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*Full Length Research Paper*

# Antibacterial and antifungal activities of *Cotula coronopifolia*

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**This study examines the antibacterial and antifungal activities of extracts, and one active principle: 6-methoxy-1-benzofuran-4-ol, isolated from the aquatic plant *Cotula coronopifolia*, against six pathogenic bacterial strains and seven pathogenic fungi. Results showed positive antibacterial and antifungal values, and underline the potential of the plant *C. coronopifolia* either as natural preservative or in pharmaceutical applications.**

**Key words:** Antibacterial activity, antifungal activity, *Cotula coronopifolia*.

## INTRODUCTION

It is well known that infectious diseases account for high proportion of health problems, especially in the developing countries. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davies, 1994). This resistance has increased due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists to search for new antimicrobial substances from various sources, such as medicinal plants (Karaman et al., 2003).

Moreover, in the face of the excessive use of synthetic antibiotics, we are interested in the possibility of their replacement with natural, biodegradable products having more specific activity. Some of the antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Mitscher et al., 1987). Consequently, search for natural additives, especially of plant origin, has notably increased. For these reasons, we initiated an investigation for the evaluation of antimicrobial properties of the aquatic plant *Cotula coronopifolia* (*Asteraceae*) from the Tunisian coast. The genus *Cotula* comprises about 55 species, most of them endemic to South Africa, two occur in Australia, two in Asia, two on the Tristan da Cunha Islands in the South Atlantic Ocean, and one each in North Africa (Jakubowsky and Mucina, 2007). The antimicrobial activity of several plants belonging to the *Asteraceae* family has been described in several studies. Indeed, Kokoska et al. (2002) reported antimicrobial activity of *Achillea millefolium, Tussilago farfara, Arctium lappa, Cichorium intybus* and *Rhaponticum carthamoides.* Concerning the genus *Cotula*, Markouk et al. (1999) announced antibacterial activity of *Cotula cinerea* extracts.

The aim of this work was to investigate the antimicrobial activities of extracts and one pure compound isolated from *C. coronopifolia* growing in Tunisia.

## MATERIALS AND METHODS

### General experimental procedures

$^{1}H$, $^{13}C$ NMR spectra were obtained with Bruker WP 300

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spectrometer at 300 MHz for $^{1}H$ NMR and 75 MHz for $^{13}C$ NMR. Measurements were made in $CDCl_3$ at 25°C and the resonances of residual solvents were used as internal references. ESIMS spectrum was recorded in positive switching mode, using a HP 1100 MSD apparatus.

**Plant material**

The plant *C. coronopifolia* was identified by Dr Fathia Harzallah-Skhiri (Laboratoire de Biologie Végétale, Ecole Supérieure d' Horticulture et d' Elevage, Chott Mariem, Tunisia).

Aerial parts (leaves, stems and flowers) of the aquatic plant *C. coronopifolia* were collected at the beginning of Mars 2008 in Monastir (Tunisian coast). A voucher specimen, Cot 2107, was deposited at the herbarium in the Faculty of Sciences, University of Monastir, Tunisia.

**Preparation of extracts**

Dried and finely powdered *C. coronopifolia* aerial part (leaves, stems and flowers) (2500 g) was immersed in methanol (MeOH) at room temperature for a 24 h and was extracted three times (3x3L). The obtained extract was filtered and evaporated to dryness. The crude methanolic extract was extracted successively with equal volume of four organic solvents of increasing polarity (Petroleum ether, Chloroform, Ethyl acetate and Butanol). Four extracts were obtained and subdivized E1, E2, E3 and E4 respectively for the petroleum ether, chloroformic, ethyl acetate and butanolic extracts. Each extract was taken to dryness under vacuum and stored at 4°C.

**Isolation and identification of the active compound 1**

Since the chloroform extract (18 g) showed promising antimicrobial and antifungal activity, it was fractioned by column chromatography over silica gel (450 g) using as eluent Chloroform/Petroleum ether; Chloroform; Chloroform/Acetone and Acetone gradients. We collected 275 fractions (each 200 ml) by increasing the polarity of solvent. Based on thin layer chromatography profile the fractions were pooled together to get 17 groups of fractions symbolized F1 to F17. The active compound was isolated from the fraction noted F4. The total structural elucidation of compound 1 was performed by combining a variety of different advanced 1D and 2D NMR experiments that allow a complete $^{1}H$ and $^{13}C$ signal assignment.

