, antifungal and antibacterial [23-25]. 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester common name is phthalic acid and it is used as a plasticizer, thus utilized in different fields namely flooring, wire & cables, shoes, cars, pipe, tubes, imitation leather, rainwear, footwear, upholstery, flooring, wire and cable, tablecloths, shower curtains, food packaging materials and children's toys [26]. Perdeuterobenzene is a deuterated form of tetraabenazine and its nontoxic hydrogen used to lower the liver metabolism, treating chorea in Huntington's disease [27]. Pentadecanoic acid is an exogenous fatty acid with anti-inflammatory, antifibrotic, red blood cell-stabilizing, and lowering cholesterol, triglycerides and glucose, and treatment of liver fibrosis [28]. 5-Methoxypyrrolidin-2-one is (pyrrolidine-2-ones) used as an antiepileptic drug [29]. Octadecanoic acid is present *Musa acuminata* colla [30]. Tricosane is a type of alkaline and is present in several plants, which are known for their antimicrobial properties [31-32]. Nonadecane is also used as a fragrance in cosmetic industries. Eicosane used in cosmetics, lubricants, plasticizers and the petrochemical industry [33]. Octacosyl acetate used as a carrier in pharmaceutical and food preparations [34]. 1- Docosanol used in the treatment of cold sores, fever blisters, and herpes simplex virus [35].

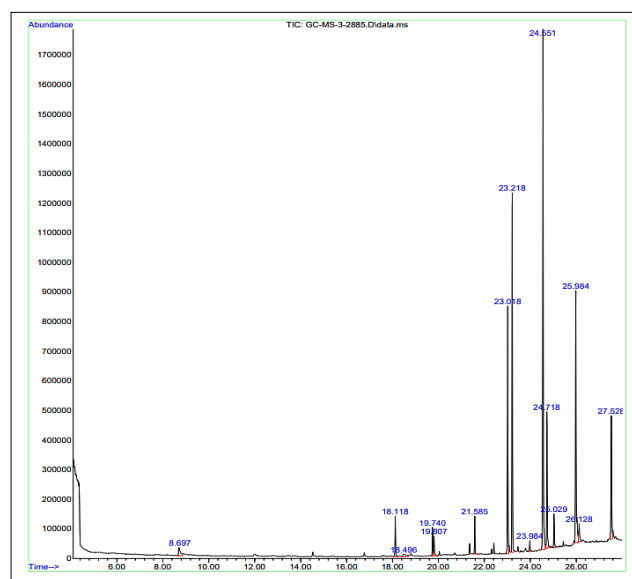


Fig 1 GC-MS peaks obtained for MAF acetone extract

#### Antibacterial activity

The agar well diffusion method was used to evaluate antibacterial activity of MAF ethanol and acetone extract against various clinical isolates (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *Salmonella sp* and *P. mirabilis*) and the results are shown in (Table 3-4). The results showed that ampicillin and diluted solvents gave no prominent zone of inhibition against the pathogens whereas MAF acetone and ethanol extract gave the most prominent zone of inhibition. Subsequently comparing MAF acetone and MAF ethanol extracts the best and overall coverage of antibacterial activity, shown in the MAF acetone extract, minimum concentration of

3mg is the best among other concentrations. The bioactive compounds present in the sample are the main source fighting against such bacterial infections [36]. Similar studies were carried out with peel, bract and inflorescence, all showed good pharmacological activity in both gram-positive and gram-negative bacteria. In that, methanol extract gave promising results [37].

