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ANTIBACTERIAL ACTIVITY OF WATER AND ETHANOLIC EXTRACTS OF *Bauhinia variegata* LEAVES

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ABSTRACT: Phytochemicals isolated from medicinal plants are known to be effective in treating bacterial infections. The antibacterial activities of the ethanol and water leaf extracts of *Bauhinia variegata* were tested. Antibacterial effects of crude extracts were performed using modified Kirby-Bauer disc diffusion technique to determine the zone of inhibition. The extracts were tested for the antibacterial activities against Gram-positive bacteria (*Sarcina ventriculi*) and Gram-negative bacteria (*Serratia marcescens).* The results demonstrated that both ethanol and water leaf extracts of *Bauhinia variegata* have shown strong inhibition zone against *Serratia marcescens* and *Sarcina ventriculi* compared to control). This medicinal plant could be developed into affordable and safe standardized herbal products and may serve as a source of new molecules for broad-spectrum antimicrobial agents. Our results revealed that *Bauhinia variegata* leaves contains total phenolic contents (453 mgGAE/ g), flavonoid 166 mg QE/g and total alkaloid 1.674 g/100g. Also *Bauhinia variegata* leaf extracts showed highly antioxidant activity.

Key words: Antioxidant, water and ethanolic extracts, antibacterial, total phenolic, total flavonoid.

INTRODUCTION

The World Health Organization reported that infectious diseases are responsible for one third of global mortality and that they still pose a major threat to public health in numerous countries (**WHO, 2017**). Their impact is more evident in developing countries due to the limited accessibility to medicines and the emergence of widespread drug resistance (**Macedo *et al.*, 2015**). The plant kingdom represents the source of various medicines. Indeed, since ancient times medicinal plants play an important role of health care population and could represent a significant source of new drugs (**Aleksic and Knezevic, 2019**). Medicinal plants are playing an important role in protecting against dreadful and dangerous microbial species.These plants are being used in various traditional systems due to having better immune potential and activity against numerous diseases, as compared to synthetic drugs. The medicinal activity may be slow with the plant extracts, but has permanent cure against various diseases **(Prakash, 2005)**. The crude extract from *Bauhinia variegata* have been shown to posses biological activities *e.g.,* Antihyper-lipidemic activity **(Kumar *et al.*, 2011)**, Antistress/ Adaptogenic Activity **(Marasani *et al.*, 2013)**, antibacterial, antioxidant (**Pandey *et al.*, 2013**), immunomodulatory activities **(Ghaisas *et al.*, 2009**), Hepatoprotective activity **(Pathak *et al.*, 2011**), hypoglycemic activity **(Chourasiya *et al.*, 2012**). The plant *Bauhinia variegata* has been reported to contain Quercetin, lupeol, β-sitosterol, tannins, kaempferol-3-glucoside, amides, carbohydrates, reducing sugar. **Bansal *et al.* (2014**) reported that the major objective of the study was to active constituent from the plant and to assess the anti-diabetic potential of *Bauhinia variegata in vivo*.

The aim of this study was to determine the antioxidant, total flavonoid, total phenolic, total alkaloid and antibacterial activity of *Bauhinia variegata* leaves.

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MATERIALS AND METHODS

Plant Material

Bauhinia variegata leaves were collected from the farm of Faculty of Agriculture, Zagazig University, Egypt. The plant was identified by botanical members of the Department of Botany, Faculty of Agriculture, Zagazig University, Egypt. The plant leaves were allowed to dry in a shady and well- aired place for 15 days, aerial parts of *Bauhinia variegata*, were cut into small pieces, ground to fine powder using blender and kept for further investigations.

Chemicals

All solvents used throughout the present work were obtained from different companies. ABts, DPPH and substrates were purchased from Sigma Chemical Co., (St. Louis, USA).

Methods

Preparation of ethanolic extracts

Bauhinia variegata leaves were extracted according to the method of **Panovska *et al.* (2005)** 50 g of dry powder were extracted with 500 ml of 70% ethanol in a screw-capped flask and shaken at room temperature for 24 hr. The extract was centrifuged at 5500 rpm for 10 min, while the residue was re-extracted under the same condition twice and filtered through filter paper. The 70% ethanolic extract was concentrated under reduced pressure, lyophilized to obtain powder and the final yield was 4.97 g, and stored at -20°C until using for further study.

