REPORT

Antimicrobial activity of *Ficus deltoidea* Jack (Mas Cotek)

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Abstract: Present study aimed to investigate the *in vitro* antimicrobial activity of the chloroform, methanol and aqueous extracts of *Ficus deltoidea* at 10mg/ml, 20mg/ml and 50 mg/ml, respectively using the disc diffusion method against 2 Gram positive {*Staphylococcus aureus* (IMR S-277), *Bacillus subtilis* (IMR K-1)}, 2 Gram negative {*Escherichia coli* (IMR E-940), *Pseudomonas aeroginosa* (IMR P-84)} and 1 fungal strain, *Candida albicans* (IMR C-44). All the extracts showed inhibitory activity on the fungus, Gram-positive and Gram-negative bacteria strains tested except for the chloroform and aqueous extracts on *B. subtilis*, *E. coli*, and *P. aeroginosa***.** The methanol extract exhibited good antibacterial and antifungal activities against the test organisms. The methanol extract significantly inhibited the growth of *S. aureus* forming a wide inhibition zone (15.67 ± 0.58 mm) and lowest minimum inhibitory concentration (MIC) value (3.125 mg/ml). *B. subtilis* was the least sensitive to the chloroform extract (6.33 ± 0.58 mm) and highest minimum inhibitory concentration (MIC) value (25 mg/ml). Antimicrobial activity of *F. deltoidea in vitro* further justifies its utility in folkleric medicines for the treatment of infections of microbial origin.

Keywords: *Ficus deltoidea,* antimicrobial activity, disc diffusion method, MIC.

INTRODUCTION

The use of herbal remedies for treatment of microbial infections is well known since ancient times, nearly all cultures and civilizations from ancient times to the present day have used herbal medicines to cure infections (Lino and Deogracious, 2006). There have been efforts to discover new antimicrobial compounds which could serves as replacement for the present day antibiotics from various kinds of sources such as micro-organisms, animals and plants owing to increase in resistance by microbes to the antibiotics commonly used today, one of such resources is folk medicines. Scientists have been prompted to explore plant sources with a view of finding new novel effective compounds which plants have in abundance (Tomoko *et al*., 2002). The presence of multidrug resistant bacterial strains and the emergence of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and add urgency to the search for new infection fighting substances especially from plant sources because compounds from plants have shown promising activities on some bacterial strains especially in areas where the conventional antibiotics have failed (Sieradski *et al*., 1999).

Ficus deltoidea, belongs to the *Moraceae* plant family and are abundantly as medicinal plants in Malaysia (Mat-Salleh and Latif, 2002). Traditional claims have shown that the plant possess antidiabetic properties and is used extensively for treatment of diabetes. However, the scientific evidence to confirm its efficacy is still lacking. This plant which is native to Southeast Asia and Philiphines (Forest *et al*., 2003) has also been used to treat other ailments such as headache and fever (Mat-Salleh and Latif, 2002). Recent study has reported on the antinociceptive activity of aqueous extract of *F. deltoidea* (Sulaiman *et al*., 2008). Hakiman and Maziah (2009) have found that aqueous extract of different *F. deltoidea* accessions possess non enzymatic and enzymatic antioxidant activities. Each part of the plant is known to have medicinal properties such as reducing level of sugar in blood, decreasing blood pressure, reducing cholesterol and lipids, migraine, contracting the vagina after delivery, delaying menopause and reducing the risk of cancer (Hamid *et al*., 2008). The main objective of this study was to assess the antimicrobial activity of chloroform, methanol and aqueous crude extracts of *F. deltoidea* against some pathogenic microbial isolates of clinical importance.

MATERIALS AND METHODS

Collection of plant material

F. deltoidea was procured from the premises of Taman Pertanian, Pahang, Malaysia. The plant was identified by the chief taxonomist, Forest Research Institute of Malaysia (FRIM), whereby a voucher specimen number was obtained and later it was deposited at the FRIM Herbarium, Kuala Lumpur, Malaysia for future reference.

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Preparation of chloroform (DCM), methanol (MeOH) and aqueous (AQ) extracts

The fresh *F. deltoidea* whole plant material (1 kg) which were dried in a protech laboratory air dryer (LDD-720) at 30°C for 5 days were grounded to powdered form (350g) using a Universal laboratory cutting mill. It was then stored in a desiccators at 2°C until further use. Two hundred grams of dry powdered plant were successively extracted with chloroform and methanol using soxhlet apparatus for 24 hours each. Each of the mixtures was filtered and concentrated using a rotary evaporator (Buchi Rotary Evaporator, R-210). Final concentrated extracts were stored at 2°C in labeled sterile bottles and kept as aliquots until further antimicrobial evaluation test. Another 100 g of powdered whole plant of the herb was extracted by soaking in distilled water in a cornical flask, stirred for about 10 minutes, closed tight using a rubber cork and left overnight on a rotating shaker. Thereafter, the solution was filtered using filter paper (Whatman No. A-1) and the extract was then freeze dried at 2°C in labeled sterile bottles. All chemicals used in this study were of analytical grade which were obtained from Fisher Scientific Chemicals, UK and Merck, Germany.

