ANTIMICROBIAL COMPOUNDS IN SOME LOCAL MEDICINAL PLANTS

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BY

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SUPERVISION SHEET

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Abstract

The antimicrobial effectiveness of 32 Egyptian medicinal plants chosen on notions including anti-microbial potency, widespread dispersal as well as some commercial judges was bio-assayed against 22-test microorganisms, 13 bacteria and 9-phytopathogenic fungi. The bacteria included 10 non-phytopathogenic Gram positive and Gram negative bacteria and 3-phytopathogenic bacteria included ***Erwinia carotovora***, ***Erwinia chrysanthemi*** and ***Xanthomonas campestris.*** The phytopathogenic fungi are affiliated to **Zygomycetes**, **Ascomycetes** and **Deuteromycetes**. n-Hexane and methanol solvents were used to extract low and high polar constituents respectively at the rate of 25% dry plant materials for each solvent in a successive manner using Soxhlet extractor and then tested for their antimicrobial potency using agar diffusion method. Results signified that the probed different parts of the tested medicinal plants exerted varied antimicrobial potency, however, at dissimilar intensities. The most effective extracts against investigated microorganisms were the n-hexane and methanol extracts of *Cymbopogon proximus*, and followed by the hexane extract of *Ammi majus* that antagonized most of non-phytopathogenic bacteria and phytopathogenic fungi. MIC were determined for the most six potent plant extracts exerting the highest activity against tested phytopathogenic microorganisms, the n-hexane extracts of *Artemisia herba-*

alba and *C. proximus* and the methanol extracts of *A. majus* and *C. proximus* antagonized ***E. carotovora*** by 9.38, 8.60, 6.70 and 4.40 $g.L^{-1}$ respectively. However, n-hexane extract of *C. proximus* and methanol extract of *Cupressus sempervirens* antagonized ***Penicillium digitatum*** by 2.20 and 5.00 $g.L^{-1}$ respectively. In a laboratory experiment the anti-citrus green mould caused by ***P. digitatum*** on navel orange fruits and anti-potato tubers soft rot caused by ***E. carotovora*** were bio-assayed by treating artificial orange and potato tubers surfaces wounds with the determined MICs of the selected extracts and then inoculated them with the caustic agent, and incubating both fruits and tubers for intervals ranging between 5 and 10 days, the disease incidence percentage and disease severity index DSI were then determined. The results in orange green mould experiment showed that the n-hexane extract of *C. proximus* and the methanol extract of *C. sempervirens* exhibited the highest incidence against citrus green mould disease, as both decreased disease incidence percentage and DSI compared to untreated fruits as well as delaying the disease symptoms for 10 days. Whereas, the results in potato tubers soft rot experiment showed that the n-hexane extract of *C. proximus*, the methanol extract of *Artemisia herba-alba* displayed the most potent effects against potato tubers soft rot, which they decreased disease incidence percentage and DSI compared with untreated tubers besides delaying the disease symptoms up to 10 days. Five extracts due of 4 medicinal plants included *A. majus*, *A. herba-alba*, *C. sempervirens* and *C. proximus* extracted by n-hexane, methanol and methylene chloride were chosen to recognize their various potent compounds using TLC technique. Bio-autographic pictures of the active ingredients towards ***P. digitatum*** and ***E. carotovora*** were pinched for each plant extract by the direct method. The active spots against tested microorganisms were then investigated to determine their chemical nature using specific chemical reagents. The results showed that the n-hexane extract of *C. proximus* had the most potent antifungal agent at R_f of 0.442 and 0.538, and they were

	identified as volatile phenolic esters and phenolic compounds respectively. Also the most potent antibacterial agent in the methanol extract of *A. herba-alba* and the n-hexane extract of C. *proximus* were identified by TLC as flavonoid and volatile phenolic esters respectively.

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Abbreviations used:

