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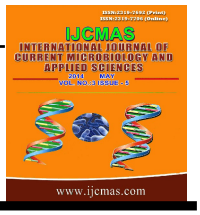

Original Research Article

Evaluation of total phenolic content and antimicrobial activities exhibited by the leaf extracts of *Musa acuminata* (banana)

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K e y w o r d s

Musa acuminata; pharmacological activities; phenolic compound; banana leaf; Folin-Ciocalteu method;

A B S T R A C T

Musa acuminata, commonly known as banana plant is vastly being consumed across the world. It is known for many pharmacological activities and reports show that phenolic compounds mainly contribute to this trait. In the present investigation, quantitative analysis of phenolic compound was carried out on ethanol (96%), acetone and petroleum ether extracts of fresh banana leaf by modified Folin-Ciocalteu method. Total phenolic recovery was found to be maximum in petroleum ether followed by acetone and ethanol. This study showed that *Musa acuminata* leaf is a good source of phenolic compounds. In addition, the leaf extracts were tested for antimicrobial activities. All the three extracts showed excellent antifungal activities against two pathogenic fungi, but failed to show any antibacterial activities.

Introduction

Globally, banana plant is the fourth most important agricultural product after rice, wheat and maize. The flower and stem region of banana plant are known for their anti-ulcer, diuretic, anti- diabetic and antiseptic properties (Lewis et al., 1999; Dhanabal et al., 2010) and they have been extensively investigated. But very limited number of studies have been carried out on its leaves. For ages, banana leaves have been used for cooking, wrapping and serving food all over the world. Reports show that they contain large amount of polyphenols, especially polyphenol oxidase which is used in the treatment of Parkinson's disorder (Chu et al., 1993). Apart from this they can treat skin ailments, it is done by simply wrapping the leaves around the burns and skin wound.

All medicinal plants produce important secondary metabolites like terpenoids, flavonoids, polyphenols, chlorophylls, betalains etc. . Among these, phenolic compounds are considered to be the chief plant constituent because of its' capacity to exhibit antioxidant, anti-cancerous and anti-inflammatory properties (Nicholson and Hammerschmidt, 1992). They inhibit

the free radicals which cause cell damage, by the formation of superoxidase anions and lipid peroxides (Yan Chun and Rong Liang, 1991).

Here the ethanol, petroleum ether and acetone extracts of the banana leaf were estimated for their total phenolic content and the best solvent for phenol extraction was monitored. Apart from this the antiseptic property of the leaf was examined by using two common fungal and bacterial cultures.

Materials and Methods

Collection of plant material

The leaves were collected from the campus of Vels University, Chennai. The collected leaves were washed thoroughly under running water and air dried for few minutes. The fresh leaves were immediately extracted with the solvents.

Preparation of leaf extracts

Two grams of fresh leaf was weighed out and added to 50ml of ethanol (96%), petroleum ether and acetone separately. It was incubated for 24 hours and then filtered. After filtration, the three supernatants were evaporated in rotary evaporator to obtain the crude extract. The extracts were suspended in 50ml of their respective extracting solvents. This was used as the stock solution.

Estimation of total phenolic content

Phenolic compounds were quantitatively estimated by modified Folin-Ciocalteu method (Makkar et al., 1983). 25ml of the stock solution of each extract was taken separately and the volume was made up to 50ml using ethanol (96%). This was used as the working solution. 0.1 ml of each working solution was taken separately and added to 0.4 ml of distilled water followed by 0.25ml of Folin-Ciocalteu reagent and 1.25ml of sodium carbonate. The blank was made using the same procedure but without the plant extract, hence 0.5ml of distilled water was added instead of 0.4ml. The tubes were incubated at room temperature in the dark for 40 minutes. The absorbance was measured at 725nm using an UV- spectrophotometer.

A standard calibration curve was constructed for tannic acid at various concentrations and the phenolic content of the three extracts were interpreted and expressed as tannic acid equivalent.

Antibacterial assay

The screening was done by disc diffusion method (Pattnaik et al., 1995).The extracts were tested against *Escherichia coli* and *Bacillus subtilis*. A loopful of the pure bacterial culture was suspended in nutrient broth and incubated for 24 hours. Nutrient agar media was sterilized and poured into plates. After solidification, 0.1ml of the inoculum was spread over the agar evenly using L rod. 6mm diameter of Whatman filter paper discs were soaked in plant extracts and dried out. Chloramphenicol and tetracycline antibiotic discs were used as the control. The discs were carefully placed on the inoculated plates and incubated for 24 hours. Later, the zone of inhibition around the disc was measured and recorded.

Antifungal assay

The assay was performed against *Aspergillus terreus* and *Penicillium solitum*. Czapek Dox Agar was used as the growth media. In each plate 15ml of

the sterile media and 5ml of the plant extract was added and the plate was gently swirled to mix the content (Raji and Raveendran, 2013). Media without the plant extract served as the control. After solidification, the fungus was inoculated at the centre of the plate and allowed to grow for 3 to 7 days. The growth was measured in diameter.

