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Research Article

In silico Docking Analysis of Secondary Metabolites of *Bauhinia variegata* and *Garcinia cambogia* With Retinol Binding Protein4 as Target for Obesity.

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ABSTRACT

Bauhinia variegata and *Garcinia cambogia* are traditionally used for treating different diseases. The present study was focused on *in silico* analysis to elucidate the inhibitory activity of retinol binding protein 4 (RBP4) using compounds from GC-MS analysis of *Bauhinia variegata* and *Garcinia cambogia*. The present study was carried out to determine the possible chemical components from *Bauhinia variegata* and *Garcinia cambogia* by GC-MS analysis. These compounds were docked with Retinol binding protein 4. These chemical components having anti-lipidemic activity were subjected to *in silico* analysis. This study evaluates the inhibitory activity of compounds with Retinol binding protein 4 using Argus lab software. Compounds like Octadecanoic acid, Tertradecanoic acid, Hexadecanoic acid and Trans-Geranylgeraniol of *Garcinia cambogia* and compounds like phytol and Hexadecanoic acid of *Bauhinia variegata* were docked with RBP4. GC-MS analysis reveal 31 compounds from *Garcinia cambogia* while, *Bauhinia variegata* exhibit 9 compounds. *In silico* analysis showed that Tetra decanoic acid has interaction energy -9.08kcal/mol, followed by Hexadecanoic acid (-9.64Kcal/mol) and then Octadecanoic acid has -9.88Kcal/mol. Trans-geranylgeraniol showed -16.45Kcal/mol. But there was no interaction with protein and ligands of *Bauhinia variegata* like Hexadecanoic acid and Phytol was studied for insilico analysis. Phytol has -3.78Kcal/mol of interaction energy similarly hexadecanoic acid has -9.88Kcal/mol. But phytol did not show any hydrogen bond between the protein and ligand but Hexadecanoic acid shows hydrogen bond. While comparing these compounds Octadecanoic acid, hexadecanoic acid and tetradecanoic acid shows better interaction with RBP4 compared to Phytol and Trans-geranylgeraniol. Molecular docking analysis proved that secondary metabolites of *Garcinia cambogia* had better inhibitory activity against RBP4 than *Bauhinia variegate* and so these compounds from *Garcinia cambogia* may act as better drug models for obesity.

Keywords: GC-MS, Retinol binding protein4, Argus Lab, *In silico*, Ligand.

INTRODUCTION

Obesity is one of the serious problems worldwide and over 1 billion people are found to be obese. Natural therapy is gaining more value as alternative medicine. India is endowed with large number of medicinal herbs in the world. *Bauhinia variegata* leaves and *Garcinia cambogia* rinds were traditionally used for treatment of many diseases.

Bauhinia variegata belongs to Fabaceae family. The leaves are traditionally used in treatment of skin diseases and stomatitis[1]. It also reported that, it useful in the treatment of tumor and obesity. Anticancer activities of *Bauhinia variegata* ethanolic extract against Dalton's ascetic lymphoma in swiss albino mice was illustrated by Rajkapoor et al., 2003[2]. Hepatic protective activity of ethanolic extract *Bauhinia variegata* leaves was investgated by Gayatri shahu, 2011[3].

Garcinia cambogia commonly known as kodampuli have been used in the Indian systems of the medicine for hundreds of years for their beneficial role in hypolipidemic agent. The fruit rind of *Garcinia cambogia* belongs to Cluciaceae family. It has been used to treat central nervous system disorders. *Garcinia cambogia* extracts are an ingredient in some herbal suppressant and energy products. It has been used as weight loss supplement[4] and traditionally used in the treatment of edema, delayed menstruation, constipation, ulcer, hemorrhoids, diarrhea, dysentery, fever, open sores, intestinal parasites, antimicrobial, antifungal and as anticancer agent[5 6 7 8].

Retinol binding protein 4 belongs to the lipocalin family. It is encoded by RBP4 gene[9]. It is newly identified adipokine, which is the major transport protein for retinol and is strongly expressed in liver but it is secreted by adipocytes[10]. RBP4 has recently has been proposed as a new potential link between obesity and insulin resistance [11]. Serum RBP4 level is increased in obesity, cardiovascular risk disorders and inversely with adiponectin as reported by previous study [12 11].

