

**Evaluation of antiurolithiatic and antioxidant activity of the Egyptian  
*Pluchea dioscoridis* L. leaves extracts in vitro**

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**ABSTRACT**

Bioactive compounds in medicinal plants are believed to be an important in the folk of medicine. Many efforts have been done to discover the safe medical treatment instead of chemical drugs which have a harmful effect on the human body. In the present study the crude extracts of *Pluchea dioscoridis* were tested to evaluate their bioactive compounds (total phenolic and flavonoid compounds) and their effects as antiurolithiasis of human stones (calcium triphosphate (CTP), cholesterol and calcium oxalate monohydrate (COM) besides, their antioxidant activities. The measured total phenolic compounds content in the methanol, ethyl acetate, and water extracts of *Pluchea dioscoridis* were found to be equal to 11.842, 9.047, and 8.126 mg/g, respectively. Also, the contents of the total flavonoid compounds (total phenol) were 19.018, 15.312, and 14.022mg/g for methanol, ethyl acetate, and aqueous extracts of *P.dioscoridis*, respectively. The results showed that all extracts of *P. dioscoridis* have a significant inhibitory effect on the dissolution of the three investigated types of formed stones (CTP, cholesterol, and COM). The inhibition effect was in the order methanol > ethyl acetate > water extract. Also, it was found that all extracts for *P.dioscoridis* were exhibited 2, 2, diphenyl-1-picryl hydrazil (DPPH) radical, reducing power, ABTS<sup>+</sup> radical, Nitric oxide radical scavenging activity. Methanol extract of *P. dioscoridis* exhibited the highest scavenging activity compared to ethyl acetate and water extracts.

Keywords: *Pluchea dioscoridis*, antiurolithiatic, antioxidant, flavonoids, phenolic compounds.

**INTRODUCTION**

Medicinal plants nowadays occupy a great position in agricultural and industrial production, and there is great interest in those countries that produce them. The cumulative synergistic action of a wide variety of antioxidants such as vitamins C and E and polyphenols, mainly phenolic acids and flavonoids, carotenoids, terpenoids, Maillard compounds and trace minerals were driven from the antioxidant capacity of plant foods (Jung, 2008). Herbal and natural plant extracts produce natural compounds that act as natural antioxidants

which detoxify reactive oxygen species and block their damage to cellular macromolecules and organelles through different mechanisms (Shahidi, 2000). Recently numerous studies have been carried out to understand and investigate the effect of various plant extracts on many diseases such as, anticancer, antioxidant, antimicrobial, Cytotoxic and Anti-Inflammatory and anticoagulation (Maridass and De Britto, 2008; (Aboul-Enein *et al.*, 2012; Hui-Yu *et al.*, 2013; Aboul-Enein *et al.*, 2014; El-Ghoraba *et al.*, 2015; Govindappa *et al.*, 2015 ).

The plants of *Pluchea* genus (family: Asteraceae) have been used traditionally as astringent, antipyretic, anti-inflammatory, hepatoprotective, diaphoretic in fevers, smooth muscle relaxant, nerve tonics, laxatives and for the treatment of dysentery, lumbago, leucorrhoea, dysuria, haemorrhoids, gangrenous ulcer and disorders causing cachexia (Chaturvedi and Singh, 1965; Farnsworth and Bunyapraphatsara, 1992; Khare, 2007; Kirtikar and Basu, 1975; Ahmad *et al.*, 1991). The phytochemical fractionation of plant extracts from this genus revealed the presence of phenolic compounds including flavonoids, tannins, phenolic acids, phenyl propanoids and chalcones in addition to eudesmane-type sesquiterpenoids, monoterpenes, protein, carbohydrates, lignin, glycosides and triterpenoids (Prasad *et al.*, 1966; Chiang *et al.*, 1979; Ahmad, 1987; Ahmed, 1990; Chawla *et al.*, 1991; Uchiyama *et al.*, 1991; Chakravarty and Mukhopadhyay, 1994).

*Pluchea dioscoridis* (L.) is a natural plant, belongs to family Asteraceae. In Egypt, it is localized mainly in the Nile region and distributed in the eastern desert, Mediterranean coastal stripes and Sinai (Boulos, 2002). It has a wide use in folk medicine, and it is used in popular medicine for rheumatic pains (Boulos and El-Hadidi, 1989). In the folk medicine however safer several herbal drugs were used against kidney stones (Ernst, 2006; Tawatsin *et al.*, 2001).

