Pharmacognosy Communications
Volume 5 | Issue 3 | Jul-Sep 2015
www.phcogcommn.org

Original Article

Phytochemical Composition and Potential Genotoxic effects of Important Egyptian Medicinal Plants

Khaled Rashed[1], Roel Anthonissen[2], Davie Cappoen[2] and Luc Verschaeve[2,3]

[1]*Pharmacognosy Department, National Research Centre, Dokki, Giza, Egypt.*
[2]*Operational directorate Food, Medicines and Consumer Safety, Toxicology Unit, Scientific Institute of Public Health, Brussels, Belgium.*
[3]*Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium.*

ABSTRACT

Introduction: We performed a phytochemical analysis and investigated the potential genotoxic properties of ten different methanol extracts of plants that are frequently used in Egypt for medicinal purposes. Such studies are important because these plants were never thoroughly investigated with respect to their possible potential side-effects and risks for humans. **Materials and Methods:** In this study we used the bacterial Vitotox test and alkaline comet assay in human C3A cells to estimate their genotoxic potential. The Vitotox test is an interesting screening test which correlates very well with the well-known Ames assay but has the advantage of being rapid, sensitive and requiring only small amounts of a test compound. The test was conducted in the presence and absence of a metabolizing S9-enzyme fraction. The comet assay is now a widely used and validated genotoxicity test which can be applied in all DNA-containing cells. In this study it was conducted in human C3A cells which conserved phase I and II biotransformation capabilities. The *in vitro* NRU assay was used to investigate toxicity and utilized as a dose-finding test. **Results:** None of the plants have shown genotoxic properties although one of them, *Derris rubusta*, showed borderline genotoxicity in both tests. This plant also contains alkaloids and coumarins, besides flavonoids, carbohydrates, tannins and triterpenes that were also found in the other plants. **Conclusion:** So far all 10 investigated medicinal plants appeared not genotoxic but due to borderline effects, *Derris robusta* deserves further complementary investigations.

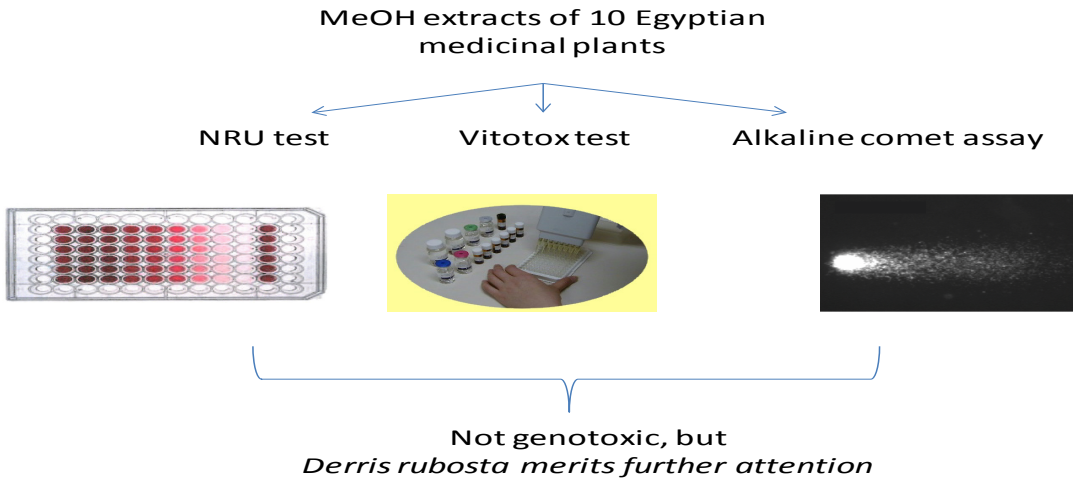
Key words: Alkaline comet assay, Egyptian medicinal plants, Methanol extracts, Phytochemical analysis, Vitotox test.

INTRODUCTION

Much attention and efforts are at present paid to the search for, and investigation of medicinal plants, including the evaluation of their potential harmful effects. Many plant species that are nowadays used in traditional medicine have proven their efficacy over the years but often such plants, or their extracts or preparations were never thoroughly tested for potential harmful side-effects. Many publications however have indicated that 'natural' does not necessarily mean 'safe'. Many traditional medicinal plants have already been identified as having genotoxic properties and other adverse health effects.[1-3] It is therefore important not only to investigate them therapeutically, but also to investigate the hazardous health effects of plants that are used or intended to be used, e.g., in traditional medicine or as food additives.

In this paper we present the results of an investigation on the genotoxicity of a number of plants that are widely used in traditional medicine in Egypt and abroad. These plants were (1) *Ficus nitida* (syn. of *Ficus benjamina* L.), (2)

Corresponding Address
Mr. Luc Verschaeve
Scientific Institute of Public Health,
Operational directorate Food, Medicines and Consumer Safety,
Toxicology Unit, Scientific Institute of Public Health,
Brussels, Belgium.
E-mail: luc.verschaeve@wiv-isp.be
DOI: 10.5530/pc.2015.3.6



Graphical Abstract

Ficus vasta, (3) *Parkia africana* (*P. biglobosa* (Jacq.) G. Don), (4) *Pistacia lentiscus*, (5) *Ampelopsis brevipedunculata*, (6) *Derris robbusta*, (7) *Bauhinia racemosa*, (8) *Lagerstroemia tomentosa*, (9) *Cichorium intybus* and (10) *Bauhinia alba* (syn. of *Bauhinia variegata* var. candida Voigt). Their medicinal use is summarized in Table 1.

