Algerian Journal of Natural Products 5:3 **(2017)** 524-529

OPEN ACCESS

Online ISSN: 2353-0391

Algerian Journal of Natural Products

www.univ-bejaia.dz/ajnp

Type of the Paper (Article)

Antibacterial Activity of Ethyl Acetate Extracts from Algerian *Cupressus sempervirens var* Against Some Human Pathogens Bacteria

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Received: 22/08/2017 /Accepted: 22/12/2017 DOI: https://doi.org/10.5281/zenodo.1127761

Abstract: *Cupressus sempervirens var.* is a plant rich in bioactive compounds and it has been a typical used in folk medicine for thousands of years. In Algeria, *Cupressus sempervirens* is widely used as traditional remedies for treatment of different complicate infectious diseases this study was conducted to evaluate antibacterial properties of ethyl acetate extracts of *Cupressus sempervirens* against gram negative and gram positive. The plant was collected from Chlef, in Tenes is a region of Algeria Mediterranean, then, the plant extract was prepared by maceration .The antibacterial activity of the extracts was investigated against 6 clinical and reference strains based on two assays: the well diffusion method and the agar dilution method. *Cuperssus sempervirence* acted as the agent antibacterial by having the highest (MIC/MBC 0.25v/v) against *B.subtilis* ATCC 6133, *E.coli* followed by Coagulase-Negative Staphylococci, *Microccocus lutea* (MIC/MBC 0.5v/v). Results of the phytochemical studies of ethyl acetate extract revealed the presence low amount of total phenolic according to the spectrophotometric method. *Cupressus sempervirens* has broad spectrum antibacterial activity and can be consider as a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control.

Keywords: *Cupressus sempervirens, antibacterial activity, ethyl acetate extracts*

I. Introduction

Cupressus sempervirens var. (Cupressaceae) is one of 20 species in the genus Cupressaceae throughout the world [1]. This shrub or tree has a typical native to eastern North America [2] also, it had been recognized in around the world, and particularly in Mediterranean basin and North Africa. crude extracts of *C. sempervirens* is also described to exert antispasmodic, astringent, antiseptic, deodorant, and diuretic effects, antioxidants, antibacterial, insecticidal activities, and inhibition of glucose-6-phosphatase and glycogen phosphorylase [3]**.**There are many reports on the ethyl acetate extracts of leaves and stems of on the various parts of *C. sempervirens* and its biologics activity as [4,5] . Methanol extract of *Cupressus sempervirens* L. inhibit microbes and biofilms [6] and MeOH extract also exhibit significant hepatoprotective and antioxidant activity [7]. Furthermore, *Cupressus sempervirens* is also used as a traditional remedy to treat various ailments of coughs, colds, parasitic infections, inflammation, hemorrhoids, and as an inflammation, hemorrhoids, and as a strong hair tonic; the fruit of the plant is used traditionally for curing diabetes and as an antiseptic [8,9,10,11].

Infectious diseases reached a higher rate in the word. Today over 17 million people years are killed by different types of micro-organisms. The World Health Organization (WHO) has been report about 52 million deaths from all causes in 1995, more than 17 million were due to infectious diseases, including about 9 million deaths in young children. Up to half the world's population of 5.72 billion is at risk of many endemic diseases. In addition, millions of people are developing cancers as a direct result of preventable infections by bacteria and viruses [12] .At present, a lot of research is committed to bacteria infection disease not only because they are major killing diseases but also because disease control becomes more difficult due to a number of factors that limit the utility of current antibiotics in remedy. Hence, the search for new molecule and drugs needs to be continued. In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to the adaptation microbes to drugs commonly used in the treatment of infectious diseases. This situation encouraged researchers for searching new molecule from various sources, like medicinal plants, which are the good sources of novel antimicrobial agents. The present study was conducted to investigate antibacterial effect of extracts of *Cupressus sempervirens* against a gram positive and negative of bacteria.

II. Materials and Methods

II.1. Collection of Plant Material

The dried leaves of *Cupressus sempervirens* were collected from Tenes, Chlef, Algeria. The dried leaves of plants were selected mainly on the basis of their local medicinal information. The plants were selected randomly in October 2014; the plants were taken and rinsed with distilled water and kept under shade till drying. The dried material was grinded finely in electric grinder.

