Antimicrobial Activity of Xerophytic Plant (*Cotula cinerea* Delile) Extracts Against Some Pathogenic Bacteria and Fungi

Djamel Bensizerara[1], Taha Menasria[2,*], Maimouna Melouka[1], Lamia Cheriet[1] and Haroun Chenchouni[2]

[1]*Department of Natural and Life Sciences, Faculty of Nature and Life Sciences and Sciences of Earth and Universe, University of Kasdi Merbah, Ouargla 30000, Algeria.*

[2]*Department of Natural and Life Sciences, Faculty of Exact Sciences and Natural and Life Sciences,*

University of Tebessa, Tebessa 12002, Algeria.

Abstract

In the present investigation, an Algerian commonly available plant namely *Cotula cinerea*, found throughout sandy desert grounds, was screening for antimicrobial activity against five different human pathogenic microbes namely, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans*. The antimicrobial activity was evaluated using the agar disc diffusion method. Aerial parts of *C. cinerea* were subjected to extraction using four solvents of different polarity (70% ethanol, *n*-butanol, ethyl acetate and petroleum ether). Petroleum ether and *n*-butanol extracts had the most effective antimicrobial activity with Gram-negative *K. pneumoniae* demonstrating the highest susceptibility. Linear regression analysis was performed to find correlations between extract concentrations and inhibition activity. Results showed a significant increase in mean diameter of inhibition zone with increasing extract concentrations of all solvents except *n*-butanol. Two-way ANOVA test was used to compare the effect of *C. cinerea* extracts on the antimicrobial properties. All plant extracts have shown significant differences in their actions as antimicrobial agents. Indeed, the *n*-butanol extract at a low concentration of 0.25 mg mL^{-1} indicated a potent antimicrobial activity of *C. cinerea* extracts.

Keywords: Antimicrobial activity, *Cotula cinerea*, Pathogenic microbes, Medicinal plant.

1. Introduction

Medicinal plants, which form the backbone of traditional medicine, have been in the last few decades the subject of very intense pharmacological studies. This has been brought by the acknowledgement of the value of medicinal plants as potential sources of new therapeutic compounds and drug development (Matu and van Staden, 2003). According to the World Health Organization, about 80% of the world's population living in developing countries rely mostly on plants for primary health care (McKay and Blumberg, 2007).

In recent years, pathogenic microorganisms have developed multiple drug resistance due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases (Katsumi *et al*., 2005). An increase in the emergence of multidrug-resistant bacteria is worrying the world population. Infection rates have greatly increased and antibiotics resistance has become an ever-increasing therapeutic problem (Shahid *et al*., 2008).Therefore, there is a need to develop alternative antimicrobial drugs from various sources such as medicinal plants (Cordell, 2000).

Plants with antimicrobial activities have become more interesting because many people are aware of problems associated with the over-prescription and misuse of traditional antibiotics. However, only about 20% of the plants found in the world have been subjected to pharmacological or biological testing (Mothana and Lindequist, 2005). Plants in the environment are exposed to a range of abiotic stresses such as osmotic stress, salinity, and temperature variations. These, in-turn, affect their growth and the metabolic processes involved in the synthesis of a wide range of secondary metabolites, such as polyphenols, tannins, terpenoids, alkaloids etc, which may have remarkable antimicrobial properties (Cowan, 1999; Marzouk *et al*., 2010; Daglia, 2012).

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* Corresponding author e-mail: tahamenasria@hotmail.com

The Algerian flora plays a key role in supporting traditional medicine, which is widely practiced over the country. This flora holds a rich diversity of medicinal and endemic plants (Beloued, 2005). Many plants used in the Algerian traditional medicine have the potential to provide pharmacologically active natural products (Maiza *et al.*, 1993; Hammiche and Maiza, 2006). Ethnopharmacological interest in the sources of these compounds has increased nationally and worldwide, particularly in the search for drugs to counter multi-drug resistant microorganisms.

