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ФИТОХИМИЧЕСКОЕ ОПРЕДЕЛЕНИЕ И КОЛИЧЕСТВЕННЫЙ АНАЛИЗ ОБЩЕГО СОДЕРЖАНИЯ ПОЛИФЕНОЛОВ, ФЛАВОНОИДОВ И АНТИОКСИДАНТНОЙ АКТИВНОСТИ ЛИСТЬЕВ СИРИЙСКОЙ СОСНЫ АЛЕПСКОЙ

Аннотация: *В данной работе было проведено исследование фитохимического скрининга и определения общего содержания полифенолов, общие флавоноиды и антиоксидантная активность метанольного (80%) экстракта, дихлорметан, этилацетат и бутанол экстракты листьев (хвои) сосны халепенсис (halepensis), количественная оценка фенолов и флавоноидов была изучена с использованием метода Фолина-Чокальтеу для оценки фенольного содержания, , метод химического детектора хлорида алюминия для определения содержания флавоноидов в обоих экстрактах метанола (80%), дихлорметановый экстракт, этилацетатный экстракт, системный бутанольный экстракт. Антиоксидантную активность предыдущих экстрактов изучали фосфатно-молибдатным методом.*

Было обнаружено присутствие флавоноидов, кумаринов, мыла, танинов, триглицеридов и стеролов. Результаты показали, что наивысшая антиоксидантная эффективность метанольного экстракта и содержание фенолов в метанольном экстракте было наибольшим (370,4 мг параболических гелей на грамм сухого экстракта), Содержание флавоноидов

в метанольном экстракте показало наивысшее значение по сравнению с предыдущими экстрактами (эквивалент 85,96 мг кверцетина на каждый грамм сухого экстракта).

Ключевые слова: *фитохимический скрининг, общие полифенолы, антиоксидантная активность, галепсия сосны.*

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PRELIMINARY PHYTOCHEMICAL SCREENING, QUANTITATIVE ANALYSIS OF TOTAL POLYPHENOLS, TOTAL FLAVONOIDS AND ANTIOXIDANT ACTIVITY OF LEAVES FROM SYRIAN PINUS HALEPENSIS

Abstract: *In this paper a study was carried out phytochemical screening and determine the content of total polyphenols, total Flavonoids and antioxidant activity of methanol(80%) extract, dichloromethane, ethyl acetate and butanol extracts of leaves (Needles) from pinus halepensis, Quantitative estimation of phenols and flavonoids have been studied using Folin-Ciocalteu method to estimate phenolic conten , method of Aluminum chloride chemical detector for determination Flavonoid content for both methanol(80%) extract, dichloromethane extract, ethyl acetate extract systemic butanol extract. The antioxidant activity of the previous extracts was studied using the phosphate molybdate method*

The presence of flavonoids, coumarins, soaps, tannins, triglycerides and sterols were all detected. the results showed that the highest antioxidant efficacy of the

methanol extract and the phenolic content of the methanol extract was the largest (370.4 mg parabolic gels per gram of dry extract), The flavonoid content of the methanol extract showed the highest value compared with the previous extracts (85.96 mg equivalent for Quercetin for each gram of dry extract).

Keywords: *phytochemical screening, total polyphenols, total Flavonoids, antioxidant activity, pinus halepensis.*

1- Introduction

Antioxidant substances can block the harmful action of the free radicals by scavenging the free radicals and detoxify the organism. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are commonly used in food processing and preservation but have been found to have side effects and have been proved carcinogenic[1]

The flavonoids present in plants possess a variety of health benefits, including activities Antioxidant and free radical scavenging, reduction of some chronic diseases, prevention of cardiovascular disorders, and certain types of cancer Thus there has been increased interest in natural antioxidants, especially those of plant origin.

