Phytochemical and Antioxidant Activity of various solvent extract from *Brachychiton populneus* leaves Deployed in Syria

Dr. Mohamad J. Khubeiz*

Abstract

This study aimed to investigate the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant potential of extract fractions (petroleum ether extract fraction-**PEef**, chloroform extract fraction-**Clef**, ethyl acetate extract fraction-**EAef**, Acetone extract fraction-**ACef**, methanolic extract fraction-**MEef**) of *Brachychiton populneus* leaves using Soxhlet apparatus. To achieve this, several parameters such as total flavonoids (TFC), and total phenolic content (TPC), scavenging activity (DPPH), Reducing power (RP) assay, using Gallic acid and ascorbic acid as standard were examined. Maximum phenolic content was exhibited by MEef extract fraction (267.07 ± 1.28), followed by EAef (208.45 ± 1.16), and ACef (159.94 ± 1.41), among Clef showed low phenolic content (18.47 ± 0.48), whereas PEef showed the least (9.83 ± 0.36) mg GAE/g dry extract. EAef showed highest flavonoid content (98.72 ± 1.18 against other fractions (PEef, CLef, ACef, MEef) whose flavonoid content reported as 26.41 ± 0.58, 81.94 ± 0.72, 41.36 ± 0.35 & 24.41 ± 0.78 mg RE/g of dried extract, respectively.

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Indeed, *B. populneus* MEef extract fraction shoots exhibited the moderately high antioxidant activity, with the lowest DPPH IC_{50} (176.12 µg/mL) and RP IC_{50} (192.024 µg Fe^{2+}/mL) values.
The results showed that the methanolic fraction reported a considerable free radicals scavenging activity and reducing effect, may be due to their richness on polyphenols. This study suggests that *B. populneus* may serve as a good source of natural antioxidants.

Keywords: *Brachychiton populneus*, Total phenolics, Total flavonoids, Antioxidant activities, Reducing power.

النشاط الكيميائي النباتي والفعالية المضادة للتأكسد لمستخلصات مذيبات مختلفة من أوراق *Brachychiton populneus* المنتشر في سوريا

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الملخص

هدفت هذه الدراسة إلى التحقق من المحتوى الفينولي الكلي (TPC)، وإجمالي محتوى الفلافونيدات (TFC) والفعالية المضادة للتأكسد لأجزاء المستخلص (الأثير البترولي – PEef ، الكلوروفورم – (Clef)، خلات الإيتيل– EAef ، الأسيتون – Acef ، ميتانول – (MEef) بالتتابع من أوراق نبات *Brachychiton populneus* باستعمال جهاز Soxhlet.ولإنجاز ذلك ، حُددت العديد من الخواص مثل الفلافونيدات (TFC)، ومحتوى الفينولات الكلي (TPC) وكبح الجذور الحرة (DPPH)، والقوة الإرجاعية، باستعمال الكورستين، وحمض الغاليك، وحمض الأسكوربيك كمواد مرجعية على التوالي. أظهر جزء المستخلص MEef القيمة العظمى للفينولات الكلية (1.28 ± 267.07), يليه EAef (1.16 ± 208.45)، و ACef (1.41 ± 159.94)، بينما Clef أظهر محتوى فينولات منخفض (0.48 ± 18.47) ، في حين أظهر PEef أقل محتوى (0.36 ± 9.83 GAE/g extract). في حين أظهر EAef أعلى محتوى من

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الفلافونيدات (98.72 ± 1.18) مقابل الاجزاء الأخرى (PEef، CLef، ACef، MEef)التي تم كان محتواها: 0.72 ,81.94 ± 0.35, 41.36± 0.78, 24.41 ± 0.58 mg/g dry extract) على التوالي.

