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PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC, ANTIOXIDANT ACTIVITY, METALS AND MINERAL CONTENTS IN SOME PARTS OF *PLANTAGO ALBICANS* GROWN IN LIBYA

Salem Abdrba Mahmoud, Dr. Hamad M. Hasan* and Noura A. Abdul Salam Mohammed

Chemistry Depart, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda, Libya.

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***Corresponding Author**
Dr. Hamad M. Hasan
Chemistry Depart, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda, Libya.

ABSTRACT

This study aimed to estimate the phytochemical screening, phenolic content, antioxidant activity and minerals content of leafs and green parts *Plantago albicans* which were collected from Deryanah region during spring seasonof 2020. The results showed that the tannins were present in high amount in the ethanolic extracts of leafs and green parts, flavonoids was present in high amount in the ethanolic extracts of green parts, anthroquinones was present in high amount in the ethanolic extracts of green parts. The data indicated that the highest amount of total phenolic was in green part (16.1 ppm) while on the leafs was 10.4 ppm), the same trend happened in the case of antioxidant activity determination which had been determined by The FRAP (Ferric Reducing Ability Power) assay. The concentrations of some elements of the studied plants were fluctuated. Sodium and potassium were found in high percent, where the other elements (iron, copper, nickel and calcium) were exist in low percent.

INTRODUCTION

Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to be harmful to humans (Schulz *et al*., 2001). These plants are either wild plant species‖ those growing spontaneously in self-maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human actions or the contrasting Domesticated plants species those that have a risen through human actions such as selection or breeding and

depend on management for their existence (Calixto, 2000). Medicinal plants are widely used all over the World as folk medicine because they still are the most effective and cheapest alternative sources of drugs (Pretorius *et al*., 2001) They produce a wide array of compounds (flavonoids, alkaloids, phenols and tannins), most of which are used in plant defense against predators. These compounds of natural origin have been a source of innumerable therapeutic agents with antibacterial, antiviral, antioxidant, antiulcer, anti- inflammatory and anticancer activities antioxidant, antiulcer, anti-inflammatory and anticancer activities. In recent decades, there has been much interest inantioxidant and antimicrobial activities of medicinal plants as the tool in discovering new biologically active molecules. Antioxidants have the capacity for scavenging free radicals, which can damage several proteins and DNA, leading togenomic instability and cancer (Laviano *et al.*, 2007; Ozben, 2007). Majority of the people living in the developing world are struggling to increase the standard of living and to improve the health care delivery in the face of increasing poverty and growing population. According to WHO survey, 80%of populations living in the developing countries merely exclusively on traditional medicine for their primary health care needs of which most involve the use of plant extracts (Sandhya, 2006).

North Africa includes Algeria, Egypt, Libya, Morocco, Mauritania and Tunisia. The region consists of the biota of semi-closed Mediterranean and Red sea, with diverse ecosystems constituting About 10,000 vascular plant species (Hejazy, 2000), It has arid, Semi-arid and a range of sub- climatic zones, The Mediterranean basin is one of the 25 internationally recognized biodiversity hot spots in the world and it has extraordinary plant diversity and species endemism. Morocco has the highest rate of species endemism in the region (WCMC, 1992). About 70% of plant species found in thewild have medicinal, aromatic and other uses, Over 10% of these have the potential for commercial exploitation as a source of drugs and pharmaceuticals (WWF and IUCN, 1994; UNESCO/UCO, 1998**).** Libya is a country in the Maghreb region of North Africa, it has an area of 176,0000 square kilometres consisting mainly of desert (more than 90%) and the Mediterranean coast, Coastal area and El-Jabal El-Akhdar known as the green mountain region which contributes to about 50% of the total plants in the whole country. *(Boulous, 1972; Feng et al., 2013*), While other concentration of plants are distributed in regions as the El- Jabel El-Garbi (Gharian), Ghadames, Awbari and Tarhona regions (Rateeb, 1999). In Libya there are about 1,825 vascular plant species, of which 134 are endemic. About 450 species are reported to be of medicinal value (Auzi, 2017), Some important plant families are Apiaceae, Asteraceae, Lamiaceae, Poaceae,

Fabaceae, Brassicaceae and Abiaceae, More than 100 species are extensively used by Bedouins and local people in folk medicine drinks, or chewed fresh or dry, They are used to cure dermal diseases, viral or bacterial infections, insect or animal bites, burns and sometimes to treat hair problems, These medicinal plants are very well documented in different floras (Kotb, 1998; EL-Gadi el al., 1992).