II.2. Extraction of plant materials

Extraction from the air-dried of aerial parts of plants of *C. sempervirens* (10 g) was carried out by simple maceration process. 10 g of powder of leaves of each plant were soaked in (30/70 v/v) of (ethanol and water) at room temperature. The poorly homogenized mixture was kept for 72 hours at room temperature (25°C) in extraction bottles with renewal of solvent every 24 hours and agitation of with occasional agitation of from time to time. After three 72 maximum the amount of solvent was separated from the mixture. The obtained ethanol extract was filtered and evaporated by using a rotatory evaporator from the extract under reduced pressure [13].The maximum amount of solvent were filtered (Whatman No. 1 filter paper), the clear phase is recovered, which was extracted by solvents of increasing polarity by ethyl acetate. The extracts were completely evaporated by rotary evaporator and were stored at 4°C in refrigerator.

II.3. Total Phenolic assay

The total phenolic content of the extract was determined by the Folin–Ciocalteu method [14]. Briefly, the reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract. Then, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$.The mixture was allowed to stand for a further 60 min in the dark, and absorbance was measured at 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of caffeic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (ug/ml).The results are expressed in mg equivalent of caffeic acid/mg of fresh material Positive control is represented by ethanol supplemented with Folin-Ciocalteu, distilled water and sodium carbonate.

II.4. Antibacterial activity assay

II.4.1. Bacterial cultures

2017 Algerian Journal of Natural Products (Online ISSN: 2353-0391), https://doi.org/10.5281/zenodo.1127761

Bacterial strains were obtained from the Laboratory of Microbiology, Groups of Antibiotical, Saidal, Medea, Algeria. Antibacterial activity of the plant extracts were investigated using reference as *E.coli, B.subtilis ATCC 6133*, *S.typhimriuim ATCC 13311*, *P. aerogenosa ATCC27853* and clinical strains as Staphylococcus a coagulase negative ,*Micrococcus luteus*. All bioassays were performed in triplicate at the Laboratory of Microbiology, Groups of Antibiotical, Saidal, Medea, Algeria.

II.4.2. Disc diffusion method

We used Agar well diffusion method to determine zone of inhibition. We used trypticase soja agar (Oxoid) for tested bacteria assay, the microorganisms were suspended in 5ml of physiologic water (institute Pasteur, Alger, Algeria) approximate by 10^5 CFU/ml for all bacteria and using sterile cotton swab for each microbe evenly spread over the entire surface of agar plate to obtain a uniform plate surface growth. Petri plates were allowed to dry. About 3-4 wells in each plate of 6 mm diameter were loaded in essential oil and were punched in agar surface with the help of a sterilized borer for placing the extracted oil samples. Therefore, antibiotics of Oxacillin, Ampicillin were used as a positive control. Dimethyl sulfoxide (DMSO) was used as negative control. The plates were then left at 4 for 30 minutes and then were incubated at for 24 hours at 37°C. After incubation, the zones of inhibition were measured using a ruler and the results reported in millimeters (mm). All the tests were run in triplicate and the average result was taken.

II.4.3. Broth dilution method

Minimum inhibition concentration was determined using the agar dilution method according Remmal *et al*. [15]. This test was performed at five concentrations of each extract (1/10, 1/25, 1/50 and 1/100) employing serial dilutions of plant extracts in Tryptic Soy Broth with 0.2% of sterile agar in agar solution. We added 13.5ml of TSB for bacteria in every test tube, we added then 1.5ml of each dilution so as to acquire final concentrations of 1/100, 1/250, 1/500, 1/1000 (v/v). After that, suitably agitated tubes before pouring into Petri dishes, then, we were inoculated bacteria onto plates using sterile cotton swab for each microbe and were dried for 5 min at 4°C. The control containing the culture medium and the agar solution at 0.2% alone are also prepared. The incubation was carried out in the dark for 24 hours at 37°C for bacteria, and each test was repeated three times. Minimum inhibitory concentrations were determined after 24h for the bacteria, the minimum inhibitory concentrations (MICs) were determined as the lowest concentration of cypress essential oil inhibiting the visible growth of each organism on the agar plate, and the minimum bactericidal concentrations (MBCs) were determined as the lowest concentration at which no growth was observed after an incubation up to 5 days.

II.5. Data Analysis

All the measurements were replicated three times for each assay. Data was analyzed using windows SPSS version 17.0 and descriptive statistic was used.

