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RESEARCH ARTICLE

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ANTIFUNGAL ACTIVITY OF *CARICA PAPAYA* SEED EXTRACT AGAINST *ASPERGILLUS FLAVUS* AS SERIOUS MYCOTOXINS PRODUCING ORGANISM AND CAUSAL ORGANISM FOR ASPERGILLOSIS

ABSTRACT:

Aqueous *Carica papaya* seed extract was investigated for its antifungal activity against *Aspergillus flavus* using cut plug method. *C. papaya* seed extract has inhibitory activity against *A. flavus* with inhibition zones ranging between 11 to 16 mm. *A. flavus* cells surviving ratio was decreased with increasing the *C. papaya* seed extract concentrations from 25 to 200 mg/ml. Treating the organism with *C. papaya* seed extract led to an external changes, irregular cell shape and disintegration of fungus cell wall under transmission electron microscope. On other hand, studying the effect of the aqueous *C. papaya* seed extract on mice lung has showed safe effect which might explore in lung disease treatment. Furthermore *C. papaya* had antioxidant characters. Analysis by GC-MS showed at least 15 components, esters were the most abundant group of compounds.

KEY WORDS:

Carica papaya, Seed extract, *Aspergillus flavus*, antioxidant characters, GC-MS

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INTRODUCTION:

Infectious diseases constitute the world major threat to human health and account for almost 50.000 deaths daily (Ahmad and Beg, 2001). Till date, natural plant extracts of various types are used in African Medicine for providing healing to various aliments even before and after the spread of modern and scientific medicine (Ogunjobi and Ogunjobi, 2011). The incidence and increasing frequency of microorganisms that are resistant to common and generally accepted effective first choice drugs is on the increase. *Aspergillus species* are ubiquitous molds found in organic matter. Although more than 100 species have been identified, the majority of human illness is caused by *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus clavatus*. The transmission of fungal spores to the human host is via inhalation. *Aspergillus* primarily affects the lungs, causing 4 main syndromes, including allergic bronchopulmonary aspergillosis (ABPA), chronic necrotizing *Aspergillus* pneumonia (or chronic necrotizing pulmonary aspergillosis (CNPA)), aspergilloma, and invasive aspergillosis (Harman*,* 2012). Also Aspergillosis is an invasive disease of the lung (Hartemink *et al.*, 2003). The development of resistance to the newer antibiotics by microbes leading us to search for newer sources of antimicrobial a global challenge involving research institutions, pharmaceutical companies and academia (Melendez and Carpiles, 2006; Adekunle and Adekunle, 2009). Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes fats, oils, minerals, vitamins, alkaloids, quinones, terpenoids, flavonoids, carotenids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols (Agbaje *et al.,* 2011).

Carica papaya linn (family caricaceae), commonly called pawpaw (English), Ibepe (Yoruba-Nigeria) or Okroegbe (Igbo-Nigeria), is a tree-like herbaceous plant, widely cultivated for its edible fruits. It originated from Southern Mexico and Costa Rica (Agbaje *et al.,* 2011).

The vegetative parts of *papaya* plant have enormous medicinal uses in various parts of Africa .The seeds of pawpaw have been reported to cure cough when eaten raw in some parts of Nigeria (Gills, 1992).

Also fruit contains certain immune-stimulating and anti-oxidant agents (Aruoma *et al.,* 2006) and the seeds are used as a potential post-testicular anti fertility drug (Lohiya *et al.,* 2000, 2005 & 2006). The latex and seeds are used in the treatment of gastrointestinal nematode infections and they have shown anthelmiintic activity (Stepek *et al.,* 2005). The seeds and immature fruit have shown inhibitory activity against human enteric pathogens (Osato *et al.,* 1993; Afolayan, 2003 and Krishna *et al.,* 2008).

The present study aimed to investigate the antifungal efficiency of aqueous dried *Carica papaya* seed extract against *A. flavus* and compared its efficiency with standard antifungal. Also, the study the efficiency of aqueous dried *C. papaya* seed extract on mycelium and conidia of *A. flavus* under transimission electron microscope. Moreover, the study aimed to the effect of *C. papaya* seed extract on lung tissue of experimental mice compared with antifungal control to investigate its safety for use.

MATERIAL AND METHODS:

Carica papaya seeds were obtained from Agriculture Unit of Tanta Agriculture Directorate which isolated from fruits, and then seeds were sun dried for several days and later oven dried at 50^{o}C for 24 hours and then blended into powder (Ogunjobi and Ogunjobi, 2011).

Seed extraction method:

The extraction of seeds was carried out using distilled sterilized water as extracting solvent. Fifty gram of seeds of the plants were weighed and dissolved in 1000 ml of distilled sterilized water inside 2 litre conical flask and covered with parafilm (Ogunjobi and Nnadozie, 2004; Ogunjobi *et al.,* 2007). The flask was shaken vigorously at 150 rpm overnight and after that left to stand 12 hours at room temperature. The resultant mixture was then filtered with whatman's No. 1 filter paper and muslin sieve to remove particles of plant sample. The clear supernatant was then collected in sterile pre-weighed tubes and stored at 4^{o}C until when needed.

Fungal isolation:

Aspergillus flavus was isolated from a patient with bronchial asthma. The isolate was maintained on 2%Potato Dextrose Agar(PDA) agar medium for 7 days at 28 ± 2^{o}C with adding 0.05g/L chloramphincol as antibacterial agent (Mahmoud *et al.,* 2011) with minor modification. The isolate was identified microscopically according to Moubasher (1993).

Molecular identification of the microorganism and accession number:

DNA extraction:

The mycelium of isolated fungus was scratched off the surface of 2% Potato Dextrose Agar PDA Petri plate. The mycelia (50 mg) were ground in liquid nitrogen using a mortar and pestle. DNA was extracted from the powdered tissue using i-genomic DNA extraction Mini Kit (INTRON Biotechnology, Inc, Cat. No. 17371) according to manufacturer's instructions. The eluted DNA was stored at -20ºC.

PCR condition:

Amplification of internal transcribed spacer (ITS) region was conducted in an automated thermal cycler (C1000TM Thermal Cycler, Bio-RAD) using ITS4 (5`-TCCTCCGCTTATTGATATGC-3`) and ITS5 (5`-GGAAGTAAAAGTCGTAACAAGG-3`) primers (White *et al.,* 1990). The following parameters were used: 35 cycles of 94ºC for 30 s, 51ºC for 1 min, 72ºC for 1.5 min, and a final extension at 72ºC for 3 min. Each PCR mixture (25 µl) as follow, (1 µl) of 25 ng nucleic acid, 1 µl of each primer (10 pmol), (12.5 µl) of GoTag® Colorless Master Mix (Promega Corporation, USA) and 9.5 µl of Nuclease free water (Promega). 15 µl of all PCR products were analyzed by electrophoresis through a 1% agarose gel, stained with ethidium bromide, and DNA bands were visualized and photographed using a UV transilluminator. The experiment was carried out at Plant Pathology and Biotechnology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Kafrelsheikh University, Egypt.

Sequencing and Sequence analysis of the ITS region:

The amplified PCR amplicon was submitted by City of Scientific Research and Technology Applications, New Borg El Arab City, Alexandria, Egypt to Macrogen Company (Seoul, Korea) to be sequenced. The DNA nucleotide sequence was analyzed using DNA BLASTn (NCBI). Pair wise and multiple DNA sequence alignment were carried out using Clustal W (1.82) (Thompson *et al.,* 1994).

Determination of antifungal activity:

The antifungal activity of seed extracts were determined by Cut plug method diffusion technique. One millilitre of *A. flavus* spore suspensions (10^{6}cells/ ml) was introduced separately and thoroughly mixed with 20 ml of Sabouraud Dextrose Agar (SDA) in triplicates (Pridham, 1956, Hugo and Russell, 1998; Anibijuwon and Udeze, 2009). A sterile 6 mm cork borer was then used to punch hole in the inoculated agar and the agar was then removed .Wells were filled with different concentrations of the seed extract which were labelled accordingly; 200 mg/ml 100 mg/ml, 50 mg/ml and 25 mg/ml compared with standard drug diflucan (fluconazole 2 mg/ml) and distilled water served as control. This compared was done in triplcate plates and was incubated at 28 ± 2^{o}C for three days. After incubation, the diameters of zones of inhibition around each well were measured and the mean were then calculated.

