Plant Archives Vol. 18 No. 1, 2018 pp. 665-668 *ISSN 0972-5210*

STUDY OF TURMERIC PLANT (*CURCUMA LONGA* L.) RHIZOMES AND MYRRH (*COMMIPHORA MYRRHA* L.) GUMS METHANOLIC EXTRACTS EFFECT ON *CANDIDA ALBICANS* ISOLATED FROM MOUTH

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Abstract

This study was conducted to turmeric plant (*Curcuma longa* L.) rhizomes and myrrh (*Commiphora myrrha* L.) gums extracts, which were extracted by maceration for 24 hours in methanol using magnetic stirrer. The plants extracts tested to determine the presence of active secondary metabolites and showed presence of saponins, phenolic compounds and flavonoids , as well as the characterization of these compounds with revelation of effective groups by Fourier Transform Infrared spectroscopy (FTIR) and (UV – spectrum).

The plants extracts have been studied by knowing the antifungal activity against *Candida albicans*, results showed the effectiveness of turmeric, myrrh against the microorganism and this may be occurred because of secondary metabolites activity as phenolic compounds presence in studied plants.

Key words : *Candida albicans*, *Curcuma longa*, *Commiphora myrrha*, extracts.

Introduction

Candida are yeast consider as a fungi member of normal flora in mucosal surfaces in oral cavity, vagina and gastrointestinal tract in the healthy humans (Shao *et al*., 2007). They are ordinarily characteristic as commensalism microorganism but in some times may cause diseases (Pahwa *et al*., 2014).

Candida albicans is the common pathogen of human belong to fungi , that it can grow in the host as yeast cells and filamentous form (hyphae) (Horn *et al*., 2009) and the fungal ability to evade the immunity of the host in addition to hydrolytic enzyme production, which lead to tissue damage (Silva *et al*., 2011).

The high diversity of *Candida albicans* infections created increasing in the countries care to find the effective antifungal treatment drugs (Lai *et al*., 2012), but these drugs may be toxic, highly cost or interactions which may occurred with other drugs, thus limitation using of synthetic antifungal drugs lead to investigate about new safe and low toxic effective pharmacological strategies especially from plants source (Sharanappa and Vidyasagar, 2013).

Plants had attended as a source of useful drugs and specific curing agents and in Iraq (*Curcuma longa* L.) and (*Commiphora myrrha* L.) are used as flavoring and curing agents in many folkloric medicine , that they used for many therapeutic treatments for example antioxidant , anti - inflammation and antibacterial effects in addition to their used in food processing (Abdulbary, 2016). Therefore, it important to investigate about their antifungal effect especially *Candida albicans*, which may contribute to the future use of the medicinal plant in pharmaceutical uses to the treatment of fungal infection.

Methods

The plants samples preparation

From Al- Najaf Al- Ashraf city markets *C. myrrha* gums and *C. longa* rhizomes were collected and identified. The plant parts were grinding well to obtain a powder by putting them in mixer grinder. Powders were stored then in sacs prior to use them for extraction (Jones and Kinghorn, 2006).

Preparation of plants extracts

The plants extracts were produced as Magnetic stirring and maceration techniques by mixing (25) grams of dry plants powder with 250 ml of solvent (methanol 95%) for 24 hours, filtration and investigation of active compounds in the extracts, then they were concentrated with removing the solvents by using rotary evaporator and were dried and stored at +4°C until using (Harborne, 1984).

Thin layer chromatographic (TLC) analysis

The samples of plants parts extracts were spotted or lined by capillary tube on glasses TLC plates, then they were positioned in glass container (jar), based on ascending chromatography with the mobile phase, which used for identifying and isolating active compounds (chloroform : ethanol: glacial acetic acid) (95:5:1) earlier to be found in the base of the jar at 10 mm depth. Then, chromatogram with samples spots examined with exposure to UV light and by iodine steam in iodine chamber (Harborne, 1984).

Ultraviolet spectra

Ultraviolet spectra of compounds were obtained on UV-1601PC UV Visible spectrophotometer (Shimadzu) using Dimethylsulfoxide (DMSO) as solvent. Ultraviolet spectra of the isolated compounds were carried out in the Central Labratory, Faculty of Pharmacy, University of Kufa.

FT-IR spectra

Spectra of the isolated compounds were recorded with (FT-IR) to determine functional groups of compounds and were recorded with a FTIR-Fourier transform infrared spectrophotometer at Central Labratory, Faculty of Pharmacy, University of Kufa.