Methanol extracts of the pre-cited plants were investigated with two complementary genotoxicity tests, i.e., the bacterial Vitotox test and the alkaline comet assay which was conducted on a human C3A cell line. The Vitotox test is based on SOS-induction following DNA damage in bacteria, and is particularly interesting as it is a rapid pre-screening test which gives results that are highly comparable to those of the well-known Ames assay. The comet assay has numerous advantages especially *in vivo*, where virtually any tissue can be examined. The status of the *in vitro* comet assay in regulatory testing is much less clear although it appears to be more specific than other *in vitro* genotoxicity tests and less prone to false positive responses.[4] The test was therefore considered an adequate complementary assay to the Vitotox test.

Table 1: Medicinal use of the tested plant extracts

	Used for/against:
Ficus nitida	This plant is widely used in hedges or columns. The ficus pseudo fruit is used in the symptomatic treatment of constipation[21] whereas other use in folk medicine is essentially against respiratory disorders and certain skin diseases.[22] Usage is essentially from the leaves and as an infusion.
Ficus vasta	*Ficus vasta* leaves are used against rheumatism, pains and intestinal worms.[23]
Parkia africana	The roots, bark, leaves, stems, flowers, fruits and seeds are used to treat a range of ailments including diarrhoea, ulcers, pneumonia, burns, coughs and jaundice.[24]
Pistacia lentiscus	Mastic gum isolated from its leaves is used as a medicine for gastrointestinal ailments for ages. It is also used in the manufacture of plasters. The resin is used as a primary ingredient in the production of cosmetics such as toothpaste, lotions for the hair and skin, and perfumes. Mastic can also heal peptic ulcers.[25,26]
Ampelopsis brevipedunculata	The fresh fruits, roots and leaves are antiphlogistic, depurative and febrifuge. Resolves clots. The plant is used externally in the treatment of boils, abscesses and ulcers, traumatic bruises and aches[27]
Derris robbusta	Root juice mixed with the juice of Sida acuta is used for sore throat. Alcoholic extract of seeds showed significant *in vitro* activity against some human and plant pathogenic bacteria. The plant also has antifungal activities against keratinophilic fungi.[28]
Bauhinia racemosa	A methanolic extract of the stem and bark are used as an anti-inflammatory, analgesic and antipyretic. A methanolic extract of the flower buds is used in the treatment of peptic ulcer.[29]
Lagerstroemia tomentosa	The bark of the plant is considered stimulant and febrifuge, leaves and flowers are used as purgative.[30]
Cichorium intybus	Tea is made from leaves, stems and roots and used against jaundice. Chicory syrup is used as a tonic and purifying medicine for infants. The flowers are used as a herbal treatment of everyday ailments such as a tonic and appetite stimulant and as a treatment of gallstones, gastroenteritis and sinus problems.[31]
Bauhinia alba	*B. alba* is used for treating skin diseases, asthma, diarrhoea and as a blood purifier and tonic.[32]

MATERIALS AND METHODS

Samples collection

The leaves of *Ficus nitida*, *Ficus vasta*, *Parkia africana*, *Pistacia lentiscus*, *Ampelopsis brevipedunculata*, *Derris robbusta*, *Bauhinia racemosa*, *Lagerstroemia tomentosa*, *Cichorium intybu*, and *Bauhinia alba* were collected from Al-Zohiriya garden, Giza, Egypt in May 2012. The plants were identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereeza Labib, consultant of plant taxonomy at the ministry of agriculture and director of the Orman Botanical Garden, Giza, Egypt. A voucher specimen was deposited in the herbarium of Al-Zohiriya Garden, Giza, Egypt.

Samples preparation

Air dried leaves of the plants (200 g) were extracted with methanol: distilled water 80:20 (v/v) 6 times at room temperature by maceration method. The extract was concentrated under reduced pressure to afford methanol extraction. Each extract was phytochemically screened according to the methods described by Yadav and Agarwala.[5]

(Geno) toxicity tests

The neutral red uptake test

The NRU test measures cell viability and is based on the property of living cells to be able to take up neutral red dye into their lysosomes[6]. Dying cells have altered membrane properties and therefore they can no longer take up neutral red (NR). The dye is given to cells in different concentrations allowing the determination of a NI_{50} concentration (50% reduction of uptake) by measuring OD_{540}.

This test was performed according to well-known standard methods[6]. Cells were plated in 96-well plates (40000 cells per well) and incubated in Dulbecco's modified eagles medium (DMEM) + 10% fetal bovine serum for 24 h at 37°C and 5% CO_2. Plant extracts were then added in different concentrations for another 24 h. Then cells were washed with phosphate buffered saline (PBS) after which 200 µL of a 0.625 µg/mL neutral red solution is added. After 3 h, cells were again washed in PBS to remove the remaining dye. Addition of 200 µL ethanol/acetic acid (50/1) results in release of the dye from the cells that were placed in a shaking bath until a homogenous color is formed (approx. 1 h). The optical density was then measured with a spectrophotometer. OD_{620} was measured as a reference value which is subtracted from the OD_{540}. This is the wavelength at which maximal absorption of NR occurs. Absorption of non-treated cells was given a 100% value to which data from exposed cells were compared. SDS (sodium dodecyl sulfate) was used as a positive control.