**Antibacterial activity**

The antibacterial activity was evaluated against two Gram negative bacteria *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and four Gram positive bacteria *Staphylococcus aureus* (ATCC 25923), *Enterococcus fecalis* (ATCC 29212) and the two clinical strains *Citrobacter freundei* and *Proteus mirabilis*. The microorganisms were obtained from the Laboratory of transmissible diseases and biologically active substances, Faculty of Pharmacy, Monastir, Tunisia.

Cultures of these bacteria were done on Nutrient Muller-Hinton medium and were incubated at 37°C for 24h. Gentamycin was used as positive control. Three replicate runs were carried out for each concentration and for each microorganism. The antimicrobial activity was evaluated by paper disc diffusion (Marmonier, 1987) and dilution methods (Gulluce et al., 2004). The minimum inhibitory concentration (MIC) values were also studied for the microorganisms which were determined as sensitive in disc diffusion assay (diameter superior to 8 mm).

**Antifungal activity**

Samples were tested against seven strains of fungi, comprising two opportunist pathogenic yeasts (*Candida albicans* and *Cryptococcus neoformans)*; three dermatophytes (*Trichophyton rubrum, Trichophyton soudanense* and *Microsporum canis*) and two hyphomycet (*Aspergillus fumigatus* and *Scopulariopsis brevicaulis*). Antifungal activity was assayed by the method of agar incorporation (dilution in a solid medium) including a negative control, as described previously (Benjilali et al., 1986; Yang et al., 1996; Griffin et al., 2000). Briefly, the test was performed in sterile Petri dishes (33mm Ø) containing sabouraud Glucose Agar (SGA). Samples were mixed aseptically with SGA (100 ml) to give stocks with concentrations of 1000, 500 and 250 $\mu l.ml^{-1}$. Stocks were dissolved in 99% EtOH, and this solvent was used as the negative control. After cooling and solidification, the medium was inoculated with a small amount (5 mm Ø) of a 7 day-old mycelium culture (for dermatophytes), a 3-day-culture suspension adjusted to $10^5$ conidies/ml (*Aspergillus* and *Scopulariopsis*) or a 3-day culture suspended in sterile distilled water and adjusted to $10^5$ spores $ml^{-1}$ (yeasts). The Petri dishes were then incubated for 7 days at 24°C for dermatophytes and *Scopulariopsis*, 24 h at 37°C for *Candida* and *Aspergillus* and 48 h at 37°C for *Cryptococcus*. The antifungal agent, Fluconazol was included in the assays as positive control. Three replicate runs were carried out for each concentration and for each microorganism. The antifungal activity of the samples was evaluated using two methods:

(1) By calculating the percentage inhibition (%I) from the diameters of the colonies in the control plate (dC) and the colonies in the treated plate (dE); %I = (dC-dE)/dC, according to the method of Singh et al. (1993).
(2) By determining the minimum inhibitory concentration (MIC), defined as the lowest concentration which inhibits the visible growth of fungi during the defined incubation period for each species.

The microorganisms were obtained from the Laboratory of Transmissible Diseases and Biologically Active Substances, Faculty of Pharmacy, Monastir, Tunisia.

## RESULTS

### Compound 1

The compound 1: 6-methoxy-1-benzofuran-4-ol ($C_9H_8O_3$) was obtained as white amorphous powder. NMR spectroscopic data run in $CDCl_3$: $^{1}H$ NMR δ 3.88 (O-CH3), 6.18 (H-3, d, J= 9.3 Hz), 6.76 (H-7, s), 7.11 (H-5, s), 7.84 (H-2, d, J= 9.3 Hz). $^{13}C$ NMR δ 57.1 (CH3-O), 104.3 (C-3), 110.2 (C-7), 112.7 (C-5), 135.1 (C-3a), 146.5 (C-2), 147.6 (C-4), 151.9 (C-7a), 164.5 (C-6). The structure of the compound 1 was established on the basic of 2D NMR spectroscopic experiments (COSY, HMBC) (Figure 1).

### Antibacterial activity

The *in vitro* antibacterial activity of the extracts and the natural product were qualitatively and quantitatively tested by using disc diffusion and liquid dilution method with the microorganisms as seen in Table 1.

