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In-vitro antioxidant properties of Indian traditional paan and its ingredients

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Indian traditional Paan is being used as a post meal digestive stimulant, astringent, aphrodisiac, nerve tonic, intoxicating agent and for several other purposes in India and is recommended in ancient scriptures of Ayurveda for normal well being. These properties might be due to the antioxidant nature of Paan. Hence, 50% methanol extracts of Paan and its ingredients were screened for antioxidant activity using DPPH method. Catechu, rose powder, betel nut and clove extracts exhibited potent antioxidant properties. Extracts of Paan, betel leaves and fennel showed moderate antioxidant activity. These results confirm the common beliefs and traditional uses of Paan in Indian tradition.

Keywords: Paan, Antioxidant activity, DPPH, Ayurveda.
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Paan is recommended in ancient scriptures of Ayurveda and is closely associated with Indian culture. In its simplest form, the Paan leaves (Piper betel) are smeared with slaked lime (chuna) and eaten with betel nut (supari, Areca catechu). This traditional combination is ideal, but many other aromatic herbs may be added. Among the different types of Paan, Meetha Paan is being used very widely in India. According to Ayurveda, Paan is a post meal digestive stimulant, oral deodorant, natural antiseptic, astringent, diuretic, mood elevator, aphrodisiac and nerve tonic. It relaxes the mind, creates a feeling of well being and improves the vocal chords. The betel nut used in Paan destroys Pitta and Kapha is intoxicating, a stimulant, laxative astringent and febrifuge. Black catethu, another ingredient of Paan is a strong, astringent, clotting agent and helps to reduce excess mucus in the nose, the large intestines and vagina. It is used in the treatment of gum bleeding, mouth ulcers, sore throat, eczema, haemorrhages, diarrhoea and dysentery. Many aromatic herbs such as cardamom, clove, fennel, gulkhand etc. used in Paan also possess potent medicinal properties. Some of these biological properties may be due to their antioxidant properties.

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Lipid peroxidation has gained more importance nowadays because of its involvement in pathogenesis of many diseases like atherosclerosis, cancer, diabetes mellitus, neurological disorders, cardiovascular diseases, rheumatoid arthritis and also in aging. Certain plants may possess sufficient antioxidant capacity that they can be used in the battle against cellular damage and diseases due to free radicals. Plant phenolics and flavonoids are known to possess strong antioxidant properties. Literature revealed the presence of these phytoconstituents in many of the ingredients of Paan. Hence, in the present investigation, the extracts of Paan and its individual ingredients were screened for their in vitro antioxidant activity using standard DPPH method.

Materials and Methods

Plant Materials
The betel leaves (Piper betel), betel nut (Areca catechu), cardamom (Elettaria cardamomum), catechu (Acacia catechu), processed cherry (Prunus cerasus), clove (Syzygium aromaticum), coconut (Cocos nucifera) and fennel (Foeniculum vulgare) were purchased from the local market of Ootacamund, India, and authenticated by Dr. S. Rajan, Medicinal Plants and Collection Unit, Govt. Arts College, Ootacamund, India, by comparing with voucher specimens. The other ingredients, viz. fruity (made of raw papaya), gulshand (a jam of rose and sugar, 1:1), lime paste (lime and water), Paan chutney (a syrupy flavoring agent made of aromatic herbs and sugar, Gopal Tobacco Limited, Kanpur, India), menthol, rose powder (a powder blend of aromatic herbs, Shiv Tobacco Ltd., Lucknow, India,) were purchased from authentic sources from the market of Raipur, Iridia.

Preparation of Paan
Paan was prepared as per the Indian tradition. Two betel leaves were washed with water and smeared with slaked lime paste and then with catechu paste. The other ingredients were added and wrapped with the same leaves. The quantities of the ingredients used are listed in Table 1.

Extraction
The Paan prepared as above (approx. 25 g) was crushed and extracted with 50% methanol (250 ml) by maceration for 15 days. Similarly, all the ingredients of Paan were crushed separately and macerated (15 g each) in 50% methanol (150 ml) for 15 days. The Paan chutney (15 g) was directly macerated similarly. After 15 days of maceration, the extracts were filtered and concentrated at low temperatures (40-50°C) under reduced pressure to remove the solvent. The nature of the extracts and their percentage yields are shown in Table 1.

Preparation of test solution
Solutions of betel nut, catechu, clove, gulshand, cherry, fruity, rose powder, Paan chutney extracts and menthol (21 mg/ml), were prepared in distilled DMSO (dimethyl sulphoxide). Solutions of betel leaves, cardamom, fennel, coconut and Paan extracts (21 mg/ml) were prepared in 50% methanol. These solutions were
serially diluted with respective solvents to obtain the lower dilutions.

**Preparation of standard solutions**

Two known antioxidants ascorbic acid and rutin solutions (21 mg/ml) were prepared in distilled DMSO. These solutions were serially diluted with DMSO separately to obtain the lower dilutions.

**Chemicals**

2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Sigma Chemicals Co., St. Louis, USA. Ascorbic acid was obtained from S. D. Fine Chem. Mumbai, India. Rutin was obtained from Acros Organics, USA. DMSO was obtained from Ranbaxy Laboratories Limited, Mumbai, India.

