

## PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT EFFECTS OF DIFFERENT SOLVENT EXTRACTS OF *PTEROCEPHALUS NESTORIANUS* NAB. GROWING IN KURDISTAN REGION-IRAQ

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Received: Sep. 2017 / Accepted: Mar., 2018 / Published: Mar., 2018

<https://doi.org/10.25271/2018.6.1.439>

### ABSTRACT:

The medicinal plants have been used as a herbal tradition in Kurdistan. Most of the medicinal plants are still not investigated from a phytochemical and biological activity. Therefore, different solvent extraction carried out to study phytochemicals and antioxidant activity of *Pterocephalus nestorianus* Nab. Through this research, it is reported for the first time that the phytochemicals and antioxidant activity on *P. nestorianus* Nab. collected at Safeen Mountain in Erbil-Iraq, which was known as a Lawa in Kurdistan. It is used against inflammation and oral diseases. The phytochemical study of four extracts (Hexane, ethyl acetate, methanol and methanol 70%) of *P. nestorianus* flowers, leaves and roots revealed that it contains rich phytochemical composition. C<sub>4</sub>H<sub>8</sub>O<sub>2</sub> and CH<sub>3</sub>OH extracts of *P. nestorianus* showed a high radical scavenging activity compared with trihydroxy benzoic acid as a standard. The flavonoid and phenolic presence showed strong correlation with the antioxidant activity.

**KEY WORDS:** Phytochemicals; Medicinal Plant; Flavonoids; Phenols; *Pterocephalus Nestorianus* Nab.

### 1. INTRODUCTION

The medicinal plants have been used as a centennial tradition in the Kurdistan Region. Most of the medicinal plants are still not investigated from a phytochemical and biological activity (Shahbaz, 2010). Phytochemicals are active compounds of plant origin. They are naturally synthesized in parts of the plant. For example, flowers, leaves, roots, fruits, pods, bark, stems, seeds, etc., any part of the plant body contain components of such phytochemicals (Tiwari et al., 2011). The quality and amount of phytochemicals contained in plant parts may differ from one part to another. In fact, there is a shortage of knowledge on the distribution of the pharmacological activity in different medicinal plant parts particularly associated with the distinction within the distribution of bioactive compounds in some plant elements than in others (Lahlou, 2004). The medicinal plants include different types of herbs used in phytotherapy and some of these plants have biological effects. Herbal medicines have a promising future due to the fact that there are 0.5 million plants round the world, and most of their medical activities have not been investigated so far, and their medical activities can be crucial in treatment of many diseases (Rasool, 2012). Polyphenolic compounds are one among the larger teams of phytochemicals contained within the plant kingdom. They are usually found in each non-edible and edible plant and are known to possess multiple biological effects as well as anti-radical activity (Kahkonen et al., 1999).

Flavonoids are a class of phytochemical that possesses a wide range of pharmacological activities. Flavonoids present an

important group and high potential of antioxidant, anticarcinogens and antimutagens (Edenharder and Tang, 1997). Antioxidants have been discovered in herbal medicine materials and supplements. Because of their natural source, the antioxidants obtained from plants have a greater advantage compared to synthetic drugs (Rohman et al., 2010). The use of natural antioxidants from healthful plants does not induce undesirable result, whereas artificial antioxidants were found to possess a genotoxic result (Chen et al., 1992; Kahl et al., 1993)

*Pterocephalus nestorianus* Nab. in Kurdistan region are known as "Lawa". This plant grows in sunny, dry, rock crevices, for the most part in Western Asia (Turkey, Iran), and it is used in Kurdistan for treating inflammation and oral diseases. *Pterocephalus* species square measure, indeed, are widely used as ornamental plants and in many of popular recipes medicines, all around the world (Kawther, 2007; Ghahreman, 1995; Vahedi et al., 2011).

