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Research Article

SCREENING OF PHYTOCHEMICAL CONTENT AND *IN VITRO* BIOLOGICAL INVESTIGATION OF *CANTHIUM DICOCCUM* (GAERTN.) AND *AMISCHOPHACELUS AXILLARIS* (L.)

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ABSTRACT

Objective: The objective of the study was to study the pet ether, ethyl acetate, and ethanol leaf extracts of *Canthium dicoccum* and *Amischophacelus axillaris* for anthelmintic activity and antihypertensive activity.

Methods: The antihypertensive activity was carried out by employing a colorimetric assay based on the hydrolysis of Histidyl-Hippuryl-Leucine and anthelmintic activity carried out against Indian earthworm *Pheritimaposthuma*.

Results: The pet ether leaf extract both the plants exhibited the maximum antihypertensive activity with a percent inhibition of 64.82 for *C. dicoccum* (*Gaertn.*) and 84.12 for *A. axillaris* (*L.*) as compared with Captopril showing percent inhibition 85.37 and for anthelmintic activity, it is found that ethanol extract of *C. dicoccum* and ethyl acetate extract of *A. axillaris* exhibited significant activity against the standard drug albendazole.

Conclusion: This study investigated the potential of *C. dicoccum* and *A. axillaris* as a new source against the antihypertensive activity. The outcome of anthelmintic activity revealed that the ethyl acetate and ethanol extracts exhibited a considerable amount of anthelmintic activity, which is mainly due to the active phytoconstituents present in the extracts.

Keywords: Antihypertensive, Anthelmintic, *Canthium dicoccum (Gaertn.)*, *Amischophacelus axillaris (L.),* Angiotensin-Converting Enzyme inhibitors.

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INTRODUCTION

Hypertension is nowadays common among people of all age groups. It does not give any early warning symptoms, but it exerts more load on heart and blood vessels due to which it is termed as a silent killer. Immense importance has been given to the study of hypertension with the development of a realistic method to measure it in the past century due to constant change in the lifestyle of people. Physicians have been working tirelessly to ascertain the relation between high blood pressure and risk of failure of heart, kidney, and even causes a stroke. Some early attempts in 1930s and 40s, including surgical procedures involving cutting nerves to blood vessels, inducing high fever and reducing sodium content in diets. Few case studies have yielded significant results proving the treatments are effective in lowering blood pressure and improving outcomes with minor setbacks. One of the proved methods of treatment of hypertension apart from drugs is by improving lifestyle and standard of living. The method of treatment associated with dietary and lifestyle measures considerably reduces the arterial pressure thereby mitigates cardiovascular morbidity and mortality [1-4]. Search for new drugs, mainly from cheap and reliable natural products, and mainly plants are of significant interest in the development of more efficient and better-tolerated drugs. Therefore, it is relatively essential to study the inhibition of angiotensin-converting enzyme (ACE) to prevent and manage hypertension. Macroparasitic disease caused by parasitic worms that are visible to naked eye affecting humans as well as other animals wherein a part of the body or an organ is infected by the worm is known as helminths. Presently, helminthiasis is one of the common agents of infection rampantly prevailing in developing as well as underdeveloped countries. The spread of helminthiasis which is a significant contributor to global diseases is worsened by prevailing malnutrition, pneumonia, anemia, and eosinophilia in underdeveloped countries [5] due to the nonavailability of basic health infrastructure and medically trained personnel to handle the situations. Helminthiasis is rarely fatal but is a major cause of morbidity [6]. The medicines available in the market which are chemically synthesized are not effective up to the mark and in some cases have developed resistance thereby causing reoccurrences of the diseases. Thus medicinal plants which are rich in botanical anthelmintics [7,3] serve as an alternate source for the development of more effective and less toxic medicines which has encouraged further research and development in analyzing new plant-derived medicines.

Canthium dicoccum (Gaertn.), the Ceylon boxwood also known as Bellachi in Kannada, belongs to the family *Rubiaceae* [8]. In India, its bark is used for fever and decoction of the root is used internally for diarrhea. Bark powder with sesame oil is used in rheumatic pain [9,10]. The plant is proved for its anti-inflammatory [11], antidiabetic, and nephroprotective activity [12].