**In vitro antioxidant activity**

The antioxidant activities of extracts of *Paan* and its ingredients were assessed on the basis of radical scavenging effect on the DPPH stable free radical\(^{10}\). The extract or standard solution (10 μl) was added to 200 μl of DPPH in methanol solution (100 μl) in a 96-well microtitre plate (Tarsons Product (P) Ltd., Kolkata, India). After incubation at 37°C for 30 min, the absorbance of each solution was determined at 490 nm using Elisa microtitre plate reader (Bio Rad Laboratories Inc., California, USA, Model 550). The corresponding blank readings were also taken and the remaining DPPH was calculated. IC\(_{50}\) value is the concentration of sample required to scavenge 50% DPPH free radical.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Materials</th>
<th>Weight taken (g)</th>
<th>Nature of extract</th>
<th>% yield of the extract</th>
<th>IC(_{50}) μg/rnl ±S.E.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Paan</td>
<td>---</td>
<td>Brown semi solid</td>
<td>26.89</td>
<td>317.10±14.54</td>
<td></td>
</tr>
<tr>
<td>2 Betel leaves</td>
<td>9.15</td>
<td>Greenish brown solid</td>
<td>7.30</td>
<td>307.09±24.34</td>
<td></td>
</tr>
<tr>
<td>3 Betel nut</td>
<td>1.57</td>
<td>Dark brown solid</td>
<td>14.72</td>
<td>14.57±1.80</td>
<td></td>
</tr>
<tr>
<td>4 Cardamom</td>
<td>0.10</td>
<td>Yellowish brown semi solid</td>
<td>9.81</td>
<td>917.40±23.91</td>
<td></td>
</tr>
<tr>
<td>5 Catechu</td>
<td>0.50</td>
<td>Brown solid</td>
<td>82.12</td>
<td>2.22±0.35</td>
<td></td>
</tr>
<tr>
<td>6 Cherry</td>
<td>2.15</td>
<td>Red semi solid</td>
<td>27.10</td>
<td>&lt;1000</td>
<td></td>
</tr>
<tr>
<td>7 Clove</td>
<td>0.03</td>
<td>Dark brown solid</td>
<td>9.10</td>
<td>16.27±1.95</td>
<td></td>
</tr>
<tr>
<td>8 Coconut</td>
<td>1.72</td>
<td>Yellowish brown semi solid</td>
<td>10.33</td>
<td>915.43±39.96</td>
<td></td>
</tr>
<tr>
<td>9 Fennel</td>
<td>1.11</td>
<td>Yellowish brown semi solid</td>
<td>12.10</td>
<td>369.14±17.70</td>
<td></td>
</tr>
<tr>
<td>10 Fruity</td>
<td>3.19</td>
<td>Red semi solid</td>
<td>22.47</td>
<td>&lt;1000</td>
<td></td>
</tr>
<tr>
<td>11 Gulkhand</td>
<td>4.57</td>
<td>Brown semi solid</td>
<td>34.37</td>
<td>842.01±27.45</td>
<td></td>
</tr>
<tr>
<td>12 Lime paste</td>
<td>0.27</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>13 Paan chutney</td>
<td>1.11</td>
<td>Yellow syrupy liquid</td>
<td>97.13</td>
<td>966.50±25.44</td>
<td></td>
</tr>
<tr>
<td>14 Menthol</td>
<td>0.01</td>
<td>—</td>
<td>—</td>
<td>&lt;1000</td>
<td></td>
</tr>
<tr>
<td>15 Rosepowder</td>
<td>0.05</td>
<td>Brown solid</td>
<td>24.12</td>
<td>11.71±0.78</td>
<td></td>
</tr>
<tr>
<td>16 Ascorbic acid</td>
<td>—</td>
<td>—</td>
<td>2.89±0.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Rutin</td>
<td>—</td>
<td>—</td>
<td>9.12±0.67</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Average of 10 determinations.

Table 1—Antioxidant activities of *Paan* and ingredients using DPPH method
Results

Among the ingredients of Paan, potent antioxidant activity was observed with the extracts of catechu, rose powder, betel nut and clove with IC_{50} values of 2.22±0.35, 11.71±0.78, 14.57±1.80 and 16.27±1.95 μg/ml, respectively (Table 1). The IC_{50} value of catechu extract was found to be lower than that of ascorbic acid and rutin, the standards used and the values in the case of rose powder, betel nut and clove were comparable to that of rutin. The 50% methanol extract of Paan exhibited moderate antioxidant activity as evidenced by its IC_{50} value 317.10±14.54 μg/ml. The betel leaves and fennel extracts also showed moderate antioxidant activity with IC_{50} values of 307.09±24.34 and 369.14±17.70 μg/ml, respectively. The remaining extracts showed low or no activity.

Discussion

In the present study, the antioxidant activity of Indian traditional Paan and its ingredients was determined using DPPH stable free radical scavenging assay. The Paan extract showed moderate antioxidant activity. However, extracts of catechu, rose powder, betel nut and clove showed potent activity. Hence, the antioxidant properties of Paan probably lies with catechu, rose powder, betel nut and clove used in Paan. The betel leaves and fennel showed moderate activity. A large number of flavonoids, phenolics and tannins are known to possess strong antioxidant properties. Literature survey indicated the presence of these compounds in clove, catechu and betel nut. Rose powder is a mixture of several aromatic and flavoring spices and it is known that the spices contain these compounds. Hence, the observed antioxidant activity of these may be due to the presence of such compounds in their extracts. The antioxidant activity of cherry, clove and fennel are reported. The present study also confirms the same. As per Ayurveda, Paan prepared and consumed according to its directions is good for health. Antioxidant principles from natural resources provide enormous scope in correcting the imbalance between free radicals and anti free radicals, which is the major cause of several diseases. The present study shows the presence of antioxidant activity in Paan and most of its ingredients and supports its ethnomedical use in India, which might be due to its antioxidant activity.

Acknowledgements

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References

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