Effect of *Carica papaya* seed extract on the cell wall of *A. flavus* under transmission electron microscope:

In this experiment, transmission electron microscope was applied to detect the effect of papaya seed extract in the conidia cell wall and mycelia of *A. flavus* at concentration 100 & 200 mg/ml compared with control (without papaw seed extract). A small portion of the fungal mat was fixed at room temperature in 2% (v/v) glutaraldehyde mixed with potassium in 2% (w/v) osmium tetraoxide buffered in 0.005 M sodium cacodylate at pH 6.5 for 40 min. After fixation, the material was washed overnight in the appropriate buffer, dehydrated at room temperature in acetone, and embedded overnight at 65°C in low viscosity epoxy resin (Spuur, 1969). At these conditions, the material was polymerized; ultrathin sections were cut by glass knives of an ULKD ultramicrotome. Sections were collected each on stabilized copper grids, stained with lead citrate and examined in a GOL 100 CX electron microscope. This method was carried out according to the instructions of Ellis and Griffiths (1974) and was applied in T.E.M. unit in Faculty of Medicine, Tanta University.

Experimental animals:

Twenty four mice were bred in the microbiology unit, mycology lab, Faculty of Science, Tanta University. Male mice aged 8 to 10 weeks and weighing 18 to 20 g were used throughout. They were maintained under standard laboratory condition at temperature of 22 ± 2^{o}C under 12 h light dark cycle and were fed with standard diet and water *at libitum*.

Experimental Treatment:

Mice were divided in to four groups (n = 6/group). The mice were injected intraperitoneal (i.p.) with 0.02 mg\24 hs of concentration 100mg and 0.04 mg/24h of concentration 200mg of *C. papaya* seed extract in 1ml of saline 0.9% for 5 days. In which each mice received doses divided on three times every 2 hrs/24 hrs. Then after 2 hrs of final dose injection mice after 5 days, the mice lungs were removed under aseptic condition. The respective control animals received 0.9% saline (non-immunized) and fourth group was received fluconazole to determine the effect of *Carica papaya* seed extract on mice lung and compared with synthetic drug as fluconazole, to evaluate whether it can be used as medicinal drug or not (its safety). The mice were bred in the microbiology unit, mycology lab, Faculty of Science, Tanta University.

Histology:

Histological examination was done in Histology Department, Faculty of medicine, Tanta University by fixing mice lungs tissues in 10% formalin in solution, processed and embedded in paraffin wax. Lung tissues blocks were sectioned at 5µm thick and stained with Haematoxylin and Eosin (H & E) (Mahmoud *et al.,* 2011) and divided to four groups as following:

Group A: Control group administered with equivalent volume of 0.9% saline for 5 days.

Group B: Mice were administered with 100 mg/ml dose for 5 days.

Group C: Mice were administered with 200 mg/ml dose for 5 days.

Group D: Mice were administered with 2 mg/ml fluconazole dose for 5 days.

All lung section samples were photographed under Carl Zeiss Axiostar light microscope connected with digital Canon camera soft program zoom browser at 40 x mags in central laboratory, Zoology Department, Faculty of Science, Tanta University.

Statistics:

Significance of variation of fungal inhibition zone under different concentration of *C. papaya* seed extract was determined. Statistical presentation and analysis of the present study was conducted using the mean, standard deviation (ANOVA) tests by SPSS.V. 16 (Pipkin, 1984) for determination of the efficiency of *C. papaya* seed extract against *A. flavus* as antifungal agent.

Measuring the *A. flavus* surviving ratio:

A cell suspension of *A. flavus* (0.5 ml) was mixed with 9.5 ml of Sabouraud medium broth in sterile test tube containing *C. papaya* seed extract. The amount of dried seed extract was adjusted to give 25, 50, 100, and 200 mg/ml of the final solution. The seeded tubes were then shaken overnight at 250 rpm at 37^{o}C. Only 0.1ml of each solution was spread onto an agar plate of Sabouraud medium. Control without the dried seed extract was prepared and the plates were incubated at 28^{o}C for 24 h and 48 h then the numbers of colony forming units (CFU) were recorded. The surviving ratio (M/C) was calculated for organism at different seed extract concentrations against that of the control, where M is the number of organism in the presence of a certain concentration of the dried seed extract, and C is the number of organism in the control as cited in (Kenawy *et al.,* 2006).

Gas chromatography:

Carica papaya seed extract content was examined by gas chromatography, Massepectroscopy in Claurs 580/560S. Work was done with column 30.0m x 250µm, Rtx–5MS (crossbond 5% diphenyl 95% dimethyl polysiloxane), Perkin ElmerCompany in Central lab, Tanta University, equipped with heated FID.

The GG conditions were employed using Helium as carrier gas (0.8ml/min) and the temperature program was 65^{o}C for 3 min, followed by an increase of 12^{o}C/min to 180^{o}C for the remainder of the run. Detector and injection point heaters were 275 and 250^{o}C respectively, and typically 0.1 or 1.0ul was injected at a 25: 1 split.

Determination of antioxidant activities by:

a- Reducing power:

The reducing activity of the samples was determined following the method of Oyaizu (1986). An equal volume (0.3 ml) of seed extract at different concentrations (1-3-5) mg/ml, 1.0% potassium ferricyanide and 0.2 M sodium phosphate buffer were mixed thoroughly. The mixture was incubated at 50°C for 20 min and then 0.3 ml of 10% trichloroacetic acid was added. The mixture was centrifuged (6000 rpm) at 4°C for 10 min. The upper layer (0.6 ml) was mixed with 0.12 ml of 0.1% ferric chloride and deionized water (0.6 ml). After 10 min of mixing, the absorbance of this mixture was measured at 700 nm. A higher absorbance of this mixture indicates a higher reducing power. Ascorbic acid was used as standard antioxidant compounds.

b- Chelating Effects on Ferrous Ions:

Fe^{2+} chelating ability of extract was determined according to the method of Dinis *et al.* (1994) and Decker and Welch (1990). The Fe^{2+} level was monitored by measuring the formation of the ferrous ion ferrozine complex. 1ml of seed extract at different concentrations (5-10-15) mg/ml was mixed with 3.7 ml methanol, 0.1 ml of 2 mM Fe Cl_2 and 0.2 ml of 5 mM ferrozine and the mixture was shaken and left at room temperature for 10 min. The absorbance of the resulting solution was measured at 562 nm. A lower absorbance indicates a stronger Fe^{2+} chelating ability. Ethylenediaminetetraacetic acid (EDTA) was used as standard compound.

The ability to chelate the ferrous ion was calculated as follows:

Chelating effect (%) = (1-absorbance $_{sample}$ / absorbance $_{control}$) x 100

c- DPPH radical scavenging activity:

1-Diphenyl-2-picrylhdrazyl (DPPH) radical-scavenging activity was examined with the method of Shimada *et al.* (1992). A total of 2 ml *papaya* seed extract of various concentrations (5-10-15 mg/ml) were mixed with 1 ml methanolic solution of 0.3 mM DPPH radical. The mixture was shaken vigorously and left to stand for 30 min in darkness. DPPH radical reduction was determined by measuring the absorbance at 517 nm against a blank, ascorbic acid are used as standard. The scavenging activity is expressed as follows:

Scavenging activity % = [(A_{517} of control – A_{517} of sample)/ A_{517} of control] x 100.

