MUTAGENICITY AND CLASTOGENICITY POTENTIAL OF DECOCTIONS AND INFUSIONS FROM PHILIPPINE MEDICINAL PLANTS

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ABSTRACT

The Philippine National Formulary lists Philippine plants whose decoctions and infusions are used for medicinal purposes. Mutagenicity potential of these decoctions and infusions were studied without metabolic activation and after metabolic activation. Without metabolic activation, decoction from leaves of *Plantage major* L. and decoctions from leaves and bark of *Pittosporum pentandrum* (Blanco) Merr. induced frameshift mutagenesis in *Salmonella typhimurium.* After metabolism in the experimental mice, these induced both base-pair and frameshift mutagenesis. Decoctions from bark of *Pitchecolobium dulce* (Roxb) Benth. induced base-pair mutagenesis without metabolic activation. Upon metabolic activation in the mice, this tendency was lost. Instead it induced frameshift mutations. Decoctions from stems of *Arcangelisia fava* (L.) Merr. induced both base-pair and frameshift mutations without metabolic activation. However, this mutagenic property was lost upon metabolism in the experimental mice.

The following are non-mutagenic before and after metabolism:

1. Decoctions from leaves of *Citrus documana* L., *Eucalyptus deglupta* Blume, *Moringa oleifera* Lam., *Pandanus odoratissimus* L. *Persea americana* Mill., *Psidium guajava* L., *Sterculea foetida* L. and *Tamarindus indica* L.
2. Decoctions from plants of *Apium graveolens* L., *Mimosa pudica* Linn., *Rosmarinus officinalis* L. and *Solanum nigrum* L.
3. Decoctions from bark of *Mangifera indica* L., and *Michelina champaca* L.
4. Decoctions from kernel of *Arecha catechu* L., from bran of *Oryza sativa* L. and from hair and cob of *Zea mays* L.
5. Infusions from leaves of *Momordica balsamina* Blanco, from bark and leaves of *Anacardium occidentale* L., from fruit of *Foeniculum vulgare* L., and from leaves of *Mangifera indica* L.

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Clastogenicity or chromosome breaking potential was exhibited by decoctions from leaves and bark of *Pittosporum pantandrum* (Blanco) Merr., decoctions from leaves of *Plantago major* L., decoctions from leaves of *Eucalyptus deglupta* Blume and decoctions from cobs of *Zea mays* L. Infusions from leaves of *Anacardium occidentale* L. also showed chromosome breaking effects.

INTRODUCTION

The use of medicinal plants in the rural areas of the Philippines is quite popular. For some plants, expressions and contusions from stem, leaves, flowers, rhizomes and seeds are utilized. In others, decoctions and infusions are used.

The Philippine National Formulary (1) lists some 84 plants of medicinal use in the Philippines. While studies on active constituents are many, those of mutagenicity and clastogenicity are only emerging. Among so many medicinal plants, so far only comfrey has been studied as regards mutagenicity and clastogenicity (2). This report deals with studies on the mutagenicity and clastogenicity potential of decoction and infusions of medicinal plants described in the Philippine National Formulary.

MATERIALS AND METHODS

The medicinal plants investigated as shown in Table 1 and 2 were gathered from Iloilo, Metro Manila and Los Baños, Laguna.

Mutant strains of *Salmonella typhimurium* were gifts from Dr. B. N. Ames, Department of Biochemistry, University of California, Berkeley.

Fetal calf serum (Lyophilized) was purchased from CALBIOCHEM. Giemsa and May-Grunwald stains are Merck products.

The experimental mice were obtained from Alabang Stock Farm.

Mutagenicity potential without metabolic activation was studied using the method of Ames (3). *Salmonella typhimurium* strains TA 1535, TA 1537 and TA 98 were used. The bacteria were grown overnight in a tryptone-yeast broth. The bacteria were incorporated into a previously melted top agar, then mixed and subsequently layered on top of a solid bottom agar. The plates were incubated at 37°C. After a period from 48 to 72 hours, revertant colonies per plate were counted.