The bacterial Vitotox test

The Vitotox test is a bacterial assay used for detecting genotoxic substances based on SOS-induction.[7-9] In short, *Salmonella typhimurium* bacteria TA104-recN2-4 (called the Genox strain) containing a modified RecN gene fused to lux genes of the luminescent marine micro-organism *Vibrio fischeri* are exposed to the test compound (extract) after which light is measured in a luminometer[7]. Indeed, when DNA is damaged RecN will, together with other genes involved in SOS-induction, be expressed and this results in light production. A signal-to-noise ratio reaching values over 1.5 is indicative of induced DNA damage and hence genotoxicity. To correct for false positives or bacteriotoxic effects, a constitutive light producing bacterial "pr1 strain", or TA 104 pr1, is included in the Vitotox test. This strain is also called the Cytox strain. Here light production should not be influenced by DNA damage whereas decreased light production is indicative of a toxic response.[8]

In the Vitotox test both strains were cultivated and then transferred into 96-well plates together with different concentrations of the test agent (here the plant extracts) and with or without a metabolizing S9-fraction (from Moltox Inc., Boone, NC, USA). Cultures were then brought into a luminometer (Modulus Microplate Multimode Reader from Turner Biosystems) at 30°C in which light emission was measured every 5 min in each well during 4 h.[8] In the present investigation plant extracts were tested in concentrations that were based on preliminary dose-finding and toxicity tests using the NRU (neutral red uptake) assay.[6] All experiments were furthermore accompanied by a negative control and a positive control which was 4-nitroquinoline-oxide (4-NQO) without S9, and benzo(a)pyrene with S9.

The alkaline comet assay

The comet assay is one of the most widely used tests for the rapid and easy screening of genotoxicity (DNA damage) in eukaryotic cells. In this test cells are embedded in an agarose gel on a microscope slide which is then placed in a lysing solution with a detergent. After this, the slides are placed in an electrophoresis tray and subjected to an electric current. As damaged DNA strands lose their 'super coiling' the electric field is responsible for the migration of the negatively charged DNA fragments toward the positive pole or anode. This results in a characteristic 'comet-like' figure.[10]

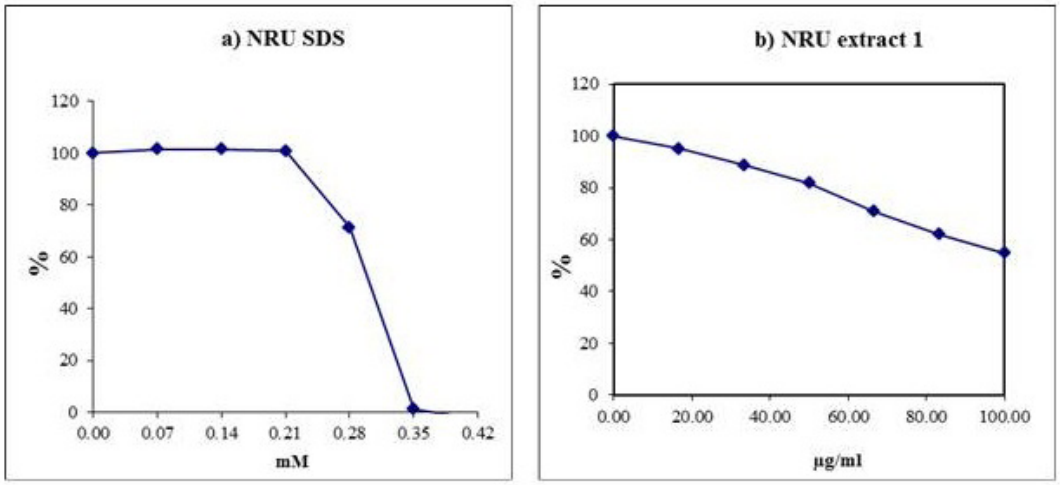


Figure 1: Example of NRU-test results for the positive control SDS (here NI_{50} = 0.301 mM) and for extract 1 (Ficus nitida; NI_{50} ~100 µg/mL). Growth inhibition is presented as % growth compared to untreated control cells

In the presently reported work we used human C3A cells that were grown in 24-well plates (1 ml/400000 cells) during a 24 h period. Then plant extracts were added in different concentrations. Concentrations were chosen based on the results of the NRU test. Cells were trypsinized after another 24 h, brought in PBS and kept on ice to prevent further DNA damage. A 10 µL cell suspension + 300 µL 0.8% LMP (low melting point) agarose was brought on pre-coated slides (1% NMP -normal melting point-agarose). Slides were kept on ice for 5 minutes and then brought in lysis buffer (2.5 M NaCl; 100 mM EDTA; 10 mM TRIS; 1 v% Triton X-100 and 10 v% DMSO). The pH was adjusted to pH=10 with NaOH pellets. The slides remained overnight into the lysing solution.

Slides were then brought into denaturation buffer (0.3 M NaOH, 1mM EDTA in water, t°=17°C, pH=13) in which electrophoresis (20 min, 1 V/m, 300 mA) occurred. After lysis, histones and nucleosomes are removed leaving super coiled DNA behind. DNA damage results in broken DNA fragments and loops that unwind and migrate in the agarose gel. A "comet like" figure is formed that can be visualized after staining with a fluorescent dye. Slides were therefore dried and renaturated in 200 µL H_2O (10 min) and stained for another 10 min. with 100 µL gelred (1:3300 stock solution). They were then analyzed with an Axio Imager.Z2 (Zeiss) fluorescence microscope with Metacyte and Metafer4 (version 3.8.5) software from Metasystems (Altlußheim, Germany). The percentage DNA in the comet tail was used as the measure of DNA damage. Ethyl methane sulfonate (0.75 mM) was used as a positive control. Two slides were prepared per exposure and a total of 100 cells (DNA comets) were measured, evenly distributed over the two slides.

