Saudi Journal of Biological Sciences 29 (2022) 103370

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Mutagenicity assessment of *Salacia chinensis* by bacterial reverse mutation assay using histidine dependent *Salmonella typhimurium* tester strains

Madhuranthakam Reddi Nagesh [a], Mansour K. Gatasheh [b], Nasrul Hoda [c], Natesan Vijayakumar [a,*]

[a] Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608002, Tamil Nadu, India
[b] Department of Biochemistry, College of Science, King Saud University, P.O.Box 2455, Riyadh 11451, Saudi Arabia
[c] Department of Neurology, Henry Ford Health System, Detroit, MI 48292, USA

ARTICLE INFO

Article history:
Received 24 March 2022
Revised 22 May 2022
Accepted 27 June 2022
Available online 1 July 2022

Keywords:
Salacia chinensis
Mangiferin
Mutagenecity
Ames test
Salmonella typhimurium

ABSTRACT

Background and objective: Genotoxicity analysis is one of the most important non-clinical environmental safety investigations required for pharmaceutical and agrochemical product registration. Any medicinal product must undergo a risk evaluation to determine its mutagenicity and carcinogenicity.
Materials and methods: The Ames test is a commonly used *in vitro* test for determining a test chemical's mutagenic activity. Histidine-dependent *Salmonella typhimurium* strains with a defective gene that causes the bacteria to synthesis the necessary amino acid histidine for life were tested for mutagenic potential. In order to reveal pro-mutagens and mutagens, the mutagenic potential of both plate integration and pre-incubation techniques was examined in the presence and absence of metabolizing system. *Salacia chinensis* has been widely used in ayurveda to treat various ailments. However, the information of mutagenicity of *Salacia chinensis* is scarce as per available literature.
Results: The mutagenicity of a *Salacia chinensis* root extract was investigated utilizing the Ames assay with plate incorporation and pre-incubation protocols using the appropriate *Salmonella typhimurium* tester strains: TA98, TA100, TA1537, TA1535, and TA102 in the presence and absence of S9. The concentrations used were 0.3123, 0.625, 1.25, 2.5 and 5 mg/plate. The extract of *Salacia chinensis* root did not show any mutagenic effect in any of the *Salmonella typhimurium* strains at the concentrations tested in the absence or presence of metabolic activation.
Conclusion: The root of *Salacia chinensis* was hence confirmed to be non-mutagenic and at least according to the results of this genotoxicity evaluation can be regarded as being safe for human use.

1. Introduction

Genotoxicity is the destruction of genetic information in somatic cells, resulting in cancer, and in germ cells, resulting inheritable mutations that cause birth defects in offspring. Duplication, insertion, and deletion of genetic material are all examples of mutations. Point mutations cause various human genetic abnormalities and have a role in the genesis of tumors in humans (OECD, 2020). Isolating the substance or chemical, i.e., antimutagens and demutagens from medicinal plants is one of the most effective ways to control the damage caused by mutagens and carcinogens (Silva et al., 2008). Genotoxicity studies are a series of *in vitro* and *in vivo* tests aimed at identifying any chemical or substances that might harm genetic material, either directly or indirectly, through a variety of ways. One of the *in vitro* tests included in the widely accepted standard test battery for genotoxicity evaluation is the Ames test. (Pfuhler et al., 2007).

The Ames test is used to find point mutations, which are responsible for a wide range of human genetic diseases. Point mutations in transcription factors and tumor suppressor genes of

* Corresponding author at: Department of Biochemistry and Biotechnology, Faculty of Science, Annamalai University, Annamalainagar 608002, Tamil Nadu, India.
E-mail addresses: tx3@diligencebio.com (M. Reddi Nagesh), mgatasheh@ksu.edu.sa (M.K. Gatasheh), nhoda1@hfhs.org (N. Hoda), nv12507@annamalaiuniversity.ac.in (N. Vijayakumar).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103370
1319-562X/

genetic material have been shown to have a role in tumor development in humans and animals. Many of the test strains include traits that improve mutation detection sensitivity like the deletion or strengthening of error-prone DNA repair mechanisms (OECD, 2020). For bacterial reverse mutation studies, a vast data bank of findings for a wide range of structures is accessible, and well-established techniques for evaluating substances with various Physico-chemical characteristics, including volatile compounds, have been created (OECD, 2020).

Salmonella typhimurium (S. typhimurium) strains are widely used in many laboratories, the Ames test has been found to be trustworthy and repeatable (OECD, 2020). Almost all of the *Salacia* genus's species, as well as complete plant sections, have been thoroughly researched for their various therapeutic properties. *Salacia chinensis* (*S. chinensis*) roots and other parts of the plant have been widely utilized in traditional medicine, notably in India, for the prevention and treatment of a variety of diseases (Gold-Smith et al., 2016). The biological activities related to *Salacia* plants include, nephroprotective, antiplasmodial, anti-inflammatory, hypotensive, anticancer, hepatoprotective, antioxidant, antigenotoxic, anticytotoxic, immunomodulatory, antimicrobial, antiabortifacient, antidiabetic, hypolipidemic, hypoglycemic, antifungal (Ramakrishna et al., 2016), antiviral, antiaging, analgesic (Imran et al., 2017), anticaries, antiulcer, antiobesity, blood purifier, skin lightening agent (Ghadage et al., 2017). Some of the primary pharmacological actions of *S. chinensis* that have been studied include the treatment of rheumatism, gonorrhoea, dysmenorrhoea, amenorrhea, venereal illness, and itches (Singh and Duggal., 2010).

