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Larvicidal Activity of *Leucas Aspera* Against the Larvae of *Anopheles, Aedes Aegypti and Culex Quinquefasciatus*

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Abstract: ***Insect-transmitted diseases cause high morbidity however they additionally embody deadly diseases that cause high mortality rates among infected individuals.Many two-winged insects species such as Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus act as vectors. Ae. aegypti, acts as a vector for yellow fever, dengue and chikungunya. Aedes aegypti is cosmopolitan within the tropical and semitropical zones. Anopheles stephensi is the primary vector of malaria in India and other west Asian countries. C. quinquefasciatus is a vector of lymphatic filariasis which is one of the widely distributed tropical diseases with around 120 million people infected worldwide. The mosquitocidal activity was analyzed by aqueous, ethanol and methanol extract of Leucas aspera plant extract against the malaria, yellow fever and dengue causing Ae.aegypti, An.stephensi. Leucas aspera is well known to possess various pharmacological activities like antifungal, antimicrobial and cytotoxic activities. Leaves of this plant are applied to treat snake bites. The result was supported by probit analyses was carried out for twenty fourth and forty eighth hour methyl alcohol extracts of L. aspera. The data showed profound larvicidal activity with methyl alcohol extract compared with the other extracts.***

Keywords: Vector control, L. aspera, Ae.a.egypti, An. stephensi, Culex quinquefasciatus.

I. INTRODUCTION

Naturally plants have pharmacological activity which can be exploited in medicine. *Leucas aspera* is a plant within the family *Lamiaceae*. Although the species has different common names depending on the region in which it is located. In India, it is commonly known as Thumbai or Thumba. **(S. Sivapriya jothi *et al*., 2014).**

A. Taxonomical Classification

Kingdom : Plantae
Class. :Dicotyledonae
Order :Tubiflorae
Family :Lamiaceae
Genus. **:** Leucas
Species. : Aspera

B. Botanical Description

1) *Leaves:* Leaves are sub sessile, linear, obtuse up to 8.0cm long and 1.25cm broad.
2) *Flowers:* White, sessile, small, axillary whorls.
3) *Calyx:* Calyx variable, tubular, 8-13mm long, the lower half usually glabrous and membranous, the upper half ribbed and hispid, mouth small, not villous.
4) *Corolla:* 1cm long, tube 5mm long, upper lip 3mm long, lower lip about twice as long.
5) *Fruit:* 2.5mm long, brown, smooth, inner face angular and outer face rounded.

Leucas aspera is mostly found in dry and open sandy soil and is abundant in areas with waste. It is a herb that is used in food to impart fragrance. *Leucas aspera* is reported to possess antifungal, prostaglandin inhibitory, antioxidant, antimicrobial and cytotoxic activities. It is used traditionally to treat snake bites in Philippines. (**Manickam Pavunraj *et al*.,2017).**

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Leucas aspera is also believed to posses antipyretic activity. It is naturally an herbal plant that helps to reduce fever. The juice of the flowers can also be used to treat intestinal worm infections in children (**Devan Elumalai *et al* 2016).** Plant extract mixed along with honey helps to treat stomach pain and indigestion. For children the flower with honey helps to cure coughs and cold. In rural areas the leaves are used as an insecticide and mosquito repellent. The juice from its leaves is used as an external utility for painful swellings. It is also used to treat Gyaenocological and obstetrical problem (**MP Singh and GS Gindha 2017).**

Mosquitoes cause vector borne disease that affects human beings and animals. In India the mosquitoes cause several diseases for eg. Malaria, dengue, chikungunya, filariasis and leishmaniasis (**S. Sivapriyajothi *et al.*, 2014)**. In India between 2000 and 2019, the malarial cases were 71.8% and 73.9% of deaths were recorded. *Anopheles stephensi* is a primary vector which causes malaria in India.

Larvae of the Anopheles species are nocturnal, crepuscular in nature. *Aedes aegypti* mosquito spreads dengue fever, chikungunya and yellow fever. These mosquitoes transmit several tropical fevers (**Devan Elumalai *et al* 2016).** Secondary plant metabolites of Saponins are steroidal in nature and that is used as an Antilarval activity. The aim of the present study is to evaluate the Larvicidal activity of the *Leucas aspera* plant with the help of methanol and ethanol extract.



