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Anti-inflammatory and Antioxidant Activities of the Methanolic Leaf Extract of *Cissus aralioides*

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Abstract Inflammation and oxidative stress are features of many degenerative diseases. This study evaluated the anti-inflammatory and antioxidant activities of the methanolic leaf extract of *Cissus aralioides in vivo* and *in vitro*. The anti-inflammatory activity was evaluated using the carrageenan and formalin-induced paw edema models and Nitric oxide (NO) scavenging ability, while the antioxidant activity was evaluated using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) photometric assay. In the anti-inflammatory study, doses of 150, 300 and 600 mg/kg of the extract were used orally. Acetylsalicylic acid (200 mg/kg) was used as a reference drug while concentrations of *Cissus aralioides* extract (CAE) ranging from 25- 800 µg/ml were used for DPPH and NO scavenging ability. In the carrageenan-induced paw edema model, CAE caused dose-dependent increase in percentage edema inhibition, increasing percentage inhibition of edema from 0% in the negative control group to 41% at 6th h at 600 mg/kg. In the formalin-induced paw edema model, CAE significantly ($p < 0.05$) and dose dependently increased the percentage edema inhibition and decreased the paw edema volumes on day 1. *Cissus aralioides* extract showed different percentage increase in the nitric oxide scavenging ability, increasing it from 24.94% at 25 µg/ml to 57.41% at 800 µg/ml while in the DPPH assay; CAE caused a concentration dependent increase in antioxidant activity from 1.88% at 25 µg/ml to 52.55% at 400 µg/ml. In conclusion, the results suggest that *Cissus aralioides* may be of benefit in ameliorating inflammatory and oxidative stress conditions.

Keywords: *anti-inflammatory, Cissus aralioides, Carrageenan, Formalin, Edema, antioxidant*

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1. Introduction

Inflammation is a complex pathophysiologic response of vascularised tissue to injury [1]. It is the body's attempt at self protection and the aim is to remove harmful stimuli, including damaged cells, irritants or pathogens and then begin the process of healing [2].

The need for anti-inflammatory drugs arises when the inflammatory response becomes inappropriate, aberrant or sustained, and when it causes tissue destruction [3].

A great number of anti-inflammatory drugs (both steroids and non-steroidal anti-inflammatory drugs) are extensively used for the treatment of acute and chronic inflammatory conditions [4]. Among these drugs, none have proved to be curative. They suppress rather than abolish the inflammatory reactions thereby providing symptomatic relief and are usually accompanied by severe adverse effects such as gastrointestinal irritations, ulcers, bone marrow depression, hypertension, myocardial infarction and muscular degenerations among others [5,6].

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS) which are formed under normal physiological conditions, but becomes injurious to the body when their action are not checkmated by antioxidant systems [7].

To protect the body against oxidative stress, antioxidant supplementation or improvement in antioxidant nutrition is essential and antioxidants from natural sources have been proved to have higher bioavailability and therefore higher protective efficacy than synthetic antioxidants [8].

The use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases. Antioxidants have also been widely used as dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease, diabetes, other degenerative diseases and even altitude sickness [9,10].

There is therefore, the need to search for more potent and less toxic anti-inflammatory and antioxidant drugs from medicinal plants as there is the worldwide green revolution which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs [11].

Cissus aralioides is a lofty climber, woody at the base with stout green succulent stems constricted at the nodes and sometimes sub-succulent leaves. Flowers are greenish

or whitish, comparatively large and horizontal. The fruit is 2½ cm long, mostly red in colour. The whole plant is covered with irritating hairs and leaves contain an acid and slightly acrid red sap They are commonly found in deciduous forests and fringing jungle across the region from Senegal to Northern and Southern Nigeria. *Cissus aralioides* is found predominantly in Tropical Africa, especially in Cameroun (Common name – Kindamina) and Nigeria (Igbo name – eriri agwo) [12].

