Biological Activities of Plants used in Egyptian Ethnopharmacology

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INTRODUCTION

The medicinal use of plants in Egypt is many thousands of years old, although the best known record, the "Ebers Papyrus", dates from approximately 1500 BC (Borchardt, 2002). Much of this ancient knowledge survived until the present time, especially in rural areas, becoming a valuable contribution to the discovery of new active compounds which may be the scaffold of new drugs. Although several works have recently been published that report studies of this huge potential (Abou Zid et al., 2011; Rashed et al., 2012; El-Seedi et al., 2013; Eissa et al., 2014; Elbanna et al., 2016), it is still a largely unexplored resource worth investigating. Research on the scientific basis of traditional medicine has gained a new impulse, due to the UN World Health Organization recommendations in the recently published document “WHO Traditional Medicine Strategy 2014-2023” (UN World Health Organization, 2013). In the present study, six plants growing in Egypt, traditionally used for medical purposes, were chosen: Diospyros lotus (Ebenaceae), Bauhinia alba (Fabaceae), Toona ciliata (Meliaceae), Alhagi maurorum (Fabaceae), Terminalia muelleri (Combretaceae) and Pistacia chinensis (Anacardiaceae). Methanol extracts of aerial parts from these plants were tested for biological activities that might corroborate their therapeutic potential. The strongest antioxidant activities, higher than or comparable to the standard compounds used, were presented by Terminalia muelleri and Pistacia chinensis (EC50 of 4.0 and 4.7 µg/mL for DPPH and 7.0 and 49.3 µg/mL for the FeCl₃ reduction assay, respectively). These results strongly correlated with the polyphenol content of the extracts. Terminalia muelleri actively inhibited acetylcholinesterase (IC₅₀=222.9 µg/mL), while the other extracts were not active in the range of concentrations tested. Alhagi maurorum presented cytotoxicity against all the cell lines tested, particularly against HeLa tumor cell line (EC₅₀ = 16.8 µg/mL), with a SI (Selectivity Index) of 3 when compared with the control non tumor cell line.

MATERIALS AND METHODS

Plant collection and identification

Aerial parts of Pistacia chinensis and Bauhinia alba leaves were collected from Al-Zohiriya garden, Giza. Diospyros lotus aerial parts, Toona ciliata stems, Alhagi maurorum herb and Terminalia muelleri stems were collected at the Agricultural Research Centre. The plants were identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. TerezaLabib consultant of plant taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt.

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ABSTRACT

In the present study, six plants traditionally used in Egypt for medical purposes were chosen: Diospyros lotus (Ebenaceae), Bauhinia alba (Fabaceae), Toona ciliata (Meliaceae), Alhagi maurorum (Fabaceae), Terminalia muelleri (Combretaceae) and Pistacia chinensis (Anacardiaceae). Methanol extracts of aerial parts from these plants were tested for biological activities that might corroborate their therapeutic potential. The strongest antioxidant activities, higher than or comparable to the standard compounds used, were presented by Terminalia muelleri and Pistacia chinensis (EC50 of 4.0 and 4.7 µg/mL for DPPH and 7.0 and 49.3 µg/mL for the FeCl₃ reduction assay, respectively). These results strongly correlated with the polyphenol content of the extracts. Terminalia muelleri actively inhibited acetylcholinesterase (IC₅₀=222.9 µg/mL), while the other extracts were not active in the range of concentrations tested. Alhagi maurorum presented cytotoxicity against all the cell lines tested, particularly against HeLa tumor cell line (EC₅₀ = 16.8 µg/mL), with a SI (Selectivity Index) of 3 when compared with the control non tumor cell line.
Extraction method

Air-dried powder of each plant (300 g) was extracted by the maceration method with 70% methanol (3 L), several times for 48 hours at room temperature (25 °C), until exhaustion. The extract was concentrated and evaporated to dryness under reduced pressure at 40°C to give 12.4, 8.5, 7.8, 4.8, 10.6 and 9.7 grams of each plant extract, respectively for Diospyros lotus, Bauhinia alba, Toona ciliata, Alhagi maurorum, Terminalia muelleri and Pistacia chinensis.

Preliminary phytochemical analysis of the extracts

One g of each extract was dissolved in methanol, adding a few drops of distilled water for complete solubility, and then the extract was subjected to different phytochemical tests according to that described by Yadav and Agarwala (2011).

Total phenols were determined by the Folin-Ciocalteau method as described by Kuda et al. (2005) and expressed as mg Gallic Acid Equivalents (GAE) per g of dry extract.

