Journal of Applied Pharmaceutical Science Vol. 3 (07), pp. 007-013, July, 2013
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2013.3702
ISSN 2231-3354

Efficacy of pyroligneous acid from *Rhizophora apiculata* on pathogenic *Candida albicans*

Darah Ibrahim[1], Jain Kassim[2], Lim Sheh-Hong*[1] and Wendy Rusli[2]
[1]School of Biological Sciences, [2]School of Chemical Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.

ARTICLE INFO

Article history:
Received on: 11/06/2013
Revised on: 29/06/2013
Accepted on: 19/07/2013
Available online: 30/07/2013

Key words:
Rhizophora apiculata,
Candida albicans,
pyroligneous acid,
candidiasis.

ABSTRACT

Rhizophora apiculata pyroligneous acid which is a crude condensate produced from the distillation of smoke generated in the process of charcoal making has the potential to be used as antifungal agent especially to treat candidal infections. In this study, pyroligneous acid (PA), concentrated pyroligneous acid (CPA), Dichloromethane extracts of CPA namely DCM A and B were tested against four pathogenic strains of *Candida albicans*. The results exhibited significant inhibition zones within the range of 7.00 -8.00 mm for PA, 16.00-17.00 mm for CPA, 16.00-18.00 mm for DCM A and 19.00-22.00 mm for DCM B. The results also revealed that extract DCM B of CPA was the most potential to be used as anticandidal agent with the minimum inhibitory concentration values between 3.13-6.25 mg/mL. Scanning electron micrographs of DCM B treated *C. albicans* cells confirmed the damaged cells caused by the extract.

INTRODUCTION

Candidal infection or also known as candidiasis is a common infection of the oral cavity, nails, skin and vagina of human (Ruhnke, 2002). It represents one of the most rapidly increasing healthcare infections with a significant mortality rate in hospitalized patients (Jarvis, 1995).

These fungal infections are becoming more prevalent worldwide because the size of the immune-compromised patient population is rising. *Candida* species especially *C. albicans* are now recognized as a major causative agent of hospital acquired infection (Douglas, 2003) and it is one of the leading causes of opportunistic fungal infections in immune-compromised individuals, including AIDS patients, transplant recipients, and cancer patients (De Repentigny *et al*., 2004; Erköse and Erturan, 2007). Even though several antifungal drugs such as griseofulvin, azole groups (fluconazole, ketoconazole and variconazole), nystatin, amphotericin B and 5-fluorocytosine are used clinically to treat pathogenic yeasts and fungi (Chen *et al*., 2013) but many of them have exhibited resistant and toxicity (Hofling *et al*., 2010). Thus, the discovery of new antifungal agents with cost-effective from natural origin are remains an important challenge for the scientific community. Pyroligneous acid, also called wood vinegar is a crude condensate produced from the distillation of smoke generated in the process of wood carbonization and charcoal making (Sameshima *et al*., 2002). It is a complex mixture of compounds derived from the chemical break-down of the components in wood through the condensation of vapors and gases generated during the pyrolysis of a limited access of oxygen. It appears to be a clear dark reddish-brown liquid which resembles to the pleasing hue of black tea, beer or wine with strong smoky aroma (Guillen and Ibargoitia, 1998; Mohan *et al*., 2006). The pH of pyroligneous acid is low ranging from 2-3, due to its high amount of volatile acids (8-10%) mainly formic and acetic acids (Sipila *et al*., 1998). Pyroligneous acid is also well-known for its organoleptic properties (Guillen and Manzanos, 2002). It is reported to contain a complex mixture of water (10-20%), a mixture of carboxylic acids among which acetic is the most prevalent, several aldehydes and alcohols. Besides that ptroligneous acid also contain formic acid, guaiacols, catechols, syringols, vanillins, methanol, acetone, furan carboxaldehydes, isoeugenol, pyrone, ketones, esters, and more than 200 organic compounds including phenolic compounds which are pyrolytic products of lignin and hemicelluloses (Ninomiya *et al*., 2004; Lee *et al*., 2010).

* *Corresponding Author*
Tel.: +6012-4629487, Fax: +604-6565125
Email: limshehhong77@gmail.com

Pyroligneous acid has been reported to posses antibacterial (Chalermsan and Peerapan, 2009) and antioxidant activities (Loo *et al*., 2007; Loo *et al*., 2008), besides exhibiting strong antifungal activity against several plant pathogenic fungi (Jung, 2007; Oramahi and Yoshimura, 2013) and also a termiticidal activity (Mitsuyoshi *et al*., 2002; Yatagai *et al*., 2002). Several researchers have reported the antibacterial activity of the pyroligneous acid against several pathogenic bacteria (Yodthong and Niamsa, 2009), including plant pathogens (Ma *et al*., 2011). Hwang *et al*., (2005) for example reported the synergistic effects among the compounds in the pyroligneous acid which exhibited antimicrobial properties. It is believed that phenolic derivatives are one of the groups of compounds being responsible for the antimicrobial activity of pyroligneous acid (Cowan, 1999). Recently, the medicinal use of the pyroligneous acid has been studied intensively in the field of oriental medical science (Kim *et al*., 2001; Park *et al*., 2003), where some natural resources has been used for investigating the biological activities (Yoo *et al*., 2005; Choi *et al*., 2006; Lee and Seo, 2006; Lee *et al*., 2006). However, its antifungal activity against pathogenic yeasts has not been studied in detail. Therefore, the present work is carried out to evaluate the anticandidal activity of the *R. apiculata* pyroligneous acid and its dichloromethane extract on four strains of pathogenic *C. albicans*. The effects of extract on the candidal cell growth and cell morphology were studied and investigated. This pyroligneous acid was selected because of its availability in Malaysia since it is a by-product of charcoal making and often considered as waste.