Cotula cinerea L., syn. *Brocchia cinerea* Del. (Asteraceae), is a xerophytic plant widely distributed in sandy and desert grounds (Markouk *et al.*, 1999a). This medicinal plant popularly known as (Gartoufa or Chouihiya), is commonly used in Algerian folk medicine, as well in the rest of the Maghreb region, as an anti-inflammatory, analgesic, antipyretic, antiseptic, and for treatment of various other diseases, including digestive problems (constipation and colic), rheumatism, and urinary and pulmonary infections. It is much appreciated in green tea or mixed with food to enhance the flavour (Maiza *et al.*, 1993; Markouk *et al.*, 1999a, 1999b; Larhsini *et al.*, 2002; Hammiche and Maiza, 2006). Several compounds have been isolated from *C. cinerea*, including flavonoids, sesquiterpene lactones, sesquiterpene coumarins and tannins (Ahmed *et al.*, 1987; Markouk *et al.*, 1999b). The objective of this work is to evaluate the antimicrobial activity of *C. cinerea* extracts, obtained using various solvents, against some pathogenic bacteria and fungi.

2. Materials and Methods

2.1. Plant Material

Cotula cinerea samples were collected from its natural range of distribution in El-Oued (Algerian Sahara Desert) (about 4 km Southeast of El-Oued city, 33°20'N to 33°19'N, 6°52'E to 6°53'E) in March 2011.

2.2. Extraction Protocol

2.2.1. Extracting Solvents

The extraction was carried out by using four solvents of increasing polarity: (i) petroleum ether (non-polar), (ii) ethanol, (iii) *n*-butanol, (iv) ethyl acetate (later three are moderately polar).

2.2.2. Preparation of Plant

The freshly picked aerial parts of the plant used in the screening were air-dried at room temperature for 2 weeks, with no direct sunlight. Once dry, plant was ground into fine powder and stored at 4 °C until time of extraction.

2.2.3. Preparation of the Extracts

The powdered plant material (20 g) was macerated for 24 h three times (3×24 h) in a mixture of ethanol/water (70:30; v/v) with frequent agitation at room temperature (25 ± 1 °C). Then the mixture was filtered using filter paper (Whatman No. 1) under the vacuum of a water pump and the ethanol was evaporated under low pressure using a rotary evaporator at 50 °C. The residue was taken as the hydro alcohol extract (Dall'Agnol *et al.*, 2003). The remaining aqueous extract was fractioned with petroleum ether, ethyl acetate and *n*-butanol (3×100 mL for each solvent). These extracts were dried under reduced pressure using a rotatory evaporator at 40 °C. The residues were taken as the petroleum ether, ethyl acetate and *n*-butanol extracts of the plant (Boligon *et al.*, 2011).

2.3. Antimicrobial Activity

2.3.1. Microbial Strains and Growth Conditions

Five clinical isolates of microorganisms were used for assessing the plant antimicrobial properties, including the Gram-positive *Staphylococcus aureus*, the Gram-negative *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, and the yeast *Candida albicans*. Table 1 lists the strains and their resistance phenotypes. All strains were obtained from the Microbiology Laboratory of Hospital Benamor Djilani (El-Oued, Algeria) and were maintained at 4 °C on slants of Nutrient Agar (NA) for bacteria and Sabouraud Dextrose Agar (SDA) for the yeast. Active cultures were prepared by transferring a loop of cells from the agar slant to a test tube containing 5 mL of Nutrient Broth for bacteria and Sabouraud Dextrose broth for the yeast. They were then incubated overnight to reach the logarithmic phase of growth; for about 6–10 hours at 37 °C for bacteria and 12–16 h at 30 °C for *C. albicans*.

Table 1. Resistance pattern of target organisms to antibiotics.

