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Antioxidant (Peroxynitrite scavenging, Hypochlorous acid scavenging) Antifungal Activity, and Screening of Bioactive Metabolites of Juniper (Juniperus communis) Using FTIR Technique

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Abstracts: Background: The use of plant-based remedies and the chemicals produced from them has a long history in traditional medicine. Traditional medicine practitioners have long relied on juniper (Juniperus communis) to alleviate symptoms such as amenorrhoea, albuminuria, bladder catarrh, acute and chronic cystitis, leucorrhea, and cystitis. Because of its high concentration of bioactive components such as phenolics, terpenoids, organic acids, alkaloids, and volatile chemicals, it finds widespread application in medicine. The coniferous tree known as Juniper communis belongs to the Cupressaceae family and the genus Juniperus. A number of recent studies have examined the vast potential of this evergreen shrub, drawing conclusions about its antimicrobial, antioxidant, anti-inflammatory, antidiabetic, antihyperlipidemic, and neuroprotective effects, as well as its antiproliferative, anticancer cell, and ability to activate inductive hepato-, renal-, and gastroprotective mechanisms. These findings are relevant to various biomedical fields. The goals of our research were to identify antioxidant (peroxynitrite and hypochlorous acid scavenging) and antifungal compounds in juniper (Juniperus communis) by means of the Fourier transform infrared spectroscopy.

Methods: Using an electric grinder, the dried material was ground into a fine powder. The stock solution was prepared by mixing 50 g of the powder with 200 ml of solvents (w/v, 50 g/200 ml). Methanol, ethyle acetate, and ethanol were the solvents utilised for the extraction process. After pouring the SDA medium on top, the dishes were let to solidify. Then, using a sterile cork borer, a 5 mL disc was removed from each fungus and deposited on top of the culture medium..

Results: Peak (Wave number cm-^{l}), Intensity, Bond, Type of Vibration, and Functional group assignment recorded [675.09, 67.825, Strong, C-Cl, Stretch and alkyl halides], [692.44, 69.075, Strong, C-Cl, Stretch, alkyl halides], [738.74, 72.075, Strong, =C–H, Bending, Alkenes], [813.96, 76.441, Strong, =C–H, Bending, Alkenes], [974.05, 65.287, Strong, =C–H, Bending, Alkenes], [1008.77, 54.765, Strong, C-F, Stretch, alkyl halides], [1049.28, 58.347, Strong, C-F, Stretch, alkyl halides], [1093.64, 64.409, Strong, C-F, Stretch, alkyl halides], [1232.51, 80.641, Strong, C-F, Stretch, alkyl halides], [1276.88, 80.140, Strong, C-F, Stretch, alkyl halides], [1606.70, 79.503, Bending, N-H, Stretch, Amide], [1647.21, 79.220, Variable, C=C, Stretch, Alkene]. Antifungal activity of secondary metabolites of Juniperus communis recorded Alternaria alternaria (15.11 ± 0.22, 12.10 ± 0.18 and 19.30 ± 0.22 respectively), Aspergillus flavus (19.07 ± 0.37, 14.25 ± 0.35 and 22.09 ± 0.37 respectively), Trichophyton rubrum (20.53 ± 0.31, 16.11 ± 0.39 and 22.94 ± 0.31 respectively), and Fusarium oxyporum (11.12 ± 0.39, 20.08 ± 0.38 and 17.62 ± 0.39 respectively). Voriconazole (VCZ) and Amphotericin B (AmB) as standard anti-fungal activity were (23.31 ± 0.38 and 27.09 ± 0.41) respectively. Juniperus communis metabolites was very highly active against Trichophyton rubrum (22.94 ± 0.31).

Conclusion: This study suggests that Juniperus communis could be a useful plant for treating fungal infections because of its medicinally active components. Findings from this study point to Juniperus communis as a potential plant source of active chemicals with medicinal value for the treatment of certain fungal diseases.

Keywords: Antifungal activity, Secondary metabolites, Juniperus communis L.