Mangiferin has been identified as a significant bioactive component in *S. chinensis* roots (Chavan et al., 2015). Mangiferin is regarded as a "super anti-oxidant," and it has piqued the curiosity of scientists all over the world. Over the last 50 years, a considerable number of publications have been written on its occurrence, chemical composition, production, and therapeutic effects (Saha et al., 2016). The study for novel medicines capable of curing major metabolic disorders, particularly those capable of lowering or regulating blood cholesterol and triglyceride levels, has gained pace in recent years, as a result, a great number of articles have been published reporting substantial activity of a wide variety of natural and synthetic compounds. Numerous clinical trials employing *S. chinensis* extracts have also been shown to lower postprandial plasma glucose levels (Koteshwar et al., 2013). Nephroprotective role in diabetic patients (Singh et al., 2010) and its antimicrobial activity against human pathogens (Kannaiyan et al., 2012) though *S. chinensis* has been studied extensively for its phytochemical constituents, pharmacology and pharmaceutical aspects, data on its mutagenicity is very scarce as per available literature.

It was thus considered important to explore whether treatment with *S. chinensis* plays any adverse role in human genetic health and hence the present study was carried out utilizing different extraction methods and the most effective and acceptable techniques available to assess the mutagenicity of *S. chinensis* root extract by Ames test.

2. Materials and methods

2.1. Plant material

The plant, *Salacia chinensis* was collected from Parvathamalai, Tiruvannamalai district, Tamil Nadu, India. The plant was given as a voucher specimen (PARC/2020/4259), which was deposited at Plant Anatomy Research Centre, Chennai, Tamil Nadu.

2.2. Bacterial tester strains

Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537 and TA102 were obtained from Moltox, USA.

2.3. Chemicals and reagents

Bacto agar was purchased from BD Chemicals; Nutrient broth No.: 2 was purchased from Oxoid Limited, UK; L-histidine, D-biotin, benzo(a)pyrene, mitomycin-c, 2-nitrofluorene, 9-aminoacredine, sodium azide was procured from Sigma Aldrich. Merck chemicals were supplied sodium chloride, sodium phosphate monobasic, sodium phosphate dibasic, and potassium chloride. D-glucose-6-phosphate and Nicotinamide Adenine Dinucleotide Phosphate were provided by HiMedia, a company based in India. Moltox USA delivered the Aroclor 1254-induced male Wistar rats' S9 liver homogenate.

2.4. Preparation of an ethanol extract

The roots of *S. chinensis* were sorted out, dried at 30^{o}C, and powdered in an ultra-centrifugal mill. 100 g of the powdered sample were extracted with 1:8 volumes of ethanol at 70 ^{o}C for 3 h. The extract was filtered and condensed under decreased pressure, then diluted in distilled water and separated with 1:3 volumes of saturated butanol. Concentrated butanol extract was dissolved in a little volume of ethanol and further non-polar solvent like hexane was added to crystallize the active compound from the root sample of *S. chinensis*. This was used as the test substance in the current study.

2.5. Bacterial reverse mutation assay (Ames test)

The Ames test was performed to assess the mutagenic impact of *Salacia chinensis* root extract on *S. typhimurium* strains and was tested using OECD Test Guideline 471. The approach mentioned in the guidelines was developed by B.N. Ames (Maron and Ames, 1983).

2.5.1. Test system

S. typhimurium TA98, TA100, TA1535, TA1537, and TA102 auxotrophs were used to evaluate the mutagenic potential of the test substance. *S. typhimurium* strains TA98 and TA1537 are reverted from auxotroph to prototroph by frameshift mutations, similarly TA1535 reverted by mutation that cause basepair substitution and TA100 is reverted by mutation that cause both frameshift and basepair substitution mutations.Tester strain TA102 reverted by mutation causing transitions or transversions.

Prior to the testing, the phenotypic features of all *S. typhimurium* tester strains were verified, including R-factor plasmids' presence or absence, the existence of distinguishing mutations (for example, *rfa* mutation via crystal violet sensitivity and *uvrB* mutation via UV light sensitivity). The mutations of the bacterial strains used in this study are listed in the table (Table 1).

The appropriate frozen permanent stock was inoculated into a flask containing around 20 mL of oxoid nutrient broth for overnight

Table 1
The mutations of the bacterial strains used in the assay.

Genotype	Tester Strain	Various types of mutations
his G 428; rfa^{-}; uvrB^{+}; R-factor	TA102	Transitions/Transversions
his C 3076; rfa^{-}; uvrB^{-}	TA1537	Frame shift mutations
his D 3052; rfa^{-}; uvrB^{-};R-factor	TA98	Frame shift mutations
his G 46; rfa^{-}; uvrB^{-}	TA1535	Base-pair substitutions
his G 46; rfa^{-}; uvrB^{-};R-factor	TA100	Base-pair substitutions

cultures. In a shaking incubator, the cultures were cultured for 16 h at 37 ± 2 ^{0}C and 100 rpm. Prior to applying each technique in the experiment, cell viability was maintained at 1–2 × 10^9 cells per mL and evaluated using nutrient agar plates.

2.5.2. Metabolic activation system

Moltox in the United States provided the metabolic activation mechanism, which was Aroclor 1254 activated rat liver S9. To achieve a 10% v/v S9 cofactor mix, the S9 fraction was combined with cofactors comprising 0.8 g D-glucose-6-phosphate, 1.75 g NADP, 1.0 g $MgCl_2$, 1.35 g KCl, 6.4 g Na_2HPO_4 and 1.4 g NaH_2PO_4-·H_2O prior to plating.

2.5.3. Solubility and precipitation

The solubility of the test material and precipitation in the final mixture was assessed before the experiment by pouring the mixture onto a minimal glucose agar plate. The test substance was found to be soluble in Dimethyl Sulfoxide at the concentration of 50 mg/mL. Concurrent negative (distilled water) and vehicle (Dimethyl Sulfoxide) controls were used during conduct of the assay.