Identification of fungal (*Candida albicans*) isolates

The fungal samples isolates from oral cavity (mouth). Identification occurred by morphological, cultural characteristics growth on Oxoid Sabouraud's Dextrose-Agar media that samples plotted with cotton swabs on the surface of the medium. *Candida albicans* was identified depending on its morphological and cultural characteristics then it was examined under the microscope with Gram's stain for observation arrangement and reaction with stain (Murray *et al*., 1999).

Screening for antifungal activity

It was carried out according to disc diffusion method (Yin *et al*., 2010); the plates of Muller – Hinton agar media with 2% glucose and 0.5 µg/ml methylene blue was inoculated with fungal study with a sterile swabs. Sterile paper discs made from (Whatman No. 1) filter paper were soaked in the plants extracts with concentration (1000 µg/ml), then allowed to desiccate beneath the laminar flow cabinet for a night. In the company of sterile forceps, the plant solutions discs were positioned on the inoculated plate and pushed gently into agar. Each plant extract was assayed in triplicate.

The control discs were soaked with DMSO, which provide a negative control. The inoculated plates were incubated at 37°C for 18-24 h. By the measurement of the zone inhibition width in the region neighboring to discs with millimeters (mm), the antifungal effects was construed.

Results and Discussion

The plants extracts tested to determine the presence of active secondary metabolites showed presence of saponins, phenolic compounds and flavonoids that the

Table 1 : FT-IR analysis, UV–Vis. Spectra and R_f values by TLC of active crude compounds of *C. longa* rhizomes and *C. myrrha* gums.

Plant solution	FT-IR analysis		UV – Vis. Spectra		R_f
	Functional group	Wave number (cm^{-1})	Wave length (nm)	λ max	
CM	O – H	3408.22	425	0.560	0.65
	C=C	1591.27& 1514	300	0.170	
	C – O	1278.81	258	0.490	
			230	0.445	
			208	0.700	
MM	O-H	3427.51	270	0.790	0.75
	C-H	2962.66	250	0.770	
	C=O	1735.93 & 1708.93	210	1.000	
	C–O	1460.11			
	C–S or C–N	1037.70			

CM= *Curcuma longa* methanolic extract. MM= *Comiphora myrrha* methanolic extract.

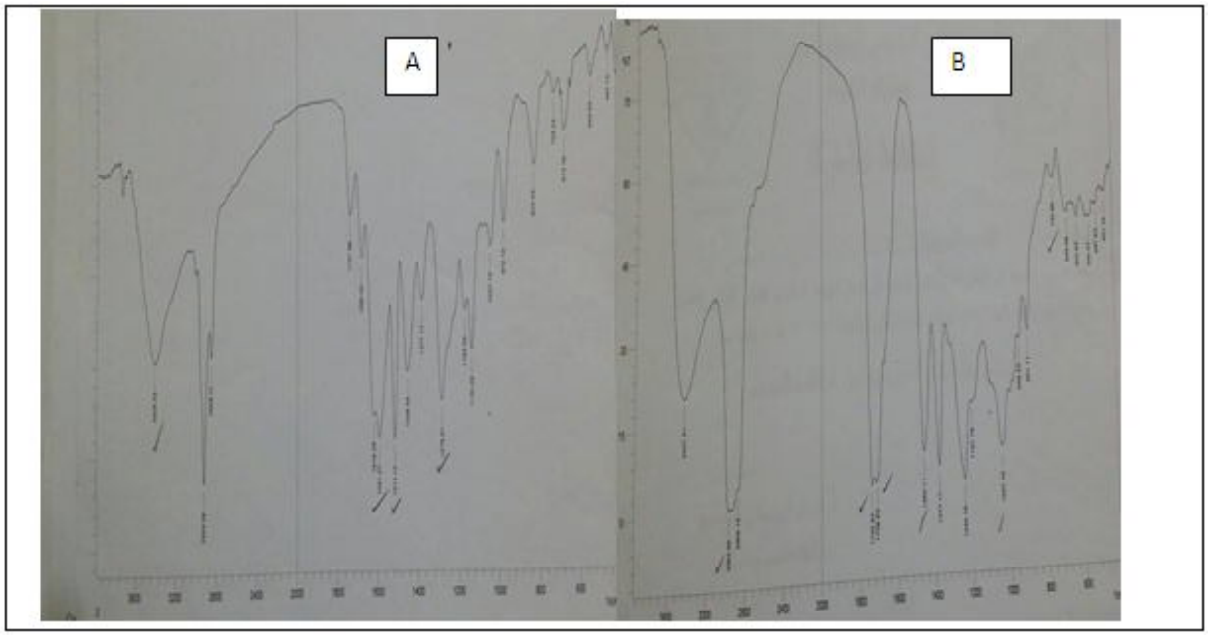

Fig. 1 : FT - IR analysis of active compounds of A. *C. longa* rhizomes and B. *C. myrrha* gums.

