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Assessment of bioactivity of *Lawsonia Inermis Linn.* using brine shrimp (*Artemia Salina*) lethality assay

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Abstract
The pharmacological evaluation of substrates from plants is an established method for the identification of compounds, which can lead to the development of novel and safe medicinal agents. The present study supports the previous that brine shrimp bioassay is simple, reliable, and convenient method for assessment of bioactivity of medicinal plants and leads support for their use in traditional medicine. The brine shrimp lethality bioassay is extensively used for the isolation of anitumour and cytotoxic agents from medicinal plants. The present study was designed to assess as well as to compare the cytotoxic activity of Lawsonia inermis Linn. using brine shrimp lethality bioassay. All the extracts of leaves were found to have promising cytotoxic activity. The highest activity was found for leaves which is followed by ethyl acetate and aqueous extract results suggest that the leaves of the Lawsonia inermis Linn. may be a promising source for novel anticancer agents.

Keywords: lawsonia inermis linn. artemia salina, brine shrimp lethality test, medicinal plants, cytotoxicity

Introduction

Lawsonia inermis Linn (Family: Lythraceae) is a much branched glabrous shrub or small tree (2-6 m in height), cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine [1]. This plant is a worldwide known cosmetic agent used to stain hair, skin and nails. The plant is reported to contain Lawsone, Esculetin, Fraxetin, Isoplumbagin, Scopoletin, Betulin, Betulinic acid, Hennadiol, Lupeol, Lacoumarin, Laxanthone, Flavone glycosides, two pentacytic triterpenes. The plant has been reported to have analgesic, hypoglycemic, hepatoprotective, immunostimulant, antiinflammatory, antibacterial, wound healing, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties [2]. Henna, a traditional product with religious associations, has been widely used over the centuries for medical and cosmetic purposes in Africa, Asia, the Middle East and many other parts of the world. Henna is a finely ground brown or green powder originating from dried leaves of the plant *Lawsonia inermis* which is grown in dry tropical and subtropical zones, including North Africa, India, Sri Lanka, and the Middle East. Microwave extraction has proved to be more effective and efficient than its conventional counterpart, the soxhlet extraction method. The Soxhlet extraction, which is a standard technique, is a continuous solvent extraction method [3, 20]. Extraction systems are used to conduct routine solvent extractions of soils, sediments, sludge, polymers and plastics, pulp and paper, biological tissues, textiles and food samples. Experiments have proved that microwaves, in comparison with the soxhlet extraction, use a lesser volume of solvent and sample and perform extraction at a much faster rate. In the discovery of effective medicines for prevention and treatment, an outbreak of coronavirus disease (COVID-19) caused by the novel extreme acute respiratory syndrome coronavirus-2 (SARS-CoV-2) poses an unprecedented obstacle. The proximity to the patient during dental care, high generation of aerosols, and the identification of SARS-CoV-2 in saliva have suggested the oral cavity as a potential reservoir for COVID-19 transmission. Soon, someday, you might be making your own drugs at home. That is because researchers have adapted a 3D printer from basic, readily available medicinal active agents fed into a drug delivery system. Brine Shrimp lethality bioassay (Luo et al., 2000; Mclaughlin et al., 1998; Meyer et al., 1982) is a rapid and comprehensive bioassay for the bioactive compounds of natural and synthetic origin [21, 32]. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their bioactivity. The method utilizes *in vivo* lethality in a simple zoological organism (Brine nauplii) as a convenient monitor for screening and fractionation in the discovery of new bioactive natural products. Brine toxicity is closely correlated with 9KB (human nasopharyngeal carcinoma) cytotoxicity ($p=0.036$ and kappa = 0.56). ED [50] values for cytotoxicities are generally about one-tenth the LC50 values found in the Brine Shrimp test. Thus, it is possible to detect and then monitor the fractionation of cytotoxic, as well as 3PS (P388) *(in vivo* murine leukaemia) active extracts using the Brine lethality bioassay (Alkofahi et al., 1988; Mclaughlin et al., 1998; Meyer et al., 1982). The Brine Shrimp assay has advantages of being rapid (24 hours), inexpensive, and simple (e.g., no aseptic techniques are required). It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample (2-20 mg or less). Furthermore it does not require animal serum as is needed for Cytotoxicities [33, 36].