Preparing aqueous extract

About 500g of the ground *Bauhinia variegata* leaves were soaked in distilled water for 72 hr., and the residue was separated for preparation of ethanolic extracts. Finally the aqueous extract was lyophilized and the final yield was 50 g. The residue was kept in a clean vial and cooled in a refrigerator until use (**Nwangwa and Ekhoye, 2013**).

Chemical composition of plant sample

Determination of moisture, crude fiber, ash, crude lipid and crude protein were determined according to the method described in **AOAC (2005)**.

Total phenolic determination

Total phenolic compounds of *Bauhinia variegata* leaves were determined according to the method described by **Ghasemzadeh *et al.* (2010).**

Determination of total flavonoids

The total flavonoid compounds of *Bauhinia variegata* leaves were determined according to the method described by **Ahn *et al.* (2007)**.

Determination of total alkaloids

The total alkaloids compounds of *Bauhinia variegata* leaves were determined according to the method described by **Adham (2015)**.

Antioxidant Activity of Studied Plant

Free radical scavenging activity (RSA) DPPH assay

The RSA of the ethanolic extract of *Bauhinia variegata* leaves was assayed with 1, 1-diphenyl-2picrylhydrazyl (DPPH) radicals dissolved in ethanol. RSA and the presence of hydrogen donors in ethanol extract were examined by reduction of DPPH in ethanol. An ethanolic solution of DPPH radicals was freshly prepared. The radical, in the absence of antioxidant compounds were stable for more than 2 hr., of normal kinetic assay. For evaluation, 100 µl (40, 100, 150, 200 µg/ml) ethanolic resideu of studied plant was mixed with 5 ml of ethanol solution of DPPH radicals and the mixture was vortexed for 20 sec. at ambient temperature. Against a blank of pure ethanol without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells. of mixing, using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA toward DPPH radicals was estimated from the differences in absorbance of ethanol DPPH solution with or without sample (control) and the inhibition percentage was calculated according to **Lee *et al.* (2002)**, from the following equation:

Inhibition (%)=[(absorbance of control– absorbance of test sample) / absorbance of control] × 100.

In vitro testing of resideu for antimicrobial activity

Antimicrobial activities of the tested samples were determined using a modified

Kirby-Bauer disk diffusion method **(Bauer *et al.*, 1996).** Plates inoculated with Gram (+) bacteria as *Sarcina ventriculi*; Gram (-) bacteria as *Serratia marcescens* at 35 – 37°C for 24 – 48 hours., and then the diameters of the inhibition zone were measured in millimetres **(Bauer *et al.*, 1996)**.

RESULTS AND DISCUSSION

Proximate Composition of *Bauhinia variegata* Leaves

The analysis of *Bauhinia variegata* leaves content are recorded in Table 1, results showed that moisture, crude protein, crude fat, crude fiber, carbohydrates and crude ash valued 9.9%, 8.55%, 1.90%. 31.60%, 41.45% and 6.90% in respective order. These results showed that the plants contained considerably high amount of crude ash, crude fiber and carbohydrates. **Kedaree *et al.* (2016)** reported that *Bauhinia variegata* leaves contained crude ash (12.72 %), crude fiber (23.79%) and crude protein (14.18%).

