Mutagenicity and Clastogenicity Potential of Five Philippine Medicinal Plants

C.Y. Lim-Sylianco, B. Basilio, L.C.V. Mendoza, D. Ang
M.C. Villena, S. del Rosario

Department of Chemistry, College of Science
University of the Philippines
Diliman, Quezon City

ABSTRACT

Expressions from rhizomes of Acorus calamus *Linn., decoctions from leaves of* Cassia tora *Linn., seeds of* Glycine max *(Linn.) Merr., tubers of* Kaempferia galanga *Linn. and roots of* Vetiveria zizanioides *Stapf. were studied for mutagenicity and clastogenicity potential using Rec assay, Ames test, host-mediated assay and micronucleus test.*

The plant test systems did not possess direct DNA damaging capacity. These were not mutagenic before and after metabolic activation. No chromosome breaking effects were observed.

INTRODUCTION

Acorus calamus Linn. is known locally as *lubigan*. Its rhizome is used as a masticatory for toothaches and as a carminative in small doses (1). It treats intermittent flatulence, colic, or dyspepsia when chewed *ad libitum* (2), and is also used in curing hemorrhagic and intestinal ulceration (3). The powdered rhizome is reported to be an excellent remedy for rheumatism and is used as an embrocation (4). It is also used for sachet and toilet powder (5).

Decoctions of leaves of *Cassia tora* Linn. (known locally as *katanda*) are used as mild laxative. A poultice of leaves is used for gout, sciatica, and pain in the joints (6).

Decoctions of roots of *Glycine max* (Linn.) Merr. possess astringent properties (7). The green bean hulls when chewed into a pulp are applied to smallpox and corneal ulcers. The dried sprouts are considered to possess laxative properties (8).

The roasted rhizome of *Kaempferia galanga* Linn., which is known as *dusol*, is utilized for rheumatism and for sore throats (9), as well as for fevers (10). Plant decoctions are used for cancerous swellings, as a tonic for dyspepsia, headaches, and malarial chills (11).

Vetiveria zizanioides Stapf., known locally as *moras*, has many medicinal uses. The oil contained in the roots is a carminative (12), and a root decoction is used as a febrifuge and an abortifacient (1).

Because of the many uses of these plants, it is imperative to find out if these are genotoxic. Genotoxicity to somatic cells induces cancer while genotoxicity to germ cells induces genetic disorders that are transmitted from one generation to the next.

Table 1. Plant samples - names, parts used and preparation.

Scientific Name	Local Name	Plant Part Used	Type of Preparation
Acorus calamus Linn.	Lubigan	Rhizomes	Expression
Cassia tora Linn.	Katanda	Leaves	Decoction
Glycine max (Linn.) Merr.	Utau	Seeds	Decoction
Kaempferia galanga Linn.	Dusol	Tubers	Decoction
Vetiveria zizanioides Stapf.	Moras	Roots	Decoction

MATERIALS AND METHODS

The plants and their respective parts that were used for the study, and the type of preparation done for each, are listed in Table 1.

The direct DNA-damaging capacity of the test plants was studied using the Rec assay. *Bacillus subtilis*, Rec^- and Rec^+ strains, were obtained from Dr. Tsueno Kada, National Institute of Genetics, Mishima, Japan. The strains were streaked separately across the surface of a top agar (13). A sterile paper disc containing the test system was placed over the starting point of each streak. The plates were incubated at 37°C for 20 hours, after which the length of the inhibition zones were measured.

To investigate mutagenicity before metabolic activation, the Ames test was used (14). *Salmonella typhimurium* strains TA 1535 and TA 1537, which were obtained from Dr. Bruce N. Ames, Department of Biochemistry, University of California, Berkeley, were used. These strains were incubated overnight at 37°C in separate test tubes, each containing 5 mL of nutrient broth. A 0.1 mL culture of each strain was transferred into a 13x100 mm test tube. Into each test tube was pipetted 2 mL of molten top agar, which was mixed with the culture. The mixture was then poured into a hardened bottom agar plate. After the top agar hardened, a sterile paper disc of 8 mm in diameter was saturated with the test solution and placed in the center of the plate. The plates were incubated for 48 hours at 37°C, after which the revertant colonies were counted.

The host-mediated assay (15) was employed to study the mutagenicity potential after metabolic activation. The indicator organism, *Salmonella typhimurium* His G 46, was a gift from Dr. M. Moriya, Institute of Toxicology, Tokyo. Albino mice of Swiss Webster strain, weighing 17-25 grams, were used. The indicator organism was injected into the peritoneal cavity followed by an oral administration of the test system. After three hours, the mice were sacrificed and peritoneal exudates were removed. The exudates were plated and the mutation frequency of the indicator organism was determined.

