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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY FOR PLANTS *DRACAENA CINNABARI, VERBENA OFFICINAL, POLYGALA TENUIFOLIA* AND *LINUX USITATISSIMUM*

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ABSTRACT

Natural compounds screened in plants with potency have protective activities contrary to knowledge and memory deficiency and other alternative. Accordingly, in response to improve and utilize this traditional medicine as a clinical remedy for instance cultivating cognition deficits, the pharmacology and manner of action of its active components must be supplementary considered. Thus, the medicinal plants of the most important strategic resources in the pharmaceutical industry are mainly important in the production. Known medicinal plant is plant that contains one or more of the numerous associates on chemical substances, one or more and low or high in concentration, and it has biological ability to inhibit or at any rate reduce the symptoms of the disease. Some medicinal plants such as *Dracaena cinnabari, Verbena officinal, Polygala tenuifolia* and *Linux usitatissimum* were studied to obtain more usefulness and lead to preferable treatment. Overall, the extracts of plants revealed significant activity on the bacterial species. Plants extracts selectively inhibited the growth of both gram positive and gram negative bacteria with zones of inhibition ranging from 7 mm to 14 mm at concentrations of 50 mg/mL. It can be concluded that the activity showed by the ethanolic extract of the plants is a result of the phytochemicals present in plants and these plants seems to be good applicants for further phytochemicals studies in an attempt to find new chemical entities combating resistant bacteria.

Key words: Medicinal plants, Phytochemical constituents, Antibacterial.

INTRODUCTION

Since prehistoric decades, human being used plants as their sources of food and later consumed it for medicinal purposes to cure different ailments and diseases[1]. Recently, medicinal plants played a very important role in the agricultural and industrial production. Many countries that produce such plants received enormously precaution believing that these medicinal plants are the leading source of therapy or it has an active substance that can be considered therapeutic. In addition to the preparation of the pharmaceutical in the form of extracts or effective constituents, otherwise raw materials for the production of some chemical compounds that are the essential of the chemical synthesis of some important materials such as pharmaceutical substance, cortisone, sex hormones, plasma and other alternative. So it was decided to study some medicinal plants in Libya such as *Dracaena cinnabari, Verbena officinal* and *Polygala tenuifolia. Dracaena cinnabari* is endemic to North Africa, Canary Islands, Cape Verde and Madeira[2,3]. The tree of *D. Cinnabari* (Family: *Agavaceae*) is known in Libya as damalachawin. It was used in traditional medicine for the treatment of gastric sores diarrhea, dysentery, hemostatic, anti-ulcer remedy, anti-

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spasmodic, analgesic, and anti-inflammatory. The aqueous extract of *D. cinnabari* resin exerts a relaxant effect on rat isolated ileum, uterus and urinary bladder. This observation is consistent with the effects of other *Dracaena* species and other Dragon's blood plants[3]. *D. cinnabari* is considered a strangest tree in the world and what distinguishes this tree is the external form of medical value, which is extracted from the bark of scarlet resin. The part of the *D. cinnabari* tree to be used is the resin, which is extracted from the plant peel and fruits where it is used in some treatments aside from dyeing[3]. The *Verbena officinal* (Family: Verbenaceae) is commonly known as Verveine is a perennial herb. It has wild and ornamental stem with aromatic custom. The flowers are used by boiling to facilitate digestion, counter pain nervous system, preserve fever, generates urine, and activates the sexual power and beneficial for pregnant women[4]. The *Polygala tenuifolia* plant grows randomly dual-blocking. It is hardy and has several medicinal uses including expectorant[5]. Recently, a number of studies have revealed that the root of *P. tenuifolia* could improve learning and memory function in animal models induced by scopolamine[6]. The root of *P. tenuifolia* Wild, or "Yanzhi", is a well-known traditional Chinese medicine that was prescribed for thousands of years for a range of nervous system disturbances; these include amnesia, neurasthenia, palpitation, and insomnia[7].

Our study also included *Linux usitatissimum*, (Family: Linaceae). Flax seeds are oily, 40% oil with a high proportion of (omega-3) which is useful in reducing cholesterol, and the oil has a distinct smell that increases with storage. Moreover, flax seed oil is rich in acid, alpha-linoleic acid (ALA) amatory bowel disease, rheumatoid arthritis, and a variety other health problems[8]. Flax seed mucilage extracts, flax seed hulls, flax seed oleosomes and flax seed alcohol extracts. Each of these products is associated with specific beneficial health effects. Although each fraction contains more than one bioactive component, reports commonly ignore the presence of a plurality of bioactive compounds in flax seed fractions or attribute the effect of a component of the flax seed on the observed effect. Whole flax seed is widely accepted as a healthy food that has anticancer activity. Controlled experimental diets have demonstrated numerous beneficial effects of flax seed consumption[9-11]. Furthermore in the present study, we consider the phytochemical and antibacterial activities of aqueous and ethanolic extract achieved from *D. cinnabari*, *V. officinal, P. tenuifolia* and *L. usitatissimum*. To justify the use of these plants in treatment of diseases and its use as preserving, therefore, no obvious study has been carried out for the phytochemical screening and antibacterial activity of these plants grown in Libya.