Results and Discussion

Total phenolic content

The total phenolic content of each leaf extract, expressed as tannic acid equivalent, was determined using the formula:

Ab= A+BX
Where,
Ab = absorbance of the leaf extract
A = intersection = 0.006
B = slope = 0.267
X = tannic acid concentration (mg/ml)

Therefore,

$$X = (Ab - A)/B$$

After obtaining the values of X, the concentration of total phenolic compound in the leaf was determined using the formula:

$$A = (XV)/m$$

Where,
A = Total phenolic content (mg/ml tannic acid equivalent),
X = Concentration of tannic acid obtained from the previous calculation (mg/ml),
V = Volume of the extract,
m = Mass of the extract (g).

The total phenolic content of the petroleum ether extract of banana leaf is 12.196mg/ml, ethanol extract is 7.0412mg/ml and acetone extract is 10.144mg/ml. The results clearly show that banana leaf is a good source of phenols which makes it a good antioxidant resource. Petroleum ether is the best solvent for extraction of phenol compounds followed by acetone and ethanol. The results with additional data are depicted in table 1.

Antibacterial activity

None of the leaf extracts showed antibacterial activity. They failed to inhibit the growth of both *Escherichia coli* and *Bacillus subtilis.* But, the extracts might have the possibility to prevent the growth of any other bacterial culture.

Antifungal activity

All the three extracts showed excellent antifungal activity against both *Aspergillus terreus* and *Penicillium solitum.* The antifungal property of the extracts was estimated by measuring the growth of fungi after 5 days of incubation. In the control plate, the fungi grew over the entire plate (9cm) in 5 days. Acetone extract showed excellent antifungal activity against *Aspergillus terreus*, i.e. it exhibited only 1.7cm of growth in 5 days.

Ethanol and petroleum ether extract also showed commendable results. Acetone extract showed good activity against *Penicillium solitum*, followed by ethanol and petroleum ether extracts. This study shows that acetone is the best solvent for extraction followed by ethanol and petroleum ether. The data summarized in table 2 shows that banana leaf is an exceptional antifungal agent.

Table.1 Total phenolic content of the leaf extracts

Extract	Absorbance	X (mg/ml)	m (g)	A = (XV)/m (mg/ml)
Petroleum ether	0.820	3.0487	0.025	12.196
Ethanol (96%)	0.476	1.7603	0.025	7.042
Acetone	0.683	2.5356	0.025	10.145

Table.2 Antifungal activity of the leaf extracts

Fungi	Mean diameter of fungal growth after 5 days of incubation (cm)			
	Control	Petroleum	Ethanol	Acetone
Aspergillus terreus	9	3.7	2.3	1.7
Penicillium solitum	9	5.9	5.7	4.9

Figure.1 Growth of *Aspergillus terreus* in control after 5 days

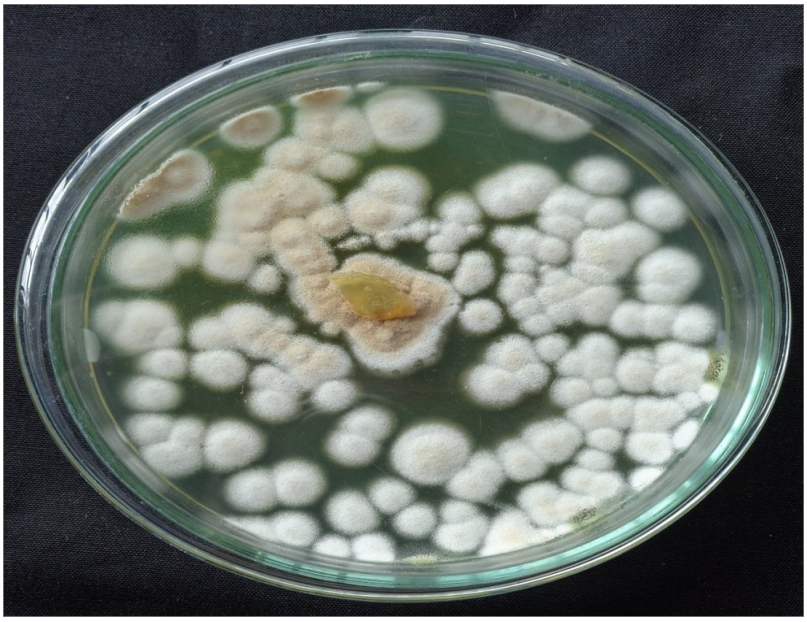
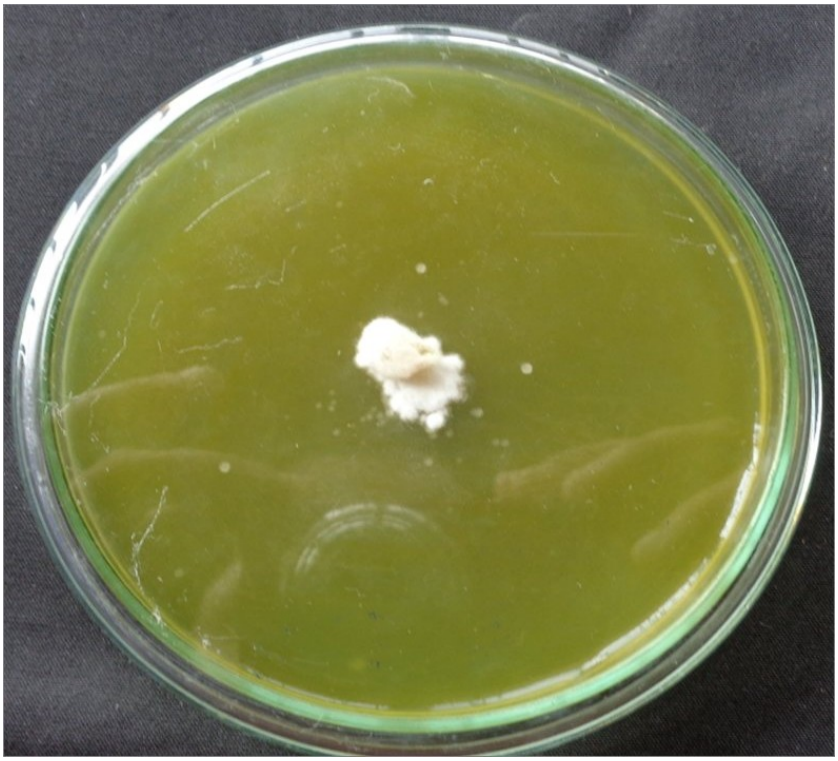


