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In-vitro hypoglycemic activity of *lawsonia inermis Linn.* Leaf extracts

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Abstract
Many of today's modern drugs have their origin in traditional plant medicine. The therapeutic efficacies of many indigenous plants for various diseases have been described by practitioners of traditional herbal medicines. *Lawsonia inermis* Linn. Is commonly known as henna, which is recognized in traditional system of medicine. *Lawsonia inermis Linn.* Extracts have been tested for their effects on glucose adsorption and diffusion. Adsorbed glucose is derived from the plant and glucose adsorption is greatly enhanced with an increase in glucose levels. The rate of diffusion from glucose in the kinetic amylolysis experimental model increased from 30 to 180 minutes, with plant extracts showing major inhibitory effects on glucose Movement through the dialysis membrane to the external solution, in comparison to control. The results confirmed *Lawsonia inermis Linn.* Anti diabetic activity. By increasing glucose adsorption, growing glucose diffusion rate and at the cellular level, the hypoglycemic effect experienced by the extracts is mediated.

Keywords: *Lawsonia inermis* linn, glucose adsorption, glucose diffusion, hypoglycemic activity

Introduction
Lawsonia inermis Linn) invites attention of the researchers worldwide for its pharmacological activities ranging from anti-inflammatory to anticancer activities. *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 nails3. 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, antiin flammatory, 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 [3]. 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 [4, 9]. 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 [10, 18]. 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 [19, 33]. That is because researchers have adapted a 3D printer from basic, readily available medicinal active agents fed into a drug delivery system [34, 36]. Diabetes mellitus is one of metabolic syndrome that alter carbohydrate, lipid and protein metabolism and additionally increased risk of complications of various vascular diseases. Hyperlipidemia associated atherosclerosis is the most common cause of death in diabetes. Insulin-dependent diabetes mellitus or type 1 diabetes is an autoimmune disorder characterized by destruction of insulin producing β-cells because auto-aggressive T-lymphocytes infiltrate the pancreas that leads to hypoinsulinemia and thus hyperglycemia [37, 39].

Material and Methods
Chemicals and Reagents
The glucose oxidase peroxidase kit was purchased from Pathozyme Diagnostics, Kagal, Maharashtra, India. Dialysis bags (12,000 MW cutoff; Himedia laboratories, India) were used in the study. All the chemicals used in the present study were of extra pure analytical grade.

Plant Material

The fresh matured leaves of the *Lawsonia inermis Linn* were collected randomly during the month of May-June, from Sangli region, Maharashtra, India. Department of Botony, Yashwantrao Chavan College of Science, Karad has identified the plant and authenticated it.

Preparation of Plant Extract

Shade drying was done for almost a month to prevent sunlight chemical degradation. The dried material was grinded and transformed in coarse powder with the aid of a grinder. The extraction of *Lawsonia inermis Linn* with solvent methanol was carried out by microwave extraction, and excess solvent present was evaporated.

Evaluation of hypoglycemic activity of plant extracts using various *in-vitro* methods

In-vitro method includes

- *Determination of glucose adsorption capacity*
- *Effect of plant extracts on in-vitro glucose diffusion*

a. Determination of glucose adsorption capacity

In addition to 25 ml of glucose, the plant extract samples (1 percent) were added. The mixture was well blended, incubated for 6 hours at 37°C in a shaker water bath, centrifuged for 20 min at 4,000×g and the glucose level was calculated in the supernatant. The bound glucose concentration was determined with the following formula,

$$\text{Glucose bound} = \frac{G_1 - G_6}{\text{Weight of the sample}} \text{ X Volume of solution}$$

Where, G_1 is the glucose concentration of the original solution.

G_6 is the glucose concentration after 6 hours.

b. Effect of plant extracts on in-vitro glucose diffusion

The samples of plant extracts (1 %) and the 25 mL of glucose solution (20 mM) were dialyzed into 200 mL of distilled water in shaker baths at a temperature of 37°C i. Diagnostic kit for glucose oxidase peroxidase was used to test glucose content in the dialysate at 300, 60, 120 and 180. A sample-free control test was performed. Glucose dialysis retardation index (GDRI) was calculated by using the following formula [40].

$$\text{GDRI} = 100 - \frac{\text{Glucose content with additional of sample (mg/dl)}}{\text{Glucose content of the control (mg/dl)}}$$

Results and Discussion

a. Glucose adsorption capacity of Lawsonia inermis Linn extract

Glucose adsorption capacity of the selected plant extracts is depicted in following table. The adsorption capacities of the samples were found to be directly proportional to the molar concentration of glucose and higher amounts of glucose was bound with increased time shown in table 1.

Table 1: Glucose adsorption capacity of *Lawsonia inermis* Linn extract

Sample	Glucose content in dialysate (mM)			
	30 min	60 min	120 min	180 min
Control	0.71±0.04	1.28±0.06	1.54±0.03	1.89±0.03
Test	0.04±0.08	1.09±0.02	1.38±0.01	1.65±0.09
Mean values (n=3)				

Mean values (n=3)

Values in parenthesis indicate glucose dialysis retardation index (GDRI)

b. Effect of Lawsonia inermis Linn extract on in-vitro glucose diffusion

The effect of the plant extracts on retarding glucose diffusion across the dialysis membrane is shown in following table. The rate of glucose diffusion was found to increase with time from 30 to 180 min. In the present study, the movement of glucose across the dialysis membrane was monitored once in 30 min till 180 min and it was found that, the samples of plant extracts demonstrated significant inhibitory effects on movement of glucose into external solution across dialysis membrane compared to control shown in table 2.

Table 2: Effect of *Lawsonia inermis* Linn extract on in-vitro glucose diffusion

Sample	Glucose content in dialysate (mM)			
	30 min	60 min	120 min	180 min
Control	0.18±0.04	0.45±0.02	1.48±0.03	1.68±0.01
Test	0.02±0.01	0.25±0.07	0.90±0.01	1.16±0.02
Mean values (n=3)				

Conclusion

The results of the current study concluded by illustrating the hypoglycemic activity of *Lawsonia inermis Linn* extract as evaluated by different in-vitro methods. The *Lawsonia inermis Linn* hypoglycemic effect was shown to be mediated by increasing glucose adsorption, increasing glucose diffusion and promoting glucose transportation over the cell membrane at cell level. These results identified can also be verified by the use of multiple in-vivo models and clinical trials, which may help to better use diabetes mellitus in an efficient way.

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