ANTI-TRICHOMEONAL ACTIVITIES OF EXTRACTS AND FUROCOUMARINS OF MURRAYA KOENIGII FRUITS

A.C. ADEBAJO1*, K.A. OLANIYAN2 and C.O. ADEWUNMI1
1. Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Nigeria.
2. Drug Research and Development Unit, Obafemi Awolowo University, Ile-Ife, Nigeria.

(Received: April, 2007; Accepted: September, 2007)

Abstract

*Murraya koenigi* has been used in ethnomedicine for its anti-infective and anti-protozoal properties and therefore the methanolic extracts of pericarps and seeds and the furocoumarins isolated from the seeds were evaluated for anti-trichomonal activities using *Trichomonas gallinae*. The demonstrated anti-trichomonal activities of the fruit extracts and isolates that were comparable to that of Metronidazole, the standard anti-trichomonal agent used, show that *furocoumarins* acting synergistically were the active constituents of this *furocoumarin-producing* *M. koenigi*. The structure activity relationships of the furocoumarins showed that a free H-8 may be important for this activity as prenyl substitution at C-5 gave a better activity than at C-5. The presence of double bond(s) in the substituents gave poorer activity while those of oxygenated groups such as epoxy, hydroxyl and another lactone significantly increased the activity. The low toxicity profile of the extracts using haemagglutination activity of formaldehyde fixed bovine erythrocytes supports the internal use of the plant in medicine. Hence, use of this plant as a spice similar to the carbazole-producing *M. koenigi* would confer on the user additional protection against trichomonads and may confirm the ethnomedicinal uses of the plants in the treatment of protozoal diseases such as amoebiasis, dysentery and trichomoniasis as well as represent a cheaper alternative to metronidazole.

Key words: Anti-trichomonal activities, toxicity, *Trichomonas gallinae*, *furocoumarins*, *Murraya koenigi*.

1. Introduction

*Murraya koenigi* (L.) Sprenger is a spicy rutaceous medicinal plant grown mostly in the tropics and subtropics for the medicinal and flavourant properties of the leaves and fruits (Dastur, 1970; Gupta and Nigam, 1971; Stone, 1985). Native to Indo-China, it is commonly called Curry leaf tree and used in most homes of the world to spice foods (Chakraborty et al., 1965; Adebajo, 1997). Volatile oils (Dutt, 1958; Nigam and Purohit, 1961; Macleod and Pieris, 1982; Wong and Tie, 1993; Onayade and Adebajo, 2000; Rana et al., 2004), monoo and dimeric carbazole alkaloids (Fiebig et al., 1985; Atta-ur-Rahman et al., 1988; Hegauer, 1990, Chakraborty and Roy, 1991; Ito et al., 1993; Reisch et al., 1992, 1994a; Bhattacharyya et al., 1994; Adebajo, 1997; Chakraborty et al., 1997; Natan et al., 1998), simple furo- and pyrano-coumarins (Gupta and Nigam, 1971; Bhattacharyya and Chakraborty, 1984; Reisch et al., 1994b, c; Adebajo, 1997; Adebajo et al., 1997; Adebajo and Reisch, 2000) have been the major constituents reported from the plant’s parts. Since it is an ancient Indian medicinal plant, its leaves have been ethnomedicinally used as tonic, febrifuge, stomachic and anti-vomiting. The leaf, stem and root are used externally in skin eruptions and bites of venomous animals while the bark and root are used as stimulant. Other uses are as carminative, hypotensive, hypoglycaemic, anti-periodic and antifungal (Chakraborty et al., 1965; Das et al., 1965; Dastur, 1970; Gupta and Nigam, 1971; Natan et al., 1998). It is also eaten raw for curing dysentery and diarrhoea (Dastur, 1970; Gupta and Nigam, 1971; Rana et al., 2004), uses probably confirmed by its anti-amoebic property and activity against *Entamoeba histolytica* (Bhakuni et al., 1969; Kapil, 1971; Kong et al., 1986). Pharmacologically and biologically, antimicrobial (Natan et al., 1998), antitumor (Fiebig et al., 1985; Chakraborty et al., 1997), α-amylase inhibitory (Bawde et al., 2002), anti-oxidative (Tachibana et al., 2001), cytotoxic, depressant, anti-trichomonal (Natan et al., 1998; Adebajo et al., 2004, 2006), anti-hypertensive, -treponemal, -spasmodytic (Bhakuni et al., 1969; Kapil, 1971; Kong et al., 1986; Adebajo, 1997) and anti-diabetic (Naraya and Sastri, 1975; Adebajo et al., 2006) activities for the extracts while antioxidative (Khan et al., 1997), hypoglycaemic (Iyer and Mani, 1990; Khan et al., 1995 a, b; Yadav et al., 2002; Grover et al., 2003), hypocholesterolemiae (Khan et al., 1996 a, b) properties for the powdered leaf have been reported. Furthermore, anti-oxidant, -tumour, -microbial, -inflammatory, trypanocidal and