Amischophacelus axillaris (L.) is a species of perennial plants in the family *Commelinaceae* commonly called Negilu there in Kannada. It is native to the Indian Subcontinent, southern China, South East Asia, and Northern Australia. It grows in monsoon forest, woodland, and wooded grassland. Traditionally plant is used for anti-inflammatory, antiparasitic, and antifungal property. In India, leaves are used for the treatment of tympanitis and as food for pigs [13].

METHODS

The *C. dicoccum* (Gaertn.) leaves were collected in the month of June-July in Hosnagar (T), Fig. 1 Shimoga district, Karnataka, and *A. axillaris* (L.) Fig. 2 leaves were collected in the month of July-August in Agumbe region, Shimoga district, Karnataka. Both the plants were authenticated and deposited in the Department of Botany Kuvempu University, Shankaraghatta, with voucher number KUAB4688 for *C. dicoccum* (Gaertn.) and KUAB4687 for *A. axillaris* (L.). The collected plant material was shade dried and coarsely pulverized. The pulverized plant material was subjected to the hot method of extraction using Soxhlet extractor. The extraction method was carried out using numerous solvents, namely, pet ether, ethyl acetate, and ethanol per their increasing polarity. The obtained extract was filtered and evaporated to dryness under reduced pressure in a rotary vacuum evaporator.

Qualitative phytochemical screening

All the extracts were subjected to preliminary phytochemical analysis using the standard procedure to identify the various phytoconstituents [14].

Antihypertensive activity

The plant extracts were tested at three concentrations dissolved in assay buffer (10 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) HEPES buffer containing 0.3M NaCl and 10 µM Zinc Sulfate) containing 20 µl of kidney cortex plasma membranes (ACE enzyme source) and 1 mM Hippuryl-His-Leuas substrate. The extracts were incubated with the enzyme for 10 min at 37°C. The substrate was added to the reaction mixture and incubated for 45 min at 37°C. The reaction was terminated by the addition of 1 M HCl. The yellow color is developed by the addition of 100 µl of pyridine and 50 µl of benzene sulfonyl chloride and was measured at 410 nm in an ELISA Plate Reader (iMARK, BIORAD). The extract block availability of substrate to the enzyme and thereby cause enzyme inhibition, by indicating no formation of yellow color. The inhibition was represented in the form of a percentage over control. Captopril, a known ACE inhibitor was tested in this assay as a standard compound [15,16]. The inhibition activity was calculated using the following equation

Inhibition activity (%) = $[(A_c - A_s/(A_c - A_b)] \times 100$

Where, A_c is the absorbance of the buffer (control), A_s is the absorbance of the reaction mixture (sample), A_b is the absorbance when the stoke solution was added before the reaction occurred (blank).

Anthelmintic activity

Indian adult earthworms *Pheritimaposthuma* were collected from earthworm rearing center, Dummalli, Shimoga (Karnataka). The worms were maintained in the cages with moderate temperatures. The worms were washed in water to remove dirt. The anthelmintic activity was evaluated on Indian adult earthworms *Pheritimaposthuma* due to its anatomical and physiological resemblances with the intestinal roundworm parasites of the human beings [17,18]. The activity was assessed using earthworms by the reported methods with small modifications [19]. The worms were washed to get rid of adhering materials and were sorted out for uniform size and length. The worms were kept in a 6% dextrose solution for acclimatization. The worms with normal motility of length having 3–5 cm and 0.1–0.2 mm in width were used for the experiment. All the worms of equal size were divided into 11 groups and each group contains three worms. I group was treated with vehicle (1% Tween-80 in normal saline) served as control, II group is treated with albendazole (Standard) 10 mg/ml, and III – XI groups were treated with different concentrations (20, 40, and 60 mg/ml in normal saline containing 1% Tween-80) of all the three extracts. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the normal group did not survive in the saline. Death was concluded when the worm lost its motility followed by the fading of their body color. The experiment was carried out in triplicate for each group and data were statistically analyzed.