The basic aim of the present study is to present quantitative and qualitative study of phytochemical screening of varied extracts of the Kurdish medicinal plant *P. nestorianus*, using spectrophotometric method, and also the analysis of antioxidant effect of plant extracts using a 2,2-diphenyl-1-picrylhydrazyl method.

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## 2. MATERIAL AND METHODS

### 2.1 Collection of Plant Material

The plant parts of *P. nestorianus* were collected in a flowering stage on Safeen Mountain, Erbil, Kurdistan Region of Iraq, in mid June 2014. The plant sample was classified and identified by Professor Abdul Hussain Al Khayat and Abdullah Saeed, botanists at the University of Salahaddin. voucher specimen plant (No. 6253) is deposited at the Salahuddin University Herbarium (SUH).

### 2.2 Preparation of Extracts

Freshly collected roots, leaves and flowers were separately dried in the shade at 25°C for one week, and then ground, using an electrical mill grinding strand mill. The sequential extraction was carried out by maceration method. In the extraction process, a set of solvents were used, according to an increasing polarity gradient. The procedure was performed in three different times. The powder (100 g) for each part was separately soaking in an n-hexane (3×400 ml) with occasional shaking in an ultrasonic bath for half an hour, then left at room temperature for 24 hrs. The mixture was filtered and the solvent was removed under vacuum (reduced pressure). Subsequently, the biomass was then macerated in EtOAc (3×450 ml), at room temperature for 24 hrs, with occasional stirring in an ultrasonic bath for half an hour. The combined EtOAc extracts were filtrated and evaporate under vacuum. The fraction insoluble in EtOAc was then taken in MeOH (3×500), at room temperature for 24 hrs, with occasional stirring in an ultrasonic bath for 30 min. Finally, the remaining constituents were extracted with 3×500 ml of MeOH: H<sub>2</sub>O (70:30). The obtained crude extracts were stored at -20°C, until further analysis.

### 2.3 Phytochemical analysis

**2.3.1 Qualitative phytochemical analysis:** Chemical tests were applied on the C<sub>6</sub>H<sub>14</sub>, C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>, CH<sub>3</sub>OH and hydro CH<sub>3</sub>OH 70% extracts of *P. nestorianus* using the standard procedure to quantify the compositions are described in different studies (Trease and Evans, 1989; Sofowora, 1993; Herborne, 1973; Raman, 2006).

- **Test for terpenoids (Salkawki's test)**

The extracts (0.2 g) were separately dissolved in 2ml of CHCl<sub>3</sub>. Then, 2 ml of conc. H<sub>2</sub>SO<sub>4</sub> was added to this solution and heated for 2 min. The grayish color indicates the presence of terpenoid compounds.

- **Test for flavanoids (Alkaline reagent)**

The extracts (10 mg) were severally treated with five drops of 2% NaOH resolution. Formation of yellow color, that becomes colorless on the addition of dilute HCl, indicates the presence of flavonoids compounds.

- **Test for alkaloids (Dragendorff's reagent)**

The extracts (50 mg) were separately stirred with a 3 ml of dilute HCl and filtered. 1 ml of dragon droff's reagent was added to the filtrate. A prominent reddish brown precipitate indicated the positive test.

- **Test for phenols (Ferric chloride test)**

Fifty mg of each sample extract was separately in 4 ml of distilled H<sub>2</sub>O, and 4 drops of neutral five percent ferric trichloride solution was added. Green color indicates the presence of phenols.

- **Test for Tannins (Gelatin test)**

The extracts (1 mg) were separately dissolved in 1 ml of distilled H<sub>2</sub>O and treated with two milliliter of gelatin solution containing 1% of NaCl. White precipitate colour indicates the presence of tannins.

- **Test for glycosides (Molish's test)**

Five drops of Molish's reagent were added to a two milliliter of the various extracts (10 mg/5 ml) individually. After that, 2 ml of conc. sulfuric acid was added to the down side of the test tube. The solution was then allowed to stand for 2-3 minutes. The appearance of a red colour at the interface of the two layers was indicated the presence of glycosidic compounds.

