www.pharmaerudition.org

ISSN: 2249-3875

International Journal of Pharmaceutical Erudition

Research for Present and Next Generation

Aug 2016

Vol: 06 Issue: 02

(01-11)

ISSN 2249-3875

International Journal of Pharmaceutical Erudition

Research Paper

Phytochemical, Pharmacognostic Investigation and Antioxidant Activity of *Lawsonia Inermis* linn. (Heena Plant)

Tailor Chandra Shekhar* , Panwar Vipin

Division of Pharmaceutical Sciences, Shri Guru Ram Rai Institute of Technology & Science, Patel nagar, Dehradun (Uttarakhand) 248001.

The present attempt is to review and compile updated information on various aspects of *Lawsonia inermis* (Linn), a plant used all over the world. This plant is commonly known as Henna or Mehandi and abundantly available in tropical and subtropical areas. Henna leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrhoea, leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent. The Physicochemical evalution of *Lawsonia inermis* Linn. have shown % loss of drying 7.36% . Total Ash value 11.5, % Acid soluble Ash value 3.06% and the extractive values were found as % Alcoholic soluble extraction value- 5.6%,% Water soluble extraction value-7.2%. The antioxidant activity *Lawsonia inermis* Linn. determind accurately, conveniently and rapidly using DPPH scavenging assay method. It is also clear that Alcoholic extract of the aerial parts i.e leaf of this plant exhibited best antioxidant potential then the other extract when compare to standard drug ascorbic acid. It showed 68.86 % inhibition at max 30 µg/ml concentration. IC 50 value was obtained as approx 23-24 µg/ml.

Key words: *Lawsonia inermis* (Henna), Antioxidant, DPPH, Ash value, Extractive values etc.

INTRODUCTION

Three decades ago, only few had any appreciation of the number of remedies that had their origin from herbal medicines, and most had vague knowledge of herbal medicines, traditional medicines or other form of complementry and alternative medicinal practices (1,2) For a variety of reasons, more individual now a days prefer to take personal control over their health with the use of herbal medicines, not only to prevent disease but also to treat them(3).

Future Prospects of herbal medicine market:

It is estimated that nearly three fourths of the herbal drugs used worldwide were discovered following leads from local medicine. According to WHO about 25% of modern medicines are descended from plants. Almost, 70% modern medicines in India are derived from natural products.

Antioxidants are the molecules that inhibit the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals(4,5).

Description of Plant:

Henna (*Lawsonia inermis* Linn.) is a tall shrub or

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small tree, 2.6 m high. It is glabrous, multibranched with spine tipped branchlets.

Fig.1: *Lawsonia inermis* Linn.(Heena plant species)

Leaves are opposite, entire, glabrous, sub-sessile, elliptical, and broadly lanceolate (1.5–5.0 cm x 0.5– 2 cm), acuminate, having depressed veins on the dorsal surface(7,8).

Morphology

Leaf- Henna leaf has an orange-red dye and leaf paste or powder is widely used for decorating hands, nails and feet with patterns.

Flowers- are very fragrant and used to extract a perfume, which is used as base for local scents. An infusion of the flowers is a valuable application to bruises. Decoction of the flowers is describes as an emmenagogue.

Seeds- are deodorant. Powered seeds with real ghee (clarified butter) are effective against dysentery.

Bark- The bark is applied in the form of a decoction to burns and scalds. It is given internally in a variety of affections, such as jaundice, enlargement of the spleen, calculus, as an alternative in leprosy and obstinate skin affections.

Root- is considered as a potent medicine for go norrhoea and herpes infection. Root is astringent may be pulped and used for sore eyes. Pulped root may also be applied to the heads of children for boils. The root is supposed to be useful in treatment of hysteria and nervous disorders(9,10,11).