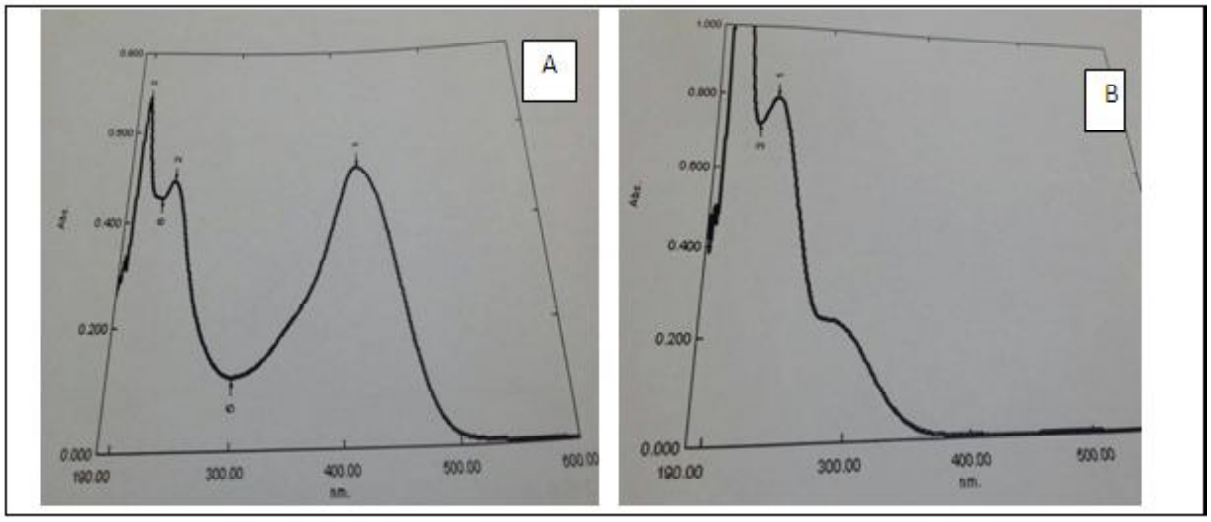

Fig. 2 : UV – Vis. Spectra analysis of active compounds of A. *C. longa* rhizomes and B. *C. myrrha* gums.

chemical tests for investigation about active constituent showed that (*Curcuma longa* L.) and (*Commiphora myrrha* L.) both had compounds have the ability of antimicrobial effect (Kareem *et al*., 2008).

FT-IR spectroscopy is used to identify unknown materials, determining the quality or stability and the amount of components in a mixture with many narrow bands (Wade, 2006) while the UV-visible spectroscopy gives a limited amount of qualitative information and the organic compounds absorption results from the presence of unsaturated bonds (Owen, 2000).

The R_f values for the active crude components are different according to function groups of each extract, the affinity between mobile phase and the samples and polarity, that highly polar compound will have strong interaction with silica and separated first and the high R_f values indicated that high dissolvability of compounds in mobile phase, but non polar compound will separate last, which would have non interaction with the stationary runs a longer distance on the plate that the low R_f values indicated low dissolvability of compounds in mobile phase (Lade *et al*., 2014).

The results of FT-IR analysis, UV – Vis. Spectra analysis and R_f value of *C. longa* rhizomes and *C. myrrha* gums illustrated in table 1. These results were in agreement with the values of some pure compounds isolated from the plants under study (Kulkarni *et al*., 2012 and Shuaib, 2014).

Identification of fungal (*Candida albicans*) isolates

The individual colonies of *Candida albicans* growing on Sabouraud's Dextrose-Agar media appeared as white to creamy, smooth, rounded colonies (Singh *et al*., 2013). While the microscopical examination showed that the single yeast appeared as spherical to oval cells and gram positive due to peptidoglycan layer in the yeast (Sudbery *et al*., 2004).

Screening for antifungal activity

The results showed that *C. longa* has high antifungal activity against *C. albicans* and the topmost inhibition zone diameter was (17 mm) and limited antifungal activity of *C. myrrha* was observed with methanolic extract (11mm).The positive results of *C. longa* and *C. myrrha* solutions samples may be because that methanolic extracts and fractions contain some anti-microorganisms effective compounds such as the flavonoids and saponins (Al-Marby *et al*., 2016).

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