Materials and Methods

Plant material

Lawsonia inermis Linn, has been collected from Karad, Maharashtra, India. Department of Botony, Yashwantrao Chavan College of Science, Karad has identified the plant and authenticated it.

Preparation of Lawsonia inermis Linn. Leaf powder

Fresh Lawsonia inermis Linn. Were collected and air dried for 10 days. The dried leaves were then crushed into a blender and churned to form a coarse powder. The powder was collected in an air-tight jar, and stored away from sunlight in a cool, dry place.

Preparation of plant extract

Extraction of Lawsonia inermis Linn, was done by microwave extraction further filtered and excess solvent present was evaporated and dried extract were collected and subjected for activity studies.

Preparation of seawater

38 gm sea salt (without iodine) was weighed, dissolved in one liter of distilled water and filtered off to get clear solution.

Hatching of Brine Shrimp

Artemia salina leach (brine shrimp eggs) collected from pet shops was used as the test organism. Seawater was taken in the small tank, and shrimp eggs were moved to one side of the tank, and sealed on this side. The shrimp was allowed to hatch for two days and be matured like nauplii. Constant supply of oxygen was rendered during the process of hatching. The hatched shrimps are drawn to the light (phototaxis), and so egg shell-free nauplii from the illuminated portion of the tank was collected. The nauplii was taken by a pipette from the fish tank and filtered to improve visibility in fresh clear sea water, and 10 nauplii was taken carefully by micropipette [77].

Preparation of Reagents

Serial dilution of extract

Clean test tubes have been taken and labelled. An analytical balance was measured against plant extract of 10mg. Dissolving 10mg of plant extract (soluble in water) in 1ml of water then prepared stock solution. Concentrations of 1 mg/ml, 100µg/ml, 10µg/ml, and 1µg/ml were prepared from stock solution using serial dilution. Then 1 ml of prepared solution was taken into the test tubes which contain 10 nauplii and 1 ml of seawater. After 24 hours the number of dead nauplii was counted [37].

Calculations

The mortality endpoint of this bioassay is defined as the absence of controlled forward motion during 30 seconds of observation. The percentage of nauplii lethality for each concentration was calculated. For each tube, count the number of dead and the number of live nauplii, and determine the % death [38, 40].

$$\% \text{ death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} + \text{Number of live nauplii}} \times 100$$

Results and Discussion

Different extracts of Lawsonia inermis Linn. used for the cytotoxicity test with help of brine shrimp. It is very useful for BSLA to screen a wide range of extract for their different bioactivities. From results it was found that the ethyl acetate extract shows less % percent death of nauplii in different concentrations compared to the other extracts.

Table 1: Results of Brine shrimp lethality assay of Lawsonia inermis Linn. Extracts

Sr. no	Extract	% death nauplii			
		1mg/ml	100 µg/ml	10µg/ml	1µg/ml
1	Methanolic extract	100	40	30	20
2	Ethyl acetate extract	70	30	20	10
3	n-hexane extract	100	50	40	30
4	Aqueous extract	80	60	50	20

Conclusion

Many plants which have been described as curative may also have harmful effects. The concentration of a substance is the most important determinant of the outcome: it could lead to toxic effects if it reaches a sufficiently high concentration in the susceptible biological system. Although the saline shrimp lethality assay is somewhat inadequate in terms of the elucidation of the mechanism of action, it is very useful to assess the bioactivity of plant extracts. In fact, in the course of our studies, the saline shrimp lethality assay proved to be a convenient system for monitoring the biological activities of a number of plant species used in traditional medicine. It was of great need to involve *in vivo* testing using animal model in order to gain relevant data which can be extrapolated to human population. Researchers have used rats and other animal models for years that exhibit a high correlation with the human population. This type of testing has recently been restricted due to ethical and economic considerations. Alternative toxicity assays are therefore widely used to test the potential for toxicity of plant products. Brine Shrimp Lethality Assay seemed a good approach, particularly since it could still be classified as *in vivo* testing. Artemia salina nauplii is one of the alternatives for herbal extracts biological toxicity assays and this test has proved to be significantly correlated with several other animal models. The preliminary toxicity data obtained through the Brine shrimp lethality assay provides a convenient platform for further study of toxicity.

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