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Introduction

The industries such as pharma, cosmetic, culinary, and medical industries are some of the networks that are interested in benefiting from the many phytochemical compounds present in plants and positive effects on health. Low molecular weight molecules are primarily categorised into three groups: are alkaloids, terpenes and phenolic compounds [1,2,3]. Second metabolites were said to involve in plants' antibacterial activities. Juniper communis L. berries and their essential oils belong to antibacterial, antiviral, antioxidant, and anti-inflammatory groups that are recognized by the European Medicines Agency. The code of federal regulation of United States also listed juniper berries, essential oils and solvent free oleoresins and natural extractives used for this purpose as GRAS [5]. Juniper has been used in traditional medicine for many years, most commonly as a herbal tea, Juniper alone or in conjunction with other plants for various complaints. Besides its application in the preparation of alcoholic drinks such as gin and beers, juniper berries are used in culinary in several civilizations [4-6]. Perhaps, one of the richest genera for the conifers is Juniperus to which both shrubs and trees that are of an evergreen type belong to. Sabina is a genus that subsumes some 75 species; there is Caryocedrus, Juniperus, and Juniperus subgenera. There are two branches of the Juniperus tree: One of them is native to the north and east and is allied with Juniperus communis, the other is native to the Mediterranean region and is connected with Juniperus oxycedrus. Jatropha communis has a single staminal band on the adaxial portion of the leaf and the seed cones or 'berries' or 'fruits' which are green in colour and black when ripened. Furthermore, it is found both in the Northern and the Southern Hemisphere; it also proves to be the most invasive of all Juniperus species. It has two stomal bands on its leaves and the mature berries can be of any colour that ranges from red, reddish brown to reddish purple [7, 8]. Some of the properties associated with juniper berries essential oil include; Diuretic properties, gastrointestinal irritant, and antiseptic properties. Specifically, terpinen-4-ol is the principal component of juniper's essential oil that makes it possess a diuretic feature. The main components of the base material, which forms the essential oil constitutes Terpenes Hydrocarbons, including – and pinene, myrcene, sabinene, thujone, limonone, etc. They stand at 0. 5 to 2. The remaining amount would be 5% (V/m) in the berries. The chief compounds identified from the oil are caryophyllene, cadinene and elemene which are sesquiterpene hydrocarbons; terpinen-4-ol is a terpen alcohol [9, 10]. Other than its efficiency in treating dyspepsia, juniper berry is also used in treating renal and bladder diseases together with other plants.

This berry is beneficial to digestion, the stomach, and rheumatic ailments. This type of plant is widely distributed in the pastures, shrub land, and patches and even on the worst of the soils of Romania in the altitudes of 700-1400m in the Carpathian hills [11]. During the second year, two kind of blossoms are formed on the branches, and the coves of those is linear with a pointed apex and 3 coves are positioned at one level. The male flowers contain yellow, round and several-staminate flowers while the female flowers are those that are also round and comprise of three whorled carpellate scales; each of the carpel possesses an ovule. The pseudo-fruits or the fruits of the plant are round and with very short stalks. These are vegetables used in this recipe, parts of the juniper tree or Baccae juniperi commonly referred to as fruits. The use of this coniferous plant has been widely acknowledged by traditional medicine for numerous advantages. The use of curcumin has targeted usage in diuretic, anti-inflammatory, antifungal, analgesic, anti hepatitis, anti diabetes, anti lipid, anti microbial, antioxidant properties and also in the treatment of nontuberculous mycobacteria, Parkinson's disease and human neuroblastoma cells. The bioactive compounds present in fruits include; Apigenin, rutin, luteolin, quertcetin-3-O- arabinosil glugoside, quercetin-3-O-rhamnosyl querrein, scutellarein, nebetin, ofutavone and bilobetaine. It is acceptable that the major components of the oil extracted from the fruits of juniper are monoterpene hydrocarbons, with the content of β-pinene being 5%, α-pinene being 51. 4%, sabinene being 5. 8%, myrcene being 8. 3%, and limonene being 5. 1% [14, 15]. Our study's objectives were: Extract, Juniper (Juniperus communis), metabolite analysis for antifungal activities, peroxynitrite, and hypochlorous acid scavenging using Fourier transform infrared spectroscopy.

Materials and Methods

Sampling Procedures

The raw Juniperus communis leaves were purchased from the Babylonian Bazaar; these samples were cleaned, and any impurities were eliminated before sample analysis inside the modern laboratory for botany at the University of Babylon's Faculty of Science. With the help of an electric grinder the powdered material obtained after the process of

drying was grinded into fine powder. The stock solution was prepared through dissolving 50 g of the powder in 200 ml of solvents; their ratio was 50 g: 200 ml. The analysed solvents used for the extraction process included methanol, ethyle acetate, and ethanol. Each of the extracts was then filtered using Whatman filter paper no. 1 after it had been shaken for not less than 6 hours. The last and fourth filtrate was so used in the studies after being concentrated to 25% crude extract using vacuum at 20°C in a rotary evaporator.

Approaches to Grown up Plants and Isolating Plant Bioactive Constituents

The contents of the culture medium were then fermented at 40°C for two days, and the culture medium was autoclave sterilisation at 121°C for 15 minutes using a 15pis/inch2 chamber according to the manufacturer's recommendation. This method has been used in isolating and culturing of the fungus.

