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Investigating Anti-mutagenic Activities of *Lantana camara* L. (Verbenaceae) Applying Salmonella *typhimurium* and the Ames Test

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Abstract

Introduction: Genetic mutations have a significant role in causing cancers, and plants are effective on cancer recovery by producing metabolites. In this regard, the present study aimed to evaluate the *Lantana camera* anti-mutation effects applying *Salmonella* typhimurium in the Ames test.

Methods: To this end, the plant was prepared from the Iran National Botanical Garden in 2018 (Tehran, Iran), and the methanolic extracts of its leaves and flowers were obtained by the percolation method. Then, anti-mutagenic activities were studied by the Ames method and the assessment of the rate of reverse mutations in mutant *Salmonella* typhimurium. Mutant strains cannot grow on minimal mineral media thus only those bacteria that have acquired a wild genotype after reverse mutation in the presence of the mutagen are able to grow on this medium. The plant extract, along with a mutagen substance was used to evaluate its anti-mutagenic effects by counting grown colonies and calculating the mean mutation inhibitory index according to the "Ong" formula. Finally, anti-mutagenic activities were retested by adding the sterile extract of the mouse liver (S_9), and the data were analyzed by SPSS statistical software, version 22.

Results: In general, the results showed that the mean number of grown colonies decreased significantly despite the plant material in comparison with the standard. According to the "Ong" formula, the percentage of inhibition was [1-T/M]×100. Based on the results, T grew a number of colonies on each petri dish despite the mutagen and extract, and M grew a number of colonies in positive control plates. Eventually, mutation inhibition percentages in leaf extracts were significantly higher than those of flower extracts, which were 75.59 ± 0.73 (+S_9) and 84.79 ± 0.17 (-S_9), as well as 49.57 ± 0.55 (+S_9) and 62.32 ± 0.23 (-S_9), respectively ($P<0.05$).

Conclusion: In general, the leaves and flowers of *L. camara* demonstrated anti-mutagenic activities with higher activities in the leaves compared to flowers.

Keywords: Anti-mutagenic activity, Anti-cancer activity, *Lantana camara* L., Ames test, *Salmonella* typhimurium

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Introduction

Cancers are known through unlimited and uncontrolled cell divisions, which can cause death.[1] Although different methods have been introduced to treat cancers, the number of cancer patients increases annually. It is estimated that nearly 16 000 000 human bodies on the earth will develop this illness by the next ten years, of which about 12 000 000 cases are fatal.[2] Accordingly, this is one of the important factors of humans dying.[3] In addition, this disease is reported to be due to the lack of the regulation of important activities of the cell, including development pathways, anti-cell programmed death processes, immune responses, and cellular microenvironment.[1,4]

In addition, damages and genetic alterations including modifications in the DNA sequence and coherence and other genetic elements have a high impact on carcinogenesis.[5]

Cancer treatment has been applied to reset cellular processes. So far, several clinical experiments have studied potential treatments for cancer through radiotherapy, chemical treatment, and immunotherapy although the first two methods have harmful and lethal effects against natural

tissues.[3] Although the third method offers highly particular and targeted therapy, it is limited and highly expensive.[6] Further, cancers recur after treatment.[3,6] Recently, new methods have been used to find new compounds with anticancer effects from difficult resources for controlling the harmful effects of anticancer medicines and finding better compounds.[3]

Medicinal plants have long been a natural resource for the treatment of many ailments. According to the World Health Organization report, many plants are currently used for medical purposes.[7]

Additionally, the metabolites of plants are useful for different therapeutic aims,[8] and plant compounds have biological roles such as pain reliever, along with anti-inflammatory and antimicrobial activities.[3] Further, they are the resources of nearly 25% of therapeutic drugs[9] and more than 60% of anticancer drugs are derived from the plants.[10]

As discussed earlier, it is essential to develop newer, safer, and more effective substances for treating cancer. Plant compounds are beneficial materials for developing other medicines with high performance while fewer side effects.[10]