RESULTS

Results showed pronounced activity for pet ether extract with percent inhibition of 64.82 for *C. dicoccum* (Table 1, Fig. 3) and 84.12 for *A. axillaris* (Table 2, Fig. 4). These activities are comparable with the activity of the positive control, captopril, which has a percent inhibition of 85.37. The preliminary evaluation of the crude extracts of *C. dicoccum* and *A. axillaris* showed that both the plants are a potential source of bioactive compounds that can inhibit the activity of ACE. The pet ether

Fig. 1: *Canthium dicoccum* (Gaertn.) plant

Table 1: Antihypertensive activity of various solvent extracts of *Canthium dicoccum* (Gaertn.) leaves with standard

Samples	Concentration	O.D.	OD-Blank	% ACE inhibition
Blank		0.405		
Control		0.985	0.580	
	20 µg	0.773	0.368	36.552±1.194
	40 µg	0.740	0.335	42.241±0.000
A	60 µg	0.687	0.282	50.977±0.583
	80 µg	0.680	0.275	56.264±5.119
	100 µg	0.609	0.204	64.827±0.895
	20 µg	0.639	0.234	59.655±0.796
	40 µg	0.652	0.247	57.414±0.298
B	60 µg	0.789	0.384	33.793±0.597*
	80 µg	0.772	0.367	36.724±0.896
	100 µg	0.758	0.353	39.138±0.497
	20 µg	0.776	0.371	36.034±0.597
	40 µg	0.844	0.439	24.310±0.696**
C	60 µg	0.954	0.549	5.344±0.597**
	80 µg	0.974	0.569	2.011±0.897**
	100 µg	1.021	0.616	0.00±0.997**
	10 nM	0.506	0.305	33.410±0.756
D	15 nM	0.412	0.211	51.966±1.134
	25 nM	0.268	0.067	85.370±0.883

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at p<0.05*, p<0.01**, *C. dicoccum*: *Canthium dicoccum*, SEM: Standard error of the mean, ANOVA: Analysis of variance, OD: Optical density extract of both the plants was found to have the highest ACE inhibitory activity. Further purification studies will be carried out to identify the bioactive compounds responsible for the observed activity.

For anthelmintic activity, the time is taken for mean paralysis and means the death of the earthworms are tabulated in Tables 3 and 4. The main effect of albendazole on the worm is to cause a flaccid paralysis that effects in exclusion of the worm by peristalsis. The data of the present study revealed that all the tested extracts of *C. dicoccum* (Gaertn.) and *A. axillaris* (L.) have anthelmintic activity in dose-dependent method giving the shortest time of paralysis and death of worms. The results showed that the ethanol extract of *C. dicoccum* (Gaertn.) and ethyl acetate extract of *A. axillaris* (L.) exhibited considerable anthelmintic activity at a concentration of 60 mg/ml by causing the death of worms in lesser time. The anthelmintic activity of all the extracts was comparable to that of the standard drug albendazole. Preliminary phytochemical screening of crude extracts of *C. dicoccum* and *A. axillaris* revealed that the presence of various phytochemical constituents is tabulated in Tables 5 and 6.

Fig. 2: *Amischophacelus axillaris* (L.) plant

DISCUSSION

A reference study showed that pet ether extract of *C. dicoccum* contains high concentration of phenolic compounds [20]. The free hydroxyl groups of phenolic compounds are the structural moieties that chelate zinc ions in the active site of ACE thereby rendering ACE inactive [21]. The present results showed that both *C. dicoccum* and *A. axillaris* showed a high concentration of phenolic groups, which is the major moiety to rendering ACE inactive. Some of the secondary metabolites that could be responsible for the observed *in vitro* ACE inhibitory activity are steroids, flavonoids, glycosides, and hydrolyzable tannins. The results of the phytochemical screening revealed the presence of alkaloids, terpenes, flavonoids, tannins, anthraquinones, and saponins in *C. dicoccum* extract [20]. Elbl and Wagner developed one of the earliest

Table 2: Antihypertensive activity of various solvent extracts of *Amischophacelus axillaris* (L.) leaves with standard