Table 2 Compounds identified in the MAF acetone extract by GC-MS

S. No.	Compounds obtained during GC-MS analysis
1	Perdeuterobenzene
2	5-Methoxypyrrolidin-2-one
3	Pentadecanoic acid, 14-methyl-, methyl ester
4	Hexadecanoic acid, methyl ester
5	n-Hexadecanoic acid
6	Octadecanoic acid
7	Pentadecanoic acid
8	Methyl 10-trans,12-cis-octadecadienoate
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
10	9,12-Octadecadienoic acid, methyl ester, (E,E)-
11	9-Octadecenoic acid (Z)-, methyl ester
12	trans-13-Octadecenoic acid, methyl ester
13	Tricosane
14	Nonadecane
15	9-Tricosene, (Z)-
16	Trifluoroacetoxy hexadecane
17	Pentadecane, 8-hexyl
18	Eicosane, 10-methy
19	Triacontane
20	Eicosane
21	Octadecane, 5,14-dibutyl
22	1-Heneicosanol
23	Behenic alcohol
24	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester
25	6-[4-Bromophenyl]-N,N-dimethyl-1,2,4,5-tetrazin-3-amine
26	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2,7-bis-[2-(diethylamino)-ethoxy]fluorine
27	1-Octacosanol
28	Octacosyl acetate
29	1-Heneicosyl formate
30	Eicosane, 9-octyl
31	Heptadecane, 2,6,10,15-tetramethyl
32	1-Docosanol, methyl ether

#### Synthesis of silver nanoparticles

Silver nanoparticles were synthesized using different concentrations of MAF acetone extract. Preliminary confirmation was done with the change of colour from ruby red to dark brown colour after 24 hrs of incubation. Thus, confirming the production of silver nanoparticles. There are various reports stating the synthesis of silver nanoparticles by the change of the solution colour to brown [22] in banana peel extract, Cavendish banana peel extract [38] and *M. acuminata* colla [39].

Antibacterial activity was performed against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *Salmonella sp* and *P. mirabilis* on muellerhinton agar plates treated with different concentrations of MAF-SNPs (Table 5-7). The most prominent zone was observed in the higher concentration of the sample 4mg and the Half-maximal inhibitory concentration to be active against pathogens is 2mg MAF-SNPs. The control samples (Ampicillin and silver nanoparticles) gave no prominent zone of inhibition when compared with other concentrations of MAF-SNPs. Similarly, MAF-SNPs gave the

most promising antibacterial activity when compared with MAF acetone extract and MAF ethanol extract. Thus, showing

the synergistic effect of MAF and SNPs gives the best antimicrobial agents.

Table 3 Antibacterial activity of MAF acetone extract against clinical isolate

Isolates	Acetone				Ampicillin	Diluted acetone
	1mg	2 mg	3 mg	4 mg		
<i>E. coli</i>	-	12 ± 1.63	15 ± 0.81	18 ± 1.63	-	-
<i>K. pneumoniae</i>	-	11.6 ± 2.05	15.33 ± 2.05	17.66 ± 2.05	-	-
<i>P. aeruginosa</i>	10.33 ± 1.20	12.66 ± 1.24	13.66 ± 1.24	17 ± 1.632	-	-
<i>S. aureus</i>	-	12.66 ± 2.05	17.33 ± 2.05	20 ± 0.25	-	-
<i>E. faecalis</i>	-	-	12.33 ± 1.69	17.33 ± 2.05	-	-
<i>Salmonella sp</i>	-	-	11 ± 1.63	13 ± 1.63	-	-
<i>P. mirabilis</i>	-	12.66 ± 2.05	15.33 ± 1.24	18.33 ± 1.24	-	-

Table 4 Antibacterial activity of MAF ethanol extract against clinical isolate

Isolates	Ethanol				Ampicillin	Diluted acetone
	1mg	2 mg	3 mg	4 mg		
<i>E. coli</i>			12 ± 1.63	14.3 ± 1.24	-	-
<i>K. pneumoniae</i>				13.6 ± 1.24	-	-
<i>P. aeruginosa</i>			12 ± 1.63	14.3 ± 1.24	-	-
<i>S. aureus</i>			11 ± 1.63	15 ± 1.63	-	-
<i>E. faecalis</i>	10 ± 0.81	12 ± 1.63	16 ± 1.63	19 ± 1.24	-	-
<i>Salmonella sp</i>	-	-	-	12 ± 1.63	-	-
<i>P. mirabilis</i>	-	12 ± 1.63	14.3 ± 1.24	18 ± 1.63	-	-

The study results compared with similar other works such as the green synthesis of silver nanoparticles from banana peel showed a larger zone of inhibition against gram-negative bacteria compared with gram-positive bacteria [40]. Naturally synthesized silver nanoparticles showed excellent antimicrobial activity against multidrug clinical isolates [38].