Abbreviation	Full name	Abbreviation	*Full name
E. faecalis	***Enterococcus faecalis***	*A. maurorum*	*Alhagi maurorum* (Medic.)
M. roseus	***Micrococcus roseus***	*A. majus*	*Ammi majus* L.
S. lutea	***Sarcina lutea***	*A. hierochuntica*	*Anastatica hierochuntica* L.
Staph. aureus	***Staphylococcus aureus***	*A. judaica*	*Artemisia judaica* L.
B. stearothermophilus	***Bacillus stearothermophilus***	*A. herba-alba*	*Artemisia herba-alba* Asso.
B. subtilis	***Bacillus subtilis***	*B. aegyptiaca*	*Balanites aegyptiaca* (L.) Del.
E. carotovora	***Erwinia carotovora***	*C. fistula*	*Cassia fistula* L.
E. chrysanthemi	***Erwinia chrysanthemi***	*C. africana*	*Cleome africana* (Botsch.)
E. coli	***Escherichia coli***	*C. droserifolia*	*Cleome droserifolia* (Forssk)
Ps. aeruginosa	***Pseudomonas aeruginosa***	*C. colocynthis*	*Citrullus colocynthis* (L.) Schard.
Ps. fluorescens	***Pseudomonas fluorescens***	*C. althaeoides*	*Convolvulus althaeoides* L.
X. campestris	***Xanthomonas campestris***	*C. sempervirens*	*Cupressus sempervirens* L.
M. phlei	***Mycobacterium phlei***	*C. arabica*	*Cuscuta arabica* Fres.
A. alternata	***Alternaria alternata***	*C. proximus*	*Cymbopogon proximus* (Hochst.)
A. faolena	***Alternaria faolena***	*C. dactylon*	*Cynodon dactylon* (L.) Pres.
A. flavus	***Aspergillus flavus***	*C. esculentus*	*Cyperus esculentus* L.
A. niger	***Aspergillus niger***	*C. papyrus*	*Cyperus papyrus* L.
B. cinerea	***Botrytis cinerea***	*C. rotundus*	*Cyperus rotundus* L.
C. albicans	***Candida albicans***	*D. peregrinum*	*Delphinium peregrinum* L.
F. solani	***Fusarium solani***	*G. capillaris*	*Gypsophila capillaris* (Forssk)
P. digitatum	***Penicillium digitatum***	*J. arabicus*	*Juncus arabicus* (Asch et Buch)
		J. phoenicea	*Juniperus phoenicea* L.
		L. farctum	*Lagonychium farctum* (Banks& Sol.)
		P. harmala	*Peganum harmala* L.
		P. australis	*Phragmites australis* (Cav.)
cv.	Cultivar variety.	*P. maritimum*	*Polygonum maritimum* L.
pv.	Pathogen variety (pathogenicity).	*R. asiaticus*	*Ranunculus asiaticus* L.
ssp.	Sub-species of microorganism.	*S. sesban*	*Sesbania sesban* L.
		S. argel	*Solenostemma argel* (Del.) Hyne.
		T. longipetalus	*Tribulus longipetalus* Viv.
		Z. spina-christi	*Ziziphus spina-christi* (L.) Willd.
		Z. coccineum	*Zygophyllum coccineum* L.

e.g	(From the Latin exempli gratia) meaning for example.	R_f	Retardation factor (TLC).
et al	(From Latin et alii/alia) used especially after names to indicate other people or things.	R_s	Solvent front (TLC).
etc	(From Latin et cetera) used after a list to show that there are other things that you could have mentioned (Remember to take some paper, a pen, etc.).	R_t	Substance distance (TLC).
i.e.	(From Latin idest) to explain exactly what the previous thing that means.	TNT	Tetranitro-tetrazolium-chloride (Bio-autogram).
NB	Written to tell someone to pay attention to something important.	TTC	2,3,5-triphenyl tetrazolium chloride (Bioautogram).
viz.	Used to introduce a list of things that explain something more clearly are given as example.	DSI	Disease severity index.
		MIC	Minimum inhibitory concentration.

*Tackholm (1974), Boulos (1983), Bedevian (1994) and Chevalier (1996).

INTRODUCTION

Since antiquity, 2900 BC, ancient Egyptians highly praised the strength of some medicinal plants in therapeutics, however, the best pharmaceutical witness might be the "Ebers Papyrus'' dating back to 1500 BC; in which they renowned over 700 drugs. In addition, Traditional African System of Medicine endows with intimations on how these plants could be worn as antimicrobial agents. Several medicinal plants illustrate impending activity as antimicrobial agents, warranting further investigation (Iwu *et al*., 1999 and Newman *et al*., 2000). Nowadays, tens of thousands of plants have already been considered for their antimicrobial potency (Rai and Mares, 2003).

From an agricultural point of view, escalating interest in the possible incidence of antimicrobial substances in higher plants is unmistakable, predominantly in medicinal plants (Hoballah, 1999). Therefore, scores of efforts have been done to ascertain new antimicrobial principals in such plants. The most important of which are folk medicines, and systemic screening of them might outcome an innovation of novel potent agents (Nitta *et al*., 2002). It is interesting to determine whether the traditional uses of the selected medicinal plants are strengthened by actual antimicrobial potency or merely based on folklore.