Studying on the Antifungal Activity of the Extract of Juniperus communis Leaf Metabolites

Twenty samples of food items which are consumed in the markets of Babylon and Najaf Province have been taken in this regard with an intention to analyse the fungi responsible for the poison. As a means of diagnosing and analyzing the samples, they were referred to the highly advanced mycology laboratory of Babylon University's science faculty. The antifungal activity of metabolite extract of Juniperus communis leave has been investigated using the mixing method alongside SDA. From each concentration, 0. Thus, 1 mL was taken out and placed in a Petri dish. Once the SDA medium was poured on top of the dishes, the dishes were allowed to set. Subsequently, a 5 mL disc was punched out from each fungus employing a sterile cork borer and inoculated on the culture medium. The dishes with the petry are then incubated at 25°C ± 2 for a period of 7 days. To determine the antifungal activity of the extracts, the diameter of the inhibitory zone obtained from the treated agar was measured with the help of a ruler for the ruler Mohammed AS in millimeters (mm).

Assessment of anti-oxidant properties

Scavenging peroxynitrite

This peroxynitrite (ONOO-) was synthesized according to the method described in the research of Beckman et al. [18]. Mix 5 millilitres of 0. 7 milliliters of H2O2 with 5 milliliters of 0. 6 M KNO2 must be quickly immersed in an ice bath for 1 sec. Splash it with 5 millilitres of ice-cold water. We added 1. 2 M NaOH. The reaction mixture was stirred for additional 3 hours at -20 C after addition of prewashed with 1. Adding 2 M NaOH to the same tube in order to neutralize excess H2O2. The peroxynitrite solution was collected from the upper layer of the frozen mixture and its concentration was measured using a spectrophotometer at 302 nm with the molar absorptivity of 1670 M-1cm-1. Determination of cell peroxynitrite-scavenging activity was done using an Evans Blue bleaching assay. The experiment was done in accordance with a standard process; however, the procedure was slightly altered [19]. In a final volume of 1 ml, the reaction mixture included the following components: Using 50 mM phosphate buffer, pH 7. 4, 0. Concentration of needed solutions: 1 mM DTPA, 90 mM NaCl, 5 mM KCl, 12. The cencentration of Evans Blue was as follows: 5μM, the concentration of the plant extract was as follows: 200 μ/ml to 0 μ/ml and peroxynitrite was at a concentration of 1mM. After the 30 minutes incubation at 25°C, an absorbance was measured at 611 nm. Hence, the proportion of ONOO-scavenging has been quantified by comparing the values obtained for the test and blank samples. We repeated all tests six times. In this sense, the reference chemical employed was gallic acid.

Sequestration of hypochlorous acid

The pH of a 10% (v/v) NaOCl solution was maintained at 6. 2 using 0. 6M H2SO4 to produce hypochlorous acid (HOCl) just before the experiment. The amount of HOCl formed was determined from the absorbance spectrum at 235nm using the molar absorptivity of 100 M-1 cm-1. Alterations were made to the design of the experiment, however, the general procedure is almost identical to that of Aruoma and Halliwell [20]. Measuring the scavenging activity was done using the decrease of catalase absorbance at 404 nm. In the last step 1 ml volume of the reaction mixture contained 50 mM phosphate buffer (pH 6. 8), 7. 2 μM catalase, 8. 4 mM HOCl, and different concentrations of plant extract (control and 100 μg/ml). Following the incubation at 25-Celsius for 20 minutes, optical density of the solution was read with reference to an appropriate control. All the tests were conducted thrice. We chose ascorbic acid as a control since it is a potent HOCl quencher.

Data Analysis by Statistic

Statistical software from IBM (New York, NY, USA) and Tukey's test for statistically significant differences (HSD) were utilised to conduct an analysis of variance (ANOVA) on the average mean values, with a confidence interval of 95% or 99%. Statistical significance was determined by a p-value lower than 0.05.

Results and Discussion

***Juniperus communis* secondary metabolites and their antifungal activity**

Bioactivity of the methanolic, ethyle acetate and ethanolic extract of Juniperus communis and standard antibiotics against four fungi and yeast. Alternaria alternaria (15.11 ± 0.22, 12.10 ± 0.18 and 19.30 ± 0.22 respectively), Aspergillus flavus (19.07 ± 0.37, 14.25 ± 0.35 and 22.09 ± 0.37 respectively), Trichophyton rubrum (20.53 ± 0.31, 16.11 ± 0.39 and 22.94 ± 0.31 respectively), and Fusarium oxyporum (11.12 ± 0.39, 20.08 ± 0.38 and 17.62 ± 0.39 respectively). Voriconazole (VCZ) and Amphotericin B (AmB) as standard anti-fungal activity were (23.31 ± 0.38 and 27.09 ± 0.41) respectively. Juniperus communis metabolites was very highly active against Trichophyton rubrum (22.94 ± 0.31).

