ANTIMICROBIAL ACTIVITY OF *Hibiscus sabdariffa* AND *Sesbania grandiflora* EXTRACTS AGAINST SOME G–ve AND G+ve STRAINS

**DOI: 10.7904/2068–4738–VII(13)–17**

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**Abstract.** Medicinal plants such as *Hibiscus sabdariffa* and *Sesbania grandiflora* are widely used to treat different diseases caused by different microbes in several countries. The antimicrobial activities of *Hibiscus* and *Sesbania* extracts were examined in present work. The antimicrobial activity of the plant extracts were investigated against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This study exhibited strong antibacterial activity of the aqueous extract of *H. sabdariffa* in comparison to *S. grandiflora* stem and root ethanolic extracts. Results obtained from this study indicate that both extracts exhibited concentration dependent effect. *Hibiscus* extract showed the highest inhibition activity against all bacterial strains at concentration 500 mg/mL in comparison to *Sesbania* extract.

**Keyword:** Antimicrobial activity, *Hibiscus sabdariffa*, Inhibition zone, *Sesbania grandiflora*.

**Introduction**

Many naturally occurring compounds found in plants have been revealed to own biological activities including anti–allergic [PANICHAYUPAKARANANT et al., 2010, ANANTAWORASAKUL et al., 2011], anti–cholinesterase [ELDEEN et al., 2005, ANANTAWORASAKUL et al., 2011], antibacterial [OZCELİK et al., 2005, ELDEEN et al., 2005, ANANTAWORASAKUL et al., 2011], antifungal [OZCELİK et al., 2005, KUETE et al., 2010, MATIVANDLELA et al., 2006, ANANTAWORASAKUL et al., 2011; BAGIU et al., 2012], antiviral [OZCELİK et al., 2005, GLATTHAAR–SAALMULLER et al., 2001, ANANTAWORASAKUL et al., 2011], antioxidant [AHMAD et al., 2005, OKONOJI et al., 2007, ANANTAWORASAKUL et al., 2011], anti–inflammatory [PANICHAYUPAKARANANT et al., 2010, ELDEEN et al., 2005, ANANTAWORASAKUL et al., 2011, BOONKAEW CAMPER, 2005], antitumor [LAMPRONTI et al., 2006, HAQUE et al., 2000, ANANTAWORASAKUL et al., 2011], cytotoxic effects [BOONKAEW CAMPER, 2005, KAMATOU et al., 2008, ANANTAWORASAKUL et al., 2011], antityrosinase [LEE et al., 2011, ANANTAWORASAKUL et al., 2011] and anti–plasmodial [KAMATOU et al., 2008, ANANTAWORASAKUL et al., 2011].

Medicinal plants are widely used in several countries such as Thailand and other Asian countries like China, Japan, and India either directly or indirectly. The world health organization (WHO) has accepted so far, an inventory of more than 20,000 species of medicinal plants.

They or their products are used in controlling various diseases and disorder symptoms as cough, fever, bronchitis, itching, pneumonia, ulcers, and diarrhea [ANANTAWORASAKUL et al., 2011].

*Hibiscus sabdariffa* is reported as one of the most important medicinal plants that have been widely used.

It is an annual, tropical or subtropical shrub grown in many countries including India, Thailand, Sudan, and Mexico.

The calyces of *H. sabdariffa* are used in the preparation of a beverage whereas many species of *Hibiscus* are used as ornamentals [HIGGINBOTHAM et al., 2014].

*Hibiscus* extracts have been demonstrated to have a wide range of antimicrobial activity against many bacterial strains, for instance.

Methanol extractions of the calyces have established to possess antimicrobial activity against each of *Staphylococcus aureus*, *Bacillus stearothermophilus*, *Serratia marcescens*, *Clostridium sporogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas* sp. at different concentrations [HIGGINBOTHAM et al., 2014, PRESCOTT et al., 2002, RASHED and BUTNARIU, 2014a].

Likewise *Sesbania* herb, which is one of the Leguminosae family, consists...
Pseudomonas aeruginosa, another G–ve bacterium, is an opportunistic pathogen that normally dwells the soil and surfaces in aqueous environments.

Its antibiotic resistance ability facilitates its survival in a wide range of other natural and artificial settings [GELLATLY HANGCOCK, 2013]. Moreover, it also owns a remarkable aptitude to develop or acquire new antibiotics resistance mechanisms [MESAROS et al., 2007]. P. aeruginosa infections are often nosocomial, and approximately all are accompanied by compromised host defenses such as in severe burns and cystic fibrosis [LYCZAK et al., 2000, GELLATLY HANGCOCK, 2013]. P. aeruginosa infections demonstrate high morbidity and mortality [GELLATLY HANGCOCK, 2013].

Furthermore, Staphylococci are Gram–positive bacteria, many of which preferentially colonize the human body [KLOOS BANNERMAN, 1994, HARRIS et al., 2002].