أظهر جزء مستخلص MEef نشاط مضاد للتأكسد مرتفع نوعاً ما، بقيمة (= IC_{50} 176.12 µg/ml)، يليه ACef (263.35 µg/ml)، وEAef (299.68 µg/ml)، باستخدام DPPH مقارنة بمحض الأسكوربيك (34.76). كما حددت القوة الإرجاعية للأجزاء المستخلصة بـ IC_{50} أدناها MEef بقيمة (192.04 µg Fe^{2+}/mL) ، يليه EAef (273.559 µg Fe^{2+}/mL)، وأخيراً ACef (388.308 µg Fe^{2+}/mL)، مقارنة بحمض الأسكوربيك (25.072 µg Fe^{2+}/mL). أظهرت النتائج أن الجزء الميثانولي أبدى نشاطاً كبيراً في كبح الجذور الحرة وخفض تأثيرها، وقد يعود السبب في ذلك لغناها بالمركبات الفينولية. تشير هذه الدراسة إلى أن نبات *B. populneus* قد يكون مصدراً جيداً لمضادات الأكسدة الطبيعية.

الكلمات المفتاحية: *Brachychiton populneus*، فينولات كلية، فلافونيدات كلية، مضادات التأكسد. قوة إرجاعية.

Introduction

Plants represent a rich source of natural compounds, which are responsible for many multifunctional biological effects. In the last few years, exhaustive research is being carried out to obtain new raw materials from plants for the development of products with healthy characteristics, which help maintain or improve health and protect against chronic diseases.

Antioxidant are important substances that have ability to protect the body from cellular damages by free radical induced oxidative stress [1]. Free radicals on human beings are closely related to toxicity, diseases like chronic renal failure, diabetes mellitus, cancer, immune dysfunction and aging [2]. Studies have shown that antioxidant properties of plants prevent oxidative stress defense and are thus important remediation for different human diseases including cancer, atherosclerosis, and aging process [3,4].

Antioxidants play an important role in inhibiting and scavenging and control the formation of free radicals thereby preventing oxidative damage to cellular components arising as a consequence of chemical reactions involving reactive oxygen species thus, providing protection against infections and degenerative diseases. They can either directly scavenge or prevent generation of oxidative stress reflects [5,6]. The two most commonly used synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have begun to be restricted because of their toxicity and DNA damage induction [7]. The plant species have been investigated in the search for novel antioxidants, but generally, there is still a demand to find more information concerning the antioxidant potential of plant species, as they are safe and also bioactive. Therefore, in recent years, considerable attention has

been directed towards the identification of plants with antioxidant activity [8]. Phenolic compounds are the plant's secondary metabolites with one or more hydroxyl groups linked to aromatic rings, perform various physiological functions which include antioxidant activity as they are excellent oxygen radical scavengers and free radical terminators. Several studies have shown the close relationship between the total phenolic amount and antioxidant activity [9]. Flavonoids are polyphenolic plant metabolites that perform different functions and provide various potential health benefits to humans because of their antioxidant nature, anti-inflammatory, anticancer, antiviral, antibacterial, vasodilator and immune stimulating activity. Flavonoids mediate their antioxidant effects by scavenging free radicals or by chelating metal ions or by inhibiting generation of free radicals [10,11].

Many researchers have been trying to isolate and identify the natural antioxidants from plants to scavenge the free radicals. *Brachychiton populneus* commonly called "kurrajong" is one of them. It belongs to the family *Malvaceae* Previously listed in the *Streculiaceae* family, is a native to eastern Australia with much value in cultivation.

Brachychiton populneus is known to possess wide range of medicinal properties including anti-inflammatory, antidiabetic potential and antipyretic activities. The researchers have used this plant to treat bacterial skin infections. hytochemical studies have shown that *Brachychiton* species are important sources of bioactive ingredients such as flavonoids, polyphenols and alkaloids. However, few reports were found regarding the phytochemical and biological properties of *B. populneus*. Recent pharmacological investigation evidenced that the seed oil has a marked antioxidant activity in vitro. This plant has also been demonstrated as potent

hepatoprotective and anti-inflammatory agents. These effects were associated with the presence of phenolic components, namely Catechin, Rutin and Myricetin. Despite, researches associated with the therapeutic uses of *B. populneus* leaves, detailed information regarding the phytopharmaceutical properties of its various plant parts are limited [12,13].