- **Test for saponins (Foam test)**

The extracts (1 g) were separately added to 5ml of distilled H<sub>2</sub>O in a test tube and then shaken vigorously. The formation of stable foam is an indication of positive aspect for the saponine.

- **Test for steroids (Liebermann Burchard test)**

Two milliliters of acetic anhydride was added to 0.5 g of each sample extract, and then 2 ml of H<sub>2</sub>SO<sub>4</sub> was added. The color converted from violet to blue or green, indicating the presence of steroid compounds.

### 2.3.2 Quantitative phytochemical screening

- **Total phenol content (TPC)**

Chlorophylls and pigments were completely removed from EtOAc and MeOH extracts by filtration of residue, which was divided into batches of 1 g each, dissolved in 50 ml of MeOH-H<sub>2</sub>O 90:10, and passed through an SPE column, fitted with RP-18 phase (10 g). The elution was made with 50 ml of MeOH-H<sub>2</sub>O, 90:10, and then with 100 ml of Me<sub>2</sub>CO before the next separation (Otto, 2008).

The total polyphenolic contents of roots, leaves and flowers of *P. nestorianus* were analysed. 0.3 ml (1mg/ml in 10% EtOH with distilled water) of the extracts, standard and blank were separately taken in a test tube and made up to a volume of 6 ml of distilled water. Then 0.5 ml of folin ciocalteau reagent was added. The mixture was stirred and left to react for 3 minutes, and then 1.5ml of sodium carbonate (20% w/v in H<sub>2</sub>O) was added, and the volume was made up to ten ml with H<sub>2</sub>O. The test tube was left in the dark place for two hours at 25°C. The absorbance was calculated at 760 nm. Gallic acid was used as standard (1 mg/ml in MeOH). All the tests were applied in duplicate. The concentration of the total phenols was calculated from the standard calibration plot and expressed as gallic acid equivalent (mg/g of sample), (Malik and Singh, 1980)

- **Total flavonoids content (TFC)**

The quantitative detection of the total flavonoids of flowers, leaves and roots of *P. nestorianus* was analyzed. 0.5ml (1 mg/ml in 80% CH<sub>3</sub>OH with distilled water) of the chlorophyll-free extracts added to a test tube. Then, 0.5ml of the CH<sub>3</sub>COOH (60%, v/v), 2 ml of the pyridine solution (20%, v/v in CH<sub>3</sub>OH), 1ml of the reagent aluminum chloride solution (5% w/v in CH<sub>3</sub>OH) and 6 ml of 80% CH<sub>3</sub>OH were added. The samples were left at 25°C for thirty minutes. The spectrophotometer was fixed to a wavelength (λ) of 420nm, and also the instrumentality was washed with H<sub>2</sub>O. All the tests were applied in duplicate. The concentration of total flavonoid content within the samples was calculated from the standardization plot and expressed as mg isoquercetin equivalent/gram of extract (Peixoto et al., 2008).

## 2.4 Evaluation of antioxidant activity

The antioxidant of each extract was analysed by using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH), method. Three milliliter of extract (2 mg/ml in CH<sub>3</sub>OH) was added to 1 ml of DPPH solution (0.2 mM in methanol) as the free radical source. Gallic acid was used as a standard (2 mg/ml in CH<sub>3</sub>OH). The mixture was shaken and kept for thirty minutes. The absorbance was calculated at 517 nm using a UV-Visible Spectrophotometer. The higher antioxidant activity was indicated by the lower absorbance of the reaction mixture. The DPPH antioxidant activity was measured using the following formula: RSA (%) = 100\* [1- (A1 /A0)], where A0 is the absorbance of blank, and A1 is the absorbance of the sample or standard (Sabrin *et al.*, 2015)