Generally, Staphylococci are able to tolerate high salt concentrations [WILKINSON, 1997] and exhibit heat resistance [ALBERT BALOWS et al., 1991].

Pathogenic staphylococci are commonly having the ability to produce coagulase, and consequently cause blood clot [KLOOS MUSSELWHITE, 1975, HARRIS et al., 2002].

S. aureus is found naturally on the skin and in the nasopharynx of the human body. As a result of the rise in antibiotic resistance, S. aureus is of increasing importance [LOWY, 2000].

This bacterium can cause local infections of the skin, nose, urethra, vagina and gastrointestinal tract [SHULMAN NAHMIAS, 1972, HARRIS et al., 2002].

The aim of the present study is to evaluate the antimicrobial activity of two selected plants; Hibiscus sabdariffa and Sesbania grandiflora. The antimicrobial activity of the plant extracts were estimated against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa, respectively.

Material and methods

Plant material

Hibiscus sabdariffa flowers were collected from local markets in Baghdad, Iraq. While, Sesbania grandiflora was

of about 60 species that are widely distributed throughout tropical and subtropical regions. Most of its species are annual herbs or shrubs, nevertheless a few are small trees [BURGESS et al., 1990, ANANTAWORASAKUL et al., 2011]. Furthermore, many species of Sesbania are playing important role in soil improvement as green manures or agroforestry trees [PALM et al., 2001, ANANTAWORASAKUL et al., 2011].

In Thailand S. grandiflora has been widely used both for food and medicine. Both, leaves and flowers of S. grandiflora are utilized as food whereas its stem has been long used as a traditional medicine for treatment of ulcers in the oral cavity.

It has been stated that all parts of S. grandiflora such as roots, bark, gum, leaves, flowers and fruits are used for medicine in Southeastern Asia and India.

The juice of the leaves has been considered as anthelmintic and tonic and has been used to treat worms, fever and itchiness [ANANTAWORASAKUL et al., 2011].

S. grandiflora powdered roots in an appropriate amount of water have been established to decrease the rheumatic swelling when applied externally with moderate rubbing to the lesion [KASTURE et al., 2002, ANANTAWORASAKUL et al., 2011].

In addition, the bark has used as astringent and utilized to treat cough, vomiting, bronchitis, ulcers, diarrhea, dysentery and dental caries [PRESCOTT et al., 2002, KACHROO VIPIN et al., 2011].

Hence Hibiscus and Sesbania extracts were used to treat different diseases caused by different microbes including Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus.

These bacterial strains cause several diseases, for example, Some of E. coli strains can cause disease in healthy individuals such as enteric/diarrheal disease, urinary tract infections (UTIs) and sepsis/meningitis [NATARO KAPER, 1998, KAPER et al., 2004].

The G–ve E. coli naturally colonizes the gastrointestinal tract of mammals, including humans in usually a commensalism relationship [KAPER et al., 2004, ELENA et al., 2005; BUTNARIU et al., 2005; SAMFIRA et al., 2013].
gathered from gardens of University of Bagdad, Iraq.

**Method**

**Preparation of Raw Material**

_Hibiscus sabdariffa_ flowers as well as stems and roots of _Sesbania grandiflora_ were washed well with tap water then dried in shade. The dried materials were eventually grounded to form a fine powder. Powdered plant material of _Hibiscus_ was extracted with water as a solvent of extraction by maceration method while powdered stems and roots of _Sesbania_ were subjected to ethanol 95% for extraction by Soxhlet apparatus.

Both crude extracts were concentrated by incubator with 40°C separately. Then the dried materials were weighted and dissolved in sterile distilled water. Two concentrations (250 and 500 mg/mL) for each extract were prepared.

**Bacterial cultures preparation**

In the meantime, growth cultures of _Escherichia coli_, _Staphylococcus aureus_ and _Pseudomonas aeruginosa_ were prepared and incubated overnight at 37°C. Each bacterial culture was then suspended in 0.9% NaCl, and diluted to a McFarland standard No. 0.5 [NCCLS, 1992, ANANTAWORASAKUL et al., 2011].

This bacterial suspension was used later to test the antibacterial activity.

**Determination of antimicrobial activity**

Disc diffusion assay [BAUER et al., 1966, ANWAR IBRAHIM et al., 2014; BUTNARIU et al., 2014] was used in order to investigate the antimicrobial activity of the tested extracts. 0.1 mL of the bacterial suspensions was spread uniformly on nutrient agar plates.

Filter paper discs in 5.5 mm diameter were prepared and sterilized. First group of these discs were loaded, by sterile micropipette, with 10 μL of 50% ethanol [ANANTAWORASAKUL et al., 2011].

Ten microliters of 30µg/mL streptomycin were loaded to a second group of discs as the inhibitory capability of streptomycin for both G–ve and G+ve was established [TODAR, 2004].