Mutagenicity potential after metabolic activation was studied using the host-mediated assay of Gabridge and Legator (4). It is a combination of *in vivo* mammalian metabolism of mutagens and the microbial mutation tests. An indicator bacteria is injected into the peritoneal cavity of the experimental mouse, and then test compounds were administered to the animals intramuscularly. After the bacteria come in contact with the metabolites of the test substance, they are withdrawn from the peritoneal cavity and

Table 1. Philippine Medicinal Plants Used as Decoctions*

English Name	Scientific Name	Parts Used
Areca Nut	*Arecha catechu* L.	Kernel
Avocado	*Persea americana* Mill.	Leaves
Black Nightshade	*Solanum nigrum* L.	Herb
Black Plum	*Eugenia cumini* (L.) Druce	Bark
Celery	*Apium graveolens* L.	Plant
Corn	*Zea mays* L.	Hair, Cob
Fragrant Screw-pine	*Pandanus odoratissimus* L.	Leaves,
Grapefruit	*Citrus decumana* L.	Leaves
Guava	Psidium guajava L.	Leaves
Horse Raddish Tree	*Moringa oleifera* Lam.	Leaves
Mango	*Mangifera indica* L.	Leaves Kernel
Ribwort	*Plantago major* L.	Leaves
Rice Polishings	*Oryza sativa* L.	Bran
Rosemary	*Rosmarinus officinalis* L.	Herb
Tamarind	*Tamarindus indica* L.	Leaves
Wild Almond	*Sterculia foetida* L.	Leaves
	Arcangelisia flava (L.) Merr.	Stem
	Eucalyptus deglupta Blume	Leaves
	Pithecolobium dulce (Roxb) Benth.	Bark
	Pittosporum pentandrum (Blanco) Merr.	Bark, Leaves
	Pandanus tectorius Soland	Roots
	Michelia champaca L.	Bark

*From Philippine National Formulary

induced mutation frequency is determined, from the number of mutants and the number of bacteria recovered. The indicator bacteria used were *Salmonella typhimurium* mutants TA 1535 and TA 1537.

Clastogenic or chromosome breaking effects were studied using the micronucleus test of Schmid (5). About 7-12 weeks old mice were used. The test substance was administered orally at 30 hours and 6 hours before the animals were sacrificed. Immediately after sacrificing the animal, both femurs

Table 2. Philippine Medicinal Plants Used as Infusions*

English Name	Scientific Name	Parts Used
Bitter guord	*Momordica balsamina* Blanco	Leaves
Cashew	*Anacardium occidentale* L.	Bark, Leaves
Fennel	*Foeniculum vulgare* Gaertn.	Fruit
Mango	*Mangifera indica* L.	Leaves

*From Philippine National Formulary

are removed. The bones are freed from muscle tissues. The marrow is forced out of the femur carefully by the use of fetal calf serum in a syringe (about 0.2 ml of fetal calf serum). The flushings were centrifuged at 1000 rpm for 5 minutes. The supernatant is drawn off by a Pasteur pipette. The cells in the sediment are carefully mixed by repeated aspirations. A small drop of the viscous suspension is put on the end of a slide and spread by pulling the material behind a polished cover glass at an angle of 45^{o}. Staining is done within 24 hours. The slides are scored for micronucleated polychromatic erythrocytes per thousand.

RESULTS AND DISCUSSION

Salmonella typhimurium strain TA 1535 is reverted to the wild type by base-pair mutagens while TA 1537 and TA 98 are reverted by frameshift mutagens. These mutants lack the lipolysaccharide barrier on their cell walls and they also lack the excision repair system. TA 98 is more sensitive to frameshift mutagens than TA 1537 because it contains an R plasmid.

Of the plants listed in Table 3, only decoctions from leaves of *Pittosporum pentandrum* (Blanco) Merr. and *Plantago major* L. showed direct mutagenic effects. Without metabolic activation, decoctions from leaves of *Pittosporum pentandrum* (Blanco) Merr. caused significant reversions in TA 98; while decoctions from leaves of *Plantago major* L. caused reversions in TA 1537 and TA 98. These observations suggest that these two plants contain direct frameshift mutagens. After metabolism in the experimental mouse the decoctions of these two plants (Table 5) induced reversions in TA 1535 and TA 1537 indicating that the tendency to induced frameshift mutations was not lost upon metabolism and that there was a production of a metabolite

Table 3. Mutagenicity Potential Without Metabolic Activation of Decoctions From Leaves of Some Medicinal Plants.

	Number of Revertants per plate		
	TA 1535	TA 1537	TA 98
Control	6.60	7.40	13.41
Citrus decumana L.	6.64	7.46	12.33
Eucalyptus deglupta Blume	6.40	5.36	8.23
Moringa oleifera Lam.	5.64	7.17	10.93
Pandanus odoratissimus L.	4.96	6.73	12.27
Persea americana Mill.	3.40	4.67	5.30
Pittosporum pentandrum (Blanco) Merr.	5.24	6.98	21.78
Plantago major L.	5.21	20.24	29.75
Psidium guajava L.	6.08	6.80	11.56
Sterculia foetida L.	6.00	5.80	12.49
Tamarindus indica L.	5.87	7.04	11.66

TA 1535, TA 1537 and TA 98 are *Salmonella typhirium* mutants.

or metabolites that induced base-pair mutations.

