International Journal of Applied and Natural Sciences (IJANS)
ISSN(P): 2319-4014; ISSN(E): 2319-4022
Vol. 3, Issue 4, July 2014, 173-180
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International Academy of Science, Engineering and Technology
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THE ANTIMICROBIAL EFFECT OF EXTRACTS OF *MELIA AZEDARACH* ON SOME PATHOGENIC MICROORGANISMS

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ABSTRACT

Our work treated *in vitro* the antimicrobial activity of essential oils (EO) extracted from tree *Melia azedarach* that has proved to be a remarkable source of molecules biologically actives through its leaves, flowers and seeds on some pathogenic bacteria and yeast. Results showed that the seed oil of *M. azedarach* is the most active because it has the best diameters of inhibition halos for the three bacteria tested, with 30 mm, 25 mm and 23 mm respectively for *S. aureus*, *E. areogenes* and *E. coli.* Leaves EO has also an antibacterial effect on the three strains with a diameter of 21 mm for *S. aureus, E. coli* and 15 mm to 20 mm for *E. areogenes.* About flower EO, the diameters of the inhibition halos vary between 17 mm and 19 mm. The best percentages of fungal growth inhibition for the EO of *Melia azedarach* were obtained for 100 µl. The greatest inhibition was obtained for *C. albicans* strains to 100 µl, respectively 75%, 70% and 75% for EO of leaves, flowers and seeds. *Saccharomyces* spp has a high percentage of 50% inhibition at a dose of 20 µl for EO leaves and seeds. EO of leaves inhibited the growth of *C. albicans* and *F. oxysporum* over 70%. The EO of flower shows growth inhibition of 70% and 50% for *C. albicans* and *F. oxysporum*.

KEYWORDS: Antimicrobial Effect, *Melia Azedarach,* Pathogenic Bacteria, Pathogenic Yeast

INTRODUCTION

Chinaberry or *Melia azedarach* L. is a tree belonging to the mahogany family (Meliaceae). *Melia azedarach* L. is native to tropical Asia. It is wide spread and naturalized in most of the tropics and subtropical countries. It was introduced and naturalized in Philippines, United States of America, Brazil, Argentine, many African and Arab countries (Khan et al., 2008). *Melia azedarach* L. is one of the most useful traditional medicinal plants like *Azadirachta indica*. Each part of *M. azedarach* has some medicinal properties like *A. indica* and thus is commercially exploitable. During the last twenty years, apart from the chemistry of this plant, considerable progress has been achieved regarding the biological activity and medicinal applications. Leaves of the Meliaceae specie *Melia azedarach* (cinnamon or Santa Barbara in Brazil) has been reported to exhibit immunomodulatory, anti-inflammatory, antihyperglycemic, anticarcinogenic, nematicidal, antiparasitic, antiviral, insecticidal and antioxidant properties (Melo et al., 2011).

There are over 50 different bioactive compounds (terpenoids and others) of these aqueous extracts, but the major components of neem and cinnamon are limonoids such as azadirachtins (AZ) (Huang et al., 1996; Nakatani et al., 1998; Kaushik, 2002; D'Ambrosio and Guerriero, 2002). The general biological action of these extracts is the induction of lipid peroxidation, generation of antiproliferative and antioxidant effects and detoxication of enzymes (Akudugu et al., 2001; Kumar et al., 2006).

This study aims to show the antimicrobial effects of extracts of *M. azedarach* on some pathogenic microorganisms.

MATERIAL AND METHODS

Collection of Plant

The leaves, flowers and seeds of *Melia azedarach* come up to trees growing in the Chlef region, Algeria. Plant Organs were collected during the period of March in May 2013, while the flowering begins until the end of April 2013 and lasts only 20 days.

Treatment Plant Organs

The flowers and leaves are washed with distilled water and dried with cotton towels before handling water. Thus, the seed was washed with distilled water, and then suffered a hand shelling to recover the fine.

Extraction of the Essential Oils

20 g of fresh leaves, flowers and seeds ground to powder of *M. azedarach* were used individually hydrodistilled for 3 h in Clevenger-type apparatus (Denny, 1989). The volatile distillate was collected over anhydrous sodium sulphate and was homogenized for 15 min. The distillate was added to 20% of dichloromethane. Drying of the organic phase was carried out using a rotary evaporator. The samples were sealed and kept in dark glass vials in the refrigerator until time of analysis. The yield of the oil was 4.22 %, 1.6 % and 0.9 % v/w respectively for seeds, flowers and leaves based on dry plant weight.

