

Formulation and Development of herbal Gel Containing Cannabis Sativa and Azadirachta Indica Leaves Extract for Anti-Inflammatory and Anti-Bacterial Activity

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Abstract:

Bacteria are everywhere and play a crucial part in the maintenance of germs that cause illness and disease. Skin infections are anticipated to occur at a rate of 24.6 per 1000 people yearly. The treatment of any skin infections primarily consists of any necessary surgical debridement; nevertheless, injecting an antibiotic directly into a wound has several potential benefits. Topical antibiotic treatment and wound care are recommended. The administration of a therapy may allow treatment with medicines that are not yet available (or safe) for systemic therapy. Gels are semi-solid systems having qualities intermediate between solids and liquids, in which the movement of the dispersing medium is constrained by an interlacing three-dimensional network of particles or solvated macromolecules of the dispersed phase. This study aimed to explore the potential of Cannabis sativa, Azadirachta indica leaves extract as anti-inflammatory and antibacterial agents. The formulation has been developed by solvent casting method. The experiment was conducted using various techniques to measure the effectiveness of each substance individually and in combination. Results showed that Cannabis sativa and Azadirachta indica leaves extract both had strong anti-inflammatory properties, while glycerol demonstrated significant antibacterial activity. When combined, these substances exhibited enhanced anti-inflammatory and antibacterial effects, indicating potential for their use in pharmaceutical and medical applications. Overall, this study highlights the importance of exploring natural products for their therapeutic potential and provides insight into the potential of these substances as novel anti-inflammatory and antibacterial agents.

Keywords: Anti-inflammatory, Novel gel, Cannabis sativa, Azadirachta indica extract, Anti-bacterial agent.

Introduction:

Herbal remedies have been used for centuries to cure various ailments and their popularity has only increased in recent years. In this, we will discuss the formulation and development of herbal gel containing cannabis sativa and Azadirachta indica leaves extract that exhibit anti-inflammatory and antibacterial activities. Inflammation and bacterial infections are common health problems that affect people world-wide. The use of natural extracts as an alternative to conventional medication has gained popularity in recent years due to their effectiveness and safety. The use of natural extracts as an

alternative to conventional medications has gained popularity in recent years due to their effectiveness and safety. Cannabis sativa and Azadirachta indica leaves are two plants that have been used for medicinal purposes for centuries. Cannabis sativa contains compounds known as cannabinoids that have anti-inflammatory and anti-bacterial properties. Azadirachta indica leaves are rich in phytochemicals such as- azadirachtin, nimbin and nimbidin; which have anti-inflammatory and anti-bacterial properties. Glycerol, a natural compound, is commonly used in cosmetics and pharmaceuticals due to its moisturizing properties. Inflammation is a biological response to injury or infection and is important in the body's defence mechanism. Anti-inflammatory drugs such as 'Non-Steroidal Anti-inflammatory Drugs (NSAIDs) and corticosteroids are commonly used to treat inflammation. In addition to inflammation, bacterial infections are also a major health concern. Therefore, there is a need for development of natural and safe anti-inflammatory agents. The topical formulation was prepared using cannabis sativa extract, Azadirachta indica leaves extract & glycerol in varying conditions. The anti-inflammatory activity was evaluated using a carrageenan-induced paw edema model in rats. The anti-bacterial activity was evaluated using the disk diffusion method against three bacterial strains: Escherichia Coli, Staphylococcus Aureus and Pseudomonas Aeruginosa.

Objective: Formulation and Development of herbal gel containing Cannabis Sativa and Azadirachta indica Leaves Extract for Anti-Inflammatory and Anti-Bacterial Activity

The plant material like Cannabis Sativa (Bhang), Family: Cannabaceae. It possesses various pharmacological activities such as Analgesic, Anti-Inflammatory, Neuro-Protective Effects, Anxiolytic, Antipsychotic, Antiemetic and Appetite stimulation. The chemical constituents Cannabidiol (CBD) and Cannflavin A and B that are present in Cannabis sativa shows anti-inflammatory pharmacological activity.

The plant Azadirachta Indica (Azadirachta indica), Family: Meliaceae. It possesses various pharmacological activities such as Analgesic, Anti-Fungal, Anti-Bacterial, Antiviral, Contraceptive and Anti-Hyperglycemic agents.