Urolithiasis defined as the urinary stone arise anywhere in the urinary tract. Calcium triphosphate (CTP) and calcium oxalate monohydrate (COM) are most common crystalline constituents of human urinary stones. Importance of aggregation in calculus formation is that it constitutes the most effective mechanism to increase the size of particle composition and structure of urinary stone (Prien and Prien,

1968). About 80% of gallstones are predominantly composed of cholesterol. Despite considerable progress in medical therapy, there is no satisfactory drug to treat kidney stones. Thus, most patients try to find an alternative therapy for many diseases including lithiasis (Atmani and Khan, 2000). Medicinal plants were founded renewable sources with antiurolithiatic effects. Many people in Egypt and the world are suffering from the urinary stones. In both analytical and biological solutions, calcium oxalate formation need for a quantitative study of the ionic interactions in solutions of this electrolyte. Many efforts have been done for the retardation and/or medication of calcium oxalate kidney stone formation. Different compounds were used to retard calcium oxalate and oxalate nephrotoxicity and increase the solubility of oxalate (Farooq *et al.*, 2006). Although dissolution and inhibition of calcium oxalate in presence of  $\alpha$ -ketoglutaric acid, amino acid, polyhydroxy carboxylic acid have been studied, dissolution of TCP, COM and cholesterol by solvent extracts of *Pluchea dioscoridis* still need more studies.

The present work aims to investigate the effect of different extracts of *Pluchea dioscoridis* on antiurolithiasis and antioxidant in vitro.

## MATERIALS AND METHODS

### 1- Plant collection:

Leaves of *Pluchea dioscoridis* were obtained from Banha, Egypt and were identified in the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Egypt.

### 2- Chemicals and reagents:

DPPH• (2,2, diphenyl-1-picrylhydrazil radical), Folin-Ciocalteu phenol reagent, gallic acid (3,4,5-trihydroxybenzoic), Folin-Ciocalteu and cholesterol, were obtained from Sigma

**Evaluation of antiurolithiatic and antioxidant activity of the Egyptian  
*Pluchea dioscoridis* L. leaves extracts in vitro**

Chemical Co. Ltd (St. Louis, MO, USA). Methanol, Ethyl acetate and Sodium carbonate, sodium oxalate, calcium oxalate, tri-calcium phosphate, were obtained from El Naser Pharmaceutical Co. Egypt). Streptokinase was purchased from Adwia Egyptian Co. for Chemical and pharmaceutical 10<sup>th</sup> of Ramadan City- El Sharkia, Egypt.

### 3- Preparation of Samples:

*P. dioscoridis* leaves were dried, crushed to powder. 20g of powder was dissolved in 100ml of each solvent (methanol, ethyl acetate and water) at room temperature under shaking. Residues were re-extracted twice with the same solvent under the same conditions then filtered using filter paper (Whatman No.4). The filtrate solutions were concentrated under reduced pressure by using rotary evaporator.

### 4. Determination of total phenolic content:

The total composition of phenolic compounds in leaves of *P. dioscoridis* extracts and their fractions were determined using the Folin-Ciocalteu method as described by Singleton and Rossi (1965), briefly, 0.5 ml of the extracts or fractions was added to 3 ml of distilled water and 0.25ml Folin- Ciocalteu reagent. The mixture was leaved to stand for 2 min at room temperature, then 0.75 ml of 20% Na<sub>2</sub>CO<sub>3</sub> was added to the mixture and the volume was completed to 5ml with distilled water. After 2 hours the absorbance of the prepared solutions was measured at 765 nm. The content of phenolic compounds was expressed as Gallic acid equivalents (GAE) in mg/g of sample.

### 5. Determination of total flavonoid content:

The total flavonoid compounds content of *P. dioscoridis* leave extracts were determined by using the modified colorimetric method described by Zhishen (1999). 25 mg of air dried plant material was crushed in a mortar with 10ml 80% methanol. The homogenous obtained mixture was allowed to stand for 20 min at room temperature, followed by filtration through filter G4. An aliquot of 0.4ml of filtrate was mixed with 0.6ml distilled water, 5% NaNO<sub>2</sub> solution (0.06 ml) and the mixture was allowed to stand for 5 min at room temperature. After 6 min, 10 % AlCl<sub>3</sub> solution (0.06 ml) was added to the mixture. Immediately, 1N NaOH (0.4 ml) and 0.45ml distilled water were added to the mixture and allowed to stand for another 30 min. Absorbance of the mixture was determined at 510nm and Quercetin was used as standard compound for the quantification of the total flavonoid content. All values were expressed as milligram of Quercetin equivalents per gram dry weight.

### 6. Dissolution Calcium triphosphate and cholesterol

#### I- Flame photometric studies:

Dissolution of calcium triphosphate (TCP) in methanolic, ethyl acetate and aqueous extracts of *P. dioscoridis* (0–3% v/v) was studied by measuring the calcium content in the filtrate after one week with the help of flame photometer. In these method 10 mg of calcium triphosphate was mixed with 10ml distilled water and *P. dioscoridis* extracts (0–3% v/v) have been added in separate test tubes wrapped with parafilm, stirred thoroughly and kept for one week. Calcium content in the filtrate was determined in each filtrate with the help of flame photometer (Ishwaret *al.*, 2008).

## II- Estimation of Cholesterol by UV-visible Spectrophotometer

Cholesterol concentration was evaluated by measuring optical density at  $\lambda_{\max}$  = 402 nm using UV-visible spectrophotometer (Ishwar and Smriti, 2008). Optical density was measured at different concentrations of cholesterol and calibration curve was obtained. Cholesterol concentration was estimated by measuring absorption and using the calibration curve. Dissolution kinetics of cholesterol was studied in extracts of *P. dioscoridis*.

