iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** IV **Month of publication:** April 2019

DOI: https://doi.org/10.22214/ijraset.2019.4375

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 7 Issue IV, Apr 2019- Available at www.ijraset.com

Antibacterial, Antioxidant and Phytochemical Analysis of Sapindus emarginatus Vahl.

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Abstract: ***Medicinal plants are gifts of nature used to cure number of human diseases. Sapindus emarginatus traditionally, used as anti-inflammatory and antipyretic. The seed is an intoxicant, and the fruit rind has oxytropic action. Its powder is used as nasal insufflations. The present finding focuses on the antibacterial, antioxidant and phytochemical analysis of Sapindus emarginatus Vahl and.the antibacterial assay was performed on various pathogens such as Escherichia coli , Pseudomonas aeruginosa , Staphylococcus aureus, salmonella typhi. With reference to standard antibiotics the inhibition of antibacterial activity were obtained by methanolic extract leaf of Sapinduus emarginatus Vahl of different concentration .the maximum zone of inhibition was observed by Salmonella typhi was found to be 2.5 ± 0.73at 10 mg concentration minimum zone of inhibition was found to be 1.1 ± 0.45 at 6mg concentration. It also showed highest antioxidant activity gave 50% inhibition of DPPH activity. The methanolic extract of per caps of leaf of Sapindus emarginatus of two tests does that were 200 mg /Kg. studies have shown that Sapindus emarginatus Vahl contains bio active compounds which having strong antibacterial, antioxidant. Further studies are being carried out on the plant to fully understand its mechanism of action and its full potency against other pathogens.***

Keywords: Antioxidant, Sapindus emarginatus, Phytochemical, Antibacterial, Methanolic extract.

I. INTRODUCTION

Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structure for synthetic fields is provided by rich natural sources. This worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. Man has used plants to treat common infectious diseases, and some of the traditional medicines are still included as part of the habitual treatment of various maladies (Heinrich *et al.*, 2004; Rios *et al.*, 2005). Scientific interest in medicinal plant has burgeoned in recent times due to increased efficiency of new plant derived drugs and rising concerns about the side effects of modern medicine. Medicinal plants are gifts of nature used to cure number of human diseases. To promote the proper use and to determine their potential as sources for new drugs, it is essential to study the medicinal plants (Parekh and Chanda, 2007). Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug. Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against microbial infections (Benkeblia , 2004). The increasingly high numbers of bacteria that are developing resistance to classical antibiotics drive much of the current interest on natural antimicrobial molecules in hope that they may provide useful leads into anti-infective drug candidates. *Sapindus* is a genus of about five to twelve species of shrubs and small trees in the Lychee family, Sapindaceae, native to warm temperate to tropical regions in both the Old World and New world . The genus includes both deciduous and evergreen species. Members of the genus are commonly known as soapberries or soapnuts because the fruit pulp is used to make soap . The generic name is derived from the Latin words *sapo*, meaning "soap", and *indicus*, meaning of "India"(Quattrocchi, Umberto 2000). *Sapindus emarginatus* traditionally, used as anti-inflammatory and antipyretic. The seed is an intoxicant, and the fruit rind has oxytropic action. Its powder is used as nasal insufflations.

II. MATERIALS AND METHODS

A. Plant Collection

Plant samples (leaves) were collected from siriya kalvarayan hills, villupuram district. Based on its morphological characteristics *sapindus emarginatus*, is classified in the following manner.

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B. Collection Of Plant Samples

Sapindus emarginatus Vahl**.** leaves were collected in the month of January 2019 from Siriya kalvarayan hills Eastern Ghats, Villupuram district. Samples were shade dried and pulverised under the room temperature for 48 hrs. The dried powders of leaf of Sapindus emarginatus were defatted with methanol (60-80ºc) in a Soxhlet Apparatus by continuous hot- percolation. The solvent was removed by kept into the incubator for 24 hrs.The method of (Brindha et al., 1982) was followed for analysing the phytochemical constitution of Sepindus emrginatus Vahl.

