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**Development, Formulation and Investigation of preliminary toxicity studies on Brine shrimp and to detect anti microbial activity by Ethanolic leaf extract of *Lantana camara***

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**Abstract**

Besides being an ornamental plant *Lantana camara* has proven several medicinal uses for different ailments to cure diseases from ancient times. Ethanolic leaf extract of *Lantana camara* was converted to tablets and compared with obtained raw extract of it. Phytochemical analysis showed the presence of tannins, saponins, glycosides and alkaloids etc. Physicochemical properties of extracts and tablets for clarity, texture, pH, color, taste, odor etc showed distinguish results. Antimicrobial activity of extract and tablets were effect in inhibiting staphylococcus and cytotoxicity test was performed on brine shrimp by extract and tablet were effective and positive potency detected by 50% mortality on naupliis.

**Key words:** Formulation, Brine, *Lantana camara*

**Introduction**

*Lantana camara* from the Latin lento means to bend, probably derived from the ancient Latin name of the genus viburnum, which it shows a little foliage and cluster of small flowers called as inflorescence. There are more than 150 species having numerous uses and application in folk medicines due to its antispasmodic, emetic, antifungal and antitumor activities. It also used against bronco pulmonary diseases.

**Characteristics**

Scientific Name : *Lantana camara*

Synonym : Camara vulgaris , Lantana scabrida

Common name; Sleeper weed, lantana wild sage

Division: magnoliopsidia

Class: lamiales

Family: Verbenaceae

Habitat: lantana generally grows best in open un shaded conditions such as waste lands and open edges of rain forests. Road sides, railway tracks and canal banks are favored. it grows to 2-4 meter in height. The leaf is ovate or ovate oblong, 2-10 cm long and 2-6cm wide. Leaves are bright green, rough, finely hairy pungent odor when crushed. Flowering occurs between August-March. The fruit is a greenish blue-black color. Mature plants produce up to 12,000 seeds annually.

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**Material and Methods**

**Plant material**

Leaves of *Lantana camara* were collected from Botanical garden and authenticated by plant botanist Dr. Madhav Chetty, Sri Venkateswara University, Andhra Pradesh, with voucher no: SVU/2017/1992.

Extraction of *Lantana Camara* Leaves

In research cell, *Lantana camara* leaves were thoroughly washed with fresh water and cleaned if any rodents or any gritty particles or any debris are present. Leaves were shredded under sun for natural dry. Dried leaves were grinded to fine powder and ethanol was added. Extraction carried for 5days i.e. 1000gm of fine powder was added to ethanol (1000ml). Filtered and filtrate was lyophilized to get marc. Marc was in semisolid to remove waxy substance further treated with pethroleum ether (40-80%). 100gm of approximate powder obtained.

**Pre formulation Studies**

Preliminary phytochemical Investigation

Test for Alkaloids

Each of the samples was extracted/ tablet with 5ml of ammonical alcohol, it was filtered and the filtrate evaporated to dryness. The residue as extracted with 1% sulphuric acid, it was filtered and the filtrate rendered alkaline with dilute ammonia solution. It was shaken with 10ml of chloroform in a separating funnel and the chloroformic extract separated. One

drop of Mayer's reagent was added to the extract and the result was observed.

#### Test for Tannins

A weight of 0.5gm of each extract/ tablet was boiled with 25ml of water for 5minutes in a 50ml baker. It was cooled; filtrated and the volume adjusted to 25ml. 1% lead acetate solution added. Color precipitated was observed.

#### Test for Glycosides

A weigh of 20mg of extract/ tablet was put into 20 ml test tube ,extract 5ml of dilute to HCL on the water bath for 2min. it was filtered and the filtrate rendered distinctly alkaline by adding , 5 drops of 20% and filtered , to filtrate 1ml of Fehling's A and B were added, heated on the water bath for 2minutes. The colour precipitate was observed.

