ANTIMICROBIAL POTENTIALITY OF PETIOLE EXTRACTS OF 
**APIUM GRAVEOLENS** L

Kiran C Nilugal*, Asif iqbal chittur, Nishaidevi, Ugandar RE

1School of pharmacy Asia Metropolitan University, G-8, Jalan Kemacahaya, Taman Kemacahaya, Batu 9, Cheras- 43200, Selangor, Malaysia

2School of Biomedicine Asia Metropolitan University, G-8, Jalan Kemacahaya, Taman Kemacahaya, Batu 9, Cheras- 43200, Selangor, Malaysia

3Faculty of Pharmacy, Masterskill Global College, Kuching, Sarawak, Malaysia.

ABSTRACT

In the present research, an aqueous and ethanol extracts of *apium graveolens* L at concentration range between 130000 µg/mL to 13 µg/mL was evaluated for antimicrobial potentiality. MIC values were determined for extracts by measuring the zone of inhibition using a disc diffusion method on direct inoculated plates of *N. gonorrhoeae* and *C. albicans*. Both extracts showed different sensitivity levels for the tested strains of microorganism and the inhibition zones ranged between 20.00±2.00 to 6.67±0.58. Among two tested strains, *N. gonorrhoeae* is more susceptible to extracts while compared to *C. albicans* and among two extracts used aqueous extract find to be more potent compared to ethanol extract as compared to the standard antibiotic used.


INTRODUCTION

World Health Organization (WHO) reported that 75 - 95% of the world populations of developing countries rely on traditional medicines and therapies involves the use of plant extract products or their active constituents. The use of an ethnomedicinal plants as a source for relief from illness can be traced back over five millennia to a written documents of
the early civilization in China, India and the Near east. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller and these plants are still widely used\[^3\] and are the invaluable sources of pharmaceutical products that have drawn the attention from around the world.\[^4\]

Apium graveolens L. or Celery (Family- Apiceae/Umbelliferae), is a native of Eurasia and is an edible vegetable was first describe by the Greeks, Egypt and it is also indigenous to India, Nepal, and China and has been cultivated for over 2000 years in the temperate zones as an important garden crop and vegetable. It boasts a very pleasant and distinct odor and this was one of the reason why celery is occupied the top rank vegetable in aromatic herb, spice in stews, in salads, in soups, as mix in cocktail drinks, in aromatherapay and celery seeds were used as a food flavoring agents.\[^5\]-\[^7\]\ The plant is also known for its numerous synonyms viz., India (Hindi): Karnauli, Ajmod; French: Celeri; German: Sellerie; Spanish: Apio; Russian: Syel’derey; Italian: Sedano.\[^8\]

A. graveolens attributed to its ethnomedicinal uses since ancient times by traditional healers and physicians. A. graveolens active ingredient from celery seeds is reported in Indian polyherbal formulations and used to treat bronchitis, asthma, liver disease, spleen disease and also screened for anticarcinogenic and life-protecting activity.\[^7\]\ Seeds decoction were used for bronchitis, rheumatism and arthritis and as a sedative, blood purifier. Juices extracted from the stem were used to treat edema, rheumatic tendencies, gout, and flatulence, overweight, lack of appetite and as a strong diuretic and an antiseptic.\[^9\]\ Chemically, A. graveolens contains a class of phenolic compounds called caffeic acid, caffeoylquinic acid, cinnamic acid, coumaric acid, ferulic acid, falvones such as apigenin and luteolin, flavonols such as quercetin and kaempferol, lunularin, dihydrostilbenoids, phytosterols, beta-sitosterol, furanocoumarins like psoralen, xanthotoxin and bergapten and phthalide derivatives such as sedanolide and senkyunolides.\[^10\]-\[^14\]\[^6\]-\[^8\]\ Further to this, celery contains vitamin A, B1, B2, B6, C, E, K and minerals such as iron, calcium, phosphorus, magnesium, molybdenum and zinc. Thus; celery has the ideal quantities of iron and magnesium to stop oncological diseases. A very good source of healthy heart dietary fiber and contains approximately 35 milligrams of sodium per stalk.\[^15\]-\[^16\]\[^9\]\ It also contains a hefty dose of the unique compound 3-n-butylphthalide, which has shown to enhance cognitive function in a mouse model of Alzheimer's disease\[^16\], primary photosynthetic products like mannitol and sucrore\[^17]\, a
pectin based polysaccharides apiuman which has shown special importance in producing anti-inflammatory benefits.\textsuperscript{[13-14]} Apart from the presence of different chemical components, the herb \textit{A. graveolens} or celery also possess a wide spectrum of pharmacological properties, which are reported in the literature viz., cardiovascular benefits\textsuperscript{[18]}, stress tolerance\textsuperscript{[17]}, ACE inhibitor\textsuperscript{[18]}, smooth muscle relaxant\textsuperscript{[20]}, anti-diabetic\textsuperscript{[21-22]}, anti- \textit{H.pylori}\textsuperscript{[22][14]}, antimicrobial\textsuperscript{[23-31]}, skin disorders\textsuperscript{[32]}, mosquitocidal\textsuperscript{[10]}, nematicidal\textsuperscript{[10]}, antifungal\textsuperscript{[10][32], antioxidant\textsuperscript{[6-7][9]}, gastric anti-ulcer, antisecretory and cytoprotective activity.\textsuperscript{[13]}

