In Vitro Antimicrobial Activity and Phytochemical Composition of Dichrostachys cinerea

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ABSTRACT

Studies on phytochemical composition and antimicrobial activity of aqueous and ethanol extracts of roots and stems of Dichrostachys cinerea against clinical isolates of Candida albicans, Streptococcus mutans and Staphylococcus saprophyticus were carried out. The main phytochemicals present in the stem and roots included alkaloids, saponins and tannins, with roots containing the greater share. Steroids and cyanoglycoside were present in the stem. Both ethanol and aqueous extracts of the tested chewing stick inhibited the growth of all three tested microorganisms. There was no significant difference (P>0.05) between the inhibitory effect of the aqueous and ethanolic extracts of the roots of D. cinerea on C. albicans. However, the ethanolic extract of the stem exhibited a significantly higher (P<0.05) bioactivity than that exhibited by the ethanolic extract of the root. The pattern of inhibition of S. mutans and S. saprophyticus by the extracts were similar. Solvent used in extraction did not produce any significant effect (P>0.05), but the stem extracts exhibited a significant inhibition (P<0.05) compared to the root extract. Our results clearly show that D. cinerea is a potential candidate plant that could be used in the development of a dentifrice.

Keywords: Candida albicans, chewing stick, dentifrice, oral pathogen, Staphylococcus saprophyticus, Streptococcus mutans

INTRODUCTION

The use of chewing sticks is deeply rooted in many cultures. The Babylonians recorded the use of chewing sticks in 7000 BC and its use ultimately spread throughout the Greek and Roman empires. Chewing sticks were also used by Egyptians, Jews and in the Islamic Empires (Almas and Al Lafi 1995). Lewis and Elvin-Lewis (1977) stated that the use of chewing sticks persists today among many African and southern Asian communities as well as in isolated areas of tropical America and southern United States. It is believed that the counterpart of the modern day toothbrush was unknown in Europe until about 300 years ago (Almas and Al Lafi 1995). However, a more recent report has indicated that a chewing stick is more effective than toothbrushing for reducing plaque and gingivitis, especially when preceded by professional instruction as to its correct application (Al-Otaibi et al. 2003).

There are various plants which are used as chewing sticks in West Africa. Lime tree (Citrus aurantifolia) and orange tree (Citrus sinensis) sometimes serve as chewing sticks. The roots of senna (Cassia vinnea) were used by African Americans and those of African laburn (Cassia sieberianba) are used in Sierra Leone. Neem (Azadirachta indica) is widely used to provide chewing sticks in the Indian sub-continent (Almas 1993). In Nigeria, the major plants that serve as source of chewing sticks include Terminalia glaucescens, Anogeissus leiocarpus and Pseudocedrella kotschyi. However, the use of Dichrostachys cinerea as a chewing stick is common in some parts of south-western Nigeria.

D. cinerea is a semi-deciduous tree up to 7 m tall with an open crown. Its native range includes Cameroon, Ethiopia, Ghana, Nigeria, Swaziland, Uganda and Zambia, among others. D. cinerea is widely used as a source of food, fuel, fibre and timber. In medicine, its bark is used to treat dysentery, headaches, toothaches, and elephantiasis. Root infusions are taken for leprosy, syphilis, coughs, as an antihelminthic, purgative and strong diuretic (World Agroforestry 2008).

Reports on the antimicrobial activity of the major chewing sticks in Nigeria against oral pathogens have been documented (Rotimi et al. 1988; Akande and Hayashi 1998; Adekunle and Odukoya 2006; Ogundiya et al. 2006) with virtually no documentation on the antimicrobial activity of D. cinerea on oral microbes. The aim of the present paper was to provide information on the phytochemical composition and antimicrobial activity of aqueous and ethanol extracts of D. cinerea on some common oral pathogens such as Candida albicans, Streptococcus mutans and Staphylococcus saprophyticus.

MATERIALS AND METHODS

Plant collection and pre-extraction preparation

Different plant parts such as leaves, stem, root and fruit of D. cinerea were collected from Oke-Ogun axis of south Western Nigeria (a woody savannah vegetation). The plant was identified by a plant Taxonomist at the Forestry Research Institute of Nigeria, Ibadan, Nigeria. All the plant parts were collected for identification purpose, only the stem and root were analysed. The stem and roots of a single plant was sun-dried for seven days then grinded using a pestle and wooden mortar.