EXPERIMENTAL

Materials

Plant material

D. cinnabari, V. officinal and P. tenuifolia, were collected from region of Alkhums and Tarhona (Libya) in spring season of 2014 *w*hile the *L. usitatissimum* was purchased from a local herb seller at Alkhums City. The identification of the plants was carried out at Biology Department, Faculty of Science, El-Mergheb University. Alkhums, Libya.

Sampling of plant material

The crude provisions of medicinal plants were washed properly with running tap water followed by distilled water for several times and air dried under shade; dried mass was grounded to fine powder. The powders obtained were kept in small dark plastic bags with proper labeling for further use.

Preparation of extract

The crude powdered plants were extracted in water and ethanol separately at a 40% (w/v) concentration (20 g crude powder in 500 mL aqueous or ethanol) by using Soxhlet procedure for 6-8 hr and then filtered. The crude extracts were kept in refrigerator at 4°C until further use.

Phytochemical screening

Phytochemical evaluates for various phytoconstituents of the extracts were undertaken using standard qualitative methods[12-18]. The extracts were screened with the presence of biologically active compounds like alkaloids, carbohydrates, glycosides, amino acid, proteins, steroids, diterpenes, flavonoids, phenolic, tannin, etc.

Detection of tannins

The extracts (4 mL) were boiled for 10 mins in 20 mL of water in a test tube. A few drops of 5% ferric chloride were added, observed for 10 min for a brownish green or a blue black coloration.

Detection of saponnins

The extracts (10 mL) were shaken and heated to boil. Frothing (appearance of creamy mass of small bubbles) shows the presence of saponnins.

Detection of alkaloids

About 4 mL each of the extracts were stirred with 10 mL of 1% aqueous hydrochloric acid on a steam bath for 10 mins. 1 mL of the extract was treated with a few drops of Mayer's reagent. Precipitation with these reagents was seen as evidence for the presence of alkaloids.

Detection of flavonoids

The extracts (5 mL) were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless the moment dilute acid was added indicates the presence of flavonoids.

Detection of phenols

The extracts (5 mL) were treated with 2-3 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

Detection of glycosides

The extracts (5 mL) were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

Detection of carbohydrates

The extracts (5 mL) were treated with a few drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of carbohydrates.

Detection of diterpenes

The extracts (5 mL) were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Detection of steroids

The extracts (5 mL) were dissolved in 10 mL of chloroform. A few drops of concentrated sulphuric acid were carefully added to form a lower layer. A reddish color formed at the interphase indicates the presence of a steroid ring.

Detection of proteins and amino acids

The extracts (5 mL) were added 0.25% w/v ninhydrin reagent and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

Test organisms

Four gram negative bacterial strains (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumonia.*) and one gram positive bacterial strains (*Staphylococcus saprophyticus*) from standard cultures were used as test strains. Standard bacterial strains were obtained from Microbiology Medical Laboratory, Central Hospital. Al- Khums, Libya.

Determination of antibacterial activity by disc diffusion method

The aqueous and ethanolic crude extracts of plants were screened for antibacterial activity by the paper disc diffusion method[22]. From the 50 mg/mL typical solution of each extracts of plants, 40 µL aliquots were transferred onto blank sterile paper discs (6 mm diameter). The dried discs were carefully and firmly placed on nutrient agar medium (UK) previously inoculated with a bacterial suspension and incubated at 37ºC for 24 hr. The appropriate solvent was used as control to determine the sensitivity of the tested strains. After incubation, plates were examined for the presence of zones of growth inhibition, and the diameters of these zones were measured in mm. Tests were performed in duplicate under sterile conditions[14,19-25].

Antibiotic assay

The effect of antibiotics on the test organism were obtained using the same procedure as that of the antibacterial susceptibility test, but instead of plant extract, antibiotics discs were hosted into nutrient agar medium. The zone of inhibition were measured and recorded. The antibiotics used were Gentamycin and Tetracycline (1 mg/mL) as control for comparison.

RESULTS AND DISCUSSION

Table 1 showed the results of preliminary qualitative phytochemical study of the plants extracts. Carbohydrates, alkaloids, tannins, steroids, and diterpenes were found present while absence of flavonoids and saponnins in the plants extracts. Glycosides were not present in alcoholic extracts of *Verbena officinalis, Dracaena cinnabari* and *Linux Usitatissimum*, but exists in *Linux Usitatissimum* aqueous extract. Proteins were absent in *Verbena officinalis* and *Dracaena cinnabari* aqueous and alcoholic extracts. Furthermore, this protein was present in *Polygala tenuifolia* and *Linux Usitatissimum*. A number of investigations were carried out to identify the compounds responsible for the biological activity of *Polygala tenuifolia* [24-27]. 80% Ethanol extract of root of *Polygala tenuifolia* could improve learning and memory in a scopolamine-induced amnesia model of rats[28]. Biologically active substances could be extracted from *P tenuifolia* roots (triterpenoid saponin) because the general saponnins from *P tenuifolia* root have shown cognition-enhancing effects, which improves hippocampal-dependent learning and memory[29]. Presence of sucrose might prevent or relieve depression[30].