Although there are many ethnomedicinal plants and plant products which are used widely to treat bacterial and fungal infection today, the search is still continue for a comprehensive, broad spectrum, potent antimicrobial drug of natural origin. In view of the above observation, we thought it was worthwhile to carry out the \textit{in vitro} study on antibacterial and antifungal activity of aqueous and ethanol extracts of the petioles of \textit{apium graveolens} L. or Celery against selected bacterial \textit{Neisseria gonorrhoeae} (\textit{N. gonorrhoeae}) and fungi \textit{Candida albicans} (\textit{C. albicans}) strains.

\section*{MATERIALS AND METHODS}
\subsection*{Plant Material and Solvent Extraction}
The herb \textit{apium graveolens} L or celery were collected locally from Klang, Selangor. Both the \textit{apium graveolens} L leaves and stem were sent to Institute of Bioscience, University Putra Malaysia for species authentication. The voucher specimen number is SK 2235/13. Any type of adulteration was strictly avoided during collection, after collection and during storage until next use. The leaves were washed thoroughly under running water, to remove any mud, clay and adhering particles and leaves were all removed immediately after washing in order to collect petioles for the study. Petioles were rinsed in distilled water, drained, cut into small pieces and air dried under shades until there is no differences in the weight. Dried petioles pieces were then grounded into coarse powder form by using a grinder and stored in a well closed container until further used.\textsuperscript{[33-34][7]}

Extraction was carried out for each solvent (750 mL) by cold maceration of coarse powder (100 g) to obtain crude extracts of distilled water and 95% ethanol. Maceration was done for 3 days at room temperature. The soaking waste residues were filtered off by using muslin cloth to obtain the crude extract filtrates. The collected filtrates were then evaporated in a waterbath at 50 °C to dryness and the solid form of crude extracts was obtained after 2 weeks of evaporation process.
Bacterial and Fungal Strains and Growth Conditions

Bacteria culture of *N. gonorrhoeae* and fungi culture of *C. albicans* obtained from Premier Diagnostic Malaysia were used in the present study. Stock cultures were maintained aseptically under optimal conditions for each microorganism and subcultured onto Thayer Martin Agar, Blood Agar and Chocolate Agar plates\(^{[35-36]}\) and were then placed into a candle jar supplied with carbon dioxide followed by incubation at 37 °C for 24 hours for *N. gonorrhoeae* strain and fungi strain was subcultured onto Sabouraud Dextrose Agar and Blood Agar plates followed by incubation at 37°C for 24-48 hours. The type of bacteria present on each agar plate was confirmed through gram staining (Figure 1) and catalase test (Figure 2)\(^{[37-39]}\) and the colonies of *C. albicans* were confirmed by conducting the Indian ink staining (Figure 3) and germ tube test (Figure 4).\(^{[40-42]}\)

Antimicrobial Activity

The initial concentration of test solution to conduct minimum inhibitory concentration (MIC) was calculated in g/L by using initial amount of plant macerated in solvent followed by calculating the concentration to obtain the extract per 10 ml.\(^{[43]}\)

\[
\text{Concentration} = \frac{\text{Weight of coarse powder (g)}}{\text{Volume of solvent (L)}}
\]

\[
= \frac{100\text{g}}{0.75\text{L}}
\]

\[
= 133.3\text{g/L}
\]

\[
= 133.3\text{g/1000ml}
\]

\[
= 0.13\text{g/ml}
\]

For 10ml = 1.30g/10ml

**Minimum inhibitory concentration (MIC)**

MIC values were determined for aqueous and ethanol extracts to evaluate the antimicrobial potentiality by measuring the zone of inhibition using a disc diffusion method on direct inoculated plates. The extracts were tested over a range of concentrations from 130000 µg ml\(^{-1}\) to 13µg ml\(^{-1}\) against 24 hours broth cultures of *N. gonorrhoeae* and *C. albicans*. A grade AA discs with 6 mm diameter (Sterile blank discs, Whatman International Ltd. England) soaked in each concentrations of extracts were placed on the plates of Mueller Hinton agar and Sabouraud dextrose agar followed by incubation at 37 °C for 24-48 hours. Plates were observed after 24 hours and also 48 hours and inhibition zones were measured (Figure 5-8). The experiments were replicated three times with duplicate samples per replicate and data was collected, analyzed and were summarized in Table 1.\(^{[44-48]}\)
Minimum bactericidal concentration (MBC)