RESULTS AND DISCUSSION:

Molecular Identification:

Nowadays, it is useful to identify fungi by molecular identification, for that 18S rRNA gene sequence offered useful method for identification of fungi. Using the universal fungal primers (ITS_4/ITS_5) for identifies the tested organism, where ITS_4, ITS_5 amplified PCR fragment was sequenced. The sequence fragment was blasted (Altschul *et al.,* 1990) to investigate whether high homology of tested *A. flavus* was found to other *A. flavus* NCBI. It was found about 98% similarity PCR sequence to species of *Aspergillus flavus*. For studied *A.flavus* showed highest similarity to *A. flavus* isolate PW2962 with accession No: KF562205.1 (Fig. 1a).

This provides additional evidence, supporting morphological identification for this species which was indeed identical to *A. flavus*. For studied *A. flavus* band size was obtained at 600 bp which resemble the other researchers (Fig. 1b). Henry *et al*. (2000) detected amplification fragments ranging from 565 to 613 bp for different *Aspergillus* species, which was 595 bp in *A. flavus*. In other fungal groups the ITS region was also very useful in resolving taxonomic difficulties, as demonstrated by Driver *et al.* (2000) in the taxanomic revision of Inglis and Tigano, (2006) to reclassify entomopathogenic species of *Paecylomyces*, previously misidentified by classical methods.

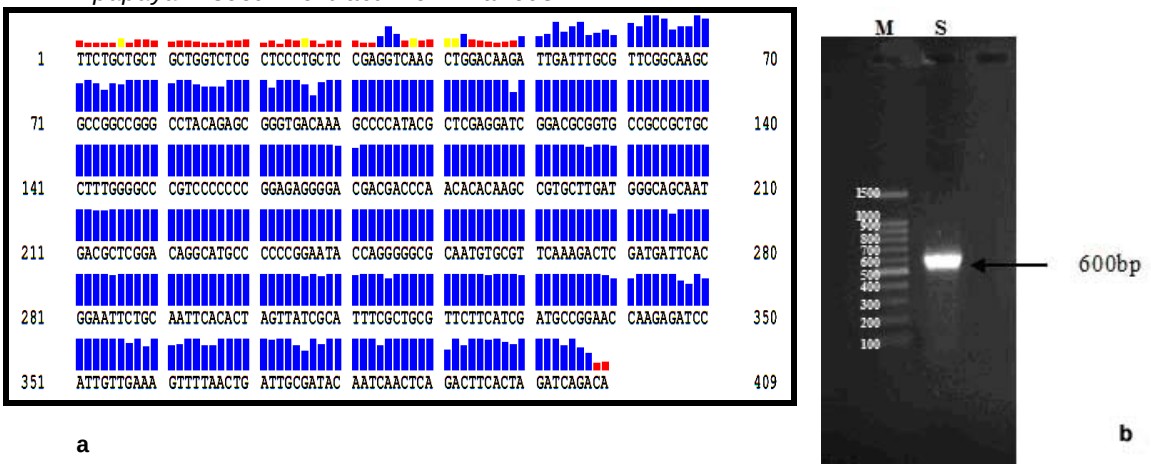


Fig. 1. a: The nucleotide sequence of the 18sr RNA gene of test *A. flavus* amplified by the PCR;
b: PCR amplification profile of the ITS region ITS4/ITS5 primers for the sample band size 600 bp

Antifungal sensitivity test:

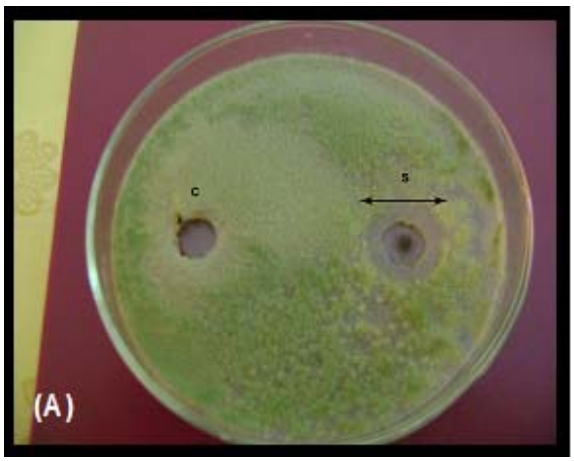
Activity of crude seed *C. papaya* extract (Fig. 2) was examined against the growth of *Aspergillus flavus* which is the causal organism of Aspergillosis in lung tissue and also secret dangerous mycotoxins so, it was important to study the efficiency of extract against this pathogen. Table 1 and figure 2 a&b showed that antifungal activity of seed *C. papaya* extract increased significantly with increasing the extract concentration from (25 to 200) mg/ml. *C. papaya* seed extract had inhibitory effect on *Aspergillus flavus* with inhibition zones ranged between 11-16 mm (Fig. 3 A&B).

Fig. 2. Seed of *Carica papaya*.

Table 1. Mean diameters of inhibition zones (mm) of different concentrations of *C. papaya* crude extract on *Aspergillus flavus*

Organism	Different concentrations of seed extract mg/ml	Mean of Inhibition Zone (mm)	*P.* value
Aspergillus flavus	25	11 ± 0.1	0.011
	50	12 ± 0.3	0.009
	100	15 ± 0.5	0.001
	200	16 ± 0.5	0.001
Fluconazole	2	21 ± 0.1	0.048

Value is the mean + SD of three replicates.
* Significant <0.05.



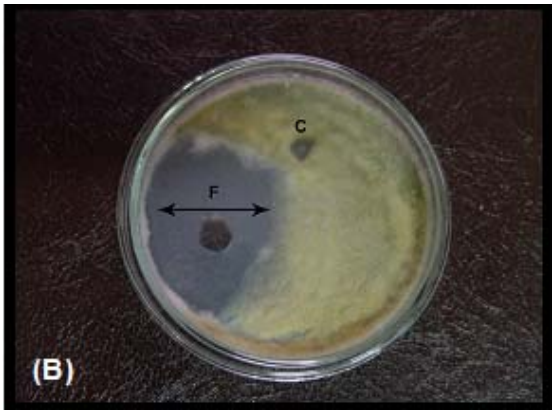


Fig. 3. A&B: Inhibition zones (mm) at S: 200 mg/ml caused by of *C. papaya* seed aqueous extract against *Aspergillus flavus* = 16 mm and C: control: distilled sterilized H_2O and F: Inhibition zones (mm) at 2 mg/ml of fluconazole = 21 mm.

Inhibition zone of *A. flavus* increased with increasing seed extract concentrations whereas 200 mg/ml of seed extract gave 16 mm almost comparable to 21 mm inhibition zone performed by fluconazole.

C. papaya contains many biological active compound, the two important compounds of which are chymopapin and papin, which are supposed to aid in digestion. The level of compound varies in the fruit, latex, leaves roots and seeds (Krishna *et al.,* 2008). It has been used for treating digestive problems and intestinal worms. The softening qualities of papin have been taken advantage of in the treatment of warts, corns, sinuses and chronic forms of scaly eczema, cutaneous tubercles and other form of the skin. Papin also is used to treat arthritis (Krishna *et al.,* 2008).

