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# Chemical Compounds and Antibacterial Activity of *Tephrosia toxicaria* Pers

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**ABSTRACT**

Eight known compounds were isolated from the shrub *Tephrosia toxicaria*. Among them, 6,7-dimethoxy-chromone (1), was described by the first time from this genus, villosinol (2), which had been previously reported without its $^{13}$C-NMR data and sumatrol (3), which has its $^{13}$C-NMR data corrected. The antibacterial activity of *Tephrosia toxicaria* extract and obovatin (6), deguelin (7), 12a-hydroxy- $\alpha$-toxicarol (8), 12a-hydroxy-rotenone (10), and tephrosin (11) is also described.

**Keywords:** Tephrosia toxicaria, Flavonoids, $^{13}$C NMR data, antibacterial activity.

## INTRODUCTION

*Tephrosia toxicaria* Pers. (Fabaceae), also referred as *T. sinapou* (Buchoz), is a shrub popularly known as “timbo de caiena” in Ceara state (Northeast of Brazil) where it is used as pesticide and fishing poison[1-2]. The phytochemical studies of *Tephrosia* genus revealed compounds with anticinoceptive, larvicidal and antiinflammatory activities[3,4]. Previous investigations of *T. toxicaria* led to the identification of flavonoids, mainly rotenoids[1-2,5-7]. In the present work, we report the isolation of eight known compounds, including the chromone,(6,7)-dimethoxy-chromone,(1) described for the first time for this genus, the flavonoid, villosinol, (2) which has been previously reported

without its $^{13}$C-NMR data and sumatrol, (3) which has its $^{13}$C-NMR data corrected. In addition, as a support of use of alternative source in the treatment of bacterial infections, we describe the antibacterial activity of ethanolic extract from its roots and of some its compounds.

## MATERIAL AND METHODS

### Plant material

Pods and roots of *T. toxicaria* Pers. were collected in Guaraciaba do Norte (Ceara state, Brazil). A voucher specimen (#32139) is kept at the Herbarium Prisco Bezerra, Universidade Federal do Ceara - Brazil.

## METHODS

The powdered pods, air-dried (180.0 g), were extracted with 95% EtOH at and provided the extract TTVE (26.0 g). An aliquot (8.7 g) of TTVE was fractionated by silicon gel column (Merck 60-120 60 Mesh) using hexane, $CH_2Cl_2$ and EtOAc as solvents. The fraction eluted with $CH_2Cl_2$ (69.0 mg) furnished, after further silicon gel column, 6.7 mg of 6a,12a-desydro-α-toxicarol[8], 4. The fraction eluted with EtOAc (800.0 mg) was purified by Si gel column, furnished 19.9 mg of luteolin[9], 5. The powdered roots (500.0 g) were extracted with ethanol to afford TTRE (14.2 g). TTRE was chromatographed on a silicon gel column with gradient mixture of hexane/$CH_2Cl_2$. Fractions eluted with hexane/$CH_2Cl_2$ (1:1) yielded 13.2 mg of obovatin[10], 6, and the fractions eluted with $CH_2Cl_2$ furnished 9.9 mg of deguelin[8], 7. Another portion of roots (649.0 g) was extracted in a Soxhlet system with water. After liophilization, the material (5.4 g) was extracted with ethyl acetate and yielded 2.1 g of organic fraction. This fraction was chromatographed on Sephadex LH-20 using EtOAc:MeOH (1:1). The fractions obtained were submitted to HPLC RP-18 (MeOH/formic acid 0.1% 4:1) and provided pure compounds villosinol[11] (2, 5.0 m g), 12a-hydroxy-α-toxicarol[8] (8, 32.8 mg), sumatrol[12] (3, 3.6 mg), α–toxicarol[8] (9, 13.0 mg), 12a-hydroxyrotenone[13] (10, 27.8 mg), tephrosin[14] (11, 62.0 mg), and 6,7-dimethoxy-chromone[15] (1, 2.0 mg). All compounds were identified by $^1$H-NMR, $^{13}$C-NMR and 2D NMR analysis and comparison with those reported in the literature.

### Antibacterial activity

### Microorganisms

The following strains used in this study were provided by the Oswaldo Cruz Foundation –FIOCRUZ: *K. pneumoniae* ATCC 10031; *P. aeruginosa* ATCC 15442; *S. mutans* ATCC 0046; *S. aureus* ATCC 6538. Strains isolated from clinical material of *Escherichia coli* 27 and *Staphylococcus aureus* 358 were also used. The bacteria were activated in Brain Heart Infusion (BHI, Himedia laboratories Pvt. Ltd., Mumbai, India) for 24 h at 35°C.