Strain	Phenotype of resistance	Phenotype of sensibility
Staphylococcus aureus	AMC, AMX, CE, CF, OX, PEN	CHL, CIP, GEN, PEF
Escherichia coli	AMC, AMX, CIP, PEF, SXT	CE, CF, CEF, C, GEN, IMI, STR
Pseudomonas aeruginosa	AMC, AMX, ATM, CF, CIP, PEF, SXT	GEN, IMI, PEF, PIP
Klebsiella pneumoniae	IMI	C, SXT
Candida albicans	ND	ND

(AMC: amoxicillin + acid clavulanic, AMX: amoxicillin, ATM: aztreonam, C: chloramphenicol, CE: cefatxin, CF: cefalotin, CEF: cefazolin, CIP: ciprofloxacin, GEN: gentamicin, IMI: impipenem, ND: not determined, OX: oxacillin, PEF: pefloxacin, PEN: penicillin, PIP: piperacillin, STR: streptomycin, SXT: trimethoprim/sulfamethoxazol)

2.3.2. Antimicrobial Assay (Disk Diffusion Assay)

The disc-diffusion assay (Qaralleh *et al.*, 2010) was used to determine growth inhibition caused by plant extracts. Inoculums, containing 10^6–10^8 CFU per milliliter, was spread on Mueller–Hinton (MH) agar plates for the four bacteria and 10^4–10^5 CFU per milliliter was poured over the base plates forming a homogenous top layer on SDA for the yeast. Using sterile forceps, Whatman's filter discs (Ø = 5 mm), impregnated with different dilutions of extracts (25, 50, 75 and 100%) from the initial concentration of 1 mg mL^{-1}, were placed on inoculated plates and left at 4 °C for 2 h before being incubated to allow the diffusion of the extract. Discs saturated with solvents (ethanol, petroleum ether, ethyl acetate, and *n*-butanol) were air-dried and used as negative controls. The plates were incubated at 37 °C for

24 h for bacteria and 48 h at 30 ℃ for the yeast, after which, inhibition zones around each disc (> 5 mm) were measured (disc diameter included).

2.4. Statistical Analysis

Linear regression analysis (LRA) was carried out to find statistically significant correlation between the different concentrations of each extract and their overall antimicrobial activity, assessed as diameter of inhibition with disregard to the tested strains. The data pertaining to antimicrobial activity of different *C. cinerea* extracts were also analysed with two-way ANOVAs, to test the effect of "tested strains" and "extract dilutions" on the levels of antimicrobial activity. Interaction between tested microbe-species and extract concentration was also included in the analysis for each plant extract. Besides the explanatory ability of LRA, it could be used, supported by ANOVA outputs, to detect potency "effectiveness" of the extract itself disregarding its concentration. Both LRA and ANOVA were considered statistically significant when P-value < 0.05.

3. Results

Hydro-alcohol extract of *C. cinerea* had a much higher extraction yield (w/w %) than the other extracts, whereas, the *n*-butanol extract had the lowest yield. Since extract yield increases with extracting solvent polarity. As a result, 70% ethanol, which was the most polar of all solvents and which was used for fractionation, has afforded the maximum yield (11.0%) compared to petroleum ether (1.0%), *n*–butanol (6.0%) and ethyl acetate (1.2%).