FIGURE:1 Image of *Leucas aspera*

II. PLANT EXTRACTS PREPARATION

The dried plant was small-grained and sieved to urge fine powder using an electrical blender. 70g of the plant powder was crammed within the thimble and extracted in turn with Aqueous, ethanol and methanol for 10h. All the extracts were subjected to rotary flash evaporator and preserved at 5°C in air tight bottle till further use. In this the Methanol extract showed good larvicidal activity (D.Elumalai *et al* 2015).

The bulked up leaves had been air dried at room temperature. One kilogram of powder was extracted with 3L of hexane (1:3 w/v) for seventy two hours. The extract is filtered by using a Buchner funnel along with Whatmann filter paper and the residue was dried. The filtrate became evaporated to dryness below decreased pressure (500 mm Hg) the usage of rotary vacuum evaporator at 40ºC. Hexane (eight g), DCM (12 g), acetone (10 g) and aqueous (eleven g) were the yield which was obtained from different extracts of *Leucas aspera*. The crude extracts had been saved at 4ºC for further use. The DCM extract exhibited good larval mortality against *Cu. quinquefasciatus* compared to other extracts (Manickam Pavunraj *et al* 2017).

Leucas aspera extract was prepared as indicated in previous research with slight modifications (Pankaj Tandon, 2010). The pulverized moisture free sample (20.0g) turned into continuously extracted with distilled water and chloroform for numerous times. The extract was filtered using Whattmann No 1 filter paper and condensed in rotary evaporator at 40rpm and stored under optimum conditions. This crude extracts of aqueous and chloroform was used to determine the larvicidal activity. In this the chloroform extract of *Leucas aspera*, leaf exhibited highest mortality rate at very low concentration than the aqueous leaf extract of *Leucas aspera* (R. Ramanibai *et al* 2011).

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The leaves of the *Leucas aspera, Vitex negundo and Eucalyptus* plant material had been shade dried for around 15 days (28±2ºC). The completely dried leaves was then been ground and sieved to get fine powder from which the extracts had been prepared. The dried leaves were converted to fine powder by crushing it in a mixer grinder. Each plant leaf powder was taken separately. To each leaf powder (500gm), 1.5 litre of petroleum ether, ethyl acetate, ethanol and water solvent was added separately. It was then left for round 24 hours with periodic shaking after being filtered. This procedure was repeated with fresh solvent until it became clear. Each solvent had been filtered with the help of whattman filter paper. The finally obtained solvents had been collected in a separate container. Finally different solvent from each plant leaves have been obtained. Totally 12 different solutions had been obtained. Each solvent solution was kept for evaporation in Soxhlet's apparatus extraction mantle at a temperature of around 60-70°C. At regular intervals it was cooled and heated within the extraction mantle. Finally crude extracts of each plant solution was obtained. In the four different extracts of 3 different plants, *Vitex negundo* exhibited good larvicidal activity when compared to other plants (V.Karthikekeyan *et al* 2012).

The entire plant *L. aspera* was washed completely with tap water and shade dried at room temperature. An electric blender powdered the dried plant materials (whole plant). From the powder, 100 g of the plant substances was extracted with 300 ml of organic solvents of ethanol for 8 h with the help of a Soxhlet apparatus (Vogel 1978). The extracts had been filtered through a Buchner funnel with Whatman number one filter paper. The crude plant extracts had been evaporated to dryness in rotary vacuum evaporator. One gram of the plant residue was then dissolved in hundred ml of methanol (stock solution) and considered as 1% stock solution. From this stock solution, different concentrations had been prepared starting from 6 to 14 %. In this the ethanolic extract of *Leucas aspera* provided an excellent potential for controlling the malarial vector, *A. stephensi* when compared to stock methanolic solution (Kalimuthu Kovendan *et al* 2012).

III. MOSQUITO CULTURE

All tests executed the usage of laboratory reared vector mosquitoes viz., *Ae. aegypti, An. stephensi and Cx. quinquefasciatus* were free of exposure to insecticides and pathogens.Cyclic generations of vector mosquitoes had been maintained at 25–29°C insectariums. Larvae was fed by larval food which contained powdered dog biscuit and yeast in the ratio 3:1 and adult mosquitoes on 10% glucose solution (D.Elumalai *et al* 2015).