Plantag albicans and Helichrysum are medicinal plants endemic to Libya, grow in the coastal Mediterranean region, commonly used for its biological properties including anti-ulcerogenic, antiinfammatory, antigiardiasic, antimalarial, anticancer, immunomo dulatory, wound healing, antioxidant, antibacterial, antihypoglycaemic, hepatoprotective, hyperlipidemic, haemopoietic and cytotoxic activities which attributed to their higher content of flavonoids, glycosides, tannins, reducing compounds, anthraquinones, anthocyanins, mucilage, polysaccharides, fatty acids, triterpenes and steroids in traditional medicinal. Plantago has been used as traditional medicinal plant for centuries, and is reported to cure numerous diseases from cold to hepatitis, skin diseases, infectious diseases, problems concerning the digestive organs, respiratory organs, reproduction, the circulation, and for reducing fever, They have been used either whole or crushed leafs, or juice from leafs of two plants has been used to treat burns, to stop bleeding and to treat all kinds of wounds to enhance the healing process (Anne, 2000).

This study was designed to study the phytochemical screening, total phenol content, antioxidant activity and some minerals content in Plantago albicans.

MATERIALS AND METHODS

Many Libyan plants are known to be of potential medicinal value and Plantago albicans is used in herbal medicine used as plant material in this study to evaluate the phytochemical screening, phenolic content, antioxidant activity and minerals content of leafs and green parts of Plantago albicans which collected from Deryanah region as shown in Figure (1) during spring seasonof 2020.

Figure (1) Deryhanah region of(*Plantago albicans*) location.

Leafs and green parts of ***Plantago albicans*** As shown in Figures (2) were washed with tape aqoues to remove dust particles and other waste material followed by distilling water. The two samples were separated then dried in open air for three weeks and grinded by blender machine (hammer). Then the powder was stored in dark glass bottles for further using.

Figure (2) *Plantago albicans*

Phytochemical Screening of Spices

Phytochemical screening was carried out on methanol extract of spices. The following tests were performed to detect various phytochemical constituents which may be present in the studied plant extracts.

Screening for Alkaloids (Mayer's Test)

To 2 ml of the extract was boiled with dilute hydrochloric acid and the mixture filtered and to the filtrate a few drops of Mayer's reagent. A cream or white color precipitate produced immediately indicates the presence of alkaloids**.**

Screening for Carbohydrate Test

To 1ml of extract, 1ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes solution appeared green showing the presence of reducing sugar.

Screening for Glycosides (Keller Kilianin Test)

5ml of each extract was added with 2 ml of glacial acetic acid which followed by the addition of few drops of ferric chloride solution and 1ml of concentrated Sulphuric acid. The formation of the brown ring at the interface confirms the presence of glycosides.

Screening for Terpanoids (Salkowski Test)

5 ml of extract was taken in a test tube and 2 ml of chloroform added to it followed by the addition of 3ml of concentrated sulphuric acid. The formation of the reddish-brown layer at the junction of two solutions confirms the presence of terpenoids.

Screening for Flavonoids (Alkaline Reagent Test)

2 ml of extracts were treated with few drops of 20% sodium hydroxide solution formation of intense yellow color, which becomes colorless on the addition of dilute hydrochloric acid, which indicates the presence of flavonoids.

Screening for Saponins (Foam Test)

2ml of the extract was taken in a test tube and 6ml of distilled water added to it. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Screening for Steroids

1ml of the extract was dissolved in 10ml of chloroform and an equal volume of concentrated sulphuric acid added by the sides of the test tube. The upper layer turns red and the sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Screening for Tannins

2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Determination of phenols Compound by Folin Ciacalteu Method

This experiment was carried out of determine Phenolic compounds, where the amount of total phenolics in the Extracts was determined by (Folin Ciocalteu) reagent according to the

method of Slinkard and Singleton**.,** ***1977*** using gallic acid as a standard. Samples (two replicates of sample) were introduced into test cuvets, then 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of Na_2CO_3 (7.5%) were added. The absorbance of all samples was measured at 765 nm using the Shimadzu UV – Vis spectrophotometer after incubating at 30 °C for 1.5 h. Results were expressed as ppm of fresh weight.