* + corresponding author (email: caadebajo@oauife.edu.ng)
mosquitoscidal activities have been indicated for some of these alkaloids isolated from the plant and other sources (Das et al., 1965; Fiebig et al., 1985; Chakrabarty et al., 1997; Nutan et al., 1998; Ramsewak et al., 1999; Higawa et al., 2000; Nakatani, 2000; Adeyemo et al., 2001). There has been no pharmacological report on furocoumarins isolated from the plant, however, activities such as the treatment of skin lesions e.g. vitiligo, feeding repellent protection against polyphageous insect larvae, inhibition to crown gall tumours and some enzymes, inhibition of the synthesis of albumins and proteins, especially of nucleic acids, anti-tumour, antimicrobial and toxic effects to some animals, have generally been reported for furocoumarins (Murray et al., 1982).

Trichomonas vaginalis and T. gallinae cause trichomoniasis in man and animals with terrible medical implications and Metronidazole is the drug of choice. Trichomonas gallinarum affects birds including poultry, causing high morbidity and mortality especially in young birds. The observations that T. vaginalis is becoming resistant to metronidazole in about 5% of the population (Murz et al., 1998) coupled with the fact that metronidazole has unpleasant adverse effects (Narcisi and Secor, 1996) have led to search for phytochemicals in African medicinal plants with potential antitrichomonal activities (Omisore et al., 2005). The furocoumarin-producing plant of M. koenigii has been reported as a geographical race of the carbazole-producing Curry leaf (Reisch et al., 1994 a, b; Adeabajo and Reisch, 2000). Since the extract of the carbazole-producing plant and its carbazole constituents has been shown to have anti-trichomonal activity (Adeabajo et al., 2004, 2006), we therefore investigated the furocoumarin-producing plant and its isolated furocoumarins for the same activity, using T. gallinae. Furthermore, since the leaf and fruit are freely eaten, the abundant pharmacological reports on M. koenigii and the report of moderately toxicity for the carbazole producing plant (Adeabajo et al., 2006), it became important to evaluate the potential toxicities of the two M. koenigii plants by determining the agglutination and haemagglutination values of their methanol extracts.

2. Materials and Methods

Plants Material, Extraction and Isolation:
The authentication, collection, extraction and isolation of the constituents of Murraya koenigii fruits were as previously reported (Adeabajo et al., 1994a,b, 1997; Adeabajo and Reisch, 2000). The dried fruits were dehulled and the pericarps separated from the seeds. Fresh cold MeOH extracts of the seeds and pericarps were made for the anti-trichomonal and cytotoxic activities determinations. The isolates were also detected by TLC in the methanolic extracts of the pericarp.

Anti-trichomonal Activity:
Trichomonas gallinae isolated from the pigeon was dropped into a test tube of normal saline. The solution was distributed into test tubes of Ringer's egg-serum culture for enteric protozoan and incubated at 37 °C for growth. Stock solutions of the isolates and Metronidazole (Flagyl, Aventis Pharma) in DMSO at the concentration of 20, 20 and 8 mg/ml, respectively, were made. Serial dilutions to 0.0, 1.953, 3.906, 7.8125, 15.625, 31.25, 62.5 and 125 mg/ml for the isolates and their derivatives, and 0.0, 0.1562, 0.3125, 0.625, 1.25, 2.5, 5.0, 10.0, 20.0 and 30.0 mg/ml for metronidazole with the fluid nutrient solution were used as the test agents. A 50 μL of each test agent and 150 μL of the nutrient solution were pipetted into the microwells and incubated in the steam incubator at 37 °C for 24 and 48 h. The number of organisms per milliliter in each well for 0, 24 and 48 h were counted using the microscope. The experiments were done in triplicates (Narcisi and Secor, 1996, Adeabajo et al., 2004, 2006). The effects of the methanolic extracts of M. koenigii pericarp and seed as well as furocoumarins isolated from the seeds on this parasite were thereby evaluated.

Cytotoxicity:
The cytotoxicities of the methanolic extracts of the carbazole-producing leaves and stems and those of the seeds and pericarps of the furocoumarin-producing M. koenigii extracts were determined by haemagglutination activity using formaldehyde fixed bovine erythrocytes (Pewman, et al., 1982; Sadiq et al., 1989; Wang et al., 1995). The isolates were not tested for cytotoxicity due to their low quantities.