The results of the bioassays showed that the four

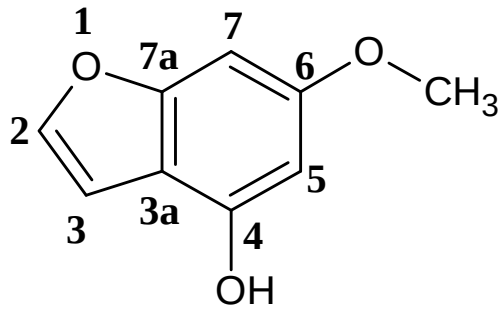


**Figure 1.** 6-methoxy-1-benzofuran-4-ol.

extracts and the natural product exhibited appreciable antibacterial activities against all bacteria. This activity varies with the species of bacteria.

The present study revealed that petroleum ether extract of *C. coronopifolia*. was more active against *E. fecalis* and *S. aureus*, presenting an important growth inhibition at lower concentrations (MIC= 31.25 µg.ml$^{-1}$).

Chloroform extract showed high antibacterial activity especially against *E. fecalis* (MIC= 15.62 µg.ml$^{-1}$). Butanol extract was less potent but effective against *E. fecalis* (MIC= 15.62 µg.ml$^{-1}$). Activity of the ethyl acetate extract was also appreciable (MIC= 250 µg.ml$^{-1}$). The natural product indicated an interesting antibacterial activity against all bacteria especially against *E. fecalis* (MIC=3.9 µg.ml$^{-1}$). In this study, the two clinical bacteria *C. freundei* and *P. mirabilis* were slightly more resistant.

### Antifungal activity

Results of the antifungal activity of samples of *C. coronopifolia* are presented in Table 2. The inhibition varied widely (0–100%) in the presence of 125 µg.ml$^{-1}$.

The results of the bioassays showed that extracts and the natural product exhibited an interesting antifungal activity against all fungi except the pathogenic yeasts *C. albicans* and *C. neoformans*. Indeed samples showed strong to very strong inhibition (52-100%). *C. albicans* showed resistance (0% inhibition) but was strongly inhibited by the chloroformic extract and the natural product (100%).

The results of MIC confirm those obtained using the percentage inhibition method. In this study, the dermatophytes were clearly the most sensitive fungal species and the yeasts were the most resistant.

## DISCUSSION

### Antibacterial activity

*C. coronopifolia* samples appeared slightly more active against Gram-positive than Gram-negative bacteria. This higher resistance among Gram-negative bacteria could be due to the differences in the cell membrane of these bacterial groups. Indeed, the external membrane of Gram-negative bacteria renders their surfaces highly hydrophilic (Smith-Palmer et al., 1998), whereas the lipophilic ends of the lipoteichoic acids of the cell membrane of Gram positive bacteria may facilitate penetration by hydrophobic compounds (Cox et al., 2000; Ultee et al., 1999).

According to several authors, *P. aeruginosa* appeared to be less sensitive to the action of many extracts (Dorman and Deans, 2000; Senatore et al., 2000; Pintore et al., 2002; Wilkinson et al., 2003).

Whereas in our research all extracts tested and the natural product were effective against these gram-negative bacteria, especially the chloroformic extract (MIC= 31.25 µg.ml$^{-1}$) and the natural product (MIC= 7.8 µg.ml$^{-1}$).

The natural product isolated (6-methoxy-1-benzofuran-4-ol) showed an interesting antibacterial activity against all bacteria, indeed benzofuran derivatives are an important class of heterocyclic compounds that are known to possess important biological properties. Widespread interest in the chemistry of benzofurans in a large number of natural products has attracted due to their biological activities and their potential applications as pharmacological agents. Indeed, some compounds have antimicrobial activity. Recently, we reported that, some of 3-substituted-5-(benzofuran-2-yl)-pyrazole derivatives showed significant antimicrobial activities towards various microorganisms (Abdel-Wahab et al., 2008).

### Antifungal activity

Our natural product isolated (6-methoxy-1-benzofuran-4-ol) showed an interesting antifungal activity against all fungi except *C. albicans*. There are well known natural products having related benzofuran ring structures, which are particularly isolated from *Machilus glaucescens*, *Ophryosporus charua*, *Ophryosporus lorentzii*, *Krameria ramosissima*, and *Zanthoxylum ailanthoidol* (Kim et al., 2001). The most recognized benzofurans are ailanthoidol, amiodarone and bufuralol compounds. Ailanthoidol, a neolignan with a 2-arylbenzofuran skeleton, was isolated from the Chinese herbal medicine *Zanthoxylum ailanthoides*. It has been reported that neolignans and lignans possess a variety of biological activities such as anticancer, antiviral, immunosuppressive, antioxidant, antifungal and antifeedant activities (Kao and Chern, 2001).