2.5.4. Positive control

Positive controls unique to each strain were employed in the experiment at the same time; the characteristics of the positive controls may be seen in the table (Table 2).

2.5.5. Plating

The *Salmonella* tester stains were exposed to the test substance with concentrations of 0.3125, 0.625, 1.25, 2.5 and 5 using spacing factor of 2 by plate incorporation and pre- incubation methods as described by Maron and Ames (Maron and Ames, 1983).

100 µL of Test solution, negative control, vehicle control, 500 µL of S9 mix or S9 mix replacement buffer, 100 µL of bacteria suspension were used in the plate integration procedure, in a sterile test tube, 2 mL of overlay agar (0.6 percent w/v bacto agar; 0.6 percent w/v sodium chloride and supplemented with 0.05 mM L-histidine/ Plates were inverted and incubated at 37 ± 2 °C for 48 h until the top agar hardened. To the pre-incubation method, 100 µL of Test solution, negative control, vehicle control, or positive control, 500 µL of S9 mix (for the test with metabolic activation) or S9 mix substitution buffer, and 100 µL of bacteria suspension were added and incubated at 37 ± 2 °C for 20 min. now at the successful completion of the incubation time, 2 mL of top agar (0.6 % w/v bacto agar; 0.6 % w/v sodium chloride and fortified with 0.05 mM L-histidine/0.05 mM biotin) was added, placed onto the MGA plates and incubated for 48 h at 37 ± 2 °C.

During the assay, separate plates were used to examine the sterility of the S9 cofactor mix, sodium phosphate buffer, vehicle, test material, top agar, and minimum glucose agar plates. In this experiment, S9 efficiency was determined using benzo(a)pyrene with S. *typhimurium* TA1535 and TA100 tester stains. After incubation, the reverted colonies were counted for treated, negative, vehicle, and positive control plates. Under a microscope, the bacterial background grass was examined for signs of test chemical toxicity. Precipitation of the test substance was evaluated after the incubation period. Toxicity in terms of the number of revertant colonies, degree of precipitation, and the background lawn inhibition (using the grades as no inhibition, slight inhibition, or complete inhibition) were scored for all plates with respect to the negative control plates.

If the number of revertants exceeds the medically significant threshold of twice or thrice, the matching solvent control's colony count is detected. When the threshold is surpassed at many doses, a dose-dependent rise is regarded as physiologically meaningful. If repeated in an independent second experiment, a rise above the threshold at just one concentration is considered physiologically significant. In a second independent experiment, a dose-dependent increased number of revertant colonies underneath the threshold is regarded to have mutagenesis potential. However, such an increase is not deemed physiologically important when colony numbers stay within the historical range of negative control.

2.5.6. Acceptability of the assay

Regulatory standards certify the Bacterial reverse mutation test if it satisfies the following conditions. Negative control and vehicle control have been steadily increasing in the background. The positive control chemicals should result in a large increase in the number of mutant colonies. The negative control and vehicle control spontaneous reversion rates are close to published numbers.

Table 2
Positive controls used in the assay.

Positive Control	Tester Strain	Concentration (µg/plate)
Absence of metabolic activation		
Sodium azide	TA100 and TA1535	0.5
2-Nitrofluorene	TA98	10
9-Aminoacridine	TA1537	50
Mitomycin-C	TA102	0.4
Presence of metabolic activation		
Benzo (a) pyrene	TA98, TA100, TA1535, TA1537, TA102	10

3. Results

3.1. Solubility and precipitation

On the basis of the solubility test performed at 50 mg/mL concentration, dimethyl sulfoxide was selected as the vehicle for the test substance in this assay. Because the highest dosage tested produced only a little amount of precipitation, the highest concentration in this study was set at 5 mg/plate.

3.2. Plate incorporation method

The results of the assay as per the plate incorporation method was summarized in (Figs. 1 and 2) and presented in (Table 3 and 4). There was no increase observed in the revertant colony count or background lawn inhibition in the Plate incorporation method at and up to the highest tested concentration i.e. 5 mg/plate, when compared to vehicle control plates. Positive control plates exhibited a much higher revertant count than vehicle control plates.

3.3. Method of pre-incubation

The findings of the test using the pre-incubation technique are summarized in (Figs. 3 and 4) and are provided in (Tables 5 and 6). There was no improvement in revertant colony count reduction in the pre-incubation procedure as compared to vehicle control plates at and up to the highest tested dose, i.e. 5 mg/plate. The positive control plates exhibited a substantial rise in revertant count when compared to vehicle control. In both methodologies used, there was no improvement in revertant colony count in the vehicle control plates as matched to negative control plates.