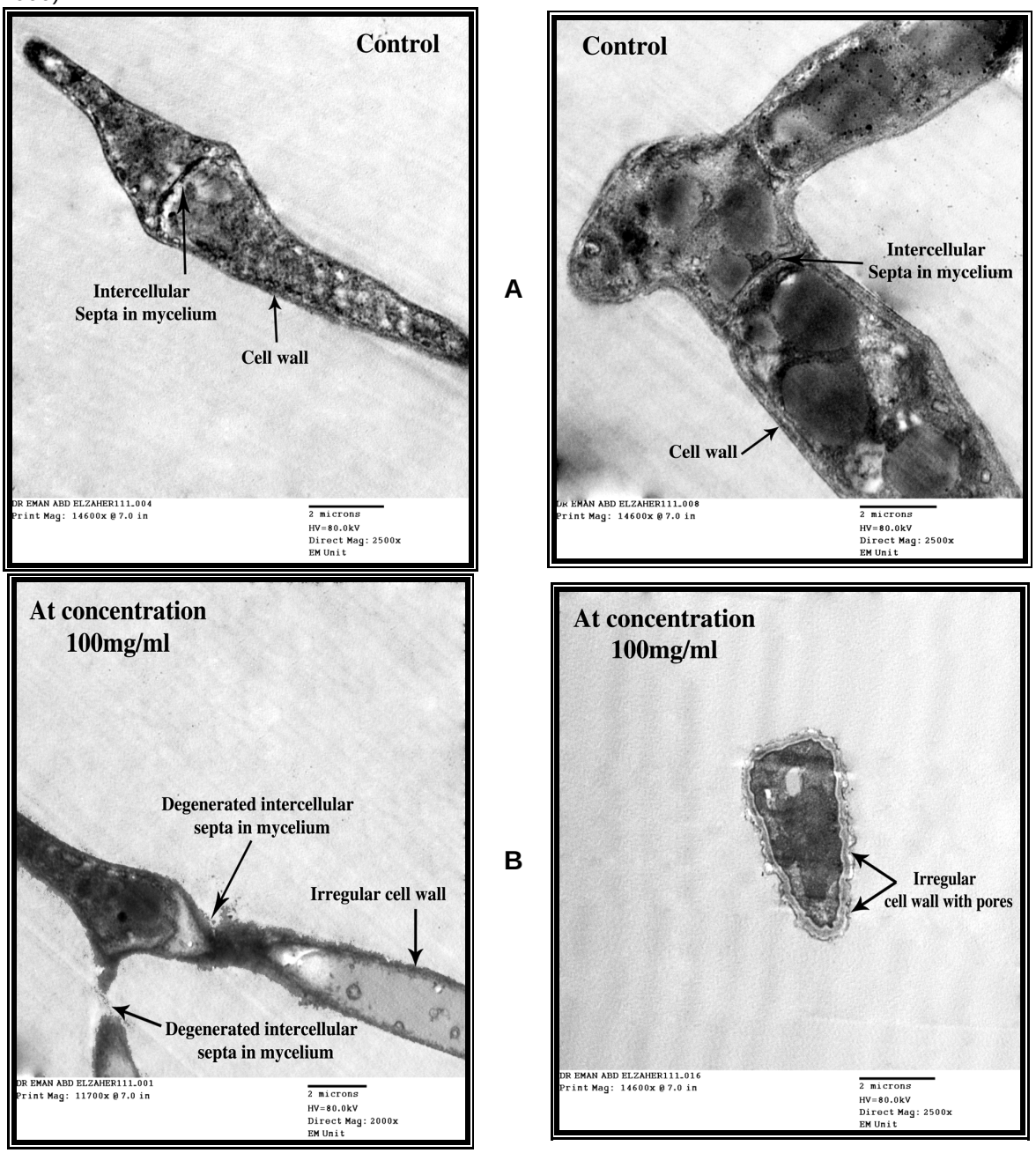
The presence of bioactive substances has also been reported to confer resistance to plants against bacteria, fungi and pests, (Srinivasan *et al.,* 2001) and therefore explains the fungal activity of seed extract against *A. flavus* studied. In present study the increasing of concentration of *C. papaya* seed extract from 25 to 200 mg/ml increase inhibitory efficiency inhibition zone against *A. flavus* from 11-16 mm, respectively. This may possibly due to the active compounds within the seed of papaw such as glycosides and caricin. Other essential biologically active compounds include alkaloids, carpaine pseudocarpaine, flavanols, butanoic acid, tannins, linalool, benzylglucosinolate, cis and trans linalool terpenoids, alpha-palmitic acid might contribute to seed inhibition efficiency (Chukwuemeka *and Anthonia,* 2010). Our results are almost similar to (Chukwuemeka *and Anthonia,* 2010) in which they found that *A. niger* mycelia were inhibited more with the semi-ripe seed extract. For *Rhizopus* spp the seed extract from unripe papaw inhibited its mycelial growth significantly when compared to *A. niger* and *Mucor* spp. Agbaje *et al.* (2011) found that the aqueous root extract of *papaya*

had inhibitory activity against *Microsporum audouinii* with inhibition zone (2.8 to 19.8 and 11.5 to 23.7 mm) against *Trichophyton rubrum* at ranged concentration from 12.5 to 400 mg/ml of papaya root extract for each fungus. On other hand, aqueous root extract of *papaya* had inhibitory activity against *Staphylococcus aureus* and *Bacillus subtilis* with inhibition zone ranged between 24 to 30 and 21.5 to 27.1 mm, respectively at different concentrations from 10 to 80 mg/ ml of aqueous root extract of *papaya* (Agbaje *et al.,* 2011). Fluconazole is very effective against filamentous fungi especially *Aspergillus flavus* that is in agreement with other study (Jons and O'day, 1999).

Effect of *C. papaya* seed extract on the cell wall of A*. flavus* by TEM:

Examining *A. flavus* morphology in the control sample indicated normal structure for mycelia and conidia of the fungus (Fig. 4A).

Concentration (100 mg/ml) of *C. papaya* seed extract had affects on cell wall; forming some external protrusion, in both conidia and mycelium, degenerated intercellular septa in mycelium with torned cell wall in conidia and cause irregular cell wall (Fig. 4B). *A. flavus* treated with 200mg/ml *C. papaya* extract highly affected the fungus morphology and led to irregular cell shape with destroyed cell wall and shrinkage of cell cavity (Fig. 4C).



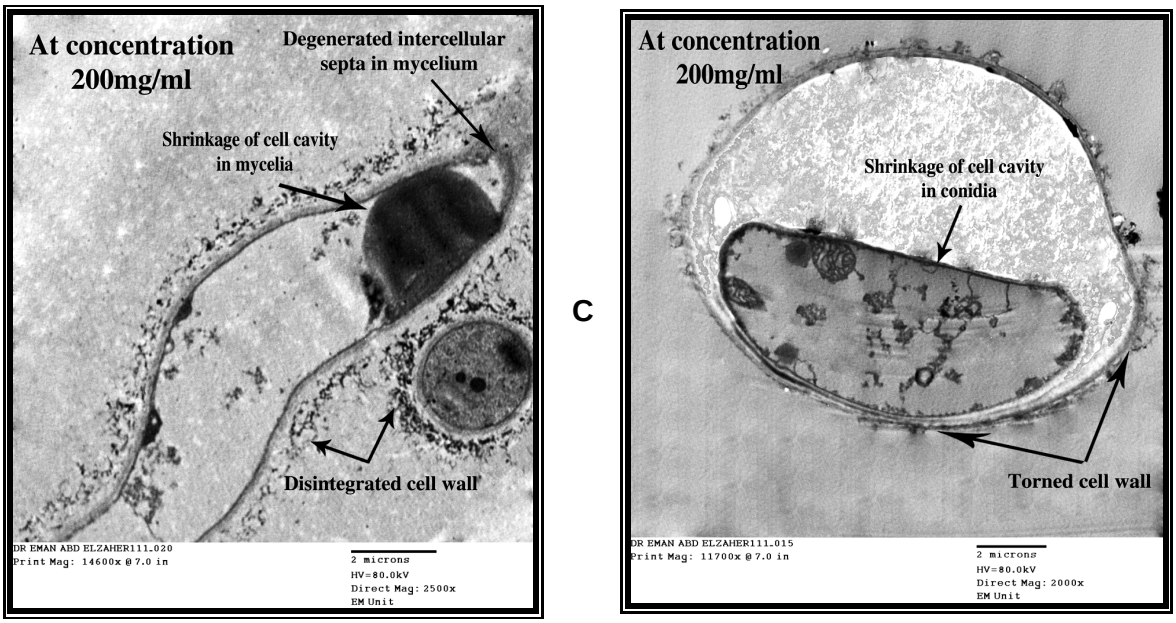


Fig. 4. Transmission electron microscope of *Aspergillus flavus* conidia and mycelia (A) Control; (B) At concentration 100mg/ml and (C) At concentration 200mg/ml of *C. papaya* seed extract.