3. Results

Anti-trichomonal Activity:
The anti-trichomonal activities of the extracts and isolates of the seeds and pericarps of this plant are shown in Table 1.

Cytotoxicities of the Methanolic Extracts of the Leaf, Stem, Seeds and Pericarps:
The results for the methanolic extracts of the leaf and stem of the carbazolic-, seeds and pericarps of the furocoumarin-producing M. koenigii are as given in Table 2.

4. Discussion

The present study reports the anti-trichomonal activities of the methanolic extracts of the pericarps and seeds of the furocoumarin-producing M. koenigii type and furocoumarins isolated from the seeds. The methanolic extracts of the furocoumarin-producing M. koenigii seeds showed similarly high
and comparable anti-trichomonal activities with those of the pericarp and Metronidazole, the standard anti-trichomonal agent used (Table 1). Similar high anti-trichomonal activities were obtained for the isolates isoimperatorin (3), isogossfrol (7) and indicolatone (9) indicating that they were the main anti-trichomonal agents of the fruits. Generally, long exposure of the parasites to Metronidazole, the extracts and these isolates did not have any significant effect on their activities as shown by similar LD₉₀ and LD₉₀ values at 24 and 48 hours probably showing that they may be good substitutes for Metronidazole. Furthermore, long exposure of the parasites to the isolates of bergapten (1), imperatorin (2), heracelenin (5), byakangelicol (6), 8-geranyloxypsoralen (8) and β-sitosterol (10) showed lowered LD₉₀ and LD₉₀ values at 48 hours than the 24 hours showing that prolonged contact time of the compounds to the organisms was beneficial (Table 1) and clearly indicating that their activities were more likely to be cidal (total death) than static (Adebajo et al., 2006).

On the other hand, 5-methoxyimperatorin (4) and 9 (a C₁₀ furoucoumarin with an 8-geranyloxy substituent bearing an extra lactone) have higher LD₉₀ and LD₉₀ at 48 hours than 24 hours. None of the furoucoumarins isolated from the furoucoumarin-producing M. koenigii gave a significantly higher activity than the MeOH extracts of the seeds or pericarps (Table 1) suggesting that these active compounds were probably working synergistically in the plant. Conversely, the carbazole alkaloids of the carbazole-producing plant with greater activities than the leaf and stem methanolic extracts were identified as the active compounds that were not working in synergism (Adebajo et al., 2004, 2006).

Prenyl substitution at C-5 gave a better activity than at C-8 as shown by the higher activity of 3 over 2 respectively, indicating the probable importance of a free H-8 for activity. Elongation of 2 with another prenyl rest substituent only gave a better LD₉₀ value for 8 (8-GOP) at 24 hours. However, the modification of the terminal prenyl rest in 8 with the introduction

### Table 1: Anti-trichomonal Activities of Fruits Extracts and Isolates of the Furoucoumarin-producing M. koenigii.

<table>
<thead>
<tr>
<th>Extracts/Isolates</th>
<th>Inhibition of <em>T. gallinae</em> at 24 h</th>
<th>Inhibition of <em>T. gallinae</em> at 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₉₀ (µg/ml)</td>
<td>LC₃₀ (µg/ml)</td>
</tr>
<tr>
<td>Seeds MeOH Extract</td>
<td>1.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Pericarp MeOH Extract</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Bergapten (C₁, 1)</td>
<td>4.0</td>
<td>30.0</td>
</tr>
<tr>
<td>Imperatorin (C₁₀, 2)</td>
<td>6.0</td>
<td>230.0</td>
</tr>
<tr>
<td>Isogossfrol (C₁₀, 3)</td>
<td>2.1</td>
<td>3.7</td>
</tr>
<tr>
<td>5-Methoxyimperatorin (C₁₀, 4)</td>
<td>15.2</td>
<td>155.9</td>
</tr>
<tr>
<td>Heracelenin (C₁₀, 5)</td>
<td>11.0</td>
<td>52.0</td>
</tr>
<tr>
<td>Byakangelicol (C₁₀, 6)</td>
<td>22.0</td>
<td>61.0</td>
</tr>
<tr>
<td>Isogossfrol (C₁₀, 7)</td>
<td>2.0</td>
<td>5.2</td>
</tr>
<tr>
<td>8-geranyloxypsoralen (C₁₀, 8)</td>
<td>22.0</td>
<td>125.0</td>
</tr>
<tr>
<td>Indicolatone (C₁₁, 9)</td>
<td>2.1</td>
<td>3.8</td>
</tr>
<tr>
<td>β-sitosterol (C₃₀, 10)</td>
<td>5.8</td>
<td>31.0</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>1.9</td>
<td>3.5</td>
</tr>
</tbody>
</table>

LC₉₀ and LC₃₀: Concentrations at which 50 and 90% parasites of *Trichomonas gallinae* were killed; N = 3.