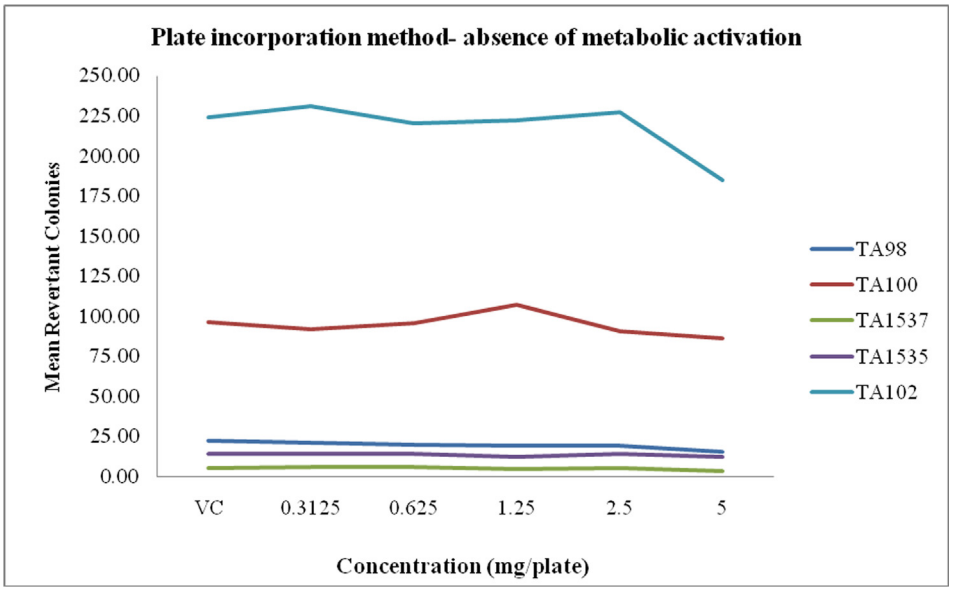

Fig. 1. Graphical representation of dose response curve of Plate incorporation method. In the absence of metabolic activation, the mean revertant colonies of all tested doses of *S. Chinensis* extract (0.3125, 0.625, 1.25, 2.5, and 5 mg/plate) were equivalent to those of VC (Vehicle control- DMSO).

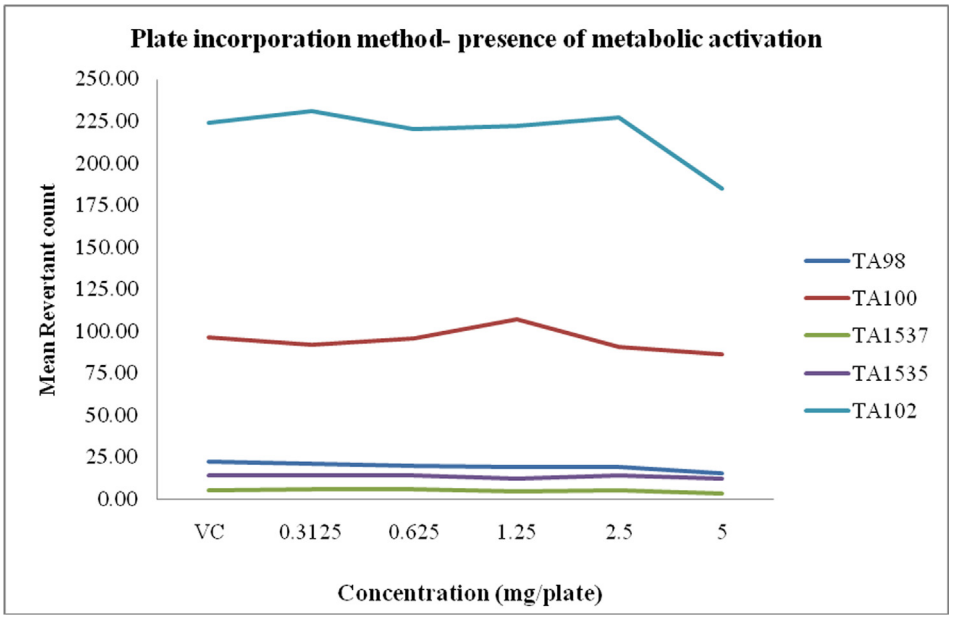

Fig. 2. Graphical representation of dose response curve of Plate incorporation method. The graph indicates that the mean revertant colonies of all the tested concentrations of *S. chinensis* extract (0.3125, 0.625, 1.25, 2.5 and 5 mg/plate) were comparable with that of VC (Vehicle control- DMSO) in the presence of metabolic activation.

3.4. Sterility test and genotype confirmation

On any of the sterility plates used for the vehicle control, test material dilutions, S9 cofactor mix, sodium phosphate buffer, or top agar, there were no contaminated colonies identified. All of the *Salmonella* strains revealed a substantial *rfa* and a crossing out in the *uvrB* gene. In test strains TA98, TA100, and TA102, the presence of the R-factor plasmid pKM101 were confirmed (Fig. 5). All requirements for a successful assay were thus satisfied, and the tester strains' susceptibility to mutagens was validated.

3.5. S9 efficiency check

In the strains TA100 and TA1535, the number of revertant colonies did not increase, when comparing positive control plates of benzo[a]pyrene (10 μg/plate) in the absence of metabolic activation to internal standard plates of benzo[a]pyrene in the presence of metabolic activation, indicating the efficiency of the S9 fraction used. In all five tester strains, the mean number of revertant colonies was equivalent to the mean value in the negative and vehicle control groups in all groups treated with varied doses of *S. chinensis*

Table 3
Revertant Colony count- Plate incorporation method (metabolic activation is not present [-S9]).

Concentration (mg/plate)	TA1537				TA1535				TA102				TA98				TA100			
	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI
NC	6.33	1.53	–	NI	15.00	1.73	–	NI	233.00	11.36	–	NI	22.67	4.16	–	NI	97.67	9.07	–	NI
VC	4.67	1.15	0.74	NI	13.33	2.52	0.89	NI	219.00	31.32	0.94	NI	20.00	4.00	0.88	NI	101.00	13.11	1.03	NI
0.3125	2.67	0.58	0.57	NI	11.67	1.53	0.88	NI	212.33	14.98	0.97	NI	24.33	0.58	1.22	NI	105.00	11.53	1.04	NI
0.625	5.00	3.61	1.07	NI	11.67	2.08	0.88	NI	226.33	8.62	1.03	NI	22.00	2.65	1.10	NI	91.33	7.37	0.90	NI
1.25	4.33	3.79	0.93	NI	13.33	1.12	1.00	NI	232.33	14.36	1.06	NI	19.33	1.15	0.97	NI	87.33	5.60	0.86	NI
2.5	6.00	2.00	1.28	NI	12.67	0.58	0.95	NI	221.33	14.74	1.01	NI	22.67	3.21	1.13	NI	88.33	6.66	0.87	NI
5	4.33	1.53	0.93	SI	12.00	1.73	0.90	SI	183.33	10.50	0.84	SI	17.33	2.31	0.87	SI	89.33	6.11	0.88	SI
PC	200.00	18.08	46.19	–	431.33	11.06	35.94	–	1729.00	89.07	9.43	–	397.00	10.58	22.91	–	746.33	76.74	8.35	–