In Nigeria folkloric medicine, the leaves are used for treatment of cuts, wounds, internal and external microbial infections and swellings. It is also used for the treatment of arthritis, rheumatism, dropsy, gout, swellings, edema, febrifuges, pain-killers, pulmonary troubles, while the sap is used for eye treatments and veneral diseases [13]. In Cameroon traditional medicine, *Cissus aralioides* leaves and roots are used as anti-microbial agents against micro-organisms of the gastrointestinal and urogenital tracts [14]. In Gabon, triturated leaves mixed with sugar cane juice are used to combat gonorrhea; the liane (freed from the leaves) is used in Congo (Brazzaville) for its analgesic and antiseptic attributes to relieve cough and by embrocating for body pain in fever, rheumatism and abdominal and kidney problems [12]. Phytochemical analysis of *Cissus aralioides* showed that it contains alkaloids, tannins, saponins, steroids, terpenes, flavonoids and cardiac glycosides [15].

The present study was therefore undertaken to evaluate the anti-inflammatory and antioxidant activities of the methanolic leaf extract of *Cissus aroloides* in consideration of its use in Nigeria folk medicine for treatment of arthritis, edema and some diseases associated with oxidative stress.

2. Materials and Methods

2.1. Solutions, Reagents Chemicals and Equipments

Freshly prepared solutions and analytical grade chemicals were used the experiments. Methanol (Sigma-Aldrich, Germany), DPPH (Sigma - Aldrich, Germany), Carrageenan (Sigma – Aldrich, Germany), Acetyl salicylic acid (Reckitt Benckiser, Pakistan), Ascorbic acid (Sigma – Aldrich, Germany. Spectrophotometer (Spectrumlabs, USA), Water bath (Uniscope, USA), Refrigerator (Haier Thermocool, Nigeria), Scientific weighing balance (Mettler, Germany), Mortar and pestle, filter paper (Whatmann), Hot air oven (Gallenkamp, Germany).

2.2. Collection and Identification of Plant Material

The leaves of *Cissus aralioides* were collected from the forest in Michael Okpara University of Agriculture, Umudike, Abia State in June, 2013 and identified by Mr. Ndukwe Ibeh of the Department of Forestry and Environmental Management, Michael Okpara University of Agriculture, Umudike. A voucher specimen catalogued MOUAU/CVM/VPP/2013/04 was deposited in the Department of Veterinary Physiology, Pharmacology and Biochemistry herbarium of the same University for reference purposes.

2.3. Preparation of the Plant Material

The leaves of the plant were dried under mild sunlight and pulverized into coarse powder of about 1 mm in diameter. The plant material was extracted using cold maceration method in absolute methanol for 48 hours with intermittent shaking at 2 hours interval. Thereafter, the extract was filtered using Whatman No. 1 filter papers into a beaker. The filterate was dried in an oven at 40°C and the extract was stored in a refrigerator at 4°C as *Cissus aralioides* extract (CAE) until when needed. The percentage yield was calculated using the formula below:

$$\% \text{ yield} = \frac{\text{Weight of extract}}{\text{Weight of plant material used}} \text{x} \frac{100}{1}$$

2.4. Experimental Animals

Wistar albino rats of both sexes weighing 100-150 g, obtained from the laboratory animal unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State were used for the study. The animals were housed in aluminum cages at room temperature and under natural light/darkness cycles. They were supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted feed (Vital feed® Nigeria). They were maintained in accordance with the recommendations of the Guide for the care and use of laboratory animals [16] and the experimental protocol was approved by the institution's ethical committee. The rats were acclimatized for two weeks prior to the study.

2.5. Acute Toxicity Test

This study was carried out using the up and down method of acute toxicity [17]. Six rats were randomly selected, weighed and placed in a cage. Three rats were treated with 5000 mg/kg of plant extract while three other rats were given equal volume of distilled water, orally by gastric gavage. The rats were observed for 72 hours for signs of toxicity and mortality.

