



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(11)60062-2 © 2011 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

A transmission electron microscopy study of the diversity of *Candida albicans* cells induced by *Euphorbia hirta* L. leaf extract *in vitro*

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ARTICLE INFO

Article history:

Received 10 December 2010

Received in revised form 25 December 2010

Accepted 28 January 2011

Available online 1 February 2011

Keywords:

*Candida albicans**Euphorbia hirta* L.

Transmission electron microscopy

Anticandidal agent

ABSTRACT

Objective: To determine the major changes in the microstructure of *Candida albicans* (*C. albicans*) after treatment with *Euphorbia hirta* (*E. hirta*) L. leaf extract. **Methods:** Transmission electron microscopy was used to study the ultrastructural changes caused by *E. hirta* extract on *C. albicans* cells at various exposure time. **Results:** It was found that the main abnormalities were the alterations in morphology, lysis and complete collapse of the yeast cells after 36 h of exposure to the extract. Whereas the control cultures showed a typical morphology of *Candida* with a uniform central density, typically structured nucleus, and a cytoplasm with several elements of endomembrane system and enveloped by a regular, intact cell wall. **Conclusions:** The significant antifungal activity shown by this methanol extract of *E. hirta* L. suggests its potential against infections caused by *C. albicans*. The extract may be developed as an anticandidal agent.

1. Introduction

Candida albicans (*C. albicans*) is a dimorphic organism that commonly inhabits in oral and vaginal mucosa and gastrointestinal tract of human beings as one of the commensal organisms. It causes opportunistic infections in immunocompromised patients, and produces allergic reactions[1]. It also causes a variety of infections that range from non-life threatening mucosal candidiasis like vaginal yeast infections, thrush, skin and diaper rash to lethal disseminated candidiasis in those with compromised immune systems who have an implantable medical device such as a pacemaker or artificial joint, or who use broad-spectrum antibiotics[2–4]. *C. albicans* infections faces a number of problems including limited number of effective antifungal agents, toxicity of the available antifungal agents, resistance of *Candida* to commonly used antifungals, relapse of *Candida* infections and non-cost effective antifungal agents[5]. Therefore, new agents from natural resources that can inhibit the growth of *C. albicans* are greatly needed and would enhance the number of effective therapeutic alternatives.

Euphorbia hirta (*E. hirta*) L. belongs to the family

Euphorbiaceae. It is a small annual herb common to tropical countries. It is usually erect, slender-stemmed, spreading up to 80 cm tall, though sometimes it can be seen lying down. The plant is an annual broad-leaved herb that has a hairy stem with many branches from the base to the top. The leaves are opposite, elliptical, oblong or oblong-lanceolate, with a faintly toothed margin and darker on the upper surface. The flowers are small, numerous and crowded together in dense cymes (dense clusters in upper axils) about 1 cm in diameter. The stem and leaves produce a white or milky juice when cut. It is frequently seen occupying open waste spaces, banks of watercourses, grasslands, road sides, and pathways[6–8].

E. hirta L. is a very popular herb amongst practitioners of traditional medicine, widely used as a decoction or infusion to treat various ailments including intestinal parasites, diarrhoea, peptic ulcers, heartburn, vomiting, amoebic dysentery, asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, sterility and venereal diseases. Moreover, the plant is also used to treat affections of the skin and mucous membranes, including warts, scabies, tinea, thrush, aphthae, fungal afflictions, measles, guinea worm and as an antiseptic to treat wounds, sores and conjunctivitis. The plant has a reputation as an analgesic to treat severe headache, toothache, rheumatism, colic and pains during pregnancy. It is used as an antidote and pain relief of scorpion stings and snakebites. The use of the latex to facilitate removal of thorns from the skin is common[6,9].

Our previous studies showed that the methanol extract

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of *E. hirta* L. possessed a good *in vitro* antifungal activity against *C. albicans* with 21 mm zone of inhibition, a minimum inhibition concentration (MIC) value of 3.125 mg/mL, and the time kill study revealed a fungicidal effect with 1 and 2 MIC values of the plant extract[6]. On the other hand, leaf extract exhibited significant antioxidant activities when evaluated by DPPH, ferric reducing power assays and also found to possess noticeable content of phenolic and flavonoid compounds. The present study was carried out to further substantiate the anticandidal activity of the leaf extract at ultrastructural level by using electron microscopy.

2. Materials and methods

2.1. Plant collection

The fresh plant was harvested from various areas in Universiti Sains Malaysia, in June 2009. The taxonomic identity of the plant was confirmed by the botanist of School of Biological Sciences at Universiti Sains Malaysia. The plant materials were washed under tap water and separated into leaves, flowers, stems and roots. The separated parts were air dried in shade for ten days and then in oven at 60 °C for one to two days, then grinded to fine powder by using electric blender and stored in clean labelled airtight bottles.