MATERIALS AND METHODS

Pyroligneous acid

The *R. apiculata* pyroligneous acid was obtained from charcoal village in Kuala Sepetang, Taiping, Perak, Malaysia. The smokes that escape from the chimneys of the charcoal's kiln at temperatures of 240-500°C were passed through a 30 meter extension of air cooled stainless steel pipe for condensation purpose. The condensed smokes or known as pyroligneous acid or wood vinegar was collected in a polyethylene container and stored at room temperature (30±2°C) and was used in the present study.

Preparation of concentrated pyroligneous acid

The pyroligneous acid was filtered through a Whatman No. 1 filter paper to eliminate any debris and oily phase. The filtrate was then concentrated using a rotary evaporator under reduced pressure at 80°C until the volume of one tenth of its original volume obtained. The resulting concentrated pyroligneous acid (designated as CPA) which was free from water and some volatile components (such as methanol, acetone etc) was collected and kept at 4°C until further used.

Preparation of the dichloromethane extract from concentrated pyroligneous acid

The extraction of the components of concentrated pyroligneous acid (CPA) was conducted using liquid-liquid extraction with dichloromethane (designated as CPM) as the extraction solvent to give DCM A extract. In order to isolate the phenolic derivatives from DCM A extract, an acid base treatment was conducted on DCM A extract by using aqueous NaOH solution, aqueous H_2SO_4 solution and $NaHCO_3$ to give DCM B extract. Figure 1 summarizes the whole procedures of getting DCM A and B (Amen-Chen *et al*., 1997).

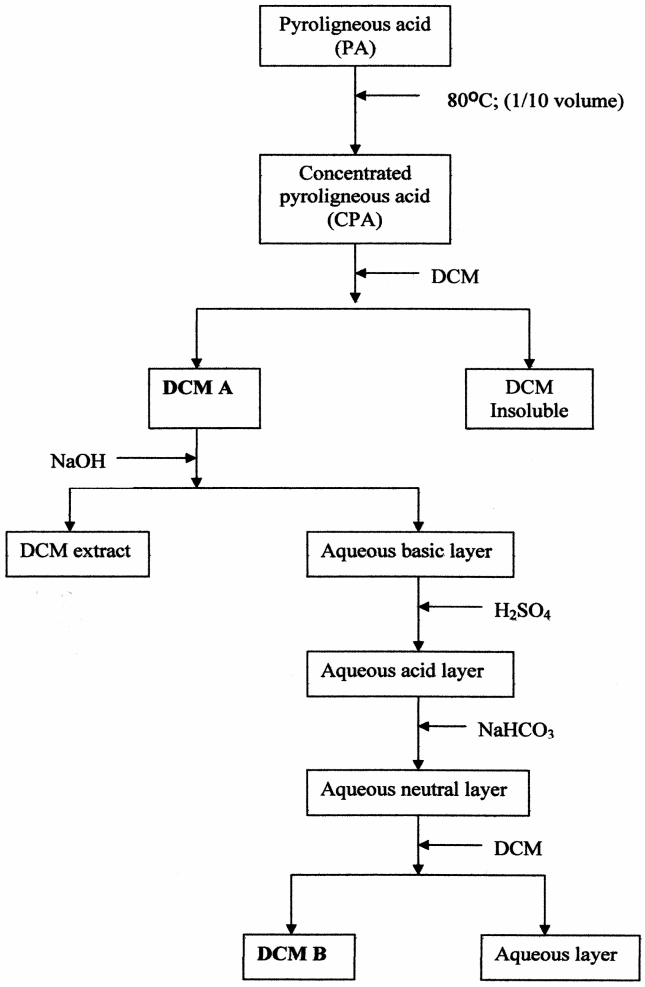

Fig. 1: the scheme for DCM extraction.

Microorganisms and cultural maintenance

Four strains of pathogenic *C. albicans* which were isolated from patients with suffering from urinary tract infection (two strains), vaginitis and onychomycosis (one each) were used in the study. The cultures were maintained on Sabouraud dextrose agar slants at 37°C for 24 h. All the cultures were kept at 4°C until further used. Subculturing was done at every four weeks to maintain their viability.