III. Result and discussion

III.1. Total Phenolic assay

The total phenolic contents in the plant extract using the Folin-Ciocalteu's reagent is expressed in terms of Gallic acid equivalent (the standard curve equation: $y = 7.026x - 0.0191$, $r2 = 0.999$). The values obtained for the concentration of total phenols are expressed as ug of GA/mg of extract

The total phenolic contents in the examined extracts ranged 08,70 ±0,01ug/mg. The lowest concentration of phenols was measured in ethyl acetate extracts. The total phenolic contents of plant extracts depend on the type of extract and the polarity of solvent used in extraction. High solubility of phenols in polar solvents provides high concentration of these compounds in the extracts obtained using polar solvents for the extraction [16, 17]. a blue-coloured solution due to the presence of phospho molybdic-phosphotungstic-phenol complex- was produced when the active extracts or fractions reacted with Folin-Ciocalteau reagent in an alkaline medium.

2017 Algerian Journal of Natural Products (Online ISSN: 2353-0391), https://doi.org/10.5281/zenodo.1127761

III.2. Antibacterial activity

The antibacterial properties of ethyl acetate extract of the leaves of *C. sempervirens* in vitro are presented in Table 1. The extracts had antibacterial effect against both Gram-positive and Gram-negative bacteria, the levels of MIC and MBC ranging from 0.25 to 0.5 v/v, respectively are shown in Table 1. The extract had the greatest activity against coagulase-negative Staphylococci and *Bacillus subtillis ATCC6133* with MIC (0.25v/v) and the least against *Micrococcus luteus* ATCC 4698 and *E.coli* with MIC (0.5v/v).

Table1: *Antibacterial susceptibility of ethyl acetate extract of cypress*

	Parameters			
Strains tested	**ZI**	**MIC (v/v)**	**MBC (v/v)**	**MIC/MBC**
coagulase-negativeStaphylococci	11	0.25	0.5	0.5
E.coli	10.4	0.5	0.5	1
Micrococcus luteus ATCC 4698	10.7	0.5	05	1
Bacillus subtillis ATCC6133	12.4	0.25	0.25	1
Salmonella typhimurium ATCC13311	00	00	00	00
Pseudomonas aerogenosa ATCC10876	00	00	00	00

The lowest value of MIC/MBC was observed in case of *Bacillus subtillis* ATCC6133 (0.25v/v). Ethyl acetate extract of *C. sempervirens* showed inhibitory activity against standard bacteria and clinical bacteria with varying strength and these effects were dose-dependent shown in Table 1. No activity of *C. sempervirens* was detected against both *Salmonella typhimurium* ATCC13311, *Pseudomonas aerogenosa* ATCC10876. It is well recognized that bacteria can develop resistance to antimicrobials drug due to continuous and prolonged adaptation to antimicrobial agents. The frequency of resistance acquisition to essential oil depends on the bacteria. The low antibacterial activity against Gram-negative bacteria was ascribed to the presence of an outer membrane which possessed hydrophilic polysaccharide chains as a barrier to phenolic molecule [18].

Phenolic compounds are one of the most important molecules of secondary metabolites found in plants. In nature they are involved in plant growth and reproduction, provide resistance from pathogens and predators and protect crops from disease and pre-harvest seed germination [19]. There are different classes of polyphenols known as tannins, lignins and flavonoids. The polyphenols possesses many characteristics and biologics activity. Flavonoids are the most widely occurring polyphenol and are present in almost every form of human consumed vegetation. In nature they are involved in plant growth and reproduction, provide resistance from pathogens and predators and protect crops from disease and pre-harvest seed germination [19].

The results of this study suggest that *C. sempervirens var* is an important source of antimicrobial molecule. These properties are due to many active phytochemicals including flavanoids, terpenoids, carotenoids, coumarins, curcumines etc. These bioactive principles have also been confirmed using modern analytical techniques. Phenolic components, present in extract, have been known to possess antimicrobial activity and some are classified as generally recognized as safe (GRAS) substances and therefore could be used to prevent post-harvest growth of native and contaminant bacteria [20,21]. The antimicrobial activity of polyphenols has been widely studied and reported of publications reporting the antimicrobial activity of polyphenols. The study of Taguri et al. [22] that demonstrated the various types of polyphenols including catechins and their oxidation products, proanthocyanidins, and hydrolyzable tannins show antibacterial activities against four groups of food-borne bacteria. The antimicrobial activity of the extract may be attributed to the high content of flavonoids which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes [23]. Flavonoids have also been reported to inhibit pathogens bacteria. More than 4000 flavonoids have been identified in fruits, vegetables, and plant-derived beverages, subdivided into many subclasses: flavonols, flavones, flavanones, anthocyanidins, flavanols, and also isoflavones [24]. The