2.6. Anti-Inflammatory Tests

2.6.1. Carrageenan – Induced Paw Edema

This was done using the method of Winter *et al.* [18]

Animals were fasted overnight and had free access to water prior to the day of the experiment but were denied access to feed and water during the experiment. Twenty five albino rats were weighed and randomly divided into five groups (A-E) of 5 rats per group. Their left paw volumes were measured using the volume displacement method, as control. Paw edema was induced by injecting 0.1 ml of 0.2% solution of carrageenan into the subplantar surface of the hind right paw. After one hour of induction, group A was given 10 ml/kg of distilled water (negative control), group B was treated with 200 mg/kg of acetylsalicylic acid (aspirin) (positive control) and groups C, D and E were treated with 150, 300 and 600 mg/kg of plant extract respectively. Thereafter, the right paw volume was determined at 1 hour, 3 hours and 6 hours post treatment.

2.6.2. Formalin – Induced Paw Edema

The method described by Turner [19] was used for this experiment.

The animals were fasted overnight and were given free access to water and feed prior to the experiment, and had access to both feed and water for the other five days of the study. Twenty five albino rats were weighed and randomly divided into five groups (A-E) of five rats per group. Their right hind paw volumes were determined using the volume displacement method. Inflammation was induced by injecting 0.1ml of 1% solution of formaldehyde (formalin) into the subplantar surface of the hind right paw of the rats. After one hour of induction, group A was given 10 ml/kg of distilled water (negative control), group B which served as positive control was treated with 200 mg/kg of acetylsalicylic acid and groups C, D and E were treated with 150, 300 and 600 mg/kg of plant extract respectively. Thereafter, the right paw volume was determined at 1 hour post treatment. The animals were treated continuously for six days. The paw volume was determined on the first day and sixth day, one hour after treatment.

Percentage edema inhibition was calculated using the formula below:

$$\% \text{ edema inhibition} = \frac{(Vc - Vt)}{Vc} x \frac{100}{1}$$

Where, Vc = paw volume of the control rats and Vt = paw volume of the treated rats.

2.6.3. Nitric Oxide Scavenging Ability

This study was carried out by the method described by Jaiswal *et al*. [21]. The nitric oxide scavenging activity of the extract was conducted based on Greiss assay method. Sodium nitroprusside (2.0 ml of 10 mM) and 5.0 ml of phosphate buffer were mixed with 0.5 ml of different concentrations (25, 50, 100, 200, 400 and 800 µg/ml) of the plant extract and incubated at room temperature for 150 minutes}. After the incubation period, 2 ml of the incubated solution was added to 2 ml of Greiss reagent (1% sulphanilamide, 0.1% α-napthyl-ethylene diamine dihydrochloride and 3% phosphoric acid) and incubated at room temperature for 30 minutes. The absorbance of the pink chromophore formed by the diazotization of nitrite with α-napthyl-ethylene diamine dihydrochloride was measured at 540 nm. Ascorbic acid was used as positive control and results were expressed as percentage inhibition of nitric oxide. All determinations were performed in triplicates. The percentage inhibition was calculated using the formula below:

$$\% \text{ inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times \frac{100}{1}$$

2.7. Antioxidant study

2.7.1. DPPH Photometric Assay

The free radical scavenging activity of the extract was analyzed by the DPPH photometric assay as described by Mensor *et al.* [20] using a spectrophotometer. The extract (2 ml) at concentrations of 25, 50,100, 200 and 400 µg/ml each was mixed with 1ml of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicates. One mililitre of methanol was added to 2.0 ml of the test extract and used as the blank while 1.0 ml of the 0.5 mM DPPH solution plus 2.0 ml of methanol was used as negative control. Ascorbic acid (vitamin C) was used as a reference standard. The percentage antioxidant activities were calculated as follows:

$$\% \text{ anti} - \text{oxidant activity} = 100 - \left(\frac{\text{ABS sample} - \text{ABS blank}}{\text{ABS control}} x100 \right)$$

2.8. Data Analysis

The results were presented as mean ± standard error of mean (SEM) where applicable (while others were presented as percent increase or decrease). Data obtained were analyzed using one way analysis of variance (ANOVA) and the variant means were separated by Least Significant Difference (LSD) of the different groups. Significance was accepted at the level of $p < 0.05$.

3. Results

3.1. Plant Extraction

The yield of *Cissus aralioides* extract was 3.48% w/w dry matter.

3.2. Acute Toxicity Test

Oral administration of 5000 mg/kg of *Cissus aralioides* and an equal volume of distilled water produced no death or any sign of toxicity after 72 hours.