Anticandidal activity by disc diffusion method

The anticandidal activity of the extract against the test pathogenic yeast strains were determined following the method described by NCCLS (2000) with slight modifications. The yeast strains were removed aseptically with an inoculating loop and

transferred to test tubes containing 5.0 mL of sterile distilled water. Sufficient inoculum was added until the turbidity was equivalent to 0.5 McFarland standards ($1x10^8$ cells/mL). One milliliter of the suspension was then added into 15.0 mL of sterilized molten Sabouraud dextrose agar aseptically. The mixtures were mixed well by swirling the plates left and right and then they were left on the bench to solidify. The commercial antibiotic disc GF A (Whatman) with 6.0 mm diameter was used to screen the anticandidal activity. Each of the sterile discs was then impregnated with 20 µL of the extracts that were pyrolineous acid (PA), concentrated pyroligneus acid, (CPA), Dichlromethane extracts (DCM A and DCM B), which corresponding to 100.0 mg/mL of extract stock. Ketoconazole at the concentration of 30 µg/mL was used as a positive control. All the impregnated disks were air dried before placing them on the agar surface. The plates were incubated at 37°C for 24 hours and the anticandidal activity was determined by measuring the diameter of the growth inhibition zones formed around the disc. The experiments were carried out in triplicate and the results were expressed as means of three experiments.

Determination of minimum inhibitory concentrations

The determination of minimum inhibitory concentration (MIC) was performed using macrodilution method (Nor-Afifah *et al*., 2010). Briefly, different extract preparations were subjected to a serial dilution using sterile Sabouraud dextrose broth medium as a diluents to give final crude extract concentrations between 0.39 and 100.00 mg/mL. The test tubes were inoculated with the yeast suspension (20 µL/mL broth), homogenized, and incubated at 37°C for 24 hours.

The lowest dilution of the extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism was recorded as the MIC value of the extract. The yeast growth was indicated by the turbidity. Each test was performed in triplicate.

Time-kill study of *Candida albicans* strain 1 in the presence of DCM B extract

C. albicans strain 1 suspension was prepared as described previously and was harvested by centrifugation, washed twice with sterile distilled water and resuspended in sterile distilled water. The suspension was adjusted using the McFarland standard. The DCM B extract was added in to 25 mL of Sabouraud dextrose broth in a 50 mL Erlenmeyer flask to achieve concentrations of 0 (control), 1.56 (1/2MIC), 3.13 (MIC) and 6.25 (2MIC) mg/mL after addition of the inoculum (Yogalatha et al., 2010). The experiments were conducted in triplicate and all the flasks were incubated in a shaker (Infors HT Ecotron) incubator at 37°C with agitation at 100 rpm. One milliliter of the mixture within each flask was withdrawn at every 4 hourly intervals starting from 0 hour until 48 hours of cultivation and the bacterial cell growth was monitored by measuring optical density at 600 nm.

Scanning and transmission electron microscope observations

The *C. albicans* strain 1 suspension was prepared as described previously. To each sample, 1.0 mL of the 24 hour old yeast suspension was inoculated in a 50.0 mL Erlenmeyer flask containing 30.0 mL of sterilized Sabouraud dextrose broth and incubated in a shaker at 37°C, 150 rpm for 24 hours. The suspension was then added to the extract stock solution (the final concentration in each flask was at the 2MIC value) and incubated at the required incubation time (0, 12, 24 and 36 hours). The SEM and TEM samples preparations were done following the method describes by Mares (1989), Borgers *et al*., (1989), Yogalatha *et al*., (2011) and Darah *et al*., (2013). The prepared samples were then viewed under a scanning electron microscope (Leica Cambridge, S-360, UK) and transmission electron microscopy (Philips CM12, Eindhoven, Netherlands).

Statistical Analysis

The data obtained were analyzed by Student *t*-test for comparing the extract on the several strains of *C. albicans* against control, using SPSS Version 12.0. Statistical significance was assumed at the 0.05 levels ($p<0.05$).

RESULTS

Pyroligneous acid from *R. apiculata* Blume barks

The physical properties of the concentrated pyroligneous acid from the Malaysia *R. apiculata* Blume were a clear to dark reddish-brown liquid that resembles black tea with low pH value of 1.8. It has a strong smoky aroma The pyroligneous acid (PA) in this study was then concentrated under reduced pressure, using a rotary evaporator at 80°C to give CPA. The removal of the volatile components in PA, such as methanol, acetone and some of the volatile acids, was another 'cleaning-up' procedure after filtration process. Those components were not the object of interests and the removal of those components would help to facilitate in the extraction and isolation of the components in PA. Additionally, at a temperature of 80°C, the desired compounds would not degrade as they were of higher boiling points (semi-volatiles compounds) and would remain in CPA.

Anticandidal activity and minimum inhibitory concentration values

Anticandidal activity of the pyroligneous acid (PA), concentrated pyroligneous acid (CPA), dichloromethane extracts (DCM A and DCM B) are shown in Table 1. The extract showed anticandidal activity with the diameters of inhibition zones ranging from 9.00 mm for PA, 16.00-17.00 mm for CPA, 16.00-18.00 mm for DCM A and 19.00-22.00 mm for DCM B (Table 1). There were significant differences ($p<0.05$) in the anticandidal activities of the extract. Subsequent experiments were conducted to determine the MIC values of the extract against the *C. albicans* strains. The minimum inhibitory concentration values ranged between 3.13 to 6.25 mg/mL (Table 2). The results obtained appeared to confirm the anticandidal potential of the extract

investigated. Based on the results on anticandidal acyivity and the MIC value, DCM B extract was chosen for subsequent experiment on time-killed study and also the observations by SEM and TEM studies.