### Antibacterial test (MIC) and resistance modulation bacterial

MIC (minimal inhibitory concentration) was determined in a microdilution assay[16] utilizing an inoculum of 100 µl of each strain, suspended in BHI broth up to a final concentration of $10^5$ CFU/ml in 96-well microtiter plates, using serial dilutions (1:1). Each well received 100 µl of each (roots ethanolic extract (TTRE) and of the compounds 6, 7, 8, 10 and 11). The concentrations of the extract and organic compounds varied 512 - 8 µg/ml. MICs were recorded as the lowest concentrations required to inhibit growth.

The minimum inhibitory concentration for antibiotics was determined in BHI by the microdilution test, using suspensions of $10^5$ CFU/mL and a drug concentration ranging from 2,500 to 2,4 µg/ml (1:1 serial dilutions). MIC was defined as a lower concentration at which growth was observed. For evaluation of the absence and presence of deguelin (7) and 12a-hydroxy-α-toxicarol (8) in *P. aeruginosa* and *Staphylococcus aureus* modulators of antibiotic resistance, a MIC of the antibiotics was determined in the presence or absence of subunits and as plates were incubated for 24 h at 37°C. Each antibacterial test for MIC determination was performed in triplicate.

## RESULTS AND DISCUSSION

It is worth to mention that villosinol, 2 has its $^{13}$C-NMR data being reported for the first time, and that the $^{13}$C-NMR assignment for sumatrol, 3 was corrected through bidimensional NMR analysis.

Villosinol (2). $C_{23}H_{22}O_8$, $^1$H-NMR (500 MHz, $CDCl_3$) $\delta_H$: 1.74 (s, 3H, $CH_3$-8'), 2.84 (dd, *J* = 15.0 and 7.5 Hz, 1H, H-4'a), 3.20 (dd, *J* = 15.0 and 9.5 Hz, 1H, H-4'b), 3.76 (s, 3H, 2-$OCH_3$), 3.83 (s, 3H, 3-$OCH_3$), 4.18 (s, 1H, 12a-OH), 4.47 (d, *J* = 12.0 Hz, 1H, $H_{eq}$-6), 4.55 (sl, 1H, H-6a), 4.58 (dd, *J* = 12.0 and 2.5 Hz, 1H, $H_{ax}$-6), 4.93 (s, 1H, H-7'), 5.05 (s, 1H, H-7'), 5.20 (t, *J* = 8.5 Hz, 1H, H-5'), 6.04 (s, 1H, H-10), 6.49 (s, 1H, H-4), 6.71 (s, 1H, H-1), 11.83 (s, 1H, 11-OH). $^{13}$C-NMR (125 MHz, $CDCl_3$) $\delta_C$: 194.4 (C-12), 170.4 (C-9), 166.1 (C-11), 156.1 (C-7a), 151.6 (C-3), 148.7 (C-4a), 144.4 (C-2), 143.1 (C-6'), 113.2 (C-7'), 109.7 (C-1), 109.0 (C-1a), 105.0 (C-8), 101.4 (C-4), 100.2 (C-11a), 92.5 (C-10), 88.7 (C-5'), 75.8 (C-6a), 67.1 (C-12a), 63.9 (C-6), 56.7 (2-$OCH_3$), 56.2 (3-$OCH_3$), 30.8 (C-4'), 17.3 (C-8').

Sumatrol (3). $C_{23}H_{22}O_7$. mp 213.6–215.1 °C. $^1$H-NMR (500 MHz, CDC13) $\delta_H$: 1.75 (s, 3H, $CH_3$-8'), 2.86 (dd, *J* = 15.0 and 8.0 Hz, 1H, H-4'a), 3.24 (dd, *J* = 15.0 and 9.5 Hz, 1H, H-4'b), 3.79 (s, 3H, 2-$OCH_3$), 3.82 (s, 3H, 3-$OCH_3$), 3.85 (d, *J* = 4.0 Hz, 1H, H-12a), 4.17 (d, *J* = 12.0 Hz, 1H, Heq-6), 4.59 (dd, *J* = 12.0 and 3.0 Hz, 1H, Hax-6), 4.88 (t, *J* = 3.0 Hz, 1H, H-6a), 4.93 (s, 1H, H-7'), 5.06 (s, 1H, H-7'), 5.20 (t, 1H, *J* = 8.5 Hz, 1H, H-5'), 6.02 (s, 1H, H-10), 6.46 (s, 1H, H-4), 6.87 (s, 1H, H-1), 12.42 (s, 1H, 11-OH). $^{13}$C-NMR (125 MHz, CDC13) $\delta_C$: 193.8 (s, C-12), 169.6 (s, C-9), 166.4 (s, C-11), 156.5 (s, C-7a), 150.1 (s, C-3), 147.6 (s, C-4a), 144.4 (s, C-2), 143.2 (s, C-6'), 112.8 (t, C-7'),110.9 (d, C-1), 105.0 (s, C-1a), 104.3 (s, C-8), 101.5 (s, C-11a), 101.4 (d, C-4), 92.1 (d, C-10), 88.4 (d, C-5'), 72.0 (d, C-6a), 66.3 (t, C-6), 56.7 (q, 2-OCH3), 56.2 (q, 3-OCH3), 44,0 (d, C-12a), 30.9 (t, C-4'), 17.3 (q, C-8').