The inhibition zone, referring to antimicrobial activity of *C. cinerea* extracts, was measured after incubation of the plates. Each of the extracts was tested three times and the average (±SD) of three values was determined. Generally, the results showed that the inhibitory effect of extracts increased with increasing of concentrations (Figure 1).
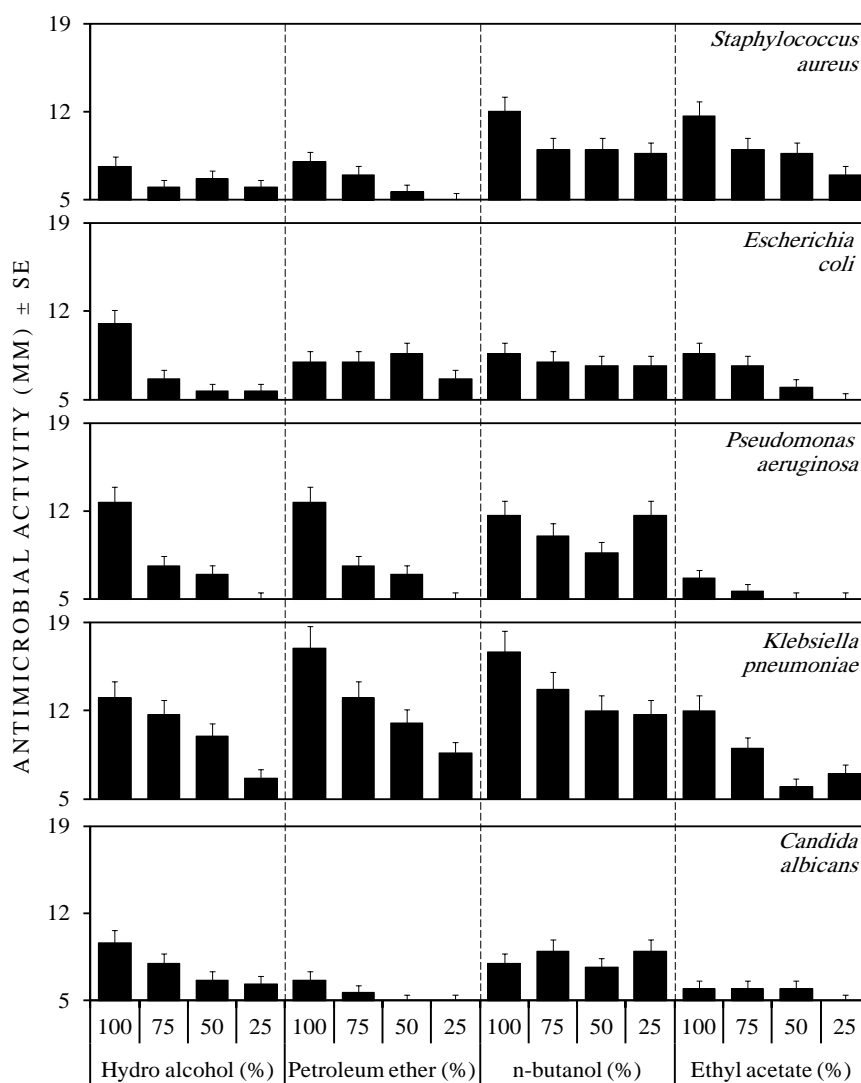
Figure 1. Antimicrobial activity of *Cotula cinerea* extracts (Extract initial concentration = 1 mg mL^{-1}, disc diameter = 5 mm).

The antimicrobial activity assayed for *C. cinerea* extracts showed an overall inhibitory effect against *K. pneumoniae* (16.67 ± 5.77 mm) with the *n*-butanol extract, and 17 ± 1.73 mm with the petroleum ether extract. Little activity was observed against *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans* at concentrations of 0.25 mg mL^{-1}. High activity against *S. aureus* was found with *n*-butanol and ethyl acetate extracts where inhibition zones equalled 12 ± 5.20 mm and 11.67 ± 3.79 mm, respectively. Moreover, the hydro alcohol extract was the most active extract against *E. coli*. Similarly, only the hydro alcohol extract of *C. cinerea* had antimicrobial activity against *C. albicans* (Figure 1). Comparing results of growth inhibition zones for the four extracts, it is evident that the petroleum ether extract possesses moderate antimicrobial properties as compared to the most active extract (*n*-butanol) and less active extracts (ethyl acetate and hydro alcohol).