Key: NC = Negative Control (distilled water), VC = Vehicle Control (Dimethyl Sulfoxide), PC = Positive Control, M = Mean, SD = Standard Deviation, FI = Fold increase, BI = Background lawn Inhibition, mg = milligram, NI = No Inhibition, SI = Slight Inhibition.
Positive controls:
TA1535 and TA100- Sodium azide 0.5 μg/plate.
TA98- 2-Nitrofluorene 10 μg/plate.
TA1537- 9-Aminoacridine 50 μg/plate.
TA102 - Mitomycin C 0.4 μg/plate.

Table 4
Revertant Colony count- Plate incorporation method (metabolic activation is present [+S9]).

Concentration (mg/plate)	TA1537				TA1535				TA102				TA98				TA100			
	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI
NC	6.00	1.73	–	NI	15.33	2.08	–	NI	233.33	14.50	–	NI	24.00	3.46	–	NI	100.67	5.86	–	NI
VC	5.33	1.15	0.89	NI	14.33	1.15	0.93	NI	224.33	11.93	0.96	NI	22.33	4.04	0.93	NI	96.33	5.03	0.96	NI
0.3125	5.67	2.31	1.06	NI	14.00	1.73	0.98	NI	231.00	13.75	1.03	NI	21.00	1.73	0.94	NI	92.00	4.36	0.96	NI
0.625	6.00	1.73	1.13	NI	14.00	3.46	0.98	NI	220.00	12.53	0.98	NI	20.00	2.65	0.90	NI	96.00	7.00	1.00	NI
1.25	4.33	2.31	0.81	NI	12.33	2.08	0.86	NI	222.00	9.54	0.99	NI	19.33	3.51	0.87	NI	107.33	4.73	1.11	NI
2.5	5.33	1.15	1.00	NI	14.00	2.00	0.98	NI	227.33	14.01	1.01	NI	19.00	1.73	0.85	NI	91.00	3.00	0.94	NI
5	3.00	0.00	0.56	SI	12.67	1.53	0.88	SI	184.67	8.62	0.82	SI	15.33	1.15	0.69	SI	86.33	6.51	0.90	SI
PC	205.33	29.26	68.44	_	392.67	24.03	30.99	_	1596.67	69.30	8.65	_	394.00	26.85	25.70	_	780.33	34.12	9.04	_

Key: NC = Negative Control (Distilled Water), VC = Vehicle Control (Dimethyl Sulfoxide), PC = Positive Control, M = Mean, SD = Standard Deviation, FI = Fold increase, BI = Background lawn Inhibition, mg = milligram, NI = No inhibition, SI = Slight inhibition.
Positive Control: TA1537, TA1535, TA102, TA98 and TA100 - Benzo(a)pyrene 10 μg/plate.

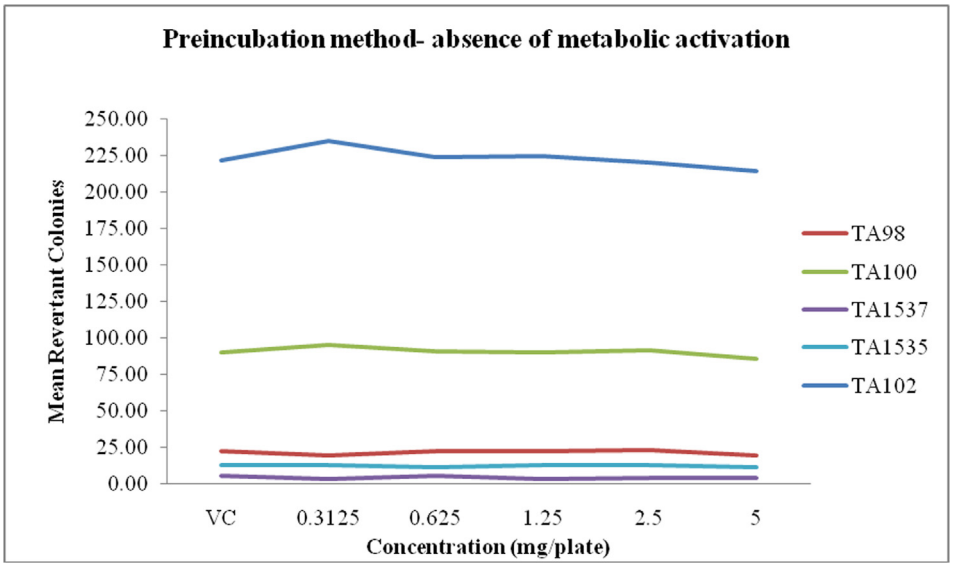

Fig. 3. Graphical representation of dose response curve of Pre incubation method. In the absence of metabolic activation, the mean revertant colonies of all tested doses of *S. chinensis extract* (0.3125, 0.625, 1.25, 2.5, and 5 mg/plate) were equivalent to those of VC (Vehicle control- DMSO).