The present work was carried out on a number of selected local medicinal plants which are usually found as wild flora in desert or uncultivated soils or introduced in crop rotation. Medicinal plant extracts were tested for their antibacterial and antifungal effects on a variety of microorganisms. Highly potent antimicrobial extracts were examined as control agents against some post-harvest diseases. Active anti-pathogenic agents were subjected to chromatographic and bioautographic studies to determine the nature of active ingredients present.

Review of literature

The ancient Egyptians put much confidence in plants for curing many diseases. Up till now, the same confidence is still existing among the contemporary Egyptians and a “turn-back” to “remedy by herbs” is now becoming a global rather than regional or national request. This is strongly favoured, since the natural drugs have little or no side effects as do the chemically synthesized medications.

The medicinal plants growing in the various habitats in Egypt represent a major and important component of these plants, which are threatened and some are on the brink of extinction.

Since times immemorial, the use of plants for curing human diseases has been in practice everywhere. Such use of plants is a part of the human history in Egypt as well as in all the countries of N. Africa and the Middle East. The people in the region depended mainly on traditional medicine for their health care needs and the ailments of their animals. The folk medicine in the region is full of receipes for curing various diseases (Batanouny *et al*., 1999).

Medicinal plants are an important health and economic component of the floras in developed as well as developing countries. Many plant species were investigated for their antimicrobial active constituents. This has been done depending on the information of the folk use of these plants, or in species with relatives of species, genera or the same family, known from other countries to have antimicrobial active constituents.

Floristic analyses show that there are about 500.000 plant species on our planet. Out of these, about 12.000 plant species can be used to create biologically active products, which are used in disease treatments. In folk medicine, thousands of years ago medicinal plants products were used in treating a wide spectrum of infectious and other diseases (Pepeljnjak *et al*., 2003).

From the agriculture point of view, the microbial infections of plants represent significant losses of agricultural products. After sustained and/ or

extensive use of existing antimicrobial agents, microorganisms exploited resistance against them. Therefore, it is logical that the search for new prototype antimicrobial products should also include natural products. In particular, medicinal plants are a logical choice, chiefly because of their seemingly infinite variety of novel molecules, which are often referred to as "secondary metabolites". Antimicrobial agents are widely distributed among medicinal plants, but only a few have been evaluated for their activity against plant pathogenic microorganisms (Mongelli, 2003).

Incidence of antimicrobial agents in selected medicinal plants and their related species:

The present literature will be limited to the investigated plants in this study.

Alhagi species:

Bonjar (2004 a) Screened the antibacterial activity of plants used in Iran as folkloric medicine. The author found that, *Alhagi maurorum* showed antimicrobial properties against ***Escherichia coli***, ***Klebsiella pneumonia***, ***Staph. aureus*** and ***Bacillus cereus***.

Ammi majus species:

Naqvi *et al*. (1990) studied antimicrobial investigations on Indian medicinal plants. They found that, *Ammi majus* extract had antifungal effect against ***Microsporum canis*** and ***Trichophyton mentagrophytes.***

Anastatica species:

Rizk *et al.* (1993) studied the constituents of *Anastatica hierochuntica* growing in Qatar. They reported that, the plant extracts possess moderate antimicrobial activity against ***Staph. aureus***.

Artemisia *species:*

Mahmoud *et al.* (1987) studied Egyptian desert plant, *Artemisia herba- alba* antimicrobial effect. They found that, *A. herba- alba* essential oil was highly effective against ***Staph. aureus*** that followed by ***Salmonella sp***., ***Pseudomonas aeruginosa***, ***E. coli*** and ***B. subtilis*** in descending order respectively. Yashphe *et al*. (1987) investigated the essential oils from *Artemisia herba- alba* populations for

their antimicrobial activity. All the oils had slight antibacterial activity against G^- bacteria (***E. coli***, ***Shigella sonni***, ***Salmonella typhosa***, ***Serratia marcescens*** and ***Pseudomonas aeruginosa***) and against G^+ bacteria (***Bacillus subtilis***, ***Streptococcus hemolyticus*** and ***Staph. aureus***). Amin *et al*. (2002) studied 250 Iranian plants for their antifungal activity. They found that *Artemisia herba-alba* extract showed antifungal activity against ***Microsporum canis***, ***Penicillium notatum*** and ***Mucor ramosissimus***. Khafagi and Dewedar (2000) evaluated Sinai medicinal plants for antimicrobial activity. They found that *Artemisia herba- alba* hexane extract showed antibacterial effect against ***Proteus vulgaris*** but it had no effect against ***Candida albicans***, ***Microsporum canis***, ***Trichophyton sp***. and ***T. mentagrophytes***, otherwise, ethanol extract had an effect against ***Staph. aureus***, ***C. albicans***, ***M. canis*** and ***Trichophyton sp***. and ***T. mentagrophytes***.

