Antimicrobial activity and chemical composition of *Pistacia chinensis* Bunge leaves

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**Abstract**

This work was carried out for determining antimicrobial activity of *Pistacia chinensis* leaves methanol extract and identifying the chemical composition of the plant extract. Methanol extract was tested for antimicrobial activity using disc-diffusion assay and the extract was fractionated on silica gel column chromatography for the isolation of the bio-active constituents. The leaves extract of *P. chinensis* showed a significant antimicrobial effect, it strongly inhibited the growth of the test bacteria and yeast studied. Chromatographic separation of the methanol extract of *P. chinensis* leaves has led to the isolation and characterization of β-sitosterol, luepol, and six flavonoids, quercetin, myricetin, quercetin 3-O-α-rhamnoside, quercetin 3-O-β-glucoside, myricetin 3-O-α-rhamnoside and myricetin 3-O-β-glucuronide using various chromatographic procedures and the interpretation of spectral data in comparison with already existing data reported in the literature. The results presented here may suggest that the leaves extract of *P. chinensis* possess antimicrobial properties, and therefore, can be used as natural preservative ingredients in food and/or pharmaceuticals.

**Introduction**

The use of herbal medicine for the treatment of diseases remains the main stay of health care system and is gaining increasing popularity in developing countries. Research programs have been going on to assay and improve the medicinal principles found in drugs for use in the development of new pharmacotherapeutic agents in the management and cure of diseases (Sofowara, 1993).

The bacterial organisms including gram positive and gram negative like different species of *Bacillus, Staphylococcus, Salmonella* and *Pseudomonas* are the main source to cause severe infections in humans (Nathan, 2004). Resistance to antimicrobials is a significant and growing problem, limiting treatment options, especially for serious gram positive infections, among them *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Enterococcus faecalis*, and gram negative bacteria such as *Klebsiella pneumonia, Escherichia coli* and *Pseudomonas aeruginosa*. Plant derived antibacterials are always a source of novel therapeutics. Historically, plants have been placed at top among the sources of novel drugs with antimicrobial activity, as traditional medicines based on plants and plant extracts have made considerable contributions to human health and well-being.

*Pistacia chinensis* is a deciduous small tree from Anacardiaceae family. It is widely distributed in China and North America. It is well known as a landscape and shade tree (Widodo, 1998). In Chinese traditional medicine, the oil from its seeds is used for biodiesel production in China and it shows a high resistance to various pests in the United States (Widodo, 1998).

There are few reports about chemical constituents and biological activities from *P. chinensis* plant, two 4-arylcoumarin moieties (neoflavone) dimers were isolated from of *P. chinensis* leaves with estrogen-like activity (Nishimura et al., 2000). Also some phenolic compounds such as gallic acid, m-digallic acid, quercetin, 6-O-galloyl arbutin-quecitrin, and quercetin -3-O(6’’-galloyl)-β-D-glucosides were isolated from the leaves (Shi and Zuo, 1992) and a new pyrrolidone derivative was isolated from *P. chinensis* tender burgeon, and anthotaxy (Jian et al., 2008). *P. chinensis* has the ability to inhibit NO production as anti-inflammatory potential of this plant (Yayeh et al., 2012). Aerial parts of the plant...
and its compounds showed an anti-HCV activity (Rashed et al., 2014). The present study aimed to investigate antimicrobial activity of methanol extract of P. chinensis leaves and to identify the constituents responsible for the observed activity.

Materials and Methods

General experimental procedures


Plant identification and collection

Leaves of P. chinensis were collected from Al-Zohiriya garden, Giza, Egypt in May 2013. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereeza Labib consultant of plant taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen is deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

Preparation of the extract

Air-dried powder of P. chinensis leaves (420 g) was extracted with methanol 80% several times at room temperature until exhaustion by maceration method. The extract was concentrated under reduced pressure to give 26 g of the crude extract.