2.2. Preparation of the plant extract

A hundred grams of each part powder was extracted by maceration in 400 mL of methanol for 14 days with frequent agitation. The mixture was filtered through clean muslin cloth followed by double filtration with Whatman No. 1 filter paper and the filtrate was concentrated by rotary evaporator with vacuum at 50 °C, poured in glass Petri dishes and brought to dryness at 60 °C oven. The percentage yield of the crude extract was determined for each part and was 11.1%, 7.3%, 4.7% and 4.1% for leaves, stems, flowers and roots, respectively. The obtained paste like mass was then stored in parafilm sealed Petri-dishes in dark cabinet. The extracts were reconstituted by dissolving in methanol to the required concentrations. The reconstituted extracts were maintained at (2–8) °C.

2.3. Transmission electron microscope (TEM)

The preparation procedure of *C. albicans* cultures for TEM was the same as described previously in SEM section. The scratched samples were fixed in in McDowell–Trump fixative prepared in 0.1 M phosphate buffer for 24 h at 4 °C, washed twice in the same buffer, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 1 h at 4 °C, then double washed in distilled water. The samples were blocked in warm agar (50 °C), dehydrated in ethanol series (50%, 75%, 95% and double 100%) and embedded in Spurr's resin. Finally, glass knives were used to obtain ultra thin sections of 90 nm that were stained with uranyl acetate and lead citrate for observations under TEM (LIBRA 120– ZEISS)[10].

3. Results

Control (untreated) *C. albicans* cell as examined by TEM showed typical morphology of *Candida* with a uniform central density, typically structured nucleus, and a cytoplasm with several elements of endomembrane system

and enveloped by a regular, intact cell wall (Figure 1). After 12 h of treatment with *E. hirta* L. leaf extract (Figure 2), the cell appeared very dense with the vesicles and membranous bodies dispositioned within the cell. The cytoplasmic volume started to decrease. After 24 h of exposure (Figure 3) the cells exhibited notable alterations in the cell membrane and the cell wall. The cytoplasmic volume decreased more with notable structural disorganization within the cell cytoplasm. Figure 4 showed the effect of *E. hirta* extract on the yeast cells after 36 h of exposure. A certain number of cells apparently underwent drastic shape changes and some lysed and collapsed. It seems that the plant extract induced dysfunctions of the cell membrane and the cell lost its metabolic functions at this stage.

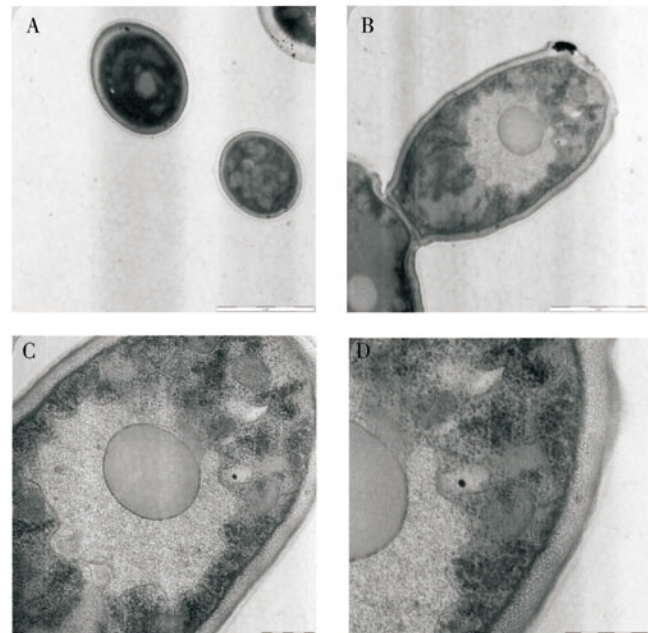


Figure 1. TEM images of the untreated cells of *C. albicans* (after 24 h). A: 4 000×; B: 5 000×; C: 10 000×; D: 16 000×.

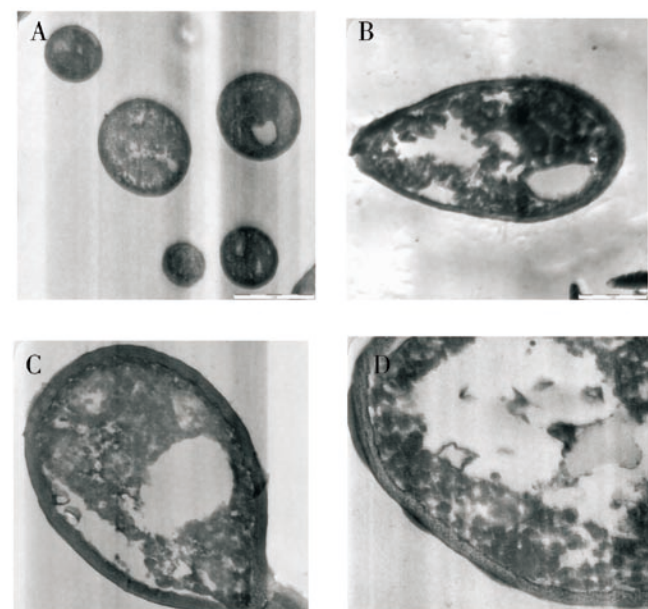


Figure 2. TEM images of *C. albicans* cells after 12 h treatment with *E. hirta* L. leaf extract. A: 4 000 ×; B: 5 000 ×; C: 10 000 ×; D: 16 000 ×.