Changes in cell wall of fungus; external, disintegration and irregular cell wall was found under TEM treated with (100 and 200) mg/ml of papaw seed extract. Also there are some deformation inside cell and shrinkage of cell cavity this may be due to presence of antioxidant properties of tested extract which are effective superoxide antioxidants with ability to inhibit mycelial growth by reacting with cell wall components (Chukwuemeka *and Anthonia,* 2010). From research findings, it has been noted that lytic enzymes found on *papaya* seed extracts may have target site on the cell wall of this fungus. The mode of action may possibly be by attack on the sugar residues on the cell wall of fungal species according to (Yoshio and Yoshio, 1981); glucose was detected as a main sugar component in the cell wall of *A. niger* whereas in *Rhizopus* spp. glucosamine and N. acetyl glucosamine were the major components hence the clue to remarkable inhibitory effects exhibited by the extracts may be attributed to this mode of action. Antibacterial activity of *C. papaya* seed could be correlated to its scavenging action on superoxide and hydroxyl radical which could be part of cellular metabolism of the enteropathogens (Osato *et al.,* 1993).

Histology:

Section of lung tissue in control mice, treated groups and fluconazole group (Figs 5-8), have similar structure because most of lung is composed of thin-walled alveoli. The alveoli are composed of a single layer of flattened epithelial cells. The extract presents a positive effect on the alveolar architecture. Treated groups indicated a presence of a dilatory effect on alveolar ducts, alveolar sacs and alveoli and there was no observable loss of alveolar architecture no emphysematous areas, and no alveolar congestion in the treated groups. In the present study the *C. papaya* seed extract has no bad abnormal effect on treated lung mice but may increase size and the dilation of alveolar sacs and alveoli (Figs 5-8) compared with control and fluconazole treated lung mice.

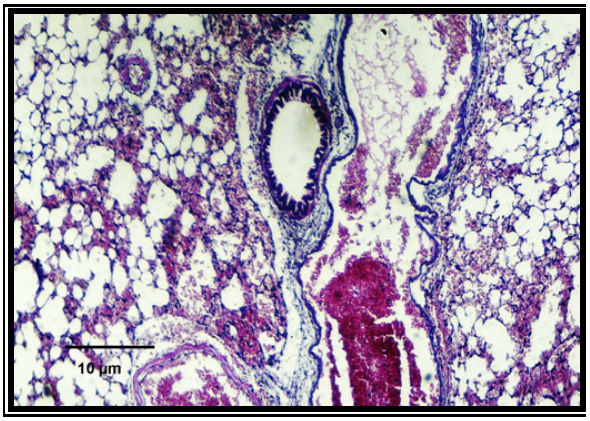


Fig. 5. Photomicrograph of H&E-stained lung of control (A) group after 5 days. x 40

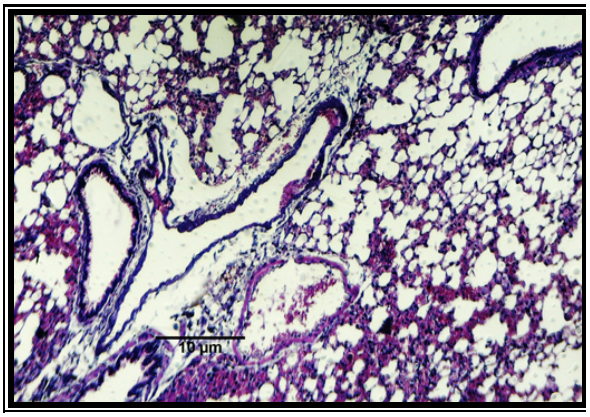


Fig. 6. Photomicrograph of H&E-stained lung of group (B) after 5 days. x 40

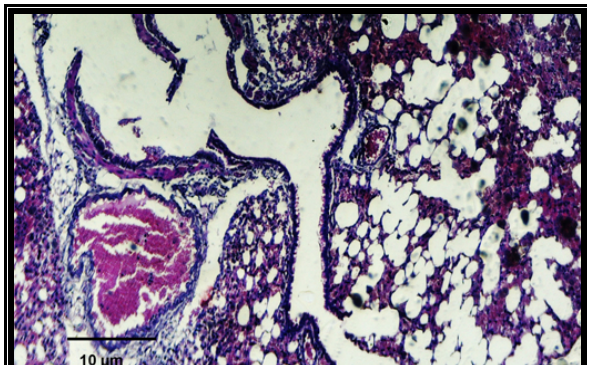


Fig. 7. Photomicrograph of H&E-stained lung of group (C) after 5days. x 40

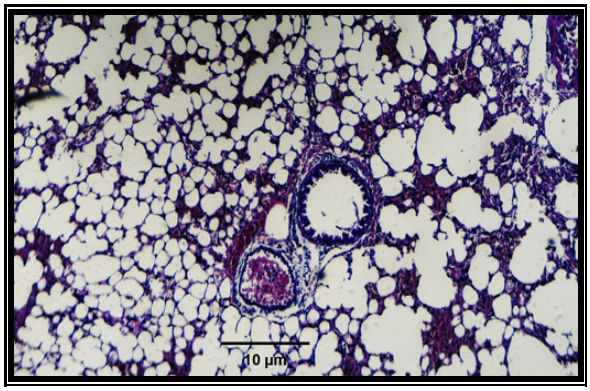


Fig. 8. Photomicrograph of H&E-stained lung of fluconazole (D) group after 5days. x 40

The dilatory effect may be due to increase elasticity of the elastic fibers of the supporting tissue surrounding the alveolar duct and the openings of the alveolar sacs and alveoli this is similar effect produced by ethanolic extract of shoot system of *Garcinia kola* (Ofusori *et al.,* 2008). In our study the seed extract effect on treated lung may be due its antioxidant properties that might be beneficial effect since there was no abnormal effect on its anatomical change. Antixoidant protect cells from toxins by moving up oxygen radicals produced from oxidative stress (Cantuti Castelvertric *et al.,* 2000). Natural nutritional supplements from plant sources origin such as seed extract of *Carcia papaya* exhibits promising pharmacological properties which lead to good recovery from respiratory diseases. The antifungal activity of pawpaw seed extract on *A. flavus* was obvious at concentrations (100 and 200) mg/ml in which M/C ratio for *A. flavus* decreased at high concentration of *C. papaya* seed extract compared with low concentration of pawpaw seed extract which give high M/C ratio and these lead us to confirm the antifungal potency of *C. papaya* seed extract against *A. flavus*.

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The effect of seed extract of *C. papaya* on surviving ratio of *A. flavus* was ranged from 0.00 surviving ratio to 0.6 and 0.8 after 24 and 48 h, respectively with tested concentrations (Fig. 9). Crude *C. papaya* seed extract killed 40% and 20% after 24 and 48 h respectively at concentration 50 mg/ml. At increasing the concentration to 100 mg/ml about 60% of *A.flavus* was killed after 24 and 48 h, 70% and 69% of *A. flavus* spores were killed after 24 and 48 h at 200 mg/ml.

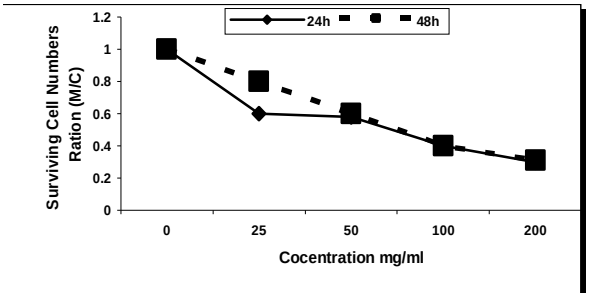

Fig. 9. Cell surviving ratio at different concentration of *C. papaya* seed extract.

Figure 10 and table 2 and show that 15 compounds were detected in Egyptian *C. papaya* seed extract. Parts of 15 compounds were similar to compounds identified for other variety in Srilanka in other search, in which they identified 37 components (Macleod and Pieris, 1983).