The pinus halepensis (Aleppo pine) plant belongs to the genus of pine species, which includes about 250 species, and this tree appears to have grown primarily in the Aleppo region in Syria, then spread around the shores of the Mediterranean (in Algeria, Morocco, southern Spain, Greece, Albania, Croatia, Ukraine, Turkey, Jordan Palestine, Libya, and Tunisia). It is grown in southern France and in the state of California[2,3.4] ., Pine trees have many uses and benefited from it in several ways (medical and non-medical), where pine tree trunks were used in construction, which gave them economic importance[5], In addition to the many uses of turpentine extracted from pine gum in the adhesive material in polyester, paper and pulp making, and making use of tar and pine oil as an insecticide[6] Pinus trees with their multi-parts (leaves - bark - fruits) were used in folk medicine to treat infections,

soothe pain in the teeth, and treat arthritis and respiratory tract infections[6] Several studies were conducted on (leaves - bark - fruits) pine trees, with the aim of detecting the effective groups (natural products) in them, as it was found that they contain both Terpenoids and flavonoids[8] These studies show [9] the plant's effectiveness as an anti-viral, anti-bacterial and antioxidant.

The extracts most active against poliovirus were those from Pinus Halepensis[10] The pinus oil extracted from the leaves has been used in medicine and treatment of arthritis[11] The oils extracted from the cones of the pinus halepensis showed high effects in the speed of wound healing[12].

This plant is considered one of the very common plants in the Syrian flora. It is worthy of us to study the Syrian medicinal plants because of the environmental richness of this plant and the wide variety that is useful in many medical and therapeutic fields, Therefore, this study focused on Preliminary Phytochemical Screening, Quantitative Analysis of Total Polyphenols, Total Flavonoids and Antioxidant Activity of Leaves from Syrian *Pinus Halepensis*.

2- Experimental:

2.1.Chemicals and reagents

aluminium chloride, sodium carbonate, sodium acetate, gallic acid, ascorbic acid and Folin & Ciocalteu's reagent were purchased from Sigma-Aldrich Chemical Co. (USA). Butanol was obtained from Merck Chemical Suppliers (Germany) . All other chemicals and solvents were of analytical grade

2.2.Plant materials

The leaves of *P. halepensis* were collected during the month of September 2017 from Syria .The plant materials were dried in shade separately.

2.3. Qualitative phytochemical analysis of plant extracts

Qualitative phytochemical analysis was carried out by using different standard methods in order to investigate the secondary metabolites presented in the ethanolic extracts of *P. halepensis*. [15-16]

2.3.1. Carbohydrates (Molish test): The extract (0.5 g) was dissolved in 2 mL of ethanol and added with 1 mL of distilled water and filtered. To this solution, 2-3 drops of α-naphthol were added followed by 1 mL of H2SO4. The formation of violet coloured ring was observed at the interface of two layers.

2.3.2. Sterols and steroids Liebermann reaction were used to indicate the presence of steroids. 10ml of ethanolic extract was vaporized. The residues was dissolved in 0.5ml of hot acetic anhydride and filtered. 0.5ml of the filtrate was treated with Libermann burrchardt. The appearance of a blue-green ring at the interphase showed a positive result

2.3.3. Resins: 0.5 g of the extract was dissolved in 2 mL of ethanol in a test tube and treated with 2 mL of distilled water and observed for turbidity **[13-14]**

2.3.4. Terpenoids 4ml of the plant extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform. Then concerted solution of sulphuric acid was added gradually and red violet color was seen for terpenoids. [15]

2.3.5. Coumarins 0.5ml 10% NH4OH was added to 5ml of ethanolic extract. On a filter paper 2 spots were added and examined under U.V light. Intense fluorescence indicates the presence of coumarins. [16]

2.3.6. **Saponins** Each of the plant extracts (0.5g) wasseparately stirred in a test tube, foaming whichpersisted on warming was taken as an evidencefor the presence of saponins [17]

2.3.7. Flavonoids (Shino or Pew test): 0.5 g of the extract was dissolved in 2 mL of ethanol and treated with few drops of conc. HCl and 0.5 g of magnesium. The pink colour was observed. [18]

2.3.8. **Tannins:** 0.5 g of the extract was dissolved in 2 mL of ethanol and added with 3 mL of hot distilled water and then filtered. Few drops of FeCl3 (0.1 g/L) were added and allowed to stand for some time and observed for brownish green or blue black colour. [16]

2.4. Extraction procedure

2.4.1. Extraction:(15 g) Fresh leaves of pinus halepensis were chopped into small pieces by hand and put into a conical flask. Volume of methanol to water was in ratio of 80 ml: 20 ml was added to the conical flask and covered with a cotton plug on the mouth of conical flask. It was kept in maceration for 4 days at 20C in order to maximize the extraction. After 4 days it was filtered through Whatman filter paper and reduced of its volume in a rotary vacuum evaporator at 35ºC , In this method we obtained a Methanol 80% extract. In the same way, we obtained all extracts of dichloromethane, ethyl acetate extract and butanol extract.