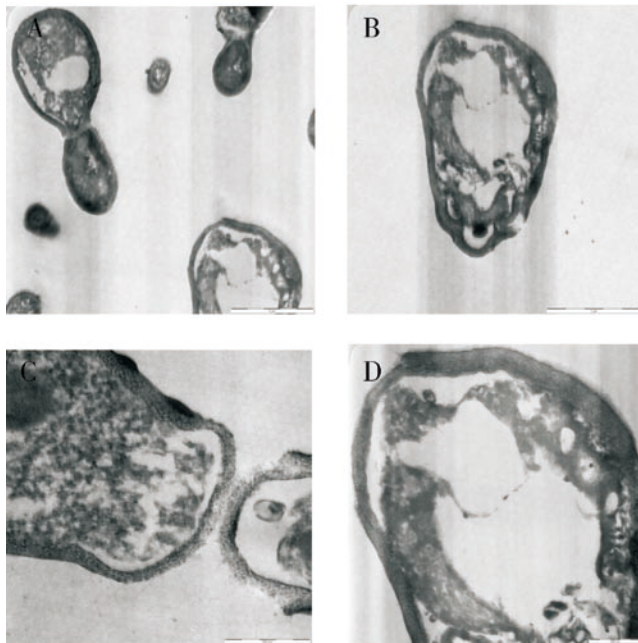


Figure 3. TEM images of *C. albicans* cells after 24 h treatment with *E. hirta* L. leaf extract.

A: 4 000 ×; B: 5 000 ×; C: 10 000 ×; D: 16 000 ×.

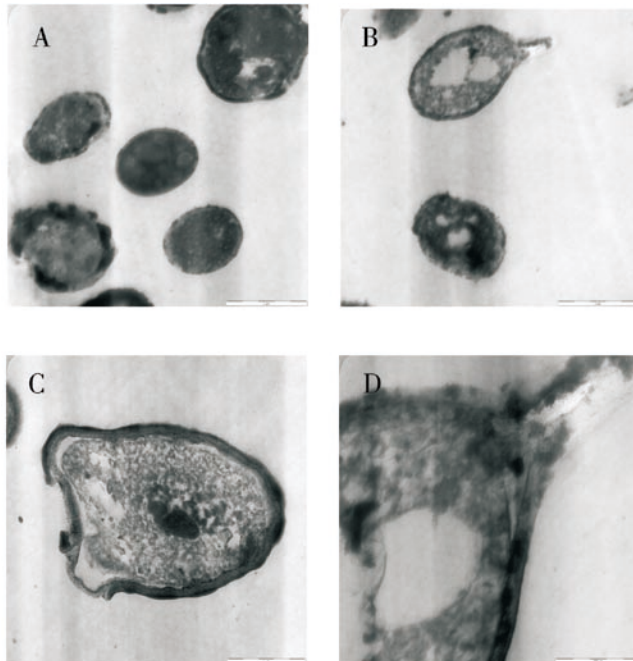


Figure 4. TEM images of *C. albicans* cells after 36 h treatment with *E. hirta* L. leaf extract.

A: 4 000 ×; B: 5 000 ×; C: 10 000 ×; D: 16 000 ×.

4. Discussion

Longer exposure to the extract inhibits the total growth of *C. albicans*. Seed extract of *E. hirta* possesses anticandidal activity against *C. albicans* strain as shown in the electron microscopy observation and further investigations are necessary on mode of action of the extract on *C. albicans* cells prior to the recommendation for its use as a safe and effective antifungal therapy.

The *E. hirta* has never been evaluated for anti-*Candida* activity before, it is the first time to study anti-

Candida activity. Therefore, further purification of active compound(s) from *E. hirta* extract and its individual antimicrobial activity are needed. The significant antimicrobial activities exerted by *E. hirta* suggest it as a potential source for developing new antimicrobial agents. Further studies of the bioactive compounds and the *in vivo* evaluation of the antimicrobial activity against *C. albicans* are therefore suggested.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

Deep thanks to Islamic Development Bank for the financial support with a master degree scholarship and to the staff of Electron Microscopy Unit, School of Biological Sciences, USM for help and facilities.

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