Peak (Wave number cm-^{l}), Intensity, Bond, Type of Vibration, and Functional group assignment recorded [675.09, 67.825, Strong, C-Cl, Stretch and alkyl halides], [692.44, 69.075, Strong, C-Cl, Stretch, alkyl halides], [738.74, 72.075, Strong, =C–H, Bending, Alkenes], [813.96, 76.441, Strong, =C–H, Bending, Alkenes], [974.05, 65.287, Strong, =C–H, Bending, Alkenes], [1008.77, 54.765, Strong, C-F, Stretch, alkyl halides], [1049.28, 58.347, Strong, C-F, Stretch, alkyl halides], [1093.64, 64.409, Strong, C-F, Stretch, alkyl halides], [1232.51, 80.641, Strong, C-F, Stretch, alkyl halides], [1276.88, 80.140, Strong, C-F, Stretch, alkyl halides], [1606.70, 79.503, Bending, N-H, Stretch, Amide], [1647.21, 79.220, Variable, C=C, Stretch, Alkene]. The following compounds are found in the plant's seeds and fruits: These are d-α-pinene, camphene, pectin, glycolic acid, malic acid, formic acid, acetic acid, cyclohexitol, terpenes, proteins, fermentable sugars, wax, gum, ascorbic acid, dihydrogen, hydro-junene, cadinene, juninene, junipere, and camphor (19, 22]. Unfortunately, the chemical profile of oils in the market for human consumption is scarce, and even more so regarding juniper berry essential oil. Necessary oils of J. communis available available in the market were effective against Staphylococcus aureus and Escherichia coli recommended by the studies of Filipowicz et al. and Gordien et al. but it was observed that Pseudomonas aeruginosa was still resistant to J. communis. As a result, there is a great deal of interest in how the various juniper EOs may be useful – given the antimicrobial activity against a number of bacteria. Some EOs from juniper trees grown in different countries of the world like Algeria, Greece, Italy, Kosovo, Macedonia, Poland, Portugal, Serbia, Spain, Slovakia, and Turkey have variations in both the quality and quantity; more researches in this direction would offer a useful background in the future [24, 25]. The antibiotic activity on multiresistant hospital isolates of S. aureus, S. marcescens, E. cloace, K. pneumoniae, P. aeruginosa, A. baumanii, L. monocytogenes, and C. albicans was higher in the extract with the closest ratio in its constituents, like α-pinene, β-pinene, p-cymeno, and limonene, or However, in comparison with the antifungal fluconazole that possess the inhibition values in the range of 16 to 128 µg/µL, the essential oils obtained from J. communis needles (var. alpina) possessed definite anti-dermatophyte activity against many dermatophyte fungi including Epidermophyton floccosum, Microsporum canis, M. gypseum, Trichophy subtleum, T.rubrum, and T. verrucosum. The active concentrations are within 0. 32 to 2. 5 µL/mL. In terms of broad-spectrum antibacterial activities, there was a slightly positive response against Candida albicans, Staphylococcus aureus, and Pseudomonas aeruginosa in J. communis ssp. Sardinian leaves and fruits' essential oils, but the MIC values were greater than 1 mg/mL. It was observed though that, juniper essential oils in their pure form exhibited less activity than in solutions made from the oil diluted in 50% ethanol. This could be due to the fact that, essential oils are best used when they are of a dilution because of their solubility nature.

Table 1. Fourier-Transform Infrared Spectroscopic peak values of solid analysis of *Juniperus communis*.

No.	Peak (Wave number cm⁻¹)	Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	675.09	67.825	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	692.44	69.075	Strong	C-Cl	Stretch	alkyl halides	600–800
3.	738.74	72.075	Strong	=C–H	Bending	Alkenes	650-1000
4.	813.96	76.441	Strong	=C–H	Bending	Alkenes	650-1000
5.	974.05	65.287	Strong	=C–H	Bending	Alkenes	650-1000
6.	1008.77	54.765	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1049.28	58.347	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1093.64	64.409	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1232.51	80.641	Strong	C-F	Stretch	alkyl halides	1000-1400
10.	1276.88	80.140	Strong	C-F	Stretch	alkyl halides	1000-1400
11.	1606.70	79.503	Bending	N-H	Stretch	Amide	1550-1640
12.	1647.21	79.220	Variable	C=C	Stretch	Alkene	1620–1680