Determination of antioxidant capacity by Prussian blue method

One gram of the powdered sample was defatted with petroleum ether. The defatted powder was then extracted sequentially by stirring with 10 ml of methanol twice, then extracted again with 10 ml 1% hydrochloric acid: methanol (v/v). The three combined extracts were evaporated under vacuum and the residue was dissolved in 10 ml methanol. Half ml of the solution was diluted with 3 ml distilled aqueous, 3 ml (0.008 M) of $K_3Fe(CN)_6$ was added,3 ml 0.1M HCl and 1 ml 1% $FeCl_3$. The blue color is allowed to develop for 5 minutes and the absorbance is measured at 720 nm ***(Wangensteen and Malterud 2004)*** in central lab of Faculty of Science, Omar Al-Mukhtar University**.**

Determination of metals and minerals

The metals of (Cu, Fe and Ni), were determined with an Atomic absorption spectrophotometer (Perkin Elmer 800) according to the method described by (Lorenz et al., 1980). Soluble sodium and potassium, calcium contents measured by a Flame Photometer (JENWAY Flame Photometer) according to the method described by (Jackson, 1958), (Nabil et al., 2018), (Hasan and Mojahulesalm, 2010), in central lab of Faculty of Science, Omar Al-Mukhtar University.

RESULTS AND DISCUSSION

Phytochemical screening assay is a simple, quick, and inexpensive procedure that gives the researcher a quick answer to the various types of phytochemical in a mixture and an important tool in bioactive compound analyses. After obtaining the crude extract or active fraction from plant material. Phytochemical screening can be performed with the appropriate tests to get an idea regarding the type of phytochemical existing in the extract mixture or fraction (*Sasidharan et al., 2011).* Such bioactive compounds have gained special attention as they can protect the human body against the oxidative stress that can cause many diseases including cancer, cardiovascular disorders, aging and antimicrobial properties (Boussoussa et al., 2014). There is an increasing interest in phytochemicals, because of their potential use in functional food products and medicines. ***Plantago albicans*** has numerous phytochemicals in

its leafs, seeds and roots, which apparently have medicinal properties and also can be used as taxonomic markers (Samuelsen, 2000).

The dried powdered plants (Plantago albicans was screened for the following constituents: carbohydrates and/or glycosides, tannins, alkaloids, flavonoids, anthraquinone and saponins. The obtained results of phytochemical screening of ethanolic and aqueous extracts for Plantago albicans (leafs and green part) were recorded in Table (1). Tannins were present in high amount in the ethanolic extracts of leafs and green parts, flavonoids was present in high amount in the ethanolic extracts of green parts, anthroquinones was present in high amount in the ethanolic extracts of green parts. On the other side, aqueous extracts whether leafs or green parts did not had high amount for all phytochemical constituents. Meanwhile, anthroquinones were absent in aqueous extract of leafs and green parts of plantago albicans, on the opposite of saponins which absent in the ethanolic extract of leafs and green parts of plantago albicans.

Table 1: Phytochemical screening of aqueous and ethanolic extracts for *Plantago albicans* (leafs and green part).

Plants Constituents	***Plantago albicans***			
	aqueous extracts		**ethanolic extracts**	
	leafs	**green part**	**Leafs**	**green part**
Tannins	++	++	+++	+++
Alkaloids	-	++	+	++
Flavonoids	++	++	+	+++
Carbohydratesand/or glycosides	+	++	++	++
Anthraquinone	-	-	+	+++
Saponin	++	++	-	-

(+: low amount, ++: moderate amount,+++: high amount and -: absent).

Gallic acid was used as a standard compound and the total phenols in extracts from ***Plantago albicans was*** expressed as (ppm) as shown in Table (2). The data indicated that the highest amount of total phenolic was in green part (16.1 ppm) while on the leafs was 10.4 ppm), the same trend happened in the case of antioxidant activity determination which had been determined by The FRAP (Ferric Reducing Ability Power) assay.

