ANTIBACTERIAL ACTIVITY OF WATER AND ETHANOLIC EXTRACTS OF Bauhinia variegata LEAVES

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Received: 19/05/2019 ; Accepted: 19/06/2019

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 mg GAE/ g), flavonoid 166 mg QE/g and total alkaloid 1.674 g/100 g. 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 -20ºC 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 residue 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:

\[
\text{Inhibition} \% = \frac{(\text{absorbance of control} - \text{absorbance of test sample})}{\text{absorbance of control}} \times 100.
\]

In vitro testing of residue 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 lipoxygenase pathway. Phenolic compounds were shown to inhibit the cyclooxygenase and lipoxygenase pathway (Ferrandiz et al., 1990; Ferrandiz and Alcaraz, 1991; Laughton et al., 1991). Flavanoids 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 variegata* 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
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•), hydrogen peroxide (H₂O₂), and superoxide anion (O₂⁻) 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 1. The proximate composition of studied leaves of Bauhinia vaiegata (g/100 g DW)

<table>
<thead>
<tr>
<th>Percentage of parameter</th>
<th>Bauhinia variegata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>9.9% (FW)</td>
</tr>
<tr>
<td>Proteins (%)</td>
<td>8.55% (DW)</td>
</tr>
<tr>
<td>Fats (%)</td>
<td>1.9% (DW)</td>
</tr>
<tr>
<td>Fibers (%)</td>
<td>31.6% (DW)</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>41.45% (DW)</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.9% (DW)</td>
</tr>
</tbody>
</table>

FW: Fresh weight    DW: Dry weight

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

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Total phenolic content (mg GAE/g sample DW)</th>
<th>Total flavonoid content (mg quercetin/g sample DW)</th>
<th>Total alkaloid (g/100 g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bauhinia variegata</td>
<td>453.672</td>
<td>166.502</td>
<td>1.674</td>
</tr>
</tbody>
</table>

### Table 3. The antioxidant activity of Bauhinia variegata leaves extract

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

<table>
<thead>
<tr>
<th>Parameters plant extracts</th>
<th>Free radical scavenging activity &quot;DPPH&quot; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40 (μg/ml)</td>
</tr>
<tr>
<td><em>Bauhinia variegata</em></td>
<td>81.990%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Inhibition zone diameter (mm/mg sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Sarcina ventriculi</em> (G+)</td>
</tr>
<tr>
<td>Control : DMSO</td>
<td>NI</td>
</tr>
<tr>
<td>1. (100 mg/ml)</td>
<td>19</td>
</tr>
<tr>
<td>2. (50 mg/ml)</td>
<td>19</td>
</tr>
<tr>
<td>3. (25 mg/ml)</td>
<td>20</td>
</tr>
<tr>
<td>4. (12.5 mg/ml)</td>
<td>18</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Inhibition zone diameter (mm/mg sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Sarcina ventriculi</em> (G+)</td>
</tr>
<tr>
<td>Control : DMSO</td>
<td>NI</td>
</tr>
<tr>
<td>1. (50 mg/ml)</td>
<td>15</td>
</tr>
<tr>
<td>2. (25 mg/ml)</td>
<td>14</td>
</tr>
<tr>
<td>3. (12.5 mg/ml)</td>
<td>10</td>
</tr>
<tr>
<td>4. (6 mg/ml)</td>
<td>NI</td>
</tr>
</tbody>
</table>

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*.

REFERENCES


Gad El-Moula, et al.


Bauhinia variegata

التأثير المضاد للبكتريا للمستخلص الماني والكحولي لأوراق نبات خف الجمل

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قسم الكيمياء الحيوية – كلية الزراعة – جامعة الزقاقون - مصر

للمركبات الطبيعية المستخرجة من أجزاء النبات المختلفة أهمية كبيرة في المجال العلاجي، ولذلك تم دراسة التأثير المضاد للبكتريا للمستخلص الإيثانيولي والماني لأوراق نبات خف الجمل على الكائنات الدقيقة (بكتريا موجبة لجرام الإيثانيولي والماني) وقد أظهرت النتائج أن كل المستخلصين (Serratia marcescens و Sarcina ventriculi) قد أظهروا تأثيرات متماثلة ضد البكتريا الموجبة والسالبة لجرام، كما أظهرت النتائج احتواء أوراق نبات خف الجمل على كمية كبيرة من المركبات الفينولية (453 mgGAE/g) وكمية من المركبات الفلافونويدية (166 mg QE/g) وكذلك أظهرت أوراق نبات خف الجمل نشاط مضاد للأكسدة، حيث يمكن استخدامها في العديد من المجالات الطبية مثل علاج العدوى الناتجة عن البكتيريا المرضية، كما يمكن أيضاً تطوير تناج هذا البذور الطبي إلى منتجات عشبية قياسية بسيطة التكلفة وأمانة وقد يكون بمثابة مصدر للعديد من المركبات المضادة للميكروبات.

المحكمون:

- أ/ د. إمام عبدالمجيد عبدالرحيم
- أ/ د. رجب عبدالفتاح المصري