Test Organisms

The choice of microorganisms has been focused on the prevalent strains in infectious diseases, these species often have natural resistance to various types of antimicrobial agents for public and plant health remains a major problem with the radical solution would be the discovery of new bioactive molecules effective, essential oils are good alternatives, we selected two groups of microorganisms:

- Gram-negative bacteria: *Escherichia coli* and *Enterobacter areogenes*
- Gram-positive bacteria: *Staphylococcus aureus*.
- Two fungus: one responsible for a devastating and destructive significant operating palm date palm plant pathology as is the case of *Fusarium oxysporum*, another responsible for Candidiasis as *Candida albicans*.

Antibacterial Activity Test

Disc-Diffusion Method *(Aromatogram)*

The basic protocol of aromatogram adopted is the one that was proposed by Khan et al. (2011) in his research on the effectiveness of seed extracts or 5 µl of the essential oil of *Melia azedarach* against some bacteria isolated from hospital.

Micro-Atmosphere *(or Vapor Phase Method) or* Disc Volatilization

This method evaluates the activity of volatiles substances on the same germs. The protocol is technically near to disc diffusion method. 20ml of Mueller-Hinton medium was poured into plastic Petri dish. After solidification, the dish

was inoculated with 5 µl containing 10^6cfu/ml of the micro-organisms and placed in the cover of each Petri dish. After incubation, the inhibition diameter is measured. Blank discs served as negative control (Abi-Ayad et al., 2011).

Antifungal Activity Test

The fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The spore suspension was adjusted with sterile saline to a concentration of approximately 10^7 spores in a final volume of 100 µl of culture. The effect of plant extracts on the growth of pathogenic strains was carried out with two methods:

- According at the method of Regnier and al. (2008), four (04) microscope slides respectively with 10, 20, 40 and 100 µl of plant extracts were fixed with glycerol in the cover of the Petri plate, we a microscope slide which was deposit 10, 20, 40 and 100 µl of oil from different types of extraction.
- In order to determine treatment dose, 0.05 % (v/v) of Tween 80 and plant extracts [0.5, 1 or 2 % (v/v)] were added at Sabouraud medium previously autoclaved. Agar thus prepared is poured into Petri plates.

For both methods, the paper disk of 5 mm diameter (Whatman filter paper n°1) with spore suspension was dried and placed on solid potato dextrose agar (PDA) surface (first method) on solid Sabouraud agar (second method) with the help of a sterile forceps. The whole is then sealed with parafilm and placed in the oven at 30°C. After 7 days, the growth diameter was measured and the results are expressed as percentage inhibition given by the following formula (Plaza et al., 2004):

$$\lfloor (\boldsymbol{C} - \boldsymbol{T})/\boldsymbol{C} \rfloor . \mathbf{100}$$

C: diameter growth of the pathogen in the control Petri plate T: diameter growth of the pathogen in Petri plate after treatment.

All data on antimicrobial activity were average of triplicate.

RESULTS

In the present investigation, the inhibitory effect of different extracts of leaves, flowers and seeds of *Melia azedarach* were evaluated against both fungicidal and bacterial strains. The antimicrobial activity was determined using disc-diffusion method and micro-atmosphere (or vapor phase method) or Disc volatilization. The activity was quantitatively assessed on the basis of inhibition zone.

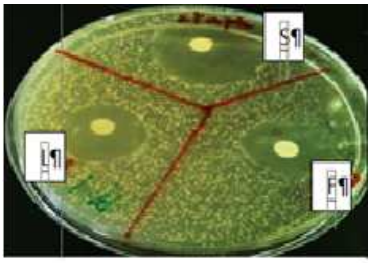
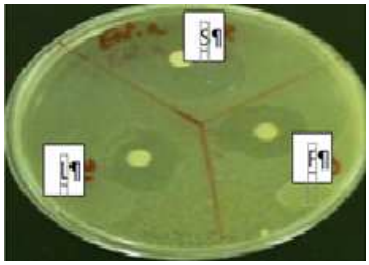
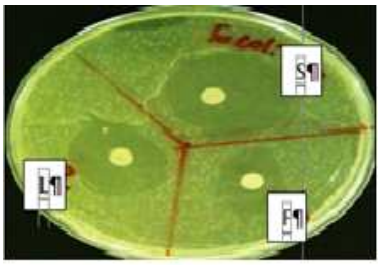
All tested essential oils exhibited antibacterial and antifungal activities (Table 1 and 2) with the two methods. The results showed an inhibition activity against all organisms.

Antibacterial Activity "in vitro"

Aromatogram

The antibacterial spectra of each essential oil showing the inhibition zone for each test bacteria are presented in Table 1. A high activity against *Escherichia coli*, *Enterobacter areogenes* and *Staphylococcus aureus* was found for seeds essential oil based on agar diffusion test. According to the classification of Ponce et al. (2003), inhibition zones, ranging between 15 mm and 19 mm, indicate that all strains are very sensitive and > 20 mm extremely sensitive to essential oil of *Melia azedarach* seeds, leaves and flowers.