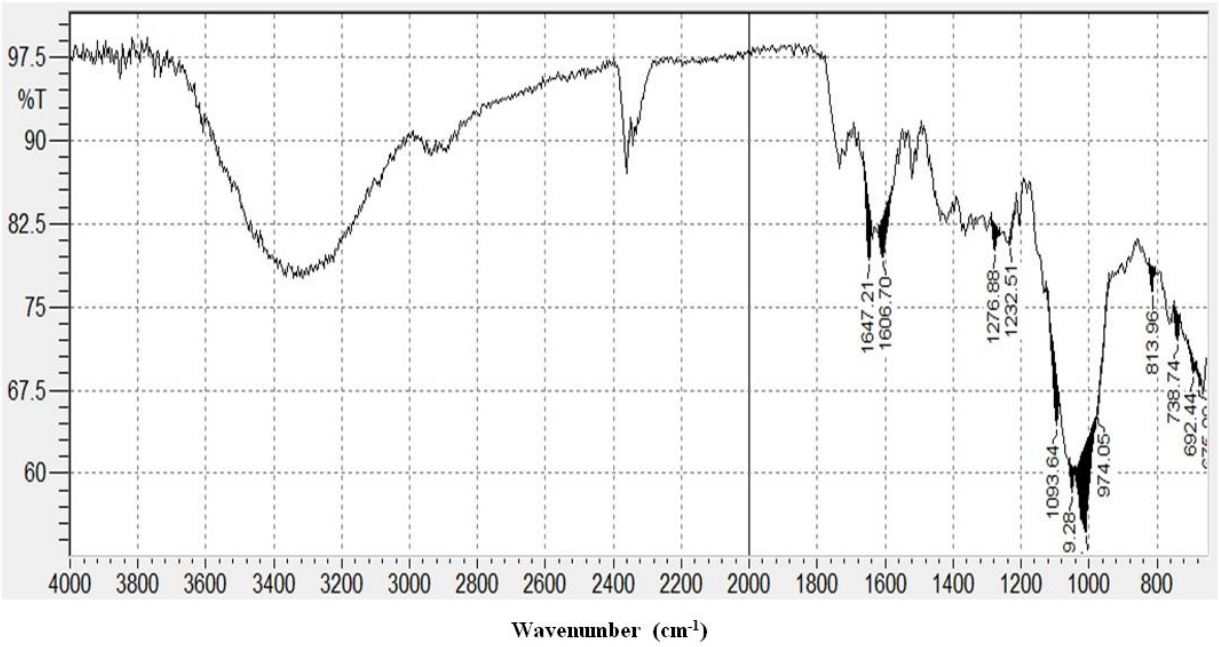

Figure 1. Fourier-Transform Infrared Spectroscopic peak values of solid analysis of *Juniperus communis*.

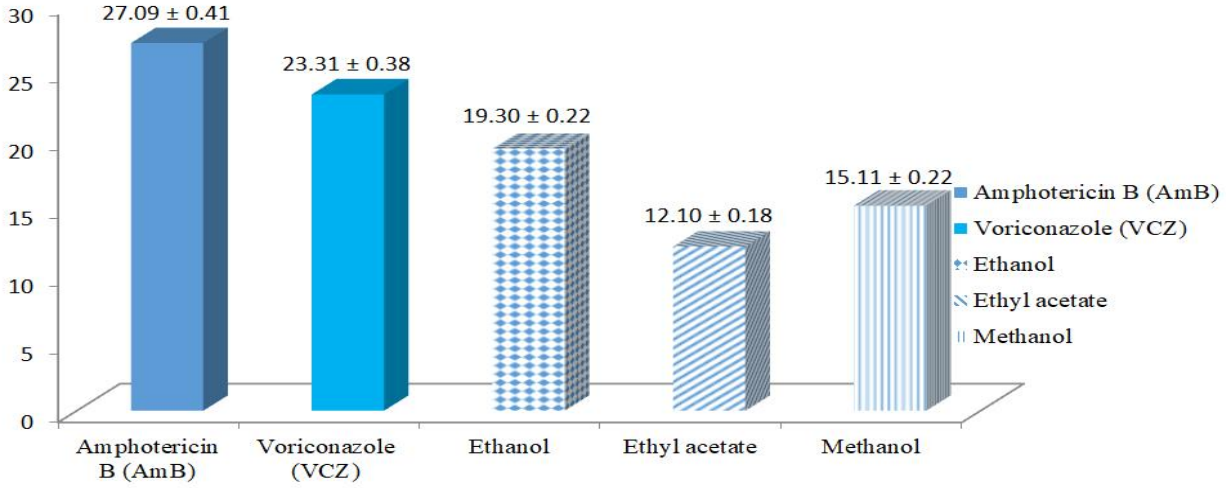

Figure 2. Anti-Fungal activity of metabolites compounds derived from *Juniperus communis* extracts against *Alternaria alternaria*