Statistical analysis

No statistics is needed for the NRU test where we only determined NI_{50} values and the Vitotox test where a dose-effect relationship and S/N ratio (Genox over Cytox strain) reaching levels over 1.5 are sufficient to decide about a compounds' genotoxicity. The Mann-Whitney's U-test was used for analysis of comet test data.

RESULTS AND DISCUSSION

We evaluated the genotoxicity of methanol extracts from the 10 Egyptian medicinal plants with the bacterial Vitotox test in our modified *Salmonella typhimurium* TA104 constructs[7,8] in the presence and absence of a metabolizing S9 fraction derived from Aroclor treated rat livers, as well as with the alkaline comet assay on a immunological competent immortalized human C3A hepatoblastoma cell line. These cells are derived from human HepG2 cells. They retain many of the properties of the normal human hepatocyte. They have the essential structural, biochemical and growth features of normal human liver cells and have conserved both phase I and phase II metabolic capacities.[11] For this reason, the comet assay was performed in the absence of S9 only.

In this study we used the neutral red uptake test in human hepatocytes (C3A cells) as a preliminary dose-finding test. The NI_{50} of the positive control SDS was approximately

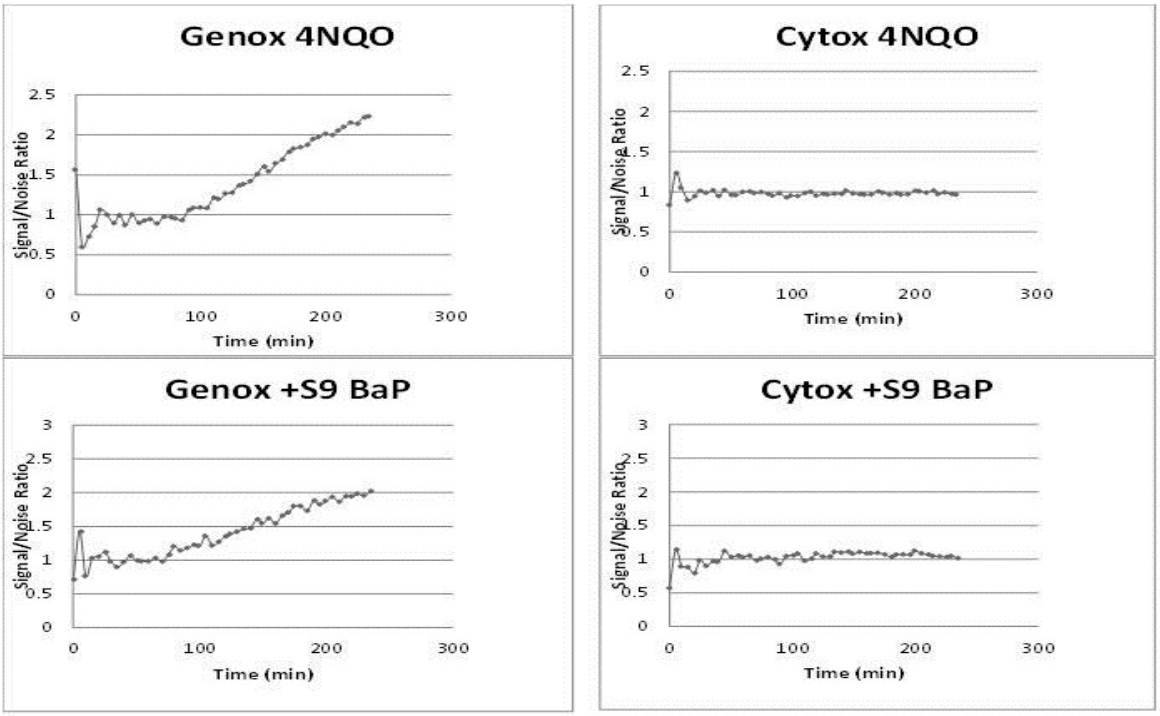


Figure 2: Examples of test results for the positive controls (0.4 mg/mL 4-NQO or 800 mg/mL benzo(a)pyrene)

0.30 mM (different tests were conducted) which is within the normal historical values of our laboratory (Figure 1). All plant extracts had NI_{50} values around 100 μg/mL. Plant extracts were therefore tested in the Vitotox and comet assay in concentrations of maximally 100 μg/mL. An example of the NRU-results is given in for sample 1 ((*Ficus nitida*) which shows a NI_{50} value close to 100 μg/mL.

We chose to conduct our genotoxicity screening with the Vitotox test because it is a suitable, simple and accurate bacterial test for prescreening purposes. The Vitotox test has furthermore the advantage of being rapid and to require minimal amounts of a test compound which gives it an advantage over most other test systems. Compared to the original Ames test the assay format is much faster and corresponds with high throughput screening procedures to a greater degree. The test correlates well with many other test systems (especially the Ames assay) and is up to 100 x more sensitive than the traditional Ames test.[9,12,13]

The other test that we employed was the comet assay. This assay has gained widespread use in various areas of research including human biomonitoring, genotoxicology, ecological monitoring and as a tool for research into DNA damage and repair in different cell types in response to a range of DNA-damaging agents.[14,15] The comet test is important because, unlike for the other cytogenetic tests, no cell cultures or dividing cells are needed. One is also not limited by the number or the appearance of chromosomes that may prevent a chromosome aberration analysis. Other advantages of the test include the fact that it detects low concentrations of a genotoxic compound, that very few cells are required for slide preparation (<10.000 and even approximately 1000 should be enough) and that relatively few cells should be studied per individual or treatment group. Often only 50 cells are studied per sample or concentration. In the early days of comet assay application 50 cells were indeed considered sufficient.[14] Nowadays most scientists agree that it would be much better to study 100 cells per group or individual (e.g., two different slides with 50 cells per slide). Results can be obtained in a few hours compared to other (cytogenetic) methods that easily takes several days. Furthermore, automation is possible.

