Extraction, Identification, Antibacterial and Antifungal activities of ALcadifa (*Tagets pltula L. Marigold*) growing in Iraq

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الخلاصة

في هذه الدراسة تم استخلاص نبات القديفة العراقي بمذيبات قطبية ولا قطبية بوساطة تقنية عمود الكروموتوغرافيا وتشخيص المستخلصات بوساطة تقنية الكروموتوغرافيا الطبقة الرقيقة وتقنية الأشعة فوق البنفسجية والمرئية وتقنية الأشعة تحت الحمراء، ومن ثم دراسة الفعالية المضادة للجراثيم.

Abstract

In this study we extract the the most important bioactive compound from Iraqi plant *Tagets pltula L.* (ALcadifa, Algafary) by using column chromatography, and detected on thin layer chromatography (TLC) plates in comparison with standard. Moreover, Fourier transform infrared (FTIR), and Ultra violet- Visible (Uv-visible) spectrometer were used to confirm the characterization of the extracted. The antimicrobial activity of extracts was tested against six species of microorganisms: The Gram-positive bacteria; *Staphylococcus aurous,* and Gram-negative bacteria included; *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, and Klebsiella pneumonia* and *Candida.*

Introduction

Tagets species are known as a rich source of natural thiophenes. At least a few thiophenes have been detected in every species [1-3]. The marigold species most often used for nematode control are *Tagetes patula*, *T. erecta*, and *T. minuta*. The key mode by which marigolds suppress plant-parasitic nematodes is through a biochemical interaction known as allelopathy. Allelopathy is a phenomenon where a plant releases compounds that are toxic to other plants, microorganisms, or other organisms, such as nematodes. Marigold plants produce a number of potentially bioactive compounds, among which α-therthienyl is recognized as one of the most toxic. This sulfur-containing compound is abundant in marigold tissues, including roots. It has nematicidal, insecticidal, fungicidal, antiviral, and cytotoxic activities, and it is believed to be the main compound responsible for the nematicidal activity of marigold. Thus, nematodes may be killed either by entering the root system of a marigold plant or contacting soil containing marigold's bioactive compounds. The nematicidal activity of marigold has been detected in roots of growing plants but not in root or leaf extracts. Some studies have shown that these nematicidal properties result from a sequence of events in the marigold roots triggered by penetration and movement of nematodes through the root tissue, and the end product of these reactions is thought to kill nematodes. Nematicidal compounds apparently permeate from marigolds' root tissues into nematodes attached to the root, but they are also believed to kill nematodes found in the rhizosphere, the soil near marigold roots. Thus, marigold is believed to be most effective in suppressing plant-parasitic nematodes when actively growing, but it is not as effective when incorporated as crop residues or root extracts. Several other plants with nematicidal properties, including sunn hemp

(*Crotalaria juncea*), are believed to release nematicidal compounds when incorporated into the soil and thus do not require root penetration to effectively kill nematodes [4-10]. The whole herb is aromatic, digestive, diuretic and sedative [11]. It is used internally in the treatment of indigestion, colic, severe constipation [11], coughs and dysentery [12]. Externally, it is used to treat sore eyes and rheumatism [11]. The leaves are harvested as required for immediate use during the growing season, whilst the flowering plant can be dried and stored for later use [11].

Materials and Methods

Chemicals: Acetone, hexane, methanol, ethanol, ethyl acetate, toluene, potassium hydroxide, sodium sulphate, potassium iodide, iodine, potassium bromide, dimethylformamide (DMF), Dimethyl sulphoxide (DMSO), were obtained from BDH Analar (England) and Aldrich Chemical Company (Germany).

Instruments: The FTIR spectra in the range (4000 – 400) cm^{-1} were recorded as KBr disc on FTIR 8300 Shimadzu Spectrophotometer. The UV-Visible spectra were measured in DMF using Shimadzu UV-Vis. 160 A spectrophotometer in the range (200-1000) nm.

Plant Material: *Tagets* were collected from natural habitat during flowering, and identified at the College of Agriculture, Baghdad University , Iraq.

Standards:

(I) Apparatus: Chromatography tube 16mm Pyrex, with 250ml volumetric flask.

(II) Reagents: hexane + acetone + absolute ethanol + toluene (10+7+6+7) ml.

Adsorbent: Mix in mechanical blender 1-2 hr. 1+1 (w/w) silica gel and extractant.

Methanolic potassium hydroxide 40%: (Dissolve 40g of KOH in methanol, cool and dilute with methanol).

Sodium sulphate solution 10%: (Dissolve 10g anhydrous Na_2SO_4 in 100mL. H_2O).