The MBC was defined as the lowest concentration of antimicrobial agent or the extract, which inhibits or killed a particular microorganism. To determine MBC, samples were swiped by using a cotton swab from plates with no visible growth or clear zone in MIC assay and subcultured on freshly prepared Mueller Hinton agar plate for *N. gonorrhoeae* and Sabouraud dextrose agar plate for *C. albicans* followed by incubation at appropriate temperature for 24-48 hours. Plates were observed after 24 and 48 hours for any visible growth and as defined formerly, the MBC was taken as the concentration of the extract that did not show any growth on new set of agar plates.[49-52]

Antimicrobial susceptibility testing (AST)

The AST was carried out by measuring zone of inhibition using disc diffusion method against standard antibiotic (Doxycycline and Amphotericin B) and control discs (discs soaked in distilled water and ethanol) which were used for comparison to detect the drug resistance in study pathogens and to assure susceptibility to drugs of choice (Figure 9-10). AST results were summarized in Table 2a-2b.[44]

RESULTS

**Confirmatory Test for *N. gonorrhoeae* and *C. albicans***

**Gram staining:** Gram staining was done to identify the morphology of *N. gonorrhoeae* and to determine the organism is of gram positive or gram negative. The microscopic examination showed the organism as gram negative. It appeared in red and was arranged in diplococcic form (Figure 1).

**Catalase test:** The catalase test facilitates the identification of enzyme catalase in the bacteria and it was done by using 3% hydrogen peroxide to see the bubble formation. Bubble formation was seen within few seconds (Figure 2).

**India ink staining:** The India ink staining revealed the clearer view of the budding formation of the yeast represented by *C. albicans* (Figure 3).

**Germ tube test:** Test was carried out by using human serum followed by incubation at appropriate temperature for 24-48 hours. The germ tube test allowed the viewing of the pseudohyphae formation of the *C. albicans* (Figure 4).
The effects of extracts on organisms were measured and summarized in Table 1 and Table 2a-2b and discussion was made in the next section.

Table 1: Minimum inhibitory concentration (MIC) of petiole extracts of *Apium Graveolens* L (Celery).

<table>
<thead>
<tr>
<th>Extract concentration</th>
<th>Zone of inhibition (in mm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>N. gonorrhoeae</em></td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td></td>
<td>20.00+2.00</td>
<td>12.67+2.08</td>
</tr>
<tr>
<td>130000µg/ml</td>
<td></td>
<td>19.33+1.53</td>
<td>12.33+2.52</td>
</tr>
<tr>
<td>13000µg/ml</td>
<td></td>
<td>18.67+1.53</td>
<td>12.67+3.06</td>
</tr>
<tr>
<td>1300µg/ml</td>
<td></td>
<td>19.00+1.73</td>
<td>7.67+2.08</td>
</tr>
<tr>
<td>130µg/ml</td>
<td></td>
<td>19.00+1.00</td>
<td>8.33+1.53</td>
</tr>
<tr>
<td>Ethanol Extract</td>
<td></td>
<td>19.67+2.52</td>
<td>10.33+4.16</td>
</tr>
<tr>
<td>130000µg/ml</td>
<td></td>
<td>18.33+0.58</td>
<td>10.67+1.53</td>
</tr>
<tr>
<td>13000µg/ml</td>
<td></td>
<td>18.67+1.53</td>
<td>6.67+0.58</td>
</tr>
<tr>
<td>130µg/ml</td>
<td></td>
<td>18.33+0.58</td>
<td>8.00+1.00</td>
</tr>
<tr>
<td>13µg/ml</td>
<td></td>
<td>18.33+0.58</td>
<td>0</td>
</tr>
</tbody>
</table>

All values are in mean ± SD. *N*=3 (the experiment was performed in three replicates)

Table 2a: Antimicrobial Susceptibility Test (AST) against *N. gonorrhoeae*.