#### Test for Saponins

A quantity of 200mg of the extract/ tablet was shaken vigorously with 10ml of distilled water and allowed to stand for about 2minutes. The extract was then filtered and the filtrate shaken vigorously.

#### Test for Flavonoids

An aqueous extract/ tablet each of the samples were prepared by maceration and filtered. A strip of filter paper was dipped in the liquid extract and dried. The paper was exposed to ammonia solution and the solution was observed for color changes.

#### Determination of Physical Characteristics of Ethanolic leaf extract of Lantana camara

Physical properties is any property that is measurable and whose value describes a state of physical systems. The changes in the physical properties of a system can be used to describe its Transformation.

Physicochemical characterization of an extract include color, taste, odour, clarity pH, extract valve, moisture content, solubility and ash value.

#### Colour, Taste and Odour

These are physical characteristics of herbal extracts/ tablet which are easily identified by simple methods as visualization, touch, smell or taste. Color is related to the part of the plant from which the extract originated. Odour comes from the essential oil depending on their components of material.

#### pH

The term pH refers to the relative amounts of hydrogen in the given chemical environment. It is a measure of the acidity or alkalinity of a given formulation or product. The pH is important in ethanolic extract/ tablet formulation, especially since it involves drug solubility, activity, absorption, stability, absorption and patience comfort.

#### Ash value

They constitute the inorganic residues obtained after completing combustion of a extract/ tablet. It includes soluble ash, acid soluble ash etc. They are criteria to judge the identity and purity of a crude drug.

#### Moisture content

Checking moisture helps reduce errors in the estimation of the actual weight of crude extract/ tablet material.

#### Extractive valve

These are indicative weights of the extractive chemical constituents of crude extract/tablet under different solution systems.

#### Flow properties of powder

Flow properties of powder are characteristics of powder that are dependent on particle size distribution, particle shape, chemical composition of the particles, moisture and temperature of storage of the powder. Several methods have been developed to assess the flow properties of powder these include angle of repose, bulk density, tapped density and Carr's index.

#### Angle of repose

The angle of repose or the critical angle of repose of a powder or granular substance is the steepest angle of descent or dip of the slope relative to the horizontal plane. When material on the slope face is on the verge of sliding. As a general guide, powders with angle of repose greater than 50° have satisfactory flow properties whereas angles close to 25° correspond to very good flow.

#### Bulk and Tap density measurement

The bulk density of a powder is the ratio of the mass of the untapped powder sample and its volume including the contribution of the inter particulate void volume. A given mass of powder in a measuring cylinder will have an initial volume  $V_0$ . After tapping for some specific amount of time, it attains a final volume  $V_f$ . The change in volume occurring when void space dimensions is known as "packing down". Hausner showed that powder with low interparticulate friction had ratios of approximately 1.2, where as more cohesive less free flowing once had ratios greater than 1.6.

#### Carr's index

Carr's developed another method of measuring powder flow from bulk density measurements called the Carr's index. Carr's index is also known as percentage compressibility and is calculated as

$$\frac{D_F - d_g}{D_F} \times 100$$

**Formulation of Ethanolic leaf extract of *Lantana camara***

Tablet extract of *Lantana camara* was made by raw ethanolic extract leaf of *Lantana camara* was sieved under sieve no: 180. Collected powder separated without adding any adjuvant as such extract pressed in manually punching tablet. Further, preserved in desiccators to carry out parameters.

**Post formulation**

Test for Antimicrobial Activity of Ethanolic extract of *Lantana camara*.

