An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

STUDIES ON THE NON-MUTAGENIC EFFECT OF *LAWSONIA INERMIS.L* ON *SALMONELLA* HISTIDINE AUXOTROPHS TA98 AND TA1535

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ARTICLE INFO

Key Words

Lawsonia inermis.L, Salmonella, TA 98, TA 1535, ATPE,

ABSTRACT

Scientific approach and sustainability are the two key strategies to attain conservation and also development in any given system .In focus to the above mentioned theme as leaves of *Lawsonia inermis* L., a promising medicinal plant, showing a huge list of medicinal properties have been processed to show a different utility in food industry. Synthetic food colourants which are proved to be carcinogenic are used in variety of foods, they have become the essential ingredient in order to fake freshness. Natural food colourants impart less pronounced colour on food products and are also considered as more expensive. Lawsone which is the main colour producing compound is extracted from *Lawsonia inermis* .L leaves by standard methods Extraction of natural food colourants from *Lawsonia inermis*.L leaves is done by Aqueous two phase Extraction (ATPE).This extract could be either used as liquid colourant or as powdered colourant. The colour extraction by (ATPE) proved to be more organic than any other standard method. The extraction method is also proved to be less laborious and less time consuming. Medicinal plants are considered as value added aspects of countries biodiversity. Hence Sustainable product development from plants is the need of the hour. All scientific approaches towards the conservation must be organic in all levels of processing plants for their useful products .Hence the above method has proved to be more organic in developing a natural food colourants.

INTRODUCTION

Health is considered as an important facet of quality living. A country's development starts with the well being of their citizens and almost all developed countries focus on preventive care ,With respect to above aspect all the government and other welfare organizations promote the ideology of being organic .The usage of eco-friendly and organic goods in daily life is been promoted and greatly appreciated. On the other hand the usage of synthetic products becomes indispensible, As the synthetic products are found to have all time availability, cheaper rates and possess well pronounced activity in little concentrations. Food industry plays key role in health of the people, and has attained a complete transformation in introducing new products. To overcome competition and to make more profit they try to impart fake freshness and taste by

adding synthetic food colourants and other artificial flavours and preservatives. The synthetic food colourants attract consumers but also causes many other health ailments .The majority of the synthetic food colourants are found to contain sunset yellow, tartrazine, brilliant blue and ponceau 4R in their formulations. Mutagenic potential of food dye (Apple green) is proved by AMES test (1) .The genotoxicity of two commonly used food colors, sunset yellow and Brilliant blue is also proved (2). Cytotoxicity of food dyes Sunset Yellow (E-110), Bordeaux Red (E-123), and Tatrazine Yellow (E-102) on *Allium cepa* L. root meristematic cells is also demonstrated (3). DNA Damage caused by food colourants is detected Using RAPD Markers (4). Genotoxicity of synthetic food colorants proved by AMES test (5). Amaranth and tartarzine are investigated for genotoxicity by AMES mutagenicity assay (6). Considering the above alarming health hazards, there is an immediate need to find a suitable organic substitute which will replace these synthetic colourants and in the same way will fulfil the needs of the manufacturer. Our aim is to develop an organic product which will have mutual benefit, with respect to the above aspect Natural colurants could be the best alternative to play dual role of being healthy and to impart colours. The natural colourants could be extracted from various plants. In the present study a member of the family Lythraceae has been exploited as a prospective natural colourant. This colourant despite having the potential to impart colour to the food items also posseses a variety of medicinal properties. L*awsonia inermis* belongs to the family Lythraceae have been chosen and to use as a food colourant. It has been used as a colorant for over three thousand years and was often mixed into a paste and used to colour skin and hair. It is still widely used in cosmetology for its counter stain was also shown by Chukwu (7). *Lawsonia inermis* used in Ayurvedic medicine for the treatments of rheumatism, insect bites, skin ailments, burns and wounds. It is also proven to have antifungal properties that are linked to the bioactive compound Lawsone, the same that also provides its colouring/dyeing properties (8). Antibacterial activity of *Lawsonia inermis* Linn. against *Pseudomonas aeruginosa* was studied (9). *In vitro* study of the effects of *Lawsonia inermis* extract on helminth parasites is also done (10). Hepatoprotective action of *Lawsonia inermis* was examined (11) Tailor Chandra Shekhar *et al* (12) have proved antioxidant Activity of *Lawsonia inermis* Linn. Application of extracts of *Lawsonia inermis*) leaves as a study and then it is used as a colorant in various confectionaries. Hence in the present investigation, non - mutagenicity of *Lawsonia inermis* was studied using AMES.