### Table 2: Agglutination and Haemaggutination values of the methanol extracts of *Muraya koenigii*.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Concentrations (µg/ml) at which agglutination occurs</th>
<th>Haemaggutination Titre Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Furocoumarin-producing type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>0.75 ± 0.03</td>
<td>0.34 ± 0.14</td>
</tr>
<tr>
<td>Pericarp</td>
<td>1.50 ± 0.00</td>
<td>0.67 ± 0.00</td>
</tr>
<tr>
<td><strong>Carbazole-producing type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Stem</td>
<td>0.00 ± 0.00</td>
<td>0.08 ± 0.00</td>
</tr>
</tbody>
</table>
of an oxygen atom and cyclisation to form another lactone ring as in 9 greatly increased its activities above those of 8 at 24 and 48 hours. In fact, this modification in the structure of 9 brought the activity of 9 close to that of 7, the most active C_16 furocoumarin with a C-8 oxygenation, the original seed MeOH extract and metronidazole. Hence, the presence of an additional α,β-unsaturated-γ-lactone in 9 (Adebajo et al., 1997b) that was absent in 8 may be responsible for the better activity of 9 over those of the other isolates. At 24 hrs, it gave same L.D_50 and L.D_{10} values as Metronidazole. Generally, the presence of double bond(s) in the substituents of 2 and 8 gave poorer activities. Replacement of the double bond in the prenyl rest of 2 by an epoxy group in 5 gave better activities, especially at 48 hours. Although, 7 has a double bond similar to 2, the presence of a free alcoholic OH in its structure might have led to its better activities. Di-substitution of the psoralene nucleus as in byakangelicol (6) reduced the activities of the monosubstituted 7 and 5.

The results of the anti-trichomonal activities obtained in this present work confirmed the similarity between the carboxal (Adebajo et al., 2005, 2006) and furocoumarinic types of M. koenigii, and may therefore show that they are probably biological equivalents. This plant has already been established as a geographically race of the Curry plant, M. koenigii (Adebajo et al., 1994a, b; Adebajo, 1997). Traditionally in Asia, the olive and fruits are eaten as spice and ethnomedicinally as anti-dysenteric preparation. This present result may partly explain why the indigene and other users did not differentiate these two races, as they would be more interested in the desired anti-dysenteric activity than in their chemical constituents. Metronidazole is the drug of choice for the treatment of amoebic dysentery. This present anti-trichomonal results for the fruits of M. koenigii coupled with its already reported activity against E. histolytica and anti-amoebic activities (Bhakuni et al., 1969; Kapil, 1971; Kong et al., 1986; Adebajo, 1997), should justify the folkloric / ethnomedical use of the plant in the treatment of dysentery in Asia, especially that caused by Entamoeba histolytica and sensitive to Metronidazole. Furthermore, the demonstrated anti-trichomonal activities of the fruit show that the use of this plant as a spice would confer on the user (both humans and birds) additional protection against trichomonads or be useful in the management of trichomoniasis, especially by the low income earners found in the developing countries.

The low haemagglutination (HA) values ranging between 0.00 and 0.67 shown by all the extracts revealed their negligible cytotoxic effects (Table 2). However, the higher values (0.34-0.67) of the furocoumarin-producing plant indicates that it is relatively more toxic than the carboxal-producing one while the pericarp extract was also more toxic than the seed. The cytotoxicity of furocoumarins is already known (Murray et al., 1982) and this may explain the relative toxicity of the extracts of the furocoumarin-producing plant. Although Nutan and co-workers (1988) have shown that the petroleum ether soluble fraction was the most cytotoxic, this relative non-toxicity of the carboxal-producing M. koenigii leaf (Table 2) confirms an earlier report of same (Adebajo et al., 2006). Therefore, the use of the furocoumarin-producing plant as a substitute for M. koenigii in spicing foods would additionally give an anti-trichomonal activity similar to the authentic Curry Leaf.

Conclusion

The anti-trichomonal activities of the seed and pericarp methanolic extracts and their isolated furocoumarins of the furocoumarin producing M. koenigii compares favourably with that of the standard drug Metronidazole. The active furocoumarins of the plant were working in synergism. This may therefore justify the folkloric use of this plant and its development as a potential chemotherapeutic agent.

References


Adebajo et al.: Anti-trichomonal activities of extracts and furocumarins of M. koenigii fruits


Adebajo et al.: Anti-trichomonal activities of extracts and furocoumarins of M. koenigii fruits