## 7. Dissolution of calcium oxalate monohydrate (COM)

### I- Flame photometric studies:

Dissolution of calcium oxalate in an aqueous solution of *P. dioscoridis* extracts (0–3% v/v) was studied by measuring the calcium content in the filtrate after one week with the help of flame photometer. In this method 0.25mg of calcium oxalate was mixed with 10ml distilled water and *P. dioscoridis* extracts (0–3% v/v) were added in separate test tubes wrapped with parafilm, stirred thoroughly and kept for one week. Calcium content in the filtrate was determined every day with the help of flame photometer (Ishwar and Smriti, 2008).

### II Conductometric titration:

Conductometric titration of  $\text{CaCl}_2$  (0.05 M) with  $\text{Na}_2\text{C}_2\text{O}_4$  (0.05 M) was carried out in the absence and presence of *P. dioscoridis* extracts using a digital conductivity meter (VSI, India). For this purpose,  $\text{CaCl}_2$  solution was admixed with *P. dioscoridis* extracts and put overnight before the titration (Ishwar *et al.*, 2005).

## 8. Antioxidant activity assays

### I-Scavenging Activity on DPPH Radicals:

DPPH• is a stable free radical due to the delocalization of the free electron on the whole molecule. The DPPH• (Purple) reacts

with hydrogen donor produce reduced form of DPPH with yellow color

$$\text{(DPPH)} + \text{(H-A)} \rightarrow \text{DPPH-H} + \text{(A)}$$

(Purple) (Yellow)

The free radical scavenging activity of *P. dioscoridis* extracts was measured using 1,1-diphenyl-2-picrylhydrazil (DPPH•) adopting the method of Shimada *et al.* (1992). 100µg/ml of different plant extracts were screened, while the most potent extracts which showed more than 90% radical scavenging activity were assayed for other antioxidant testing assays. 0.1mM solution of DPPH• in methanol was prepared and then 1ml of this solution was added to 3ml of extract solution. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The reaction was carried out with three replicates for each extract.

DPPH scavenging effect (%) =  $[(A_0 - A_1)/A_0] \times 100$  where,  $A_0$  was the absorbance of the control reaction and  $A_1$  was the absorbance in the presence of the sample (Oktayet *al.*, 2003).

### II-Reducing power assay

The reducing power for *P. dioscoridis* extracts was determined according to the method of Oyaizu (1986); 1ml of test sample solution (20 µg/ml) was mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5ml). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5ml) was added to the mixture, and centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and a freshly prepared ferric chloride solution (0.5ml). The absorbance was measured at 700 nm. Ascorbic acid (20µg/ml) was used as standard. A blank was prepared without adding standard or test compound. Increased

## Evaluation of antiurolithiatic and antioxidant activity of the Egyptian *Pluchea dioscoridis* L. leaves extracts in vitro

absorbance of the reaction mixture indicates increase in reducing power (Khanam *et al.*, 2004). The percent increase in reducing power was calculated using the following equation;

Increase in reducing power % =  $\{ [A_{\text{test}} - A_{\text{blank}}] / A_{\text{blank}} \} \times 100$

Where A test' is absorbance of test solution;  
A blank' is absorbance of blank.

### III-ABTS<sup>•+</sup> radical scavenging activity

The method of Re *et al.* (1999) was adopted for the ABTS<sup>•+</sup> assay. The stock solutions were 7 mM/L ABTS<sup>•+</sup> solution and 2.4 mM/L potassium persulphate solution. The solution was prepared by mixing in equal quantities of the stock solutions and allowing them to react for 12–16 h in the dark. One mL of the ABTS<sup>•+</sup> solution was diluted with 60 mL of methanol. ABTS<sup>•+</sup> solution was prepared freshly for each assay. 10mL of each clove extract (10 mg extraction + 10 mL solvent) was reacted with 5 mL of ABTS<sup>•+</sup> solution for 7 min then the absorbance was recorded at 734nm. A control without extract was also analyzed. Antiradical activity was calculated as follows:

ABTS radical-scavenging activity (%) =  $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$

Where in A control is the absorbance of ABTS radical in methanol and A sample is the absorbance of ABTS<sup>•+</sup> radical with the added *P.dioscoridis* extract.

### IV-Nitric oxide radical (NO<sup>•</sup>) scavenging assay

NO<sup>•</sup> generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al.* (1994). Briefly, the reaction mixture (5.0ml)

containing SNP (5mM) in phosphatebuffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25°C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The generated NO<sup>•</sup> radicals interacted with oxygen to produce the nitrite ion (NO<sup>-</sup>) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was measured at 546nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data was presented as an average of three independent determinations.

## RESULTS AND DISCUSSION

### Total phenolic and flavonoid contents

The contents of the total phenolic compounds were 11.842, 9.047 and 8.126 mg/g in ethanol, ethyl acetate and water extracts, respectively. The flavonoid contents of *P. dioscoridis* extracts in terms of quercetin equivalent mg/g dry weight were 19.018, 16.312 and 14.022mg/g for in ethanol, ethyl acetate and water extracts, respectively. It was obvious that the concentration of phenolic content was lower than that of flavonoid in most cases.