In the Vitotox tests, we always include a solvent control (untreated bacteria) and a positive control which was 4-nitroquinoline-oxide (4-NQO; without S9) or benzo (a) pyrene (with S9). They both gave the expected response (Figure 2). Indeed, there was no influence on light emission in the Cytox strain and hence no toxicity or a direct (DNA-damage independent) influence on 'lux', whereas there was an increased S/N ratio in the Genox strain reaching values well above 1.5, indicating genotoxicity. All

plant extracts were tested at least twice in the Vitotox test. Only extract 1 (*Ficus nitida*) gave contradictory results in the absence of S9 in the first two assays and therefore 4 different tests were performed with changing concentrations of the extract. For this extract, repeat tests did not always give the same result in terms of genotoxicity and toxicity and interpretation of the data was therefore difficult. Figure 3a shows a genotox effect at the highest concentration (100 µg/mL; S/N> 1.5). This concentration also gave a toxic response as indicated in Figure 3b (S/N <<0.8). However, three repeat studies showed no genotoxicity, but toxicity was sometimes found at concentrations as low as 5 µg/mL (an example is given in Figure 3c-d). Because of absence of a genotoxic response in all but one test we yet concluded that this extract was not genotoxic but only low doses could be accurately tested. This sample was also non genotoxic in the presence of S9, and S9 apparently had a detoxifying effect (S/N ~ 1, Figures 3e-f).

The other samples were clearly non-genotoxic but some concentrations often also presented a toxic response. This is indicated in Table 2 which gives a summary of the results based on interpretation of graphs similar to those of Figures 2 and 3. When light emission was lowered to values well below S/N =0.8 we concluded that the concerned concentration of the extract was toxic although decreased light production may potentially have other, unknown reasons. The Vitotox test thus indicated toxicity at concentrations that were sometimes well below

Table 2: Synthesis of Vitotox test result for all 10 extracts

Ficus nitida (sample 1) Tested at concentrations of 1, 5, 10, 25, 50, 75 and 100 µg/mL. (distributed over different experiments)	No S9: Not genotoxic at doses below 25 µg/ml. At higher doses a toxic response may hinder proper evaluation of genotoxicity. With S9: S9 is apparently detoxifying the sample as toxicity was not or less present. CONCLUSION: not genotoxic but higher doses are toxic.
Ficus vasta (sample 2) Tested at concentrations of 1, 10 and 100 µg/mL.	No S9: Highest concentration is clearly toxic. The other concentrations are not genotoxic. With S9: There is a small increase in light production but it remains below 1.5. CONCLUSION: Not genotoxic at doses below 100 µg/ml.
Parkia africana (sample 3) Tested at concentrations of 1, 10 and 100 µg/mL.	No S9: Highest concentration is clearly toxic. Other concentrations are not genotoxic. With S9: Not genotoxic. There is a small increase in light production but it remains below 1.5. CONCLUSION: Not genotoxic at doses below 100 µg/ml.
Pistacia lentiscus (sample 4) Tested at concentrations of 0.5, 5 and 50 µg/mL.	No S9: All concentrations tested are highly toxic. Therefore we cannot evaluate genotoxicity. With S9: The highest dose is still very toxic. Other doses are not toxic and not genotoxic. CONCLUSION: Toxicity prevents to adequately evaluate genotoxicity of the sample in the absence of S9. With S9 the sample is not genotoxic.
Ampelopsis brevipedunculata (sample 5) Tested at concentrations of 0.5, 5 and 50 µg/mL.	No S9: Only the lowest concentration (0.5 µg/mL) is not toxic. This is also not genotoxic. Other doses are difficult to evaluate because of the toxic response. With S9: Highest dose is still toxic. Remaining doses are not genotoxic. CONCUSION: 0.5 µg/ml is not genotoxic but higher doses cannot be adequately evaluated due to a toxic response.
Derris robbusta (sample 6) Tested at concentrations of 1, 10 and 100 µg/mL.	No S9: Highest concentration is toxic. Other concentrations are not genotoxic. With S9: Borderline genotoxicity at highest concentration (100 µg/mL; S/N in Genox strain ~ 1.5) but because there is some increased light production also in the Cytox strain (and S/N of Genox over Cytox < 1.5) the conclusion remain "not genotoxic". CONCLUSION: not genotoxic at doses less than 100 µg/ml.
Bauhinia racemosa (sample 7) Tested at concentrations of 1, 10 and 100 µg/mL.	No S9: Here only the lowest tested concentration (1 µg/mL) is not toxic and also not genotoxic. Higher concentrations (10 and 100 µg/ml) cannot be evaluated with respect to genotoxicity because they are too toxic. With S9: Not genotoxic but highest dose shows signs of toxicity. CONCLUSION: Not genotoxic at lowest dose. The conclusion on higher doses may be questionable due to toxicity.
Lagerstroemia tomentosa (sample 8) Tested at concentrations of 1, 10 and 100 µg/mL.	No S9: Highest concentration (100 µg/mL) is toxic. Other doses (10 µg/mL) are not genotoxic. With S9: not genotoxic. CONCLUSION: Not genotoxic at concentation of 10 µg/mL).
Cichorium intybus (sample 9) Tested at concentrations of 1, 10 and 100 µg/mL.	No S9: Only the lowest dose (1 µg/mL) can be studied (others are toxic). 1 µg/ml is not genotoxic. We cannot say for higher concentration because of too much toxicity. With S9: highest dose toxic. Other concentrations were not toxic and not genotoxic.
Bauhinia alba (sample 10) Tested at concentrations of 1, 10 and 100 µg/mL.	No S9: Only lowest concentration is not toxic. Lowest concentration (1 µg/ml) is not genotoxic. With S9: Highest concentration is toxic. Lowest two concentrations are not genotoxic.