(III) Sample preparation: Air dried plant sample rinsed with acetone to dissolve the exudates material. After evaporation of the solvent, the residues were chromatogaphed. Weigh 2 g sample in to 100 ml volumetric flask, pipit 2ml 40% Methanolic potassium hydroxide in to flask and swirl 1min. then pipit 1ml. H_2O swirl 1min. and let stand in dark 1hr. pipit 30 ml hexane in to flask, dil to vol. with 10% Na_2SO_4, and shake vigorously for 1min. let stand in dark for 1hr. before chromatography.

(IV) Chromatography: With column on filtration, place glass wool plug in bottom and add 12 cm layer absorbent. Apply full vacuumed and add more absorbent to give 7cm layer. Use flat instrument to press firmly. Place 2cm layer anhydrous Na_2SO_4 above absorbent and press.

(V) Thin layer chromatography: The extracted compounds were dissolved in an appropriate solvent, applied to silica gel plates, Merck (Germany) 20 × 20 cm,

0.25mm in thickness, and developed using the solvent system : ethyl acetate : toluene : methanol (9 : 1.5 : 1).

Antibacterial Activity: Five strains of bacteria and one yeast were used as test microorganisms. The bacterial strains included Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*; and the yeast *Candida*. All microorganisms were clinical isolates, obtained from the biochemical technology division, department of applied sciences, university of technology, Iraq, and very carefully identified using standard microbiological methods.

Antimicrobial assay: Extracts were diluted two-fold and used at a concentration starting from 100% to an end dilution of 6.25% where 25 mg/mL in 10% DMSO were prepared using vortex mixture. Whatman paper discs were injected with 20 µL of different *N. Tagets* extracts using a micropipette and were dried in the biosafety cabinet for 30 min. Negative control disc were prepared using a 10% DMSO. All the bacteria were cultured on Nutrient Agar (NA) (Merck). Inoculums were prepared in 5 mL Mueller Hinlon broth with 3-5 colonies of each bacterial strain. The inoculums were incubated at 35°C for 2-3 h to get an approximately close to 0.5 McFarland standard for susceptibility testing [13]. Two methods were used- 1) Disc diffusion method: Sterile paper discs impregnated with each extract were placed aseptically over the bacterial cultures and incubated at 37°C for 24 h. 2) Well diffusion method: wells were made on inoculated agar and each well filled with 25 µL of extract. Each inoculated plate was incubated with a positive-control antibiotic disc for each of the bacteria.

Results and Discussion

Mainstream medicine is increasingly receptive of the use of antimicrobial and other drugs derived from plants, as traditional antibiotics become ineffective and because of the rapid rate of plant species extinction. There is a feeling among natural-products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures, which could be synthesized chemically, is at risk of being lost irretrievably [14].

The determination of the MIC; (Table 1) showed that 3 plant extracts tested by column chromatography exhibited an antimicrobial effect against some of the six tested micro-organisms.

Table 1: The MIC values in mg/ml of *Tagets pltula* extracts in Agar diffusion assay.

Ext.	Microorganisms					
	Staphylococcus aureus	***Escherichia Coli***	***Proteus vulgasis***	***Pseudomonas aeruginosa***	***Klebsiela***	***Candida albicans***
1	0.71	0.70	0.70	0.83	1.10	-
2	0.15	0.22	0.15	0.15	0.19	0.17

3	0.16	0.13	0.12	0.20	0.17	0.13
Gen	0.5	1.56	8	3.5	3.0	-
Amp	-	-	-	-	-	0.15

Gen = Gentamicin, Amp = Amphotericin, Ext = Extractants

In disc diffusion method, the α-erthienyl extract of the plant showed considerable activity against all bacteria (Table 2).

Table 2: Antibacterial activity of *Tagets pltula* extracts against bacteria and yeast in Disc diffusion method.

Ext.	Microorganisms					
	S. aureus	***Escherichia Coli***	***Proteus vulgasis***	***Pseudomonas aeruginosa***	***Klebsiela***	***Candida***
1	19.8	20.4	21.4	19.2	19.3	-
2	28.5	30.5	25.4	27.5	28.7	26
3	26.5	26.5	24.7	23.5	25.7	24
Gen	19	15	18	17	16	-
Amp	-	-	-	-	-	18

Gen = Gentamicin, Amp = Amphotericin, Ext = Extractants

It seems that the MIC values of the extracts as well as amphotericin and gentamicin do not depend on the type of the media used and also the antibacterial activity of quercetagetin is lower than α-erthienyl and 5-(but-3-en-1-ynyl)-2,2'-bithiophene.