Table 1. Total phenolic and total flavonoid contents in *P. dioscoridis* extracted of methanol, ethyl acetate and water solvents.

EXTRACT	Total phenolic(mg/g dw)	Total flavonoid(mg/g dw)
Methanol	11.842	19.018
Ethyl acetate	9.047	16.312
water	8.126	14.022

### Dissolution of CTP and COM:

#### i) Flame photometric studies:

Figure (1) Showed that dissolution of CTP increases with time which may be described as due to the coordination of  $Ca^{2+}$  ion with the constituents of the *P. dioscoridis* extracts such as carbohydrate,

and protein (Kretsinger and Nelson, 1976). This indicated that *P. dioscoridis* extracts can help in prevention the formation of kidney stones and also in treating them. Methanol extract was more effective than other extracts.

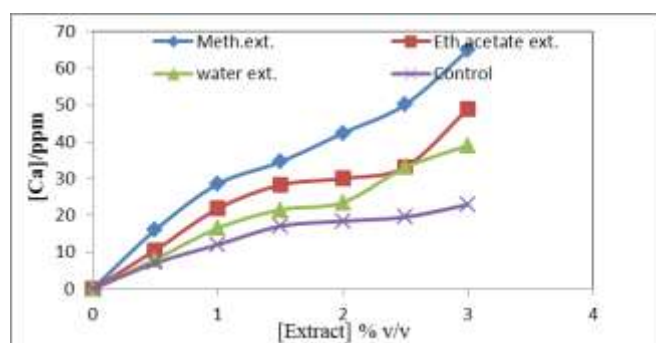


Fig. 1. Plot of calcium concentration vs volume of extracts for dissolution of tricalcium phosphate in methyl, ethyl acetate and water extracts.

### Estimation of Cholesterol by UV-visible Spectrophotometer

Cholesterol concentration was evaluated by measuring optical density at  $\lambda_{max}=402nm$  using UV-visible spectrophotometer. Optical density was measured at

different concentrations of cholesterol and calibration curve was obtained (Fig. 2). Cholesterol concentration was estimated by measuring absorption and using the calibration curve.

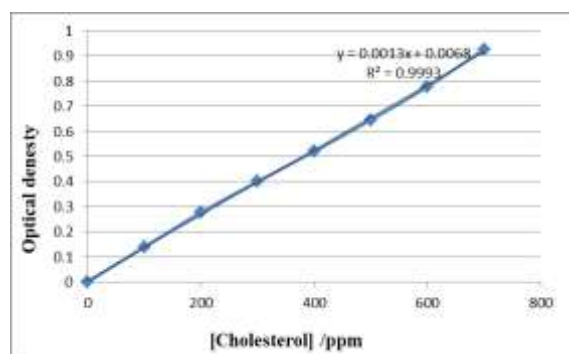


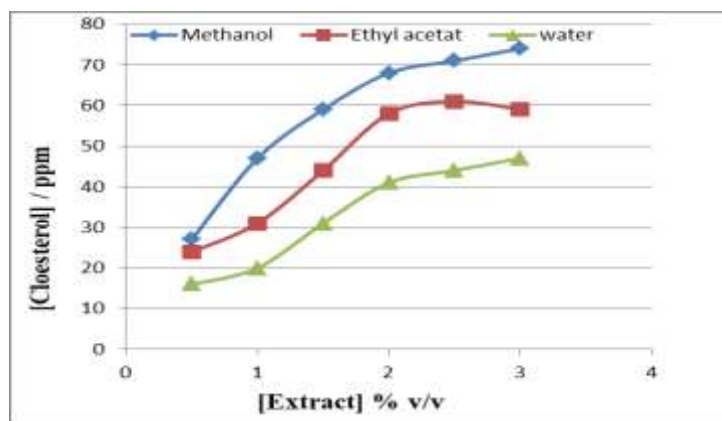
Fig. 2. Calibration curve for determination of [Cholesterol]

## Evaluation of antiurolithiatic and antioxidant activity of the Egyptian *Pluchea dioscoridis* L. leaves extracts in vitro

### Cholesterol in methanol, ethyl acetate and water extracts

Because the insolubility of cholesterol in water it is held in solution by detergent action of which it forms micelles with bile acids and phospholipids, there was no induction period in case of methanol extract, but in other cases induction period with sigmoidal curves was observed. Induction period was found to increase as: methanol < ethyl acetate < water (Fig. 3). Methanol extract is found to be most

effective than ethyl acetate for the dissolution of cholesterol. The results indicated that the major polyphenolic compounds present in *P.dioscoridis* have cholesterol-lowering activity by inhibiting and reducing solubility of cholesterol in micelles which may result in delayed cholesterol absorption. Ngamukote *et al.* (2011) found that polyphenolic compounds in grape seed extract bind bile acids may be linked to the gallic acid, catechin, and epicatechin content.