Culex quinquefasciatus larvae have been collected from close to stagnant and drainage water. Larvae have been colonized and maintained within the laboratory at 27±2º C and 75- 85% relative humidity. The larvae were been fed with dog biscuits. Once it attains pupal level they have been transferred and maintained in a mosquito net cage (18×18.5 cm) for adult emergence. Adults were been maintained within the same cage and continuously supplied with 10% sucrose solution to prevent from microbial infection. It was periodically blood fed on restrained rats. Glass petridish with 50 ml of tap water lined with filter paper was kept inside cage for oviposition. Eggs have been collected from the filter lined in petridish and transferred to enamel trays. They have been maintained under the same condition in laboratory and used for larvicidal bioassay(R.Ramanibai *et al* 2011).

The important vector species of mosquitoes such as *Ae.aegypti, An. stephensi, and C. quinquefasciatus* were selected and identified within the Zonal Entomological Research Centre, Vellore, Tamil Nadu, India. *An. stephensi* acts as a vector of malaria in India and larvae of these species are usually found in distinctly different habitat. *Ae. aegypti* is a vector for transmitting numerous tropical fevers such as dengue fever, chikungunya, yellow fever and different diseases. *C. quinquefasciatus* acts as a vector for filariasis in India (Deven Elumalai *et al* 2018).

IV. LARVICIDAL BIOASSAY

Elumalai D *et al.*,2015 evaluated the larvicidal activity of whole *Leucas aspera* plant against *Ae. Aegypti, An. Stephensi and Cu. quinquefasciatus* using different solvent aqueous, ethanol, methanol, chloroform and petroleum ether. Methanol extract of *Leucas aspera* found to be more potent and showed 100% mortality at 80ppm. Other extracts showed 100% mortality against 3 mosquito species at 100ppm and 120 ppm. Methanol extract of Leucas aspera was found to be potent Larvicidal agent when compared to the other extracts .

R Ramanibai *et al* 2011 evaluated the larvicidal activity of aqueous and chloroform leaf extract of *Leucas aspera*(willd) against mosquito larvae *Culex quinquefasciatus*. The aqueous extract of *Leucas aspera* exhibited 76%(1^{st} instar),64%(2^{nd} instar),61.33%(3^{rd} instar) and 52% (4^{th} instar) of mortality at 3% concentration of the extract. Even at low concentration, the chloroform extract of *Leucas aspera* leaf displayed higher mortality rate than the aqueous *Leucas aspera* leaf extract. The highest dose of 5% concentration of chloroform extract *Leucas aspera* exhibited 100% mortality to all the four instars of *Culex quinquefasciatus.*

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Manickam Pavunraj *et al* 2017 evaluated *Leucas aspera* leaves using different solvent extract of hexane, aqueous, DCM and acetone which were screened for their antibacterial, antifungal and larvicidal activities against selected bacterial, fungal strains and mosquito larvae of *Culex quinquefasciatus* respectively. DCM extract exhibited a broad spectrum of antibacterial activity against *B. subtilis* (17.1 ± 2.04 mm), *S. aureus* (23.4 ± 2.90 mm), *E. coli* (20.3 ± 1.56 mm), *P. aeruginosa* (16.5 ± 1.05 mm), *P. vulgaris* (16.1 ± 2.56 mm) and *K. pneumonia* (15.1 ± 3.66 mm) at 10 mg/disc. Acetone extract inhibited the growth of *B. subtilis* (15.3 ± 1.85 mm), *S. aureus* (21.8 ± 2.61 mm), *E. coli* (17.5 ± 1.25 mm), *P. aeruginosa* (14.0 ± 0.57 mm), *P. vulgaris* (13.4 ± 0.50 mm) and *K. pneumonia* (14.3 ± 0.68 mm) at the concentration of 10 mg/disc. The activity of DCM extract of *L. aspera* (Willd.) of leaves were found to be more profound than the hexane, acetone and aqueous extracts against all the tested microorganisms. In comparison, the aqueous extract showed less profound antibacterial activity at 1 mg/disc. Among the extracts tested, DCM extract of *L. aspera* (Willd.) leaves were found to be most potent and exhibited the maximum zone of inhibitions against *T. viride* (29.2 ± 2.00 mm), *C. albicans* (24.4 ± 0.80 mm), *A. flavus* (22.8 ± 0.36 mm), *E. floccosum* (19.5 ± 2.17 mm) at 10 mg/disc than the other extracts . The acetone extract was found to be moderately effective against the four tested fungal pathogens. In addition, the hexane and aqueous extract at a concentration of 10 mg/disc were relatively found to be less effective. The DCM extract of *L. aspera* (Willd.) exhibited 100% larval mortality at 1000 ppm against *C. quinquefaciatus* followed by acetone (74.0%) and hexane (65.18%) extracts. On the contrary, the aqueous extract at 1000 ppm was found to be less effective versus 4th instar larvae of *C. quinquefasciatus.*

V. DOSE RESPONSE

From the stock solution, different concentrations (1.0%, 3.0% and 5.0%) have been prepared. Based on the screening results, aqueous and chloroform solvent extract of *Leucas aspera* leaves have been subjected to dose response bioassay for larvicidal activity against the larvae of *Culex quinquefasciatus.* The number of dead larvae was counted after forty eight hrs of exposure and the percentage mortality was changed which was reported from the average of 3 replicates(R.Ramanibai *et al* 2011).