The Verbenaceae plant family includes various plant genus and species, most of which have been traditionally utilized as remedies for some disease.[11] *Lantana camera* from the plant Verbenaceae family is endemic of Africa and America. The leaves of this plant are effective on the treatment of bellyache, wounds, rheumatism, pain in a tooth, pneumonia, and other ailments.[12] Furthermore, *L. camara* has several biologically active compounds. Moreover, many terpenes, fatty acids, and flavonoids have been extracted from this plant in phytochemical studies.[13,14] Additionally, this plant is claimed to have anti-protozoal,[12] anti-bacterial, anti-fungal,[12,13] anti-oxidant,[14] insecticidal,[15] and anti-viral[16] activities, as well as allelopathic properties.[17] Similarly, the major essential compounds of *L. camara* are γ-curcumin (6.3%), Davanone (7.3%), germacrene D (10.9%), α-humulene (11.5%), and β-caryophyllene (23.3%).[18]

Considering the above-mentioned explanations, this research aimed to investigate the anti-mutagenic activities of the *L. camera* extract applying mutant *Salmonella* typhimurium through the Ames test.

Materials and Methods

Plant Material

Different parts of *L. camara* were prepared from the National Botanical Garden of Iran (Tehran Iran) in Spring 2018.

Plant Extract

Different parts of the plant were prepared and dried in shadow. Then, they were powdered, and 50 g of them were converted to the extract by adding alcohol (Methanol 80%) using the percolation method. In addition, the extracts were concentrated by a rotary system at 40°C (the concentrated extract was about 5 g), dehydrated in the oven (40°C), and finally, their anti-mutagenic activities were investigated based on the aim of the study.[19]

Bacterial Strains

The histidine auxotrophic mutant strains (His-) of *Salmonella* typhimurium (TA100) were obtained from the Laboratory of Microbiology of Kharazmy University (Tehran, Iran) and used to determine the occurrence of base-pair mutations. These mutant strains cannot grow on a minimal mineral medium, and only those bacteria having mutated to wild (His+) type by the reverse mutation in the presence of a mutagen (Sodium azide, NaN_3) can grow on this medium. Therefore, the presence of an anti-mutagenic substance (e.g., a plant methanolic extract), along with the mutagen (i.e., NaN_3) can reduce the rate of the reverse mutation.

Anti-mutagenic Activity Assay

The anti-mutagenic effect of the extract was evaluated by the Ames method using the mutant strain of *Salmonella* typhimurium (TA100) in the presence of NaN_3 and counting grown colonies indicating the incidence of a reverse mutation. The mutant *Salmonella* typhimurium strain (TA100) that requires histidine for growing in minimal media is suitable for measuring the anti-mutagenic activity of mutagenic substances.[20-22]

In this phase, the anti-mutagenic effect of the extract was evaluated by adding S_9 (The sterile extract of the mouse liver containing microsomal enzymes). The cytochrome oxidase enzyme (P450), which inactivates oxidant and toxic compounds, can be found in the membrane of liver cells, especially the endoplasmic reticulum membrane). Thus, the metabolic and antimutagenic activities of compounds are strengthened in the presence of the microsomal extract of the liver (S_9).

The concentration of 1% or 1 µg/mL of the concentrated extract was used because of its suitability for assaying anti-mutagenic activity without killing the bacteria. Then, the anti-microbial activity of the methanolic extract against *Salmonella* typhimurium was assessed by the microbial culture and the disk diffusion method to obtain the minimum inhibitory concentration, which was obtained 6.25 µg/mL for both leaf and flower extracts. Further, Dimethyl sulfoxide was considered as the solvent.[19]

Next, the anti-mutagenic test was performed by adding the plant extract (0.5 mL) to the fresh overnight culture (0.5 mL) and the Histidine-Biotin solution (0.5 mL) containing top agar (10 mL) and NaN_3 (1.5 µg) in a test tube. The contents of this tube were shaken for 3 seconds by a shaker and then evenly spread on the entire surface of the minimal glucose agar medium. Then, the experiment was repeated three times, and petri dishes were placed in an incubator (at 37°C for 24 hours).[21,22]

The positive control contained NaN_3 (1.5 µg) as a

mutagen per plate, and plates without any NaN_3 or the plant extract, which only consisted of 0.5 mL sterile distilled water, were considered as the negative control. After the incubation, grown colonies were counted per plate.[21-23]

In the second experiment, 0.5 mL of the S_9 compound (prepared from the laboratory complex of the Islamic Azad University of Tehran, Science and Research Branch) was added to all plates.