Isolation of the compounds from P. chinensis leaves methanol extract

Twenty five grams of the extract was subjected to silica gel column chromatography eluting with n-hexane, dichloromethane, ethyl acetate and methanol gradually. One hundred and forty fractions of 100 ml conical flask were collected. The fractions that showed similar Paper Chromatography (PC) in Butanol–Acetic acid–Water 4:1:5 (BAW) and 15% acetic acid were combined to give 4 fractions (I, II, III, and IV). Fraction I (2.45 g) was subjected to sub–column of silica gel eluted with n-hexane: dichloromethane (50:50) gave compound 1 and elution with dichloromethane: n-hexane (80:20) gave compound 2. Fraction II (1.85 g) was subjected to sub–column of silica gel eluted with dichloromethane: ethyl acetate (90:10) yielded compound 3 and elution with ethyl acetate solvent gave compound 4. Compound 5 yielded from elution with ethyl acetate:methanol (90:10) and compound 6 was obtained from elution with ethyl acetate: methanol (80:20) from fraction III (2.75 g). Compound 7 yielded by elution with ethyl acetate: methanol (75:25) and compound 8 was obtained by elution with ethyl acetate: methanol (60:40) from fraction IV (3.22 g). All the isolated compounds were purified on sephadex LH–20 column using different systems of methanol and distilled water.

General method for acid hydrolysis of flavonoid glycosides

Five milligram of each flavonoid glycoside 5, 6, 7 and 8 in 5 ml 10% HCl was heated for 5 h. The aglycones were extracted with ethyl acetate and identified by co-TLC with authentic standards. The sugars in the aqueous layer were identified by co-paper chromatography (co-PC) with authentic markers on Whatman No. 1 sheets in solvent system (n-BuOH-AcOH-H2O 4:1:5 upper layer).

Antimicrobial tests

The bacterial and yeast strains used are described in Table 1. These microbial strains were isolated from human and food beings and belong to the microbiological laboratory collection of the department of microbiology from Suez Canal University, Egypt. Nutrient agar (for bacterial strains) and Sabouraud dextrose agar media (for yeast strains) were inoculated with this suspension of the respective organism and poured into a sterile petri dish.

The agar diffusion assay was performed according to the modified Kirby-Bauer disc diffusion method (Selim et al., 2013). One ml of each test organism liquid culture was individually suspended in 3 ml of a 0.9% NaCl solution. The essential oil (EO) and methanol extract were dissolved in 10% dimethylsulfoxide (DMSO) to a final concentration of 30 mg/ml as stock solution and sterilized by filtration through 0.45 μm Millipore filters. Antimicrobial tests were then carried out using 100 μl of suspension containing 10⁸ cfu/ml of bacteria and 10⁶ cfu/ml of yeast spread on nutrient agar and Sabouraud dextrose agar media, respectively. The discs (6 mm in diameter) were impregnated with 100 μg of the essential oil and methanol extract, and then placed onto inoculated agar. Negative controls were prepared using the same solvent employed to dissolve the extract. The
inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains and 48 h for yeast isolates. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

Results and Discussions

The present study was focused on evaluation of the antimicrobial activity of *P. chinensis* leaves methanol extract as can be seen in table 1 as well identification of the main constituents of *P. chinensis* leaves methanol extract which were, β-sitosterol, lupeol, and six flavonoids, quercetin, myricetin, quercetin 3-O-α-rhamnosoide, quercetin 3-O-β-glucoside, myricetin 3-O-α-rhamnosoide, and myricetin 3-O-β-gluronide. The chemical structures of the isolated compounds are shown in Figure 1.

<table>
<thead>
<tr>
<th>Gram Positive Bacteria</th>
<th>Inhibition zone in diameter (mm)</th>
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<tbody>
<tr>
<td><em>Bacillus cereus</em></td>
<td>Food</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Human</td>
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<tr>
<th>Gram Negative Bacteria</th>
<th>Inhibition zone in diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aeromonas hydrophila</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Brevundimonas vesicularis</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em></td>
<td>Food</td>
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<tr>
<th>Yeast</th>
<th>Inhibition zone in diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>Human</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em></td>
<td>Human</td>
</tr>
</tbody>
</table>

*Inhibition zone in diameter (mm) around the discs impregnated with extract (50 µg/disc).

Structure Elucidation of the isolated compounds

β-sitosterol (1): 17 mg, white needles. 1H-NMR (CDCl₃, 400 MHz): δ ppm 5.37 (IH, m, H-6), 3.52 (IH, m, H-3), 1.09 (3H, s, CH₃-19), 0.98 (3H, d, J= 6.5, CH₂-21), 0.92 (3H, t, J= 7.4, CH₂-29), 0.85 (3H, d, J= 6.7Hz, CH₂-26), 0.81 (3H, d, J= 6.7Hz, CH₂-27), 0.75 (3H, s, CH₃-18). 13C-NMR(CDCl₃,100 MHz): δ ppm 140.46 (C-5), 121.52 (C-6), 71.64 (C-3), 57.25 (C-7), 56.48 (C-14), 50.35 (C-9), 46.38 (C-24), 42.82 (C-13, 4), 39.88 (C-12), 37.64 (C-1), 36.75 (C-10), 35.92 (C-20), 34.24 (C-22), 31.78 (C-8, 7), 31.45 (C-2), 29.24 (C-25), 28.46 (C-16), 26.24 (C-23), 24.54 (C-15), 23.48 (C-28), 21.14 (C-11), 19.82 (C-26), 19.58 (C-19), 19.24 (C-27), 18.68 (C-21).