Samples	Concentration	O.D	OD-Blank	% ACE inhibition
Blank		0.308		
Control		0.914	0.693	
	20 µg	0.527	0.219	68.398±0.416
	40 µg	0.541	0.233	66.378±0.831
A	60 µg	0.498	0.190	72.583±0.166
	80 µg	0.412	0.104	84.993±0.833**
	100 µg	0.418	0.110	84.127±0.249**
	20 µg	0.576	0.268	52.236±0.333
	40 µg	0.544	0.236	44.589±0.333
B	60 µg	0.534	0.226	45.021±0.416
	80 µg	0.512	0.204	47.474±0.916
	100 µg	0.501	0.193	49.495±0.249
	20 µg	0.639	0.331	61.327±0.166
	40 µg	0.692	0.384	65.945±0.499
C	60 µg	0.689	0.381	67.388±0.499
	80 µg	0.672	0.364	70.562±0.416
	100 µg	0.658	0.350	72.150±0.831
	10 nM	0.506	0.305	33.410±0.756
D	15 nM	0.412	0.211	51.966±1.134
	25 nM	0.268	0.067	85.370±0.883

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at p<0.05*, p<0.01**, *A. axillaris*(L.): *Amischophacelus axillaris* (L.), SEM: Standard error of the mean, ANOVA: Analysis of variance, OD: Optical density

Table 3: Anthelmintic activity of various solvent extracts of *Canthium dicoccum* (Gaertn.) leaves with standard

Treatment groups	Concentration (mg/ml)	Mean paralysis time (min)±SEM	Mean death time (min)±SEM
Control/Vehicle		----------	----------
Standard	10	7.13±0.008	13.09±0.039
	20	14.13±0.20	22.13±0.014
A	40	12.12±0.023	20.10±0.017
	60	10.08±0.017	20.08±0.020
B	20	12.14±0.012	24.10±0.008
	40	10.06±0.026	22.13±0.026
	60	10.10±0.015	18.12±0.011
	20	12.06 ±0.008	15.10±0.008
C	40	9.09±0.020	18.14±0.014
	60	9.08±0.008	16.15±0.017

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at p<0.05*, p<0.01**, *C. dicoccum*: *Canthium dicoccum*, SEM: Standard error of the mean, ANOVA: Analysis of variance