**Agar Diffusion method**

In *-in vitro* antimicrobial activity of the extracts was evaluated using the agar well diffusion technique. Nutrient agar was used as the medium. The test bacteria used is *Staphylococcus aureus*, agar medium was wetted and added to test tube and stabilized at 45°C for 15min in a reciprocal water bath shaker. The stabilized agar was seeded with 0.1ml of a 24hours broth culture of the test organism. The seeded agar was rolled palms to allow mixing of the organisms and then powder aseptically into sterilized petridishes transferred and allow to set room temperature. Wells were bored using a sterile borer size 5mm with internal diameter of 10mm external and labeled. Each

well was filled with the different concentrations of the ethanolic extract of powder and tablets. The concentrations maintained for both is same 105w/v, 5% w/v, 2.5% w/v, 1.25% w/v. Plates were allowed to stand for one hour on the bench to enable diffusion of the extract, in to the agar in room temperature. The plates were incubated over night (24hours) at 37°C and the Zones of the inhibition were measured and recorded.

**Brine shrimp Lethality Test**

Bioactivity of the *Lantana camara* ethanol leaf extract was measured by the preliminary toxicity test.

**Procedure**

Brine shrimp eggs were hatched in artificial seawater which was prepared by dissolving 38gm of NaCl in 1litre of distilled water. After 28-36hours incubation at room temperature (22-27°C) under light source, the larvae (nauplii) were separated from shells. Later nauplii's were separated and transferred in test tubes 20 each and different concentration of 0.2%, 0.4%, 0.6%, 0.8% with respect to time 3hr, 6hr, 9hr and 12hrs. The number of live and dead nauplii's was counted and % mortality was calculated.

**Results****Table 1: Phytochemical Screening of Ethanolic leaf extract and tablet of *Lantana camara***

Bioactive compounds	Raw powdered extract	Ethanolic extract of leaf <i>Lantana camara</i>	Ethanolic extract of leaf <i>Lantana camara</i> Tablet
Glycosides	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Flavanoids	+	+	+
Alkaloids	+	-	-

**Table 2: Phytochemical Properties of Ethanolic leaf extract and tablet of *Lantana camara***

Physical properties	Characteristics of ethanolic leaf extract	Characteristics of ethanolic leaf extract tablet
Colour	Greenish brown	Greenish brown
Odour	sharp	Sharp
Taste	bitter	Bitter
pH	3.68	3.68
Texture	powdery	Flat and spherical tablet
Clarity	No particles	Stuffed mass

Table 3: Anti microbial activity of ethanolic leaf extract and tablet of *Lantana camara*

Mean Zone of inhibition (mm) $\pm$ S.D against <i>Staphylococcus aureus</i>		
Concentration ( w/v)	Ethanolic extract (A/B)	Ethanolic extract tablet(C/D)
1.25%	26.67 $\pm$ 0.58	26.53 $\pm$ 0.58
2.5%	21.67 $\pm$ 0.58	22.12 $\pm$ 0.55
5%	16.33 $\pm$ 0.58	17.33 $\pm$ 0.58
10%	0	0