Lupeol (2): 15 mg, white powder. 1H-NMR (CDCl₃, 400 MHz): δ ppm 0.75, 0.8, 0.85, 0.96, 0.98, 1.08, 1.75 (each 3H, s), 3.25 (1H, dd, J = 5.6, 10.8 Hz, H-3), 4.58 (1H, s, H-29a), 4.68 (1H, s, H-29b), 13C-NMR(CDCl₃, 100MHz): δ ppm 151.46 (C-20), 108.78 (C-29), 78.64 (C-3), 55.82 (C-5), 50.75 (C-9), 48.72 (C-18), 48.42 (C-19), 43.25 (C-17), 43.28 (C-14), 40.82 (C-8), 39.76 (C-22), 38.75 (C-4), 38.58 (C-8), 38.72 (C-13), 37.65 (C-10), 35.78 (C-16), 34.55 (C-7), 29.42 (C-21), 28.46 (C-23), 27.68 (C-2), 27.65 (C-15), 25.48 (C-12), 21.42 (C-11), 19.45 (C-30), 18.72 (C-6), 18.44 (C-28), 16.56 (C-25), 16.27 (C-26), 15.75 (C-24), 15.28 (C-27).

Quercetin (3): 9 mg, yellow powder. UV λmax (MeOH): 255, 267, 371; (NaOMe): 270, 320, 420; (AlCl₃): 270, 455; (AlCl₃/HCl): 264, 303sh, 315sh, 428; (NaOAc): 257, 274, 318, 383; (NaOAc/...
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Quercetin 3-O-α-rhamnoside (5): 16 mg, yellow crystals. 1H–NMR (DMSO–d6, 400 MHz) δ ppm 7.26 (2H, m, H–2`, 6`), 6.83 (1H, d, J=9 Hz, H–5’), 6.49 (1H,d, J=2.5 Hz, H–8),6.14 (1H, d, J=2.5Hz, H–6), 5.25 (1H, s, H–1’’) 0.78 (3H, d, J=6Hz). 13C–NMR (100 MHz, DMSO–d6): δ ppm 177.42 (C–4), 167.45 (C–7), 161.40 (C–5), 157.01 (C–2), 157 (C–9), 149.19 (C–4’), 145.57 (C–3’), 134.12 (C–3), 131.97 (C–6’), 121.40 (C–1’), 115.71 (C–2’), 115.40 (C–5’), 103.10 (C–10), 101.97 (C–1’’), 99.98 (C–6), 94.47 (C–8), 71.47 (C–4’’), 70.94, 70.85, 70.62 (C–2’’, C–5’’, C–3’’), 17.76 (C6’’).

Myricetin 3-O-β-glucuronide (8): 19 mg, yellow amorphous powder. UV λmax (MeOH): 262, 298sh, 349; (NaOMe): 272, 324, 392; (AlCl3): 272, 312, 428; (AlCl3/HCl): 270, 310, 404; (NaOAc): 270, 318, 366; (NaOAc/H3BO3): 260, 300, 374. 1H-NMR (CD3OD, 400 MHz): δ ppm 7.42 (2H, s, H-2’, 6’), 6.45 (1H, d, J = 1.2 Hz, H-8), 6.22 (1H, d, J = 1.2 Hz, H-6), 5.47 (1H, d, J = 7.5 Hz, H-1’’). 13C-NMR (CD3OD, 100 MHz ): δ 177.52 (C-4), 174.25 (C-6’’), 165.84 (C-7), 162.68 (C-5), 158.46 (C-9), 148.28 (C-2), 146.92 (C-3’’, 5’’), 137.55 (C-3), 137.15 (C-4’’), 123.32 (C-1’’), 108.86 (C-2’, 6’’), 104.74 (C-10), 104.18 (C-1’’), 99.52 (C-8), 94.64 (C-6), 78.26 (C-3’’), 78.42 (C-5’’), 75.66 (C-2’’), 73.48 (C-4’’).