Several benzofuran are widely distributed in nature and has been reported to have antiviral, antioxidant and antifungal activities (Fuganti and Serra, 1998). Two benzofuranes from *Eupatorium aschenbornianum* presented antimicrobial activity (Rios et al., 2003).

**Table 1.** Antibacterial activity of *C. coronopifolia* L.

| Microorganisms[a] | | E1 | E2 | E3 | E4 | Compound 1 | GTb |
|---|---|---|---|---|---|---|---|
| EC | IZ* | 10 | 12.5 | 10.5 | 10 | 14 | 19 |
| | MIC** | 125 | 125 | 250 | 250 | 62.5 | 2 |
| EF | IZ* | 12 | 12.5 | 10 | 13 | 14 | 20 |
| | MIC** | 31.25 | 15.62 | 250 | 62.5 | 3.9 | 0.75 |
| SA | IZ* | 9 | 10 | 11 | 10 | 12 | 19 |
| | MIC** | 31.25 | 31.25 | 250 | 250 | 15.62 | 1 |
| PA | IZ* | 9.5 | 11 | 10 | 9.5 | 13 | 15 |
| | MIC** | 62.5 | 31.25 | 250 | 250 | 7.8 | 1 |
| CF | IZ* | 9 | 9.5 | 10 | 9 | 10.5 | 17 |
| | MIC** | 500 | 250 | 250 | 500 | 250 | 0.75 |
| PM | IZ* | 10 | 9 | 10.5 | 11 | 12 | 17.5 |
| | MIC** | 250 | 250 | 250 | 125 | 125 | 0.75 |

*IZ: inhibition zone (mm) including disk diameter of 6 mm, **MIC: minimum inhibitory concentration (µg.ml$^{-1}$), [a]Microorganisms: EC, *Escherichia coli* (ATCC 25922); EF, *Enterococcus fecalis* (ATCC 29212); SA, *Staphylococcus aureus* (ATCC 25923) ; PA, *Pseudomonas aeruginosa* (ATCC 27853); CF, *Citrobacter freundei*; PM, *Proteus mirabilis*. nt: not tested, [b]GT: Gentamycin.

**Table 2.** Antifungal activity of *C. coronopifolia* L.

| Micro-organisms[a] | | E1 | E2 | E3 | E4 | Compound 1 | FCb |
|---|---|---|---|---|---|---|---|
| TR | I%* | 61 | 66 | 72 | 72 | 56 | 100 |
| | MIC** | 250 | 250 | 250 | 250 | 250 | 75 |
| TS | I%* | 59 | 55 | 60 | 95 | 50 | 100 |
| | MIC** | 250 | 250 | 250 | 125 | 250 | 75 |
| MC | I%* | 56 | 55 | 56 | 100 | 50 | 100 |
| | MIC** | 250 | 250 | 250 | 125 | 250 | 75 |
| SB | I%* | 59 | 100 | 100 | 58 | 50 | 100 |
| | MIC** | 250 | 125 | 125 | 250 | 250 | 75 |
| AF | I%* | 100 | 100 | 100 | 100 | 100 | 100 |
| | MIC** | 125 | 125 | 125 | 125 | 125 | 50 |
| CA | I%* | 100 | 0 | 100 | 0 | 0 | 100 |
| | MIC** | 125 | >103 | 125 | >103 | >103 | 65 |
| CN | I%* | 0 | 0 | 100 | 0 | 100 | 100 |
| | MIC** | >103 | >103 | 125 | >103 | 125 | 75 |

*I%: Percentage inhibition of microorganisms in the presence of 125 µg.ml$^{-1}$ of samples (0-25%,no or little inhibition; 25-50%,average inhibition; 50-75%, strong inhibition; 75-100%, very strong inhibition), **MIC: minimum inhibitory concentration (µg.ml$^{-1}$), [a]Microorganisms : TR, *Trichophyton rubrum*; TS, *Trichophyton soudanense*; MC, *Microsporum canis* ; SB, *Scopulariopsis brevicaulis*; AF, *Aspergillus fumigatus* ;CA, *Candida albicans*; CN,*Cryptococcus neoformans*, [b]FC: Fluconazol.

## Conclusion

This preliminary screening is an interesting evaluation of the potential antimicrobial activity of the plant *C. coronopifolia*.

The results obtained indicate that further assays will be worthwhile to search for more eventual activities of this plant. Our next approach will be focused on isolating and testing pure active compounds. These active metabolites should provide models for the synthesis of better bactericides and fungicides from *C. coronopifolia.*

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