Table 1: Antibacterial Activity (Zone of Inhibition, Mm) by Aromatogram of Organs Extracts of *M. Azedarach* against Some Clinical Pathogens

Diameter of Inhibition Zone (Mm)								
Staphylococcus Aureus			Enterobacter Areogenes			Escherichia Coli		
Seed	Leaf	Flower	Seed	Leaf	Flower	Seed	Leaf	Flower
30 ± 0.07 (+++)	21 ± 0.15 (+++)	19 ± 0.05 (++)	25 ± 0.01 (+++)	15 ± 0.35 (++)	17 ± 0.5 (++)	23 ± 0.25 (+++)	20 ± 0.1 (+++)	17 ± 0.06 (++)
								

S: seed, **F:** flower, **L:** leaf, **(+++)**: extremely sensitive, **(++)**: very sensitive

The Microatmosphere Test

This test indicated *Melia azedarach* leaves and flowers oils as highly active Gram-negative bacteria as *Escherichia coli* and *Enterobacter areogenes* (Table 2). The results revealed that leaves oil had a major contribution to the vapour activity against *Enterobacter areogenes* followed by flowers oil against *Escherichia coli*. While seeds oil has an activity lower than leaves and flowers oils against two strains. In contrast, *Staphylococcus aureus* showed complete resistance for essential oils of leaves, flowers and seeds of *M. azedarach*, contrariwise the method aromatogram showed an inhibition effect on *S. aureus* for essential oils organs of *M. azedarach*.

Table 2: Antibacterial Activity (Zone of Inhibition, Mm) by Microatmosphere Test of Organs Extracts of *M. Azedarach* against Some Clinical Pathogens

Diameter of Inhibition Zone (Mm)								
Staphylococcus Aureus			Enterobacter Areogenes			Escherichia Coli		
Seed	Leaf	Flower	Seed	Leaf	Flower	Seed	Leaf	Flower
00 (R)	00 (R)	00 (R)	12 ± 0.05 (+)	19 ± 0.16 (++)	16 ± 0.03 (++)	12 ± 0.25 (+)	16 ± 0.5 (++)	13 ± 0.35 (+)

(+): sensitive, **(++)**: very sensitive, **(R)**: resistant

Results of antibacterial activity of *M. azedarach* depict that all the extracts effectively inhibited the growth of both gram positive and gram negative strains used by aromatogram method. In contrary with microatmosphere method, extract inhibited the growth only Gram-negative bacteria.

Antifungal Activity *"in vitro"*

Phytopathogenic fungi (*Fusarium oxysporum*) and one clinical fungus (*Candida albicans*) were used as test organisms in the screening. All tested essential oils showed high antifungal activity against the both strains by both agar diffusion and microatmosphere methods.

The Microatmosphere Test

Inhibition of fungal growth was noted at every dose (Figure 1) and that the strains reacted differently to each volume of treatment.

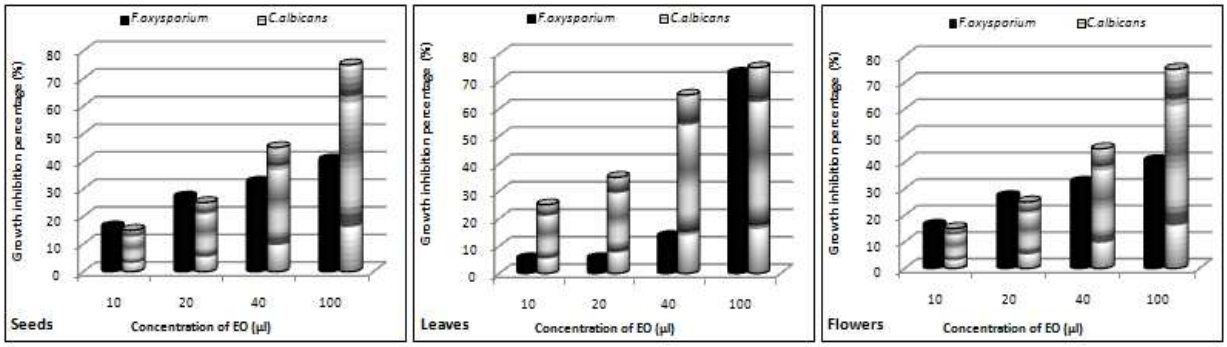


Figure 1: Growth Inhibition Percentages obtained of Fungal Strains Exposed to Seeds Leaves and Flowers EO of *Melia Azedarach*

The highest inhibition of both strains growth was obtained for 100 µl dose. The greatest inhibition was obtained for *C. albicans* strain respectively 75%, 70% and 75% for leaves, flowers and seeds EO. 100 µl dose of three organs essential oils showed a complete inhibition on *C. albicans.*

The Disc-Diffusion Tes*t*

There were significant effect ($p<0.05$) of the different oil volume used for the treatment. After an incubation of 7 days at 30 °C, we noticed a significant inhibition and relatively proportional to the amount incorporated into the culture medium. 2 % showed the higher inhibition for both strains.