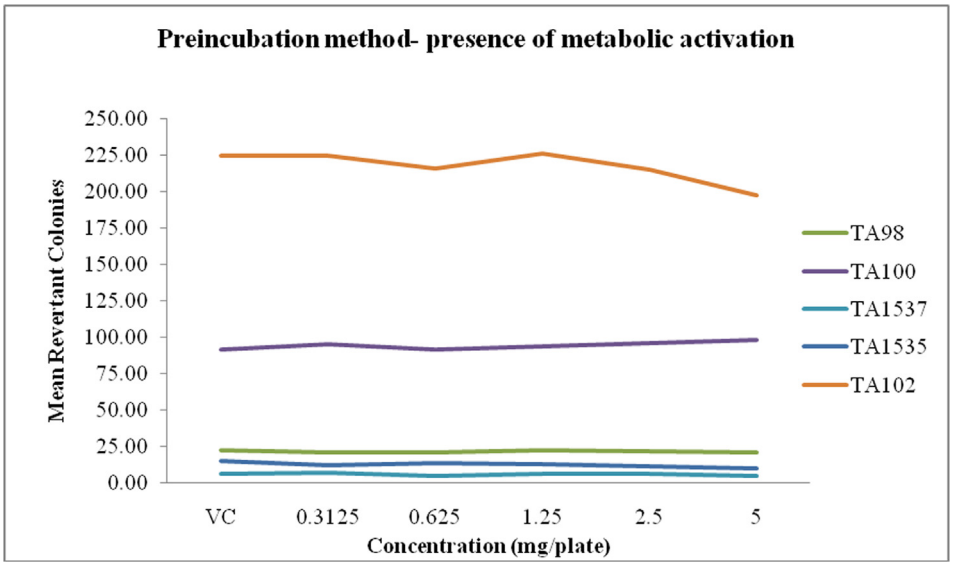

Fig. 4. A graphical depiction of the Pre incubation method's dosage response curve. In the presence of metabolic activity, the mean revertant colonies of all tested doses of *S. chinensis* extract (0.3125, 0.625, 1.25, 2.5, and 5 mg/plate) were equivalent to those of VC (Vehicle control-DMSO).

Table 5
Revertant Colony count- pre incubation method (metabolic activation is not present [-S9]).

Concentration (mg/plate)	TA1537				TA1535				TA102				TA98				TA100			
	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI
NC	5.33	1.15	-	-	14.33	2.89	-	-	231.67	12.06	-	-	23.00	2.08	-	-	91.00	3.00	-	-
VC	6.00	1.73	1.13	NI	13.33	1.53	0.93	NI	222.00	21.63	0.96	NI	22.67	2.52	0.99	NI	92.00	2.65	1.01	NI
0.3125	3.67	0.58	0.61	NI	13.33	3.06	1.00	NI	235.00	6.43	1.06	NI	19.67	3.46	0.87	NI	95.33	11.59	1.04	NI
0.625	6.00	0.00	1.63	NI	11.67	1.15	0.88	NI	223.67	8.08	0.95	NI	22.33	2.65	1.14	NI	90.67	3.51	0.95	NI
1.25	4.00	0.00	0.67	NI	13.00	1.73	1.11	NI	224.67	10.82	1.00	NI	22.67	4.36	1.02	NI	90.33	2.08	1.00	NI
2.5	4.67	2.89	1.17	NI	13.33	1.53	1.03	NI	220.00	23.03	0.98	NI	23.33	2.08	1.03	NI	92.00	9.85	1.02	NI
5	4.67	1.53	1.00	SI	12.00	1.73	0.90	SI	214.33	16.82	0.97	SI	19.67	2.52	0.84	SI	86.00	4.58	0.93	SI
PC	207.67	9.07	44.47	-	390.67	22.50	32.56	-	1687.33	23.16	7.87	-	275.00	14.11	13.98	-	708.33	31.39	8.24	-

Key: NC = Negative Control (Distilled Water), VC = Vehicle Control (Dimethyl Sulfoxide), M = Mean, SD = Standard Deviation, FI = Fold increase, BI = Background lawn Inhibition, mg = milligram, NI = No Inhibition, SI = Slight Inhibition.
Positive controls:
TA1535 and TA100- Sodium azide 0.5 μg/plate.
TA98- 2-Nitrofluorene 10 μg/plate.
TA1537- 9-Aminoacridine 50 μg/plate.
TA102 - Mitomycin C 0.4 μg/plate.

Table 6
Revertant Colony count- Pre incubation method (metabolic activity is present [+S9]).

Concentration (mg/plate)	TA 1537				TA 1535				TA 102				TA 98				TA 100			
	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI	M	SD	FI	BI
NC	6.00	0.00	–	NI	15.67	0.58	–	NI	236.00	16.97	–	NI	22.33	2.08	–	NI	98.33	12.66	–	NI
VC	6.67	1.15	1.11	NI	15.67	2.31	1.00	NI	225.00	7.00	0.95	NI	22.67	2.52	1.02	NI	92.33	8.50	0.94	NI
0.3125	7.33	0.58	1.10	NI	12.33	2.31	0.79	NI	224.67	7.57	1.00	NI	21.00	3.46	0.93	NI	96.00	12.77	1.04	NI
0.625	5.33	2.31	0.73	NI	14.00	3.61	1.14	NI	216.33	23.50	0.96	NI	21.00	2.65	1.00	NI	92.00	8.19	0.96	NI
1.25	6.33	2.31	1.19	NI	13.00	3.00	0.93	NI	226.33	28.36	1.05	NI	23.00	4.36	1.10	NI	94.00	11.14	1.02	NI
2.5	6.67	1.15	1.05	NI	11.67	2.31	0.90	NI	215.67	14.50	0.95	NI	22.33	2.08	0.97	NI	96.67	9.29	1.03	NI
5	5.33	2.31	0.80	SI	10.00	1.00	0.86	SI	197.67	14.57	0.92	SI	21.33	2.52	0.96	SI	98.67	15.31	1.02	SI
PC	217.00	22.07	40.71	–	412.67	30.99	41.27	–	1598.67	71.93	8.09	–	395.00	14.11	18.52	–	759.00	38.57	7.69	–

Key: NC = Negative Control (Distilled Water), VC = Vehicle Control (Dimethyl Sulfoxide), PC = Positive Control, M = Mean, SD = Standard Deviation, FI = Fold increase, BI = Background lawn Inhibition, mg = milligram, NI = No Inhibition, SI = Slight Inhibition.
Positive Control: TA1537, TA1535, TA102, TA98 and TA100 - Benzo(a)pyrene 10 μg/plate.

root extract. According to the results of this Ames experiment with *S. chinensis* root extract, under the conditions of this assay, *S. chinensis* root extract did not generate a positive reaction in either the presence or absence of a metabolic activation system.