**Fig. 3.** Plots of Cholesterol vs volume of extracts for dissolution

### Dissolution of calcium oxalate monohydrate (COM)

#### i) Flame photometric studies:

Figure (4) indicated that all extracts have inhibitor effect on the dissolution of calcium oxalate monohydrate. It was obvious that the order of inhibition was as the follow; methanol > ethyl acetate > water extracts. The dissolution effect of all extracts could be attributed to the active group on the chemical constituents of

extracted compound such as  $\text{COO}^-$ ,  $\text{NH}$ ,  $\text{OCH}_3$ ,  $-\text{N}-$ ,  $\text{OH}$  groups, which acts as good inhibitors (Grases *et al.*, 1988).

On the other hand, the present result showed that the higher effect of all extract at (1.5 v/v) extract then decreased. This result was in agreement with that reported by Das *et al.* (2005) who showed that the increase of calcium content in the filtrate up to 1 v/v then decrease. Dissolution can be attributed to the phenolic compound which can produce high dissolution of COM stone.

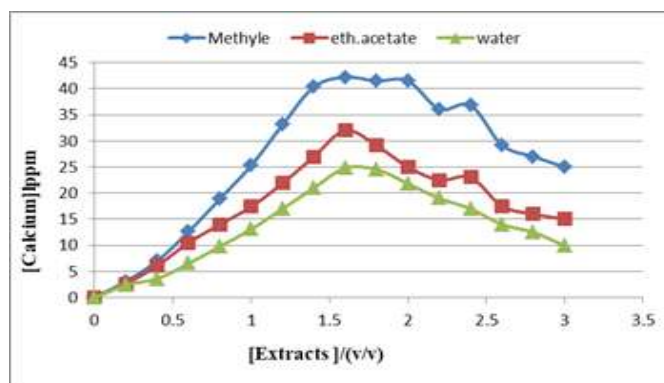


Fig.(4).Plot of calcium concentration vs. volume of extracts for dissolution of calcium oxalate monohydrate in methyl extract, ethyl acetate and water extract.

### (ii) Conductometric titration

It was clear from Figure (5) that the calcium dissolution of COM in case of using methanol extract was higher than that by using ethyl acetate and water extracts. Generally, the reduction in concentration of COM using the three investigated plant extracts may be attributed to the nature of the extracted compounds and the active groups, as well as due to the reduction in the size of calcium oxalate particle. The chemical analysis of *P. dioscoridis* indicated the presence of phenolic and flavonoids compounds in addition to protein, carbohydrates, especially disaccharides

which form complex with calcium ion.

The aqueous extract of *P. dioscoridis* contains anionic groups which adsorbed effectively on cationic sites and reducing the rate of dissolution of calcium oxalate monohydrate crystals (Nema *et al.*, 2009). As the negativity of the additive increases on the surface of COM crystal,  $\text{Ca}^{2+}$  active sites nearly completely blocked and the dissolution rate of COM crystal decrease. The high presence of  $-\text{OH}$  group in the component of *P. dioscoridis* extracts make a good inhibition for dissolution of calcium oxalate monohydrate crystals (Grases *et al.*, 1988).

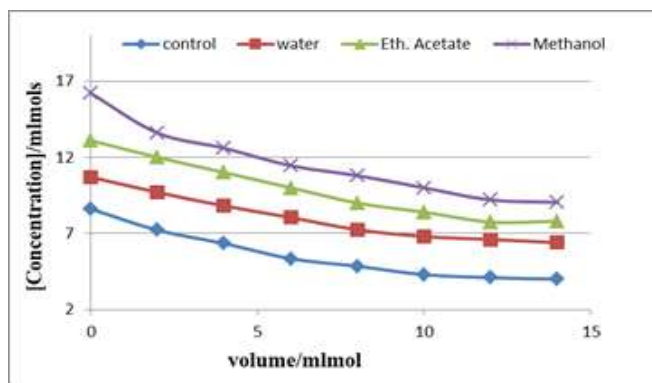


Fig. (5).Dissolution of COM in the absence and presence of *P. dioscoridis* extracts.



## Evaluation of antiurolithiatic and antioxidant activity of the Egyptian *Pluchea dioscoridis* L. leaves extracts in vitro

### Determination of antioxidant activity assays

Four methods were used to determine the antioxidant activities of *P. dioscoridis* extracts, DPPH<sup>•</sup> free radical, reducing power assay, ABTS<sup>•+</sup> radical scavenging activity and nitric oxide radical (NO<sup>•</sup>) scavenging and Hydrogen peroxide scavenging activity. The term antioxidant is used to describe any substance or compound that has activity against oxidative damage and delaying or preventing the action of free radicals, antioxidants act to protect in several ways either by directly inhibiting ROS production, preventing their spread, or destroying them (MIQUE, 2002).

#### 1. DPPH scavenging activity assay

Scavenging DPPH<sup>•</sup> radical model is the largest used to determine the free radical activities of antioxidant. In the present study the antioxidant activity of extracts from *P. dioscoridis* was determined by using DPPH free radical. The free radical scavenging activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH (Blois, 1958).

Antioxidant assay of DPPH free radical is developed based on the ability of 2, 2-diphenyl- 1-picryl-hydrazyl (DPPH), a

stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for a visible deep purple color of DPPH in alcoholic solution and the color intensity can be measured at absorbance 515nm (Kong, 2003).