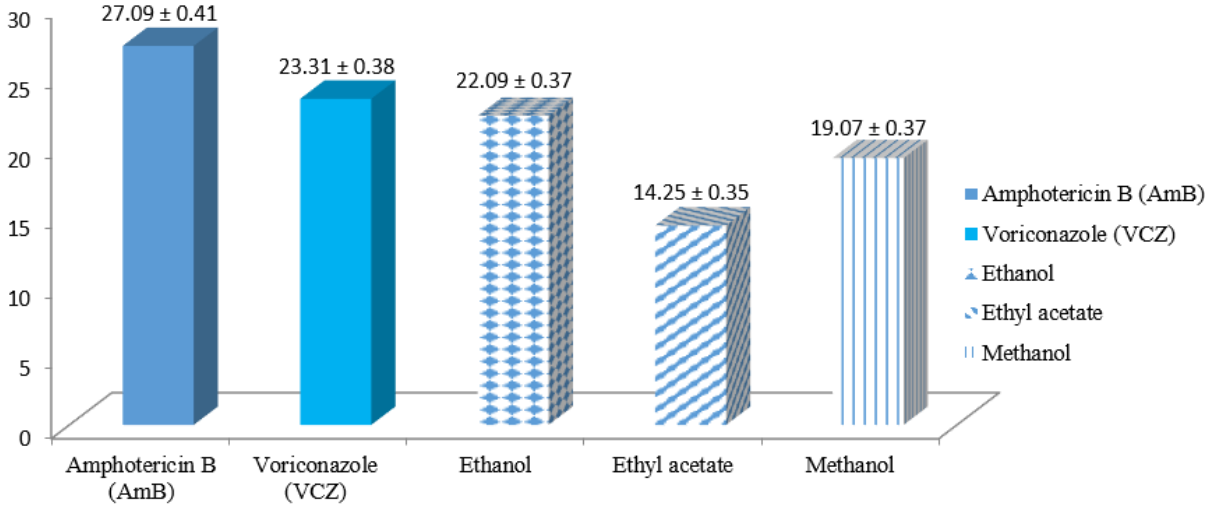

Figure 3. Anti-Fungal activity of metabolites compounds derived from *Juniperus communis* extracts against *Aspergillus flavus*

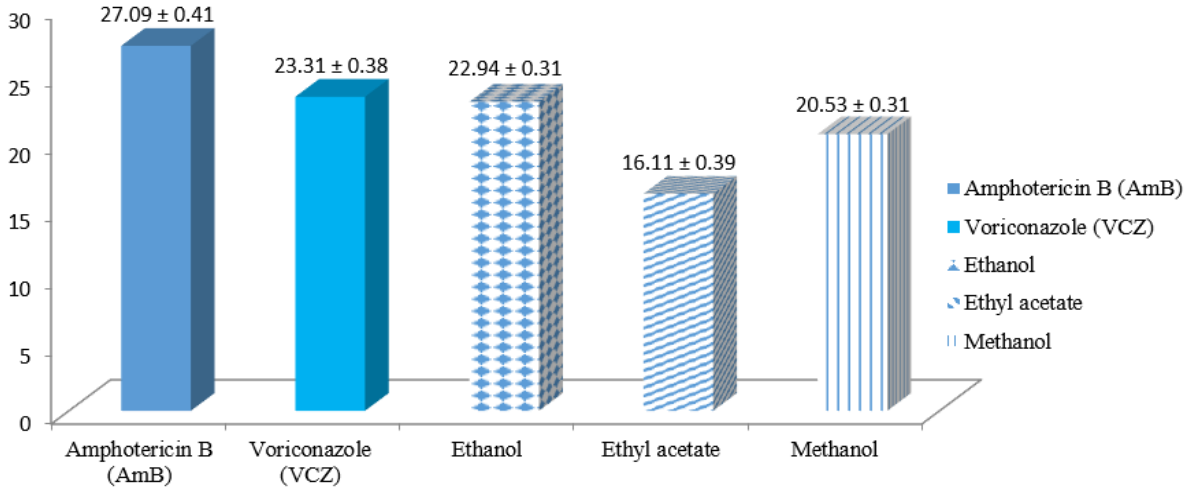

Figure 4. Anti-Fungal activity of metabolites compounds derived from *Juniperus communis* extracts against Trichophyton rubrum