Table. 1: Anticandidal activity of the *R. apiculata* pyroligneous acid and its dichloromethane extracts

Strains	Diameter of inhibition zones (mm)				
	PA (100 mg/mL)	CPA (100 mg/mL)	DCM A (100 mg/mL)	DCM B (100 mg/mL)	Ketoconazole (30 µg/mL
Candida albicans strain 1	9.0	17.0	16.0	22.0	24.0
Candida albicans strain 2	8.0	17.0	17.0	20.0	24.0
Candida albicans strain 3	7.0	16.0	18.0	21.0	23.0
Candida albicans strain 4	8.0	17.0	16.0	19.0	25.0

PA pyroligneous acid; CPA concentrated pyroligneous acid; DCM dichloromethane extract.
C. albicans 1- isolated from urinary tract infection, *C. albicans* 2- isolated from vaginitis, *C. albicans* 3- isolated from yeast onychomycosis, *C. albicans* 4- isolated from urinary tract infection

Table. 2: Minimal Inhibitory Concentration values of the Dichloromethane B extract against strains of *Candida albicans*

Strains	MIC values (mg/mL)
Candida albicans strain 1	3.13
Candida albicans strain 2	3.13
Candida albicans strain 3	6.25
Candida albicans strain 4	3.13

C. albicans 1- isolated from urinary tract infection, *C. albicans* 2- isolated from vaginitis, *C. albicans* 3- isolated from yeast onychomycosis, *C. albicans* 4- isolated from urinary tract infection

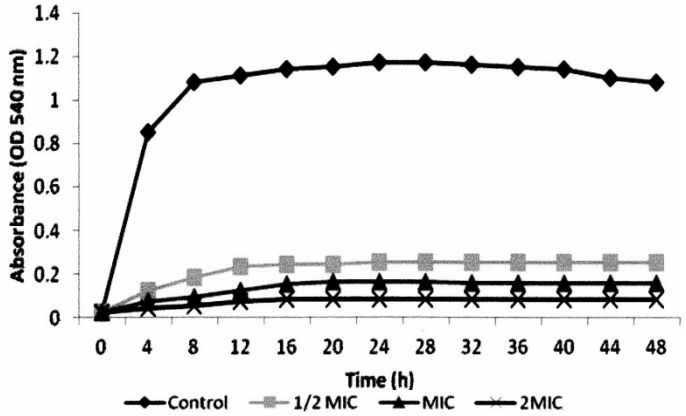

Fig. 2: growth profiles of *Candida albicans* strain 1 after treated with 1/2MIC (1.56 mg/ ml), MIC (3.13 mg/ml) and 2MIC (6.25mg/ ml) of the DCM B ectract.

Time-kill study

Time-kill study was conducted over a period of 48 hours using DCM B extract with the aim to assess the anticandidal activity with ½ MIC (1.56 mg/mL), MIC (3.13 mg/mL) and 2MIC (6.25 mg/mL) values over time and the results are shown in Figure 2. There was a drastic dropped in OD after 12 hours of cultivation, which leads to the stationary phase of yeast growth compared to control (untreated cell). At MIC and 2MIC values, the extract eradicated the cell numbers. The results showed the potency of the extract as an anticandidal agent against *C. albicans*.

Scanning (SEM) and transmission (TEM) electron microscopy observations

A clearer view on the effect of the DCM B extract against the *C. albicans* cells are shown in Figure 3. Figure 3A shows many oval and smooth cells in appearance and some at a budding stage (control or untreated cells). After 12 hours of exposure (Figure 3B), there was some effect of the extract observed on the cells compared to the control cells. Figure 2C shows the 24 hours treated cells with a distinct cell formation, with cavitations and shrunken cells. Finally, after 36 hours of exposure to the extract (Figure 3D), completely collapsed and cavitated cells were observed. At this stage the damage of the cells was beyond repair and the cells lost their metabolic functions completely.

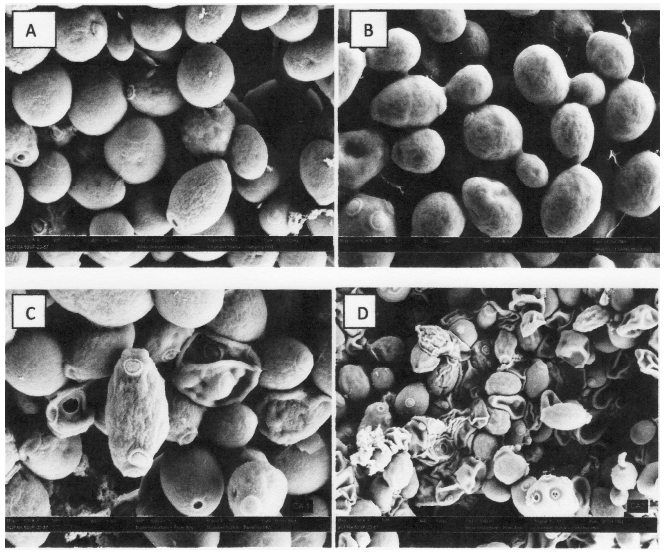

Fig. 3: SEM micrographs of the untreated and DCM B extract treated *C. albicans* strain 1cells. (A) Untreaed cells, (B) 12 hours, (C)24 hours and (D) 36 hours of exposure.