Table 1: Chemical constituents (12,13,14)

S no.	Plant Parts	Chemical constituents
1.	Leaves	2-Hydroxy-1,4-napthoquinone, 1,4dihydroxynaphthalene, 1,4-naphthoquinone, 1,2-dihydroxy-glucoyloxynaphthalene, luteolins, apigenin, and their glycosides, esculetin, fraxetin, scopletin, β-sitosterol, tannin, gallic acid, glucose, mannitol, fat, resin and mucilage.
2.	Barks	napthoquinone, isoplumbagin, triterpenoids-Hennadiol, aliphatics (3-methylnonacosan-1-ol)
3.	Flowers	essential oil (0.02 %) rich in ionones (90 %), β-ionones.
4.	Roots	24β-ethylcholest-4-en-3β-ol
5.	Seeds	Linoleic acid, Arachidic acid, Stearic acid, Palmitic acid
6.	Whole plant	Laxanthone I, Laxanthone II, Laxanthone III, n-Triacontanol

Traditional uses (15, 16, 17)

1. It is used for the treatment of epilepsy and jaundice, and for dyeing grey hair.
2. It is used as a remedy for malignant ulcers.
3. The Ayurvedic Pharmacopoeia of India indicated the use of leaves in dysuria, bleeding disorder, prurigo and other obstinate skin diseases.
4. The leaf is used in vulnerary, diuretic, headache, hemicranias, lumbago, bronchitis, boils, Ophthalmia, Syphilitis, Sores, Amenorrhoea, Scabies, and spleen diseases and favours the growth of the hair.
5. The bark is given in jaundice and enlargement of the spleen, also in calcalous affections and as an alternative in leprosy and obstinate skin diseases.
6. It is used as medicinal plant because of its attributed antibacterial, antifungal, anti amoebiasis, astringent, antihemorrhagic, hypotensive and sedative effect.

Materials and Method:

Collection & Authentification: The Rhizomes of crude drugs of *Lawsonia inermis* Linn. was collected carefully from local areas of Dehradun (Uttarakhand) and authenticated from Division of life sciences, SGRRITS, Patel nagar, Dehradun. A voucher specimen herbarium of this Plant was also submitted in this department of college.

Standardization of *Lawsonia inermis* Linn :

The evaluation of crude drugs involves the determination of identity, purity, quality. Purity depends upon the absence of foreign matter whether organic or inorganic, while quality refers essentially the concentration of the active constituents in the drugs that makes it valuable to medicine. The following standardization parameters were evaluated.

Determination of the foreign matter:

Foreign matter in herbal drugs consists of either parts of the medicinal plant or it may be any organism, part of product of an organism. It may also include minerals admixture not adhering to the medicinal plant material e.g., soil, stone, dust etc.

Determination of physical constants:

Loss on drying at 105 °C:

Loss on drying is the loss of mass expressed as per cent w/w. The test for loss on drying determines both water and volatile matter in the crude. Moisture is an inevitable component of crude drug, which must be eliminated as far as possible.

Ash values:

Ash values are helpful in the determining the quality of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic

matter, which may otherwise interfere in an analytical determination.

Extractive Values:

Alcohol soluble and water soluble extractive values were calculated by macerating 5 gm. Coarsely powdered crude drug with 100 ml of 90% v/v ethyl alcohol and chloroform water in a stoppered flask for 24 hours separately. Then, these solutions were filtered through filter paper and concentrated and calculated their values accordingly. These values provide the quantity of Phytoconstituents present in the crude drug. Then, these Extracts were subjected to preliminary qualitative Phytochemical screening or investigation..

Thin layer Chromatography (TLC)

Studies were also carried out then for various extracts to confirm the presence of different Phytoconstituents in these extracts. TLC is mode of liquid chromatography, in which,the extracts is small spot or band at the origin of thin sorbent layer supported on a glass plates. The mobile phase migrates through the stationary phase by capillary action. The separation of solutes takes place due to their differential adsorption/ partition co efficient with respect to both mobile or stationary phases. The mobile phase consists of a single solvent or a mixture of solvent. Although, a number of sorbents like silica gel, cellulose, polyamide, alumina, chemically modified silica gel etc. are used, silica gel (type 60) is most commonly used sorbent handmade plates are prepared by using techniques like, pouring dipping or spraying. Now-a- days, ready made precoated plates are also available. The plates need to be activated at 110°C for 1 hr. this removes water/moisture lossely bound to silica gel surface (18,19,20).