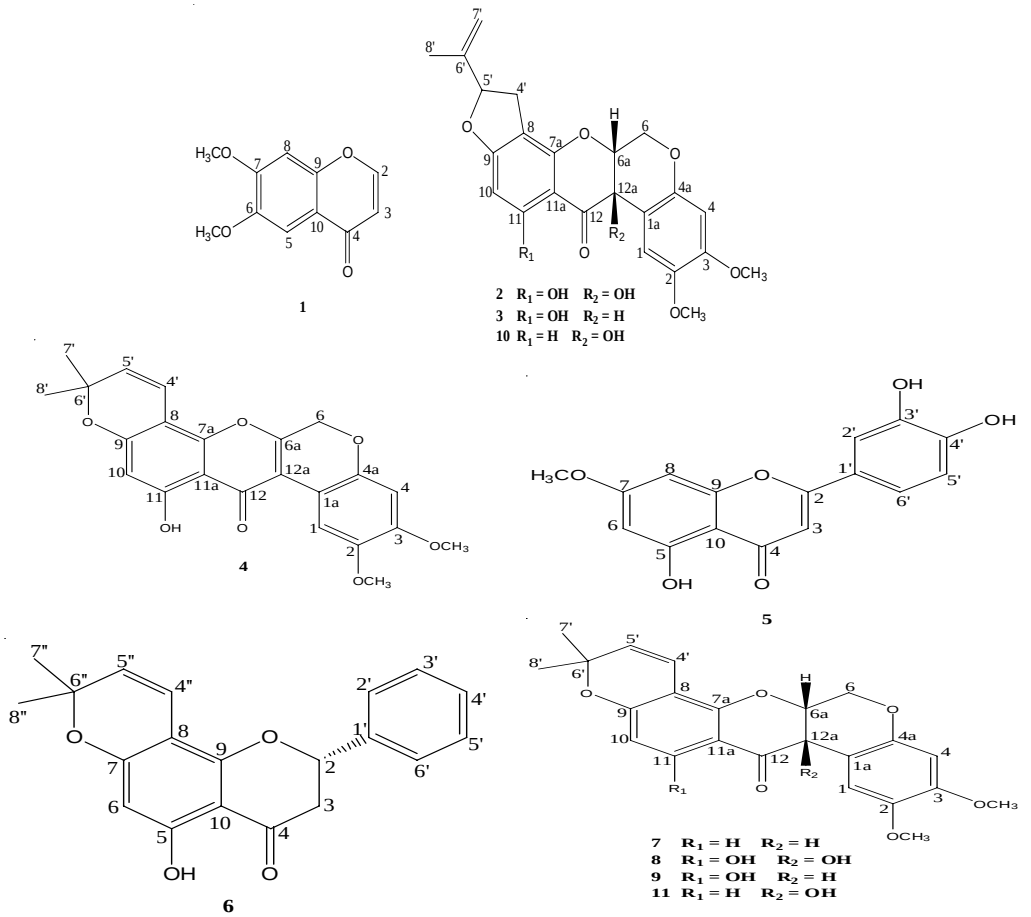


**Fig. 1. Compounds isolated from *T. toxicaria.***

**In vitro antibacterial activity**

The MICs of *Tephrosia toxicaria* extract, TTRE, obovatin, (6), deguelin (7), 12a-hydroxy-α-toxicarol (8), 12a-hydroxy-rotenone (10), and tephrosin (11) against six bacterial strains are presented in Table 1. All tested compounds and extract showed antimicrobial activity against Gram-positive and *Gram-negative* bacteria, with the best effect of 12a-hydroxy-α-toxicarol against to the grown of *Gram-positive S. aureus* 358 with MIC 256 µg/mL, while Deguelin is responsible for the best result, the *Gram-negative* bacteria, *P. aeruginosa* was inhibited at 64 µg/ml.