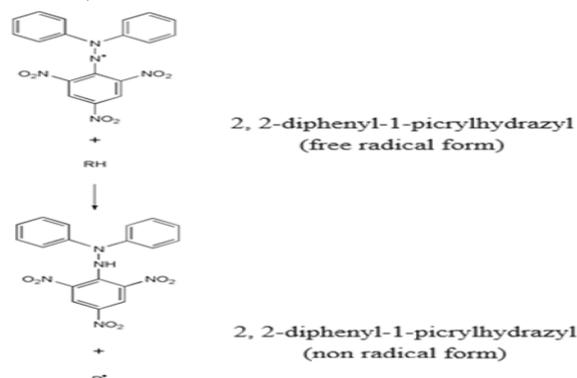


Figure 1. Structure of DPPH and its reduction form by the antioxidant RH.

## 3. RESULTS AND DISCUSSION

We report, for the first time, the screening of phytochemicals and antioxidant activity of roots, leaves and flowers from *P. nestorianus*. Eight phytochemical were screened; six of them existed in various solvent extracts. They are saponins, glycosides, terpenoids, phenols, flavonoids, steroids (Table 1). These phytochemicals may be responsible for the medicinal value of *P. nestorianus*. However, alkaloids and tannins were not existed in all extracts. The logic in using various solvent when screening for secondary metabolisms in medicinal plant extract was clearly determined in this investigation. For instance, the non-polar compounds were present in n-hexane extracts, but absent in polar solvents. This corroborates the reports (Solomon *et al.*, 2013). The presence of flavonoids has medicinal applications such as: antibacterial, cytotoxic antitumor, anti-allergic, antiviral, treatment of neurodegenerative illness, anti-inflammatory (Sandhar *et al.*, 2011). Furthermore, flavonoid compounds are known to prevent lipid-peroxidation, cyclo-oxygenase and lipoxygenase enzyme activities (Cook and Samman, 1996). They exert these effects as free radical scavengers, divalent cation chelators (Sandhar *et al.*, 2011). The phenolic compounds are one of the largest groups of the plant secondary metabolites. The antioxidant properties of plant extract which are present in phenolic compounds (Brown and Rice-Evans, 1998) that acts as primary free radical scavengers or antioxidants. Many medicinal plants were found in this type of phytochemicals.

Table 1: Phytochemical study of various solvent extracts of flowers, leaves and roots from *P. nestorianus*.

Phytochemicals	Flowers extract				Leaves extract				Root extract			
	Hex	Eth	Me	Me70%	Hex	Eth	Me	Me70%	Hex	Eth	Me	Me 70%
Saponins (Foam method)	-Ve	-Ve	-Ve	+Ve	-Ve	+Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve
Tannins (Gelatin method)	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve
Glycosides (Molish's method)	+Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve
Terpenoids (Salkawki's method)	+Ve	+Ve	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve	+Ve	+Ve	+Ve	+Ve
Phenols (Ferric chloride method)	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve
Flavanoids (Alkaline reagent method)	-Ve	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve	+Ve	-Ve	-Ve	-Ve	-Ve
Steroids (Liebermann Burchard method)	+Ve	+Ve	+Ve	-Ve	+Ve	+Ve	-Ve	-Ve	+Ve	+Ve	+Ve	+Ve
Alkaloids (Dragendorff's reagent method)	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve	-Ve

Hex: Hexane, Eth: Ethyl acetate, Me: Methanol, Me 70%: methanol: H<sub>2</sub>O (70:30), +Ve: present, -Ve: absent

Also, the present investigation revealed that the leaves and flowers of *Pteroccephalus nestorianus* contain significant amount of TPC and TFC (Table 2). The TPC values were found ranging from 200 to 61 mg. The maximum quantity contained in ethyl acetate flowers extract, while the minimum quantity was present in the ethyl acetate roots extract of the phenolic compounds. The TFC of flowers, leaves and roots from ethyl acetate and methanolic extract were found between 8 to 80 mg isoquercetin equivalent / g of extract. The flavonoid content of

the flowers was high compared to the roots and the leaves. The secondary metabolites (phytochemicals) and other chemical are constituents of *P. nestorianus* account for their medicinal value. For instance, saponins are cardiodepressant and hypotensive (Olaleye, 2007). The plant samples also contained steroids which have been reported to have antibiotic activity (Raquel, 2007) and they are very useful compounds which are related to sex hormones (Okwu, 2001). Furthermore, Glycosides are known to reduce the blood pressure (Addy and