Antioxidant and anti-acetylcholinesterasic assays

The antioxidant activity of the extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and FeCl₃ reduction assays (Barreto et al., 2012) and the in vitro anti-acetylcholinesterasic (anti-AChE) activity was assessed by a modification of the Ellman method (Arruda et al., 2012). Results for the DPPH and anti-AChE assays are expressed as EC₅₀ and IC₅₀, respectively, which is the concentration of extract which caused an effect of 50% (50% DPPH radical scavenging compared with the control without extract or 50% enzymatic inhibition in the case of anti-AChE activity). In the case of FeCl₃ reduction, EC₅₀ was calculated as the concentration of extract which yielded an Absorbance of 0.5 at 700 nm.

Antibacterial and antitumor activity

Antibacterial activity against Gram-positive Bacillus subtilis DSM10 and Gram-negative Escherichia coli DSM498 was assessed by the broth microdilution method, as described by De León et al. (2005). Bacteria were added to the microwells containing varying concentrations of extract dissolved in nutrient broth (up to 200 µg / mL), resulting in a concentration of 5x10⁴ CFU / mL, and the effect of the extracts on bacterial growth, when compared with untreated controls, was assessed after 24 h at 30°C (B. subtilis) or 37°C (E. coli) by measuring turbidity at 550 nm. In vitro cytotoxicity against human tumor cell lines HeLa (cervix), MCF7 (breast), A549 (lung) and Vero cell line (non-tumor control, Cercopithecus aethiops kidney) was carried out in 96-well microplates according to a previously published method (Moujir et al., 2008).

Briefly, 50,000 cells/well were seeded in 100 µL DMEM containing 2% FBS, in the presence of varying concentrations of extract, and cell viability was assessed by the MTT method after 48h at 37 °C, 5% CO₂ and 98% humidity. Results are expressed as EC₅₀, which is the concentration of extract which caused 50% cell cytotoxicity when compared with non-treated control cells.

Statistical analysis

Results were obtained from at least three independent experiments and were expressed as mean ± standard deviation. The one-way analysis of variance (ANOVA) was performed followed by Dunnett test, using XLStat. Differences were considered significant at p<0.05. Correlation analysis and linear regression was carried out using Microsoft Excel.

RESULTS

All the extracts analyzed contained carbohydrates and/or glycosides, condensed and hydrolysable tannins, flavonoids, sterols and/or triterpenes. Alkaloids and/or nitrogenous bases, saponins and coumarins were either absent or present in levels under the detection limit of the qualitative tests used in this work. Concerning polyphenols, levels varied more than ten-fold among the six extracts, between 39.8 and 470.9 GAE / mg dry extract for A. maurorum and T. muelleri, respectively. The strongest antioxidant activities (Table 1), comparable to quercetin, the standard compound used, were presented by T. muelleri and P. chinensis. The antioxidant activity of the extracts by the DPPH and FeCl₃ reduction methods were correlated (r²=0.900).

T. muelleri actively inhibited acetylcholinesterase (AChE), while the other extracts were not active in the range of concentrations tested (Table 1). It should be pointed out that the IC₅₀ of T. muelleri, although approximately 100-fold the result of berberine, an extremely active AChE inhibitor, is a good result for a whole extract containing a wide array of compounds.

Regarding antimicrobial activity, none of the extracts was active against B. subtilis or E. coli in the range of concentrations tested, i.e., up to 200 µg/mL and in 24h of exposure.

Considering that both D. lotus and A. maurorum have been described as being used in traditional medicine to treat tumors in Europe and India (US Department of Agriculture, 2016), the in vitro cytotoxicity of the extracts from these two plants against tumor cell lines was tested (Table 2).

A. maurorum presented cytotoxicity against all the cell lines tested, particularly against HeLa tumor cell line, with SI=3 for this cell line (where SI, Selectivity Index, is the ratio between EC₅₀ values for the Vero reference cell and tumor cell lines, respectively). Although this selectivity was not found for the other cell lines, this is often the case in chemotherapeutic agents, which are often specific for a particular type of cancer.
DISCUSSION