Table 2 shows the results concerning the modulation tests of bacterial resistance to aminoglycosides. When (7) and (8) compounds are combined with the antibiotic amikacin, tested against *P. aeruginosa* and *S. aureus* strains, the isolated and combined MIC values were the same. Synergism was observed in the combinations of (7) with gentamicin against the two bacterial strains used in the test and in the combination of (7) with neomycin against *P. aeruginosa*, characterized by reduction of MIC by 50% compared to MIC of the antibiotics tested alone. Only the (8) combination with neomycin against *S. aureus* showed antagonism, increasing the MIC by 50% compared to the MIC of the neomycin tested alone.

Some natural products of origin plant and phytochemicals are known to have antimicrobial properties, which may be of great importance in treatment against infections. With the increased incidence of antibiotic resistance, alternative natural plant products may be of interest[17]. Several studies have been conducted in different countries, demonstrating the efficacy of this type of treatment. Many natural products of plants were evaluated not only for direct antimicrobial activity, but also as resistance modifying agents[18,19].

Various chemical compounds (synthetic or natural) have direct antibacterial activity against many species, expanding the activity of an antibiotic, reversing the natural resistance of bacteria to specific antibiotics, causing the inhibiting the active efflux of antibiotics through the plasma membrane and / or elimination of plasmids. Potentiation of antibiotic activity or reversion of resistance to antibiotics allows the classification of these compounds as modifiers of antibiotic activity[20,21].

The mechanism described as “synergistic multi-effect targeting” or “Herbal shotgun” is a possible strategy that explains modulatory effects and refers to the use of plants and drugs in an approach using various-substance combinations, which may not only affect a single target, but several ones, where the different therapeutic components contribute in a synergistic-agonistic effect. This approach is not only for combinations of extracts: Combinations between complex mixtures (extracts and/or oil) and compounds chemical isolated synthetic or naturals or antibiotics are also possible [22,23].

**Table. 1: Values of the minimal inhibitory concentration (MIC) (µg/ml) of *Tephrosia toxicaria* extract and obovatin (6), deguelin (7), 12a-hydroxy-α-toxicarol (8), 12a-hydroxy-rotenone (10), and tephrosin (11)**

| | ***E. coli*** **(27)** | ***K. pneumoniae*** **(10031)** | ***P. aeruginosa*** **(15442)** | ***S. mutans*** **(0046)** | ***S. aureus*** **(6538)** | ***S. aureus*** **(358)** |
|---|---|---|---|---|---|---|
| TTRE | 512 | 512 | ≥1024 | 512 | 512 | ≥1024 |
| 6 | ≥1024 | ≥1024 | ≥1024 | 512 | 512 | ≥1024 |
| 7 | ≥1024 | 512 | 64 | ≥1024 | 512 | 512 |
| 8 | 512 | 512 | ≥1024 | ≥1024 | 512 | 256 |
| 10 | 512 | 512 | 512 | 512 | 512 | ≥1024 |
| 11 | 512 | 512 | 512 | ≥1024 | 512 | ≥1024 |

**Table. 2: MIC values (µg/mL) of aminoglycosides in the absence and presence of compounds (7) and (8) in *P. aeruginosa* and *Staphylococcus aureus.***

| Antibiotics | *P. aeruginosa* (15442) | | *S. aureus* (358) | |
|---|---|---|---|---|
| | MIC alone | MIC combined (7) | MIC alone | MIC combined (8) |
| Gentamicin | 64 | 32 | 64 | 32 |
| Amicacin | 32 | 32 | 64 | 64 |
| Neomycin | 32 | 16 | 32 | 64 |

## CONCLUSION

In conclusion, the prospective health-promoting effects of plant secondary metabolites have encouraged the research about their chemical constitution and their potential to treat several diseases, among them bacterial infection. The plant substances and their extracts display rarely toxic side effects when compared in treatments with conventional drugs, so these results could be leads for the development of new antibacterial agent, or the compounds from *T. toxicaria* even may be used associates with standard antibiotics.

FRLS, MVST, JNV, JQL (Master and PhD sudents), IGP (Graduate student) contributed running the laboratory work and drafted the paper; AMCA, JM, MRS, RBF, GMPS did the analysis and interpretation of data of RMN, critical revision of the manuscript. JGMC, EFFM and FFGR contributed with the microbiological tests.

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