The sequential extraction method ensures the extraction of active compounds from plant material according to their polarity, and also reduces the antagonistic effect of compounds in the extract [14]. Multiple solvents have been commonly used to extract phytochemicals, and scientists usually employed a dried powder of plants to extract bioactive compounds and eliminate the interference of water at the same time. Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield. The polarity, from least polar to most polar, of a few common solvents is as follows: Hexane < Chloroform < Ethylacetate < Acetone < Methanol < Water [15].

This study included successive extraction of *B. populneus* leaf powder with solvents of increasing polarity, and in vitro antioxidant activity assay for the successive extracts as a rapid screening tool for extract activity.

Material and methods:

Preparation of the *B. populneus* leaves extract:

Healthy and mature leaves of *B. populneus* were collected in the month of June 2020 from the medicinal plant garden of Department of Botany, University of Damascus. The plant was authenticated by the center of Plant taxonomy, Department of Botany, University of

Damascus. The leaves were dried in a room under ambient condition, then pulverized with an electric grinder. The resulting powder was stored at -18°C in a deep freezer until needed for extraction.

Soxhlet Extraction

Fifty gr powdered leaf material was successively extracted with organic solvents, with increasing polarity index, like petroleum ether, chloroform, ethyl acetate, Acetone, and methanol using soxhlet apparatus continuously for 12 h with 500 ml of the various solvents each. After extraction, each time the residue was dried and extracted with the next solvent. The extract fractions (petroleum ether extract fraction-PEef, chloroform extract fraction-Clef, ethyl acetate extract fraction-EAef, Acetone extract fraction-ACef, methanolic extract fraction-MEef) were obtained.

The extracts were condensed using rotary vacuum evaporator and kept for evaporation to remove solvents in laboratory temperature until dried completely. The dried extract fractions were then stored in 4^{o}C for future use. The dried extracts were weighed to determine the percentage yield of the soluble constituents using the formula, % Yield: (Weight of dry extract/ Weight taken for extraction) × 100. Part of the extracts were dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1mg/ml and stored at 4 ^{o}C for future tests [16].

Determination of Total Phenolic Content (TPC)

The concentration of total phenolic in the extracts was estimated by a colorimetric assay based on procedures described by [17] with some modifications. Briefly, 1 mg/ml standard solution of Gallic acid was made in methanol and total phenolic contents were expressed as mg Gallic acid equivalent (GAE) per g of plant extract. The reaction mixture was prepared by mixing 0.1 ml (1

mg/ml) of each sample with 1.0 ml of 10 % Folin-Ciocalteu's reagent dissolved in distilled water. After kept at room temperature for 6-8 min, 1 mL of 20% Na_2CO_3 solution and 1.9 ml distilled water were added to the mixture. The same procedure was repeated for standard solution of Gallic acid with all the reagents. Here, blank as except test sample (extracts, standard). The samples were thereafter incubated in a thermostat at 40 ± 1 ^oC for 60 min and then cooled. Finally, the absorbance of reaction mixture was measured at λ = 760 nm by double beam UV-visible spectrophotometer. Standard curve was plotted using Gallic acid (7.813, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml) fig 1. The samples were prepared in triplicates for each analysis and the mean value of absorbance was obtained.