Figure (6) represents the activity of the studied extracts in the inhibition of free radical, through which the values of the free radical were calculated for the extracts of methanol, ethyl acetate, water and ascorbic acid as a standard substance. It was found that all the extracts in addition to ascorbic acid inhibit the free radical DPPH<sup>•</sup>. The highest inhibition of DPPH compound occurs when using extraction with methanol solvent, and it reached 82.023, 74.123 and 64.364 for methanol, ethyl acetate and water extracts of *P. dioscoridis*, respectively at concentration 100µg/ml. But the lowest scavenging activity was found when using the solvent methanol, ethyl acetate and water and amounted to 24.36, 34.23 and 37.89µg/ml, respectively at concentration 20 µg/ml compared with scavenging activity of ascorbic acid as standard. These results are in agreement with those obtained by Ahmed *et al.* (2015) on using ethanol as a polar solvent.

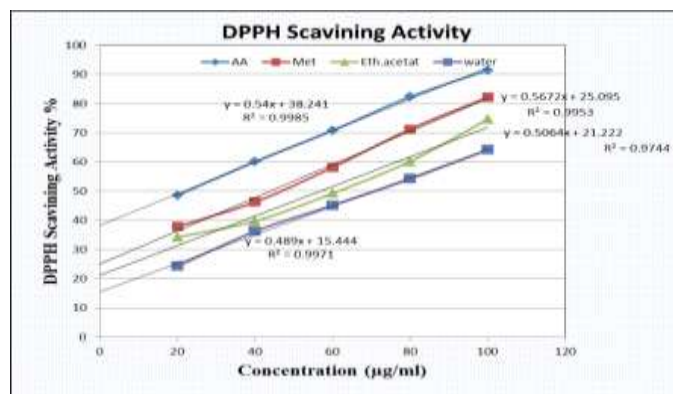


Fig. (6). DPPH scavenging activity (%) for methanol, ethyl acetate and water extracts of *P. dioscoridis*

Debouba *et al.* (2012) mentioned that phenolic acids and their esters as well as flavonoids are active antioxidants compound that combat and neutralize a large variety of free radicals including, the hydroxyl radical and singlet oxygen. Flavonoids produce their antioxidant effects by neutralizing all types of oxidizing radicals and Flavonoids can also act as powerful chain breaking antioxidant due to the electron-donating capacity of their phenolic groups. Al-Jaber (2011) found that TPC and TFC work as potent scavenger against the artificial radical DPPH and physiological radicals including

$ROO^{\cdot}$ ,  $OH^{\cdot}$  and  $O^{\cdot}$ . In a way it is directly proportional to the increase in concentration.

From the values of  $IC_{50}$  free radical inhibition DPPH $\cdot$  (Table 2), the methanol extract showed more effective in inhibiting free radical DPPH $\cdot$ . It was estimated that the  $IC_{50} = 43.91, 56.82$  and  $70.67 \mu\text{g/ml}$  for methanol, ethyl acetate and water extracts, respectively, these values remain ineffective compared to ascorbic acid, which has a value of  $IC_{50} = 21.78 \mu\text{g/ml}$ . The current results indicated that the values of  $IC_{50}$  were increased with the increase of phenols and flavonoids contents.

**Table (2).  $IC_{50}$  values of the antioxidant activity of methanol, ethyl acetate and water extracts of *P. dioscoridis* and ascorbic acid as stander against DPPH scavenging capacity.**

Extract Concentration mg/mL	Ascorbic acid $\mu\text{g/ml}$	Methanol $\mu\text{g/ml}$	Ethyl acetate $\mu\text{g/ml}$	Water $\mu\text{g/ml}$
20	48.54	37.89	34.23	24.23
40	60.11	46.25	39.73	36.22
60	70.79	58.13	49.23	45.13
80	82.32	71.24	60.21	54.2
100	91.43	82.11	74.63	64.14
$IC_{50}$	21.78	43.91	56.82	70.67

Flavonoids have a special structure that enables them to interact with all kinds of free radicals and make them stable. The hydroxyl groups on the flavonoids return the oxygen radicals by transferring hydrogen or free electron. The products (flavonoid radicals) react with each other to produce the stable quinone. In the present work there were significant differences between the antioxidant activity of methanol solvent extract compared with the ethyl acetate and water solvent extracts. The active compounds found in *P. dioscoridis* are responsible for the therapeutic effects. The oxidation and reduction properties of phenolic compounds are responsible for the anti-oxidant, anti-cancer, anti-fungal and anti-microbial activity (Aboul-Enein *et al.*, 2012; Aboul-Enein, 2014; Carlos, *et al.*,

2014) Also, many researchers showed the inhibitory ability of the extracted compounds on the DPPH free radical. Free radicals have a great relationship with the constituents of plant extracts and the antioxidant activity of these extracts which can be attached to their content of phenolic compounds, and the efficiency of these compounds as antioxidants depends on the number of hydroxyl groups linked in the aromatic ring (Debouba *et al.*, 2012) as well as its content of flavonoid and phenols (Nazirolu *et al.*, 2010).