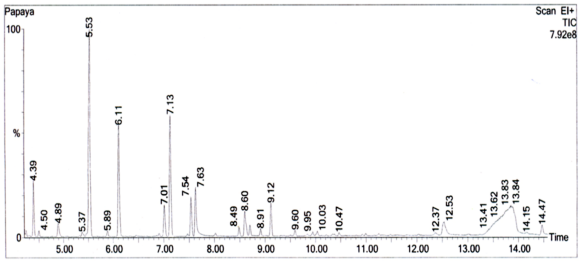

Fig. 10. Components of *C. papaya* by GC.MS.

Table 2. Components of *C. papaya*

No.	RT	Compound Name	Height	Area	Area %
1	4.393	Butane, 2,2, 3, 3-tetramethyl	211.854.352	5.659.569.5	3.731
2	4.894	Ethane, 1,1-dithoxy	50.738.708	1.927.515.5	1.271
3	5.529	Toluen	789.093.312	26.911.348.0	17.741
4	6.114	Butyl ester	423.112.864	14.519.853.0	9.572
5	7.015	β-Xylene	120.600.968	4.072.026.2	2.684
6	7.135	o-Xylene	461.919.104	15.353.781.0	10.122
7	7.540	m-Xylene	147.941.488	4.715.013.5	3.108
8	7.630	Ethanol, 2-butoxy	181.952.176	7.218.099.5	4.758
9	8.485	Benzene	33.071.004	1.051.683.8	0.693
10	8.600	1-ethyl-3-methyl	93.238.192	3.909.948.5	2.578
11	8.700	Benzene, 1,2,3-trimethyl	39.603.660	1.182.598.2	0.780
12	9.115	1,2,4-trimethyl	125.216.864	3.824.446.0	2.521
13	12.532	Thiocyanic acid	48.933.072	4.159.325.8	2.742
14	13.842	Phenylmethyl ester	110.488.032	40.895.164.0	26.960
15	14.467	Isothiocyanatomethyl	36.178.500	1.572.235.0	1.036

In the present work, a fairly wide range of different types of compounds was detected, including hydrocarbons, alcohols, aldehyde, acids, esters and ketons. In our study, *C. papaya* seed extract was dominated by esters. Also, the volalites of Srilankan *papaya* were also dominated by esters (about 53% w/w of the sample) with the major compounds (51% w/w of the sample) being a complete range of six methyl esters of even carbon numbered carboxylic acids from futanoate to tetradecanoate inclusive (Macleod and Pieris, 1983).

In our results, it were found toluene, o-xylene, p-xylene, Benzen propyl and Butane 2, 2, 3,3– tetramethyl in *C. papaya* seed extract which were similar to components of Srilankan *papaya* which was contain toluene, o-xylene and methyl butanoate (Macleod and Pieris, 1983).

The presence of isothiocyanatomethyl, thiocyanic acid and 1,2,4 trimethyl, at retentiontime 14,467, 12,532 and 9,115 min respectively, in *C. papaya* seed extract may be due the reason to its antifungal activity against *A. flavus*. This similar to other searches finding, who found that in seed herb extract of cleomechrysantha 2-methybutyl isothiocyante, isothiocyanatomethyl benzene and 4-methyl thiobutyl isocyanate which had antibacterial activity against *Pseudomonas putida* and *E. coli* (Hashem and Wahba, 2000).

Presence of thiocyanic acid, phenylmethylester at retention time 12, 532 and 13, 842 min respectively which coincided with other result (Gayosso *et al.,* 2010), who found cartenoid specially β-cryptoxanthin in *C. papaya* fruit extract previous studies demonstrated the presence of carotenoids as esters in different fruits (Ornelas-Paz *et al.,* 2008).

Andersson *et al.* (2009) observed that the content of esterified caratenoids in cherries increased in ripening, which allows esterified carotenoids to integrate more quickly to the membranes increasing the color of the fruit and its accumulation in chromoplasts (Yahia and Ornelas-Paz, 2010).

As shown in figures 11 and 12, antioxidant of *C. papaya* seed extract assayed by determining its reducing power in which its absorbance was 0.44 , 0.46, and 0.55 at (1, 3, and 5) mg/ml respectively and ferrous ion chelating ability percentage were 65.3, 72.3, and 80% at (5, 10, and 15) mg/ml respectively. *C. papaya* seed extract reducing power was higher compared to Ascorbic acid. Chelating power percentage of *C. papaya* seed extract increased from 65.3 to 80% at (5 and 15) mg/ml respectively. Antioxidant property increased with increasing its reducing power and ferrous ion chelating ability percentage. Phenolic compounds are usually found in plants in conjugated forms through hydroxyl groups with sugar as glycosides (Robbins, 1980). Natural phenolics are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α - tocopherol radicals and inhibit oxidases (Oboh and Rocha, 2007). The reducing power of aqueous *C. papaya* seed extract was assayed to confirm its antioxidant property in which most antioxidant compounds convert the oxidized form of iron (Fe^{3+}) in ferric chloride to ferrous (Fe^{2+}). Then Fe^{2+} was then monitored by measuring the formation of Perls Prussian blue at 700nm (Oyaizu, 1986), because reducing capacity of compound may serve as significant indicator of its antioxidant potential. Also, antioxidant activity of chloroform seed extract of *C. papaya* was confirmed by other researchers showing highest total phenolic content and antioxidant activity which was higher than acetone extract (Kothari and Seshadri, 2010).

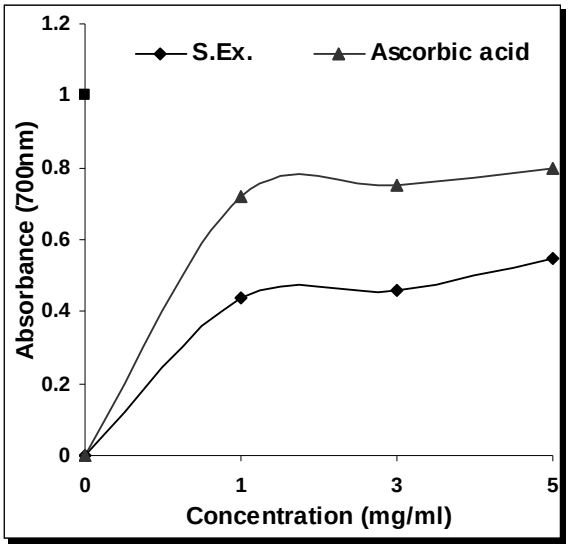

Fig. 11. Reducing power of *C. papaya* seed extract and Ascorbic acid by spectrophotometric detection of Fe^{3+}-Fe^{2+} transformation

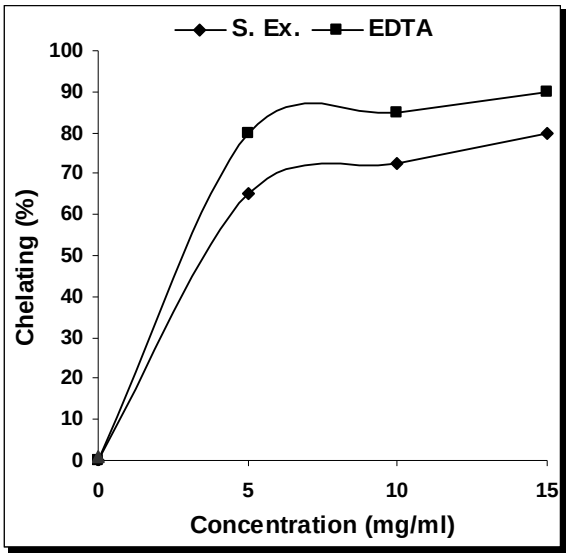

Fig. 12. Chelating ability of *C. papaya* seed extract and Ethylenediamine-tetraacetic acid (EDTA)

Ferrous ion chelating ability of aqueous *C. papaya* due to its ability to chelate and deactivate transition metals, prevent such metals from participating in initiation of lipid peroxidation and oxidative stress through metal catalysed reaction which investigated by Oboh *et al.* (2012).