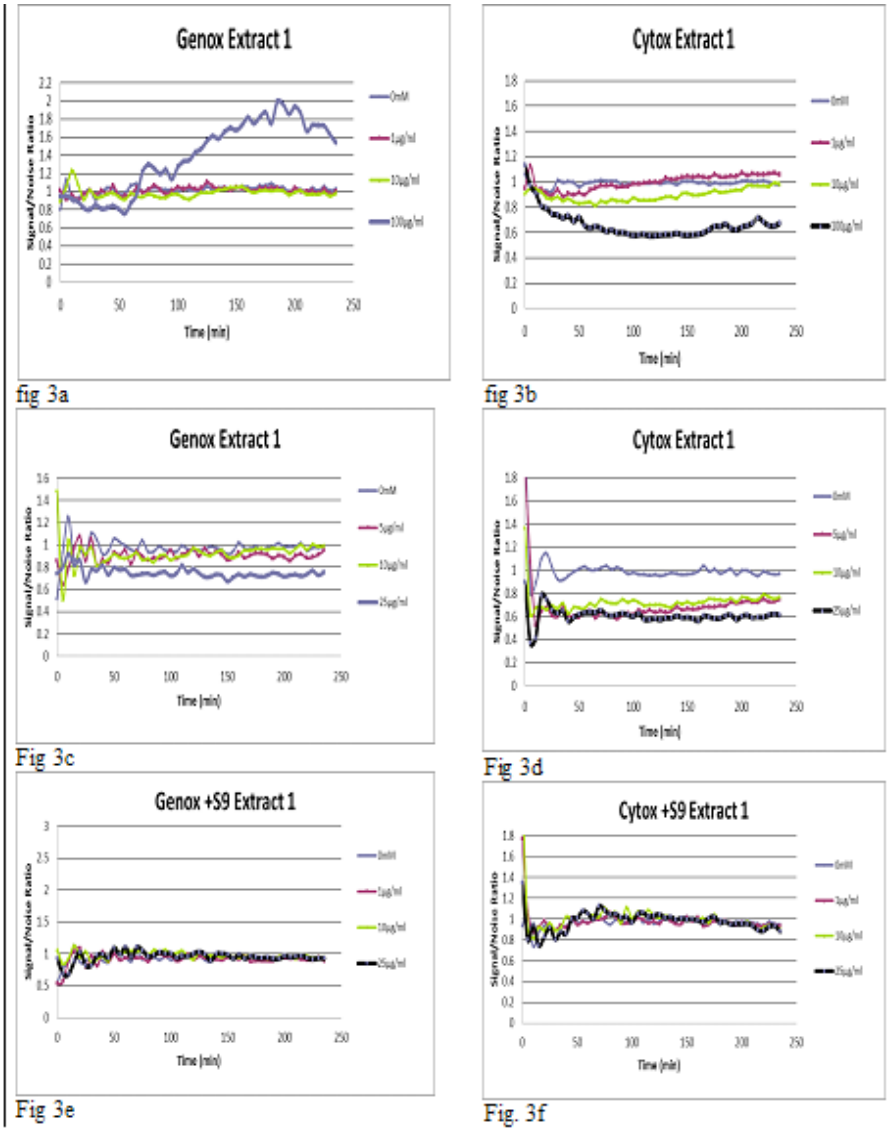


Figure 3: Examples of Vitotox results (S/N) for two tests with extract 1 in the absence of S9 showing deviating results, and a test in the presence of S9.

the NI_{50} value as determined according to the NRU test. This is not surprising as it is established that this bacterial genotoxicity test is very performing when pure single compounds are tested but that the interpretation of results from tests of complex mixtures, as are plant extracts, sometimes are more difficult due to an apparent toxic response and/or irregular dose-response curves.[9,16] Toxicity according to the Vitotox test may also be due to another mechanism as that detected by the NRU assay. The Vitotox test never the less remains an interesting screening test which correlates very well with the Ames assay.[8,12,13] Our results indicate that none of the extracts are mutagenic (genotoxic). This should however be confirmed by another test as reliability at higher concentrations was not always certain. This is another reason why we also conducted the alkaline comet assay in C3A cells.

In the comet assay different endpoints can be investigated, especially when analysis is done with an automated or semi-automated image analysis system. Examples are the total intensity of each comet, its tail length, % DNA in the comet head, % DNA in the comet tail and tail moment. It is our experience that the % tail DNA gives the most reliable measure. This is confirmed by literature data and is probably due to the fact that generally, % tail DNA covers the widest range of DNA damage.[17,18]