3.3. Carrageenan – Induced Paw Edema

The result of the carrageenan-induced paw edema is presented in Table 1. The result showed that *Cissus aralioides* extract (300 and 600 mg/kg) and the reference drug (aspirin) significantly ($p < 0.05$) reduced the paw edema in the rats at 1 h, 3 h and 6 h, when compared to the negative control group. Reduction in paw volume of the rats was in a dose dependent manner at the 3rd and 6th h. Though CAE at the dose of 150 mg/kg reduced the rats' paw edema throughout the study, the reduction was not statistically significant ($p > 0.05$) when compared to the distilled water treated group. There was also a dose dependent increase in percentage edema inhibition, from 0% in the distilled water treated group to 41% by the extract at 600 mg/kg at the 6th h as compared to 44% by aspirin.

Table 1. Effect of *Cissus aralioides* extract on carrageenan-induced paw edema in rats

Treatment	Mean increase in paw volume in ml ± SEM (% inhibition)		
	1 h	3 h	6 h
Distilled water (10 ml/kg)	0.44 ± 0.13 (-)	0.85 ± 0.11 (-)	0.78 ± 0.14 (-)
Aspirin (200 mg/kg)	0.25 ± 0.12* (43)	0.41 ± 0.19* (52)	0.44 ± 0.18* (44)
CAE (150 mg/kg)	0.37 ± 0.07 (16)	0.77 ± 0.04 (9)	0.74 ± 0.09 (5)
CAE (300 mg/kg)	0.29 ± 0.07* (34)	0.68 ± 0.08* (20)	0.57 ± 0.09* (27)
CAE (600 mg/kg)	0.31 ± 0.09* (30)	0.54 ±0.10* (36)	0.46 ± 0.09* (41)

*= $p < 0.05$ when compared to distilled water

3.4. Formalin-Induced Paw Edema

The result of the formalin-induced paw edema is presented in Table 2. The result showed that CAE at different doses (150, 300 and 600 mg/kg) just like the reference drug; aspirin (200 mg/kg) significantly ($p < 0.05$) reduced the paw edema in the rats when compared to the negative control group on the first day of treatment. The extract also caused reduction in the paw edema of the rats on day 6 but the reduction was not statistically significant ($p > 0.05$) except at the dose of 600 mg/kg where the reduction was up to 30%. Also, percentage inhibition of paw edema increased with increase in the dose of the extract. The reference drug had a better effect on paw edema reduction when compared to CAE in this study causing 63% reduction on day 1 as against 35% by the extract at the 600 mg/kg CAE.

Table 2. Effect of *Cissus aralioides* extract on formalin-induced paw edema in rats

Treatment	Mean increase in paw volume in ml ± SEM (% inhibition)	
	Day 1	Day 6
Distilled water 10 mg/kg	0.52 ± 0.08 (-)	0.30 ± 0.04 (-)
Aspirin 200 mg/kg	0.19 ± 0.03* (63)	0.27 ± 0.07 (10)
CAE 150 mg/kg	0.37 ± 0.03* (28)	0.27 ± 0.04 (10)
CAE 300 mg/kg	0.34 ± 0.02* (35)	0.23 ± 0.08 (23)
CAE 600 mg/kg	0.34 ± 0.06* (35)	0.20 ± 0.03 (33)*

*= $p < 0.05$ when compared to Distilled Water.

3.5. Nitric-oxide Scavenging Ability

The result of the nitric oxide scavenging ability of *Cissus aralioides* is presented in Figure 1. Percentage nitric oxide inhibitions were 24.94% and 26.30% at the concentrations of 25 µg/ml and 50 µg/ml respectively. There was a decrease in a percentage inhibition at 100 µg/ml (21.48%) but there was an increase in percentage antioxidant activity in a concentration dependent manner from the concentration of 200 µg/ml to 800 µg/ml), with CAE having its highest percentage inhibition (57%) at 800 µg/ml.