most important type of flavonoids that possess antimicrobial activity flavan-3-ols such as gallocatechin-3-gallate, epigallocatechin-3-gallate, catechin-3-gallate, and flavonols such as rhamnetin, myricetin, morin, and quercetin. A different mechanism of action of flavonols, among which the most convincingly identified, is the aggregatory effect on all the bacterial cells [25].
The mechanism of the toxicity of polyphenols against microbes may be related to inhibition of hydrolytic enzymes (proteases) or other interactions that inactivate microbial adhesins, cell envelope transport proteins and non-specific interactions with carbohydrates [26]. The mechanism of flavonols due to their hydrophobicity, flavonols are capable of penetrating cell phospholipid membranes, being therefore able to exert their antibacterial activity also inside the cell. Moreover, rhamnetin resulted to be more active than quercetin and morin, probably because of the methoxy group in the A-ring, which makes this molecule more hydrophobic [27].However, activities against the other three bacterial groups appeared to depend on the structures of the polyphenols, the presence of a galloyl group (3,4,5-trihydroxybenzoyl group) increases antibacterial activity [22].
The results of this study indicated that ethyl acetate extracts of *Cupressus sempervirens var* exhibited antibacterial activity of *Cupressus sempervirens var* using agar well diffusion method was also reported. According to study of Shahid et al [27] the antibacterial activity of ethyl acetate extracts of *Cupressus sempervirens var* on *S. aureus, P. aeruginosa, B. subtilis and K. pneumoniae* were ranged from 10 mm to 28 mm against gram positive and negative bacteria.
The best activity of ethyl acetate extracts of *Cupressus sempervirens var* against gram positive bacteria and this result in accordance to the study of Mothana et al. [29] about 64 methanol and aqueous extracts of 30 Yemeni plants *Cupressus sempervirens* in which pronounced antimicrobial activity was observed only against Gram-positive bacteria [29].

IV. Conclusion

The ethyl acetate extracts of *Cupressus sempervirens var* showed in vitro antibacterial activity against majority of the isolated strains. Ethyl acetate extract of plants displayed higher activity against tested bacteria at lowest concentration. The antibacterial effect has been attributed to the presence of some active molecules in the extracts as flavonoids, On the basis of these data presented; these flavonoids may be considered new chemical classes of antibacterial substances for infections that may be caused by bacteria in the future. Therefore, further work is under way to identify their precise antibacterial mechanism.

V. Acknowledgements

The author would like to thank the University Hassiba Ben Bouali – Chlef, Algeria, for support in this study.

V. References

[1] James AD. Dake›handbook of medicinal plants of the bible.CRC press, Tylor & Francis group. NY, USA,(2008). 161 p
[2] Amouroux P, Jean D, Lamaison J. Antiviral activities in-vitro of Cupressus sempervirens on two human retroviruses HIV and HTLV. Phytother. Res. (1998)12,367–68.
[3] Rawat P, Khan MF, Kumar M, Tamarkar AM, Srivastava AK, Arya KR, Maurya R. Constituents from fruits of *Cupressus sempervirens*. Fitoterapia (2010)81,1 62-166.
[4] Chaudhary, H. J., Shahid, W., Bano, A., Ullah, F., Munis, F., Fahad, S., & Ahmad, I. In vitro analysis of *Cupressus sempervirens* L. plant extracts antibaterial activity. Journal of Medicinal Plants Research, 6(2012), 273-276.
[5] Koriem, K. M., Gad, I. B., & Nasiry, Z. K.Protective effect of *Cupressus sempervirens* extract against indomethacin-induced gastric ulcer in rats. Interdisciplinary toxicology, 8(2015), 25-34.
[6] Selim, Samy A., et al.Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (*Cupressus sempervirens* L.). BMC complementary and alternative medicine, 1(2014), 179.
[7] N. A. Ibrahim, H. R. El-Seedi, and M. M. D. Mohammed, Nat. Prod. Res., 21 (2007), 857 (2007).
[8] A. Bruni, M. Ballero, and F. Poli, J. Ethnopharmac., 57, (1997)97p.
[9] R. E. Uncini Manganelli, F. Camangi, and P. E. Tomei, J. Ethnopharmac., 78,(2001)71p.

