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# Comparison of Classical and Ultrasound-Assisted Extractions of *Hyphaene thebaica* Fruit and Evaluation of Its Extract as Antibacterial Activity in Reducing Severity of *Erwinia carotovora*

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***Abstract*—***Erwinia carotovora* var. c*arotovora* is the main cause of soft rot in potatoes. *Hyphaene thebaica* was studied for biocontrol of *E. carotovora* which inhibited growth of *E. carotovora* on solid medium, a comparative study of classical and ultrasound-assisted extractions of *Hyphaene thebaica* fruit. The use of ultrasound decreased significant the total time of treatment and increase the total amount of crude extract. The crude extract was subjected to determine the *in vitro*, by a bioassay technique revealed that the treatment of paper disks with ultrasound extraction of *Hyphaene thebaica* reduced the growth of pathogen and produced inhibition zones up to 38mm in diameter. The antioxidant activity of ultrasound-ethanolic extract of Doum fruits (*Hyphaene thebaica*) was determined. Data obtained showed that the extract contains the secondary metabolites such as Tannins, Saponin, Flavonoids, Phenols, Steroids, Terpenoids, Glycosides and Alkaloids.

***Keywords*—**Ultrasound, classical extract, Biological control, *Erwinia carotovora, Hyphaene thebaica.*

## I. Introduction

THE traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of compounds and the rate of mass transfer. Usually, these techniques require long extraction time and have lower yield. Moreover, many natural products are thermally unstable and may degrade during thermal extraction [1]. The use of ultrasound resulted in increased component extraction in a shorter time and at lower temperatures. The mechanical extract of ultrasound is able to accelerate the extraction of active plant compounds, contained within the body of plants, due to disruption of the cell walls and enhanced mass transfer of cell contents [2]. Nowadays, sonication has been employed to extract pharmaceutically active compounds [3], polysaccharides [4], cellulose [5], flavonoids [6], saturated hydrocarbons, fatty acid esters and steroids [7] from plant materials. *Erwinia carotovora* var. *carotovora* is a broad host-range pathogen causing seed decay, blackleg and aerial soft rot, especially in potatoes [8], [9]. The bacterial soft rot was found to occur universally and the diversity of soft - rot bacteria (including pectolytic *Erwinia* spp.) associated with postharvest losses of horticultural commodities [10]. Soft rot is one of the destructive diseases of vegetables. It occurs worldwide wherever fleshy storage tissue of vegetables and ornamentals are found. The disease can be found on crops in the field, in transit and in storage or during marketing making economic loss and post harvest bacterial soft rot losses have been estimated to vary between 15-30% of the harvested crop [11]. Plants remain the most common source of antimicrobial agents [12]. Doum palm fruit (*Hyphaene thebaica*) is a desert palm tree with edible oval fruit, originally native to the Nile valley. It is a member of the palm family, *Arecaceae* [13]. Doum is one of commonly consumed beverages, and is rich in polyphenolic compounds. The current study focus on natural antioxidant especially plant polyphenolics [14]. The fruits of Doum showed antimicrobial and antihypertensive activities, these activities were attributed to the presence of flavonoids [15]. Also, the aqueous extract of Doum fruits showed an antioxidant activity; this is due to the substantial amount of their water-soluble phenolic contents [16].The medicinal values of the plant lie in their phytochemical components which produce definite physiological actions. The most important of these components are alkaloids, tannins, flavonoid and phenolic compounds [17].

The aim of this study was to investigate the activities of this fruit ultrasonic extract as antimicrobial activities. In addition, some antioxidant compounds content were determined.

## II. Experimental

### *A. Materials and Methods:*

All other organic solvents used in the study were analytical grade.

#### 1. Pathogenic Strain

*Erwinia carotovora* var. *carotovora* (119a) strain was isolated from infected melon plants in Egypt and identified [18].

#### 2. Plant Material

Fruits of Doum *Hyphaene thebaica* (*Palmae*) were collected from Jazan area, Saudi Arabia (2011). The fruits

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were dried in shade at room temperature for 15 days and powdered.

*B. Soxhlet Extraction (SE)*

Fruit powder were extracted in solvents such as Ethyl alcohol, ether and ethyl acetate; solvent to solid ratio of 10 ml/g; by using soxhlet apparatus. The exhaustive extraction was performed for 6h at 85°C. After extraction, samples were cooled and filtered under vacuum through Whatman No. 1 paper. The extract was stored at 4ºC for phytochemical screening and antimicrobial analysis.

*C. Ultrasound-Assisted Extraction (UAE)*

The extraction method was performed by Fischer sonicator (with a frequency of 25 kHz and a nominal power 600 W). Temperature in the bath was controlled at 30°C). Extractions were carried out on the same solvent to solid ratio of 10 ml/g; of each sample for several interval time (cf. Table I) with the same solvents. The extracts filtered and concentrated under reduced pressure. This experiment was performed in triplicate.

*D. Bioassay Techniques*

A bioassay based on clearing zone agar plate was used to determine the antibiotic activity of the metabolite produced by *Hyphaene thebaica* against the phytopathogen *E. carotovora* var. *carotovora*.

The suspension of tested *Erwinia* was prepared by adding 10 ml sterilized distilled water to each Petri dish of 2 days- old *Erwinia* cultures grown on nutrient agar medium. The suspension of *Erwinia* was added to nutrient agar medium before solidification, then, discs (2mm in diameter) of filter paper were impregnated with the extract of the tested *Hyphaene thebaica* and transferred onto nutrient agar plates under aseptic conditions. Another filter paper discs (2mm) were impregnated with sterilized distilled water and transferred onto nutrient agar plates inoculated with *Erwinia* suspension only and used as control [19]. All plates were incubated at 25±2°C for two days and then, the inhibition zone was measured in mm.

