LYOPHILIZED AQUEOUS EXTRACT OF PINUS HALEPENSIS (MILL.) RESIN: CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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ABSTRACT

In traditional Algerian medicine, Pinus halepensis (Mill.) resin is used as antimicrobial, anti-inflammatory, analgesic, wound healer, for the treatment of respiratory and urinary diseases. In the present study, a lyophilized aqueous extract (LAE) of Pinus halepensis (Mill.) resin is subjected to chemical composition analysis by gas chromatography coupled to mass spectrometry (GC/MS), antioxidant activity was evaluated in vitro using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching assays. LAE of Pinus halepensis (Mill.) resin was also tested for its potential activity against four dermatophytic fungi: Trichophyton rubrum, Trichophyton equinum, Trichophyton mentagrophytes, Trichophyton tonsurans. Results showed that LAE of Pinus halepensis (Mill.) resin contained 64.57% of aromatic compounds. This extract contains also succinic acid, sugars and alcohol sugars, alkaldoids and other compounds that represent respectively 0.58%, 6.45%, 0.87% and 2.05%. The obtained extract exhibited satisfying antioxidant activity with an inhibition percentage of 93.76±0.41 at a concentration of 300 µg/mL and IC50 of 83.64µg/mL by the DPPH method. The tested extract showed also a good antioxidant activity using β-carotene test. At resin concentration of 100 µg/mL, antifungal tests showed, inhibition rates of 58.9%, 58.44%, 41.36% and 34.43% against T. tonsurans, T. equinum, T. rubrum and T. mentagrophytes respectively. Upon the obtained results, this extract can be used as external treatments for wounds to prevent oxidation and fungal infections.

Keywords: Pinus halepensis, resin, GC/MS, antioxidant activity, antifungal activity

INTRODUCTION

Plants have been used as an important source of medicine since ancient times and their products are being used for different purposes such as medicine, food, health care, agriculture, agrochemicals, pharmaceutical, etc. The medicinal properties of different plant species have contributed to the origin and evolution of many traditional herbal therapies (Malik et al., 2012). The importance of medicinal plants lies in their biological active principles, which are the real healers in the process of medication (Kumar, 2009). The Aleppo pine or Pinus halepensis (Mill.) is a tree species of primary importance in the Mediterranean by the area it occupies and the role it plays in the economy of countries in this region (Nahal, 1962). The resins are considered to play a role in the defence of the coniferous trees against insects and microbial pathogens (Trapp and Croteau, 2001). Natural coniferous resins extracts are raw materials for various products in the industry and have been used as traditional medicines as a homemade salve for skin wounds and infections (Sipponen, 2013). Natural resins have a very wide range of applications, mainly used in industries coatings (varnishes, lacquers, paints, sealants, cements, adhesives...), printing (printing inks...), stationery (paper sizing), polymers (adhesives, rubber, various materials). Gums and many resins are also the sources of varied products for the food industry, perfumery, cosmetics, pharmacy and medicine (Delmond, 2002; Ghammi et al., 2007).

Several medical traditionally uses of P. halepensis (Mill.) were recorded in Algeria according to regions and diseases. Different parts of pine were used in decoction: infusion, pine resin powder mixed with honey or olive oil (Meddour and Meddour-Sahar, 2015). Fruits infusion for the treatment of arterial hypertension (Sarri et al., 2012), buds, leaves and resin decoction and poultice for the treatment of respiratory and urinary tract disorders, antiseptic and stimulant of adrenalglands (Bendradjid et al., 2014), fruits infusion and powder for haemorrhoids, tuberculosis, ulcer treatments and pulmonary conditions (Sarri et al., 2015). Seeds decoction for the treatment of gastric and intestinal ulcers, respiratory infections, prostate infection, sterility, toothache (Bousala and Bousala, 2017), stomachache and kidney inflammation (Sennaoui et al., 2019). Roots decoction for the treatment of respiratory disorders (Chelghi and Ferchichi, 2019). The resin was used against rheumatism (Rebeus et al., 2012), to treat asthma (Meddour and Meddour-Sahar, 2015), as antifungal (Chernat and Gharsouli, 2015) and to treat mainly burns, wounds and skin inflammation, in flu and cough cases (Meddour and Meddour-Sahar, 2015). Leaves, resin infusion and decoction for the treatment of respiratory ailments (bronchitis, pneumonia and colds), urinary problems, parasitosis and wounds (Miara et al., 2019; Bendif et al., 2020). Several studies reported that the most important fungal species implied in dermatophytic diseases in Algeria belonged to Trichophyton genus. They were predominant in the Northeast, 66.68% including Trichophyton rubrum and Trichophyton tonsurans (Hannali et al., 2007), Trichophyton rubrum was the predominant pathogen of feet and the inguinal folds in Sétif (Ilham and Touabti, 2013), Trichophyton mentagrophytes primarily responsible for the inflammatory disease in Batna (Chelgham et al., 2012). While in 2016, Ennaghra et al. found that the occurrence of trichophytic was 72% among this percentage there were 24% T. rubrum and 16% T. mentagrophytes.