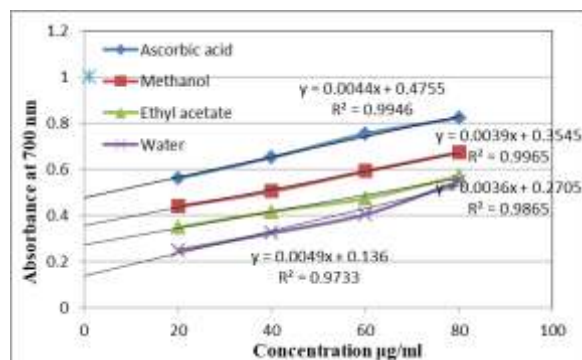
## 2- Reducing power assay

Many of antioxidant compounds, in reducing power assay convert the oxidation form of iron ( $Fe^{+3}$ ) in ferric chloride to ferrous ( $Fe^{+2}$ ). The presence of reductant

## Evaluation of antiurolithiatic and antioxidant activity of the Egyptian *Pluchea dioscoridis* L. leaves extracts in vitro

extracted from *P. dioscoridis* extracts would result in the reducing of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by donating an electron. The complexed quantities of  $\text{Fe}^{2+}$  during reaction can be measured at 700 nm. Increase in absorbance at 700 nm indicates an increase in reductive ability by measuring the formation of Perl's ability

(Nabavi *et al.*, 2008). In the present study Figure (7) shows that the highest value of reducing power is for methanolic extract which corresponds to the highest absorbance. It was found that the reducing powers of extracts also increased with the increase of sample concentrations.



**Fig. (7). Reducing power assay of (methanol, ethyl acetate and water) extracts of *P. dioscoridis*.**

In this work the results showed that there was no high difference between the ascorbic acid as standard and methanolic extract in reducing power, so the methanolic extract of *P. dioscoridis* is effective and comparable with the standard.

### 3. ABTS<sup>•+</sup> radical scavenging activity:

ABTS free radicals are one of the most commonly used methods to evaluate antioxidant activity. ABTS free radicals become stable by accepting a hydrogen ion from the antioxidant, losing their blue color. On the other hand, in the ABTS assay as well as in the DPPH assay, when antioxidant activity occurs, the ability to remove the hydroxyl radicals or superoxide radicals through physiologic action or oxidation is evaluated with a high index indicating a strong antioxidant activity. ABTS radical-scavenging through antioxidant activity is well known to be attributable to their hydrogen-donating ability (Kwang *et al.*,

2015). The results of ABTS scavenging activity, which is expressed as the extract concentration providing 50% inhibition of ABTS scavenging activity ( $\text{IC}_{50}$ ), and it was found that the highest activity value of ( $\text{IC}_{50}$ ) = 39.95 µg/ml for ascorbic acid, followed by 56.18, 71.60 and 104.6 µg/ml for methanol, ethyl acetate and water extracts, respectively (Table 2).

The results of ABTS<sup>•+</sup> radical scavenging activity (Fig. 8) showed a wide range of ABTS<sup>•+</sup> scavenging activities from 18.23% to 82.34%. The highest radical scavenging activity was recorded for methanolic extract, followed by ethyl acetate and water extracts with respective values of 69.64, 60.23 and 47.13.5% compared with 82.34% for ascorbic acid. ABTS<sup>•+</sup> + protonated radical, has an absorbance maximum at 734 nm which decreased with the quenching of proton radicals (Mathew and Abraham, 2006).

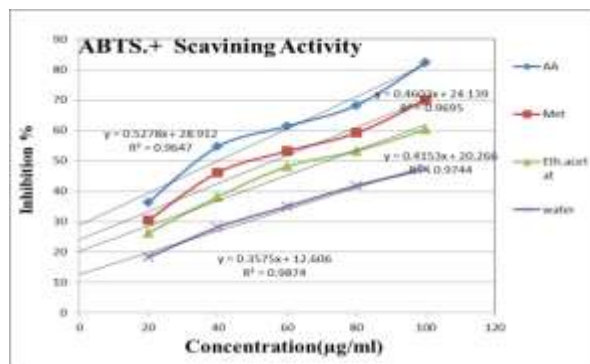


Fig. (8). ABTS+ scavenging activity (%) for methanol, ethyl acetate and water extracts of *P. dioscoridis*.

#### 4-Nitric oxide free radical scavenging activity

Nitric oxide (NO) is a potent wide range inhibitor of physiological processes such as regulation of cell mediated toxicity, neuronal signaling and inhibition of platelet aggregation. Nitric Oxide (NO) scavenging assay is based on the scavenging ability of the extracts as well as ascorbic acid, which is used as standard. In this study the maximum inhibition of NO was observed in the extracts of highest concentration (100µg/ml). The methanol, ethyl acetate and water extracts showed the maximum activity of 86.24, 70.41 and 63.43µg/ml, respectively

at (100 µg/ml), while it was found to be 94.34 µg/ml for ascorbic acid as stander at the same concentration as show in Figure (9). The IC<sub>50</sub> values were found to be 51.22µg/ml, 56.23µg/ml and 84.31µg/ml for methanole, ethyl acetate and water extracts, respectively, while it was 0.053µg/ml for ascorbic acid (Table 2). The methanole, ethyl acetate and water extracts of *P. dioscoridis* exhibited significant antioxidant activity but less than ascorbic acid. The results are compared with ascorbic acid and the activity may be related to the phenolic contents and flavonoids in this plant extract.