The *n*-butanol extract at 25% of concentration demonstrated moderate to high antimicrobial activity against *K. pneumoniae*, *P. aeruginosa* and *S. Aureus*, whereas *C. albicans*, *E. coli* and *P. aeruginosa* remained uninhibited at an equal concentration of ethyl acetate extract. The hydro alcohol extract at 25% of concentration also demonstrated low antimicrobial activity against all tested species.

The susceptibility of microbial species to crude *C. cinerea* extracts was in the following descending order: For hydro-alcohol extract, *K. pneumoniae > P. aeruginosa > E. coli > C. albicans > S. aureus*. For petroleum ether extract, *K. pneumoniae > P. aeruginosa > E. coli > S. aureus > C. albicans*. For *n*-butanol extract, *K. pneumoniae > S. aureus > P. aeruginosa > E. coli > C. albicans*. For ethyl acetate extract, *K. pneumoniae > S. aureus > E. coli > P. aeruginosa > C. albicans*.

LRA determined that the slopes were statistically significantly non-zero, i.e. one can assume that a relationship exists between inhibition activity and extract concentrations of hydro alcohol ($P < 0.001$), petroleum ether ($P < 0.001$), and ethyl acetate ($P < 0.001$). However, the linear regression slope for *n*-butanol was not statistically significantly different from zero ($P = 0.187$), indicating there was no significant trend between *n*-butanol extract concentrations and inhibition zone (Table 2). Additionally, comparison of regressions showed that slopes were not significantly different ($P = 0.232$) while the intercepts were highly significantly different ($P < 0.001$).

Table 2. Linear regression analysis applied for extract dilutions and antimicrobial activity.

Extracts	Slope	Intercept	R²	S(C/I)	SS(I)	F	P
Hydro alcohol	6.16	4.13	0.304	28.88	584.98	25.34	<0.001
Petroleum ether	5.60	4.57	0.224	26.25	655.73	16.76	<0.001
n-butanol	2.37	8.53	0.030	11.13	886.94	1.78	0.187
Ethyl acetate	4.29	4.47	0.192	20.13	449.65	13.80	<0.001

(C/I: Concentrations/Inhibition, SS: Sum square).

Two-way ANOVA revealed that all plant extracts had significant differences in their actions as antimicrobial agents with either tested strains or extract concentrations or even their interaction. Fisher-values in "Strain test" and "Extract dilutions" factors showed all highly or very highly significant effects for the four extracts (except for dilutions of *n*-butanol). Thus antimicrobial activity of *C. cinerea* varied significantly between tested strains (in particularly for the petroleum ether extract, $P < 0.001$) and according to dilutions (especially within the petroleum ether extract, $P < 0.001$). In general, the interaction effect of the two factors (Strain test * Extract concentration) had no statistical significance on the variation of antimicrobial activity in all extracts except that of petroleum ether ($P = 0.002$) (Table 3).

Table 3. Two-way analysis of variance (ANOVA).

Extracts	Effect	SS	Df	MS	F	P
Hydro alcohol	Strain test	97.6	4	24.4	4.1	0.007
	Extract dilutions	191.3	3	63.8	10.6	<0.001
	Interaction	56.2	12	4.7	0.8	0.667
	Residuals	240.0	40	6.0		
	Total	585.0	59	9.9		
Petroleum ether	Strain test	334.4	4	83.6	39.2	<0.001
	Extract dilutions	152.4	3	50.8	23.8	<0.001
	Interaction	83.6	12	7.0	3.3	0.002
	Residuals	85.3	40	2.1		
	Total	655.7	59	11.1		
n-butanol	Strain test	229.4	4	57.4	4.1	0.007
	Extract dilutions	45.5	3	15.2	1.1	0.370
	Interaction	48.7	12	4.1	0.3	0.988
	Residuals	563.3	40	14.1		
	Total	887.0	59	15.0		
Ethyl acetate	Strain test	120.9	4	30.2	6.0	<0.001
	Extract dilutions	90.2	3	30.1	6.0	0.002
	Interaction	38.6	12	3.2	0.6	0.793
	Residuals	200.0	40	5.0		
	Total	449.7	59	7.6		

(SS: Sum of squares, MS: Mean of the Sum of Squares, *Df*: Degrees of freedom).