<table>
<thead>
<tr>
<th>Extract and Disc</th>
<th>Zone of inhibition (in mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>N. gonorrhoeae</em></td>
<td></td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Distilled Water</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>18.8+1.30</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>37.6+3.91</td>
<td></td>
</tr>
<tr>
<td>Ethanol Extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>3.00+4.12</td>
<td></td>
</tr>
<tr>
<td>Ethanol Extract</td>
<td>19.20+1.30</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>35.80+5.02</td>
<td></td>
</tr>
</tbody>
</table>

All values are in mean ± SD. *N*=5 (the experiment was performed in five replicates)
Table 2b: Antimicrobial Susceptibility Test (AST) against *C. albicans*.

<table>
<thead>
<tr>
<th>Extract and Disc</th>
<th>Zone of inhibition (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>C. albicans</em></td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>0</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td>11.8±2.05</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>14.2±2.28</td>
</tr>
<tr>
<td>Ethanol Extract</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0</td>
</tr>
<tr>
<td>Ethanol Extract</td>
<td>15.4±3.21</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>13.8±2.59</td>
</tr>
</tbody>
</table>

All values are in mean ± SD. N=5 (the experiment was performed in five replicates)

Figure 1

Figure 2

Figure 3

Figure 4
Figure 5: Zone of inhibition of aqueous extract of Aegium Gerardii L against N. gonorrhoeae

Figure 6: Zone of inhibition of ethanol extract of Aegium Gerardii L against N. gonorrhoeae
Figure 7: Zone of inhibition of aqueous extract of *Aegium Cretaceum* L. against *C. albicans*

Figure 8: Zone of inhibition of ethanol extract of *Aegium Cretaceum* L. against *C. albicans*
DISCUSSION

In this study, the antimicrobial potentiality of petiole extracts of *Apium Graveolens. L* (Celery) was studied using disc diffusion method against *N. gonorrhoeae* and *C. albicans*. The aqueous and ethanol extracts (130000µg/ml - 13µg/ml) of *Apium Graveolens. L* showed different sensitivity levels for the tested strains of microorganism and the inhibition zones ranged between 20.00±2.00 to 6.67 ± 0.58 (Table 1). Among two tested strains, *N. gonorrhoeae* is more susceptible to extracts while compared to *C. albicans* (Figure 5-8) and
among two extracts used aqueous extract (130000µg/ml) find to be more potent compared to ethanol exact (130000µg/ml). This clearly shows petiole extracts of *Apium Graveolens. L* has potential antimicrobial property. At this point we can conclude that the type of solvent used to extract appeared to have impact on their activity. However, bear in mind that the extracts used were in crude forms. Generally, it is known through the literatures that crude extracts might contains various types of active compounds or wide variety of secondary metabolites, such as tannins and quinine which are responsible for plant pigment, terpenoids which give plants their odours and flavour, alkaloids, glycosides, saponins, flavonoids, lectin, to some extent aromatic substances, most of which are phenols or their oxygen substituted derivatives. These substances serve as plant defense mechanisms or known to be synthesized by plants in response to microbial infection or against predation by microorganisms, insects, and herbivores \[53\][28-29][34] and it shouldn’t be surprising that the presence of the above mentioned phytoconstituents naturally in all type of plants might help explain the observed inhibition zone.

Various biologically active substances from celery were identified, isolated and have been studied and found very interesting results against tested microorganism viz., sedanolide and two senkyunolides were active against nematode, mosquito larvae and fungi, furanocoumarins were inhibited *L. monocytogenes, E. coli* and *M. luteus* \[26\], essential oil, seeds and seed oil of celery found active against *Malassezia furfur* \[32\], *L. innocua, E. coli, S. aureus* and *P. fluorescens* \[40\] *C. jejuni* \[24\], *B. subtilis, E. coli* and *S. cerevisiae*. \[23\] Similarly, Mubarak et al., found celery seed extracts active against *H.pylori* and *S.aureus*. \[27\] The amount of nutrients and phytoconstituents present in celery is producing exciting opportunities to researchers for the expansion of modern chemotherapies against wide range of microorganism and this study warranted these claims.

**CONCLUSION**

It is plausible to conclude that the observed antimicrobial activity could be attributed to the presence of above mentioned chemical constituents and secondary metabolites of celery and we believe that purified forms of isolated compounds from these two extracts would show more potential activity.
ACKNOWLEDGEMENT

We would like to thank the Department of Pharmacy and Microbiology, Asia Metropolitan University, Cheras, Selangor Malaysia for providing us the laboratory facilities to conduct this study.

REFERENCES


27. Mubarak N, Thomas J Smith. Antimicrobial compounds from celery seeds (Apium graveolens L); towards a new generation of broad spectrum antibiotics., 2011; 1-3.


31. Zizovic. Antibacterial potential of celery and parsley fruit extracts obtained by supercritical fluid extraction for food industry applications. Institute for Medicinal Plant Research., 2012; 78-80.