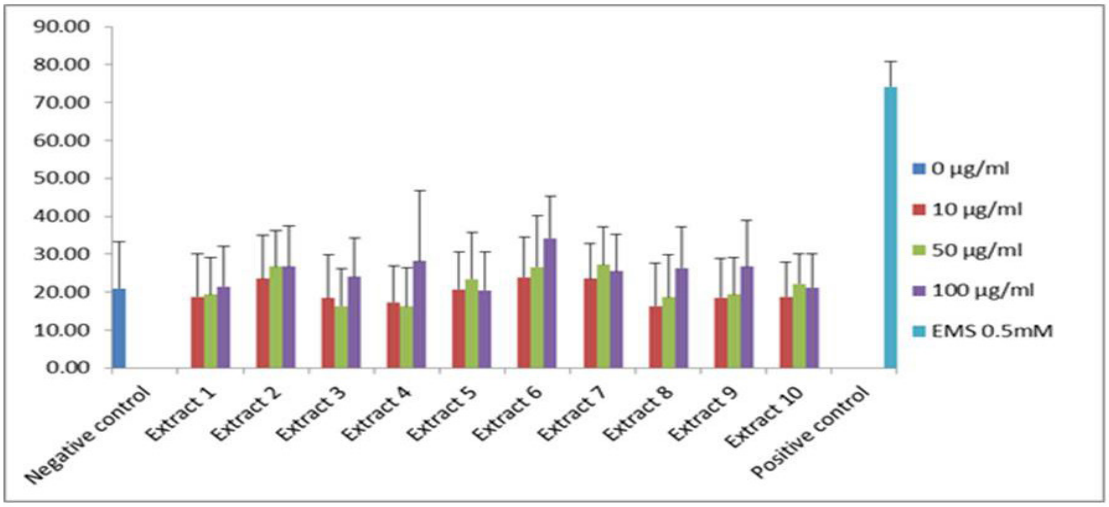


Figure 4: Mean percentage of 'DNA comets' according to the alkaline comet assay performed with different concentrations of methanol extracts of the 10 Egyptian plants. Bars are standard deviations.

Table 3: Phytochemical screening of the methanol extracts. (+) presence of constituents, (-) absence of constituents

Phytoconstituents	Plant extract number									
	1	2	3	4	5	6	7	8	9	10
Triterpenes and /or sterols	+	+	+	+	+	+	+	+	+	+
Carbohydrates and/ or glycosides	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Coumarins	-	-	-	-	-	+	-	-	-	-
Alkaloids and/ or nitrogenous compounds	-	-	-	-	-	+	-	-	-	-
Tannins	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-

Figure 4 gives the mean DNA comet tail frequencies (%) in function of the concentration tested. Standard deviations were rather large but this is quite normal as tail DNA contents can theoretically be from 0 up to 100% and a few cells with deviating tail DNA contents are sufficient to produce large standard deviations. The positive control was ethyl methane sulfonate (EMS; 0.5 mM). For each data point 100 cells were studied. It can be seen that all extracts in the three tested concentrations did not induce DNA damage as comet tail DNA contents were similar to those of the solvent control. None of the extracts and concentrations were found genotoxic according to the Mann-Whitney U-test ($p>0.05$). It may yet be noted that most DNA damage was seen for extract 6 (*Derris rubusta*) which was also 'borderline-genotoxic' in the Vitotox test. Both, the bacterial Vitotox test and comet assay thus reached similar conclusions.

None of the extracts were thus found genotoxic but extract 6 was borderline genotoxic in both tests and hence it merits further attention and should possibly be further investigated. It should be noted that this extract was also different from the others in terms of their phytochemical analysis. Indeed, preliminary to our genotoxicity studies we conducted a phytochemical analysis of the extracts. We did not presented the results in this paper but a summary is given in Table 3. It shows that the extracts contain several interesting bio-active compounds such as flavonoids, carbohydrates, tannins and triterpenes. *Derris robbusta* however, which proved to be the most active extract also contains alkaloids and coumarins. The significance of this in terms of toxicity and genotoxicity remains unclear,

but coumarin was for example found to be a carcinogen in rodents although it appears not genotoxic *in vivo*.[19,20]

CONCLUSION

The genotoxicity of ten methanol extracts of Egyptian medicinal plants were investigated with two complementary *in vitro* genotoxicity tests. None were shown to be genotoxic but Derris *robbusta* may need further investigations due to borderline genotoxicity in both tests.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

Highlights of Paper

- None of the plants were genotoxic.
- Derris rubosta showed borderline genotoxicity.
- This plant contains alkaloids and coumarins that were not present in the other plants.

Author Profile

• **Dr. Khaled Rashed:** Received his Ph. D. Degree in Science at the Organic Chemistry Department, Faculty of Science from the Menoufia university. He is a researcher in the pharmacognosy department. His scientific research interests go to the extraction and isolation of bio-active chemical constituents of medicinal plants such as as flavonoids, alkaloids, terpenoids, by different chromatographic methods, and also the chemical structural elucidation of natural products by techniques of spectroscopic analyses. He published more than 77 articles in international journals and has many international cooperation's.

• **Prof. Luc Verschaeve:** Presently in charge of the Toxicology laboratory of the Scientific Institute of Public Health, Brussels and Professor at the Department of Biomedical Sciences, Antwerp, Belgium. He has over 200 scientific papers of which more than 100 are references in PubMed. He is co-author of several books and author of a handbook on Genetic Toxicology. His expertise is especially on genetic toxicology. Present research topics are 'medicinal plants' and 'non-ionizing radiations'.