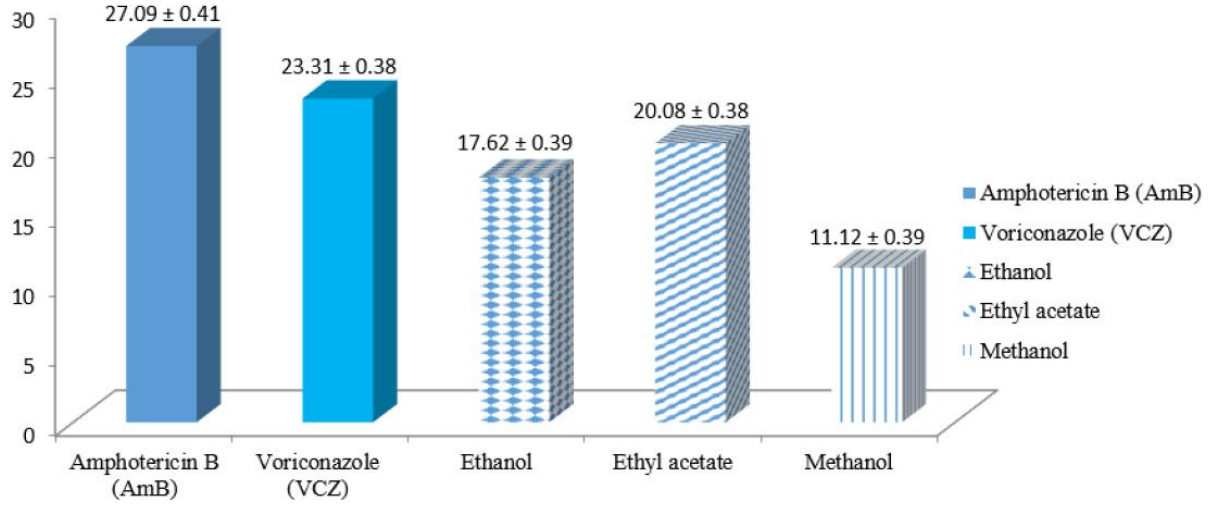

Figure 5. Anti-Fungal activity of metabolites compounds derived from *Juniperus communis* extracts against *Fusarium oxyporum*

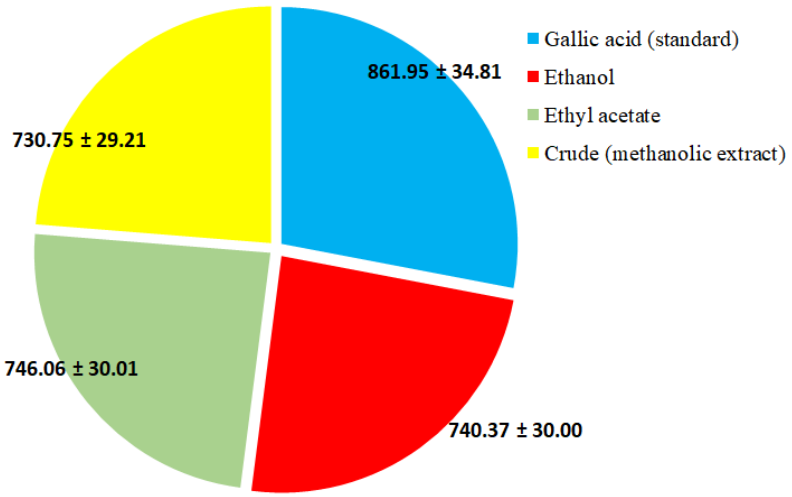

Figure 6. Antioxidant activity (Peroxynitrite scavenging) of Juniperus communis extract (methanol, Ethyl acetate , Ethanol fraction) and Gallic acid (standard)