S.no	Test	Observation	Inference
1.	To 2ml of the test solution, a few micro litres of chloroform was mixed with 3 to 4 drops of acetic anhydride and 1 drop of conc. H_2SO_4.	Colour change from purple to blue or green	Steroids present
2.	A piece of tin and 2 drops of thionyl chloride was added to 2 ml of the plant extract.	Development of violet/purplish colour	Triterpenoids present.
3.	2ml of test solution was mixed with a very small quantity of anthrone reagent and a few drops of conc. H_2SO_4 and heated.	Green or purple colour developed.	Sugar present.
4.	To 2ml of plant extract, 2ml of Fehling's reagent and 3 ml of water was added.	Formation of reddish-orange colour	Presence of reducing sugar.
5.	To 2 ml of the respective plant extract, 2N HCl was added. The resulting aqueous layer was decanted. To this, a few drops of Mayer's reagent was added.	Turbidity or white precipitate	Alkaloids present.
6.	A drop of neutral ferric chloride (5% solution) was added to 2 ml of the plant extract mixed with a bit of alcohol.	Formation of intense blue-coloured solution.	Phenolic compound(s) present.
7.	To 2 ml of the solution mixed with alcohol, a pinch of magnesium metal and 1-2 drops of concentrated HCl was added and the test tube was heated.	Formation of red to orangish-red colour	Presence of flavonoids.
8.	To 2 ml of alcoholic plant extract, a few drops of Ehrlich's reagent and a few drops of conc. HCl was added.	Development of pink colour.	Presence of catachins
9.	To 2 ml of test solution, a few ml of water was added and the tube was shaken.	Formation of foamy lather upon shaking.	Saponins present.
10.	To 2 ml of test solution, a few ml of water and a few ml of lead acetate solution was added.	Formation of a white precipitate	Tannins present.
11.	To 2 ml of test solution, a few ml of magnesium acetate solution was added.	Pink colour developed.	Anthraquinones present.
12.	To 2 ml of plant extract, a solution of 1% ninhydrin (in alcohol) was added.	Development of blue or violet colour	Presence of aminoacids.

C. Collection Of Test Microorganisms

Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi, were used for testing antibacterial activity of leaves extracts. These cultures were obtained from K.A.P. Viswanathan Govt. Medical College, Trichy, India. The bacterial species were sub-cultured on nutrient broth and incubated at 37°C for 18-24 hrs for reviving the organisms. Later, fresh overnight cultures were used for the experiment.

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D. Anti Bacterial Assay

In the present investigation, well diffusion method (Perez *et al.*, 1990) was used for the antibacterial assay. Sterile Petri plates containing 20 ml of Nutrient agar medium were seeded with 0.01ml (bacteria 108 CFU/ ml) of 18 hours old test bacterial culture with calibrated loop (Himedia) and lawned evenly using sterile cotton swabs. Wells were made using well cutter and added with one drop of sterile agar at the bottom of the well to seal it. Plant extracts were added at different concentrations. Wells with 6mm diameter were loaded with different concentrations. Incubation was made at 37° C for 24 hours. Standard antibacterial agents were used as positive control. The plates were incubatedat37^{0}Cfor24hours,andantibacterial activity was measured through the diameter of the zone of inhibition formed using Hi-media scale.

E. DPPH Radical Scavenging Activity

1 ml of 0.1 mM DPPH radical solution was combined with 1 ml of the leaf extracts of *Sapindus emarginatus* (prepared using different solvents) at varying concentrations (50-500µg/ml). We prepared corresponding blank solutions and employed L-ascorbic acid (of equal concentrations as the test solutions, in the range of 50-500 µg/ml) as a positive control antioxidant known to rapidly scavenge free radicals. We used a mixture of 1 ml methanol with 1 ml DPPH as control. The disappearance of DPPH radical was monitored at 520 nm using a spectrophotometer, after incubation at room temperature in the dark place for 30 minutes. % inhibition was calculated using the formula given below:

Inhibition % = (Ac-As/Ac) × 100

III. RESULT AND DISCUSSION

In the organism *Escherichia coli*, the antibiotic chloramphenicol showed zone of inhibition (Table: 1) of 2.2 ± 0.44 cm in 30mg concentration. The antibiotic Bacitracin showed zone of inhibition of 1.3 ± 0.13 cm in 10mg. The antibiotic Amikacin showed zone of inhibition of 2.5 ± 0.76 cm in 30mg. The antibiotic Ampicillin and Methicillin showed no zone of inhibition. In *Pseudomonas aeruginosa*, *the* antibiotic chloramphenicol showed zone of inhibition of 2.3 ± 3.11 cm in 30mg. The antibiotic Amikacin showed zone of inhibition of 3 ± 1.32 cm in 30mg. The antibiotic Bacitracin, Amphicillin and Methicillin showed no zone of inhibition (Fig-4). The antibiotic Amikacin showed the highest zone of inhibition in *Pseudomonas aeruginosa*. In *Staphylococcus aureus*, the antibiotic chloremphenicol showed zone of inhibition of 2.2 ± 1.22 cm in 30mg. The antibiotic Bacitracin showed zone of inhibition of 1.7 ± 0.34 cm in 10mg. The antibiotic Amikacin showed zone of inhibition of 2.5 ± 0.22 cm in 30mg. The antibiotic Ampicillin, Methicillin showed no zone of inhibition. *Salmonella typhi*, the antibiotic chloramphenicol showed zone of inhibition of 3.5 ± 2.42 cm in 30mg. The antibiotic methicillin showed zone of inhibition of 3.0 ± 1.56 cm in 5mg. The antibiotic Amikacin showed zone of inhibiton of 2.7cm in 30mg.

Table 1: Inhibition of bacterial zone obtained from different organisms against antibiotic discs (positive controls)

S.No	Name of the organisms	Ampicillin (10mcg)	Chloramphenicol (30mcg)	Methicillin (5mcg)	Bacitracin (10mcg)	Amikacin (30mcg)
1.	Escherichia coli	-	2.2 ± 0.44cm	-	1.7 ± 0.13cm	2.5 ± 0.76cm
2.	Pseudomonas aeruginosa	-	2.3 ± 3.11cm	-	-	3 ± 1.32cm
3.	Staphylococcus aureus	-	2.2 ± 1.22cm	-	1.7 ± 0.34cm	2.5 ± 0.22cm
4.	Salmonella typhi	-	3.5 ± 2.42cm	3.0 ± 1.56cm	-	2.7 ± 0.51cm

The inhibitions of antibacterial zone were obtained by methanolic extract leaf of Sepindus emarginatus Vahl of different concentration. In Escherichia coli, the zone of inhibition was found to The maximum zone of inhibition shown by Escherichia coli was 1.5 ± 0.272 at 6mg concentration and the minimum zone of inhibition was 0.9 ± 0.52 at 10 mg concentration. In Pseudomonas aeruginosa the zone of inhibition was found to the maximum zone of inhibition shown by Pseudomonas aeruginosa was 1.7 ± 0.318 at 9mg concentration and minimum zone of inhibition was 1.1 ± 0.575 at 10mg concentration. In Staphylococcus aureus, the zone of inhibition was found to At 10mg, the zone of inhibition was found to be 1.5 ± 0.487. The maximum zone of inhibition shown by Staphylococcus aureus was 1.5 ± 0.592 at 6mg and minimum zone of inhibition was found to be 0.9 ± 0.48 at 7mg concentration. In Salmonella typhi, the zone of inhibition was found to be 1.1 ± 0.457 at 6mg. At 9mg, the zone of inhibition was found to be 1.5±0.493. At 10mg, the zone of inhibition was found to be 2.5 ± 0.732. The maximum zone of inhibition shown by Salmonella typhi was found to be 2.5 ± 0.73 at 10mg and minimum zone of inhibition was found to be 1.1 ± 0.457 at 6mg concentration(Table 2).

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Table 2: Inhibition of antibacterial zone obtained methanolic extract leaf of sepindus emarginatus Vahl.