2.4.2. The content of total phenolics

Determination of total phenolics was determined spectrophotometrically by using the Folin-Cioalteu's assay with some modification. Briefly, to appropriate volume of undiluted extracts 7.5 ml of water was added. The mixture was vortexed for 20 s and 500 µl of FC reagent was added. The mixture was vortexed for additional 20-30 s and 1.5 ml of filtered 20% sodium carbonate solution was added in time interval from 1 min to 8 min after addition of the FC reagent. The mixture was placed in a water bath at 40o C for 30 min. The absorbance of the colored product was measured at 765 nm. Different concentrations of gallic acid were used to prepare a calibration curve, and the level of total phenolics was calculated. Results are expressed in mg of gallic acid equivalents per gram extract.

2.4.3. Determination of total flavonoids

Total flavonoids in plant extracts were determined using spectrophotometric method by Briefly, equal volumes of plant extract and 2% aluminum chloride (AlCl3) solution dissolved in methanol were mixed. The samples were incubated for an hour at room temperature, and after that absorbance was measured at 415 nm. Sample blank was used in the same procedure, but without addition of aluminum chloride. The same procedure was repeated for the standard solutions of quercetin, and the calibration curve was constructed. Results are expressed in mg quercetin equivalents per g. extract.

2.4.4. Total antioxidant capacity

Total antioxidant activity was estimated by phosphomolybdenum assay [17]Preparation of Molybdate Reagent Solution 1ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water.

method

various extract of in concentration 100 µl were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95 ºC for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Different concentrations of ascorbic acid were used to prepare a calibration curve, and the level of total Total antioxidant was calculated. Results are expressed in mg of ascorbic acid equivalents per gram extract.

3-Results and Discussion

3.1. The results of Qualitative phytochemical :

The results of Qualitative phytochemical Contain the Resins, Saponins, Flavonoids, Carbohydrates, tannins, Coumarins, Sterols and steroids Table 1.

Table 1. Results of phytochemical analysis of *pinus brutia*

Test	Stem extract
Coumarins	++
Terpenoids	++
Sterols and steroids	+
Resins	+
Saponins	+++
Flavonoids	++
Carbohydrates	+
Tannins	++

+ = Present - =Absent

3.2. Total phenolics, flavonoids and antioxidant

The level of phenol, flavonoids and antioxidant compounds in different solvent extracts of the leaves of *p.halepensis* are shown in Table 2. The results indicated that the TP content of various extract ranging from 200.16 to 370.40mg GAEgr-1 dry weight for those of solvents extracts. The results showed that the phenolic content of the Methanol 80%extract was the largest (370.40mg GAEgr-1 dry extract), The flavonoid content of the Methanol 80% extract showed the highest value compared with the previous extracts (85.96mg quercetin .gr-1 dry extract) and the Total antioxidant of the Methanol 80% extract showed the highest value compared with the previous extracts(195.33mg ascorbic acid.g-1 dry extract).

Table 2. Total phenolics content, total flavonoids content, Total antioxidant capacity of leaves *pinus halepensis* extracted with different extraction systems.

Solvent	Total phenolics [a]	Total flavonoids[b]	Total antioxidant[c]
Methanol80%	370.40	85.96	195.33
Ethyl acetate	236.48	44.13	85.35
butanol	200.16	56.12	98.56
dichloromethane	209.44	20.43	10

Expressed as: a - mg equivalents of gallic acid g-1; b - mg equivalents of quercetin g-1;c- ascorbic acid.

4. Conclusion

Chemical detection tests showed that the leaves extracts of *p.halepensis* contained flavonoids and Carbohydrates ,tannins, Resins, saponins, Coumarins, Sterols and steroids

The results of Molybdate phosphate test showed that the methanol 80% extract has a greater effectiveness in return molybdate phosphate.

The results showed that the phenolic content of the methanol 80% extract was the largest (370.40 mg gallic acid per gram of dry extract), The flavonoid content of the methanol extract showed the highest value compared with the previous extracts

(85.96mg equivalent for quercetin for each gram of dry extract). There is a direct proportion between extracts content of phenolic compounds and flavonoids inhibitory and antioxidant capacity.

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