

Bioassay Research Laboratory
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Bioassay Research Laboratory
report of
BACTERIAL MUTAGENICITY TEST

For

SMITH NATURALS Co., Ltd.
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Bacterial Mutagenicity Test

Samples

The genotoxicity of plants extract by Ames test [1,2] was performed in *Salmonella* mutation assay using *Salmonella typhimurium* TA98 and TA100 as tester strain with and without metabolic (S9 mix [3]) activation.

The tested plant extracts are:

1. Butea superba Powder extract, Lot No. SBS-SA-04-PE
2. Pueraria mirifica Powder extract, Lot No. SPM-SA-25-PE
3. Terminalia Chebula Powder extract, Lot No. STC-SA-01-PE
4. Butea superba liquid extract, Lot No. SBS-SA-04-FE
5. Pueraria mirifica liquid extract, Lot No. SPM-SA-25-FE

Powder extracts were dissolved in Dimethyl sulfoxide (DMSO), sterilized and kept in refrigerator. Liquid extracts were sterilized and kept in refrigerator until analysis.

Bacterial Strains.

Salmonella typhimurium strains TA98 and TA100 were obtained from Dr. Taijiro Matsushima (Japan Bioassay Research Center, Japan Industrial Safety and Health Association, Kanagawa, Japan).

Methodology

The preincubation procedure was performed as described by Matsushima et al (1980) [4]. The test extracts solution at different doses per 50 µl were preincubated with 0.5 ml of S9 mix (metabolic activation method, S9 mix contained 4 mM NADPH, 4 mM NADH, 5mM G-6-P, 8mM MgCl₂, 33 mM KCl, 100 mM sodium phosphate buffer pH 7.4, and 10 % S9 fraction : S9 fraction prepared according to Matsushima et al. (1976) [3] or 0.1 M phosphate buffer pH 7.4 (direct method) and 0.1 ml of bacterial tester strains which had been cultured in nutrient broth. The mixture was preincubated for 20 min at 37 °C, then mixed with 2 ml molten top agar containing 0.05 µmol/ml of L-histidine and biotin. Then the top agar was rapidly

pouring onto a 30 ml of Vogel-Bonner minimal agar plate. All plates were incubated for 48 hours at 37 °C after that the number of revertant colonies were scored.

Determination of Toxicity (Growth inhibition)

For determination of the toxicity of test compounds, the growth of the background lawn of tester strains on each plate was examined under a stereo microscope. The back-ground growth consists of micro colonies visible with aid of the stereo microscope. Bactericidal compound lead to a decrease in the number of the micro colonies and to an increase in their diameter. When the toxicity was observed, an asterisk was placed to the right of the number of the colonies expressed in the data sheet.

Controls

The following standard mutagens served as positive control for test;

| Tester strain | Without activation (- S9 mix) | | With activation (+ S9 mix) | |
|---------------|-------------------------------|--------------------------|----------------------------|--------------------------|
| | Name | Concentration (µg/plate) | Name | Concentration (µg/plate) |
| TA 98 | AF-2 | 0.1 | 2-AA | 0.5 |
| TA 100 | AF-2 | 0.01 | 2-AA | 1.0 |

Control values (mean ± standard deviation) of solvent controls and positive controls on the study are shown as follows:

| Tester strain | Solvent control | | Positive control | |
|---------------|-----------------|----------|------------------|----------|
| | - S9 mix | + S9 mix | - S9 mix | + S9 mix |
| TA 98 | 20 ± 8 | 28 ± 7 | 435 ± 82 | 947 ± 56 |
| TA 100 | 143 ± 30 | 144 ± 29 | 658 ± 63 | 786 ± 89 |

Results

Table 1 Mutagenicity results of the plant extracts in Ames test (preincubation method)

| Concentration (µg/plate) | Number of Revertants /plate | | | |
|--|-----------------------------|----------|--------|--------|
| | TA 100 | | TA 98 | |
| | + S9 | -S9 | + S9 | - S9 |
| DMSO | 146 ± 15 | 121 ± 11 | 30 ± 4 | 24 ± 5 |
| <i>Butea superba</i> powder extract; lot No. SBS-SA-04-PE | | | | |
| 13.75 | 177±19 | 154±23 | 30±2 | 28±6 |
| 6.875 | 164±20 | 128±10 | 37±3 | 27±6 |
| 3.4375 | 162±12 | 150±13 | 27±2 | 26±2 |
| 1.71875 | 146±23 | 124±14 | 31±7 | 19±2 |
| <i>Terminalia chebula</i> powder extract; lot No. STC-SA-01-PE | | | | |
| 2500 | 135±10 | 151±15 | 25±1 | 27±2 |
| 1250 | 161±14 | 145±21 | 28±5 | 31±5 |
| 325 | 155±16 | 151±19 | 30±3 | 23±3 |
| 312.5 | 149±20 | 136±19 | 23±5 | 32±5 |
| <i>Pueraria mirifica</i> powder extract; lot No. SPM-SA-25-PE | | | | |
| 13.75 | 180±3 | 166±27 | 31±8 | 26±1 |
| 6.875 | 152±24 | 151±10 | 34±3 | 20±1 |
| 3.4375 | 158±29 | 139±16 | 35±1 | 22±1 |
| 1.71875 | 149±20 | 142±20 | 38±3 | 21±2 |

| Concentration (μ l/plate) | Number of Revertants /plate | | | |
|---|-----------------------------|--------------|-------------|------------|
| | TA 100 | | TA 98 | |
| | + S9 | -S9 | + S9 | - S9 |
| Puraria mirifica liquid extract; lot No. SPM-SA-25-FE | | | | |
| 50 | 167 \pm 16 | 143 \pm 13 | 44 \pm 7 | 31 \pm 7 |
| 25 | 165 \pm 25 | 150 \pm 33 | 35 \pm 2 | 28 \pm 6 |
| 12.5 | 165 \pm 14 | 159 \pm 23 | 34 \pm 6 | 25 \pm 5 |
| 6.25 | 135 \pm 12 | 127 \pm 13 | 36 \pm 3 | 22 \pm 4 |
| Standard Butea superba lot No. SSS-SA-04-FE | | | | |
| 50 | 171 \pm 19 | 163 \pm 23 | 58 \pm 10 | 21 \pm 6 |
| 25 | 191 \pm 20 | 123 \pm 10 | 42 \pm 8 | 23 \pm 8 |
| 12.5 | 100 \pm 24 | 132 \pm 19 | 36 \pm 8 | 22 \pm 3 |
| 6.25 | 188 \pm 14 | 137 \pm 13 | 27 \pm 3 | 19 \pm 2 |

The results are mean \pm standard deviation of 2 plates from 2 independent experiments.

Positive control [+S9: TA100, 2AA (0.5) =535 \pm 71, TA98 =487 \pm 19,

-S9: TA100,AF-2 (0.1) =907 \pm 78, TA 98 =447 \pm 5];

Data evaluation

All tested plant extracts are considered to be non-mutagenic and no toxicity to bacterial tester strains were observed. The number of revertant colonies per plate with the test extracts was in the value that of the negative control (solvent control).

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Signature: _____



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References:

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