The Total Active Components in *Bauhinia variegata* Leaves

Rice-Evans *et al.* (1996) and **Mattei *et al.* (1998)** reported phenolic compounds have been widely studied; phenolic compounds have at least one aromatic ring which can carry the hydroxyl groups which can known as reducing agents. The natural antioxidants such as phenolics and flavonoids compounds have wide spectrum pharmacological effects like antibacterial, anti-allergic, neuroprotective activities, anti-inflammatory and anticancer, also protect plants from the attack of pathogenic microbial. **Khaga *et al.* (2015)** reported that plant extract of *Bauhinia variegata* is a source of potent antioxidant with the strongest DPPH radical scavenging activity (IC50 = 6.48±0.08 µg/ml) whereas standard ascorbic acid has IC50 of 45.93 µg/ml. They also recorded that the extract of *Bauhinia variegata*, contain high value of phenolic (156.30±0.3 mg GAE/g) and flavonoid content (14.04±1.4 mg QE/g) exhibited the greatest antioxidant activity. The medicinal properties of plants are due to the phytochemicals. These phytochemicals are the secondary metabolites which are produced in adequate quantity under stressed conditions, which allows the plant to protect itself from adverse environmental factors. Dietary intake of these phytochemicals may promote health benefits, protecting against chronic degenerative disorders, such as cancer, cardiovascular and neurodegenerative diseases. This study reported that the phenolic contents was (453 mgGAE/ g), flavonoid was 166 mg QE/g. and total alkaloid was 1.674 g/100 g). **Krishnaveni (2014)** reported that the *Bauhinia variegata* flavonoid content was (160.0±6.9 mg/g) and phenolics was (126.66±6.11 mg/g).This study revealed the presence of medicinal active constituents of *Bauhinia variegata*. The phytochemicals like flavonoids, phenols and terpenoids (Table 2) which are rich in *Bauhinia variegata* may be contributing in providing the medicinal properties to the plant. The inflammation is mainly caused by cyclooxygenase and lipooxygenase pathway. Phenolic compounds were shown to inhibit the cyclooxygenase and lipooxygenase pathway **(Ferrandiz *et al.*, 1990; Ferrandiz and Alcaraz., 1991; Laughton *et al.*, 1991)**. Flavonoids are found to inhibit Ornithine decarboxylase enzyme, it is rate-limiting enzyme in polyamine biosynthesis, which has been correlated with the rate of DNA synthesis and cell proliferation in several tissues, hence inhibiting cell proliferation **(Tanaka *et al.*, 1997a; Tanaka *et al.*, 1997b; Makita *et al.*, 1996).** Flavonoids inhibit growth of microorganism by depolarizing membrane, inhibiting DNA, RNA and protein synthesis **(Dzoyem *et al.*, 2013).** Study of flavonoids, phenols and terpenoids from this plant may help to exploit more of the medicinal properties of *Bauhinia variegate* leaves.

Radical Scavenging Activity (RSA) of *Bauhinia variegata* Leaves Extract

Many studies in the last ten years interested in the theory of free radical disease causation, especially in certain forms of cancer and vascular diseases. Because of the developments in the free radical field have guided us to the consideration on dietary agents, the natural antioxidant (especially vitamins E, A and C), in a possible prophylactic and the role of the disease process. A free radical is a chemical species that has unpaired electrons (**Pryor *et al.*, 2006**). These electrons, which made free radicals are

Table 1. The proximate composition of studied leaves of *Bauhinia vaiegata* (g/100 g DW)

Percentage of parameter	*Bauhinia variegata*
Moisture (%)	9.9% (FW)
Proteins (%)	8.55% (DW)
Fats (%)	1.9% (DW)
Fibers (%)	31.6% (DW)
Carbohydrates (%)	41.45% (DW)
Ash (%)	6.9% (DW)

FW: Fresh weight DW: Dry weight

Table 2. The total phenolic, total flavonoid and total alkaloid contents of *Bauhinia variegata* leaves extract

Plant extract	Total phenolic content (mg GAE/g sample DW)	Total flavonoid content (mg quercetin/g sample DW)	Total alkaloid (g/100 g FW)
Bauhinia variegata	453.672	166.502	1.674

very reactive and take section in chemical reactions with other components in cell such as proteins, complex carbohydrates, nucleic acids and lipids) in the body (**Kohen and Nyska, 2002**). In the biological systems, free radicals are referred to reactive oxygen species (ROS), as the most biologically significant free radicals. ROS produced in cells include hydroxyl radical ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), and superoxide anion ($O^{\bullet}_2$) **Pryor *et al.* (2006)**.

Table 3 show the antioxidant activity of *Bauhinia variegata* leaves extract. All the samples analyzed showed an increase in antioxidant activity with increasing concentrations, thus exhibiting a concentration dependent pattern of free radical scavenging ability. The extract of *Bauhinia variegata* is the potent antioxidant with DPPH scavenging IC50= 6.48±0.08 µg/ml with respect to the standard ascorbic acid of IC50 of 45.93 µg/ml. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological system, acting as scavengers of singlet oxygen and free radicals (**Khaga *et al.* 2015)**. Antioxidant activities greatly associated with the presence of phenolic compound (**Shahwar *et al.*, 2010**).

Antimicrobial Activity

The intensive use of antibiotics is often followed by the presence of resistant strains of microorganisms. In view of the resistance of bacteria to drugs, the search for natural compounds having antibacterial activity is an urgent one in order to cope with the harmful effects of these microorganisms. For these reasons, accordingly, in this research two extracts *i.e.* by ethyl alcohol and water were tested against different microorganisms Gram-positive; *Sarcina ventriculi* (G^+) and negative bacteria; *Serratia marcescens* (G^-). Inhibition zones are recorded as shown in Tables 4 and 5. Control was in the same conditions. It was observed that control (DMSO) did not produce any inhibition zones. It is showed that all extracts gave rise to concentration dependent inhibition zones in the two types of bacteria (positive and negative Gram).