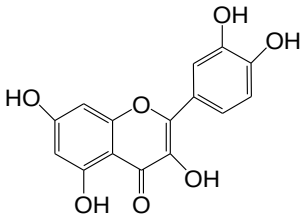
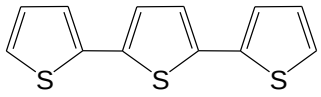
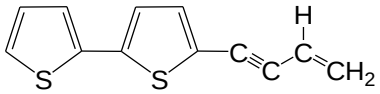
Regarding antifungal activity, Quercetagetin extract from *Tagets pltula* were resisted by *Candida*. The strongest antifungal activity was observed using the α-terthienyl and 5-(but-3-en-1-ynyl)-2, 2'-bithiophene extracts from *Tagets pltula* with an MIC value of 0.17, and 0.13 mg/ml. The standard drug Amphotericin achieved the highest antifungal activity against *Candida* (*MIC*=0.15 mg/ml).

Table 3: Identification of marigold.

No.	***Retention time(min)***	***R_f value***	***$\lambda_{max\ (nm)}$***	***Extractant***
1	17.5	R_f 1 = 0.86	281, 335	Quercetagetin
2	35	R_f 2 = 077	293, 367	Alfa-Terthienyl
3	55	R_f 3 = 0.72	299, 415	5-(but-3-en-1-ynyl)-2,2'-bithiophene

Table 4: The physiochemical data of extractants.

No.	**Structures**	**IR cm^{-1}**				
		O-H	**C=C**	**C-S**	**C-O-C**	**C=O**

1		3420	1608	-	1235asy 1122sy	1667
2		-	1630	735	-	-
3		-	1635	700	-	-

Conclusion

The activity of the plant against both gram-positive and gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. Since *Tagets pltula* demonstrates activity against the most prevalent gram-negative bacteria in urinary infections namely E. coli, the use of the plant as a urinary anti-infective is validated. Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases.

References

1) Sorensen, N. A. (1977), Polyacetylenes and conservatism of chemical characters in the *Compositae.* In: The Biology and Chemistry of the *Compositae* I. (Heywood V. H. Harborne J. B. and Turner B. L. eds.), Academic Press, London, New York, San Franscisco, pp. 385D 409.

2) Bohlmann, F. and Zdero C. (1985), Naturally occurring thiophenes. In: Thiophene and its Derivatives I. (Gro- nowitz J. ed.), John Wiley, Chichester, pp. 261D323.

3) Kagan J. (1991), Naturally occurring di- and trithiophenes. In: Progress in the Chemistry of Organic Natural Products **56,** (Herz, W. Kirby, G. W. Tamm, Ch. eds.). Springer, Wien, New York, pp. 87D169.

4) Hooks and Fallon. 2006. Anally in the war against nematode pests: Using sunnhemp as a cover crop to suppress root-knot nematodes. University of Hawai‘i at Mänoa, College of Tropical Agriculture and Human Resources, Plant Disease publication PD-32.

5) Lehman, P.S. (1979), Factors influencing nematode control with marigolds. Florida Dept. of Agriculture Conservation Service, Nematology Circular no. 50.
6) Natarajan, N. Cork, A. Boomathi, N. Pandi, R. Velavan, S. and Dhakshnamoorthy, G. (2006), Cold aqueous extracts of African marigold, *Tagetes erecta*, for control of tomato root knot nematode, *Meloidogyne incognita*. Crop Protection 25: 1210–1213.
7) Ploeg, A.T. (2002), Effect of selected marigold varieties on root-knot nematodes and tomato and melon yields. Plant Disease 86: 505-508.
8) Ploeg, A.T. and Maris, P.C. (1999), Effect of temperature on suppression of *Meloidogyne incognita* by *Tagetes*cultivars. Supplement to the Journal of Nematology 31: 709–714.
9) Sipes, B.S. and Arakaki, A.S. (1997), Root-knot nematode management in dryland taro with tropical cover crops. Supplement to the Journal of Nematology 29:721–724.
10) Topp, E. Miller, S. Bork, H. and Welsh, M. (1998), Effects of marigold (*Tagetes* sp.) roots on soil microorganisms. Biology and Fertility of Soils 27: 149–154
11) Bown, D. (1995), *Encyclopaedia of Herbs and their Uses.* Dorling Kindersley, London. ISBN 0-7513-020-31
12) Duke, J. A. and Ayensu, E. S.(1985), *Medicinal Plants of China* Reference Publications, Inc. ISBN 0-917256-20-4
13) National Committee for Clinical Laboratory Standards (NCCLS). 2002. Performance standards for antimicrobial susceptibility testing. NCCLS approved standard M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.
14) Cowan, M. (1999), Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12(4): 564-582.