Calculating the Percentage of Mutation Inhibition

The average number of grown colonies per plate was determined, and the mean mutation inhibitory activity was calculated by the "Ong" formula.[24] This formula computes the percentage of mutation inhibition based on the number of the grown colonies per plate as follows:

$$Percentage\ of\ inhibition = [1- T/M] \times 100$$

where T denotes the numbers of the grown colonies each petri with the mutagen and the plant extract, and M represents the number of grown colonies in the plates of the positive control. The mutagenicity of NaN_3 without the extract (positive control) was considered as 100% growth (i.e., 0% mutation inhibitory activity).[21]

Finally, the anti-mutagenic activity was categorized as moderate (25%-40%) or strong (>40%).[18,21,25]

Analysis of Data

The findings were presented as the mean ± standard deviation of three replications per sample in each experiment. Furthermore, any significant difference between the mean of the grown colonies per petri dish was analyzed by SPSS statistical software (version 22) using the one-way analysis of variance, and the significance level was considered as $P < 0.05$.

Results

The present study assessed the anti-mutagenic activities of plant extracts applying the mutated *Salmonella* typhimurium (TA100) strain.

Positive control plates, including sodium azide (NaN_3), were used to induce reverse mutations. NaN_3 converts several mutant bacteria into wild types (these bacteria can grow on the minimal mineral medium without histidine). Moreover, negative control plates, including distilled water without the presence of NaN_3, were applied to induce spontaneous mutations, and the resulting colonies indicated that several bacteria in the medium spontaneously mutated and became wild. In this case, the number of colonies is extremely low compared to the positive control (Figure 1, a-b) and is negligible.

The number of the grown colonies in plates containing the extracts was lower compared to the positive control due to the anti-mutagenic activities of the plant extracts that inhibited the reverse mutations of bacteria in the presence of NaN_3 (Figure 1, c-f).

The number of the grown colonies and the percentage of mutation inhibition calculated by the Ong formula[24] are presented in Table 1.

Anti-mutagenic Activity of Lantana camara Leaf Methanolic Extract

The statistical results of the anti-mutagenic activities of the leaf methanolic extract in the absence of S_9 showed that the number of the colony-forming unit (CFU) of the mean grown colonies (201.66 ± 4.83) significantly decreased compared to the control ($P < 0.05$), and

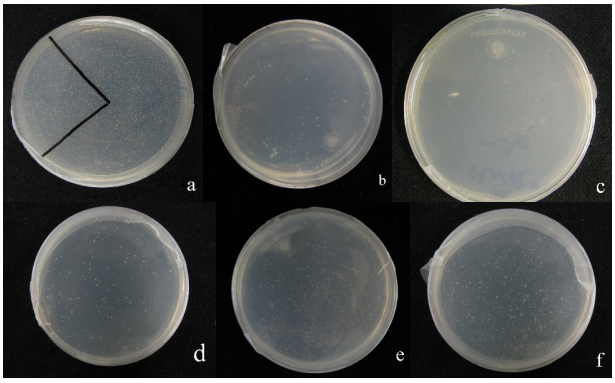

Figure 1. Anti-mutagenic Activity of the *Lantana camera* Extract. The images of the grown colonies in the positive control (a), negative control (b), treatment with the leaf extract in the presence of S_9(c), treatment with the leaf extract without S_9(d), treatment with the flower extract in the presence of S_9(e), and treatment with the flower extract without S_9 (f).