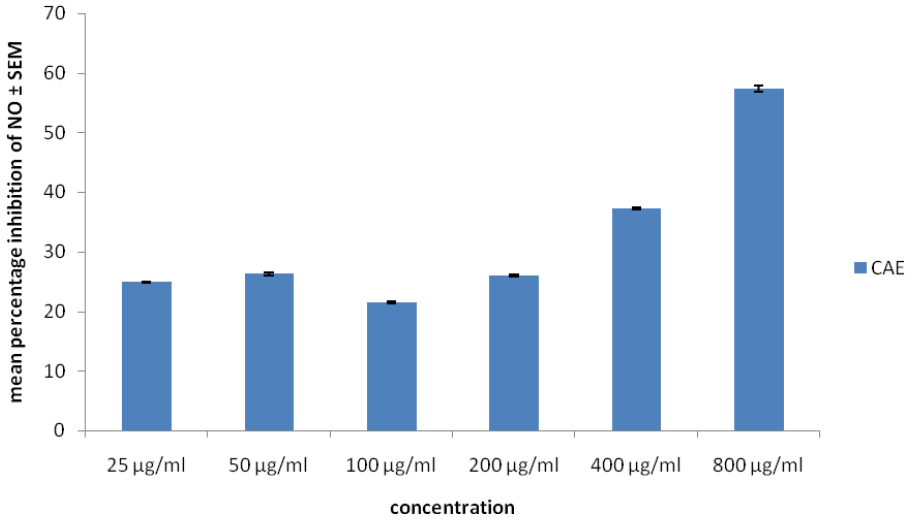

Figure 1. Nitric oxide scavenging ability of methanolic leaf extracts of *Cissus aralioides*

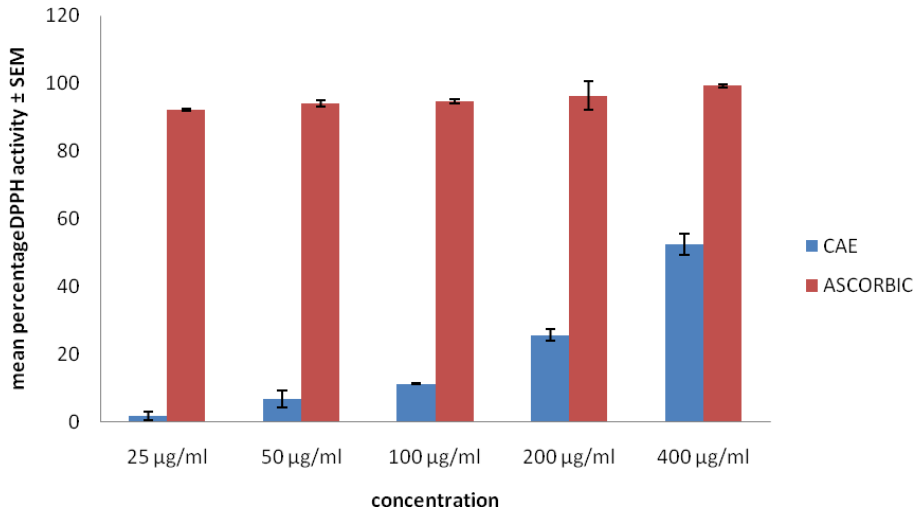

Figure 2. Percent antoxidant activity of *C. aralioides* in DPPH photometric assay

3.6. DPPH Photometric Assay

The result of the DPPH photometric assay of *Cissus aralioides* is presented in Figure 2. *Cissus aralioides* extract caused a concentration dependent increase in percentage antioxidant activity increasing the antioxidant activity from 1.88% at the concentration of 25 µg/ml to 52.6% at the concentration of 400 µg/ml while ascorbic acid had 92.15% at the concentration of 400 µg/ml.

4. Discussion

This study evaluated the anti-inflammatory and antioxidant activities of methanolic leaf extact of *Cissus aralioides.* The anti-inflammatory activity was evaluated using acute and chronic inflammatory models while the antioxidant activity was evaluated using DPPH photometric assay.

Acute toxicity test in rats for 72 hrs produced no death or signs of toxicity which suggests that the extract was well tolerated and the doses used for the study were safe in the animal models used and the LD_{50} is greater than 5000 mg/kg.