The larvae in every solution had been then left for twenty-four hrs and forty eight hrs, the number of dead larvae was counted after 24 hrs and 48 hrs of exposure, and the percentage mortality was mentioned from the average of 5 replicates. Mortality was then recorded when control mortality ranged from 5% to 20%, and it was corrected by Abbott's (1925) formula. Based at the percentage mortality values, LC50 and LC 90 values of plant extract of *L. aspera* against *An. stephensi, Ae. aegypti, and Cx. quinquefasciatus* had been recorded by calculating the regression line using probit analysis of Finney (1971) as defined by Busvin (1971) (D.Elumalai *et al* 2015).

VI. STATISTICAL ANALYSIS

The evaluation program probit (Finney, 1971) was used for the determination of LC 50 and LC 90. The lethal concentrations (µg/ml) for 50% and 90% of the mortality i.e LC 50 and LC 90 had been noted at 48 hrs after treatment. Mortality was corrected using Abott's (1925) formula. All data are mean ± SE of three replicates. Statistical evaluation turned into finished using SPSS version 10 (R. Ramanibai *et al* 2011).

The common larval mortality data had been subjected to probit analysis for calculating LC50, LC90 and other statistics at 95% fiducial limits of upper confidence restriction and lower confidence restriction and chi-square values had been calculated with the help of the SPSS 11.5 (Statistical Package of Social Sciences) software. Results with $P < 0.05$ were taken into consideration to be statistically significant (D.Elumalai *et al* 2018).

The data associated with zone of inhibition activity was analysed using one-way ANOVA. Significant differences among treatments had been determined using Tukey's HST multiple range tests ($P \leq 0.05$) (Manickam Pavunraj *et al* 2017).

VII. RESULT AND DISSCUSSION

Kovendan et al result interpreted that after 24 hours of treatment, the crude extract of the plant displayed larvicidal and pupicidal activities. The mortality of all larval instars and pupae is moderate (Kalimuthu kovendan *et al* 2012). In contrary to the aqueous leaf extract of *Leucas aspera*, Ramanibai et al found that the chloroform extract of *Leucas aspera* had good larvicidal activity even at low concentrations(Ramanibai .R *et al* 2011) .According to Karthikeyan's studies, the four different solvent extracts of Vitex negundo showed good larvicidal activity. The greatest mortality percentage of mosquito larvae was measured in a 1% concentration of crude extracts. In the control group, there was no mortality. ANOVA for the death percentage of mosquito larvae in varying concentrations and solvents reveals significant differences at the 5% level (Karthikeyan .V *et al* 2012).

Human populations in many regions of the world have long employed plant products to combat vectors and insect infestations . The use of these plant derivatives in mosquito control as an alternative to synthetic insecticides could lessen environmental risks while

also lowering production costs . Many researchers have pointed out that phytochemicals produced from plants can operate as larvicides, insect development regulators, repellents, ovipositional attractants, and deterrents.(Manickam Pavunraj *et al* 2017)

Mosquito larvae management with larvicidal chemicals is an important part of vector disease control. Plants as possible Larvicidal are seen as a realistic and preferable option for controlling mosquito species at the community level. Some phytochemicals derived from plants operate as general toxicants against adult and larval mosquitoes, while others act as growth inhibitors, chemosterilants, repellants, or attractants. Although several plant extracts have been claimed to exhibit mosquitocidal or repellant properties against mosquito vectors, only a few plant products have proven useful in mosquito control.

Many researchers have noticed that phytochemicals derived from plant sources operate as larvicides, insect development regulators, repellents, and ovipositor attractants, among other things. Triterpenoids are thought to have larvicidal properties against mosquitos . The presence of terpenoids, triterpenoids, and alkaloids in *L. aspera* could explain its potent larvicidal activity. Due to their larvicidal toxicity, natural products such as extracts from portions of plants with insecticidal and therapeutic qualities have better efficiency in reducing mosquito menace. Against *An. Stephensi, Aedes aegypti, and Cx. Quinquefasciatus,* crude leaf extracts of *L. aspera* showed effective larvicidal properties (Elumalai .D *et al* 2015).