In the last decade most research done on pine in Algeria were focused on the organic solvent extraction of essential oils from aerial parts of the tree, spines (Abi-Ayad et al., 2011), needles (Fekih et al., 2014; Dob et al., 2005), twigs and buds (Fekih et al., 2014), flowering aerial parts (Haichour et al., 2020) or pine...
extracts, leaf and bud decoction (Berroukche et al., 2014), bark aqueous extract (Kaouache and Derouiche, 2018), young ovulate cones methanolic extract (Mzizi et al., 2019) and seeds polysaccharides (Aboub et al., 2019). On the bases of the available literature about the extracts and the ethnomedicinal use of Pinus halepensis (Mill.) and the biological activities focused on antioxidant and antifungal especially phytopathogenic fungi (El Omari et al., 2021), the extract and the use of pine resin aqueous extract seem to be more close to the folk use of pine. Thus the aim of this work, which is in our knowledge the first study done in Algeria, was to determine the chemical composition as well as the antioxidant and antifungal activities of a lyophilized aqueous extract of Pinus halepensis (Mill.) resin collected in Sétif region (Northeast of Algeria).

**MATERIAL AND METHODS**

**Preparation of lyophilized aqueous extract (LAE) of Pinus halepensis (Mill.) resin**

Resin samples are collected in the north of Sétif region (in the Northeast of Algeria). A sample of 1.9 g of air-dried resin is ground into a fine powder in a blender and mixed with 90 mL boiling water by a magnetic stirrer for 4h. The aqueous extract was then filtered over cheesecloth and Whatman paper No. 1, respectively. The filtrates were frozen at -20°C in ultra-low temperature freezer and lyophilized in a dry freezer machine (Cryoviroire) under 260-mTorr pressure at -81°C (Gülçin et al., 2007).

**Chemical composition of LAE of Pinus halepensis (Mill.) resin by GC / MS**

The identification of the components of LAE of P. halepensis resin was performed based on the gas chromatography coupled to mass spectrometry (GC / MS) as described by Mansouri et al. (2011) and Soltani et al. (2017). Five (5) mg of pine resin samples of LAE were mixed with 50µL of dry pyridine and 35µL of N,O-Bis (trimethylsilyl) trifluoroacetamide, heated at 80°C for 20 min and analyzed by GC-MS. This technique is performed using a Hewlett Packard Gas Chromatograph 6890 Series II Plus linked to Hewlett Packard 6972 mass spectrometer system equipped with capillary column HP-MS 30 m long, 0.25 mm id and 0.5 µm film thicknesses. The column temperature is programmed from 100 to 325°C at a rate of 5°C/min. The carrier gas is helium with a flow rate set at 20 mL/min. The injection mode is Split (Split ratio 50:1, injector temperature 280°C). The identification of the compounds present in pine resin LAE samples is accomplished using computer searches on commercial libraries. In some cases, when identical spectra have not been found, only the structural type of the corresponding component was proposed based on its mass-spectral fragmentation. If available, reference compounds were co-chromatographed to confirm GC retention times. Components identification is verified by search in NIST Data Base using CAS numbers (http://webbook.nist.gov/chemistry/).