Chromatographic separation of P. chinensis leaves methanol extract resulted in the isolation and identification of compound 1 which gave dark spot under short UV light on thin layer chromatography (TLC) that changed to violet colour on spraying with vanillin sulphuric and heating in an oven at 110°C for 5 min. NMR spectral data has shown signals very close to β-sitosterol (Patche et al., 2009). Compound 2 afforded a dark spot under short UV light and changed to pink to violet upon spraying with vanillin-sulphuric acid and heating in an oven at 110°C for 5 min. NMR spectral data showed signals very similar to lupeol (Abdullahi et al., 2013). Compounds 3, and 4 gave a yellow spot and appeared as fluorescence yellow colour after spraying with AlCl3 and its spectral data are very similar to that of quercetin 3-O-α-rhamnoside (Lawrence et al., 2005). Compound 5 yielded deep purple spot under UV light and changed to yellow when subjected to ammonia and AlCl3, and complete acid hydrolysis gave quercetin as an aglycone and rhamnose as sugar moiety and its spectral data was very similar to that of quercetin 3-O-α-rhamnoside (Lawrence et al., 2005). Compound 6 (quercetin 3-O-β-glucoside) is obtained as a deep purple spot and gave yellow colour when exposed to ammonia vapour and a bright yellow colour when spraying with AlCl3.

Complete acid hydrolysis of the compound gave quercetin as an aglycone and glucose as sugar moiety.
Spectral data is very close to spectra of quercetin 3-O-β-glucoside (Song et al. 2007). Compounds 7, and 8 are obtained as deep purple spot and both gave yellow colour when exposed to ammonia vapour and gave a bright yellow colour when spraying with AlCl3. Complete acid hydrolysis of the both compounds gave myricetin as an aglycone, rhamnose and glucuronic acid as sugar moieties, respectively. Spectral data of both compounds are very close to spectra of myricetin 3-O-α-rhamnopyranoside, and myricetin 3-O-α-glucuronide (Manguro et al., 2005).

Antimicrobial activity of P. chinensis leaves methanol extract

The in vitro antimicrobial potential of P. chinensis leaves methanol extract against a panel of microorganisms is shown in Table 1. The extract showed a significant antimicrobial activity against all tested bacteria, including gram positive, and gram negative ones with diameter zones of inhibition 9 to 28 mm. In the comparison of microbial sensitivity to the extract, Serratia marcescens seems to be more sensitive than other infectious pathogens such as E. coli. No remarkable activity was observed against S. aureus. The antimicrobial activity of P. chinensis leaves methanol extract was more pronounced against gram-positive than gram-negative bacteria. Generally, the higher resistance among gram-negative bacteria could be described to the presence of their outer phospholipidic membrane, almost impermeable to lipophilic compounds (Burt, 2004). The absence of this barrier in gram-positive bacteria allows the direct contact of the hydrophobic constituents with the phospholipids bilayer of the cell membrane, where they bring about their effect, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacteria enzyme. The antimicrobial activity of P. chinensis leaves methanol extract could, in part, be associated with their major constituents such as triterpenes and flavonoids. These components have been reported to bring about their effect, causing either an increase in ion permeability and leakage of vital intracellular constituents, or impairment of the bacteria enzyme. The antimicrobial activity of P. chinensis leaves methanol extract has a significant antimicrobial activity against some clinical bacteria. The phytochemical results showed that the ethanol extract contained mainly five flavonoids identified as luteolin-7-O-glycoside, luteolin-7, 3’-O-diglycoside, apigenin, quercetin-3-O-glycoside and kaempferol-3-O-glycoside. Among these molecules, quercetin-3-O-glucoside had the highest antibacterial activity, but the synergism between apigenin, quercetin-3-O-glycoside and kaempferol-3-O-glycoside afforded the best minimal inhibitory concentration for each tested species. The substitution type of flavonoids had and impact on the antibacterial activity (Souâd et al., 2009).

Conclusion

This present study provided scientific evidence that P. chinensis leaves methanol extract has a significant antimicrobial activity and this activity may be due to the presence of the constituents isolated from the extract. As the industries tend to reduce the use of chemical preservatives in their products, P. chinensis leaves methanol extract with potentially active antimicrobial properties might be considered as a natural source for the maintenance or extension of the shelf life of the products. These results indicate the possibility of exploitation of P. chinensis leaves extract as effective inhibitor of microorganisms. This is the first report on the antimicrobial activities of P. chinensis extract in the literature. The isolated compounds are expected to be useful for the study of antimicrobial agents in the future.

References