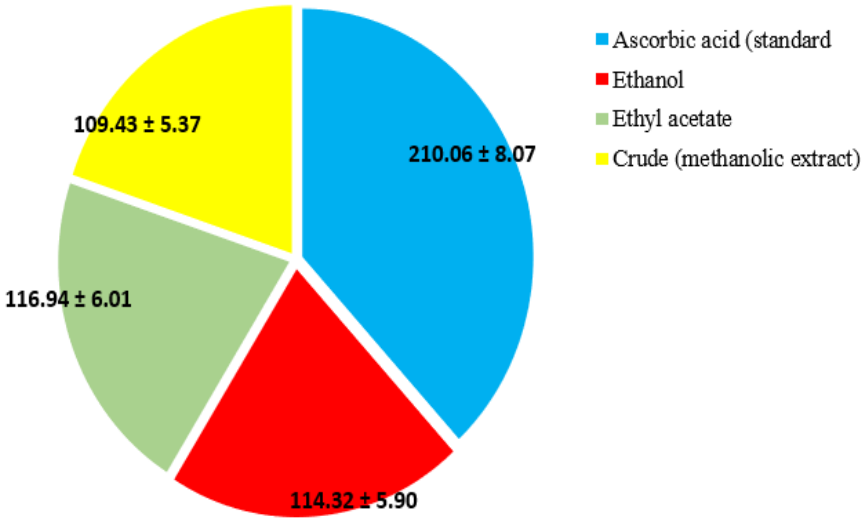

Figure 7. Antioxidant activity Hypochlorous acid scavenging) of Juniperus communis extract (methanol, Ethyl acetate , Ethanol fraction) and Ascorbic acid (standard)

Antioxidant [Peroxynitrite scavenging and Hypochlorous acid scavenging] activity of *Juniperus communis*

Antioxidant activity (Peroxynitrite scavenging and Hypochlorous acid scavenging) of (methanol, Ethyl acetate, Ethanol extract and standards) of *Juniperus communis*. recorded 730.75 ± 29.21, 746.06 ± 30.01, 740.37 ± 30.00 and Gallic acid (standard) 861.95 ± 34.81 respectively of Peroxynitrite scavenging. Although peroxynitrite (ONOO-) is not very unstable on its own, it becomes the extremely reactive peroxynitrous acid (ONOOH) when protonated. Oxidative stress and tissue damage result from the production of an excess of ONOO-. Evans Blue is bleached by oxidising it with peroxynitrite. Here we show that the plant extract scavenges peroxynitrite to limit Evans Blue bleaching, and it does it more effectively than the standard gallic acid Figure 6. 109.43 ± 5.37, 116.94 ± 6.01, 114.32 ± 5.90 and Ascorbic acid (standard) 861.95 ± 34.81 respectively of Hypochlorous acid scavenging Figure 7.

The ingredients of juniper berries are list as follows: juniper essential oil, juniper resin, invert sugars, catechin, organic acid, terpenic acids, leucoanthocyanidin, tannins, gum, lignen, wax, and flavine. Studies regarding some Juniper extracts have revealed that they contain tannins, alkaloids, flavonoids, and phenolic compounds; all of these compounds have been discovered to possess the ability to inhibit bacterial growth to certain extents [26-29]. MAPs are under the research focus of organisations due to having dropped in efficiency and increased worry of synthetic compounds especially on antibiotic-resistant microorganisms. While manufactured substances that form the counterpart of MAPs usually have a single major bioactive ingredient present in the substance, the MAPs derived from essential oils or extracts of natural raw materials have a rich composition of bioactive components as mentioned above that contribute towards the desired end use in the target application [30, 31]. There are different ways of extracting the bioactive compounds of plants as has been advanced by different scientific researchers over the years. Such processes are hydro distillation, maceration, Soxhlet extraction among others. Others are ultrasound, microwave, pulsed electric field, enzymatic and super and sub critical solvent extraction. The conventional methods employ routine extraction procedures, however, they require a huge amount of solvent and the process takes a long time. On the other hand the combination of the two are greener because they require little solvent, comparatively take shorter time to be completed and also because they are effective some of them even do not use toxic solvents. Research to establish the presence of antimicrobial and antifungal uses of the essential oils and their compounds revealed. In clinical microbiology, pharmaceutical preparations, food preservation, essential oils or their components have been applied in their antimicrobial activity. Most works have been devoted to the detection of oils with antibacterial activity; considering the results of investigations, oils with high antibacterial and antifungal activity can be selected for further in vivo research and experimental studies in the future [32–35]. Juniper berry essential oils (Juniperus communis L. , Cupressaceae) are one of the kinds of such oils. Hence, the juniper plant is commonly found in Croatia; however, its natural habitat is in North America, temperate Asia, and Europe. The usual products that are derived from juniper are gin, spice and essential oil which are gotten from the berry that is the female cone of the tree [36, 37]. After the investigation of bio-composition and antibacterial efficiency of Juniper communis oil from the Western Romanian Carpathians we stated that β-pinene, 1α-pinene, p-cymol and β-myrcene are four major volatile compounds.

Conclusion

The study's findings suggest that Juniperus communis is an excellent plant source for chemicals with medicinal activity that may be useful in treating some fungal diseases. The current study's findings provide more evidence that Juniperus communis, a common tree in the forest, contains active chemicals with potential medical use in the treatment of various fungal infections. It is clear that the primary chemicals in the oil, terpen hydrocarbons, had potent antifungal activity, since the essential oil had the lowest MIC values against fungal strains.

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