Source of microbial strains

Five microbial isolates were chosen for antimicrobial investigation: two Gram positive bacteria{*S. aureus* (IMR S-277), *B. subtilis* (IMR K-1)}, two Gram negative bacteria {*E. coli* (IMR E-940), *P. aeroginosa* (IMR P-84)} and a fungus {*C. albicans* (IMR C-44)}. All microbial strains were purchased from the Institute of Medical Research (IMR), Kuala Lumpur, Malaysia and American Type Culture Collection (ATCC), USA respectively.

Preparation of standard bacterial and fungal suspensions

The surface viable counting technique of Miles and Misra (1938) were adopted to determine the *S. aureus, B. subtilis*, *E. coli P. aeruginosa* and *C. albicans* organisms per ml of the stock suspensions. About (10^6-10^8) colony-forming units per ml was used for all experiments. All bacterial stock cultures were maintained on nutrient agar (NA) slants while *C. albicans* stock cultures were maintained on Sabouraud dextrose agar (SDA) slants at 4°C. These were subcultured prior to each experiment and were used for antibacterial, antifungal tests and MIC determination, respectively.

Testing for antibacterial activity

The antibacterial activities of the prepared extracts on the test strains were assessed by the disc diffusion method (NCCLS, 2006). The three concentrations of the extracts were prepared by dissolving in 10% DMSO. Ten microliters of the test microorganisms from a 24 hours nutrient broth medium (10^6-10^8 CFU/ml) were inoculated onto nutrient agar by the spread plate method and were allowed to solidify at room temperature. Filter paper discs (5 mm in diameter) were impregnated with the test extracts, allowed for 30 minutes at room temperature to dry and placed on the inoculated test plates. Tetracycline (10µg) served as the positive control. The antibacterial assay plates were incubated at 37°C for 24 hours. At the end of incubation time, the diameters of the inhibition zones formed were measured, averaged and the mean values were tabulated in mm.

Testing for anti-fungal activity

The NCCLS method as used for antibacterial assay was adopted to assess the antifungal activity of the prepared extract against *C. albicans*. Instead of nutrient agar, yeast and mold extract agar were used. The inoculated medium was incubated at 25ºC for two days and 10 µg nystatin was used as positive control (NCCLS, 2006).

Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

The micro-well dilution method was used for determination of MIC and MFC values for each crude extracts showing antimicrobial activity against test pathogens (Zgoda and Porter, 2001). Two fold serial dilution of the crude plant extracts was carried out; 25mg/ml - 0.195mg/ml in the respective micro-plates. Thereafter, 100µl inoculum (adjusted to 10^6-10^8 CFU/ml) was added to each well. The microbial suspensions were used as negative control, while broth containing standard drugs (nystatin and tetracycline) were used as positive controls. The micro-titer plates were incubated in a Heraeus Incubators (BK-6160) at 37ºC for 24 hours (bacteria) and 25ºC for 48 hours (fungi). Each extract was assayed in duplicate; one was kept for incubation while another set was kept at 4°C for comparing the turbidity in the wells of micro-plate. MIC/MFC is the lowest concentration of the extracts in the wells of the micro-titer plate that showed no turbidity or visible growth after incubation.

RESULTS

The results of the present investigations revealed that the three crude extracts from *F. deltoidea* whole plant possess potential antimicrobial activities against some of the tested organisms at the three concentrations (10, 20 and 50 mg/ml) used (table 1).

The crude extracts activities were observed to be strain specific and concentration dependent. The crude extract activities were best on Gram positive followed by the fungal and lastly the Gram negative strains tested. The chloroform, methanol and aqueous extracts of *F. deltoidea* were found to be active on at least one of the tested microbial strains. The extracts displayed relative antimicrobial activities against most of the tested microorganisms with the diameter of inhibition zones