Extraction procedure

The ethanol extract preparation was done as previously described by Ogundiya et al. (2006). However, for water extraction, the procedure was basically the same except that soaking was done for 48 h and the filtrate was evaporated to dryness. The crude extracts were reconstituted into an aqueous solution using sterile distilled water to obtain extract concentrations of 0.4 and 0.2 g/ml.
Microorganisms

Pure cultures of Candida albicans, Streptococcus mutans and Staphylococcus saprophyticus isolated from patients with dental diseases were obtained from the Medical Microbiology Department of the University College Hospital (UCH) Ibadan, Nigeria. Bacterial cultures were maintained on Nutrient agar slants and the fungus on Potato dextrose agar slants, both at 6-8°C (Acheampong et al. 1988).

Phytochemical studies

Both qualitative and quantitative analyses of the phytochemicals present were carried out using methods described by Fadeyi et al. (1987) and Harbone (1998).

Antimicrobial assay

The antimicrobial activity of different concentrations of both ethanolic and aqueous extracts was determined by a modified agar-well diffusion method of Perez et al. (1990) as described by Popoola et al. (2007). The bacterial plates were incubated at 37°C (fungal plates at 28°C) and the zone of inhibition measured in mm after 24, 48 and 72 h of growth. A control experiment was set up by using an equal amount of sterile distilled water in place of different extract concentrations.

Statistical analysis of data

Data were expressed as mean ± standard deviation. The statistical significance of differences was assessed using analysis of variance. A two-tailed P value of less than 0.05 was considered to be statistically significant. Values that were significantly different were separated using Duncan’s Multiple Range test using SPSS for Windows ver. 11.0 statistical package.

RESULTS

Table 1 shows the result of phytochemical analysis of the stem and root of D. cinerea. The prominent phytochemicals present in the stem and root of the tested plant included alkaloids, saponins and tannins, with the root containing a significantly higher amount (P<0.05) of these phytochemicals. Steroids were present in appreciable amounts in the stem while the root contained only trace amounts. In a similar trend, cyanoglycosides were present in the stem but not in the root.

The results of the antimicrobial assay of both the root and aerial parts of the plant against selected microorganisms are presented in Table 2. The root showed significantly (P<0.05) higher inhibition zones than the aerial part.

Table 1 Results of the quantitative estimation of the phytochemicals (mg/100 g) present in the ethanol extracts of Dichrostachys cinerea.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Alkaloids</th>
<th>Steroids</th>
<th>Phenols</th>
<th>Tannins</th>
<th>Cyanoglycosides</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>105.5 ± 1.5 b</td>
<td>11.5 ± 2.5</td>
<td>Trace</td>
<td>49.2 ± 3.5 b</td>
<td>1.5 ± 0.1</td>
<td>108.3 ± 0.7 b</td>
</tr>
<tr>
<td>Roots</td>
<td>127.4 ± 0.9 a</td>
<td>Trace</td>
<td>80.0 ± 1.9 a</td>
<td>ND</td>
<td>117.6 ± 0.4 a</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± standard deviations. ND implies not detected.

Within column values with different letters are statistically significant (P<0.05)

Means were separated using Duncan’s Multiple Range test using SPSS for Windows ver. 11.0 statistical package.

Table 2 Inhibition of Candida albicans by aqueous and ethanol extract of Dichrostachys cinerea.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Incubation period (h)</th>
<th>Aqueous extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract concentration (g/ml)</td>
<td>0.4</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Roots</td>
<td>24</td>
<td>22.5 ± 0.5 c</td>
<td>17.5 ± 2.5 c</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>32.0 ± 2.0 a</td>
<td>32.0 ± 1.0 a</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>20.5 ± 0.5 c</td>
<td>17.5 ± 0.5 c</td>
</tr>
<tr>
<td>Stem</td>
<td>24</td>
<td>22.5 ± 0.5 c</td>
<td>17.5 ± 2.5 c</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>29.5 ± 3.5 a</td>
<td>31.0 ± 4.0 a</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>26.0 ± 1.6 b</td>
<td>23.0 ± 2.7 b</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n=3)

Within column values with different letters are statistically significant (P<0.05)

Means were separated using Duncan’s Multiple Range test.

Table 3 Inhibition of Streptococcus mutans by aqueous and ethanol extract of Dichrostachys cinerea.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Incubation period (h)</th>
<th>Aqueous extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract concentration (g/ml)</td>
<td>0.4</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Roots</td>
<td>24</td>
<td>39.0 ± 0.0 a</td>
<td>28.5 ± 1.5 a</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>32.5 ± 1.5 bc</td>
<td>28.0 ± 1.5 a</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>16.5 ± 1.5 d</td>
<td>14.0 ± 1.0 b</td>
</tr>
<tr>
<td>Stem</td>
<td>24</td>
<td>39.0 ± 0.0 a</td>
<td>28.5 ± 1.5 a</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>35.0 ± 4.0 b</td>
<td>28.0 ± 0.0 a</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>31.0 ± 2.0 bc</td>
<td>27.1 ± 0.3 a</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n=3)

Within column values with different letters are statistically significant (P<0.05)

Means were separated using Duncan’s Multiple Range test.