The retardation factor (R_f) is calculated using following formula-

$$R_f = \frac{\text{Distance travelled by solute from the origin}}{\text{Distance travelled by solvent from the origin}}$$

Qualitative TLC analysis:-

The preliminary phytochemical investigation of PE,CE,AlcE and AqEof leafs of *Lawsonia inermis revaled* the presence of saponins, glycosides, fats and volatile oil.

Antioxidant activity by Invitro DPPH Scavenging assay method:

Antioxidant compound play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological system from a wide variety of sources. DPPH is widely used to test the ability of compound to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. The DPPH method can be used for solid or liquid samples and is not

specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample. A measure of total antioxidant capacity helps understand the functional properties of compound.

An easier way to present antioxidant activity of compound would be to reference a common reference standard.

$\%\ Inhibition = (Abs_{control} - Abs_{test}) / Abs_{control} \times 100$

This method has been developed to determine the antioxidant activity of compound utilizes the stable2, 2-dipheny-1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. Antioxidant compounds may be water-soluble lipid soluble, insoluble, or bound to cell walls (21,22,23).

Results & Discussion:

Table 1: Observation of Physiochemical parameter of the Roots of *Lawsonia inermis* Linn.

S. NO.	ORGANOLEPTIC EVALUATION		OBSERVATION
1.	Parameter	Nature	Coarse powder
		Colour	Dark brown
		Odour	Aromatic
		Taste	Bitter
2.	Physiochemical Evalution	% loss of drying	7.36%
		% total ash value	11.5%
		% water insoluble ash value	3.06%
		% acid insoluble ash value	5.01%
3.	Extractive value	%alcohols soluble Extractive value	5.6%
		%water soluble extractive Value	7.2%

The Preliminary Phytochemical tests revealed that leaves contained Saponin glycosides, fats and volatile oil. Aqueous extract contain Saponin glycoside and volatile oil.

Table 2: Phytochemical Screening Data results

S.NO.	PHYTOCHEMICAL CONSTITUENT	CHEMICAL TEST	EXTRACTS			
			PEE	CE	ALCE	AQE
1.	Carbohydrate test	Molish's Test	+	–	+	+
		Benedict's Test	+	+	+	+
		Fehling's Test	+	–	+	+
2.	Protein test	Million's Test	–	–	–	–
		Biuret Test	–	–	–	–
		Xanthoprotein Test	–	–	–	–
3.	Amino acid	Ninhydrine Test	–	–	–	–
		Cystein Test	+	+	+	+
4.	Fats and Oil	Filter paper stain Test	+	+	–	–
		Solubility Test	–	–	–	–

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		Saponification Test	–	–	–	–
5.	Steriod test	Salkowaski Reaction	+	–	+	–
		Liebermann-Burchard Reaction	+	–	+	–
		Liebermann-Burchard	+	–	+	–
6.	Volatile Oil	Characteristic odour	+	+	+	+
		Filter paper not stain	–	–	–	–
		Solubility Test				
7.	Glycoside					
	Caradiac Glycocides	Legal test	–	+	–	–
		Keller-Killani Test	–	–	–	–
	Anthraquinone Glycosi	Borntrager's Test	–	–	–	–
		Modified Borntrager'sTest	–	–	–	–
	Saponin Glycosides	Foam Test	+	+	+	+
		Heamolytic Test	–	+	–	–
	Cynogenetic Glycosides	Sodium picrate Test	–	+	–	–
	Coumarin Glycosides	Aromatic Odour	–	–	–	–
		Flourescence Test	–	–	–	–
	Flavonoids Test	Lead Acetate Test	–	–	–	–
		Sodium Hydoxide Test	–	–	–	–
8.	Alkaloid Test	Dragendroff's Test	–	–	–	–
		Mayer ' s Test	–	–	–	–
		Wagner ' s Test	–	–	–	–

PEE =Petroleum ether extracts **CE** = Chloroform extracts **AlcE** = Alcoholic extracts **AqE** = Aqueous extract

Qualitative TLC

The qualitative TLC analysis results in separation of different phytoconstituent in different solvent system and they were identified by their characteristic colour band with corresponding visualizing reagent.