Glycerol, also known as glycerin, is a colorless, odorless, and sweet-tasting liquid that is commonly used as a solvent, lubricant, and humectant. It possesses various pharmacological activities such as Anti-Inflammatory, Antimicrobial, Wound Healing activity, Laxative and Moisturizing activity.

Materials and Methods:

Aqueous extract of Cannabis sativa, Azadirachta indica leaves in a concentration of 0.1gm, 0.3gm, 0.5gm respectively along with different polymers. To prepare 100gm of gel by adding a sufficient quantity of distilled water. Water required for these solutions was divided into two parts. In one part, the defined amount of extract was dissolved and to this calculated quantity of Polyethylene Glycol 400 and Ethanol were added. Carbolpol 934 was dissolved, and to the solution Benzalkonium Chloride was added. Both of the solutions were mixed in a beaker and triethanolamine was added dropwise to obtain the gel consistency.



(1)



(2)



(3)

i.e. 1. Aqueous extract of *Azadirachta indica* and *Cannabis Sativa* Leaves

2. Formulation of Gel base on Magnetic Stirrer with Hot Plate

3. Best Formulation (Formulation C - 0.5% extract of Active Ingredients)

Collection and identification of medicinal plants: Leaves and flowers of *Cannabis Sativa* and leaves of *Azadirachta indica* were collected and dried from local areas of Roorkee. Herbal species were identified and authenticated at College of Pharmacy Roorkee, Roorkee.

Chemicals: Carbopol 934, Glycerol, Dimethyl Sulphoxide, Triethanolamine, Methyl paraben, Benzalkonium Chloride were issued from the laboratory of College of Pharmacy Roorkee. Ethanol 96% was used in this research.

Extraction: Fresh *A.indica* and *C.sativa* were collected from local areas near campus, Roorkee and washed in distilled water till the surface dust is completely removed and dried under shade. The aqueous leaf extract was prepared as described earlier, briefly, 50 gm of *A.indica* powder and 50 gm of *C.sativa* powder was mixed with 500 ml of distilled water respectively and boiled for about 45 min. The boiled solution was filtered using Whatman No. 1 filter paper and clear aqueous leaf extract was obtained. The extract was stored at 4°C until further use.

Formulation of Gel: Formulation A, B, C were prepared which comprised ethanolic extract in a concentration of 0.1%, 0.3% and 0.5% respectively as shown below. Carbopol 934, Polyethylene Glycol

400, Ethanol, Dimethyl Sulphoxide, Triethanol Amine, Benzalkonium Chloride were used to prepare 100gm of the gel by adding a sufficient quantity of distilled water.

Ingredients	Qty. in (%) for gel base	Qty. in (%) Formulation A	Qty. in (%) Formulation B	Qty. in (%) Formulation C
Carbopol 934	1.0	1.0	1.0	1.0
Cannabis sativa Extract	—	0.1(gm)	0.3(gm)	0.5(gm)
Azadirachta indica Extract	—	0.1(gm)	0.3(gm)	0.5(gm)
Glycerol	—	2.0	4.0	6.0
Propylene Glycol 400	4.0	4.0	4.0	4.0
Ethanol	3.0	3.0	3.0	3.0
Dimethyl sulfoxide	0.3	0.3	0.3	0.3
TriethanolAmine	1.2	1.2	1.2	1.2
Methyl paraben	0.2	0.2	0.2	0.2
Benzalkonium Chloride	1.0	1.0	1.0	1.0
Water	Q.S. 100	Q.S. 100	Q.S. 100	Q.S. 100

Table 01: Ingredients used in the formulation

Preparation of gel formation: We used concentrated extract of *A.indica* and *C.sativa* for preparation of gel formulations. Carbopol 934, Glycerol were applied as gelling agents in formulations. Among 3 formulations, 1 formulation had better properties compared to others, so it was selected for further tests.

Carbopol 934: Methyl paraben was dissolved in purified water 50°C. 1.0 gm of Carbopol 934 was dispersed in purified water 40°C respectively by a mixer at 1200 rpm for 30 mins. Herbal extracts and essential oils were dispersed separately in Dimethyl sulfoxide and added to gel base and mixed well. The pH was then adjusted to pH 6 using triethanolamine and stirred slowly until a clear and transparent gel was obtained.