Table 1: Antimicrobial activity of *F. deltoidea* extracts

Zones of Inhibition (mm)					Antibiotics (10 µg/disc)
Bacterial strains	Plant Extracts	10 mg/ml	20 mg/ml	50 mg/ml	
Gram Positive Strains					Tetracycline
S. aureus (IMR S-277)	Chloroform	8.33 ±0.58	10.00±1.00	11.50±1.00	20.50 ± 1.00
	Methanol	10.67± 0.76	12.00±0.50	15.67±0.58	
	Aqueous	7.33±0.76	8.17±1.53	9.00±1.00	
B. subtilis (IMR K-1)	Chloroform	6.33±0.58	9.40±1.15	10.50±1.00	17.00 ± 0.50
	Methanol	9.50±0.58	11.00±1.00	12.33 ±0.58	
	Aqueous	-	-	-	
Gram Negative Strains					Tetracycline
E. coli (IMR E-940)	Chloroform	-	-	-	15.33±0.89
	Methanol	8.00±1.73	8.67±0.58	9.33±0.58	
	Aqueous	-	-	-	
P. aeruginosa (IMR P-84)	Chloroform	7.33±1.04	8.00±0.29	8.00±1.15	16.50 ± 0.50
	Methanol	7.00±1.50	9.00±0.76	10.50±0.50	
	Aqueous	-	-	-	
Fungi					Nystatin
C.albicans (IMR C-44)	Chloroform	6.67±1.15	8.00±1.15	9.00±1.04	18.33 ± 0.58
	Methanol	7.00±1.50	10.00±1.26	11.50±1.00	
	Aqueous	7.33±0.58	8.00±1.00	10.00±1.73	

- No activity, Values are mean inhibition zone (mm) ± S.D of three replicates.

ranging between 6.33 ± 0.58 to 15.67 ± 0.58 mm (bacteria) and 7.33 ± 0.58 to 11.50 ± 1.00 mm (fungi). Highest antibacterial activity was exerted by MeOH extract against *S. aureus* at 50 mg/ml (15.67 ± 0.58 mm) and the least by the chloroform extract against *B.subtilis* at 10 mg/ml (6.33 ± 0.58 mm). Highest antifungal activity was exerted by the MeOH extract on *C. albicans* at 50 mg/ml (11.50 ± 1.00 mm) and the least by the chloroform on *C. albicans* at 10 mg/ml (6.67±1.15 mm), respectively (table 1). However no activity was exerted by the chloroform and aqueous extracts at all test concentrations on *B. subtilis, E. coli* and *P. aeroginosa* (table 1). All *F. deltoidea* whole plant extracts exhibited a broad spectrum on the tested bacteria and fungi strains. The highest MIC value was observed at 25 mg/ml exerted by the chloroform extract on *B. subtilis.* The least was 3.125 mg/ml exerted by the methanol extract on *S. aureus* (table 2).

Table 2: Minimum inhibitory concentration (MIC) of the plant extracts on the bacterial and fungal strains

	MIC (mg/ml)		
	Chloroform	Methanol	Aqueous
Gram Positive			
S. aureus	6.25	3.125	12.5
B.subtilis	25	12.5	-
Gram Negative			
E. coli	-	-	-
P. aeruginosa	12.5	12.5	-
Fungi			
C. albicans	12.5	6.25	12.5

- = No Activity

DISCUSSION

In the present era, plant and herb resources are abundant, but these resources are dwindling fast due to the onward march of civilization (Vogel, 1991). Although a significant number of studies have been used to obtain purified phytochemicals, very few screening programmes have been initiated on crude plant materials. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants (Veeramuthu *et al*., 2006).

The activity of the plant extracts was best on the Gram-positive strains tested followed by the fungal strains and lastly the Gram negative strains, this is in agreement with previous reports that plant substances are more active against Gram-positive bacteria than Gram-negative bacteria (Waseem *et al*., 2010; Saify *et al*., 2005). The results obtained is indicative of the presence of broad spectrum antimicrobial compounds or metabolic toxins in *F. deltoidea* whole plant that could be exploited in treating infections associated with the aforementioned bacterial and fungal strains. Previous studies on antimicrobial activities of medicinal plants have indicated that inhibition zones of 10 mm or greater were taken to represent good activity of such plants (Bukola *et al*., 2008; Ramesh *et al*., 2011). If extracts displayed an MIC less than 100 mg/ml, the antimicrobial activity was considered as good; from 100 mg/ml to 500 mg/ml the antimicrobial activity was moderate; from 500 mg/ml to 1000 mg/ml the antimicrobial activity was weak; over 1000 mg/ml the extract was considered inactive.

Chloroform, methanol and aqueous extracts of *F. deltoidea* whole crude plant presented a good activity against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *C. albicans* with MIC values of 25, 12.5, 6.25 and 3.125 mg/ml, respectively.

CONCLUSION

The results obtained from the *in vitro* study of *F. deltoidea* whole plant corroborates its use in folkloric medicine. *F. deltoidea* extracts were effective against three or more of the pathogenic microorganisms, however, *E. coli*, *P. aeroginosa* and *B. subtilis* were resistant to the chloroform and aqueous extracts at all test concentrations used which may be unsuitable for their medicinal use in infections associated with these species or strains. This study also showed that *F. deltoidea* could be potential sources of new antimicrobial agents.

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