**Antioxidant activity**

**Radical scavenging by DPPH method**

Antioxidant activity of pine resin LAE is evaluated according to the DPPH (1,1-diphenyl-2-picrylhydrazyl) method proposed by Chen et al. (2004) and Que et al. (2006). Two (2) mL of LAE of P. halepensis (Mill.) resin solution (2mg/mL) and different concentrations of BHT (butylated hydroxyltoluene) in distilled water are mixed with 2 mL of 0.1 mM DPPH solution in methanol. Control is prepared with the same method but the tested substance is substituted by distilled water. After 30 min in dark, DPPH discoloration is measured at 517 nm with a spectrophotometer (thermo- Sientific: HEIOS®490) using distilled water as blank. All tests are performed in triplicate. Extracts scavenger activity in % is calculated according to the following formula: scavenger activity in % = [(Ac – Ae)/Ac] x 100 where: Ac = (Absorbance of control), Ae = (Absorbance of tested extract).

The graph of the inhibition percentage variation as a function of tested extract concentrations allows determining the IC50, concentration corresponding to 50% inhibition of DPPH radicals. This value is compared to that recorded for the reference compound (BHT).

**β-carotene bleaching test**

The antioxidant capacity is determined by measuring inhibition of volatile organic compounds production and the formation of conjugated dienehydroperoxides arising from linoleic acid oxidation which results in the discoloration of β-carotene (Krishnaiah et al., 2011). β-carotene bleaching test is realized according to the method described by Sun and Ho (2005). In a flask, 1mL of β-Carotene solution (1 mg/mL of pure chloroform) is added to 25 µL of linoleic acid and 200 mg of the Tween 40 emulsifier mixture. After evaporation of the chlorof orm by rotary evaporator (Rotamantle) at 42°C, 100 mL of oxygen-saturated distilled water are added with vigorous shaking until emulsification.

Next, 10 mL of this mixture are transferred into test tubes containing 2mL of distilled water (control), 2 mL of BHT solution (positive control, standard antioxidant) at 2mg/mL of methanol or 2 mL of different concentrations of the tested extract; all tests are performed in triplicate. As soon as the emulsion is added to test tube, the zero time point absorbance is measured at 490 nm by a spectrophotometer (thermo- Sientific: HEIOS®490), blank contains all emulsion components but devoid of β-carotene. After incubating emulsion for 2 h at 50°C, absorbance readings are taken at regular intervals (15min) until 2h (decolorized β-carotene).

**Antifungal activity of resin LAE of P.halepensis (Mill.) resin**

The LAE of Pinus halepensis (Mill.) resin was tested in plate based poisoned bait assays against four dermatophytic fungi Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton tonsurans obtained from the laboratory of Dr. Jane Nicklin, School of Biological and Chemical Sciences, Birkbeck College, University of London, UK.

The dermatophytic fungi are cultivated on Sabouraud dextrose agar (Oxoid LTD, Basing Stoke, Hampshire, England). Resin LAE dissolved in methanol is added according to the concentrations of 12.5 µg/mL, 25 µg/mL, 50 µg/mL and 100µg/mL. The medium containing the appropriate concentration of MeOH without pine resin extract is used as control. Plates are incubated at 25°C for 9 days. The fungal growth inhibition rates are calculated according to the equation: Inhibition % = [(Cd-Td)/Cd]x100 where Cd: control colony diameter, Td: test colony diameter. Results converted to prohibit values (Finney, 1971), are plotted against log2 of the dilution factor in order to determine the dilution required to inhibit germation by 50%.

**Statistical analysis**

Results are expressed as mean ± SD. Graphs were realized by Graph Pad Prism 6.0 (GraphPad Software, USA). Statistical significance of the difference was assessed by a two-way analysis of variance. Differences were considered significant at p<0.05.

**RESULTS AND DISCUSSION**

**Chemical composition of resin LAE of P.halepensis (Mill.)**

Analysis of the chemical composition of the LAE of pine resin by GC/MS resulted in the identification of 53 compounds. The most representative results according to the NIST database (Table 1), are aromatic compounds such as cinnamic and benzoic acids derivatives that represent, quantitatively the most important fraction. Among phenolic compounds, caffeic acid area is 35.52%. Methyl ester of salicylic acid, 3,5-Bis (1,1-dimethylthyl) catechol, vanillin, [1,1’-biphenyl]-4,4-diol, 3,3-dimethoxy-, isorhamnetin, coumarin 3-(1,1-dimethoxy) -7-hydroxy-6-methoxy-, p-salicilic acid (4-hydroxybenzoic acid), protocatechuic acid and even thymol are respectively present at percentages of 5.53, 5.09, 3.84, 3.05, 2.73, 1.80, 1.78, 0.97 and 0.63. Besides, the analysis reveals the presence of a variety of sugars representing 6.45%. This aqueous extract also contains succinic and phosphoric acids, D-verbene and alkaloids as tetra hydroxarame. Total percentage area of identified compounds, referring to the NIST database, is 74.52%.