Table 3: R_f values of different extracts of *Lawsonia inermis*

SOLVENT SYSTEM	PEE (R_F VALUE)	ALcE (R_F VALUE)	CE (R_F VALUE)
Chloroform	0.72	0.8	0.76
Pet ether	0.5	0.56	0.64
Alcohol	0.88	0.78	0.74
Chloroform: pet ether (5: 5)	0.6	0.5	0.62
Pet ether: alcohol (5: 5)	0.8	0.66	0.7
Alcohol: chloroform (5: 5)	0.9	0.78	0.68
Chlorofrom : pet ether (7:3)	0.86	0.8	0.9
Pet ether: alcohol (7:3)	0.8	0.7	0.76
Alcohol: chloroform (7:3)	0.78	0.9	0.86
Chlorofrom : pet ether (3:7)	0.8	0.6	0.5
Pet ether: alcohol (3:7)	0.58	0.68	0.62
Alcohol: chloroform (3:7)	0.78	0.82	0.8

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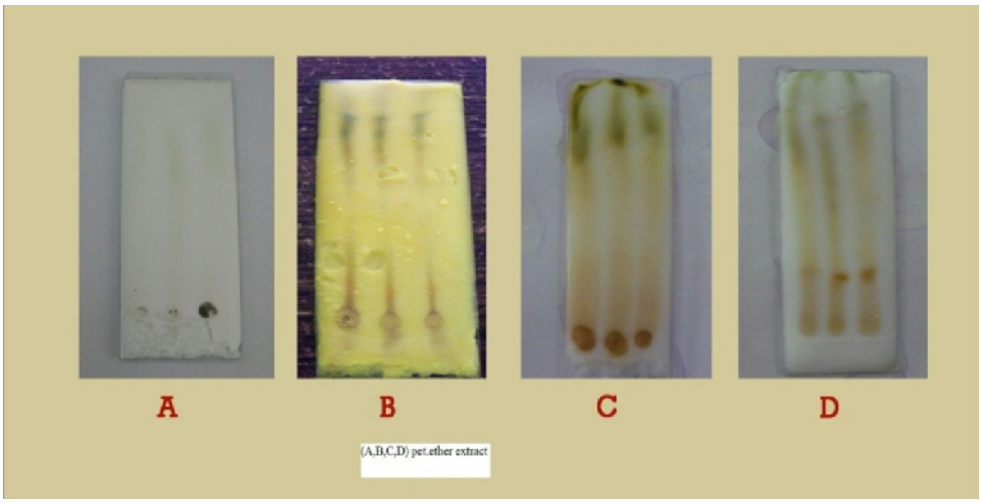