REFERENCES

1. Verschaeve L, Van Staden J. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. J. Ethnopharmacol. 2008; 119(1): 575- 87.
2. Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. Toxicol. Appl. Pharmacol. 2010; 243(2):198-216.
3. Ouedraogo M, Baudoux T, Stévigny C, Nortier J, Colet JM, Efferth T, *et al*. Review of current and "omics" methods for assessing the toxicity (genotoxicity, teratogenicity and nephrotoxicity) of herbal medicines and mushrooms. J. Ethnopharmacol. 2012; 143(2): 492-512.
4. Burlinson B. The *in vitro* and *in vivo* comet assays. Methods Mol. Biol. 2012; 817(1): 143-63.
5. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J. Phytol. 2011; 3(12): 10-4.
6. Repetto G, Del Peso A, Zurita JL. Neutral red uptake assay for the estimation of cell viability/cytotoxicity. Nature Prot. 2008; 34(12): 1125–31.
7. van der Lelie D, Regniers L, Borremans B, Provoost A, Verschaeve L. The VITOTOX test, a SOS-bioluminescence *Salmonella typhimurium* test to measure genotoxicity kinetics. Mutat. Res. 1997; 389(2): 279-90.
8. Verschaeve L, Van Gompel J, Regniers L, Van Parijs Ph, Van der Lelie D. VITOTOX® genotoxicity and toxicity test for the rapid screening of chemicals. Environmental and Molecular Mutagenesis 1999; 33(3): 240–8.
9. Verschaeve L. High-throughput bacterial mutagenicity testing: Vitotox™ assay. In: Steinberg P. (ed.), High Throughput Screening Methods in Toxicity Testing. Wiley, Hoboken, NJ; 2013. 213-32.
10. Olive PL, Banáth JP. The comet assay: a method to measure DNA damage in individual cells. Nature protocols 2006; 288(1): 23–9.
11. Kelly JH. Permanent human hepatocyte cell line and its use in a liver assist device (LAD). US Patent Nr. 1994; 5(8): 290: 6.
12. Westerinck WM, Stevenson JC, Horbach GJ, Schoonen WG. Evaluation of the Vitotox TM and Radarscreen assay for the assessment of genotoxicity in the early research phase of drug development. Mutat. Res. 2009; 676 (1-2) 113-30.
13. Westerinck WM, Stevenson JC, Horbach J, van de Water FM, van de Waart B, Schoonen WG. Genotoxicity and carcinogenicity: regulatory and novel test methods. In: Steinberg P. (ed.), High Throughput Screening Methods in Toxicity Testing, Wiley, Hoboken, NJ; 2013. 233-69.
14. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, *et al*. Single cell gel/comet assay: guidelines for *in vitro* and *in vivo* genetic toxicology testing. Environ. Mol. Mutagen. 2000; 35(3): 206-21.
15. Collins AR. The comet assay for DNA damage and repair: principles, applications, limitations. Mol. Biotech. 2004; 26(3): 249-61.
16. Verschaeve L. The VITOTOX® genotoxicity test. In Recent Research Developments in Applied Microbiology and Biotechnology, Pandali SG (ed.). Research Signpost: Trivandrum; 2005. p. 33–49.
17. Kumaravel TS, Jha AN. Reliable comet assay measurements for detecting DNA damage induced by ionising radiation and chemicals. Mutat. Res. 2006; 605(1): 7–16.
18. Collins AR, Oscoz AA, Brunborg G, Gaivão I, Giovannelli L, Kruszewski M, *et al*. The comet assay: topical issues. Mutagenesis 2008; 23(3): 143-51.
19. da Silva, FR, Erdtmann B, Dalpiaz T, Nunes E, Ferraz A, Martins TLC, *et al*. Genoxicity of Nicotiana tabacum leaves on *Helix aspersa*. Genet. Mol. Biol. 2013; 36(2): 269-75.
20. Coumarin. IARC Monograph on the Evaluation of Carcinogenic Risks to Humans. 2000; 77: 193-225.

21. Bruneton J. Pharmacognosy, phytochemistry medicinal plants. Intercept, Paris; 1999. 224.
22. Mousa O, Vuorela P, Kiviranta J, Abdel Wahab S, Hiltunen R, Vuorela H. Bioactivity 360 of certain Egyptian *Ficus species*. J. Ethnopharmacol. 1994; 41(1): 71–6.
23. Dawit Abebe, Asfaw Debella, Kelbessa Urga. Medicinal Plants and Other Useful Plants of Ethiopia, Camerapix publishers international, Addis Ababa, Ethiopia; 2003. 269-74.
24. Adetutu A, Morgan W, Corcoran O. Ethnopharmacological survey and *in vitro* evaluation of wound-healing plants used in South-western Nigeria. J. Ethnopharmacol. 2011; 1379(3): 50-6.
25. Loughlin MF, Ala'Aldeen DA, Jenks PJ. Monotherapy with mastic does not eradicate *Helicobacter pylori* infection from mice. J. Antimicrob. Chemother. 2003; 51(12): 367-71.
26. Paraschos S, Magiatis P, Mitakou S, *et al*. *In vitro* and *in vivo* activities of chios mastic gum extracts and constituents against *Helicobacter pylori*. Antimicrob. Agents Chemother. 2007; 51(2): 551–9.
27. Yabe N, Matsui H. *Ampelopsis brevipedunculata* (*Vitaceae*) extract inhibits a progression of carbon tetrachloride-induced hepatic injury in the mice. Phytomedicine 2000; 7(6): 493-8.
28. Asolkar LV, Kakkar KK, Charke OJ. Glossary of Indian Medicinal plants with active principles. New Delhi Publication and Information Directorate; 1992. 72-3.
29. The Wealth of India. A dictionary of Indian raw materials and industrial products. Vol. 2, CSIR, New Delhi, India; 1959. p. 56-7.
30. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. New Delhi: NISCAIR, CSIR; 2002. p. 546-54.
31. van Wyk BE, van Oudtshoorn B, Gericke N. Medicinal Plants of South Africa, Briza Publications. Pretoria, South Africa; 1997.
32. Vishnu Mittre. Wild plants in Indian folk life–A historical perspective. In: Jain SK. (ed.), Glimpses of Indian ethnobotany. Oxford and IBH Publishing Co, New Delhi; 1981. p. 37-58.