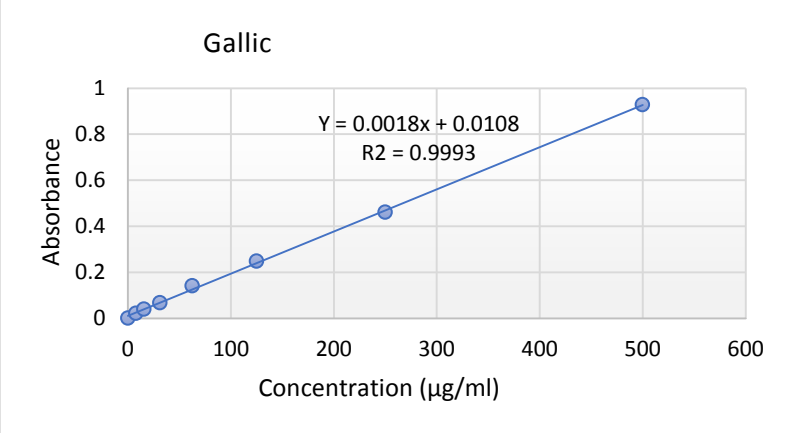

Fig 1: Gallic Acid Calibration curve

Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by aluminum chloride colorimetric assay adapted from Chatatikun *et al* [18] with slight modification. Briefly, 0.1 ml of each extract was mixed with 2.7 ml methanol, and then 0.1 ml 10% aluminum chloride-hexa hydrate, 0.1 ml 1M potassium acetate were added. Standard solution of Rutin (1mg/ml) in concentration 7.813, 15.625, 31.25, 62.5, 125, 250 and 500 µg/ml were prepared in 95% methanol. Methanol was used as reagent blank. The reaction mixture was incubated at room temperature for 40 minutes protected from light. The absorbance of the reaction mixture was measured at 415 nm. Total flavonoid contents were expressed as mg Rutin Equivalents (RE) per gr of plant extract fig 2.

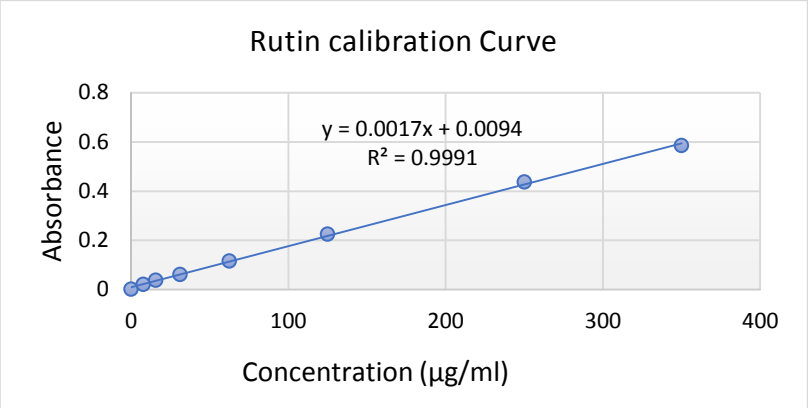

Fig 2: Rutin Calibration Curve

DPPH Radical Scavenging Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was determined using the method as described by Blois (1958) [19] with some modification. The stock solution was

prepared by dissolving 25 mg DPPH with 100 ml methanol, and then stored at -18 °C until needed. The working solution was obtained by diluting DPPH solution with methanol to obtain an absorbance of about (0.6 – 0.8) at 517 nm using the spectrophotometer. An aliquot of 0.5 ml of the fractions extract (50, 100, 200, 400, 600, 800 and 1000 µg/ml) was added to 2.5 ml of DPPH solution in a test tube. These solution mixtures were shaken vigorously. After incubation at 40 ± 1 °C for 20 minutes in the dark, the absorbance of each solution was determined at 517 nm using spectrophotometer. The blank was prepared by using methanol in place of sample/standard. The scavenging activity on the DPPH radical was expressed as inhibition percentage using the following equation:

$$\mathbf{I\ \% = [(A_B - A_S)/A_B] \times 100}$$

Where: A_B is the absorbance of the control reaction (containing all reagents except the test compound), and A_S is the absorbance of the test compound. The experiment was carried out in triplicate. Ascorbic acid was used as positive control (6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml). Results were expressed as mg sample/mL in the form of IC_{50} values were estimated from the % inhibition versus concentration sigmoidal curve, using a non-linear regression analysis.