Verbena officinal was used as herbal medicine or health supplement in different countries earlier. It was found in moderate climatic region and is known for its anti-inflammatory, diuretic and expectorant properties as folk medicine. Pharmacological activities were found for several of its constituents. The plant has been used to treat acute dysentery, enteritis and amenorrhea. The plant was also used for its anticonvulsant effect as well as it is used for the treatment of jaundice, cough, cold and digestive problems. It has also been used for healing liver and gall bladder diseases and nervous exhaustion. Various extracts of this plant have shown antibacterial, antioxidant, analgesic, anti-rheumatic and nerve growth factor-

potentiating activities[31-41]. *Dracaena cinnabari* showed significant effects against different pathogenic microorganisms such as, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *S. saprophyticus.* The encouraging objects clear the zone of inhibition, which has shown the statistical efficiency. The phytochemical screening revealed the presence of different types of phytochemical constituent. Due to the existence of phenols that were observed in the extracts throughout the attained results, it could be concluded that the phytochemical bioactive compounds are factors for antimicrobial activities of *D. cinnabari* resin. Also, it was shown that proper potential interaction with antimicrobial activities, which provides the interest for considering, whether the isolated purified compounds from *Dragon's blood* may have better therapeutic potential effectiveness as compared to crude extract or not. Consequently, there is considerable variation in the chemical composition among various samples of *Dragon's blood*; quality control assurance needs to be established for the traditional medical trade[42].

Table 1: Phytochemical results

Name of the compd.	Name of the test	Name of the plants							
		Polygala tenuifolia		Verbena officinal		Dracaena cinnabari		Linux Usitatissimum	
		Aq. extract	EtOH extract	Aq. extract	EtOH extract	Aq. extract	EtOH extract	Aq. extract	EtOH extract
Tannins	5% Ferric chlori	+	++	++	++	+	+	++	+
Flavonoids	Alkaline reagent test	-	-	-	-	-	-	-	-
Carbohydrates	Molisch's test	+	+	++	+	+	+	+	+
Phenols	Ferric chloride test	++	+	++	+	+	+	+	+
Alkaloids	Mayer's test	+	+	+	+	+	+	+	+
Steroids	Chloroform + Acetic acid + H_2SO_4	+	+	+	++	+	+	+	+
Glycosides	Legal's test	+	++	+	-	-	-	+	-
Proteins	Ninhydrin test	+	+	-	-	-	-	+	+
Diterpenes	Copper acetate test	+	+	+	+	+	+	+	+
Saponnins	Distilled water	-	-	-	-	-	-	-	-

Absent = –, Present = +, More quality = ++

The aqueous extract of *D. cinnabari* resin have relaxant effects on ileum, uterus and urinary bladder smooth muscle, increased heart contractility of the isolated perfused heart, reduced blood pressure of anesthetized normotensive rats and increased urine excretion in conscious rats. These effects may be taken into consideration, when such resin is being used in folk medicine[43]. Table 1 showed the bioactive constituents such as tannins, carbohydrates, phenols, alkaloids, steroids and glycosides in aqueous extract, proteins and diterpenes while flavonoids, saponnins and glycosides (ethanolic extract) were not present for *Linux Usitatissimum* flax seed, which is one of the oldest cultivated crops, which is widely grown for oil, fiber, and food[44]. The antimicrobial effect of Usitatissimum flax seed showed in Table 2 highly regarded results against bacterial strains compared to the other plants results and as stated above to antibiotics.

Table 2: Zones of antibacterial inhibition of reference antibiotics

Plants name / Bacterial strains	Polygala tenuifolia		Verbena officinal		Dracaena cinnabari		Linux usitatissimum	
	Aq. extract	EtOH extract	Aq. extract	EtOH extract	Aq. extract	EtOH extract	Aq. extract	EtOH extract
Escherichia coli	9	10	-	-	13	14	7	8
Proteus vulgaris	-	-	-	-	9	10	9	10
Pseudomonas aeruginosa	-	-	7	-	8	9	8	7
Klebsiella pneumonia	-	-	-	-	7	8	7	9
S. saprophyticus	9	-	-	-	10	11	7	8

Table: Zones of antibacterial inhibition of reference antibiotics

Bacterial strains	Diameter of zones of inhibition (mm) concentration of antibiotics (40 mg/mL)	
	Gentamiycin	Tetracycline
Escherichia coli	15	15
Proteus vulgaris	16	23
Pseudomonas aeruginosa	Nil	Nil
Klebsiella pneumonia	13	17
S. saprophyticus	16	15

CONCLUSION

Results conclude that the crude extracts of *D. cinnabari* and *L. Usitatissimum* exhibited significant antimicrobial activity than *P. tenuifolia* and *V. officinal*, whereas its properties support folkloric use in treatment of some diseases accordingly antimicrobial agents. This doubtless explains the use of these plants by the indigenous people against numerous infections since generations.

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