Observation on SEM micrographs suggested the cells had undergone distinct morphological and cytological alterations. Further evidences of these changes can be clearly observed from the TEM micrographs which reaffirm some form of disorganization of the yeast cells and also destruction of its cytoplasm and organelles.

Figure 4A shows a typical structure of *C. albicans* cells with nucleous and organelles. The cytoplasm contains element of cell membrane system and enveloped by a typical cell wall of yeast cells. Figure 4B shows a 12 hour of exposure to the extract, cells were dense with the vesicles, membrane cell and cell wall disposition and altered within the cells. After 24 hours of exposure (Figure 4C), the cells exhibited notable alterations in the cell membrane and cell wall.

The cytoplasmic volume decreased and there was deformed or stunted budding formation with disorganization within the cell cytoplasm. Figure 4D shows the effect of a prolonged exposure to the extract (36 hours), where the cells undergoing severe disorganization within the cells which led to collapsed cells and lysed.

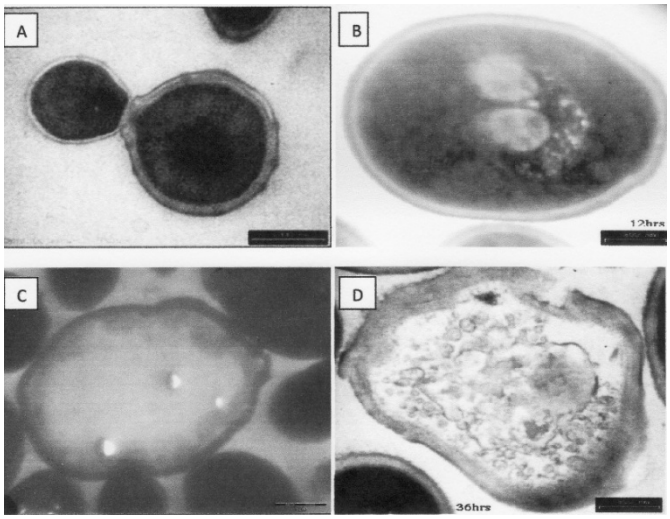

Fig. 3: TEM micrographs of the untreated and DCM B extract treated *C. albicans* strain 1cells. (A) Untreaed cells, (B) 12 hours, (C)24 hours and (D) 36 hours of exposure.

DISCUSSION

Many antifungal including anticandidal agents are available to treat superficial and systemic candidiasis (Hofling *et al*., 2010; Chen *et al*., 2013). The emergence of drug-resistant strains and dose-limiting toxic effects has impeded antifungal treatment. Moreover, immune-compromised and hospitalized patients are more susceptible to severe candidal infections. Many investigators have searched for new compounds with anticandidal action in natural products (Rukayadi *et al*., 2008; Agarwal *et al*., 2010; Lim *et al*., 2011; Boroujenis *et al*., 2012).

R. apiculata pyroligneous acid is a by-product of charcoal making industry from the billets of *R, apiculata*, a mangrove plant which were planted widely for charcoal industry in Larut-Matang district, Malaysia. The freshly collected reddish-brown distilled solution from the charcoal kiln at the temperature of 240 -500°C has a smoke aroma (Loo *et al*., 2007; Loo *et al*., 2008). *R. apiculata* pyroligneous acid has been reported to consist of 5.5% acetic acid, 3.4% methanol and 6.5% wood tar. Due to its high amount of volatile acids (8-10%), pyroligneous acid is acidic with pH ranging from 2-3. These acids contribute to its mild corrosive properties. *R. apiculata* pyroligneous acid from Matang, Malaysia has been studied for its phenolic content and antioxidant properties (Loo et al., 2007). However, the detail study on anticandidal activity of the *R. apiculata* pyroligneous acid is none. To the best of our knowledge this is the first time that anticandidal activity (on human pathogenic *C. albicans*) of *R. apiculata* pyroligneous acid is described.

The present study has shown that pyroligneous acid has promising antibacterial activity and this is probably why it is widely used in traditional oriental medicine (Kim *et al*., 2001; Park *et al*., 2003). The activity of the four extracts (PA, CPA, DCM A and DCM B) against four strains of pathogenic *C. albicans* can be indicative of the presence of broad spectrum antifungal compounds or simply general metabolic toxins in the solution. The anticandidal and antifungal activities of the extracts may be due to the presence of lignans (like phyllanthin and hypophyllanthin), flavonoids (like quercetin), astragalin, triterpenoids, glycosides, and tannins (ellagitannins), in the extract. Phytochemical constituents like flavonoids are known to prevent gastric ulcer due to the astringent and antimicrobial properties, which appear to be responsible for gastro-protective activity, as reported by Okolo *et al*., (2012). P-cymene, a monoterpenoid has also been reported to have a good antimicrobial properties (Paithankar *et al*., 2011; Selvamohan and Sandhya 2012).