Characterization of physicochemical properties of Gel Formulations:

Macroscopic study: Formulations were checked within 48 hrs of preparation and macroscopic balance (the absence of palpable and follicular particles, color, and transparency).

Microscopic study: Formulations were checked in terms of uniformity, gel texture, and air bubble by

optical microscope with a magnification of 10 and 40 within 48 hrs.

Determination of Physical Appearance: All the gel formulated was tested for a physical appearance by visual inspection. They were tested for their appearance such as color, odor, consistency, homogeneity and greasiness with no lumps.

Determination of pH: Since the formulation was a topical formulation to be applied to the skin, therefore the pH of the gels was determined using a digital pH meter. 1 gm of the gel was stirred in 10 mL distilled water till a uniform solution is formed and the glass electrode completely into the gel solution to cover the electrode. The pH meter was prior standardized with standard buffers of pH 4 and pH 7.

Determination of Viscosity: Viscosities of gels were determined using Brookfield viscometer. Gels were tested for their rheological characteristics at 25°C using Brookfield viscometer. The measurement was made over the whole range of speed settings from 10 rpm to 100 rpm with 30 seconds between 2 successive speeds and then in descending order.

Determination of Spreadability: Spreadability denotes the extent of the area to which the gel readily spreads on application to the skin or the affected part. The bioavailability efficiency of a gel formulation also depends on its spreading value. The spreadability is expressed in terms of time in seconds taken by two slides slipping off from the gel, placed in between the slides, under a certain load. The less time is taken for separation of two slides, the better the spreadability. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation (about 1 g) was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 6 cm along with the slide. A 500-gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times both formulated gels and marketed gel and the meantime is taken for the calculation.

Formula: $S=M \times L/T$

S=Spreadability

M= Mass in gm (30gm)

L=Length of the glass (6cm)

T= Time in sec.

Determination of Drug Content: 1 g of the gel was taken in 10 ml of the volumetric flask containing 5 ml of Ethanol and diluted to 5 ml with the same solvent. From the above solution, 1ml was further diluted with 10 ml ethanol. The resultant solution was filtered through Whatman filter paper and absorbance of the solution was measured at 268 nm using UV visible spectroscopy.

Determination of Extrudability: The formulated gel was filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weight of filled tubes was recorded and the tubes were sandwiched between two glass slides and were clamped. 500 gm weight was placed over the slides and then the cap was removed to extrude. The amount of extruded gel was collected and weighed. Extrudability was determined by calculating the percentage of extruded gel.

When it is greater than 90% then extrudability is excellent.

When it is greater than 80% then extrudability is good.

When it is 70% then extrudability is fair.

Evaluation of physicochemical properties of Gel Formulations:

Homogeneity:

The visual inspection of all the prepared gel formulations was carried out and it was concluded that all the gel formulations showed good appearance and homogeneity.

Formulation	Homogeneity
F1	Good
F2	Good
F3	Good

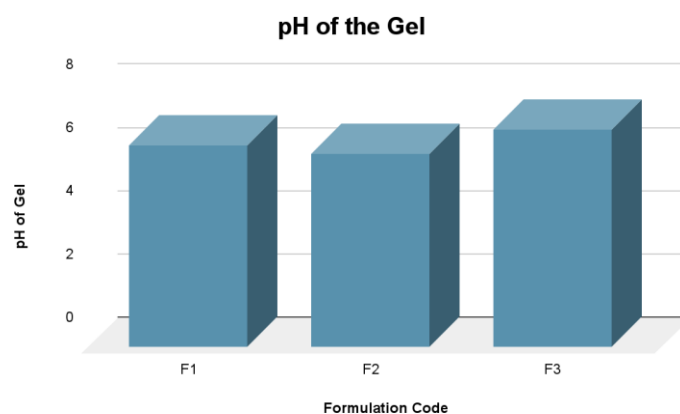
Table 02: Homogeneity of the formulation

pH Determination:

The pH value for the optimized gel (F1, F2, and F3) formulation was recorded 5.87 to 6.11 respectively. The pH of the gel was found to be within the range of pH of the skin and would not cause any irritation to the skin. Thus, prepared gel formulations are suitable for skin application.