Table 1. The Effects of the Leaf and Flower Methanolic Extracts of *Lantana camera* on the Number of the Grown Colonies of *Salmonella* typhimurium TA100 (by Reverse Mutations) and Mutation Inhibition Percentage With or Without S_9

	TA100 + s_9		TA100 - s_9	
Sample	**Mutation inhibition percentage**	**Mean number of grown colonies**	**Mutation inhibition percentage**	**Mean number of grown colonies**
Positive control	-	826.33 ± 3.02	-	826.33 ± 3.02
Negative control	-	102.66 ± 4.98	-	102.66 ± 4.98
Leaves extract	84.79 ± 0.17*	125.66 ± 1.37	75.59 ± 0.73**	201.66 ± 4.83
Flower extract	62.32 ± 0.23*	311.33 ± 1.68	49.57 ± 0.55**	416.66 ± 3.38

Note. S_9: Sterile extract of the mouse liver containing microsomal enzymes.
*A significant difference was at $P = 0.026$, at the level of 5% ** Significant difference was at $P = 0.018$ at the level of 5%.

mutation inhibition percentage was calculated as 75.59 ± 0.73. Additionally, strong anti-mutagenic activity (above 40%) was observed according to the standard "Ong" formula.[24]

In addition, the mean number of the grown colonies of this extract in anti-mutagenic studies in the presence of S_9 demonstrated a significant decrease (125.66 ± 1.37 CFU, $P < 0.05$) compared with the positive control, showing a percentage of mutation inhibition of 84.79 ± 0.17 (Figures 2 and 3). As shown in Figure 2, the anti-mutagenic effect with S_9 was higher compared to the absence of S_9.

Anti-mutagenic Activity of Lantana camara Flower Methanolic Extract

Based on the results, the mean grown colonies significantly decreased (416.66 ± 3.38) CFU in the presence of the flower methanolic extract and the absence of S_9 (P <0.05) compared with the positive control. Further, the mutation inhibition percentage was 49.57 ± 0.55 and anti-mutagenic was above 40% although it was significantly lower compared to the leaf extract ($P = 0.018$).

In the presence of S_9, the mean number of the grown colonies of the flower extract showed a significant decrease (311.33 ± 1.68 CFU and $P < 0.05$) compared with the control. Furthermore, the percentage of mutation inhibition was 62.32 ± 0.23, and anti-mutagenic activity was also above 40% although it was significantly lower than that of the leaf extract ($P = 0.026$).

Discussion

The bacterial reverse mutation assay is a simple, rapid, and inexpensive assay for the detection of the mutagenic and anti-mutagenic activities of different substances. The damage of DNA by mutagens may be the main cause of most genetic defects and cancer. In addition,

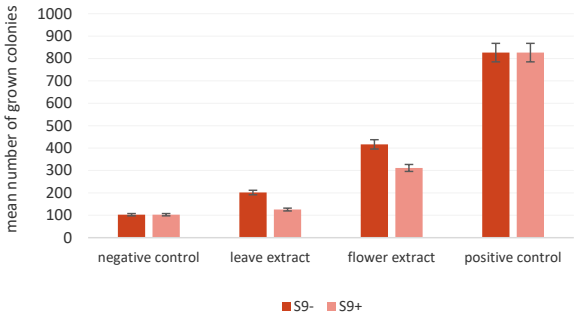

Figure 2. Comparison of the Mean Number of Grown Colonies Between the Extracts of *Lantana camara* With the Control With S_9 (+ S_9) and Without S_9 (-S_9) ± standard error.
Note. The comparison was made at the 0.05 level. S_9 is a sterile extract of the mouse liver containing microsomal enzymes such as the cytochrome oxidase enzyme (P450) which causes the anti-toxic action and inactivates oxidant and cancer compounds. In addition, the positive control contained sodium azide (NaN_3) as a mutagen per plate, and plates consisting of only sterile distilled water without any NaN_3 or the plant extract were considered as negative control.