The results of our study showed the MIC values of the extracts were between 3.13 to 6.25 mg/mL which is considered higher than the MIC values for bacteria (Darah *et al*., 2011, Supardy *et al*., 2011). Usually higher concentrations of extract are needed to inhibit the growth of yeast including *Candida* spp. Based on the time-killed studies the DCM B extract of pyroligneous acid showed fungicidal activity on *C. albicans* cells at 2MIC values (6.25 mg/mL) after 24 hours of treatment. In antimicrobial treatment, it is clinically more important to find fungicidal than fungistatic agents, especially in immunocompromized patients such as HIV patients. The reason is because the prophylactic use of fungistatic drugs has been associated with increased frequency of innate or acquired drug resistance in clinical isolates.

Prabhakar et al., (2008) reported that the ethanolic extracts of *Syzygium jambolanum* and *Cassia siamea* exhibited anticandidial activity at 100 mg/ml, whereas *Odina wodier* was at 500 mg/ml. This could be due to yeasts are eukaryotic cells and their cell wall contains chitin, which totally different from cell wall component of bacteria. Furthermore, *C. albicans* has isotropic growth by budding and several antifungal drugs can inhibit the budding formation in them. In this study the treatment of the *C. albicans* strains 1 with DCM B extract, decreased blastoconidia budding at MIC value (3.13 mg/mL) compared to the control ($p<0.05$). The MIC results had quantitatively demonstrated the anticandidal activity of the extract towards the susceptible cells. The succession of anticandidal activity of any plant extract depends on the ability of the antimicrobial agent to penetrate the cell wall. There are specific interactions between the bioactive compounds with the cell wall compartments (Hyldgaard *et al*., 2012). This interaction with the constituents' target site will either aid or hinder the penetration of the bioactive compounds into the cells. Previous studies have suggested that the deleterious effect of the extract on the cell wall of the fungus could be the main reason for the decrease in the rate of yeast budding, because the cell wall is necessary for cell division (Darah *et al*., 2013).

Morphogenesis which is the transition of unicellular yeast cells to the filamentous form (pseudohyphae), is an attribute of *Candida* species such as *C. albicans*. The presence of the filamentous form and budding is associated with virulence and pathogenicity, but both forms may be involved in the development and progress of disease. Several antifungal drugs and plant extracts can inhibit germ-tube formation and budding of yeast cells.

The pyroligneous acid and its DCM extracts at the concentration between 3.13 to 6.25 mg/mL inhibited morphogenesis of the yeast cells. These morphological changes can be observed from SEM and TEM micrographs. It was suggested that the effects of the extract were exerted on the outer membrane of the cell wall which then altered the cell membrane or plasmalemma structure and also the permeability of the cells. At the early stage (12 hour) the treated cells exerts a slight difference in their morphologies compared to control cells (untreated). However, the cells started to show more aberrant morphology over time. It can be summarized that the lethal action of the treated cells started with the shrinkage of the cells and then followed by the accelerated pores or cavity formations and unusual cell morphogenesis. It might have been due to the induced membrane permeability changes that occurred to the membrane cell structure resulted from the breakage of the hydrogen bonds that functions in keeping the rigidity of the membrane (Neu and Gootz, 1996; Rao *et al*., 2010). Another possibility is the potassium leakage and amino acid influx of the treated cells as suggested by Gopala-Rao *et al*., (2010). Thus, the results suggested that membrane cell is the major target site of the extract (Ogundare, 2006).

CONCLUSION

The present study conclusively demonstrates the anticandidal potential of the *R. apiculata* pyroligneous acid, an untapped source of compounds with anti- *C. albicans* activity that could be a resource in the development of therapeutically natural products. The extract was proven to be fungistatic at lower concentration and fungicidal at higher concentration. In addition, phytochemical studies will be necessary to isolate the active constituents and evaluate the antiyeast activity against a wide range of yeast population.

ACKNOWLEDGEMENT

The authors would like to thanks MOSTI of Malaysia and Universiti Sains Malaysia for the research grant awarded to complete this study.

REFERENCES

Agarwal V, Lal P, Pruthi V. Effect of plant oils on *Candida albicans*. J Microbiol Immunol Infections, 2010; 43(5): 447-451.

Amen-Chen C, Pakdel H, Roy C. Separation of phenols from Eucalyptus wood tar. J Biomass Bioenergy, 1997; 13: 25-37.

Boroujenis HAR, Pirbalouti AG, Hamedi B, Abdizadehi R, Malekpoor F. Anti-candida activity of ethanolic extracts of Iranian endemic medicinal herbs against *Candida albicans*. J Med Plants Res, 2012; 6(12): 2448-2452.

Borgers M, De Ven MA, Van Cutsem J. Structural degeneration of *Aspergillus fumigates* after exposure to saperconazole. J Med Vetenary Mycol, 1989; 27: 381-389.

Chalermsan Y, Peerapan S. Wood vinegar: by-product from rural charcoal kiln and its role in plant protection. Asian J Food and Agro-Industry, 2009; Special Issue: S189-S195.

Chen YL, Lehman VN, Averette AF, Perfect JR, Heitman J. Posaconazole exhibits *in vitro* and *in vivo* synergism: antifungal activity with caspofungin or FK506 against *Candida albicans*. PLOS ONE, 2013; 8(3): e57672. doi:10.1371/journal.pone.0057672.