Of the plants in Table 4, decoctions from bark of *Pithocolobium dulce* (Roxb) Benth. induced reversions in TA 1535 suggesting the presence of direct base-pair mutagens. Upon metabolism in the experimental mouse, this tendency to induce base-pair mutations was lost (Table 6). Instead, it induced frameshift mutations as indicated by reversions induced in TA 98. This shows that a frameshift mutagen was produced upon metabolism, while the direct based-pair mutagen was transformed to a non-mutagen.

Decoctions from stems of *Arcangelisia flava* (L.) Merr. (Table 4) induced both base-pair and frameshift mutations without metabolic activation. However, this mutagenic property was lost upon metabolism in the experimental mouse (Table 6). Decoctions from bark of *Pithecolobium dulce* (Roxb) Benth. induced both base-pair and frameshift mutagenesis without metabolic activation. However, the tendency to induce base-pair mutations was lost upon metabolism (Table 6).

None of the infusions (Table 7, and 8) induced mutations in TA 1535, TA 1537 and TA 98 before metabolic activation indicating the absence of direct mutagens. The absence of mutagenic effects on TA 1535 and TA 1537

Table 4. Mutagenicity Potential Without Metabolic Activation of Decoctions from Parts Other Than The Leaves of Some Medicinal Plants

	Parts Used	Number of Revertants per plate TA 1535	TA 1537	TA 98
Control	–	6.60	7.42	13.41
Apium graveolens L.	plant	6.16	7.53	13.68
Arcangelisia flava (L.) Morr.	stem	10.44	6.36	33.98
Arecha catechu L.	kernel	7.08	6.80	7.50
Mangifera indica L.	bark	6.80	5.62	7.02
Michelia champaca L.	bark	6.40	4.70	13.34
Mimosa pudica Linn.	plant	4.67	6.58	11.21
Oryza sativa L.	bran	6.72	6.40	12.22
Pithecellubium dulce (Roxb) Benth.	bark	22.23	7.26	13.36
Pittosporum pentandrum (Blacno) Merr.	bark	6.85	6.08	34.54
Rosmarinus officinalis L.	plant	4.86	5.78	11.98
Solanum nigrum L.	plant	5.41	7.61	13.56
Zea mays L.	hair	6.68	6.78	9.00
	Cob	6.44	5.48	7.86

TA 1535, TA 1537 and TA 98 are mutant straints of *Salmonella typhimurium.*

Table 5. Mutagenicity Potential After metabolic Activation of Decoctions From Leaves of Some Medicinal Plants

	Mutation Frequency TA 1535	TA 1537
Control	1.00	1.00
Citrus decumana L.	1.03	0.30
Eucalyptus deglupta Blume	0.43	0.29
Moringa elifera Lam.	0.06	0.36
Pandanus odoratissimus L.	0.55	0.24
Persea americana Mill.	0.34	0.65
Pittosporum pentandrum (Blanco) Merr.	3.08	4.41
Plantago major L.	3.72	3.75
Psidium guajava L.	0.98	0.76
Sterculia foetida L.	0.48	1.04
Tamarindus indica L.	1.00	0.30

TA 1535 and TA 1537 were the indicator organisms used in the Host-Mediated Assay

Table 6. Mutagenicity Potential After Metabolic Activation of Decoctions of Parts Other Than The Leaves of Some Medicinal Plants.

	Parts Used	Mutation Frequency TA 1535	TA 1537
Control	–	1.00	1.00
Apium graveolens L.	plant	0.31	1.01
Arcangelisia flava (L.) Merr.	stem	1.09	1.07
Arecha catechu L.	kernel	1.05	0.62
Mangifera indica L.	bark	1.08	0.06
Michelia champaca L.	bark	1.03	0.64
Mimosa pudica Linn.	plant	1.01	0.87
Oryza sativa L.	bran	0.78	0.69
Pithecolobium dulce (Roxb) Benth.	bark	0.85	3.98
Pittosporum pentandrum (Blanco) Merr.	bark	0.06	1.05
Rosmarinus officinalis L.	herb	1.01	0.08
Solanum nigrum L.	plant	0.24	1.10
Zea mays L.	hair	0.09	0.07
	cob	0.64	0.17

TA 1535 and TA 1537 are the indicator organisms in the Host-Mediated Assay

Table 7. Mutagenicity Potential Without Metabolic Activation of Infusions From Some Medicinal Plants.