S.No	Name of the organisms	6mg	7mg	8mg	9mg	10mg
1.	Escherichia coli	1.5 ± 0.272	1.0 ± 0.206	1.1 ± 0.327	1.0 ± 0.374	0.9 ± 0.525
2.	Pseudomonas aeruginosa	1.6 ± 2.58	1.2 ± 0.2	1.5 ± 0.334	1.7 ± 0.318	1.1 ± 0.575
3.	Staphylococcus aureus	1.5 ± 0.592	0.9 ± 0.468	1.5 ± 0.586	1.3 ± 0.581	1.5 ± 0.487
4.	Salmonella typhi	1.1 ± 0.457	1.2 ± 0.25	1.4 ± 0.411	1.5 ± 0.493	2.5 ± 0.732

The present study Sepindus emarginatus showed the good antioxidant activity. 100 µl and 150 µl concentration of the sample gave the 50% inhibition of DPPH activity while other concentration shows moderate antioxidant properties. Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals. Similarly Divya Teja Banda studied In-Vivo Anti-oxidant activity of Methanolic extract of Sapindus emarginatus in mono sodium glutamate induced obesity rats. After the treatment with the methanolic extract of pericarps of leaf of Sapindus emarginatus of two test doses that were 200mg/kg, 400mg/kg. In MSG induced obesity rats shows significant results(Table 3). After the completion of 28 days the five group's rats were sacrificed and their livers were collected and the homogenised liver extract was subjected to test for the antioxidant parameters like SOD, GSH, Catalase.

Table 3; DPPH radical scavenging activity for methanolic leaf extract of Sepindus emarginatus Vahl.

S.No.	Concentration of the sample	Methanol solvent	Ascorbic acid
1.	50µl	21.64 ± 5.81	86.11 ± 2.53
2.	100µl	52.81 ± 2.16	88.66 ± 5.48
3.	150µl	53.96 ± 1.22	90.55 ± 11.28
4.	200µl	38.96 ± 8.26	92.45 ± 6.12
5.	250µl	48.48 ± 7.65	95.87 ± 7.42

In the present analysis of Triterpenoids and sugar test showed positive, whereas catachins test showed negative result. In the same way the test which showed positive result were flavanoids, saponoins, tannin, anthraquinones test and amino acid test. Phytochemcial analysis of the *Sapindus emarginatus* Vahl. extract was conducted by this analysis, the presence of several phytochemicals like, flavonoids, tannins, saponins, sugars, glycosides and acids were tested(Table 4). The phytochemical screening for aqueous plant extract *Sapindus emarginatus* leaf shows presence of phytosterols, phenolic compounds, tannins, flavonoids, coumarin glycosides and terpenoids. *Sapindus emarginatus* leaf shows absence of catachin , Steroid, Carbohydrate, in methanol extract.

Table 4: Preliminary phytochemical analysis of Serpindus emarginatus Vahl. methanolic leaf extract.

S. No	Name of the test	Results
1	Triterpenoids test	+
2	Sugar test	+
3	Catachins test	-
4	Flavenoids test	+
5	Saponins test	+
6	Tanins test	+
7	Anthraquinones test	+
8	Amino acid test	+
9	Steroids test	-
10	Carbohydrate test	-

The antibacterial activity of Sepindus emarginatus leaf extract were tested by the disc diffusion assay showed that there has been an increasing effect on bacterial growth inhibition with increasing concentration of the extract. And the extract showed good inhibitory activity on almost all the bacteria tested. It has been found that among all the tested organisms, the Gram negative bacterial strain, Escherichia coli was found to be more susceptible to the plant extract by showing inhibition zone ranging from 8.9 – 16.1 mm and the gram positive strain Staphylococcus aureus was least susceptible with the inhibition zone ranging from 6 – 11.8 mm. The