S.No.	Formulation	pH of Gel
1.	F1	6.37
2.	F2	6.11
3.	F3	6.87

Table 03: pH determination of the formulation



Graphical Representation of the pH value of Polyherbal gel

Viscosity Measurement:

A Brookfield Viscometer was used to measure the viscosity of the gel. Viscosity reveals the rheological properties of all formulations. It should be optimized not too high or not too low according to topical applications.

Viscosity of Gel			
S.No.	Formulation	Spindle Speed(rpm)	cP
1.	F1	20	29128
2.	F2	20	30526
3.	F3	20	28525

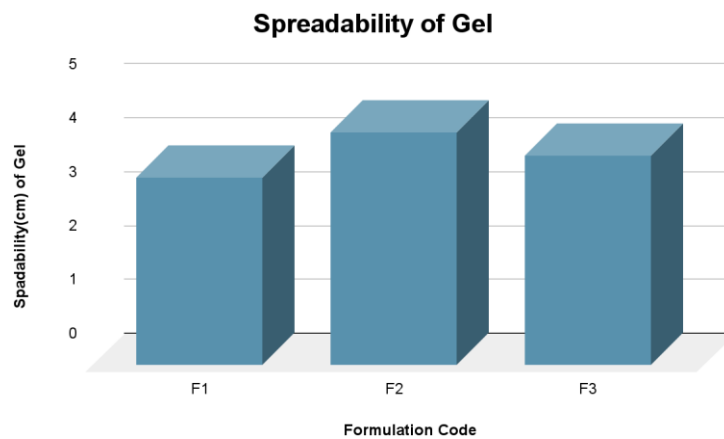
Table 04: Viscosity of the formulation

Spreadability of Gel:

The spreadability of Gel was measured based on 'Slip' and 'drag' characteristics of gels. Spreadability is an important property of topical formulation from a patient compliance point of view.

Spreadability of Gel		
S.No.	Formulation	Spreadability
1.	F1	3.48
2.	F2	4.32
3.	F3	3.90

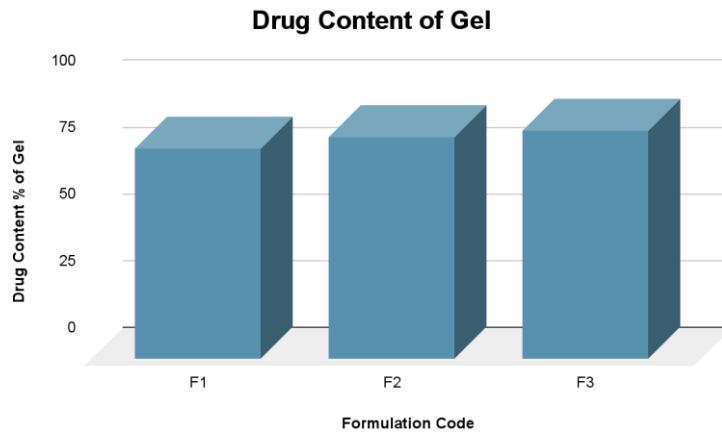
Table 05: Spreadability of the formulation



Graphical Representation of Spreadability of Polyherbal gel

Drug content of Gel:

Drug content is the drug concentration of Gel, which was measured by a UV spectrophotometer. The drug content percentage of the gel was found to be within the range of 78.92% to 85.34%.



Extrudability of the gel:

The extrusion of the polyherbal gel from the tube is important during its application and in-patient compliance. Polyherbal gel with high consistency may not extrude from the tube whereas the low viscous gel may flow quickly, and hence suitable consistency is required to extrude the polyherbal gel from the tube. The conventionally thickened polyherbal gel has good extrudability.

Extrudability of Gel		
S.No.	Formulation	Extrudability (g/cm ²)
1.	F1	9.3
2.	F2	8.9
3.	F3	10.3

Table 06: Extrudability of the formulation

The extrudability of the best formulation F3 was found to be 10.3g/cm². Thus, the prepared gel possesses optimum extrudability.

