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Phytochemical Analysis, Determine the Content of Total Polyphenols, Total Flavonoids and Antioxidant Activity of Leaves from Syrian Pinus Brutia

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Abstract
The aim of this study was to 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 from pinus brutia ,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 and systemic butanol extract.The antioxidant activity of the previous extracts was studied using the phosphate molybdate method and the results showed that the highest efficacy of the methanol 80% extract .The results showed that the phenolic content of the methanol extract was the largest (429.44 mg gallic acid per gram of dry extract), The flavonoid content of the methanol extract showed the highest value compared with the previous extracts (98.27 mg equivalent for quercetin for each gram of dry extract). The antioxidant activity of the methanol extract was the largest(113.32 mg equivalent for ascorbic acid for each gram of dry extract).
Keywords: phytochemical screening, total polyphenols, total Flavonoids, antioxidant activity**,** *pinus brutia*

1- Introduction

Phenolic compounds are natural antioxidants and they are considered to have a preventive role in the development of cancer and heart disease [1]. Phenolic acids are a group of phenolic compounds biosynthesised by the shikimate pathway [2]. This class of phenolic compounds exhibits various physiological activities, including antibacterial, anti-inflammatory and anticarcinogenic [3,4].

Researches about biological and pharmacological activities have also been documented for phenolic compounds, including free radicals scavenging, apoptosis of cancer cells [5,6, antiherpectic, antihuman immunodeficiency virus (HIV) reverse transcriptase and anti-HIV activity [7,8].

Pinus (Pinaceae), with over 100 widely known species, is the widest extant genus of conifers [9, 10, 11]. Pines are economically an important source of wood, paper, resins, charcoal, food (particularly seeds), and ornamentals, In folk medicine, various parts of Pinus species (bark, needle, cone and resin) have been used for rheumatism or as anti-inflammatory, antioxidant and antiseptic [12] Pinus brutia is used internally to treat diarrhoea [13]. For the treatment of peptic ulcers[14].

Figure 1. photo of *pinus brutia*

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. brutia* 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

The steam bark extracts were analyzed for the Flavonoids, Pholabatannins, Resins, Tannins, Phenols, Carbohydrates as follows .[15-16]

2.3.1. **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.

2.3.2. 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

2.3.3. **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.

2.3.4. 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.

2.4.Extraction procedure

2.4.1.Extraction:(15 g) Fresh leaves of pinus brutia 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% aluminium 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, Glycosides, Tannins Table 1.

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

Test	Stem extract
Resins	+
Saponins	+++
Flavonoids	++
Glycosides	+
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.brutia are shown in Table 2. The results indicated that the TP content of various extract ranging from 179.38 to 429.44 mg GAEgr-1 dry weight for those of solvents extracts. The results showed that the phenolic content of the Methanol 80%extract was the largest (429.44 mg GAEgr-1 dry extract), The flavonoid content of the Methanol 80% extract showed the highest value compared with the previous extracts(98.27 mg quercetin .gr-1 dry extract) and the Total antioxidant of the Methanol 80% extract showed the highest value compared with the previous extracts(113.32 mg ascorbic acid.g-1 dry extract).

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

Solvent	Total phenolics [a]	Total flavonoids[b]	Total antioxidant[c]
Methanol80%	429.44	98.27	113.32
Ethyl acetate	240.56	64.44	58.02
butanol	179.48	49.55	36.02
dichloromethane	163.10	20.13	13

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.brutia* contained flavonoids and , . Carbohydrates and tannins, and Resins

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 (429.44 mg gallic acid per gram of dry extract), The flavonoid content of the methanol extract showed the highest value compared with the previous extracts (98.27 mg 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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