Ferenczy *et al.* (1972) revealed the screening of antimicrobial effect in higher plants. They found that, *Artemisia vulgaris* and *A. cardunculus* showed antibacterial activity towards ***B. cereus*** and ***C. albicans***. Valsaraj *et al*. (1997) studied the antimicrobial effect of some selected medicinal plants from India. They found that, *Artemisia vulgaris* had antibacterial effect against ***Staph. aureus***, ***B. subtilis***, ***Ps. aeruginosa*** and ***E. coli***. Dulgar and Gonuz (2004) investigated the antimicrobial activity of certain plants used in Turkish folkloric medicine. Their results revealed that, *Artemisia vulgaris* showed no effect towards ***E. coli***, ***Staph. aureus***, ***Ps. aeruginosa***, ***B. subtilis***, ***Micrococcus luteus*** and ***Candida albicans***.

Naqvi *et al.* (1990) studied antimicrobial investigations on Indian medicinal plants. They found that, *Artemisia scoparia* showed antimicrobial effect against ***Staph. aureus***, ***Streptococcus pyogenes*** , ***S. viridans***, ***Diplococcus pneumonia***, ***Microsporum canis***, ***Trichophyton mentagrophytes***, ***C. albicans*** and ***C. tropicalis***.

Also, Badran and Aly (1995) found that, the plant extracts of *Artemisia cina* showed an inhibitive effect on ***Alternaria alternata***, ***Aspergillus flavus***, ***A. niger*** and ***Penicillium chrysogenum***. On the other hand, the medicinal plants *Artemisia molinieri, A. selengensis* and *A. stolonifera* were studied for the antifungal effect against some phytopathogenic fungi. Swiader and Zarawska (1996) found that, all species of *Artemisia* tested were active against the tested phytopathogenic fungi ***C.***

albicans, Aspergillus fumigatus, Fusarium culmorum and ***Alternaria solani***. Vajs *et al.* (2004) studied the antifungal activity of davanones and coumarins isolated from *Artemisia lobelia*. They found that, all the compounds inhibited the growth of ***A. niger***, ***A. flavus***, ***Alternaria alternata***, ***Penicillium ochrochloron*** and ***P. funiculosum***.

Dulgar *et al*. (1999) studied the antifungal activity of *Artemisia absinthium*. They found that, *A. absinthium* had antimicrobial activity against ***E. coli***, ***B. cereus***, ***B. subtilis***, ***Mycobacterium smegmatis***, ***Sarcina lutea***, ***M. luteus***, ***Staph. aureus*** and ***Erwinia amylovora***. Mangena and Muyima (1999) studied the antimicrobial activity of *Artemisia afra* essential oil. They found that, *A. afra* has a broad spectrum of inhibitory activity against ***Streptococcus pyogenes***, ***Micrococcus luteus***, ***Staph. aureus***, ***B. subtilis*** and ***Erwinia carotovora***. Stavri *et al.* (2005) studied the bioactive constituents of *Artemisia monosperma*. They found that, only eriodyctiol-7-methyl ester has activity against ***Mycobacterium sp***. and ***Staph. aureus***.

Balanites species:

Adamu *et al*. (2005) investigated the antimicrobial screening of some Nigerian plants. They found that, *Balanites aegyptiaca* extracts showed antimicrobial activity against ***Proteus mirabilis***, ***Ps. aeruginosa***, ***Staph. aureus*** and ***E. coli***.

Cassia species:

Valsaraj *et al.* (1997) studied the antimicrobial effect of some selected medicinal plants from India. They found that, *Cassia fistula* had antibacterial effect against ***Staph. aureus***, ***B. subtilis***, ***Ps. aeruginosa*** and ***E. coli***. Samy *et al*. (1998) found that, 34 plant species, selected on the basis of folklore medicinal reports in India were assayed for antibacterial activity against (G^- bacteria) ***E. coli***, ***Klebsiella aerogenes***, ***Proteus vulgaris*** and ***Ps. aeruginosa***. They found that, *Cassia fistula* showed significant antibacterial activity against the tested bacteria. Bonjar (2004 b), evaluated the antibacterial properties of some medicinal plants in Iran. He found