Antibacterial activity:

Name of organism and code	Zone of Inhibition (mm) Well diffusion method		
	F1	F2	F3
Pseudomonas aeruginosa (MTCC 424)	-ve	-ve	-ve
Staphylococcus aureus (MTCC 737)	+ve	+ve	+ve

Table 07: Antibacterial activity by Well diffusion method (-ve no result; +ve good antibacterial activity)

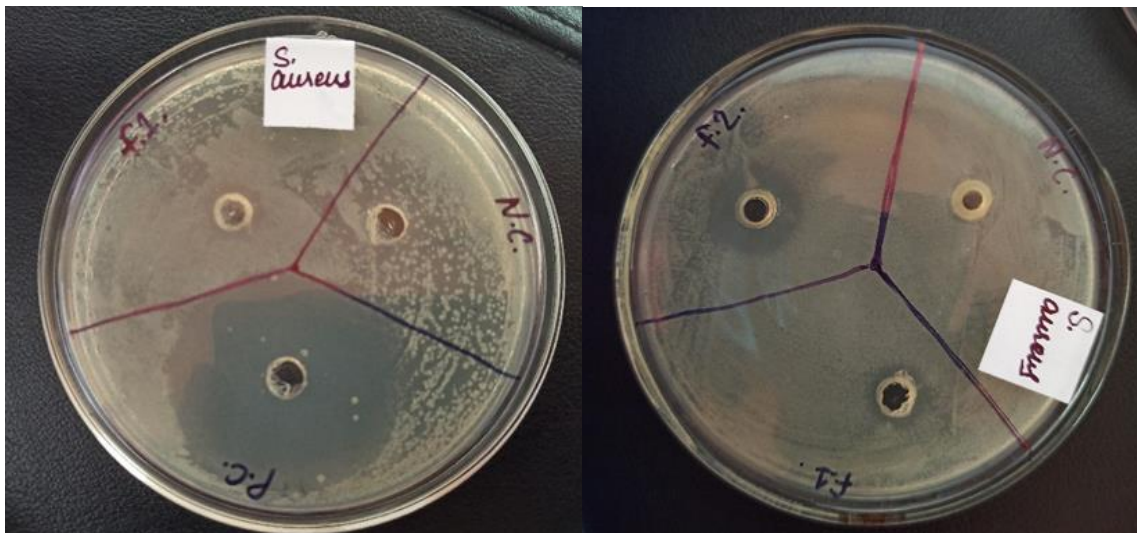
In well diffusion assay, formulation F3 showed comparatively good antibacterial activity as compared to formulation F1 and F2.

Determination of relative percentage inhibition:

Test organism	Inhibition Zone Diameter (mm)		
Staphylococcus aureus (MTCC 737)	F1	F2	F3
	19%	25%	45%

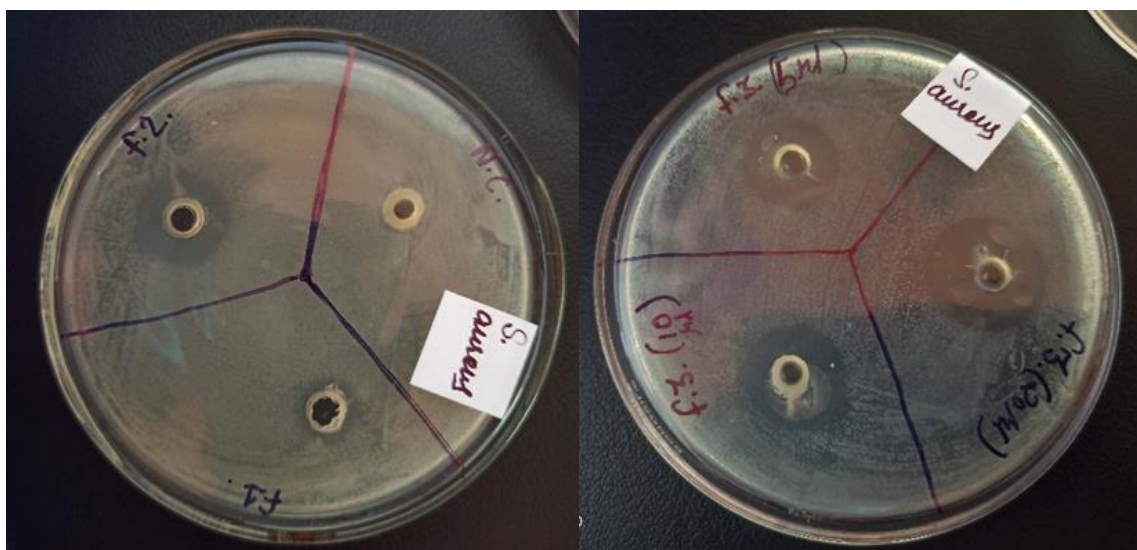
Table 08: Relative percentage inhibition of different gel formulations