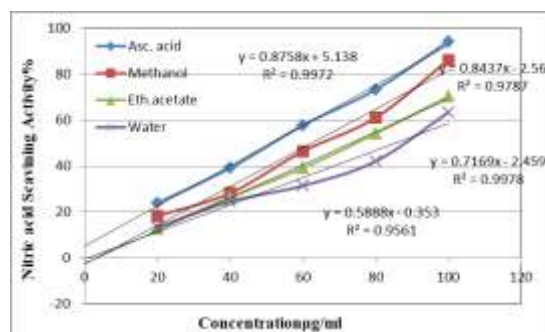


Fig. (9). Nitric oxide free radical scavenging activity (%) for methanol, ethyl acetate and water extracts of *P. dioscoridis*.

**Evaluation of antiurolithiatic and antioxidant activity of the Egyptian  
*Pluchea dioscoridis* L. leaves extracts in vitro**

### Conclusion

The idea of this work aims to evaluate the ability of different extracts of the Egyptian *Pluchea dioscoridis* L. on the inhibition crystal growth of tri calcium phosphate, calcium oxalate mono hydrate crystals, cholesterol and antioxidant. The experimental result showed that all *P. dioscoridis* extracts has significant effect on the inhibition of tri-calcium phosphate, calcium oxalate monohydrate and cholesterol. On the other hand, all extract have high effect on the elimination of free radical in all methods in these article. The result showed the high effect for methanol extract due to its high polarity compared to ethyl acetate and water extracts.

The possible economic utilization of this plant calls for information on its autoecology, which would include phytosociological relations with associated species and the prevailing environmental conditions, and the explanation of such behavior by laboratory experiments. Information on its reproductive capacity and population attributes (e.g. size structure, survival, natality and mortality) enables us to explain its ecological and sociological behavior. Therefore it is recommended to carry out extensive phytochemical and pharmacological studies on *P. dioscoridis* in *vivo*.

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**Evaluation of antiurolithiatic and antioxidant activity of the Egyptian  
*Pluchea dioscoridis* L. leaves extracts in vitro**

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## Evaluation of antiurolithiatic and antioxidant activity of the Egyptian *Pluchea dioscoridis* L. leaves extracts in vitro

تقييم النشاط المضادة للتحصى (حصوات الكلى) ومضادات الأكسدة لمستخلصات اوراق نبات البرنوف المصرى  
*Pluchea dioscoridis* في المختبر

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### المستخلص

يعتقد أن المركبات النشطة بيولوجيا في النباتات الطبية مهمة في الطب الشعبي. لقد تم بذل العديد من الجهود لاكتشاف العلاج الطبي الآمن بدلاً من الأدوية الكيميائية التي لها تأثير ضار على الجسم. تم اختبار [مستخلصات الميثانول وأسيئات الأيثيل والماء للبرنوف *Pluchea dioscoridis* وذلك لتقدير المركبات النشطة بيولوجيا (مركبات الفينول والفلافونويد الكلية) وتأثيرها على تكوين حصوات الكلى (الكالسيوم ثلاثي فوسفات (CTP) والكوليسترول وأكسالات الكالسيوم أحادي الهيدرات (COM) ، والنشاط المضاد للأكسدة . وقد وجد أن المحتوى الكلي للمركبات الفينولية في المستخلصات يساوي (11.842 ملجم/جم ، 9.047 ملجم/جم ، 8.126 ملجم/جم) في مستخلصات الميثانول ، أسيئات الإيثيل، ومستخلصات الماء للبرنوف على التوالي . وفيما يتعلق بالمركبات الفلافونيدية الكلية ، أظهرت ا لنتائج أن إجمالي الفينول الموجود ( 19.018 مجم/جم ، 15.312 مجم/جم ، 14.022 مجم/جم) للميثانول ، أسيئات الإيثيل والمستخلصات المائية للبرنوف على التوالي . أظهرت النتائج أن كل المستخلصات للبرنوف لها تأثير مثبت كبير على إذابة ثلاثي فوسفات الكالسيوم (CTP) والكوليسترول وأكسالات الكالسيوم أحادي الماء. وقد وجد من النتائج أن معدل تثبيط حصوات الكلى الكوليسترول والمستخلص الميثانول اعلى من الأيثيل اسيئات والماء على التوالي. كما وجد أن جميع مستخلصات البرنوف *P.dioscoridis* لها تأثير مضاد للشقوق الحرة لجذور DPPH ، وقدرة لاخترال ABTS بلاضافة الى نشاطها في مهاجمة شقوق اوكسيد النيتريك. أظهر مستخلص الميثانول من *P. doscoridis* أعلى نشاط مضادة للأكسدة مقارنة لاسيئات الأيثيل والمستخلص المائي.