Fig. 2 : PEE showed the presence of saponin glycoside

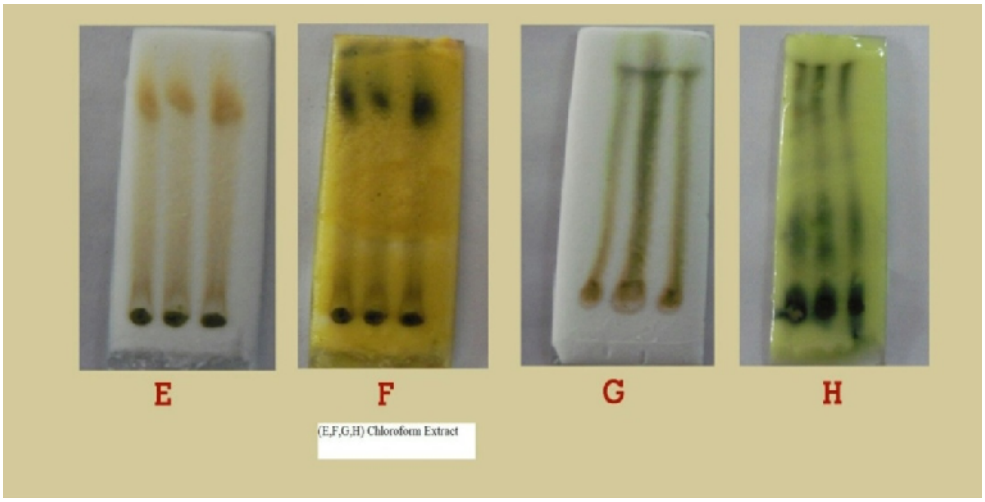

Fig. 3 : Chloroform extract showed the presence of fats

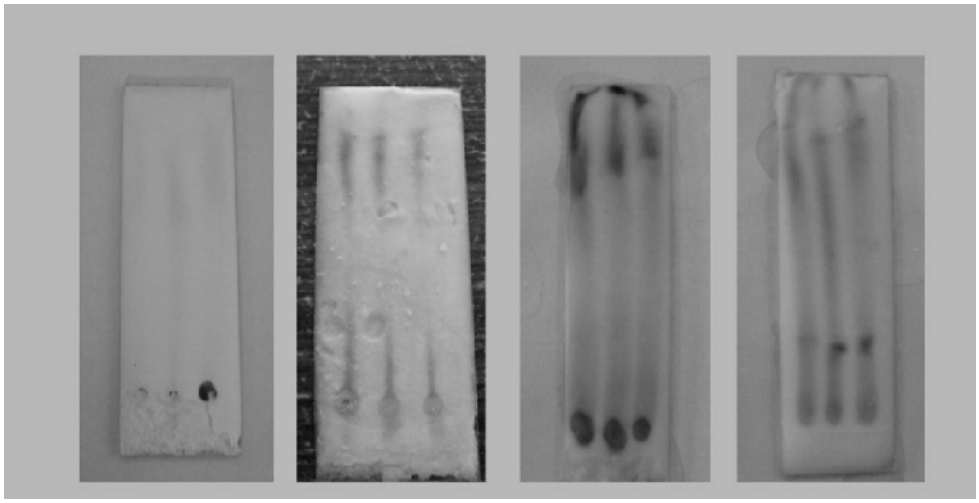

Fig. 4 : Alcoholic extract showed the presence of flavonoids

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RESULTS OF ANTIOXIDANT ACTIVITY

Table 4: Absorbance of different extracts of *Lawsonia inermis* with ascorbic acid

CONTROL: - 0.2444

CONC. µg / ml	ASCORBIC ACID (Abs)	PEE (Abs)	CE (Abs)	ALcE (Abs)
5	0.2380	0.2428	0.2343	0.2405
10	0.1719	0.2386	0.1720	0.2365
15	0.0469	0.2350	0.2420	0.2204
20	0.0415	0.2341	0.2245	0.1729
25	0.0410	0.2323	0.2348	0.1393
30	0.0390	0.2232	0.2128	0.0761