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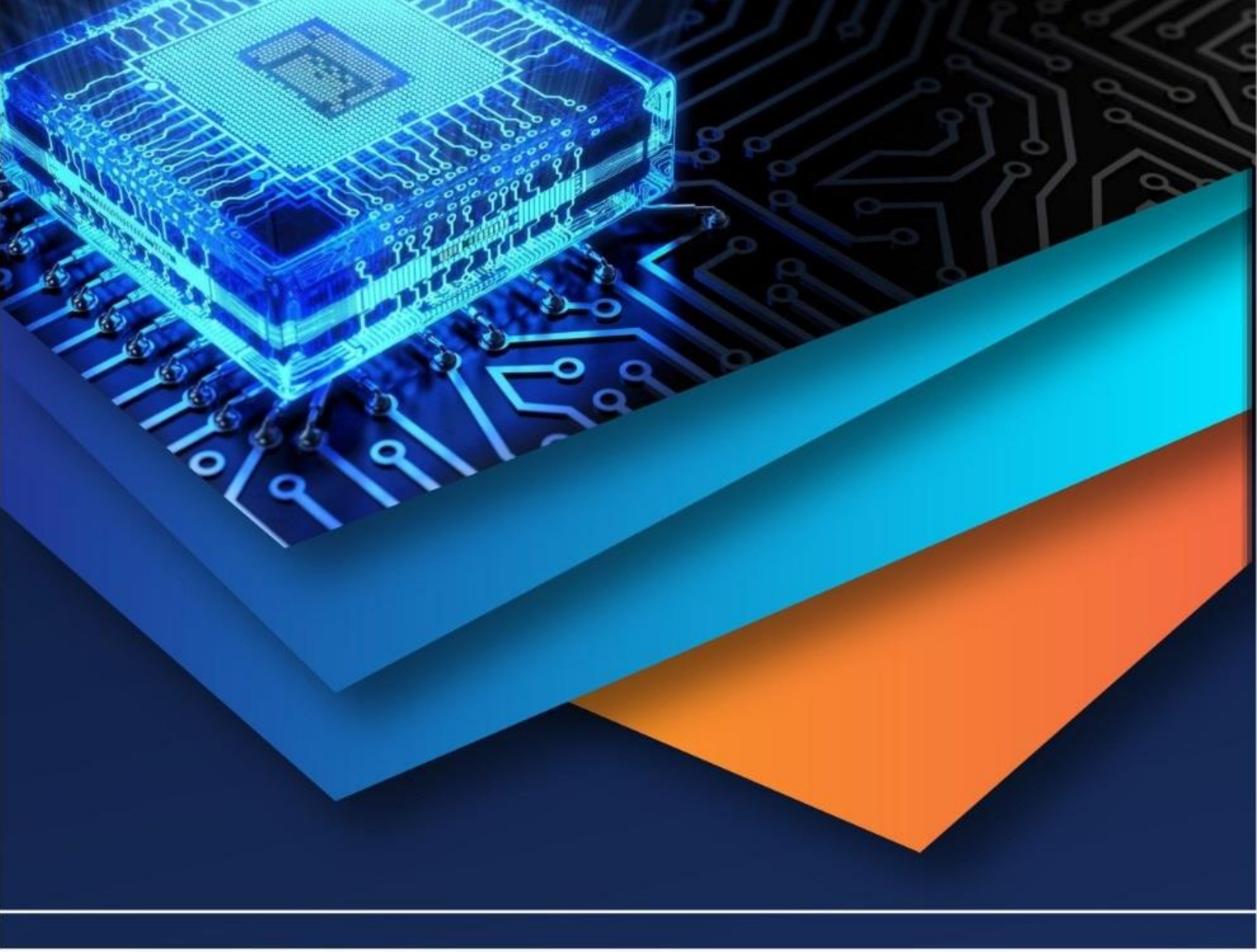
antimicrobial activity in terms of zone of inhibition was presented. The observed activity may be due to the presence of potent phyto constituents in the leaf extracts (Sathiya et al., 2008). Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins, tannins etc. and getting these chemicals out into the herbal remedy depends upon the solubility of these compounds in various solvents. Against all the tested bacterial strain, we observe methanol extract of all the samples showing much better antibacterial activities in contrast to aqueous extract, which may be because of organic nature of methanol and also for the reason of its high capacity to dissolve more organic and active antimicrobial compounds (Cowan, 1999). The antimicrobial action of the aqueous extracts could be ascribed to the anionic components such as thiocyanate, nitrate, chlorides and sulfates besides other water soluble component swhich are naturally occurring in the plant material (Darout et al.,2000).

Antioxidants mostly known to protect our body from the formation of free radicals. Ascorbic acid is not synthesized in human being and dietary or oral consumption only provide this vitamin. The high quality of ascorbic acid was found in fresh leaves of *Adhatoda vasica* showed 1200 µgm of ascorbic acid content. The normal human body when fully saturated contains about 5000 mg of vitamin C, at which 30mg found in adrenal glands, 200mg in extra cellular fluids & really distributed in varying concentrations throughout the cells at the body. (Danne, 1990). Lycopene is one of the over 600 or more carotenoids pigments. Some studies reported that lycopene could inhibit the growth of cancer and endometric cancers (Rao and Agarwal 2000).

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