4. Discussion

Infectious diseases represent a serious public health problem and remain the major cause of death throughout the world. Alternative natural products of plants could be of high interest to attenuate the increasing incidence of antibiotic resistance. Some phytochemicals and plant extracts are known to have antimicrobial properties, which could be of great importance in the therapy of microbial infections. Recently, various studies have been conducted over different countries, and have demonstrated the efficacy of this type of treatment (e.g. Coutinho *et al.*, 2008; Habbu *et al.*, 2009). Algeria has also recently increased research on traditional herbal medicines following scientific findings that verified their effectiveness in healing several health issues.

The present investigation explored the use of one such plant, *C. cinerea* Del., endemic in North Africa, for treating infectious diseases. The assay of antimicrobial activity of *C. cinerea* extracts showed that the hydro

alcohol extract was least active against the tested strains, and only the relatively polar fraction (*n*-butanol) had high activity against the strains. ANOVA analysis confirmed that *n*-butanol had significant variation in its antimicrobial activity against the tested strains, however there was no significant variation in activity for its tested concentrations, which was also demonstrated by LRA. These findings support the conclusion that the active antimicrobial compounds are highly concentrated in this fraction. The other extracts (hydro alcohol, petroleum ether and ethyl acetate) had the best antimicrobial activity when used at high concentrations. This was clearly revealed by both LRA and ANOVA. The wide range of antimicrobial activity shown by *C. cinerea* extracts might reflect the differences in chemical concentrations and composition obtained by each solvent. Indeed, the successful extraction of active botanical compounds from plant materials is dependent on the type of solvent used in the extraction procedure (Parekh *et al.*, 2005; Hayouni *et al.*, 2007).

The plant extracts were active against both Gram-positive and Gram-negative bacteria, though they were more active against the later. Concerning the antimicrobial activity against *C. albicans*, the present study revealed low to no activity by the plant extracts. In our study, the highest activity was recorded against the Gram-negative bacteria: *K. pneumoniea*, which was the most susceptible bacterium of all the tested strains. These results may be of great importance in infection therapy since *K. pneumoniae* can be commonly involved in urinary, intra-abdominal, and respiratory infections (Lavender *et al.*, 2005; Keynan and Rubinstein, 2007; Ahmad *et al.*, 2012).

Markouk *et al.* (1999a) reported that the acetate extract of *C. cinerea* collected from Zagora (Sothern Morocco) exhibited an antibacterial effect with a minimum inhibitory concentration (MIC) of 200 µg/mL against all tested bacteria, and that the *n*-butanol extract was highly effective too, especially against *Pseudomonas fluorescens* and *Bacillus* sp., with an MIC of 12 µg/mL. In the same study, the ethyl ether extracts of *C. cinerea* were found to be inactive against all tested bacteria. These results are in agreement with ours, and confirm that bioactive components of any plant may differ in their solubility based on: (i) the extracting solvents (Hayouni *et al.*, 2007; Hassan *et al.*, 2009), (ii) the nature of biologically active components such as alkaloids, saponins, tannins, phenols, etc. (Hassan *et al.*, 2009; Marzouk *et al.*, 2010), and (iii) the geographical origin of the plant material (Seidel *et al.*, 2008), because ecological conditions in general (including abiotic factors "edaphic, climatic, water stress…" or biological interactions "such as intra and/or interspecific competitions…") may have a large impact on growth and fitness of vegetation species (Cordell, 2000; Seidel *et al.*, 2008), particularly by affecting their metabolism and secondary metabolites production (Cowan, 1999). Moreover, Ahmed *et al.* (1987) reported that *C. cinerea* is particularly rich with flavonic compounds besides sesquiterpene-lactone and sesquiterpene coumarins, which have been also isolated of this plant.