The micronucleus test was used to study chromosome breaking effects (16), using Swiss Webster mice weighing 20-25 grams. The test system was administered twice: 30 hours, and six hours, prior to the extraction of the bone marrow of the femur and its flushing

Table 2. DNA damaging potential using the Rec assay.

SAMPLE	Zone of Inhibition (mm) Rec^-	Rec^+
Positive control, 4-NQO	17.9 ± 1.22	22.4 ± 1.02
Negative control, dist. H_2O	0	0
Acorus calamus Linn.	0	0
Cassia tora Linn.	0	0
Glycine max (Linn.) Merr.	0	0
Kaempferia galanga Linn.	0	0
Vetiveria zizanioides Stapf.	0	0

Table 3. Mutagenicity potential before metabolic activation using Ames test.

SAMPLE	Revertants Per Plate TA 1535	TA 1537
Positive control, EMS	TNTC*	TNTC*
Distilled H_2O	4.5	5.7
Acorus calamus Linn.	5.0	5.1
Cassia tora Linn.	4.9	5.0
Glycine max (Linn.) Merr.	2.1	5.2
Kaempferia galanga Linn.	3.8	4.2
Vetiveria zizanioides Stapf.	4.2	5.6

*Too numerous to count

into a test tube containing fetal calf serum (obtained from Grand Island Biological Supply, N.Y.). The air-dried smear was stained and examined for micronucleated polychromatic erythrocytes.

RESULTS AND DISCUSSION

Table 2 shows that no zones of inhibition were formed by the test plant systems in both strains of *Bacillus subtilis*, suggesting that the decoctions and expressions did not possess direct DNA damaging capacity. The Rec strain does not have the recombination repair system, thus, if its DNA were damaged, a zone of inhibition should be formed. This, however, was not observed.

The results of the Rec assay were strengthened by the finding that before metabolic activation, the test systems did not cause reversions of *S. typhimurium* mutants TA 1535 and TA 1537 (Table 3). The expressions and decoctions from the test plants did not contain base-pair or frameshift mutagens. TA 1535 is reverted to the wild-type by base-pair mutagens while TA 1537 is similarly reverted by frameshift mutagens. With the test systems from the five medicinal plants, no significant reversions were observed. The plant preparations are therefore not genotoxic before metabolic activation.

Table 4 shows that the indicator organism was not mutated, implying that the plant preparations were not mutagenic even after metabolic activation. The mutation frequency for the plant preparations was at the same range as the negative control, which was distilled water.

The preparation from *Glycine max* had the lowest mutation frequency (even lower than that of distilled water), suggesting that this preparation reduces the spontaneous mutation of the indicator organism.

Table 4. Mutagenicity potential after metabolic activation using the host-mediated assay.

SAMPLE	Mutation Frequency*
Control	1.00 ± 0.02
Acorus calamus Linn.	0.99 ± 0.03
Cassia tora Linn.	1.05 ± 0.07
Glycine max (Linn.) Merr.	0.45 ± 0.01
Kaempferia galanga Linn.	1.29 ± 0.10
Vetiveria zizanioides Stapf.	1.12 ± 0.23

*Mutation frequency of the indicator organism *Salmonella typhimurium* His G 46.

Table 5. Clastogenicity potential using the micronucleus test.

SAMPLE	No. of micronucleated polychromatic erythrocytes per thousand
Positive control, DMN	14.34 ± 1.22
Distilled H_2O	2.34 ± 0.09
Acorus calamus Linn.	1.66 ± 0.03
Cassia tora Linn.	1.33 ± 0.11
Glycine max (Linn.) Merr.	1.33 ± 0.08
Kaempferia galanga Linn.	1.26 ± 0.03
Vetiveria zizanioides Stapf.	2.67 ± 0.14

Table 5 shows that the plant preparations do not possess chromosome-breaking effects. This means that they do not contain substances that can fragment the chromatin material of the bone marrow cells of mice. If fragmentation took place after telophase, when the nucleus is expelled, some fragments would have been left behind, forming micronuclei in the cytoplasm of the cells. This is not seen in the values of micronucleated polychromatic erythrocytes given in Table 5. These

recorded values fall within the range of distilled water, which is the negative control.

Damage to the DNA of somatic cells can induce cancer while damage to the DNA of germ cells can induce sterility and genetic defects which can be transmitted from one generation to the next. The findings in this study can provide assurance that the preparations commonly used from the plants concerned do not induce damage to the DNA of the cells of the users. ■

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