Reducing power Test

The reducing power of the extracts was evaluated according to the protocol of [20] with some modification. Different concentration (50, 100, 200, 400 and 800 µg/ml) of extract and fractions were prepared with methanol and 0.4 ml of each taken in test tube as triplicates. To the test tubes 1 mL of sodium phosphate buffer (0.2 M, pH= 6.6) and 1 mL of 1% potassium ferric cyanide solution [$K_3Fe(CN)_6$] was added. These contents were mixed well and were

incubated at 50°C for 20 minutes. After incubation 1 mL of 10% Trichloroacetic acid was added and were kept for centrifugation at 3000 rpm for 10 minutes. After centrifugation, 2 ml of the supernatant solution was mixed with distilled water (2 ml) and 0.1% ferric chloride $FeCl_3$ (0.2 ml) solution, and was incubated at 35 ± 1 °C for 20 minutes. The absorbents measured at 700 nm. The same procedure was applied to ascorbic acid (Ascorbic acid) solutions (standard), and the blank was prepared by adding every other solution but without extract and ferric chloride, and the control was prepared by adding all other solution but without extract. Increased absorbance of the reaction mixture indicated increased reducing power. The percentage inhibition was calculated by using the following equation:

$$\% \text{ Reducing power activity} = \left[\frac{A_{sample} - A_{control}}{A_{sample}}\right] \times 100$$

where A_{sample} and $A_{control}$ are the absorbance of the sample and the control, respectively. The IC_{50} value (µg/mL) is the effective concentration giving an absorbance of 0.5 for reducing power and was obtained from non-linear regression analysis.

Statistical analysis

The experiments were carried out in triplicate and results are given as the mean ± standard deviation SD. Statistical analysis was performed and graphs were obtained using Microsoft Excel (Microsoft Inc. 2013).

Results and Discussion

Each plant has a characteristic chemical composition, with uniform phenolic groups present in the same species. Similar chemical structures may show the same chemical interactions with specific reagents during the reaction, and most of the natural antioxidants perform different functions. Therefore, a reliable antioxidant

evaluation protocol requires different assessments of antioxidant activity to account various mechanisms of action. In this study, several techniques have been used to determine the in vitro antioxidant activity to allow rapid screening of substances such as DPPH scavenging activity and reducing power assay. The fractions were also evaluated for the presence of phenolic and flavonoid content which are responsible for antioxidant activity. The extraction yields of various fractions are depicted in Table 1.

The yield produced by different solvents during fractionation indicated that it did not follow the polarity of solvents. The maximum yield 7.215 g was obtained for MEef whereas the yield of other fractions; PEef, CLef, EAef and ACef was found to be 4.174 g, 2.091 g, 3.347 g and 0.196 g, respectively.

Total phenolic content (TPC)

Phenolic content in the given plant extracts was determined by using Folin-Ciocalteu reagent. This is also called as Gallic acid equivalence method as the total phenolic amount was calculated from the equation ($y = 0.0018x + 0.0108$, $R^2 = 0.9993$) obtained from Gallic acid standard curve Fig 1 (a) and results were expressed as mg Gallic acid equivalents (GAE)/g dry extract. This method is preferred more because of its accuracy. In the present study, the methanolic extract (MEef) of the extract fraction showed comparatively high phenolic content (267.07 ± 1.28 mg GAE/G dry extract) than ethyl acetate extract fraction (EAef) (208.45 ± 1.16 mg GAE/G dry extract) respectively. It follows Acetone extract fraction (ACef) (159.94 ± 1.41 mg GAE/G dry extract), among (Clef) showed low phenolic content whereas (PEef) showed the least. The results were given in Table 1.

Table 1: Extraction yield, Total phenolic and Total flavonoid content of the plant extract fractions

Sample Extract	Extraction yield %	TPC mg of GAE/g of dry extract	TFC mg of RE/g of dry extract
PEef	4.174	9.83 ± 0.36	26.41 ± 0.58
Clef	2.091	18.47 ± 0.48	81.94 ± 0.72
EAef	3.347	208.45 ± 1.16	98.72 ± 1.18
Acef	0.196	159.94 ± 1.41	41.36 ± 0.35
MEef	7.215	267.09 ± 1.28	24.41 ± 0.78

Total flavonoid content (TFC)