[10] O. Said, K. Khalil, S. Fulder, and H. Azaizeh, J. Ethnopharmac., 83, (2002)251p.
[11] A. D. James, Handbook of Medicinal Herbs (2nd edn), CRC Press, Boca Raton, FL, (2002), 338p.
[12] WHO.,1997. Infectious diseases kill over 17 million people a year: WHO warns of global crisis.
[13] Merghem R., Jay M., Viricel M. R., Bayet C. et Voirin B. Five 8-C benzylated flavonoids from Thymus hirtus (Labiateae). Phytochemistry., 38 (1995) ,637-640.
[14] SINGLETON, V.L., ORTHOFER, R., LAMUELA-RAVENTOS, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299(1999), 152-178.
[15] Remmal A., T-elarkia ., Bouchikhi T., Rhyyour K ., Rhyyour K ., Ettayebi M..Improved method for the determination of microbial activity of essential oils in Agar Medium, J. Essent. Oil. 5(1993),179-184.
[16] MOHSEN, M.S., AMMAR, S.M.A. Total phenolic contents and antioxidant activity of corn tassel extracts. Food Chem. 112(2008), 595-598.
[17] ZHOU, K., YU, L.Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT. 37, (2004), 717-721.
[18] Hu, Q. P., Cao, X. M., Hao, D. L., & Zhang, L. L. Chemical Composition, Antioxidant, DNA Damage Protective, Cytotoxic and Antibacterial Activities of Cyperus rotundus Rhizomes Essential Oil against Foodborne Pathogens. Scientific Reports, 7(2017).
[19] Ross J., Kasum C.Dietary Flavonoids: Bioavailability, Metabolic Effects, and Safety. Annual Review of Nutrition 22(2002),19-34.
[20] Kabara, J. J. Phenols and chelators. In N. J. Rusell & G. W. Gould(Eds.), Food preservatives London: Blackie(1991), 200p
[21] Singh, A., Singh, R. K., Bhunia, A. K. & Simmon, J. E. Use of plant essential oils as antimicrobial agents against Listeria monocyt-ogenes in hotdogs. In Program listing. The 2001 IFT Annual Meeting New Orleans, LO.(2001).
[22] Taguri T, Tanaka T, Kouno I. Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-borne disease. Biol Pharm Bull. 27(2004),1965–1969.
[23] T.P.T. Cushnie, A.J. Lamb.Antimicrobial activity of flavonoids Int. J. Antimicrob. Agents, 26 (2005), 343-356.
[24] Daglia, M.Polyphenols as antimicrobial agents. Current opinion in biotechnology, 23(2012), 174-181.
[25] Cushnie TPT, Hamilton VES, Chapman DG, Taylor PW, Lamb AJ: Aggregation of Staphylococcus aureus following treatment with the antibacterial flavonol galangin. J Appl Microbiol 103(2007),1562-1567.
[26] R. Pyla, T.J. Kim, J.L. Silva, Y.S. Jung Enhanced antimicrobial activity of starch-based film impregnated with thermally processed tannic acid, a strong antioxidant Int. J. Food Microbiol., 28 (2010), 154-160p.
[27] Alvesalo J, Vuorela H, Tammela P, Leinonen M, Saikku P,Vuorela P: Inhibitory effect of dietary phenolic compounds on Chlamydia pneumoniae in cell cultures. Biochem Pharmacol .71(2006),735-741.
[28] Shahid, W., Durrani, R., Iram, S., Durrani, M., & Khan, F. A. Antibacterial activity in vitro of medicinal plants. Sky J Microb Res, 1(2013), 5-21.
[29] Mothana RA, Gruenert R, Bednarski PJ, Lindequist U .Evaluation of the in vitro anticancer, antimicrobial and antioxidant activities of some Yemeni plants used in folk medicine. Pharmazie.,64(2009), 260-268.

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BOUDJEMAA Hadjer, Antibacterial Activity of Ethyl Acetate Extracts from Algerian *Cupressus sempervirens var* Against Some Human Pathogens Bacteria, ***Algerian J. Nat. Products,*** 5:3 **(2017)** 524-529

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Online ISSN: 2353-0391

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DOI: https://doi.org/10.5281/zenodo.1127761	