Choi JH, Yoon SK, Lee KH, Seo Y, Kim H, Hong SB, Kim JY, Paik HD, Kim CH. Antitumor activity of cell suspension culture of green tea seed (*Camellia sinensis* L.). Biotechnol Bioprocess Eng, 2006; 11: 396-401.

Cowan MM (1999). Plant products as antimicrobial agents. Clinical Microbiol Review, 1999; 12(4): 564-582.

Darah I, Lim CL, Nurul Aili Z, Nor Afifah S, Shaida Fariza S. Effects of methanolic extract of a soft sponge, sp. on bacterial cells: structural degenaration study. Int J Comprehensive Pharma, 2011; 2: 1- 6.

Darah I, Lim SH, Kuppan N. Antimicrobial activity of crude methanolic extract from *Phyllanthus ninuri*. Natural Product Communications, 2013; 8(4): 493-496.

De Repentigny L, Lewandowski D, Jolicoeur P. Immunopathogenesis of oropharyngeal candidiasis in human immunodeficiency virus infection. Clinical Microbiol Reviews, 2004; 17: 729-759.

Douglas LJ. *Candida* biofilms and their role in infection. Trends in Microbiol, 2003; 11: 30-36.

Erkose G, Erturan Z. Oral *Candida* colonization of human immunodeficiency virus infected subjects in Turkey and its relation with viral load and CD4+ T-lymphocyte count. Mycoses, 2007; 50: 485-490.

Gopala-Rao TV, Sen SK, Samal A, Satpathy S. Nystatin induced changes in growth, viability and amino acid influx of yeast *Saccharomyces cerevisiae*. Int J Chem Res, 2010; 2(1): 8-17

Guillen MD, Ibargoitia ML. New components with potential antioxidant and organoleptic properties, detected for the first time in liquid smoke flavoring preparations. J Agric Food Chem, 1998; 46: 1276-1285.

Guille'n MD, Manzanos MJ. Study of the volatile compositions of an aqueous oak smoke preparation. Food Chem, 2002; 79: 283-292.

Hofling JF, Anibal PC, Obando-Pereda GA, Peixoto IAT, Furletti VF, Foglio MA, Goncalves RB. Antimicrobial of some plant extracts against *Candida* species. Brazilian J Biol, 2010; 70(4): 1065-1068.

Hwang YH, Matsushita YI, Sugamoto K, Matsui T. Antimicrobial effect of the wood vinegar from *Crytomeria japonica* sapwood on plant pathogenic microorganisms. J Microbiol Biotechnol, 2005; 15: 1106- 1109.

Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiol, 2012; 3: 12.

Jarvis WR. Epidemiology of nosocomial fungal infections with emphasis on *Candida* species. Clinical Infectious Diseases, 1995; 20: 1526-1530.

Jung KH. Growth inhibition effect of pyroligneous acid on pathogenic fungus, *Alternaria mali*, the agent of alternaria blotch of apple. Biotechnol Bioprocess Eng, 2007; 12: 318-322.

Kim JS, Choi JS, Kim JJ, Kim SM, Cho KY, Kim JC. Isolation and identification of herbicidal substances from wood vinegars. Korean J Weed Sci, 2001; 21: 357-364.

Lee, HJ, Seo Y. Antioxidant properties of *Ergeron annuus* extract and its three phenolic constituents. Biotechnol Bioprocess Eng, 2006; 11: 13-18.

Lee HJ, Kim YA, Ahn JW, Na HJ, Kim HM, Seo Y. Screening of Korean marine plants for their inhibitory effect on histamine release from RPMC in vitro. Biotechnol Bioprocess Eng, 2006; 11: 80-83.

Lee SH, H'ng PS, Lee AN, Sajap AS, Tey BT, Salmiah U. Production of pyrolignrous acid from lignocellulosic biomass and their effectiveness against biological attacks. J Appl Sci, 2010; 10(20): 2440-2446.

Lim SH, Darah I, Jain K. Assessment of *in vivo* and *in vitro* cytotoxic activity of hydrolysable tannin extracted from *Rhizophora apiculata* barks. World J Microbiol Biotechnol, 2011; 27(11): 2737-2740.

Loo AY, Jain K, Darah I. Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, *Rhizophora apiculata*. Food Chemistry, 2007; 104: 300–307.

Loo AY, Jain K, Darah I. Antioxidant activity of compounds isolated from the pyroligneous acid, *Rhizophora apiculata*. Food Chemistry, 2008; 107: 1151–1160.

Ma X, Wei Q, Zhang S, Shi L, Zhao Z. Isolation and bioactivities of organic acids and phenols from walnut shell pyroligneous acid. J Analytical Appl Pyrolysis, 2011; 91(2): 338-343.

Mares D. Electron microscopy of *Microsporum cookie* after in vitro treatment with protoanemonin: A combines SEM and TEM study. Mycopathologia, 1989; 108: 37-46.

Mitsuyoshi Y, Madoka N, Keko H, Tatsuro O, Akira S. Termiticidal activity of wood vinegar, its components and their homologues. J Wood Sci, 2002; 48 (4): 338-342.