Table 2: Total phenol content (ppm) and Frap (ppm) in Ethanol extracts of *Plantago albicans.*

Plants Parameters	*Plantago albicans*	
	leafs	Green Part
Total Phenol (ppm)	10.4	16.1
Antioxidant Activity (ppm)	5.59.	9.75

Mineral and metal contents

Most of plant tissues contain varying amounts and proportions of elements, which largely remain as oxides. The element constituents (Cu, Fe, Na, K, Ni and Ca) of the studied plants were shown in Table (2). The concentrations of the elements of the studied plants were fluctuated as following: Sodium were found as a high percent in the two studied parts of ***Plantago albicans*** followed by potassium contents which exist in high percent. While, the other elements (Fe, Cu, Ni and Ca) exist in low percent in all the studied parts of ***Plantago albicans***.

Table 3: The elements content (ppm) in plantago albicans parts.

Elements Plants	leafs	Green part
Na	24	54
K	23	26
Fe	2.70	2.72
Cu	1.48	1.37
Ni	1.25	1.25
Ca	1	3

DISCUSSION

Phytochemical screening

These phytochemical compounds are known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plantextract used in this study. Tannins Are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer. (Ruch *et al*., 1989). Abd El-Gawad *et al.,* 2015, Nofal *et al*., 2016, Lukova *et al.,* 2017, Fayera *et al.,* 2018 and, Getahun 2020 reported that tannins one of the main phytochemical found in some species of plantago. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity (Nobori *et al.,* 1994). Abd El-Gawad et al., 2015; Nofal et al., 2016; Lukova et al., 2017; Fayera et al., 2018 and Getahun, 2020 reported that alkaloids one of the main phytochemical found in some species of ***plantago albicans.*** Flavonoids have been shown to exhibit their actions through effects on membrane

permeability, and by inhibition of membrane-bound enzymes such as the ATP and phospholipase A_2. Flavonoids serve as health promoting compound as a results of its anion radicals *(Hausteen, 1983)*. These observations support the usefulness of this plant in folklore remedies in the treatment of stress-related ailments and as dressings for wounds normally encountered in circumcision rites, bruises, cuts and sores. Lourens et al., 2004; Fergusion, 2001; Grierson and Afolayan, 1999). Abd El-Gawad et al., 2015, Lukova et al., 2017, Fayera et al., 2018 and, Getahun, 2020 reported that flavonoids one of the main phytochemical found in some species of plantago. Carbohydrates and/or glycosides. Lukova et al., 2017 and Getahun, 2020 found carbohydrates in the phytochemical constituents in some species of plantago. Saponin. Saponins, known to produce inhibitory effect on inflammation. (Just et al., 1998) and are major ingredients in traditional Chinese medicine and thus responsible for most of the observed biological effects. (Liu and Henkel, 2002). Abd El-Gawad et al., 2015; Nofal et al., 2016 and Fayera et al., 2018 found saponins in the phytochemical constituents in some species of plantago.

Total Phenolic Content

The antioxidant properties of polyphenols are due to their redox properties, which allow them to act as reducing agents, hydrogen donators, metal chelators and single oxygen quenchers. Polyphenolics exhibit a wide range of biological effects including antibacterial, anti inflammatory, anti allergic, hepato-protective, antithrombotic, antiviral, anticarcinogenic and vasodilatory actions; many of these biological functions have been attributed to their free radical scavenging and antioxidant activity ***(Soobrattee et al., 2005).*** Phenolic and flavonoid molecules are important antioxidant components which are responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. They also have ideal structural characteristics for free radical scavenging (Benzie and Strain, 1996). Different literature reports indicate a linear correlation of total phenolic and flavonoid content with antioxidant capacity). Antioxidant activity is a complex procedure usually happening through several mechanisms and is influenced by many factors, which cannot be fully described with one single method, Therefore, it is essential to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action. (George, 2008; Okhale et al., 2001) Our results of total phenols were lower than Tegin et al., 2018 where their result was 32.66 ppm, Krima et al., 2015 who reported that total phenol of aqueous extract was 114.45 ppm and Pourmorad et al., 2006 who determined the total phenolic content of selected Iranian medicinal plant species' extracts varied from 24.1 289.5

ppm Other results were agreement of our result as Kobeasy et al., 2010 (13.05 ppm). while other results were more than our results as Pereira et al., 2017 who evaluated the total phenolic content of P. coronopus leafs extracted by hexane, ethyl acetate and methanol were 2.07, 2.43, and 28.1 ppm respectively, while aqoues extract was 9.98 ppm.

