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A New Anticancer Tannin and Known Tannins from *Terminalia cattapa*†

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The structures of the new tannin and the known compounds were established on the basis of ^{1}H and NMR analysis. The new tannin was found to have high activity against *Ehrlish ascita* carcinoma cells.

INTRODUCTION

Terminalia cattapa L. (Family Combretaceae), a large tree found along canals and road sides, occurs from tropical Asia to Northern Australia and in Polynesia as well as in Egypt. Leaves, fruit and bark are astringent and all have medicinal properties. For example, extracts and raw material from *T. cattapa* have been used in treating dysentery, swollen rheumatic joints, leprosy, yaws, diarrhea, thrush, abscesses, bilious fever, putrid exudation and bloody feces, as well as galactagogue for women. Furthermore, extracts also serve as a pain killer, mild laxative, and numbing agent[3] Additionally, extracts of some species of *Terminalia* show antiviral activity and anti-HSV-1 activity[4]. Previously, the known tannins, punicalin, punicalagin, chebulagic acid, geraniin, grantin B, 1-desgalloyleugeniin, 2,3-(s)-hexahydroxydiphenoyl-D-glucose, along with four new hydrolyzable tannins, terflavin A, terflavin B, tergallogin and teractain were isolatd from *T. cattapa*[5, 7–11]. Here, five tannins were isolated from *T. cattapa* found in Egypt, one being new; three were tested for anticancer activity.

RESULTS AND DISCUSSION

Two dimensional paper chromatography of the aqueous acetone extract of fresh leaves of *T cattapa* indicated the presence of tannins[5–10]. From the mixture, a new tannin and four known tannins were isolated and identified.

The ^{1}H-NMR spectrum of the new compound (**1**) showed a duplication of signals due to the presence of a free anomeric hydroxyl group. The presence of three galloyl groups was established by three singlets at δ 6.98, 7.01 and 7.10. Also, the presence of signals at δ 2.4 and 7.5 suggested that a chebulic acid moiety was also present. The low field anomeric protons of the sugar moiety in the α, β isomers were detected at 5.8 (d, J = 4.0 Hz) and at 4.98 (d, J = 8.0 Hz), respectivley.

The structure of **1** was confirmed by ^{13}C-NMR data. The presence of two downfield signals of δ 172.5 and 174.0 ppm correspond to the two carbonyl signals of chebulic acid. This was supported by the presence of another two upfield signals at 39.5 and 40.6 ppm for C-3 and C-2, respectively. Also, three downfield signals at δ 164.5, 166.5, and 166.9 ppm were found to represent carbon signals of the three carbonyl groups of the galloyl moieties.

†Part 3 in a series on anti-cancer tannins; see Ref. 1 and 2.

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The presence of signals in the sugar region, starting from the anomeric centre, proved the unesterified anomeric centre of the sugar moiety. The spectrum exhibited two signals at δ 91.5 ppm (α-C-1) and 95.2 (β-C-1). In addition, other signals were present in the region for esterified hydroxyl groups at 62.5–77.8 ppm for (C-6, -4, -2, -3, and -5).

From the above data, **1** is 2-O-chebuloyl 3,4,6-tri-O-galloyl (α, β)-glucopyranose, a new natural product.

Other tannins, chebulinic acid (**2**), chebulagic acid (**3**), punicaligin (**4**), and punicalin (**5**), were isolated and identified; the data for these compounds were identical to those reported in the literature[10–12]

EXPERIMENTAL

The leaves of *Terminalia cattapa* were collected in December 1997 from Giza, Egypt, and a voucher specimen is deposited in the NRC Herbarium, Cairo, Egypt. The air-dried leaves were powdered and extracted with 80% aqueous acetone. The extract was concentrated under reduced pressure. The concentrated material was fractionated over a Sephadex LH-20 column using ethanol and ethanol/water/acetone mixtures for gradient elution to yield eight fractions. Each fraction was further analyzed with preparative paper chromatography using either BAW or 6% HOAc. Further purification over Sephadex LH-20 was employed.

The phenolics were identified bhy co-chromatography with authentic samples and color reactions. The structures were confirmed by ^{1}H- and ^{13}C-NMR spectroscopy.

Compound **1**: an off-white amorphous powder R_f-values 26 (6 : 1 : 2)-40 (6% HOAc).