4. Discussion

S. chinensis roots were extracted and evaluated for mutagenic potential using well-established and scientifically supported pro-

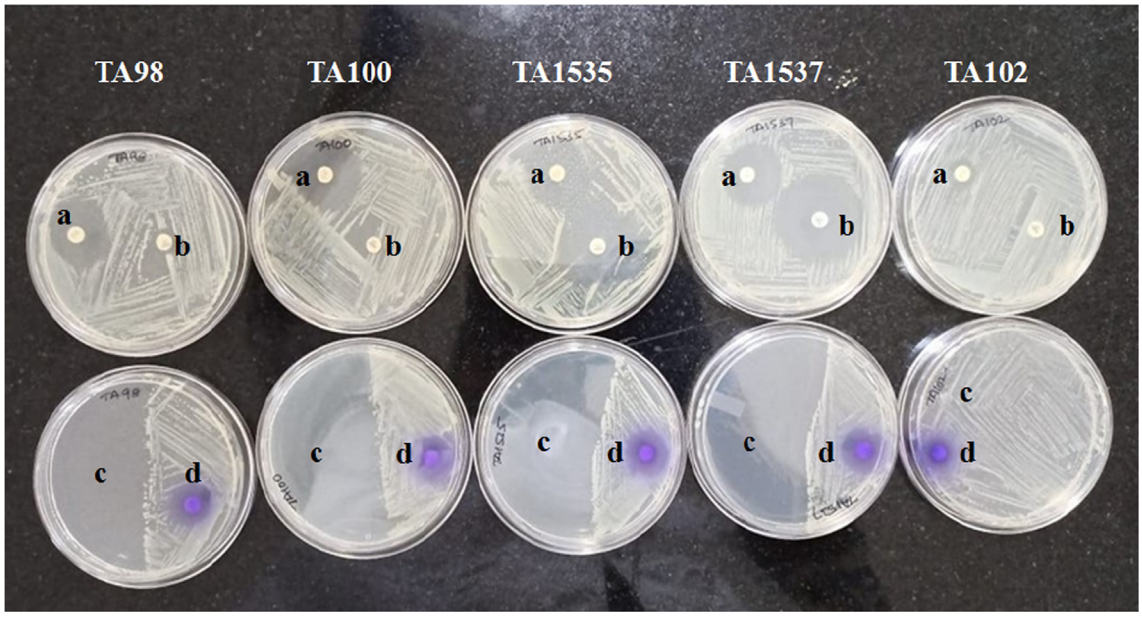

Fig. 5. Photograph of the plates of genotypic confirmation of *Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537 and TA102. a.No zone of inhibition observed in the strains TA98, TA100, TA102 which carries plasmid pKM101 (Ampicillin resistance).b. No zone of inhibition observed in the strains TA102 which carries plasmid pAQ1 (Tetracycline resistance).c. Due to the deletion of *bio-uvrB* region, no growth was observed in the UV exposed area except TA102.d. Since, *rfa* mutation is present; all *Salmonella* strains have shown a zone of growth inhibition surrounding the Crystal Violet disk.

cedures approved by international regulatory organizations. The current investigation was conducted using all five *S. typhimurium* tester strains, TA98, TA100, TA1535, TA1537, and TA102, in the presence and absence of metabolic activation and utilizing two distinct exposure techniques. Because the number of revertant colonies in the treatment and vehicle control groups was not significantly different ($p > 0.05$), all of the *S. chinensis* extract doses tested (0.3125, 0.625, 1.25, 2.5, and 5 mg/plate) were found to be non-mutagenic in the tester strains, both in the presence and absence of metabolic activation. However, the negative response seen in the plate inclusion method was verified by subsequent testing that used a modification in experimental conditions in the pre-incubation procedure.

To the best of our knowledge, no research has shown *S. chinensis's* mutagenic effects. The results of this genotoxicity test employing *S. chinensis* root extract match those obtained in *S. typhimurium strains*, after isolation of mangiferin up to 5 mg/plate (Govindaraj et al., 2009; Sellamuthu et al., 2011). However, similar effects have been observed in investigations with other species of the same genus (Flammang et al., 2006). As a consequence, our findings are consistent with those of *Salacia oblonga* (Flammang et al., 2006). The results of the current study's *S. chinensis* root extract also agrees with the mutagenic assessment of *Salacia crassifolia*. When tested using the Ames experiment, additional species in the Celastraceae family, including *Salacia oblonga* and *Salacia crassifolia*, yielded comparable findings to those found in this work (Carneirol et al., 2013). A similar effect seen in this investigation was seen in a hydroalcoholic extract of *Salacia oblonga* root bark, which has antimutagenic characteristics and protects against Mitomycin-C-induced cyto-nuclear damage (Singh et al., 2009).