2. MATERIALS AND METHODS

Preparation of crude extract

Fresh lawsone leaves were collected from the garden. Lawsone extract was prepared. Ascorbic acid (0.1%) was added to arrest the activity of polyphenoloxidase which otherwise causes enzymatic browning. The lawsone extract was stored at 4 oC, and required quantities were taken as and when required for different experiments and directly subjected to Aqueous two phase extraction (ATPE). Predetermined quantities of PEG 6000 and ammonium sulphate (13) were weighed and added to crude extract to make the total weight of the system 100% (w/w). The contents were mixed thoroughly using a magnetic stirrer for equilibration and were allowed for phase separation for 4–5 h. After the separation of two phases, the volumes of top and bottom phases of the system were noted and analyzed for lawsone content. All the experiments were carried out at 25 ± 1 C and the pH of the system was maintained in the range of 5–5.5. The extraction

experiments were carried out in duplicates and the average values are reported.

UV–VISIBLE SPECTROSCOPY ANALYSIS

UV-visible spectroscopy analysis was carried out by using UV-Visible absorption spectrophotometer between 200 to 700 nm. The colourant was diluted with distilled water and the spectra were determined.

Non- mutagenicity of *Lawsonia inermis* on *Salmonella* histidine auxotrophs TA98 and TA1535

The Ames test to check the non-mutagenicity of the *Lawsonia inermis* is carried out using the standard protocol of Maron and Ames (14). Tester strains used were *Salmonella* histidine auxotrophs TA98 and TA1535. Non- mutagenicity of the extracted *Lawsonia inermis* of present study are subjected to the Ames test. Different concentrations (0.312, 0.625, 1.25, 2.5 and 5.0 mg/ml) of *Li*AqE was prepared and incubated. The response of *Li*AqE was compared with control and the plates were evaluated and number of revertant colonies was tabulated .

3. RESULTS AND DISCUSSION

The lyophilized ethanolic extract of *Lawsonia inermis* thus obtained gives the maximum absorption peak at 450 nm. The graph shows the absorption range of the colourant. (Fig.1 & Table 1). The dyeing principle is *Lawsonia inermis* (lawsone) that is contained in dried leaves in concentration of 0.5-2%". But lawsone is not present freely. (15). Lawsone is not present as a free molecule in the leaves, but it is derived from its precursors, the hennosides, during henna preparation. Hennosides are three isomers derived from the keto-enol interconversion of the naphtoquinone structure. In this case the second ring is thrice oxygenated, that give rise to three possible hydroxyl groups and consequently change to the diketonic form. Each of the hydroxyls can be glucosidated, giving rise to the three isomers. The aglycone, derived from their hydrolysis, is further converted by oxidation into lawsone that is the active dyeing compound.The colour that lawsone produce is found to be very appealing and it is very easy to handle in the food industry. According to the earlier literature lawsone is used as a dye but not as a food colourant as some of the reports have showed that has mild toxicity. The toxicity of root extract of this plant is also shown (16).There are many number of reports showing the medicinal properties of the leaf extract. Apart from antibacterial activity this plant is also found to show anticancerous activity (17, 18). The use of organic products is always ideal. There are several reports supporting the concept of colouring the food in the natural way (19). The natural colourant from *Li*AqE tested for the mutagenicity strain TA 98 and TA 1535 without metabolic activation are shown in the table 2. Different concentrations of (0.312, 0.625, 1.25, 2.5 and 5 mg/mL) of *Li*AqE significantly increases the number of his^+ revertant colonies in the strain TA 98 and in TA 1535 when compared to Sodium azide as positive control without metabolic activation. In this test both the strain TA 98 and TA 1535 was found to be very less sensitive to the *Li*AqE at all test concentrations when compared with the positive control. The percentage of inhibition was maximum up to 98% for the strain TA 98 (0.625 mg/ ml) whereas in TA 1535 shows the 92% at 0.3125 mg/ml concentration thereby decreasing the number of his+ revertant colonies were observed in the present investigation. Similarly evaluation of the food dye namely apple green for its mutagenic potential reveals the moderate mutagenic effect of the colourant at highest concentration (1). Elizabeth *et al* (20) proved that the reduction products of Azo dyes are potential genotoxicants through AMES test.

Table 1: Absorbance (OD) of the extract

S.no.	Wavelength	Absorbance
1	400	0.1604
2	410	0.1624
3	420	0.1626
4	430	0.1622
5	440	0.1610
6	450	0.1652
7	460	0.1643
8	470	0.1637
9	480	0.1624
10	490	0.1599
11	500	0.1599
12	510	0.1599
13	520	0.1563
14	530	0.1536
15	540	0.1521