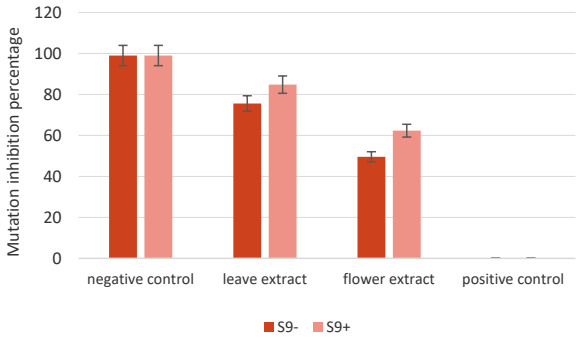

Figure 3. Comparison of the Percentage of Mutation Inhibition Between the Extracts of *L. camara* With the Control With S_9 (+ S_9) and Without S_9 (-S_9) ± Standard Error
Note. *L. camera*: *Lantana camera*. The comparison was made at the 0.05 level. S_9 is a sterile extract of the mouse liver containing microsomal enzymes such as the cytochrome oxidase enzyme (P450) which causes the antitoxic action and inactivates oxidant and cancer compounds. Further, the positive control contained sodium azide (NaN_3) as a mutagen per plate, and plates consisting of only sterile distilled water without any NaN_3 or the plant extract were considered as negative control.

the anti-mutation and anti-cancer activities of plants are due to their secondary metabolites.[26] Further, the plant structures of *L. camara* have many of these compounds which are accountable for several medical properties for treating diseases such as cancers, measles, chickenpox, asthma, edema, blood pressure, eczema, eye infections, tetanus, and malaria.[27]

These research findings represented that *L. camara* methanolic extracts had anti-mutation activities by applying the *Salmonella* typhimurium reverse mutation assay and the Ames test, which is in line with the results of Zare et al on the anti-mutation and anti-cancer activities of two species from the Verbenaceae family (*Lippia* genus), namely, *Lippia citriodora* and *Lippia nodiflora*, which were attributed to their flavonoids and essential oil components.[28] Furthermore, Begum et al reported the existence of flavonoids as the components of *L. camara*.[29]

Our results are also in conformity with those of Ghasemian et al, demonstrating the effects of secondary metabolites on the anti-mutagenic and anti-oxidant activities of the pomegranate peel extracts of two cultivars (from Iran) using the Ames test. They also suggested that the existence of flavonoid compounds in these plants was responsible for these activities.[21] Additionally, Ruberto and Baratta reported the anti-oxidant and anti-cancer activities of phenolic compounds.[30] Meanwhile, phenolic compounds are found to be the major constituents in the plants of the Verbenaceae family, including *L. camara*,[31] which can explain its anti-mutagenic activity.

In another study, Vicuña et al concluded that essential oils or fatty compounds (e.g., terpenoids) in the Verbenaceae family are responsible for anti-tumor and anti-carcinogenic effects by augmenting DNA repair mechanisms.[32] Moreover, Sefidkon indicated

that the vegetative and reproductive parts of *L. camara,* which were planted in Iran, contained essential oils and fatty compounds including β-caryophyllene (14.0% and 22.5%), sabinene (16.5% and 7.3%), 1,8-cineole (10.0% and 6.0%), humulene (6.0% and 10.8%), and bicyclogermacrene (8.1% and 18.5%).[33] Therefore, the existence of the essential oils can partly be involved in the observed anti-mutagenic and anti-cancer activities of *L. camara* as well.

According to our results, the leaf extract demonstrated the highest anti-mutagenic effects in the presence of S_9 ($+S_9$). Effective compounds (i.e., essential oil, flavonoid, and the like) are probably more abundant in the leaves as compared to the flowers of the plant, or the types of the compounds in the flowers probably differ from those of the leaves, which needs to be studied and analyzed in the future.

Conclusion

Generally, the findings of the research showed that the *L. camara* methanolic extracts of its flowers and leaves had potent anti-mutagenic activity against *Salmonella* typhimurium. These activities are probably related to the existence of flavonoids and different fatty compounds in this plant. The findings revealed that anti-mutagenic activity was higher in the leaf extract compared to the flowers. Thus, it is suggested that future studies directly investigate the anti-cancer activities of this plant on human and animal cancer cell lines.

Ethical Approval

Not applicable. There was no need for moral confirmation considering that bacterial samples were used in this study.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interests.

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