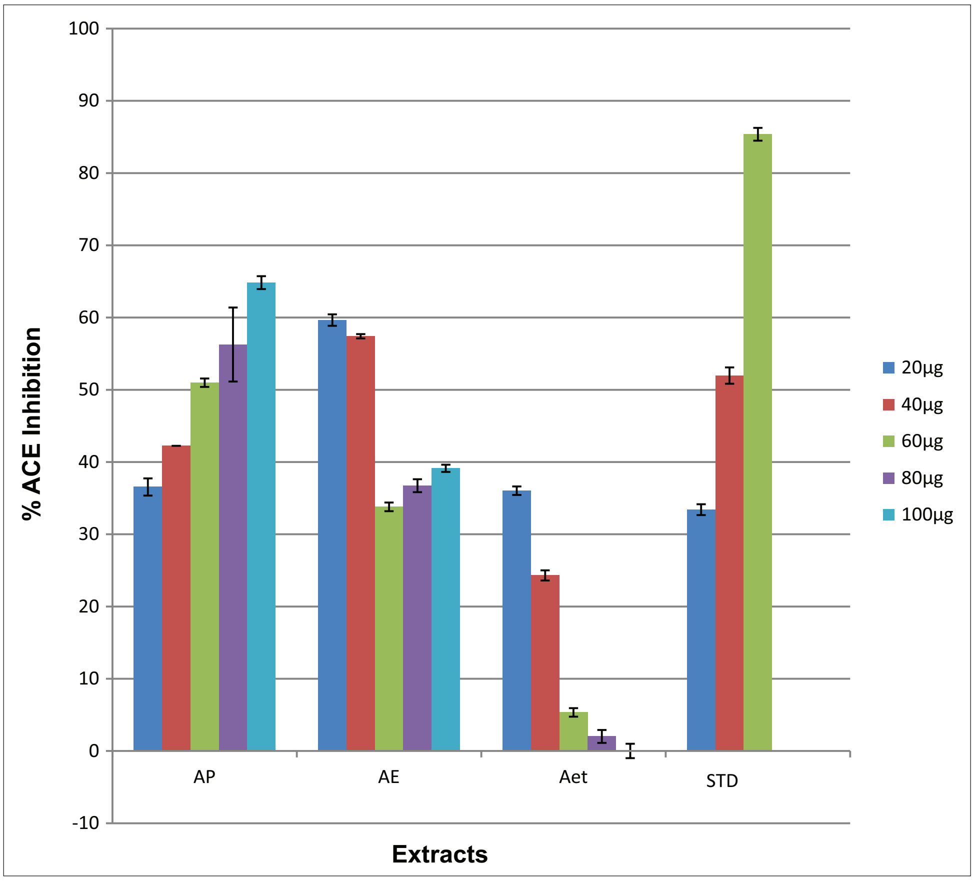

Fig. 3: Antihypertensive activity of *Canthium dicoccum* (Gaertn.) of various solvent extracts in angiotensin-converting enzyme inhibitors method. AP- pet ether extract, AE- ethyl acetate extract, Aet- ethanol extract, STD: Standard, Standard = Captopril in 10 nM, 15 nM, 25 nM

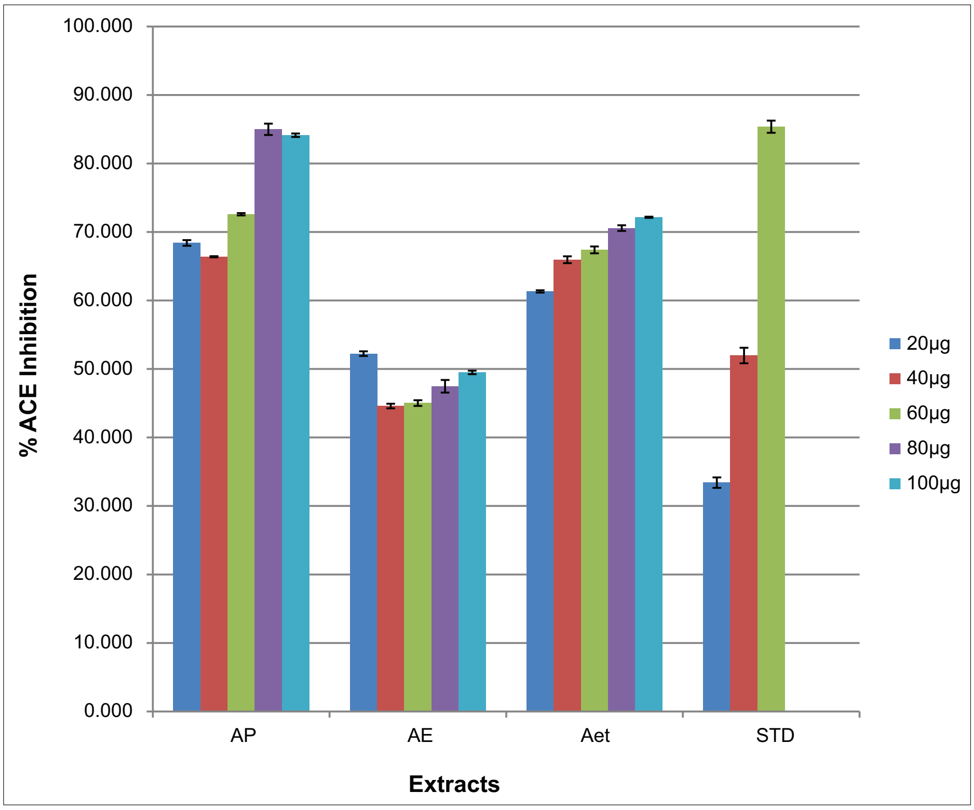

Fig. 4: Antihypertensive activity of *Amischophacelus axillaris* (L.) of various solvent extracts in angiotensin-converting enzyme inhibitors method. AP- pet ether extract, AE- ethyl acetate extract, Aet- ethanol extract, STD – standard. Standard = Captopril in 10 nM, 15 nM, 25 n