Figure.2 Growth of *Aspergillus terreus* in acetone extract after 5 days



References

Adewoye, E.O., V.O. Taiwo and Olayioye, F.A. 2010. Anti-oxidant and anti-hyperglycemic activities of musa sapientum root extracts in alloxan-induced diabetic rats. J Med Chem. 28;53(20):7365-76.

Bentley, R. and Trimen, H. 1980. Medicinal Plants. J. & A. Churchill. Vol. I-IV.

Chu, H.L., D.B. Yeh and Shaw, J.F. 1993.Production of L-DOPA by banana leaf polyphenol oxidase. Bot. Bull. Acad. Sin. 34: 57–60.

Dhanabal, S.P., M. Sureshkumar, M. Ramanathan and Suresh, B. 2010. Hypoglycemic effect of ethanolic extract of Musa sapientum on alloxan induced diabetes mellitus in rats and its relation with antioxidant potential. J Nutr. 140(8):1462-8.

Entaz Bahar, Joushan Ara, Mofasser Hossain and Obayed Raihan. 2013. Antioxidant (In-Vitro) Effect of Methanol& Petroleum Ether Extracts of the Aerva lanata. The pharma innovation. IC Journal No: 7725, Vol. 2.

Jamuna, J., Mysore Shobha, Kari Sambaiah and Paramahans Salimath. 2011. Beneficial effects of banana (*Musa* sp. var. elakki bale) flower and pseudostem on hyperglycemia and advanced glycation end-products (AGEs) in streptozotocin-induced diabetic rats. J Physiol Biochem. 67(3):415-25.

Lewis, D.A., W.N. Fields and Shaw, G.P. 1999. A natural flavonoid present in unripe plantain banana pulp (Musa sapientum L. var. paradisiaca) protects the gastric mucosa from aspirin-induced erosions. J Ethnopharmacol. 65(3):283-8.

Makkar, H.P.S., M. Bluemmel, N.K. Borowy and Becker, K. 1993. Gravimetricdetermination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric. 61: 161–165.

Mukhopadhyay, S., D. L. Luthria and Robbins R. 2006. Optimization of ExtractionProcess for Phenolic Acids from Black Cohosh (*Cimicifuga racemosa*) by Pressurized Liquid Extraction. J. *Sci. Food Agric*. 86: 156–162.

Nicholson, R.L. and Hammerschmidt, R. 1992. Phenolic Compounds and Their Role in Disease Resistance. Annual Review of Phytopathology. Vol. 30: 369-389.

Pattnaik, S., V.R. Subramanyam and Kole, C. 1996. Antibacterial and antifungal activity of ten essential oils in vitro. Microbios. 86(349):237-46.

Qian Zhang and Wenyi Kang. 2010. Active compounds from rhizomes of Musa basjoo. Zhongguo Zhong Yao Za Zhi. 35(18):2424-7.

Raji, R. and Raveendran, K. 2013. Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger*. Asian Journal of Plant Science and Research. 3(1):13-15.

Suhad Humadi and Viorica Istudor. 2009. *Lythrum salicaria* (Purple loosestrife). Medicinal use, extraction and identification of its total phenolic compounds. Farmacia. Vol. 57, 192-200.

Thakorlal, J., C.O. Perera, B. Smith, L. Englberger and Lorens, A. 2010. Resistant starch in Micronesian banana cultivars offers health benefits. Pac Health Dialog. 16(1):49-59.

Zhou Yan-Chun and Zheng Rong-Liang. 1991. Phenolic compounds and an analog as superoxide anion scavengers and anti oxidants. Biochemical Pharmacology. Vol. 42: 1177-1179.

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