5. Conclusion

The root of *S. chinensis* extracted with ethanol, butanol, and hexane showed no significant increase in revertant colony count in the absence of metabolic activity or in the presence of metabolic activation in the current investigation; thus, under the experimental circumstances, the extracts of *S. chinensis* roots were shown to be non-mutagenic. As a result of the observations, *S. chinensis* root extract did not cause base-pair or frameshift mutations, suggesting that DNA does not appear to be a significant target for this medicinal plant. More research will be needed to discover the bioactive components in plant extracts that are responsible for medicinal efficacy. This plant is a wonderful natural source of anti-diabetic and anti-obesity agents.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors acknowledge the support of King Saud University's Researchers Supporting Project Number (RSP-2021/393) in Riyadh, Saudi Arabia.

References

Carneirol, C.C., Silva, C.R., Menezes, A.C.S., Pérez, C.N., Chen-Chen, L., 2013. Assessment of genotoxic, cytotoxic, and protective effects of *Salacia crassifolia* (Mart. Ex. Schult.) G. Don. stem bark fractions in mice. Genet. Mol. Res. 12 (3), 2167–2177.

Chavan, J.J., Ghadage, D.M., Bhoite, A.S., Umdale, S.D., 2015. Micropropagation, molecular profiling and RP-HPLC determination of mangiferin across various regeneration stages of Saptarangi (*Salacia chinensis* L.). Ind. Crops Prod. 76, 1123–1132.

Flammang, A.M., Erexson, G.L., Mecchi, M.S., Murli, H., 2006. Genotoxicity testing of a *Salacia oblonga* extract. Food Chem. Toxicol. 44 (11), 1868–1874.

Ghadage, D.M., Kshirsagar, P.R., Pai, S.R., Chavan, J.J., 2017. Extraction efficiency, phytochemical profiles and antioxidative properties of different parts of Saptarangi (*Salacia chinensis* L.) - an important underutilized plant. Biochem. Biophy. 12, 79–90.

Gold-Smith, F., Fernandez, A., Bishop, K., 2016. Mangiferin and cancer: mechanisms of action. Nutrients 8 (7), 396.

Govindaraj, Y., Melanaphuru, V., Agrahari, V., Gupta, S., 2009. Genotoxicity studies of mangiferin isolated from *Salacia chinensis* Linn. Acad. J. Plant Sci. 2, 199–204.

Imran, M., Arshad, M.S., Butt, M.S., Kwon, J.H., Arshad, M.U., Sultan, M.T., 2017. Mangiferin: a natural miracle bioactive compound against lifestyle related disorders. Lipids Health Dis. 16, 1–17.

Kannaiyan, M., Manuel, V.N., Raja, V., Thambidurai, P., Mickymaray, S., Nooruddin, T., 2012. Antimicrobial activity of the ethanolic and aqueous extracts of *Salacia chinensis* Linn. against human pathogens. Asian Pac. J. Trop. Dis. 2, S416–S420.

Koteshwar, P., Raveendra, K.R., Allan, J.J., Goudar, K.S., Venkateshwarlu, K., Agarwal, A., 2013. Effect of NR-Salacia on post-prandial hyperglycemia: A randomized double blind, placebo-controlled, crossover study in healthy volunteers. Phcog Mag. 9, 344-349

Maron, D.M., Ames, B.N., 1983. Revised methods for the *Salmonella* mutagenicity test. Mutat. Res. 113 (3-4), 173–215.

OECD (Organisation for Economic Co-operation and Development). OECD guidelines for the testing of chemicals, Guideline 471. Bacterial Reverse Mutation test, adopted 26 June 2020, Paris.

Pfuhler, S., Albertini, S., Fautz, R., Herbold, B., Madle, S., Utesch, D., Poth, A., 2007. Gesellschaftfuer Umwelt-Mutations for schung, Genetic toxicity assessment: employing the best science for human safety evaluation part IV: recommendation of a working group of the Gesellschaftfuer Umwelt-Mutationsforschung (GUM) for a simple and straight forward approach to genotoxicity testing. Toxicol. Sci. 97 (2), 237–240.

Ramakrishna, D., Tidke, S.A., Shinomol, G.K., Kiran, S., Ravishankar, G.A., 2016. A source of herbal drug for several human diseases and disorders. Int. J. Curr. Pharm. Rev. Res. 7, 122–133.

Saha, S., Sadhukhan, P., Sil, P.C., 2016. Mangiferin: a xanthonoid with multipotent anti-inflammatory potential. Biofactors 42, 459–474.

Sellamuthu, P.S., Arulselvan, Muniappan, B.P., Fakurazi, S., Kandasamy, M., 2011. Mangiferin from *Salacia chinensis* prevents oxidative stress and protects pancreatic β-cells in streptozotocin-induced diabetic rats. J. Med. Food. 16, 719-727.

Silva, R.M.G.d., Sousa, N.C.d., Graf, U., Spanó, M.A., 2008. Antigenotoxic effects of *Mandevilla velutina* (Gentianales, Apocynaceae) crude extract on cyclophosphamide-induced micronuclei in Swiss mice and urethane-induced somatic mutation and recombination in *Drosophila melanogaster*. Genet. Mol. Biol. 31 (3), 751–758.

Singh R.G., Rathore, S.S., Usha, R.K., Agarwal, A., Dubey, G.P., 2010. Nephroprotective role of *Salacia chinensis* in diabetic CKD patients: A pilot study. Indian J Med Sci, 64, 378-384

Singh, A., Duggal, S., 2010. Salacia species: hypoglycemic principles and possible role in diabetes management. Integrative Med. 7, 374–382.

Singh, N.K., Biswas, A., Rabbani, S.I., Devi, K., Khanam, S., 2009. Hyrdoalcoholic root bark extract of *Salacia oblonga* prevented Mitomycin-C induced sperm abnormality in wistar rats. Phcog. Mag. 5, 254–259.