Mohan D, Pittman CU Jr., Steele PH. Pyrolysis of wood/biomass for biooil: A critical review. Energy Fuels, 2006; 20(3): 848-889.

NCCLS, (National Committee For Clinical Laboratory Standards) (2000). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. 3rd ed. Wayne, P. A. : Approved Standard, NCCLS document M100-S12.

Neu HC, Gootz TD. 1996. Antimicrobial Chemotherapy. In: Baron S. (eds.), *Medical Microbiology*, Galveston (TX): University of Texas Medical Branch at Galveston.

Ninomiya Y, Zhang L, Nagashima T, Koketsu J, Sato A. Combustion and de-sox behavior of high-sulfur coals added with calcium acetate produced from biomass pyroligneous acid. Fuel, 2004; 83: 2123-2131.

Nor-Afifah Supardy, Darah I, Shaida FS, Mohd Jain NMK, Nurul AZ. (2010). Antimicrobial activity of various extracts of a tropical Chlorophyta macroalgae, *Halimeda discoidea*. J Appl Sci, 2010; 10(23): 3007-3013.

Ogundare AO. The pattern of potassium and protein leakage from microbial cells by *Vernonia tenoreana* leaf extract. Int J Tropical Med, 2006; 1(3): 100-192.

Okolo SC, Okoh-Esene RU, Ikokoh PP, Olajide OO, Anjorin ST. (2012). Phytochemicals, mineral content and antimicrobial screening of *Phyllanthus amarus* Schum and Thonn in Abuja, Nigeria. J Microbiol Biotechnol Res, 2012; 2(2): 17-22.

Oramahi HA, Yoshimura T. Antifungal and antitermitic activities of wood vinegar from *Vitex pubescens*Vahl. J Wood Sci, 2013; DOI: 10.1007/s10086-013-1340-8.

Paithankar VV, Raul KS, Charde R. M. and Vyas, J. V. (2011). *Phyllanthus ninuri*: A magic herb. Research in Pharmacy 1(4): 1-9

Park C, Choi YH, Lee WH, Choi BT, Lee YT, Kim CG. (2003). Up-regulation of Bax and downregulation of Bcl-2 in oak smoke flavoring (Holyessing)- induced apoptosis of human prostate carcinoma cells. Koren J Oriental Medicine, Physiol Pathol, 2003; 17: 85-90.

Prabhakar K, Kumar LS, Rajendran S, Chandrasekaran M, Bhaskar K, Khan AKS. Antifungal activity of plant extracts against *Candida* species from oral lesions. Indian J Pharma Sci, 2008; 70(6): 801-803.

Ruhnke M. 2002. Skin and mucous membrane infections. *In Candida and Candidiasis* (Calderone, R.A., ed.) ASM Press, Washington, D.C., U.S.A: 307-325.

Rukayadi Y, Shim J, Hwang J. Screening of Thai medicinal plants for anticandidal activity. Mycoses, 2008; 51: 308-312.

Sameshima K, Sasaki M, Sameshima I. 2002. Fundamental evaluation on termicidal activity of various vinegar liquids from charcoal making. Proceedings of the 4th International Wood Science Symposium, September 2-5, Serpong, Indonesia: pp: 134-138

Selvamohan T, Sandhya V. Studies on bactericidal activity of different soaps against bacterial strains. J Microbiol Biotechnol Res, 2012; 2(5): 646-650.

Sipilä K, Kuoppala E, Fagernäs L, Oasmaa A. Characterization of biomass based flash pyrolysis oils. Biomass and Bioenergy, 1998; 14 (2), 103-113.

Supardy NA, Darah I, Shada Fariza S, Nurul AZ. Inhibition of *Klebsiella pneumoniae* ATCC 13883 cells by hexane extract of *Halimeda discoidea* (Decaisne) and the identification of its potential bioactive compounds. J. Microbiol Biotechnol, 2011; 22(6): 872-881.

Yatagai M, Nishimoto M, Hori K, Ohira T, Shibata A. (2002). Termicidal activity of wood vinegar, its components and their homologues. J Wood Sci, 2002; 48: 338-342.

Yodthong B, Niamsa N. Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets. Biomass and Bioenergy, 2009; 33(6-7): 994-998.

Yogalatha L, Darah I, Jain K, Sasidharan S. Effects of *Vernonia cinerea* methanol extract on growth and morphogenesis of *Candida albicans*. European Rev Med Pharmacol Sci, 2011; 15(5): 543-549.

Yogalatha L, Darah I, Jain K, Sasidharan S. Toxicity of *Vernonia cinerea*. Pharma Biol, 2010; 48(1):101-104.

Yoo EJ, Lim HS, Park KO, Choi MR. Cytotoxic, antioxidative, and ACE inhibiting activities of Dolsan leaf mustard juice (DLMJ) treated with lactic acid bacteria. Biotechnol Bioprocess Eng, 2005; 2005; 10: 60-66.

How to cite this article:

Darah Ibrahim, Jain Kassim, Lim Sheh-Hong and Wendy Rusli., Efficacy of pyroligneous acid from *Rhizophora apiculata* on pathogenic *Candida albicans*. J App Pharm Sci, 2013; 3 (07): 007-013