The antioxidant activity of the extracts was extremely high, with the exception of *A. maurorum*, which presented low activity and also low level of polyphenols. *T. muelleri* and *P. chinensis* stand out as being particularly active, both in the DPPH and in the FeCl₃ reduction assays. The antioxidant potential of the Terminalia genus corroborates the results of Steekamp et al. (2004), who reported that methanol and water extracts of *T. sericea* had a strong oxidant-scavenging effect in FMLP-stimulated neutrophils, and also of Janporns et al. (2015), who detected high amounts of tocopherol and other antioxidants in the seeds of *T. catappa*. *Pistacia* and *Diospyros* species have also been cited as excellent sources of antioxidants (Krishnaiah et al., 2011). The antioxidant activity may be an important factor in the medicinal properties of these plants, especially when used topically to disinfect and heal wounds. Antioxidant activity could be partially explained by the polyphenol content of the extracts, although the *B. alba* extract, which contained the second highest polyphenol level, corroborating the richness in these compounds reported for other *Bauhinia* species (Elbanna et al., 2016), was only the fourth strongest antioxidant by both assay methods. It is likely that, in addition to polyphenols, other groups of compounds also contributed to the strong antioxidant activity detected in *P. chinensis* extracts, considering that the level of activity detected was approximately the same as *T. muelleri*, which had 1.65 times more polyphenols, and that the extracts of *B. alba* and *T. ciliata*, also very rich in polyphenols, were much weaker antioxidants. The high polyphenol content, almost half of the dry weight in *T. muelleri*, but still extremely high in *B. alba*, *P. chinensis* and *D. lotus*, is also a positive feature in medicinal plants, since polyphenols are also known as presenting other bioactivities besides antioxidant, such as anticancer and antibacterial (Li et al., 2014). Concerning AChE inhibition, the fact that most extracts did not inhibit this enzyme in the range of concentrations tested was not wholly unexpected, considering that the most potent AChE inhibitors known are alkaloids (Barbosa-Filho et al., 2006, Murray et al., 2013), a chemical group which was not detected in these samples. However, phenolic compounds may also present anti-AChE activity (Murray et al., 2013), and *T. muelleri*, which contained the highest amount of polyphenols, was the only extract which presented an inhibition above 50%.

### Table 1: Antioxidant activities, polyphenol content and anti-AChE activity of the plant extracts.

<table>
<thead>
<tr>
<th>Extract / compound</th>
<th>DPPH (EC₅₀ µg/mL)</th>
<th>FeCl₃ reduction (EC₅₀ µg/mL)</th>
<th>Polyphenols (mg GAE/g)</th>
<th>Anti-AChE (IC₅₀ µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. lotus</em></td>
<td>37.8 ± 0.66</td>
<td>118.5 ± 0.87</td>
<td>235.1 ± 11.36</td>
<td>&gt;250</td>
</tr>
<tr>
<td><em>B. alba</em></td>
<td>75.3 ± 2.32</td>
<td>255.6 ± 1.63</td>
<td>385.1 ± 42.67</td>
<td>&gt;250</td>
</tr>
<tr>
<td><em>T. ciliata</em></td>
<td>188.9 ± 8.30</td>
<td>356.7 ± 14.94</td>
<td>87.1 ± 2.34</td>
<td>&gt;250</td>
</tr>
<tr>
<td><em>A. maurorum</em></td>
<td>&gt;250</td>
<td>&gt;1000</td>
<td>39.8 ± 2.14</td>
<td>&gt;250</td>
</tr>
<tr>
<td><em>T. muelleri</em></td>
<td>4.0 ± 0.03</td>
<td>7.0 ± 0.30</td>
<td>470.9 ± 8.74</td>
<td>229.9 ± 19.22</td>
</tr>
<tr>
<td><em>P. chinensis</em></td>
<td>4.7 ± 0.09</td>
<td>49.3 ± 0.91</td>
<td>285.3 ± 15.67</td>
<td>&gt;250</td>
</tr>
<tr>
<td>Quercetin</td>
<td>4.5 ± 0.08</td>
<td>18.4 ± 0.39</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Berberine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.00 ± 0.22</td>
</tr>
</tbody>
</table>

Results of n=3 experiments are presented as mean ± standard deviation. Values in the same column sharing different letters are significantly different at p<0.05.

**GAE**, Gallic Acid Equivalents. Quercetin was used as standard antioxidant and berberine as standard AChE inhibitor.

### Table 2: In vitro cytotoxicity of *D. lotus* and *A. maurorum* extracts (48h exposure) against HeLa, MCF7 and A549 human tumor cell lines; Vero non-tumor cell line was used as control.