The results reported here can be considered as the first report on the antimicrobial properties of *Cotula cinerea*, an endemic species of the Algerian Saharan flora. Our findings also contribute to the knowledge of antimicrobial properties reported elsewhere for other *Cotula* species. Based on these antimicrobial results obtained using the disk diffusion method, it appears that this technique could not always be a reliable and sure method for screening the antimicrobial activity of plant extracts. As indicated by Moreno *et al.* (2006), the absence of an inhibition zone did not necessarily mean the compound was inactive, especially for the less polar compounds, which diffuse more slowly in the culture medium.

Consequently the analysis of the present results offers a simple scientific basis for traditional use of *C. cinerea* against microbial pathogen. However, *in vivo* studies on this medicinal plant are necessary and should seek to determine toxicity of different active compounds, their side effects, pharmacokinetic properties and reach their required minimum inhibitory concentration (MIC) in tissues and organs having the infection. The antimicrobial activities could be enhanced if active components are purified and adequate dosage determined for proper administration.

5. Conclusion

In the light of this study, *C. cinerea* is a prospective wild plant for the isolation of new antimicrobial substances. Although further investigations are clearly necessary to clarify and identify the bioactive constituents, we believe that our results presented herein represent a solid stepping-stone for other researchers in the field. Moreover, our antimicrobial assays results has justified and supported, at least in part, the Algerian common usage of the plant. The screening of some medicinal plant crude extracts has shown that some of those were potentially rich sources of antimicrobial agents. Finally, promoting human well-being deserves joining efforts in considering and valorising Saharan natural patrimony, as well as carrying out more scientific research on plants living in drylands by conducting chemical, biological, toxicological and pharmacological investigations as well as investigating therapeutic potential.

References

Ahmad TA, El-Sayed LH, Haroun M, Hussein AA and El-Ashry EH. 2012. Development of immunization trials against *Klebsiella pneumoniae*. *Vaccine*, **30**: 2411-2420.

Ahmed AA, El-Sayed NH, El-Negoumy SI and Mabry TJ. 1987. Flavonoids of *Cotula cinerea*. *J Nat Prod*, **50**: 519-520.

Beloued A. 2005. **Les Plantes Médicinales d'Algérie (Medicinal Plants of Algeria).** Ed Office des Publications Universitaires, Algiers, 284 p.

Boligon AA, Agertt V, Janovik V, Cruz RC, Campos MMA, Guillaume D, Athayde ML and dos Santos ARS. 2011.

Antimycobacterial activity of the fractions and compounds from *Scutia buxifolia*. *Rev Bras Farmacogn*, **22**: 45-52.

Cordell GA. 2000. Biodiversity and drug discovery - a symbiotic relationship. *Phytochem*, **55**: 463-480.

Coutinho HDM, Costa JGM, Siqueira-Junior JP and Lima EO. 2008. *In vitro* anti staphylococcal activity of *Hyptis martiusii* Benth against methicillin-resistant *Staphylococcus aureus-* MRSA strains. *Rev Bras Farmacogn*, **18**: 670-675.

Cowan MM. 1999. Plant products as antimicrobial agents. *Clin Microbiol Rev*, **12**: 564-582.

Daglia M. 2012. Polyphenols as antimicrobial agents. *Curr Opin Biotech*, **23**: 174-181.

Dall'Agnol R, Ferraz A, Bernardi AP, Albring D, Nör C, Sarmento L, Lamb L, Hass M, von Poser G and Schapoval EES. 2003. Antimicrobial activity of some *Hypericum* species. *Phytomed*, **10**: 511-516.

Habbu PV, Mahadevan KM, Shastry RA and Manjunatha H. 2009. Antimicrobial activity of flavonoid sulphates and other fractions of *Argyreia speciosa* (Burm.f) Boj. *Indian J Exp Biol*, **47**: 121-128.