Nyarko, 1990). The terpenoids are a large group of natural compounds in plants. Many of them have various biological

activities, e.g. antiviral, virostatic, antitumor, antibacterial, anti-inflammatory and others (Petr, et al., 2006).

Table 2. Quantitative evaluation of total phenols content and total flavonoids content in flower, leaves and roots extract of *p. nestorianus*

Extracts	TPC	TFC
	mg gallic acid equivalent/g	mg isoquercetin equivalent/g
Ethyl acetate extract (F)	200 ± 13	80 ± 4
Ethyl acetate extract (L)	104 ± 3	13 ± 2
Ethyl acetate extract (R)	61 ± 3	22 ± 9
Methanolic extract (F)	156 ± 11	73 ± 1
Methanolic extract (L)	130 ± 6	39 ± 3
Methanolic extract (R)	78 ± 6	8 ± 3

Data represents mean ± SD. Each experiment was executed in duplicate; F: flowers, L: leaves, R: roots.

On the other hand, in the present study antioxidant was performed with flowers, leaves and roots and revealed that, the methanol and ethyl acetate extracts of *P. nestorianus* exhibited strong antioxidant activity. The extracts of flowers and leaves are stronger antioxidant than ethyl acetate extracts of roots, appeared to be as potent as gallic acid with the maximum inhibition around (87%), except ethyl acetate roots extract shows lower antioxidant activity (29.6%), (Table 3) which compared to (89.9%) gallic acid. Their antioxidant effects were related which contained significant amounts of flavanoids and phenolic compounds (Breyera et al., 2007)

Table 3: DPPH antioxidant activity of methanol and ethyl acetate extracts from flowers, leaves and roots of *Pterocephalus nestorianus*.

Extracts	Nab.	
	DPPH FRS activity%	RSD%
Eth. flowers	87.3	1.0
Eth. leaves	87.0	2.6
Eth. roots	29.6	1.9
Me flowers	87.5	2.8
Me leaves	88.2	2.0
Me roots	87.6	1.9
Gallic acid	89.9	2.4

Each value represents the relative standard deviation (RSD%) (n = 3); F: flowers, L: leaves, R: roots; FRS: free radical scavenging; Eth: ethyl acetate; Me: Methanol.

The inhibitor compositions have chemical element donating category (RH) like phenolic and flavonoid compounds because of the formation of non-radical DPPH-H form. The reaction that takes place is the formation of radical and decreased form of DPPH, (Figure 1). The formed free radical can undergo further reactions which manages the amount of the molecules of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) reduced by one molecule of the reductant.

The phytochemical screening tests and antioxidant activity are beneficial in the identification of the phytochemicals and may lead to the new medicine and modification of the structure. Further, these tests facilitate their isolation and identification of pharmacologically active chemical compounds.

#### 4. CONCLUSION

The phytochemical investigation and antioxidant activity of *p. nestorianus* is significant for us in determining new sources of therapy and pharmacology. The present study was to evaluate the standing of phytochemical properties and inhibitor activity, particularly in flowers and leaves of *P. nestorianus* to enhance the health standing of individuals. The results indicate that the plant extracts might become a crucial supply of phytochemicals with a high inhibitor activity.

#### 5. ACKNOWLEDGMENTS

This investigation was supported by the Salahaddin University-Erbil/Iraq, the Pavia University-Pavia/Italy, and Gasha Medical Institute-Erbil/Iraq. The Authors are grateful to Michael Smith (University of Oxford, UK) for the plant identification.

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