_Hibiscus_ and _Sesbania_ extracts were added to third and fourth group of sterile discs, respectively, with concentrations of (250 and 500 mg/mL) for each extract.

The loaded discs were allowed to dry, placed on the plates and subsequently incubated overnight at 37°C. The diameter of inhibition zone was measured in order to evaluate the antimicrobial activity.

The experiment was carried out in duplicate and the mean of diameter of the inhibition zone was calculated.

**Results and discussion**

Each extract was tested for its antibacterial activity against _E. coli_ and _P. aeruginosa_ as representations of Gram negative, while _S. aureus_ represented Gram positive bacteria.

**Table 1.**

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Concentration mg/mL</th>
<th>Diameter of Inhibition zone (mm)ᵃ</th>
<th><em>Escherichia coli</em></th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Pseudomonas aeruginosa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hibiscus sabdariffa</em></td>
<td>250</td>
<td>13.55</td>
<td>11.61</td>
<td>8.45</td>
<td>8.52</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>17.47</td>
<td>15.34</td>
<td>9.52</td>
<td>9.52</td>
</tr>
<tr>
<td><em>Sesbania grandiflora</em></td>
<td>250</td>
<td>21.71</td>
<td>29.51</td>
<td>26.67</td>
<td>26.67</td>
</tr>
<tr>
<td>stems</td>
<td>500</td>
<td>7.45</td>
<td>6.67</td>
<td>7.83</td>
<td>7.83</td>
</tr>
<tr>
<td><em>Sesbania grandiflora</em></td>
<td>250</td>
<td>9.46</td>
<td>8.91</td>
<td>9.81</td>
<td>9.81</td>
</tr>
<tr>
<td>roots</td>
<td>500</td>
<td>Antibiotic</td>
<td>24.97</td>
<td>31.8</td>
<td>27.95</td>
</tr>
<tr>
<td></td>
<td>N.Dᵇ</td>
<td>8.51</td>
<td>N.D</td>
<td>6.96</td>
<td>6.96</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>N.D</td>
<td>10.72</td>
<td>31.71</td>
<td>28.69</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>Antibiotic</td>
<td>N.D</td>
<td>10.72</td>
<td>6.96</td>
</tr>
</tbody>
</table>

ᵃ data are represented as mean of 2 replications; ᵇ the used antibiotic is Streptomycin (30μg/mL); ᵆ N.D represents none determined inhibition zone
Streptomycin was used as a positive control. On the other hand, 50% ethanol was considered as a negative control.

Results shown in Table 1 indicate that both extracts exhibited concentration dependent effect. Hibiscus extract showed the highest inhibition activity against all bacterial strains at concentration 500 mg/mL in comparison to Sesbania extracts with a diameter of inhibition zone of 17.47, 9.52 and 15.34 mm for E. coli, P. aeruginosa and S. aureus, respectively.

In general, the H. sabdariffa aqueous extract has shown more biological activity than the corresponding concentration of S. grandiflora.

Even thought, a recent study revealed that Hibiscus sabdariffa did not produce antibacterial activity against tested bacterial strains [ANWAR IBRAHIM et al., 2014].

This might be related to the differences in the extraction methods that have been used.

In contrast, [NDUKWE et al., 2005] suggested that the susceptibility of Gram–negative organisms were less than Gram–positive organisms to activity of different plants [KADHEM ABDUL et al., 2007; BUTNARIU and CAUNII, 2013; RASHED and BUTNARIU, 2014b; BUTNARIU and SAMFIRA, 2012].

However, the results of the present work propose different patterns ignoring bacterial type. S. grandiflora stem extract showed a close influence to H. sabdariffa at concentration of 500 mg/mL against P. aeruginosa.

It exhibited the second impact towards bacteria under investigation except against S. aureus where the Sesbania roots extract revealed higher effect than stems.

Even if, [KACHROO VIPIN et al., 2011; BUTNARIU, 2014; IANCULOV, et al., 2005] has demonstrated higher inhibition of E. coli and P. aeruginosa at 250 mg/mL.

Worth to mention the differences in the extraction could affect the results.

Conclusions

A wide range of safe alternative medications using for preventing, curing infections and to get rid of the problematic multi–resistant microbes are investigated especially from plant resources due to their effectiveness.

As conclusion from the results mentioned above, H. sabdariffa extract revealed an effective biological activity against G+ve and G–ve strains compared with S. grandiflora extracts.

The results of this work afford a high light of the potentiality of these plants in drug improvement and provide valuable information in this field.

Nevertheless, investigations are in demand to determine that these effects in vitro are achievable in vivo.

Acknowledgment

The author is so grateful to Miss Farah J. Hashim from University of Baghdad and Dr. Raed M. Qadir from University of Duhok for their encouragement and support in carrying out and revising the work.

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Received: December 29, 2015
Article in Press: April 23, 2016
Accepted: Last modified on: May 20, 2016