Table 4 Inhibition of Staphylococcus saprophyticus by aqueous and ethanol extract of Dichrostachys cinerea.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Incubation period (h)</th>
<th>Aqueous extract</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract concentration (g/ml)</td>
<td>0.4</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Roots</td>
<td>24</td>
<td>35.0 ± 5.0 a</td>
<td>25.5 ± 2.5 b</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>28.5 ± 2.5 b</td>
<td>26.0 ± 0.0 b</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>21.0 ± 1.0 c</td>
<td>15.0 ± 0.0 c</td>
</tr>
<tr>
<td>Stem</td>
<td>24</td>
<td>35.0 ± 5.0 a</td>
<td>25.5 ± 2.5 b</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>35.5 ± 0.0 a</td>
<td>31.0 ± 1.0 a</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>26.0 ± 0.1 b</td>
<td>24.0 ± 3.0 b</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation (n=3)

Within column values with different letters are statistically significant (P<0.05)

Means were separated using Duncan’s Multiple Range test.
and stem extracts of *D. cinerea* are presented in Tables 2-4. At the tested concentrations, results obtained indicated that both ethanolic and aqueous extracts of the tested chewing sticks had an inhibitory effect on the growth of the three tested microorganisms. There was no significant difference (P>0.05) between the inhibitory effect exhibited by aqueous and ethanolic extracts of the roots of *D. cinerea* on *Candida albicans*. However, the ethanolic extract of the stem exhibited a significantly higher (P<0.05) bioactivity than that exhibited by the ethanolic extract of the root.

The result of antimicrobial assay of the different extracts of *D. cinerea* on *Streptococcus mutans* is shown in Table 3. ANOVA test of the data depicted that the solvent used in the extraction process did not produce any significant effect (P>0.05) on the bioactivity of the extract, although the stem extracts produced a significant effect (P<0.05) compared to the bioactivity exhibited by the root extract. The sensitivity of *Staphylococcus saprophyticus* to the aqueous and ethanolic extracts of the root and stem of the tested plant is presented in Table 4. Statistical analysis of the data obtained depicted that the patterns of sensitivity of *S. saprophyticus* to both aqueous and ethanolic extracts was similar to those observed for *C. albicans* and *Strep. mutans*.

**DISCUSSION**

Well known examples of constitutive plant compounds with antimicrobial properties include phenols, unsaturated lactones, saponins, cyanogenic glycosides, glucosinates, alkaloids, tannins, and linoleic and stearic acids (Ingham 1973; Osbourn 1996; Darout et al. 2000; Abd El Rahman et al. 2003). The presence of some of these phytochemicals in an appreciable amount in the investigated plant parts may have contributed to the observed anti-oral pathogenic activities of the stem and root extracts of *D. cinerea*. The alkaloid, saponin and tannin contents of the stem extract were lesser than those of the root extract, although the former had a significantly higher antimicrobial activity. It seems that the additional bioactivity contributed by steroids and cyanoglycosides might have been responsible for this. This becomes more plausible as cyanoglycosides and steroids have been reported to have antimicrobial activity (Ingham 1973; Osbourn 1996).

Many studies have demonstrated antimicrobial, anti-caries, anti-periapical and antifungal properties of both aqueous and ethanol extracts of various chewing sticks (Buada and Boak-Yiadom 1973; Rotimi et al. 1998; Akande and Hayashi 1998; Ugoji et al. 2000; Adekunle and Odukoya 2006). Prominent examples of such chewing sticks include *Anogeissus leiocarpus*, *Terminalia glaucescens*, and *Pseudocedrela kotschyi*. Results from the present study have placed *D. cinerea* in the same group of those other chewing sticks.

The African continent is a continent endowed with a wide variety of microorganism (Dzink and Socransky 1985). Similarly, fagaronine, a compound extracted from *Fagara zanthoxyloides* (a Nigerian chewing stick) has been shown to provide beneficial effects for oral hygiene of some rural natives (Odebiyi and Sofowora 1979). Results from this study have clearly shown that *D. cinerea* is a potential candidate plant that could be used in the development of dentifrice.

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