The biosynthesized silver nanoparticles of MAF exhibited higher inhibitory concentration against both gram-positive and gram-negative bacteria at 0.5mg/mL. As shown in (Table 5a-b), minimum inhibitory concentration ranges against pathogens (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, *Salmonella sp* and *P. mirabilis*) were ranged from 0.062-1mg/ml and minimum inhibitory concentration point

for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, and *P. mirabilis* -0.5mg/ml and *Salmonella sp* -2.5mg/ml. Control sample without test sample showed growth in all wells whereas ciprofloxacin used as positive control showed no growth of bacteria in all wells.

The above results, it showed that *Salmonella spp.* was less susceptible to silver nanoparticles due to lacking power of to penetrate through the bacteria lipopolysaccharide [41]. Therefore, resazurin salt used in the study converts to resorufin within the viable cells by oxidoreductases enzyme and which indicated by the colour change from blue to pink. This dye has been widely used for decades to determine the bacterial contamination of milk and cheese [42].

Table 5 Antibacterial activity of MAF-SNPs against clinical isolates

Isolates	Acetone				Ampicillin	Diluted acetone
	11 ± 1.63	13.3 ± 1.69	16.6 ± 1.24	20 ± 0.81		
<i>E. coli</i>	12.3 ± 1.24	13.6 ± 1.24	16.6 ± 1.24	20.6 ± 1.24	-	-
<i>K. pneumoniae</i>	14 ± 0.81	16.3 ± 1.24	17.8 ± 1.43	21.3 ± 1.24	-	-
<i>P. aeruginosa</i>	13.3 ± 1.24	15 ± 0.81	18 ± 0.81	21 ± 0.81	-	-
<i>S. aureus</i>	10 ± 0.81	14 ± 0.81	17.3 ± 1.24	18.6 ± 1.24	-	-
<i>E. faecalis</i>	-	-	12.3 ± 1.24	14.6 ± 1.24	-	-
<i>Salmonella sp</i>	12 ± 0.81	14.6 ± 1.24	18.3 ± 1.24	20.3 ± 1.24	-	-
<i>P. mirabilis</i>	11 ± 1.63	13.3 ± 1.69	16.6 ± 1.24	20 ± 0.81	-	-

Table 6 MIC of MAF-SNPs

Isolates	Acetone extracts of SNP (mg)						C1	C2
	1	0.75	0.5	0.25	0.125	0.062		
<i>E. coli</i>	-	-	-	+	+	+	-	+
<i>K. Pneumoniae</i>	-	-	-	-	+	+	-	+
<i>P. aeruginosa</i>	-	-	-	-	-	+	-	+
<i>S. aureus</i>	-	-	-	-	+	+	-	+
<i>E. faecalis</i>	-	-	-	+	+	+	-	+
<i>P. mirabilis</i>	-	-	-	+	+	+	-	+

Table 7 MIC of MAF-SNPs

Isolates	Acetone						C1	C2
	3	2.75	2.5	2.25	2	1.75		
<i>Salmonella sps</i>	-	-	-	+	+	+	-	+



## CONCLUSION

In the present study, we focused on identifying the phytochemical constituents by the standard protocol and analytical method such as GC-MS and also ascertaining the

potentiality of MAF extracts in antimicrobial activity and synthesizing silver nanoparticles. Overall, MAF acetone extract was best in extracting the bioactive and volatile compounds with antibacterial activities. Hence, acetone extract used for synthesizing SNPs, and further synthesized MAF-SNPs show promising antimicrobial agents.

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