<table>
<thead>
<tr>
<th>Extract / compound</th>
<th>HeLa (µM)</th>
<th>MCF7 (µM)</th>
<th>A549 (µM)</th>
<th>Vero (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. lotus</em></td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td><em>A. maurorum</em></td>
<td>16.8 ± 1.83</td>
<td>50.0± 2.76</td>
<td>61.4 ± 2.60</td>
<td>51.2± 3.98</td>
</tr>
<tr>
<td>Colchicine</td>
<td>1.7± 0.06</td>
<td>0.02 ± 0.00</td>
<td>1.4± 0.16</td>
<td>1.4± 0.06</td>
</tr>
</tbody>
</table>

Results of n=4 experiments are presented as mean ± standard deviation. Colchicine was used as standard chemotherapeutic compound.
roots from this plant. However, this effect could be caused by an interference with nicotinic receptors and not by inhibition of the enzyme. Also, Benamar et al (2010) report AChE inhibition by leaf aqueous extracts of P. atlantica and P. lentiscus, whereas the methanol extract of P. chinensis in the present work was not active, but besides being different species, the extraction solvent was not the same.

The fact that we did not detect antimicrobial action against E. coli and B. subtilis in plants which are traditionally used to treat bacterial and fungal infections is not surprising, since the maximum concentrations used in the present work (200 mg/mL) were much lower than the effective concentrations reported by other authors, which are in the order of magnitude of 1 mg/mL or even 20 mg/mL (e.g., see Steenkamp et al., 2004, for Terminalia sericea, Bibi et al., 2011, for Pistacia integerrima and Toona ciliata, and Panda et al., 2016, for Bauhinia variegata, several species of Terminalia and of Diospyros). It should also be pointed out that, in medicinal plants used to treat wounds, there are often several other effects acting concomitantly besides antimicrobial, such as anti-inflammatory, immunomodulatory and antioxidant activities, therefore a lower antibacterial activity may contribute to the healing potential of the herb, especially when used in a complex mixture, as is the case of extracts (Dhama et al., 2014).

The high cytotoxic activity of the A. maurorum extract corroborates its traditional use in the treatment of glandular tumors and nasal polyps (U.S. Department of Agriculture, 2016). Our results indicate a higher susceptibility of HeLa cancer cell line, furthermore with a high selectivity index when compared with Vero non tumor cell line. This indicates that further investigation on the active compound responsible for this activity may yield the scaffold for a drug with application against cervix cancer and/or other human carcinomas, presenting lower toxicity to healthy tissue. Although the D. lotus extract was not active against the cell lines used in the present study, a recent study by Rauf et al (2015) isolated dimeric naphthoquinones from the methanol extract of this plant which were quite active as antiproliferative agents against mouse lymphoma cells (L5178), and also against a multidrug resistant cell line (L5178Y MDR).

This result is not surprising, since the cell lines tested in the present work are epithelial adherent cells and therefore quite different from lymphoma cells.

Another aspect which needs to be addressed, especially if the medicinal use of these plants is to be continued, is the safety of their administration. Some studies on the safety of extracts from these and/or closely related plants have been reported. D. lotus roots and leaf extracts from D. kaki did not present toxicity to mice (Uddin et al., 2014; Xie et al., 2015). Although no studies on the safety of B. alba were found, B. vahlii leaves are eaten as vegetables in India and used to treat diarrhea and dysentery (Sowndharajan et al., 2013), and studies with mice confirmed their low toxicity (Narayan et al., 2012). Toona ciliata was safe against rats, in doses effective against ulcers (Malairajan et al., 2007). Leaf extracts of Terminalia muelleri did not cause acute toxicity in mice (Fahmy et al., 2015), and extracts of a plant from the same genus, T. arjuna, were found to produce no negative effects in long-term clinical trials in humans (Bhawani et al., 2013). P. chinensis bark extract did not cause any significant effect on biochemical and histological parameters in rats (Sattar et al., 2016), whilst its leaves have for a long time been used in tea without any references to negative effects (Bi et al., 2016). Concerning A. maurorum, no toxicity studies have been published so far, although it has also been traditionally used medicinally for many years without reports of negative effects. Although the ethnomedicinal use of the plants reported suggests their toxicity is not very significant, systematic acute and long-term toxicity studies, and also the effects on beneficial microbiota, need to be carried out.

The results obtained in the present work confirm the potential of these medicinal plants, not only as an alternative to conventional treatments by populations with low income, but especially as possible sources of active molecules which may be the scaffolds of future drugs. In this sense, we would like to highlight the interest to carry out further research to isolate and identify the compounds responsible for the activities, in particular the antitumor activity of A. maurorum and the antioxidant activities of T. muelleri and P. chinensis.

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