*E. Phytochemical Screening of Fruit Extracts*

The presence of various phytochemical compounds in the fruits of *Hyphaene thebaica* were carried out on the extract and on the powdered specimens using standard procedures to identify the constituents of the secondary metabolites such as Tannins, Saponin, Flavonoids, Phenols, Steroids, Terpenoids, Glycosides and Alkaloids of *Hyphaene thebaica* as described [17], [20]-[24].

*F. Statistics Analysis*

The resultant clear zones around the discs were measured in mm. The antimicrobial activity of fruit extract was indicated by clear zone of growth inhibition. Data of experiments are represented by three replicates from each experiment.

## III. Results and Discussion

*A. Effects of Different Solvents and Extraction Method*

Conventional extraction methods using organic solvents (Ethyl acetate, and ethanol) were compared with ultrasonic extraction in the same solvents.

It is well-known that different solvent will yield different amount and composition of extract. The selection of solvent is the essential to extract objective constituents. In this study, we investigated the effects of ethanol and ethyl acetate on the yield of the crude extract under the same extracting condition which are the ratio of solid/liquid is 1:10 (g/ml), extracting temperature is 30°C and extracting time is 30min or at refluxing temperature and extracting time is 6h in sonication or soxhlet extraction respectively. Mass yield of the extracts obtained by each method and using Ethanol or Ethyl acetate are shown in Table I.

TABLE I
The Yield Obtained with Ultrasound or Soxhlet Extraction Using Ethanol or Ethyl Acetate

| Solvent | Yield (%) | |
|---|---|---|
| | US (30 min) | Soxhlet (6 H) |
| Ethanol | 90 | 65 |
| Ethyl acetate | 54 | 42 |

The mass yield of the extracts obtained by sonication within 30min was higher than that obtained by classical method within 6h. This means that, the extraction rate of the ultrasound-assisted process was many times faster than that of the conventional method. On the other hand change the solvent of extraction from ethyl acetate to ethanol increase the yield significantly

*B. Effect of Extraction Time*

We investigated the effect on extraction efficiency of ethanol and ethyl acetate under the different ultrasonic time (0, 15, 30, 60, 75, 90min) with the ratio of solid/water at 1:10 (g/ml) at 30°C.

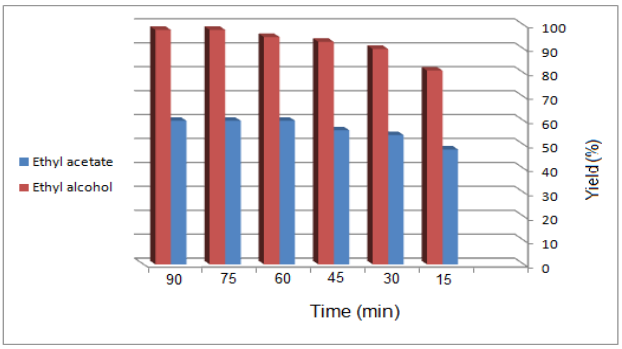


Fig. 1 Effect of time of Doum extraction on yield obtained using ethyl alcohol or Ethyl acetate

The results showed the extraction yield has a significant increase from 15 to 30 min in both ethanol and ethylacetate (Fig. 1). However, a minor increase in the yield was observed upon increasing the time from 30 to 75 and 60 in ethanol and ethylacetate respectively. Extraction yield has no change in the prolonged time periods. Therefore, 30min was best time selected for the extraction in ethyl alcohol or ethylacetate. (cf.

Fig. 1).

### C. Bioassay Techniques

The results of antibacterial activity of the extracts of fruit part of *Hyphaene thebaica* against the phytopathogen *Erwinia carotovora* var. *carotovora* are shown in (Table II). The results showed that the production of clear inhibition zone which is due to the production of either antibiotics, toxic metabolites and compounds similar to antibiotics as mechanisms for biological control possibility of the use of this plant as a biocontrol agent of *Erwinia carotovora* var. *carotovora* at the field level.

TABLE II
EFFECT OF ANTIMICROBIAL EXTRACT ACTIVITY OF *HYPHAENE THEBAICA* ON THE *ERWINIA CAROTOVORA* VAR. *CAROTOVORA* BY BIOASSAY TECHNIQUE

| *Hyphaene thebaica* (ethanolic extract) | Antimicrobial activities (mm) |
|---|---|
| Dist. Water | 0 |
| classical extract | 16 |
| Ultrasound extract | 38 |

### D. Phytochemical Screening of Fruit Extracts

The results indicate that the phytochemical screening of *Hyphaene thebaica* showed the presence of eight phytoconstituents, these are Tannins, Saponin, Flavonoids, Phenols, Steroids, Terpenoids, Glycosides and Alkaloids. This is coinciding with Ahmed et al who reported that total antioxidant capacity of the aqueous extract of doum fruit was highly possible due to substantial amount of water-soluble [25]. This investigation has opened up the presence of saponins and flavonoids, which act to prevent or reduce oxidative stress by scavenging free radicals [26]. Also Doum palm fruit is a source of potent antioxidants [27]. These results are in conformity with the observation of other workers [28], [29]. Phytochemical constituents such as alkaloids, flavanoids and glycosides are secondary metabolites in plants that have alleviated the pathogenic and environmental stress.

## IV. CONCLUSION

We have modified a method for extraction of doom fruit under ultrasonic radiation. There was a remarkable increase in the yield of the crude extracted material in addition to decrease in time of extract. The maximum yield was obtained after 1 h. The crude ethanolic extract was the more effective on bacteria than the ethyl acetate extract. the phytochemical screening of *Hyphaene thebaica* showed the presence of 8 phytoconstituents, Flavonoids, Phenols, Glycosides and Alkaloids, …etc

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