VIII. CONCLUSION

This plant, *Leucas aspera,* can be used to develop a new biocontrol agent that can successfully deal with the obstacles or threats of mosquito larvae. The planned investigation will serve as a baseline. However, more research is needed to isolate the active ingredients responsible for this activity and to determine the precise mechanisms of action.

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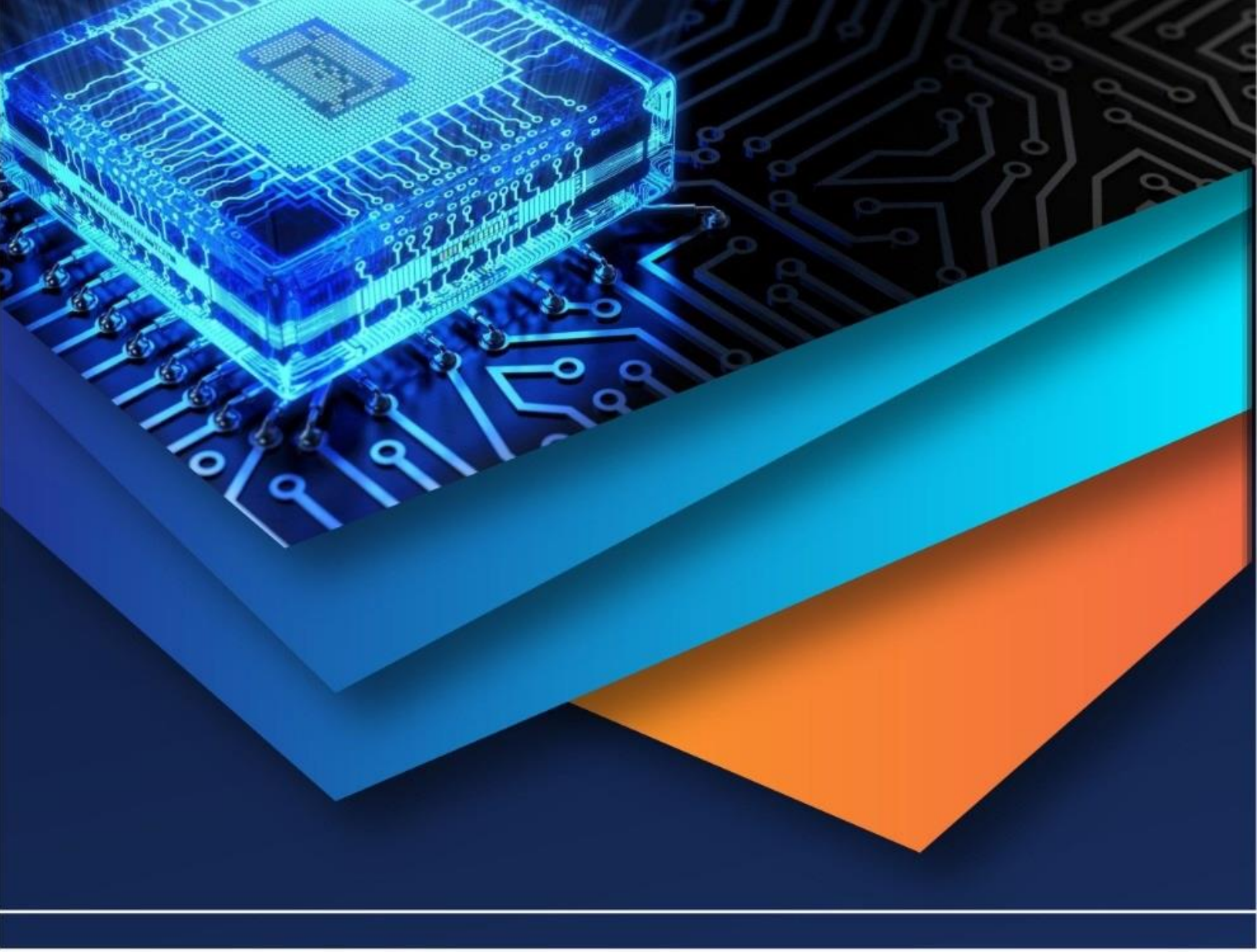
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