Free radical-scavenging activities of *C. papaya* seed extract samples were determined by the method of Shimada *et al.* (1992). DPPH method is usually used to evaluate antioxidant activity of various natural compounds by reducing stable DPPH radicals to yellow-coloured diphenylpicrylhydrazine. DPPH radical scavenging ability is responsible for hydrogen-donating efficiency of antioxidants. As shown in figure 13, the DPPH radical scavenges ability of *Papaya* seed extract increases gradually with concentration increase. At 15 mg/ml scavenging effect of seed extract are 88%. Ascorbic acid showed scavenging effects of 98% at 15 mg/ml. So from this result, the *Papaya* seed extract may contain lots of reductones to react with radicals to stabilize and terminate radical chain reactions.

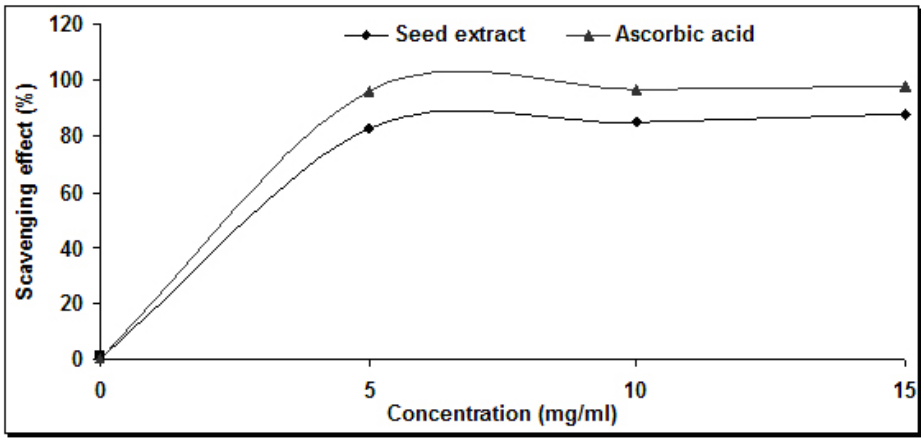

Fig. 13. Scavenging effect of *C. papaya* seed extract.

CONCLUSION:

The current study indicates efficient activity of *C. papaya* aqueous seed extract as antifungal against *Aspergillus flavus* espically at concentration 200mg/ml and safe use on lung tissue in treating fungal lung diseases when compared with fluconazole. *C. papaya* seed extract had antioxidant characters. Importance of *C. papaya* seed extract is becoming due to two effects, firstly, because of its antifungal activity against *A. flavus* due to presence bioactive components as free groups in ester and hydroxyl radical which could be part of cellular metabolism effect on pathogens. Secondly, because it had antioxidant characters so, it can be used medically in future, this information can be useful in determining the possible role of components found in *C. papaya* seed extract which can be important role in prevention of different health disorders. Further studies are needed to evaluate the phytochemicals found in *C. papaya* for using it as pharmaceutical compound.

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REFERENCES:

Adekunle AS, Adekunle OC. 2009. Preliminary assessment of antimicrobial properties of aqueous extract of plants against infectious diseases. Biol Med., 1(3): 20-24.

Afolayan AJ. 2003. Extracts from the shoots of *Arctotis artotoides* inhibit the growth of bacteria and fungi. Pharm. Biol., 41(1): 22-25.

Agbaje EO, Adejuwon AO, Idika N. 2011. Antifungal and Antibacterial activities of aqueous and methanolic root extracts of *Carica papaya* linn. (Caricaceae). Int. Res. J. Microbiol., 2(8): 270-277.

Ahmad I, Beg AZ. 2001. Antimicrobial and phytochemical studies on 45 indian plants against multi-drug resistant human pathogens. J. Enthnopharmacol., 74(2): 113-123.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J. Mol. Biol., 215(3): 403-410.

Andersson SC, Olsson ME, Johansson E and Rumpunen K. 2009. Carotenoids in sea buckthorn (*Hippophae rhamnoides* L.) berries during ripening and use of pheophytin a as a maturity marker. J. Agric. food chem., 57(1): 250-258.

Anibijuwon II, Udeze AO. 2009. Antimicrobial activity of *Carica papaya* (Pawpaw leaf) on some pathogenic organisms of clinical origin from south-western Nigeria. Ethnobot. Leaf., 13: 850-864.

Aruoma OI, Colognato R, Fontana I, Gortlan J, Migliore L, Koike K, Coecke S, Lamy E, Mersch-Sundermann V, Laurenza I, Benzi L, Yshino F, Kobayashi K, Lee MC. 2006. Molecular effects of fermented papaya preparation on oxidative damage, Map kinase activation and modulation of the benzo(a) pyrene mediated genotoxicity. Biofactors, 26(2): 147-159.

Cantuti Castelvertric I, Shukitt-Hale B, Joseph JA. 2000. Neurobehavioural aspects of antioxidants in aging. Int. J. Dev. Neurosci., 18(4-5): 367-381.

Chukwuemeka NO, Anthonia AB. 2010. Antifungal effects of pawpaw seed extracts and papain on post harvest *Carica papaya* L. fruit rot. Afr. J. Agric. Res., 5(12): 1531-1535.

Decker EA, Welch B. 1990. Role of ferritin as lipid oxidation catalyst in muscle food. J. Agric. Food Chem., 38(3): 674-677.

Dinis TCP, Madeira VMC, Almeida LM. 1994. Action of phenolic derivates (acetoaminophen, salicylate, and5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scanvenger. Arch. Biochem. Biophys., 315(1): 161-169.

Driver F, Miliner RJ, Trueman JWH. 2000. A taxonomic revision of Metarhizium based on a phyllogenetic analysis of rDNA sequence data. Mycol. Res., 104(2): 134-150.

Ellis PH, Griffiths DA. 1974. The location and analysis of melanins in the cell walls of some soil fungi. Can. J. Microbiol., 20(10): 1379-1386.

Gayosso-García Sancho LE, Yahia EM, Martínez-Téllez MA, González-Aguilar GA. 2010. Effect of maturity stage of papaya Maradol on physiological and biochemical parameters. Am. J. Agric. Biol. Sci., 5(2): 199-208.

Gills LS. 1992. Enthnomedical uses of plants in Nigeria University of Berin Press, Nigeria, pp. 276.

Harman EM. 2012. Aspergillosis emedicine. Medscape Reference, Drugs, Diseases and Procedures, Aspergillosis Clinical Presentation, http://emedicine.medscape.com/ article/ 296052- overview.

Hartemink KJ, Paul MA, Spijkstra JJ, Girbes AR, Polderman KH. 2003. Immunoparalysis as a cause for invasive aspergillsis? Intensive Care Med., 29(11): 2068-2071

Hashem FA, Wahba HE. 2000. Isothiocyanate in myrosinase treated herb extract of Cleome chrysanthadecne and their antimicrobial activities. Phytother. Res., 14(4): 284-287.

Henry T, Iwen PC, Hinrichs SH 2000. Idetification of *Aspergillus* species using internal transcribed spacer regions 1 and 2. J Clin Microbiol. 38: 1510-1515.

Hugo WB, Russell AD. 1998. Pharmaceutical Microbiology, 6th Edition, Blackwell Sciences Ltd, Oxford, pp. 510.

Inglis PW, Tigano MS. 2006. Identification and taxonomy of some entomopathogenic *Paecilomyces Spp.* (Ascomycota) isolated using rDNA-ITS sequences. Genet. Mol. Biol., 29(1): 132-136.

Jons KJ, O'day DM. 1999. Pharmcologic management of Keratomycosis. Surv. Ophthalmol., 33:178-188.

Kenawy ER, Abdel-Hay FI, Abou El-Maged A, Mahmoud Y. 2006. Synthesis and antimicrobial activity of some polymers derived from modified aminopolyacrylamide by reacting it with Benzoate esters and Benzaldehyde derivatives. J. Appl. Polym. Sci., 99(5): 2428-2437.