Table 4: It shows anthelmintic activity of different extracts of *Canthium dicoccum* (Gaertn.) and *Amischophacelus axillaris* (L.) leaves with standard

Treatment groups	Concentration (mg/ml)	Mean paralysis time (min)±SEM	Mean death time (min)±SEM
Control/Vehicle		----------	----------
Standard	10	7.13±0.008	13.09±0.039
	20	18.09±0.005	22.14±0.012
A	40	15.10±0.008	20.09±0.005
	60	13.14±0.014	18.11±0.008
B	20	17.11±0.008	23.08±0.005
	40	10.08±0.014	20.11±0.020
	60	9.05±0.012	15.05±0.026
	20	16.14±0.015	21.14±0.028
C	40	15.03±0.173	20.01±0.046
	60	13.02±0.023	18.02±0.00

Significance level: The data were analyzed using ANOVA and expressed as Mean±SEM followed by Dunnett's test and differences between means were regarded significant at $p<0.05$*, $p<0.01$**, *A. axillaris* (L.): *Amischophacelus axillaris* (L.), SEM: Standard error of the mean, ANOVA: Analysis of variance

Table 5: Phytochemical screening of various solvent extracts of *Canthium dicoccum* (Gaertn.) leaves

Phytoconstituents	Pet. ether extract	Ethyl acetate extract	Ethanol extract
Alkaloids	−ve	+ve	+ve
Steroids	−ve	+ve	+ve
Carbohydrates	+ve	+ve	+ve
Flavonoids	−ve	+ve	−ve
Phenolics/Tannins	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Glycosides	−ve	+ve	+ve
Coumarin	−ve	+ve	+ve

Table 6: Phytochemical screening of various solvent extracts of *Amischophacelus axillaris* (L.) leaves

Phytoconstituents	Pet. ether extract	Ethyl acetate extract	Ethanol extract
Alkaloids	−ve	+ve	+ve
Steroids	−ve	+ve	+ve
Carbohydrates	+ve	+ve	+ve
Flavonoids	+ve	+ve	−ve
Phenolics/Tannins	+ve	+ve	+ve
Saponins	+ve	+ve	+ve
Glycosides	+ve	−ve	+ve
Coumarin	−ve	+ve	+ve

assays for ACE inhibition, stated that an extract is considered active if it is able to inhibit the enzyme by more than 50% [22,23]. The pet ether extract of *C. dicoccum* and ethanol extract of *A. axillaris* plant showed proper inhibition of more than 50% result when compared with the standard drug. In the anthelmintic activity, the ethanol and ethyl acetate extracts of both the plant not only established paralysis but also caused the death of worms, particularly, at a higher concentration of 60 mg/ml in a shorter time as compared to that of albendazole. Phytochemical screening of the extracts showed the presence of flavonoids, alkaloids, steroids, and tannins [24]. Polyphenolic compounds were mainly present in tannins; these compounds are the main reason to show anthelmintic activities [25,26]. Phytochemical screening showed the presence of tannins in the plant extracts (Tables 5 and 6). Tannins can bind to free proteins in the gastrointestinal tract of host animals that may lead to death. Local healers from Karnataka used *Canthium* species and *A. axillaris* (L.) for many medicinal purposes, these plants are unknown as an antihypertensive and anthelmintic agent.

CONCLUSION

The present study suggests that the leaves of *C. dicoccum* and *A. axillaris* possess ACE inhibitory that might be helpful in treating hypertension. Further investigations on the isolation of active compounds present in the extracts and *in vivo* studies are essential to identify a potential chemical entity for clinical use in the treatment of hypertension and further related cardiovascular disorders. This study investigated the potential of *C. dicoccum* and *A. axillaris* as a new source against ACE. The outcome of anthelmintic activity revealed that the ethyl acetate and ethanol extracts exhibited a considerable amount of anthelmintic activity, which is mainly due to the active phytoconstituents present in the extracts. The present study gives proof that it may be a productive medicine in the upcoming days. Further, the plant extracts will be explored for its phytochemical outline to identify the active component, which is responsible for anthelmintic activity.

AUTHOR'S CONTRIBUTIONS

All authors have equally contributed to making this report to be successful.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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