The three oils gave the higher inhibition percent for the *C.albicans*. For the *Fusarium oxysporium*, the leaves and flowers oils gave the same results. The percent inhibition of growth is most important than the percent inhibition of *C. albicans* growth (Figure 2).

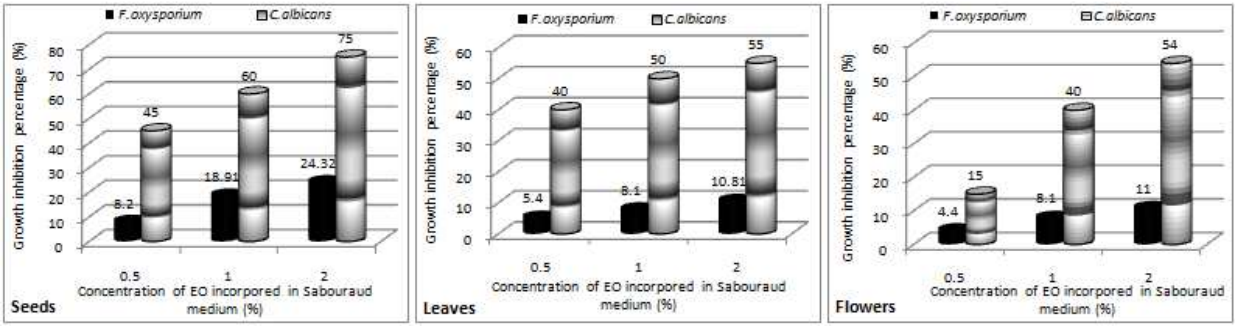


Figure 2: **Growth Inhibition Percentages Obtained of Fungal Strains Exposed to Three Concentrations of Seeds, Leaves and Flowers EO of *Melia azedarach***

DISCUSSIONS

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism (Sen and Batra, 2012). Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Guleria and Kumar, 2006).

In Algeria, *Melia azedarach* resources are abundant but these resources are neglecting. Although a significant number of studies have been used to obtain purified chemical constituents of *Melia azedarach* extracts. In the present investigation, different extracts of *M. azedarach* was evaluated for exploration of their antibacterial and antifungal activity against certain human and plant pathogenic microorganism. Susceptibility of each organ of this plant extract (seed, leaf and flower) was tested by microatmosphere and disc-diffusion methods.

From the above results, it can be concluded that all microorganisms used are susceptible to seeds oil essential more as compared to leaves and flowers extracts. The difference in sensitivity might be ascribed to the difference in chemical composition between organs of plant. Khan and al. (2011) have reported that seeds of *Melia azedarach* constitued of β-sitosterol, vanillin, benzoic acid, vanillic acid, daucosterol, α-D-glucopyranose, limonoid glycoside viz 6,11-diacetoxy-7-oxo-14beta, 15beta-epoxymeliacin (1,5-diene-3- O-beta-D-glucopyranoside) and scopoletin, a hydroxylcoumaramin, melianol meliacin, meliacarpin, meliartenin vanillin, hydroxyl-3-methoxcinnamaldehyde and (+-) pinoresinol. The extract leaves was also phytochemically screened for alkaloids, tannins, saponins, flavonoids, cardiac glycosides and phenols (Sultana et al., 2013).

Our investigation showed that aqueous extracts of *M. azedarach* were active against the locally isolated human and plant pathogens except for *Staphylococcus aureus* by microatmosphere test with greater inhibition zones than those reported by the literature. This analysis of using several extracts so as to study the efficacy of plant for antimicrobial activity has also been realize by many scientist in many species (Khan et al., 2011; Kaneria et al., 2009; Suresh et al., 2008; Saleem et al., 2002; Abdul Viqar et al., 2008; Carpinella et al, 1999-2003; Carpinella and Ferravoli, 2005).

Many plant species present inhibition zones of differing diameters; however, size difference of the inhibition zone depends primarily upon many factors for e.g. diffusion capacity of substances (present in the extracts) in the agar medium, antimicrobial activity of diffused substances, growth and metabolic activity of microorganisms in the medium.

CONCLUSIONS

Based on these results, it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms such as *S. aureus* and *C. albicans* and they can be used in the treatment of infectious diseases caused by resistant microorganisms.

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