$\%\ Inhibition = (Abs_{control} - Abs_{test}) \div Abs_{control} \times 100$

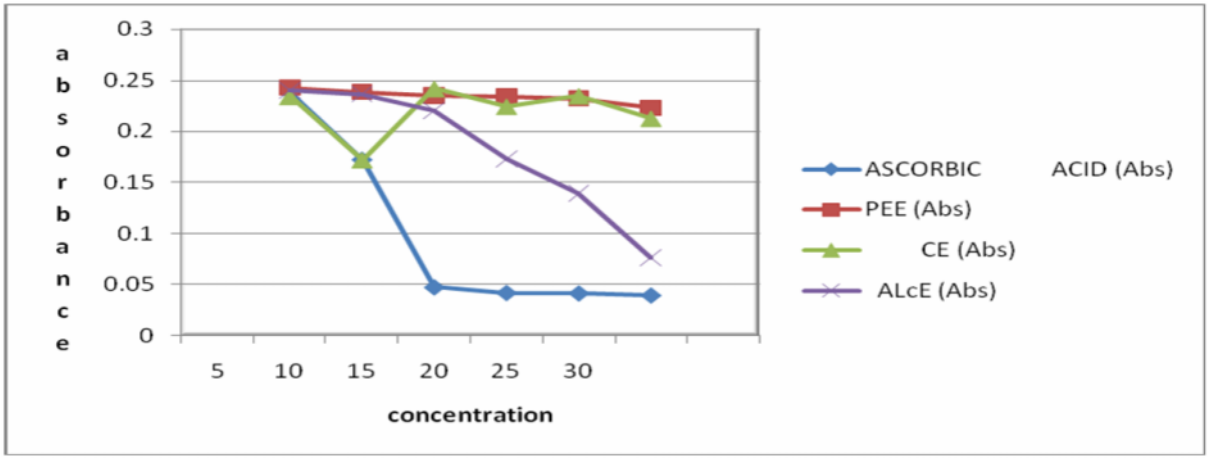

Fig.5 : Absorbance in different concentration

Table 5: % Inhibition of different extracts of *Lawsonia inermis* with ascorbic acid

Conc. µg/ml	Asc acid(% inhibition)	PEE (% inhibition)	CE (% inhibition)	AlcE (% inhibition)
5	2.62%	0.65%	4.13%	1.59%
10	29.66%	2.37%	29.62%	3.23%
15	80.81%	3.84%	0.98%	9.81%
20	83.01%	4.21%	8.14%	29.25%
25	83.22%	4.95%	3.92%	43%
30	84.04%	8.67%	12.92%	68.86%

ISSN 2249-3875

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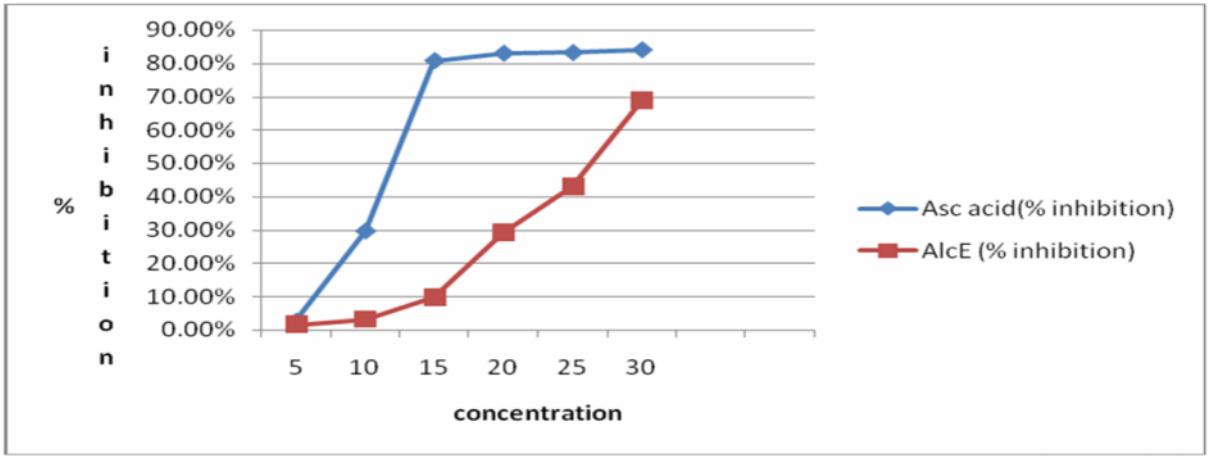

Fig. 6 : % inhibition in different concentration

Thus it is clear from graph that alcohol extract of the undergraound parts i.e leaf of this Plant exhibited best antioxidant activity than the other extracts when compare to standard durg ascorbic acid also. It showed 68.86% inhibition at max 30 µg/ml concentration. IC 50 value was obtained as approx 23-24 µg/ml.

Acknowledgements: The authors are thankful to the Management and Staff members of Shri Guru Ram Rai institute of technology & science, Patel nagar, Dehradun for their support and Mahant Shri Devendra Das Ji Maharaj, Chairman, SGRR Education Mission, Dehradun (Uttarakhand) for providing me necessary facilities in the college

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Source of Support: Nil, Conflict of Interest: None