	Parts Used	Number of Revertants per plate TA 1535	TA 1537	TA 98
Control	–	6.60	7.42	13.41
Momordica balsamina Blanco	leaves	4.80	6.78	10.98
Anacardium occidentale L.	bark	6.78	7.54	11.52
	leaves	5.56	6.98	12.96
Foeniculum vulgare Gaertn.	fruit	5.48	7.38	13.28
Mangifera indica L.	leaves	5.23	7.64	11.45

TA 1535, TA 1537 and TA 98 are mutant strains of *Salmonella typhimurium*

Table 8. Mutagenicity Potential After Metabolic Activation of Infusions from Some Medicinal Plants.

	Parts Used	Mutation Frequency TA 1535	TA 1537
Control	–	1.00	1.00
Momordica balsamina Blanco	leaves	1.04	1.02
Anacardium occidentale L.	bark	1.05	0.09
	leaves	1.59	1.41
Foeniculum vulgare Gaertn.	fruit	1.00	1.00
Mangifera indica L.	leaves	1.04	1.02

TA 1535 and TA 1537 are the indicator organisms in the Host-Mediated Assay

was also observed after metabolism suggesting that none of the infusions are pro-mutagens.

The micronucleus test is an in vivo test for mutagenicity and clastogenicity. Mutagenic substances cause the formation of micronuclei in bone marrow cells of experimental mice. Mitotic bone marrow cells with chromatid breaks and chromatid exchanges suffer from disturbance in anaphase distribution of chromatin. Chromosome pieces lag in the anaphase. After telophase, a sizable protion of the displaced chromatin is not included in the nucleus of daughter cells. Instead they form single or multiple micronuclei in the cytoplasm of these cells. Aside from mutagens, spindle posions results in micronuclei formation (5).

Decoctions from bark and leaves of *Pittosporum pentandrum* (Blanco) Merr. induced micronuclei formation in erythrocytes of bone marrow cells (Table 9). This clastogenic property could be a consequence of its tendency to induce base-pair and frameshift mutagenesis after metabolic activation (Table 5). Decoctions from leaves of *Plantago major* L. which induced base-pair and frameshift mutations after metabolic activation (Table 5) caused the formation of micronucleated polychromatic erythrocytes.

Two plants, the decoctions of which were shown to be non-mutagenic exhibited clastogenic effects. These are decoctions from leaves of *Eucalyptus deglupta* Blume and decoctions from cobs of *Zea mays* L. (Table 9). These were not shown to contain mutagenic substances either before or after metabolism. It is possible that their clastogenic effects were not due to mutagens but to some constituents that affect the spindle apparatus. The same expla-

Table 9. Clastogenicity Potential of Decoctions and Infusions from Some Medicinal Plants.

	Parts Used	No. of micronucleated polychromatic crythrocytes per thousand
Control	—	2.00
Decoctions		
Apium graveolens L.	plant	2.00
Arcangelisia flava (L.) Merr.	stem	1.51
Arecha catechu L.	kernel	1.43
Eucalyptus deglupta Blume	leaves	8.10
Eugenia cumini (L.) Druce	bark	1.72
Mangifera indica L.	bark	2.11
Mimosa pudica Linn.	plant	1.51
Oryza sativa L.	bran	1.23
Pitthecolobium dulce (Roxb) Benth.	bark	1.21
Pittosporum pentandrum (Blanco) Merr.	bark	7.93
	leaves	8.56
Plantago major L.	leaves	9.20
Rosmarinus officinalis L.	herb	1.87
Solanum nigrum L.	herb	1.64
Zea mays L.	hair	2.08
	cob	9.87
Infusions		
Momordica balsamina Blanco	leaves	1.25
Anacardium occidentale L.	bark	2.00
	leaves	8.54
Foeniculum vulgare Gaertn.	fruit	2.21
Mangifera indica L.	leaves	1.11

nation is offerred for the clastogenic effects of infusions from leaves of *Anacardium occidentale* L.

Decoctions from bark of *Pithecolobium dulce* (Roxb) Benth. induced frameshift mutations after metabolic activation but did not induce the formation of micronucleated erythrocytes. It is possible that the mutagenic metabolite could have been short-lived and did not reach the bone marrow cells.

The following are non-mutagenic and non-clastogenic:

1. Decoctions from plants of *Apium graveolens* L., *Mimosa pudica* Linn., *Rosmarinus officinalis* L., and *Solanum nigrum* L.
2. Decoctions from bark of *Euginia cumiai* (L.) Druce, and *Mangifera indica* L.
3. Decoctions from kernel of *Arecha catechu* L. from bran of *Oryza sativa* L. and from hair of *Zea mays* L.
4. Infusions from leaves of *Momordica balsamina* Blanco, and *Mangifera indica* L.
5. Infusions from bark of *Anacardium occidentale* L. and from fruit of *Foeniculum vulgara* Caertn.