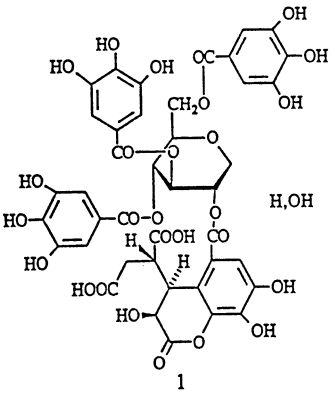
^{1}H-NMR spectral data: (ppm) 7.10, 7.01, 6.98 (each 2H, s, gallic H-2, 6), 7.5, 2.4 (each 1H, s chebuloyl moiety H-2 and H-3) 5.8 (d, J = 4.0 Hz, H-1), 4.98 (d, J = 8.0 Hz, H-1).

^{13}C-NMR spectral data: (ppm). 174.0, 172.5, (carbonyl groups), 164.5, 166.5, 166.9 (carbonyl groups of galloyl moiety), 95.2 (β-C-1 glucose), 91.5 (α-C-1 glucose), 77.8–62.5 (C-2, 3, 4, 5 and 6, sugar carbons), 40.6, 39.5 (C-2 and C-3, chebulic acid).

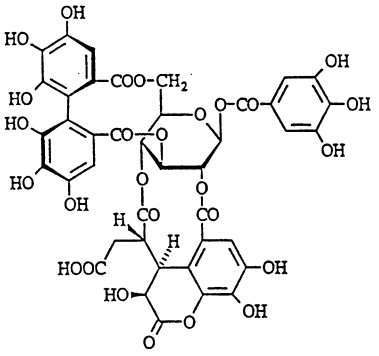
Materials and Methods

Compound **1**, **2** and **3** were tested against *Ehrlich ascites* carcinoma (EAC) cells in mice. Female Swiss albino mice (weighing 18–20 g) obtained from the breeding unit of the National Research Centre, Cairo, Egypt, were used in this study. *Ehrlich ascita* carcinoma cells were supplied by Dr. C. Benckukh, Netherlands Cancer Institute, Amsterdam.

The tumor line was maintained in female mice by weakly intraperitoneal transplantation of 2.5×10^6 cells. The ascitic fluid was diluted with normal saline so that each 0.2 mL contained 2.5×10^6 of cells. The cells were counted microscopically using a hemacytometer, and the mice were inoculated with the tumor cells in a concentration of 2.5×10^6 cells/mouse. After 1 h of tumor inoculation, the mice were divided into eleven groups of 10 mice each.



1



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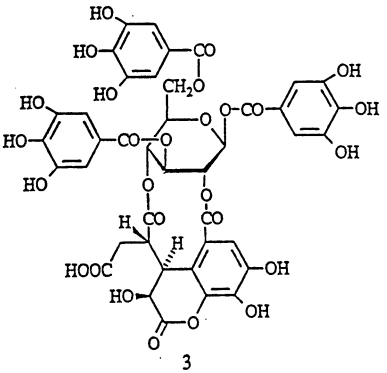

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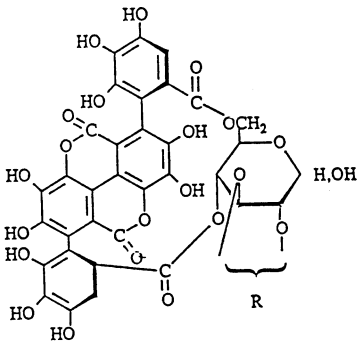
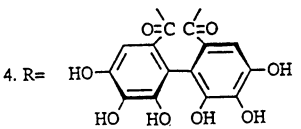


R



4. R=

5. R=H,H

Treatment

The group of ascitic mice were given injections with 0.2 mL using different concentrations of the tested extract and of geraniin to study the growth inhibition effect. One group served as a control. After 24 h of inoculation all animals were weighed daily.

Biological Activity

ANTITUMOR ACTIVITY OF *T. CATTAPA* COMPOUNDS AGAINST EAC CELLS IN MICE

Sample no.	MST ± S.D.	T/C (%)
Control	10 ± 0.8	100
Compound 1	35 ± 0.9	350
Compound 2	25 ± 0.8	250
Compound 3	23 ± 0.7	230

MST : mean survival time
S.D.: Standard deviation
T/C: MST (standard)/MST (control) × 1000

The table above illustrates the effects of tannins **1**, **2** and **3** on the growth rate of EAC cells in the female Swiss albino mice. The compounds exhibited significant anti-tumor activity against EAC cells in mice. The new compound **1** and the known compounds **2** and **3** increased the mean survival time to 35, 25 and 23 days, respectively (*i.e.*, T/C = 350, 250 and 230%).

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