Hammiche V and Maiza K. 2006. Traditional medicine in Central Sahara: Pharmacopoeia of Tassili N'ajjer. *J Ethnopharmacol*, **105**: 358-367.

Hassan A, Rahman S, Deeba F and Mahmud S. 2009. Antimicrobial activity of some plant extracts having hepatoprotective effects. *J Med Plant Res*, **3**: 20-23.

Hayouni EA, Abedrabba M, Bouix M and Hamdi M. 2007. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. *Food Chem*, **105**: 1126-1134.

Katsumi S, Kazshi T and Hiroshi O. 2005. Pathogen occurrence and antimicrobial susceptibility of urinary tract infection cases during a 20-year period (1983-2002) at a single institution in Japan. *Jpn J Infect Dis*, **58**: 303-308.

Keynan Y and Rubinstein E. 2007. The changing face of *Klebsiella pneumoniae* infections in the community. *Inter J Antimicrob Agents*, **30**: 385-389.

Larhsini M, Markouk M, Jaouhari JT, Bekkouche K, Lazrek HB and Jana M. 2002. The antipyretic activity of some Moroccan medicinal plants. *Phytother Res*, **16**: 97-98.

Lavender H, Jennifer J and Clegg JS. 2005. *Klebsiella pneumoniae* type 3 fimbria-mediated immunity to infection in the murine model of respiratory disease. *Inter J Med Microbiol*, **295**: 153-159.

Maiza K, Brac de la Perrière RA and Hammiche V. 1993. Pharmacopée traditionnelle saharienne: Sahara septentrional. *2nd Proc of European Conf on Ethnopharmacology & 11th Int Conf of Ethnomedecine*, Heidelberg France, pp 169-171.

Markouk M, Redwane A, Lazrek HB, Jana M and Benjama A. 1999a. Antibacterial activity of *Cotula cinerea* extracts. *Fitoterapia*, **70**: 314-316.

Markouk M, Lazrek HB and Jana M. 1999b. Analgesic Effect of Extracts from *Cotula cinerea* (L). *Phytother Res*, **13**: 229-230.

Marzouk B, Marzouk Z, Décor R, Mhadhebi L, Fenina N and Aouni M. 2010. Antibacterial and antifungal activities of several populations of Tunisian *Citrullus colocynthis* Schrad. immature fruits and seeds. *J Med Mycol*, **20**: 179-184.

Matu EN and van Staden J. 2003. Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *J Ethnopharmacol*, **87**: 35-41.

McKay DL and Blumberg JB. 2007. A review of the bioactivity of South African herbal teas: Roobos (*Aspalathus linearis*) and Honey comb (*Cyclopia intermedia*). *Phytother Res*, **21**: 1-16.

Moreno S, Scheyer T, Romano CS and Vojnov AA. 2006. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radic Res*, **40**: 223-231.

Mothana RA and Lindequist U. 2005. Antimicrobial activity of some medicinal plants of the island Soqotra. *J Ethnopharmacol*, **96**: 177-181.

Parekh J, Jadeja D and Chanda S. 2005. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish J Biol*, **29**: 203-210.

Qaralleh H, Idid S, Saad S, Susanti D, Taher M and Khleifat K. 2010. Antifungal and antibacterial activities of four Malaysian sponge species (Petrosiidae). *J Med Mycol*, **20**: 315-320.

Seidel V, Peyfoon E, Watson DG and Fearnley J. 2008. Comparative study of the antibacterial activity of propolis from different geographical and climatic zones. *Phytother Res*, **22**: 1256-1263.

Shahid M, Malik A, Akram M, Agrawal LM, Khan AU and Agrawal M. 2008. Prevalent phenotypes and antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* at an Indian tertiary care hospital: plasmid-mediated Cefoxitin resistance. *Inter J Infect Dis*, **12**: 256-264.