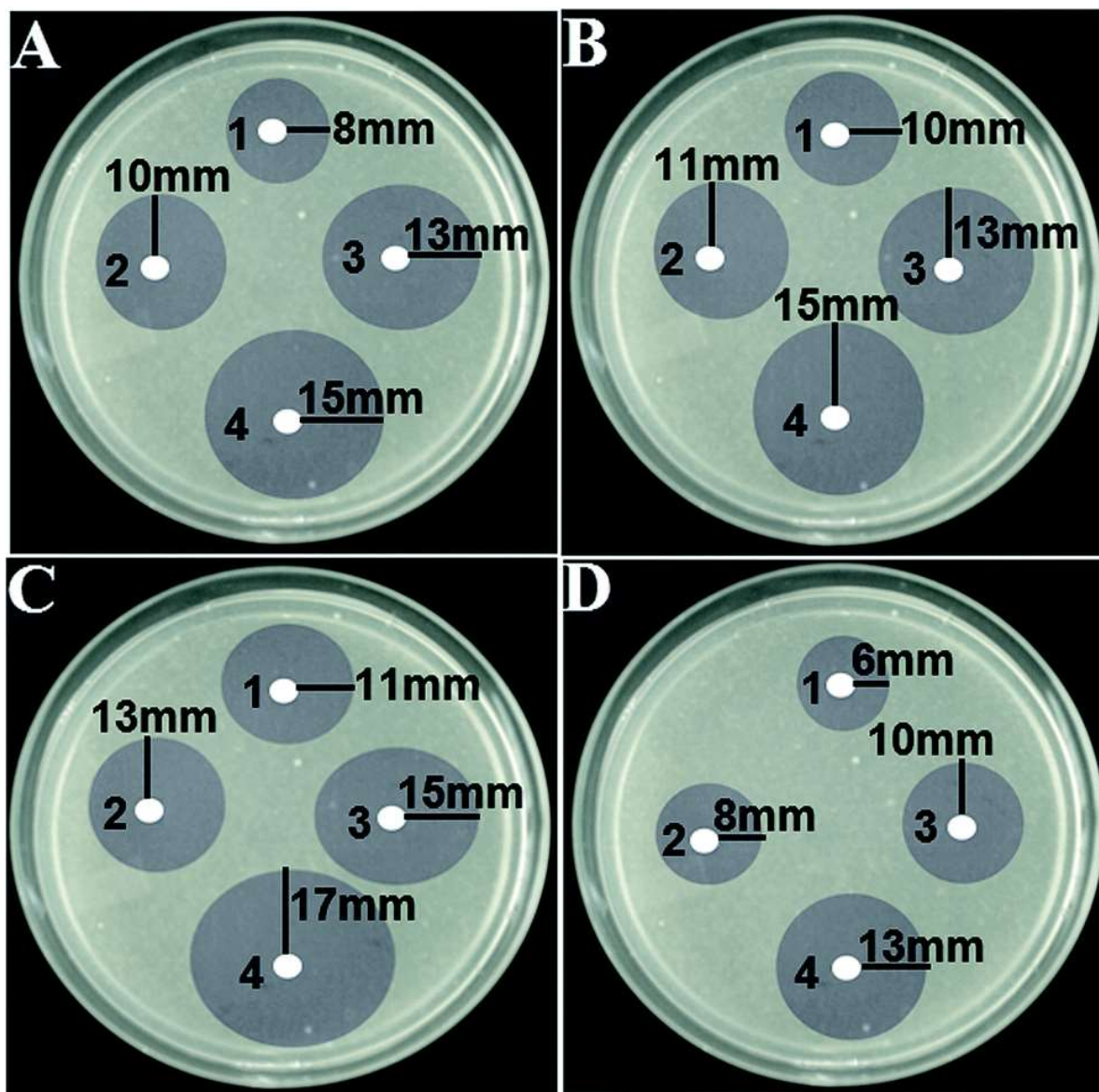


Fig. 1: Anti microbial activity of ethanolic leaf extract and tablet of *Lantana camara*



Table 4. Brine shrimp Lethality Test of Ethanolic extracts and tablet of *Lantana camara*

Sl No	Time(hrs)	Concentration	No of nauplii's	No of surviving nauplii		Total no of surviving nauplii's		% Mortality
1	0	0	10	Extract of <i>Lantana camara</i>		Extract tablet of <i>Lantana camara</i>		
2	3	0.2%	10	10	10	10	10	10%
3	6	0.4%	10	10	10	10	10	10%
4	9	0.6%	10	10	10	10	10	10%
5	12	0.8%	10	10	10	10	10	10%

### Conclusion

Extraction of *Lantana camara* leaf extract gave a higher percentage of yields. It contains phytochemical like tannins, glycoside, saponins etc. Ethanolic extract and formulated tablet of *Lantana camara* yields good powder and perfect texture with flat, spherical surfaces. Later extract and tablet were experimented against toxicity studies on brine shrimp which prove to be cytotoxic in nature as detected to be active to 50% mortality on nauplii's and also experimented on *Staphylococcus aureus* for antimicrobial activity which proved to be inhibiting maximum area covering towards petridish.

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