Natural resins are complex mixtures of several classes of compounds, the main ones as described by Delmond (2002) are in addition to essential oils and polysaccharides, acid constituents, aliphatic as succinic acid, aromatic as benzoic and cinnamic acids and phenolic acids as salicylic, p-coumaric and ferulic acids combined as esters to resin alcohols (resinols and neosalinols) and phenolic compounds as urushiol. Natural resins contain also neutral constituents, essentially triterpenic in the majority (Ghanmi et al., 2007), resin alcohols, free or as esters and are generally phenolic. The compounds present in greater quantities in the Pinus genus are mono or sesquiterpenes (Ghanmi et al., 2007; Simard, 2007; Ali-Ayd et al., 2011), their concentrations vary with species. Several diterpenes were isolated from different parts of pines (Simard, 2007). Earlier Karepova et al. (1983) reported that the aqueous extract of the woody verdure of Pinus sylvestris contained aliphatic mono-, di- and tricarboxylic acids and benzene carboxylic acids. Among aromatic acids, they found benzoic acid in the largest amounts. These results are consistent with the obtained results, Pinus halepensis (Mill.) resin LAE also contained aliphatic acids such as succinic acid and a significant amount of aromatic compounds such as benzoic and cinnamic acids derivatives greatly represented by caffeic acid. Sugars are present at 6.45%; these sugars derived probably from the hydrolysis of polysaccharides following sample boiling. This is reinforced by data of Sipponen (2013), who found constantly coumaric acid (hydroxycinnamic acid), a group of resin acids (hydroxyabietic and abietic acids) and a group of lignans specially pinorresinol natural coniferous resins and other terpenic wood extracts.
Table 1 Components of Pinus halepensis (Mill.) resin lyophilized aqueous extract identified by GC/MS

| Compounds                    | %area | Total % area |
|------------------------------|-------|--------------|
| Linear hydrocarbon acids     |       |              |
| succinic acid                | 0.58  | 0.58         |
| 2,6-Difluoroaniline or 2,6-difluoro Benzenamide | 0.58 |
| Vanillin                     | 3.84  |              |
| 3',5'-Bis(trifluoromethyl) acetophenone | 0.3  |
| 4-Hydroxybenzoyc acid p-Sahlic acid | 1.78 |
| vanillic acid                | 1.00  |              |
| Protocatecholic acid         | 0.97  |              |
| Aromatic compounds and derivatives |     |              |
| Coumarin, 3-((1,1-dimethylallyl)-7-hydroxy-6-methoxy-) | 1.80 |
| Isoferulic acid              | 2.73  | 64.57        |
| Caffeic acid                 | 35.52 |              |
| 1',3'-Biphenyl-4,4'-diol, 3,3'-dimethoxy- or 4,4'-Biphenyldiol, 3,3'-dimethoxy- | 3.05 |
| 3-Methyl-2-phenylindole (3-Methyl-2-phenylindole | 0.72 |
| Thymol                       | 0.63  |              |
| M-methoxymandelic acid ommethyl ester of salicylic acid | 5.53 |
| 2-(1H-benzimidazol-2-yl) aniline | 1.03 |
| 3,5-Bis(1,1-dimethylthyl) catechol | 5.09 |
| Sugars and their alcohols    |       |              |
| Xyliot                       | 0.97  |              |
| D-Xylopyranose               | 0.59  | 6.45         |
| D-Glucose                    | 2.20  |              |
| Alkaloids                    |       |              |
| Tetrahydrolaromane           | 0.87  | 0.87         |
| Others                       |       |              |
| Phosphoric acid              | 0.69  |              |
| D-Verbenone or 2-Pinen-4-one ether of glycerol | 0.49 |
|                               | 0.49  | 2.05         |
|                               | 0.87  |              |

Antioxidant activity

Plants rich in flavonoids and phenolic acids are a good source of natural antioxidants. A positive and significant correlation existed between antioxidant activity and total phenolics (Wojtyło et al., 2007; Ignat et al., 2011). The pine bark provide a readily available source of dietary antioxidants (Walia et al., 2019).