The results of present study showed that the water and ethanol extract of *Bauhinia variegata* inhibited the growth of tested isolate strongly; this may be due to presence of the phytochemical groups as mentioned in Table 2.

Table 3. DPPH scavenging activity of *Bauhinia variegata* leaves extract

Parameters plant extracts	Free radical scavenging activity "DPPH" (%)			
	40 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)
Bauhinia variegata	81.990%	92.398%	95.102%	95.153%

Table 4. Effect of ethanolic resideu on the diameter of inhibition zone (mm) of microorganisms

Concentration	Inhibition zone diameter (mm/mg sample)	
	***Sarcina ventriculi* (G+)**	***Serratia marcescens* (G-)**
Control : DMSO	NI	NI
1. (100 mg/ml)	19	15
2. (50 mg/ml)	19	10
3. (25 mg/ml)	20	9
4. (12.5 mg/ml)	18	9

Table 5. Effect of water resideu on the diameter of inhibition zone (mm) of microorganisms

Sample	Inhibition zone diameter (mm/mg sample)	
	***Sarcina ventriculi* (G+)**	***Serratia marcescens* (G-)**
Control : DMSO	NI	NI
1. (50 mg/ml)	15	12
2. (25 mg/ml)	14	10
3. (12.5 mg/ml)	10	10
4. (6 mg/ml)	NI	9

According to these results, *Sarcina ventriculi* were found to be more sensitive to the extract than *Serratia marcescens*. These results were agreed with **Mishra *et al.* (2014)** they found that the presence of variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids that are found to have effective as antimicrobial properties. The probable mechanism of phenolic compounds activity includes enzyme inhibition by oxidizing compounds, possibly through reaction with sulphedral groups or through more nonspecific interaction with proteins**.** Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection **(Dixon *et al.* 1983).** Their antibacterial activity is probably due to their ability to form complexes with extra cellular and soluble proteins and to complex with bacterial cell walls leading to disruption of microbial membranes **(Tsuchiya *et al*., 1996)**. Many plants contain non toxic glycosides which can get hydrolyzed to release phenolic which are toxic to microbial pathogens **(Aboaba and Efuwape, 2001).**

Conclusion

The results of this study indicated that *Bauhinia variegata* leaves have potential to inhibit two pathogenic bacteria. *Bauhinia variegata* leaves show much promise in the development of phyto-medicine having antimicrobial properties and the drug derived from *Bauhinia variegata* may have the possibility of alternative medicinal source because of their antibacterial activity. This study also indicated that ethanol and water extract of this plant has highest capability to antibacterial activity against *Serratia marcescens* and *Sarcina ventriculi*.

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التأثير المضاد للبكتريا للمستخلص المائي والكحولي لأوراق نبات خف الجمل *Bauhinia variegata*

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للمركبات الطبيعية المستخرجة من أجزاء النبات المختلفة أهمية كبيرة في المجال العلاجي، ولذلك تم دراسة التأثير المضاد للبكتريا للمستخلص الإيثانولي والمائي لأوراق نبات خف الجمل على الكائنات الدقيقة (بكتريا موجبه لجرام *Sarcina ventriculi* وبكتريا سالبه لجرام *Serratia marcescens*)، وقد أشارت النتائج إلى أن كلا المستخلصين الإيثانولي والمائي قد أعطوا نتائج جيدة ضد البكتريا الموجبة والسالبة لجرام، كما أظهرت النتائج احتواء أوراق نبات خف الجمل علي كمية كبيرة من المركبات الفينولية (453 mgGAE/g) وكمية من المركبات الفلافونويدية 166 mg QE/g والمركبات القلويدية 1.674 g/100g، وكذلك أظهرت أوراق خف الجمل نشاط مضاد للأكسدة، وبذلك يمكن استخدامها في العديد من المجالات الطبية مثل علاج العدوى الناتجة عن البكتيريا الممرضة، كما يمكن أيضًا تطوير نتائج هذا النبات الطبي إلى منتجات عشبية قياسية بسيطة التكلفة وآمنه وقد يكون بمثابة مصدر للعديد من المركبات المضادة للميكروبات.

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