Flavonoids, including flavonols, flavones and condensed tannins, are a class of plant phenolics, which contain hydroxyl groups, are responsible for the radical scavenging and chelating properties [21]. Flavonoid content in plant extracts was obtained by Aluminum chloride colorimetric method using $AlCl_3$. It involves complex formation between the aluminum ion, Al (III), and the carbonyl and hydroxyl groups of flavonoids to produce characteristic yellow color [22]. Total flavonoid content was calculated from equation ($y = 0.0017x + 0.0094$, $R^2 = 0.9991$) obtained from the standard calibration curve of Rutin Fig 2 (a). The ethyl acetate extract fraction (EAef) showed highest flavonoid content (98.72 ± 1.18 mg RE/g dry extract) against other fractions (PEef, CLef, ACef, MEef) whose flavonoid content reported as 26.41 ± 0.58, 81.94 ± 0.72, 41.36 ± 0.35 & 24.41 ± 0.78 mg RE/g of dried extract, respectively. The results were seen in Table 1.

DPPH free radical scavenging activity

Benefits of topical application of herbal compounds are influenced by biologically active components, such as antioxidants. Due to great structural diversity, the antioxidant profiles differ greatly from one plant to another. Activity of natural extracts depends on the plant compounds as well as type and polarity of the extraction solvent and the isolation procedure [23].

DPPH (2, 2-diphenyl-1-picryl-hydrazyl) is a stable, free radical that produces purple color in alcohol. This color fades in presence of antioxidant molecule as it converts into 2, 2-diphenyl-1-picryl-hydrazine. More the scavenging ability less the absorbance detected at 517 nm. This method was chosen as it is easy to perform and gives accurate results [24].

Scavenging activity of extract fractions was determined based on their DPPH neutralization. The percentage scavenging activity of various concentrations (1000, 800, 600,400, 200,100 and 50 µg/ml) of all extract fractions and ascorbic acid (400, 200, 100, 50 ,25, 12.5 and 6.25 µg/ml) determined, was found to be concentration dependent. More the scavenging activity, less is the IC_{50} value. Results of free radical scavenging activity of plant extracts are presented in Fig 3.

Table 2: DPPH IC_{50} values of the plant extract fractions and standard

Plant Extract	PEef	Clef	EAef	ACef	MEef	Asc. acid
IC_{50} µg/ml	-	-	299.68	263.35	176.12	34.76

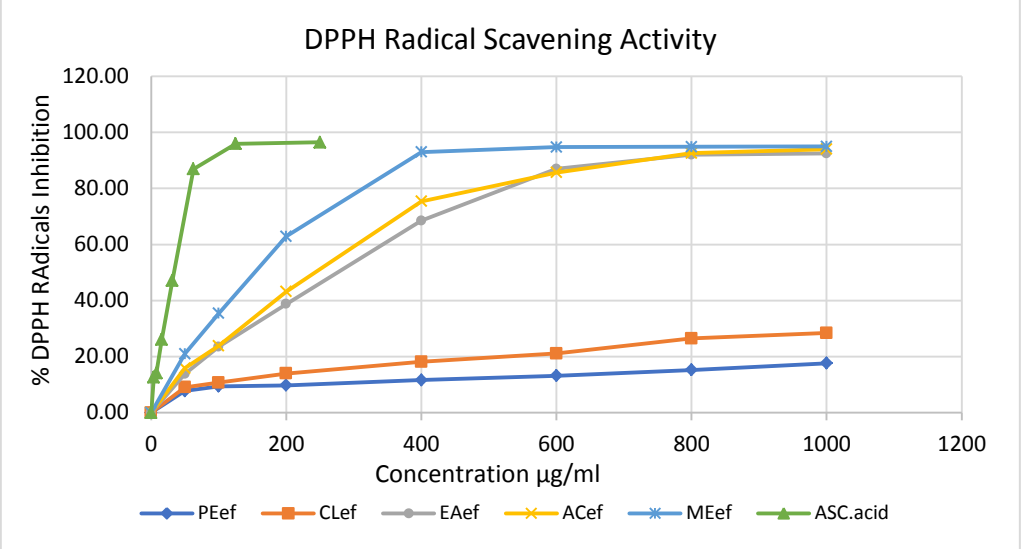

Fig. 3 Antioxidant activities of different extract fractions of *B. populneus* leaves