Antioxidant activity

The obtained results were lower than results of Abate et al., 2017 who found higher ferric reducing activity ranging from 336.58 ppm (90% methanol) to 172.94 ppm, Lukova *et al.*, 2018 found FRAP content were 51.85 P for plantago to 97.66 ppm for *plantago major* and 159.48 P ppm for plantago media and Vicas *et al.*, 2015 recorded FRAP content were 25.639 and 29.771 ppm in some plantago species. Some authors reported that phenolic compounds are unstable and readily become non-antioxidative under heating (Barros et al., 2007; Yen and Hung CY, 2000). Phenolic and flavonoid molecules are important antioxidant components which are responsible for deactivating free radicals based on their ability to donate hydrogen atoms to free radicals. They also have ideal structural characteristics for free radical scavenging (Amarowicz et al., 2004).

Minerals

Copper is required for adequate growth, cardiovascular integrity, lung elasticity, neovascularization, neuroendocrine function, and iron metabolism. An average adult human ingests about 1 mg of copper per day in the diet; about half of which is absorbed**.** Our results were higher than (Pereira et al., 2017) results who reported that Copper in Plantago coronopus was below of detection limit, and (Guil- Guerrero 2001) found Copper was in similar amounts in the three species,ranging from 0.14 ppm (P.mlanceolata***)*** to 0.22 ppm***(***P. media).

Iron has several vital functions in the body. It serves as a carrier of oxygen to the tissues from the lungs by red blood cell haemoglobin, as a transport medium for electrons within cells, and as an integrated part of important enzyme systems in various tissues. The physiology of iron has been extensively reviewed. Bothwell, 1997; Hallberg, 1982; Dallman, 1986; Brock *et al.*, 1994; Kühn, 1996 and Mascotti *et al.*, 1995). Our results were higher than the results of Guil-Guerrero 2001 who found Iron values ranged from 1.54 ppm (P. lanceolata) to 2.62 ppm (P. media).

Sodium is one of the body's electrolytes, which are minerals that the body needs in relatively

large amounts. Electrolytes carry an electric charge when dissolved in body fluids such as blood. Most of the body‘s sodium is located in blood and in the fluid around cells. Sodium helps the body keep fluids in a normal balance. Sodium plays a key role in normal nerve and muscle function. Our results were lower than the results of Guil-Guerrero, 2001) who determined sodium content ranged from 29 ppm (P. lanceolata) to 124 mg (P. major***).***Concentrations for P. lanceolata from Wilman and Derrick, 1994) varied between 109 and 315 ppm on a dry matter and Pereira *et al.*, 2017 revealed that sodium content was 50 ppm.

Potassium is a mineral found in foods. It is also an electrolyte, which conducts electrical impulses throughout the body. It is an essential nutrient because it is not produced naturally by the body. Therefore it is important to consume the right balance of potassium-rich foods and beverages. Our results were lower than Guil-Guerrero (2001) who estimated potassium content ranged from 318 ppm (P. major***)*** to 440 ppm (P. media). But higher than Pereira *et al.*, 2017 who content was 8.02 ppm. Nofal et al., 2016 who revealed that potassium content was 1.173%. Wilman and Derrick, 1994 found for P. lanceolata values ranged from 2620 to 3500 ppm on a dry matter.

Nickel is a transition element extensively distributed in the environment, air, aqoues, and soil. It may derive from natural sources and anthropogenic activity. Although nickel is ubiquitous in the environment, its functional role as a trace element for animals and human beings has not been yet recognized. Pereira et al., 2017 who found nickel content was below detection limit in plantago coronopus leafs. Calcium is a nutrient that all living organisms need, including humans. It is the most abundant mineral in the body, and it is vital for bone health. Humans need calcium to build and maintain strong bones, and 99%. Trusted Source of the body‘s calcium is in the bones and teeth. It is also necessary for maintaining healthy communication between the brain and other parts of the body. It plays a role in muscle movement and cardiovascular function. Pereira et al., 2017 found Calcium content were 60, 108 and 143 ppm in P. major, P. media P. lanceolate respectively. This result were higher than our results. Guil- Guerrero,2001 who determined Calcium content ranged from 60 mg in P. lanceolata to 143 ppm in P. media.

CONCLUSION

The study revealed that medicinal plants in the study area are rich in phytochemicals and mineral content. The medicinal plants have a wide range of potential applications in the

manufacture of new drugs, nutraceuticals, and healthcare products. The medicinal plants comprise a variety of pharmacologically active phytochemicals that have been used to treat different types of diseases.

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