DPPH test

The test results of the free radicals scavenging effect from the LAE of pine resin and BHT (Fig. 1) demonstrate a fairly significant capacity for trapping the DPPH radical and are concentration-dependant. Maximum inhibition (93.76%±0.41) was recorded at 300µg/mL, however, at 200 µg/mL inhibition is already almost in a maximum of 92.23%±0.13. According to the results, the IC50 as determined from the trend curve is 83.64µg/mL (R² = 0.9624). The positive control BHT, also shows a scavenging DPPH power with an IC50 of 45.11µg/mL (R² = 0.9324). It is also noteworthy that the inhibition rate was over 90% at only 150 µg/mL and as determined from the trend curve is 83.64µg/mL (R² = 0.9642). The positive control BHT, also shows a scavenging activity of samples extracted from leaves richer in polyphenols and galls of Gutiera senegalensis by measuring the inhibition of β-carotene oxidative degradation.

β-carotene bleaching test

Based on the results of β-carotene bleaching assay of pine resin LAE (Fig. 2), the OD recorded at T=0min is higher than those recorded for other time intervals. The decrease in OD occurred between 0 and 15 minutes and then became slower for the resin and BHT. The decrease in absorbance is however very marked for the aqueous and methanolic controls devoid of resin extract following the disappearance of the β-carotene, the oxidation of linoeleic acid is at a maximum after 60 min. BHT appeared the most potent antioxidant; the LAE of P. halepensis resin also showed a remarkable and significant antioxidant effect by inhibiting β-carotene oxidation. Neacsu et al. (2007) isolated six knotwood flavonoids, two flavonoid glucosides and one cinnamic acid derivative from Jack pine and European aspen knotwood, all compounds inhibited lipid peroxidation and scavenged peroxy radicals, their antioxidant properties are close to that of the reference compound Trolox, Kounamé et al. (2009) highlighted the radical scavenging activity of samples extracted from leaves richer in polyphenols and galls of Gutiera senegalensis by measuring the inhibition of β-carotene oxidative degradation.

Pycnogenol containing organic acids- caffeic, cinnamic, fumicar, gallic, vanillic, ferulic, and protocatechuic, extracted from French Maritime pine tree bark, (Simpson et al., 2019), provided photoprotection, reduce hyperpigmentation of human skin and improved skin barrier function and extracellular matrix homeostasis (Grether-Beck et al., 2016). Several research studies have demonstrated the antioxidant potential of derivatives of the components of Pinus genus as proanthocyanidin, cinnamic acid and its derivatives. The tested LAE pine resin extract contained a relatively large fraction consisting of cinnamic acid derivatives which are more active antioxidants than those of benzoic acid derivatives (Eom et al., 2012). According to Ku et al. (2007), hot water extracts from Pinus densiflora, P. radiata and P. rigida indicated greater antioxidant activity owing to high proanthocyanidin content. The same authors also suggest that P. radiata bark can be considered as a natural resource for biological and pharmaceutical applications why not the LAE resin; which was also obtained by hot water extraction. The present results showed also the presence of caffeic, vanillic, isoferric and protocatechuic acids in P. halepensis resin LAE obtained by hot water that can be used as a potent radical scavenger and consequently as potent antioxidant.
The results of this study showed that Pinus halepensis (Mill.) resin lyophilized aqueous extract has considerable anti-oxidant and antifungal activities in vitro. Also, it contains important amounts of phenolic compounds and can be used as a natural source of antifungal and antioxidants valuable in food supplement. It is hoped that efforts will continue to discover natural active compounds derived from plants with potent therapeutic action devoid of toxicity. We should then maintain our efforts in protecting and valorizing our natural patrimony, as well as scientific research on Pinus halepensis (Mill.) resin from chemical analysis, biological, toxicological and pharmacological investigations to therapeutic aspects.