Kothari V, Seshadri S. 2010. Antioxidant activity of seed extracts of *Annona squamosa* and *Carica papaya*. Nutr. Food Sci., 40(4): 403-408.

Krishna KL, Paridhavi M, Jagruti AP. 2008. Review on nutritional, medicinal and pharmacological properties of Papaya *Carica Papaya* Linn. Indian J. Nat. Prod. Resour., 7(4): 364-373.

Lohiya NK, Manivannan B, Garg S. 2006. Toxicological investigations on the methanol sub-fraction of the seeds of *Carica papaya* as a male contraceptive in albino rats. Reprod. toxicol., 22(3): 461-468.

Lohiya NK, Mishra PK, Pathak N, Manivannan B, Bthande SS, Panneerdoss S, Sriram S. 2005. Efficacy trial on the purified compounds of the seeds of *Carica papaya* for male contraception in albino rat. Reprod. toxcol., 20(1): 135-148.

Lohiya NK, Pathak N, Mishra PK, Maniyannan B. 2000. Contraceptive evaluation and toxicological study of aqueous extract of the seeds of *Carica papaya* in male rabbits. J. Enthnopharmacol., 70(1): 17-27.

Macleod AJ, Pieris NM. 1983. Volatile components of Papaya (Carica papaya L.) with reference to Glucosinolate products. J. Agric. Food Chem; 31(5): 1005-1008.

Mahmoud YA1, Al-Ghamdi AY, Abd El-Zaher EH. 2011. A protective mechanism in lungs of rats experimentllay infected with *Aspergillus fumigatus*. Mycobiology, 39(1): 40-44.

Meléndez PA, Capriles VA. 2006. Antibacterial properties of tropical plants from Puerto Rico. Phytomedicine, 13(4): 272-276.

Moubasher AH. 1993. Soil fungi in Qatar and other Arab countries. University of Qatar, 1st ed. Center for Scientific, Appl. Res. Qatar, pp. 78-81.

Oboh G, Ademosun AO, Lajide. 2012. Improvement of the nutritive value and antioxidant properties of citrus peels through *Saccharomyces cervisiae* solid substrate fermentation for utilization in livestock feed. Livest. Res. Rural Dev., 24(1): 1-9.

Oboh G, Rocha JBT. 2007. Polyhenols in red pepper [*Capsicum annuum var. aviculare* (tepin) and their protective effect on some pro-oxidants induced lipid peroxidation in brain and liver. Eur. Food Res. Technol., 225(2): 239-247.

Ofusori DA, Ayoka AO, Adelakun BA, Falana OA, Adeeyo OA, Ajeigbe KO, Yusuf UA. 2008. Microanatomical effect of ethanolic extract of *Garcinia kola* on the lung of Swiss albino mice. Internet J. Pulm. Med., 10(1): 301-309.

Ogunjobi AA, Fagade OE, Dravid OO. 2007. Antimutagenic and potential anticarcinogenic and potential anticarcingenic activites of aloe-vera gel and aqueous garlic extract in the bacterial reverse mutation test (Ames assay). Afr. J. Biomed. Res., 10(3): 275-278.

Ogunjobi AA, Nnadozie N. 2004. Comparative effect of antimicrobial activites of Ocimum gratissium

and Vernoniaamygdalina. Bull. Sci. Assoc. Nigeria, 25: 165-170.

Ogunjobi AA, Ogunjobi TE. 2011. Comparative study of antibacterial activities of ethanol extracts of the bark and seeds of *Garcinia kola* and *Carica papaya*. Afr. J. Biomed. Res., 14(2): 147-152.

Ornelas-Paz JJ, Yahia ME, Gardea A. 2008. Changes in external and internal color during post harvest ripening of "manila" and "Ataulfo" mango fruit and relationship with carotenoid content determined by liquid chromatography-APcl time-of-filght mass spectrometry. Postharvest Biol. Technol., 50(2-3): 145-152.

Osato JA, Santiago LA, Remo GM, Cuadra MS, Mori A. 1993. Antimicrobial and antioxidant activities of unripe *papapya*. Life Sci., 53(17): 1383-1383.

Oyaizu M. 1986. Studies on the product of browning reaction prepared from glucose amine. Jpn J. Nutr., (44): 307-315.

Pipkin FB. 1984. Medical statistics made easy. Churchill livingstone. Edinburgh London Melpourne and New York, pp. 137.

Pridham TG, Shotwell OL, Stodola FH, Lindenfelser LA, Benedict RG, Jackson RW. 1956. Antibiotics against plant disease. I. Laboratory and green house survey. Phytopathology, 46: 575-581.

Robbins R. 1980. Medical and nutritional aspects of citurs bioflavonoids. In: "Citrus Nutrition and Quality. (Nagy S, Attaway J. eds)". American Chemistry Society, Washington, DC. USA, pp. 43-59.

Shimada K, Fijikawa K, Yahara K, Nakamura T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem., 40(6): 945-948.

Spuur AR. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res., 26(1): 31-41.

Srinivasan D, Perumalsamy L, Nathan P, Sures T. 2001. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J Enthno Pharmacol; 94: 217-222.

Stepek G, Buttle DJ, Duce IR, Lowe A, Behnke JM. 2005. Assessment of anthelmintic effect of natural plant cysteine proteinases against the gasterointestinal nematode, Heligmosomoides-polygrus, in-vitro. Parasitolology, 130(Pt2): 203-211.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22(22): 4673–4680.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: "PCR protocols: a guide to methods and applications. (Innis MA, Gelfand DH, Sninsky JJ, White TJ. Eds)". New York: Acad. Press. pp. 315–322.

Yahia ME, Ornelas-Paz JJ. 2010. Chemistry, stability and biological actions of carotenoids. In: Fruit and vegetable phytochemicals. (de la Rosa IA, Alvarez-Parrilla E, Gonzalez-Aguilar GA. Eds)". USA: Wiley-Blackwell, pp. 177-222.

Yoshio T, Yoshio T. 1981. Investigation of the structure of *Rhizopus* cell wall with lytic enzymes. Agric. Biol. Chem., 45(7): 1569-1575.

النشاط الضد فطريات لمستخلص بذور الباباظ ضد الاسبرجلس فلافس ككائن منتج للسموم الفطرية وكائن مسبب للأسبرجلوسيس

إيمان حسن فتحي عبد الظاهر

قسم النبات، كلية العلوم، جامعة طنطا

تم دراسة النشاط المضاد للفطريات للمستخلص المائى لبذور الباباظ ضد الاسبرجلس فلافس باستخدام طريقة الآجار كت بلج وقد وجد أن مستخلص بذور الباباظ له نشاط تثبيطى ضد الاسبرجلس فلافس بقدر يتراوح بين 16 إلى 21 مم وبتناقص النشاط الحيوى لخلايا فطر الاسبرجلس فلافس بزيادة تركيز مستخلص بذور الباباظ من 25-200 ملجم/مل .وقد أدى معاملة الفطر بمستخلص بذور الباباظ إلى تغيرات خارجية وتكوين شكل غير منتظم للخلايا وأيضا اختفاء للجدار الخلوى للفطر وذلك بفحصه تحت الميكرسكوب القاطع. وجد أيضا أن مستخلص بذور الباباظ له نشاط مضاد للاكسدة كما أنه له قوة اختزالية وله نشاط اختزالى على مادة 1 داى فينيل 2 بكريل هيدرزيل . وقد تم فحص تأثير مستخلص بذور الباباظ على رئة فئران التجارب ووجد أن له طبيعة آمنة ولذا يمكن استخدامه طبياً فى المستقبل بآمان. وبتحليل مستخلص بذور الباباظ بواسطة جهاز الفصل الكروماتوجرافى (GC-MS) وجد أن به 15 محتوى داخلى فى البذور مثل الاستير الذى سجل الانتشار كبير بين هذه المحتويات.

المحكمون:

أ.د. محمد إبراهيم أحمد علي قسم النبات، علوم القاهرة

أ.د. يحي أحمد الظواهري قسم النبات، علوم الزقازيق