CONCLUSION

No mutagenic effects were observed in decoctions from leaves of *Citrus decumana* L., *Moringa oleifera* Lam., *Pandanus odoratissimus* L., *Persea americana* Mill., *Paidium guajava* L., *Sterculea footida* L. and *Tamarindus indica* L. either before or after metabolism.

Likewise, no mutagenic effects were observed before or after metabolic activation of decoctions from plants of *Apium graveolens* L., *Mimosa pudica* Linn., *Rosmarinus officinalis* L. and *Solanum nigrum* L.; decoctions from bark of *Mangifera indica,* L., and *Michelia champaca* L.; decoctions from kernel of *Arecha catechu* L.; decoctions from bran of *Oryza sativa* L. and from hair and cob of *Zea mays* L.

Infusions from leaves of *Momordica balsamina* Blanco, from bark and leaves of *Anacardium occidentale* L., from fruits of *Foeniculum vulgare* L., and from leaves of *Mangifera indica* L., did not induce mutations either before or after metabolic activation.

Without metabolic activation, decoctions from leaves of *Plantago major* L., and decoctions from leaves and bark of *Pittosporum pontandrum* (Blanco) Merr. induced frameshift mutations in *Salmonella typhimurium.* After metabolism in the experimental mice, these induced both base-pair and frameshift mutations. Decoctions from bark of *Pithocolobium dulce* Roxb) Benth. induced base-pair mutations without metabolic activation. Upon metabolic activation in the experimental mice, this tendency was lost. Instead, it induced frameshift mutageneisis. Decoctions from stems of *Arcangelisia flava* (L.) Merr. induced both base-pair and frameshift mutations

Table 10. Summary of Mutagenicity and Clastogenicity Potential of Some Philippine Medicinal Plants.

Scientific Name	Local Name	Preparation	M*	C*
Citrus decumana L.	Suha	Decoction, leaves	–	–
Eucalyptus deglupta Blume	Eucalyptus	Decoction, leaves	–	+
Moringa oleifera Lam.	Malunggay	Decoction, leaves	–	–
Pandanus odoratissimus L.	Pandan-mabango	Decoction, leaves	–	–
Persea americana Mill.	Abukado	Decoction, leaves	–	–
Pittosporum pentandrum (Blanco) Merr.	Mamalis	Decoction, leaves	+	+
Plantago major L.	Lanting	Decoction, leaves	+	+
Psidium guajava L.	Bayabas	Decoction, leaves	–	–
Sterculia foetida L.	Kalumpang	Decoction, leaves	–	–
Tamarindus indica L.	Sampalok	Decoction, leaves	–	–
Apium graveolens L.	Kintsay	Decoction, plant	–	–
Arcangelisia Flava (L.) Merr.	Abutra	Decoction, stem	+**	–
Areca catechu L.	Bunga	Decoction, kernel	–	–
Mangifera indica L.	Mangga	Decoction, bark	–	–
Mitchelia champaca L.	Tsampakang pula	Decoction, bark	–	–
Mimosa pudica Linn.	Makahia	Decoction, plant	–	–
Oryza sativa L.	Darak	Decoction, bran	–	–
Pithecelobium dulce (Roxb) Benth.	Kamatsile	Decoction, bark	+	–
Pittosporum pentandrum (Blanco) Merr.	Mamalis	Decoction, bark	+	+
Rosmarinus officinalis L.	Romero	Decoction, herb	–	–
Solanum nigrum L.	Kama-kamatisan	Decoction, plant	–	–
Zea mays L.	Mais	Decoction, cob	–	–
Anacardium occidentale L.	Kasuy	Infusion, bark	–	–
		Infusion, leaves	–	+
Foeniculum vulgare Gaertn.	Anis	Infusion, fruit	–	–
Mangifera indica L.	Mangga	Infusion, leaves	–	–
Momordica balsamina Blanco	Ampalaya	Infusion, leaves	–	–

M* – mutagenicity potential

C* – clastogenicity potential

+** – Mutagenicity disappears upon metabolism

without metabolic activation. However, this mutagenic property was lost upon metabolism.

Clastogenic or chromosome breaking property was exhibited by decoctions from leaves and bark of *Pittosporum pentandrum* (Blanco) Merr., decoctions from leaves of *Plantago major* L., decoctions from cobs of *Zea mays* L. Infusions from leaves of *Anacardium occidentale* L. also showed chromosome breaking effects. The rest were found to be non-clastogenic.

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