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REFERENCES

Abou, A., Kadri, N., Debbache, N., Dairi, S., Remini, H., Dahmoun, F., Berkani F., Adel, K., Belbahi, A. & Madani, K. (2019). Effect of precipitation solvent on some biological activities of polysaccharides from Pinus halepensis Mill. seeds. International Journal of Biological Macromolecules, 141, 663–670. doi: 10.1016/j.ijbiomac.2019.08.266

Abi-Ayad, M., Abi-Ayad, F.Z., Lazzouni, H.A., Rebiahi, S.A., Ziani-Cherif, C. & Bessiere, J. (2011). Chemical composition and antifungal activity of Aleppo pine essential oil. Journal of Medicinal Plants Research, 5(22), 5433-5436. doi: 10.5897/JMPR.K000340

Benderradji, L., Rebbas, K., Ghadbane, M., Boumar, R., Brini, F. & Bosseznor, H. (2014). Ethnobotanical and antifungal activities of medicinal plants in Djebel Medjerda region (M’SILA, ALGERIA). Global Journal of Research on Medicinal Plants and Indigenous Medicine, 3(12), 445-459. https://search.proquest.com/docview/1644784454?accountid=854

Bouaoula, A. & Bouaoula, I. (2017). Ethnobotanical survey of medicinal plants in northeastern of Algeria. Phytomedecine, 36, 68–81. https://doi.org/10.1016/j.phymed.2017.09.007

Bougandoura, N. & Bendimerad, N. (2013). Evaluation de l’activité antioxidante des extraits aqueux et méthanolique de Satureja calamintha ssp. Nepeta (L) Briq. Revue «Nature & Technologies». B-Sciences Agronomiques et Biologiques, 9, 14–19. (French) https://www.univ-chief.dz/revueenateau/Issue_09.Art_B.03.pdf

Chang, K.P., Rossal, S. & Chang, H. (2011). Pharmacological studies on the leaf of Xanthosoma sagittifolium. Phytotherapy Research, 25(3), 435-439. doi: 10.1002/ptr.3518

Chenc, C.N., Wu, C.L. & Lin, J.K. (2004). Propolin C from propolis induces apoptotic cell death in melanoma cells. Biochemical Pharmacology, 67, 53–66. doi: 10.1016/j.bcp.2003.07.020

Chermet, S. & Gharzouli, R. (2015). Ethnobotanical Study of Medicinal Flora in the North East of Algeria - An Empirical Knowledge in Djebel Zdimm (Setif). Journal of Materials Science and Engineering, A 5 (1-2), 50-59. doi: 10.17262/jmse2015.1.2-0001

Chehra, D. & Ferche, L. (2019). Ethnobotanical study of Belezma National Park (BNP) plants in Batna: East of Algeria. Acta Scientifica Naturalis, 6, 40–54. doi: 10.2478/asn-2019-0017

D'Alonzo, A., de Lira Mota; K.S., de Oliveira Pereira, F., Mendes, J.M. & Haichour, R. (2015). Investigation on mechanism of antifungal activity of eugenol against Trichophyton rubrum. Medical Mycology, 53, 507–513. doi: 10.1099/ijmm.0.000034-0
Sipponen, A. (2013). Coniferous resin salve, ancient and effective treatment for chronic wounds – Doctoral dissertation (article-based), Helsinki University Hospital, University of Helsinki, Helsinki, Finland.

Soltani, E., Cerezuela, R., Mezaache-Aichour, S., Angeles Esteban, M. & Zerroug, M.M. (2017). Algerian propolis extracts: Chemical composition, bactericidal activity and in vitro effects on gilthead seabream innate immune responses. *Fish & Shellf Immunology, 62*, 57-67. [http://dx.doi.org/10.1016/j.fsi.2017.01.009](http://dx.doi.org/10.1016/j.fsi.2017.01.009)

Sun, T., & Ho, C.T. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry, 90*, 743–749. [https://doi.org/10.1016/j.foodchem.2004.04.035](https://doi.org/10.1016/j.foodchem.2004.04.035)

Trapp, S., & Croteau, R. (2001). Defensive resin biosynthesis in conifers. *Annual Review of Plant Biology, 52*, 689–724. doi: [10.1146/annurev.arplant.52.1.689](https://doi.org/10.1146/annurev.arplant.52.1.689)

Walia, A., Gupta, A.K. & Sharma, V. (2019). Role of Bioactive Compounds in Human Health”. *Acta Scientific Medical Sciences* 3(9), 25-33. [https://www.actascientific.com/ASMS-3-9.php#popup1](https://www.actascientific.com/ASMS-3-9.php#popup1)

Wojdylo, A, Oszmiański, J, & Czemerys R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry, 105*, 940–949. doi: [http://dx.doi.org/10.1016/j.foodchem.2007.04.038](http://dx.doi.org/10.1016/j.foodchem.2007.04.038)