The examination of antioxidant activity of extract from *B. populneus* leaves showed different values. The highest antioxidant activity was observed in the methanolic extract than other extracts studied. The data obtained showed that the methanolic extract presented a high activity, while the lowest activity was shown by the leaves extract in petroleum ether. To scavenge DPPH free radicals IC_{50} values of extract fractions, were found to be EAef (299.68 µg/ml), ACef (263.35 µg/ml), MEef (176.12 µg/ml) and Ascorbic acid (34.76 µg/ml) respectively (Table 2 and fig. 4). All the fractions showed higher IC_{50} values than ascorbic acid. Due to low activity of petroleum ether and Chloroform extract fractions, IC_{50} are not calculated for them.

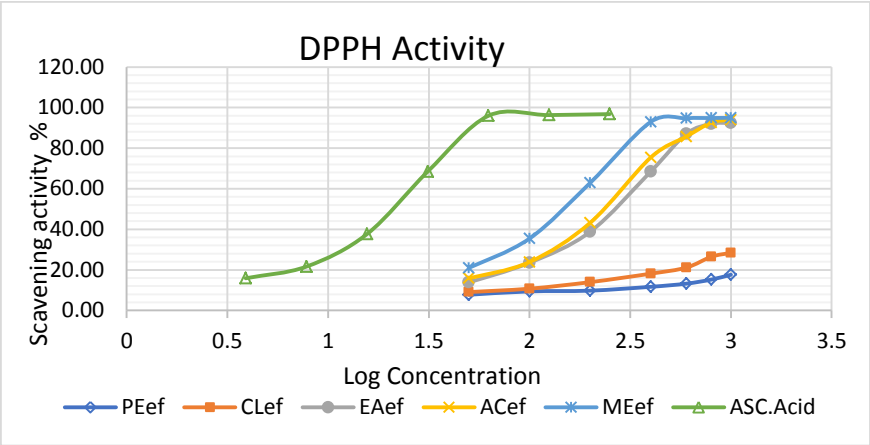

Fig. 4 non-linear regression analysis of different extract fractions of *B. populneus* leaves

In comparison to IC_{50} values of Ascorbic acid, methanolic extract fraction manifested the strongest capacity for neutralization of DPPH radicals.

Reducing Power assay

Measurement of reducing power gives antioxidant activity in the extract. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{3+}/ ferri cyanide complex used in this method to the ferrous form. By measuring the formation of Pearl's Prussian blue at 700 nm, it is possible to determine the concentration of Fe^{2+} ion [25]. The reductive capacity of EAef, ACef and MEef was compared to that of ascorbic acid in (Table 3). In order to compare of reducing ability of samples can be used a factor as IC_{50} value.

Table 3 Reducing power IC_{50} values of the plant extract fractions and standard

Plant Extract	PEef	Clef	EAef	ACef	MEef	Asc. acid
IC_{50} µg Fe^{2+}/ml	-	-	273.559	388.308	192.024	25.072

Like the antioxidant activity study, reducing power of EAef, ACef and MEef also increased with increasing concentrations of the extract fractions. The reducing power abilities of different extracts and reference, ASC.Acid are shown graphically in (Fig. 5). The absorbance of all extracts and standard is a function of their concentrations, and generally, increases linearly with the increase in concentration.