Carrageenan-induced paw edema model is widely used for the study of the effects of drugs and extracts on inflammation especially at the acute phase [22]. On injection of carrageenan in the subplanter areas of rats' hind paws, development of edema results from the release of serotonin, histamine and prostaglandins [23]. The development of carrageenan-induced paw edema in rats is biphasic. The first phase occurs within one hour and is attributed to release of cytoplasmic enzymes such as histamine, serotonin and kinins from the mast cells while the second phase, which occurs after one hour is mediated by the release of prostaglandin-like substances within the inflammatory area [24].

Cissus aralioides extract significantly ($p < 0.05$) reduced the rat paw edema from the first to the sixth hour in this study. This suggests that the anti-inflammatory property of CAE could be due to its effect on both phases of the inflammatory reactions. This may have been brought about by inhibition of the mediators of inflammation such as serotonin, histamine and prostaglandins by the extract through its stabilizing effects on basophils and mast cells with decrease in cellular infiltration and release of mediators of inflammation [25]. Also the decrease in rat paw edema by CAE may be attributed to inhibition of cyclooxygenase and lipooxygenase enzymes since inhibitors of these enzymes play important roles in carrageenan-induced paw edema in rats [26].

Formalin-induced paw edema resembles human arthritis and is one of the most suitable methods used to screen anti-arthritic and anti-inflammatory agents (especially against chronic inflammation). The development of edema in this model involves infiltrations of neutrophils, macrophages and proliferation of fibroblasts [27]. Formalin produces localized inflammation and pain when injected into the paw of rats which is biphasic, comprising of early neurogenic component and later tissue mediated response [28]. The result of the experiment showed that CAE caused a dose-dependent and significant reduction of the paw edema of rats on day 1 while on day 6 the reduction was more pronounced at the dose of 600 mg/kg in the formalin-induced paw edema model. This suggests that CAE may be effective in the neurogenic phase of the inflammation at all the doses used but requires high doses in the later stage. This also suggests that CAE may possess anti-proliferative property and that the use of the extract for management of chronic inflammation requires higher doses.

Nitric oxide (NO) is a key mediator in the phenomenon of inflammation and is an important model for screening anti-inflammatory drugs [29]. *Cissus aralioides* extract caused a concentration dependent inhibition of Nitric oxide up to the concentration of 800 µg/ml. The ability of the extract to scavenge NO is an indication of its anti-inflammatory potentials.

The antioxidant activity of *Cissus aralioides* was evaluated using DPPH assay. This assay has been used for the evaluation of antioxidant activities of natural compounds [30]. In this study, CAE caused a concentration dependent increase in percent antioxidant using DPPH assay. DPPH assay is a fast, reliable and reproducible method widely used to measure the *in vitro* general antioxidant activity of pure compounds as well as plant extracts [31]. According to Ramdas and Seema [32], substances that increase percentage antioxidant activity in DPPH photometric assay, as seen in this experiment (Figure 2), is assumed to have antioxidant activity. This antioxidant activity could slow or terminate the production of Reactive oxygen species (ROS), thereby reversing oxidative stress.

Phytochemical analysis of *Cissus aralioides* showed that it contains alkaloids, tannins, saponins, steroids, terpenes, flavonoids and cardiac glycosides [15]. Tannins are well known for their antioxidant and anti-inflammatory properties [33]. Steroids possess anti-inflammatory properties [15]. Also a number of flavonoids including quercetin have been shown to inhibit both cyclooxygenase and lipooxygenase pathways and flavonoids can also inhibit nitric oxide synthase [34]. The presence of these phytochemicals can explain the anti-inflammatory activity of this plant.

In conclusion, methanolic leaf extract of *Cissus aralioides* have demonstrated significant anti-inflammatory and antioxidant potentials, thereby establishing the pharmacological basis for its use in the treatment of arthritis, edema and some diseases associated with oxidative stress in Nigeria ethnomedicine. However, further work is required to isolate and characterize the active compound(s) responsible for these activities and to determine the exact mechanism of action.

Statement of Competing Interests

The authors have no competing interests.

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