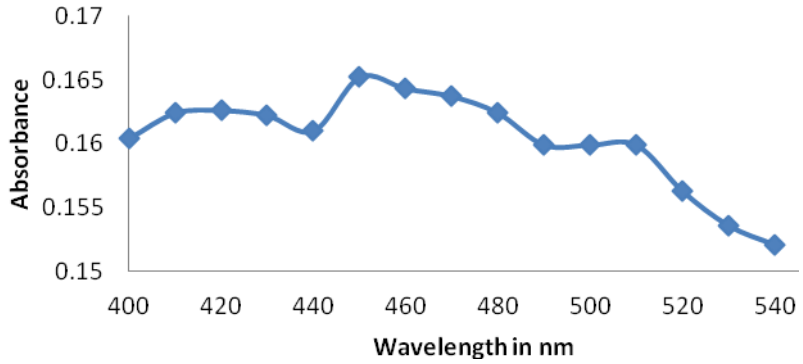

Fig.1: Absorption spectra of the *lawsonia inermis* aqueous extract

Table 2: Means colony count - Strain TA 98 and TA 1535- spontaneous mutation of *li*aqe

Concentration (mg/ml)	Histidine Revertant Colonies		Percentage of Inhibition	
	TA 98	TA 1535	TA 98	TA 1535
Positive control Sodium azide (1.5 µg/plate	289 ±18.4	347.5 ±		
0.312	0 ± 0	11.5 ± 2.1	0	96.69
0.625	4.5 ± 3.5	26.5 ± 7.8	98.44	92.37
1.25	16 ± 1.4	46 ± 1.4	94.46	86.76
2.5	34.5 ±3.5	52.5 ±2.1	88.06	84.89
5	66 ±17	72.5 ± 2.1	77.16	79.13

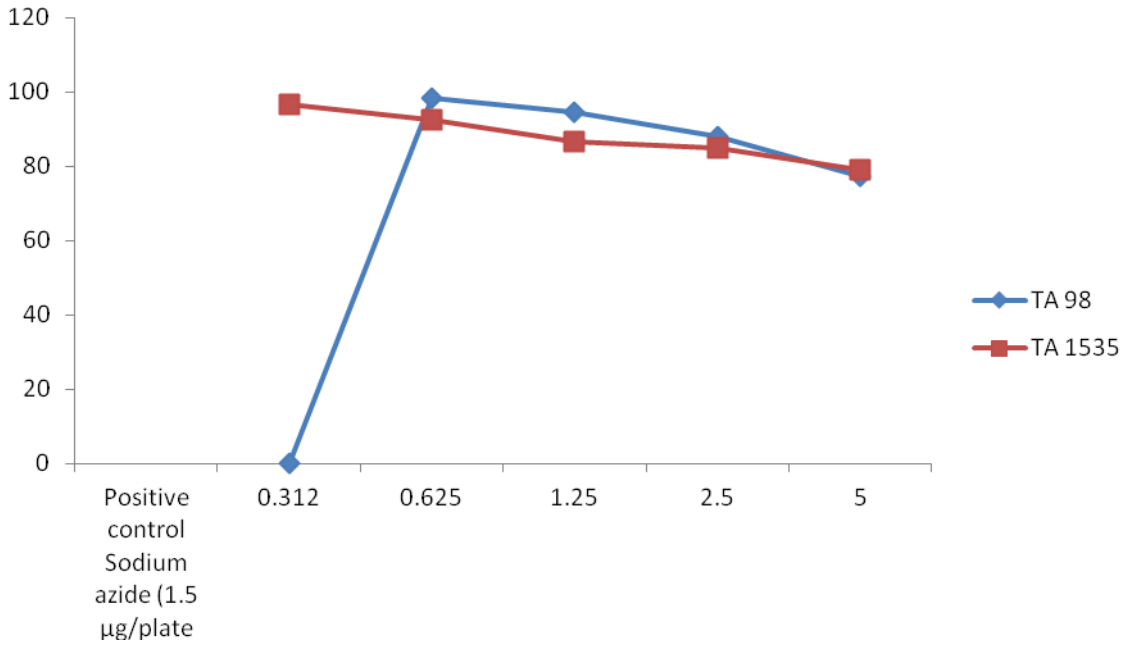

Fig.2: Strain TA 98 and TA 1535- Spontaneous mutation of *li*aqe

Toxicity is always dose dependant hence the usage of lawsone as a colourant must always accompany safe dosage use. The synthetic colourants are found to cause various toxic response even at lower concentrations (21). So it is always better to include organic products in all possible ways in our life.

4. CONCLUSION

Biocolors are natural, prepared from renewable sources that are easily degradable and without production of recalcitrant intermediates when they enter in the environment. Natural colours have grown importance in food industry. The awareness among people towards natural pigments and their therapeutic uses are increasing because of their nontoxic or less toxic properties. On the other hand, synthetic colours are based on toxic raw materials. The continuous use of synthetic colours not only causes considerably environmental pollution but also many health related problems in human beings i.e. carcinogenic effects etc. It is therefore, essential to explore various natural sources of food grade colorants and their potential uses. It is conclude that *Lawsonia inermis* (Lawsone) used as a alternative colour in food industry.

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