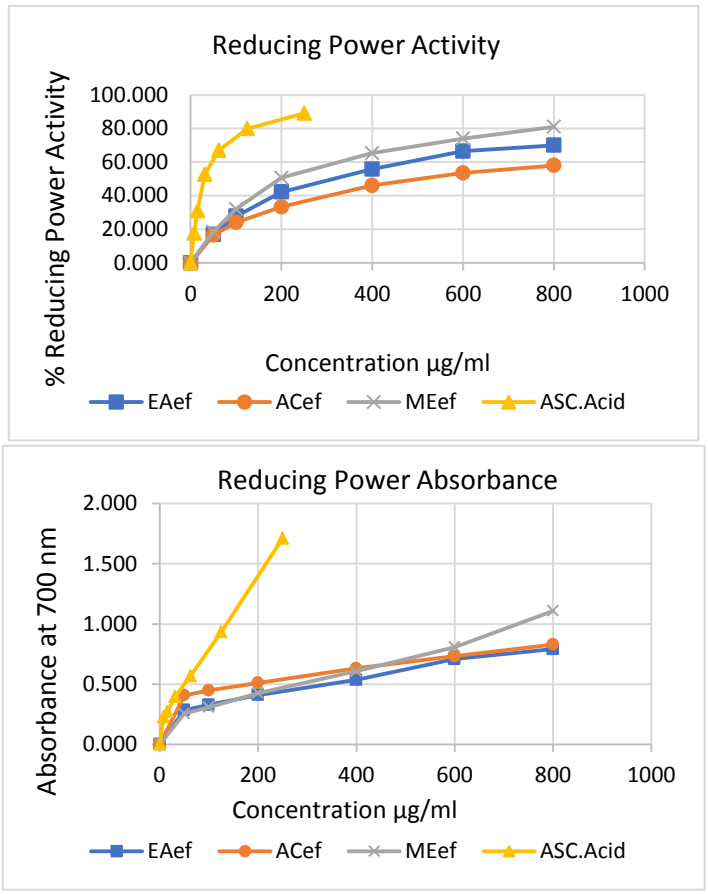

Fig. 5 Reducing power abilities of different extract fractions of *B. populneus* leaves

In the present work, different solvent extracts of *B. populneus* leaves were evaluated for their reducing power activity. Ascorbic acid was used as standard and its IC_{50} values was 25.072 µg

Fe^{2+}/ml. Methanolic extract (MEef) showed good activity with IC_{50} values of 192.024 µg Fe^{2+}/ml. Ethyl acetate extract (EAef) showed moderate activity and its IC_{50} values was 273.559 µg Fe^{2+}/ml, while Acetone extract (ACef) showed weak activity with IC_{50} values of 388.308 µg Fe^{2+}/ml.

The highest reducing power was found in Methanolic extract, followed by Ethyl acetate extract. From the results, it is plausible that, the high antioxidant activity observed in the MEef fraction due to its phenolic contents.

Many reference studies indicate a strong relationship between antioxidant activities and higher phenolics content [26], which means that phenols compounds are the main agents responsible and contribute largely in the antioxidant activities of medicinal plants [26, 27]. Moreover, the anti-radical ability of phenolic compounds is due to their capacity to trap free radicals through the transfer of the hydrogen atom then transformed into a stable molecule [28], and their reducing power is due to the presence of hydroxyl group in their structure that can serve as an electron donor [29].

The results are a contribution to the valorization of some medicinal plants from southern Syria, which have never been published. To the best of our knowledge, data on the composition of *B. populneus* and extracts are scarce. Therefore, this investigation can be evaluated as the first report about their antioxidant properties in respect to polyphenol and flavonoid content. It is extremely important to point out that there was a positive correlation between antioxidant potential and phenolic content estimated by the assays. The high content of total phenols in extract fractions and synergistic interactions might explain the strong antioxidant properties of this plant. Additional biological testing will be necessary to ascertain new and beneficial activities of these extract fractions.

Consequently, phytochemical investigations should be planned to identify and characterize active principles, and assess its benefits by laboratory assays.

Conclusions

To conclude, the polarity of the extracting solvent plays a significant role in contributing antioxidant activities from *B. populneus* leaves. The present study indicated the presence of higher polyphenol and flavonoid content assortment of *B. populneus* might be the key players in scavenging of oxidative stress inducing species. The results from this study hence can be employed as a milestone in the further investigation particularly in isolating and characterizing the active principles that contribute to antioxidant activities. The extensive study will maximize the therapeutic effects of *B. populneus* as a natural antioxidant for use in pharmaceutical, food, and cosmetic industries. Accordingly, more in-depth studies in vivo are necessary to develop these plants as potential botanical components for disease treatment.

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