The relative percentage inhibition of gel formulations containing *A.rachta* and *C.sativa* for Staphylococcus aureus was found to be 19%, 25%, and 45% respectively. From the study F3 formulation was considered as the best antibacterial formulation, since it shows maximum inhibition.



(A)

(B)



(C)

(D)



(E)

(A), (B) and (C) positive control v/s DMSO v/s formulation F1, F2, and F3 respectively in well diffusion assay; (D) Evaluation of different concentrations (5 μ L, 10 μ L, and 20 μ L) of F3 in well diffusion assay.

Result and Discussion:

Plants are seen as an important source of potentially beneficial ingredients for creating new medicinal treatments, as they are generally safe with minimal to no negative effects. The application of gels on affected areas provides faster drug release directly to the site of action compared to creams and ointments. Currently, gels are extensively utilized for administering drugs topically. Plant and herb extracts known for their medicinal properties can be added to these gels to offer extra advantages. Common pathogens like *S. aureus*, *E. coli*, *B. subtilis*, and *A. niger* are responsible for skin infections. Previous studies have explored the antibacterial effects of *Azadirachta indica* and the antiinflammatory effect of *Cannabis sativa* on various plant and human pathogens. Yet, applying these plants directly to the skin surface is challenging, so their extracts were created in gel form. The chemical components of *Azadirachta indica*, *Cannabis sativa* are believed to have antiseptic properties and act as natural preservatives in cosmetics. Herbal beauty products are thought to be safe for prolonged use. Nonetheless, it is crucial to prioritize quality control for the effectiveness and safety of herbal cosmetic items; hence, conducting quality control assessments for such products is necessary. Conducting stability studies and patch tests are established methods to demonstrate the effectiveness and efficiency of cosmetic herbal formulations. Short-term stability studies following ICH guidelines showed variations in pH levels of all formulations and bases under different storage conditions.

pH, Viscosity, Spreadability, Extrudability showed minimal variations in the results which proved that all the prepared formulations are suitable for 8 and 40°C. Applicability of the herbal formulation was proved to be satisfactory from the results of viscosity and spreadability. In our studies it was observed that the prepared formulations readily spread on application to the skin or affected part and homogeneity confirmed no lumps, respectively. Also, the physicochemical parameters applied in the testing of stability of cosmetics formulations made apparent consequences that formulations C is much better than

formulations A, B and base due to its relatively higher concentration of active constituents. Literature surveys revealed that individually all extracts have potentially been known for their antibacterial and anti-inflammatory activity respectively. However, no literature is available related to the formulation of a polyherbal formulation containing the extracts of *Azadirachta indica*, *Cannabis sativa*. This study clearly indicated that formulations A, B and C which possessed plant extracts were more potent than the base. The possible explanation for this is the presence of active constituents of plants which exhibit antibacterial and anti-inflammatory activity. But as compared with other formulations the potency of Formulation C was found to be greater.

Conclusion:

1. Formulation F3 has shown comparable good antibacterial and anti-inflammatory efficacy in the well diffusion assay when compared to formulations F1 and F2.
2. The formulations were tested for visual impact, pH, spreadability, and antimicrobial activity. Formulation F3 with 0.5gm of herbal extracts was the best formulation, having good in-vitro activity outside the body. As each of the ingredients are natural, no side effects are to be expected from this formulation.
3. As a result, we can draw the conclusion that the herbal gel formulation including *Azadirachta indica* and *Cannabis Sativa* extract can be used to treat skin conditions.
4. Furthermore, after the completion of animal clinical studies, the product's industrial manufacturing can begin.

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