Our preliminary studies on the leaf extract of *Bauhinia variegata* leaves and *Garcinia cambogia* rinds showed the

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Table 1: Gc-Ms Analysis Of Ethylacetate Extract Of *Garcinia Cambogia*

S.No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area%
1.	3.75	2(3H)-Furanone	$C_4H_4O_2$	84.07gmol^{-1}	1.55
2.	5.49	Methylmalonic acid	$C_4H_6O_4$	118.09gmol^{-1}	2.43
3.	8.43	Dodecane	$C_{12}H_{26}$	170.33gmol^{-1}	0.19
4.	8.57	Catechol	$C_6H_6O_2$	0.1gmol^{-1}	3.85
5.	8.83	Pyridazinone	$C_5H_6N_2O$	110.11gmol^{-1}	2.84
6.	9.11	2 amino pyridine 3ol	$C_6H_6O_2$	110.11gmol^{-1}	1.45
7.	11.29	Tetradecane	$C_{14}H_{30}$	198.39gmol^{-1}	0.31
8.	12.97	Benzoic acid, 4-ethoxy, ethyl ester	$C_7H_6O_2$	122.12gmol^{-1}	0.48
9.	13.61	Caryophellenyl alcohol, 1(2H)Naphthalenone,	$C_{15}H_{24}$	204.36gmol^{-1}	0.21
10.	13.77	Octahydro-8amethyl, Hexadecane	$C_{16}H_{34}$	226.44gmol^{-1}	0.26
11.	15.59	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37gmol^{-1}	0.49
12.	16.11	Trans-Geranylgeraniol Hexadeca-2,6,10,14-tetraen-1-ol,	$C_{20}H_{34}O$	290.48gmol^{-1}	1.24
13.	16.82	Cetene, Z-8-Hexadecene	C_2H_2O	42.03gmol^{-1}	0.60
14.	17.65	n-Hexadecanoic acid,	$C_{16}H_{32}O_2$	256.42gmol^{-1}	4.87
15.	18.78	E-15-Heptadecenal Cyclohexadecane n-Nonadecanol-1	$C_{16}H_{32}$	224.42gmol^{-1}	1.00
16.	19.32	Oleic acid, 9-Octadecanoic acid, Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.46gmol^{-1}	4.06
17.	19.51	Octadecanoic acid	$C_{18}H_{36}O_2$	284.47gmol^{-1}	3.06
18.	21.42	3,5 Diamino-6-1,2,4-triazine Benzaldehyde, 2,4,7-Trinitrofluorenone	$C_4H_7N_5$	125.13gmol^{-1}	0.17
19.	21.66	Naptho[1,2-b]quinoxaline,5-(4-morpholyl)	$C_3H_4N_2$	68.07gmol^{-1}	0.55
20.	22.14	Imidazole,4,5-di(2-furyl)-2-(3-in dolyl) 1H-Indole, 2-methyl Tridecanoic acid	$C_9H_9N_O$	147.18gmol^{-1}	0.36
21.	22.35	Acetic acid,4 acetoxy-3-phenyl ester 2-Dodecen-1-yl succinic anhydride	$C_{16}H_{26}O_3$	266.37gmol^{-1}	0.59
22.	22.54	Supraene Anthracene	$C_{14}H_{10}$	178.23gmol^{-1}	0.34
23.	22.72	Phthalic acid Disooctylphthalate	$C_6H_4(COOH)_2$	166.14gmol^{-1}	0.60
24.	23.02	Acetic acid, 4-acetoxy-3-phenyl ester	$C_2H_4O_2$	60.05gmol^{-1}	0.52
25.	23.68	4-Cyanobenzoic acid, Bicyclo hept-2-en-6-ol, 1-Methylene-2b-hydroxymethyl	$C_8H_5NO_2$	147.13gmol^{-1}	0.52
26.	24.13	Trichothec-9-en-4-ol, Benzene, Tetramethylhexadeca pentaene	$C_{10}H_{18}O$	154.25gmol^{-1}	1.39

Table 1: Gc-Ms Analysis Of Ethylacetate Extract Of *Garcinia Cambogia*

S.No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area%
		Supraene			
27.	25.51	Tricyclo decan-1-amine	$C_{10}H_{17}N$	151.25gmol^{-1}	61.83
		Trichothec-9-en-4-ol			
28.	26.41	Supraene	$C_{10}H_{18}O$	154.25gmol^{-1}	2.04
29.	26.91	Vitamin E	$C_{31}H_{52}O_3$	472.74gmol^{-1}	0.25
		9-Isopropyl-6-methyl-			
		6,7-dihydro-9 H-5-oxa-			
30.	28.29	9-azabenzocyclohepten,	C_4H_8O	72.11gmol^{-1}	1.32
		4-butanal			
		Ethanone,			
		5-Methyl-2-			
31.	28.66	phenylindolizine,	$C9H_{10}O_2$	150.17gmol^{-1}	1.13
		Benzoquinoline			

Fig. 1: GC-MS Chromatogram of Ethyl acetate extract of *Garcinia combogia*

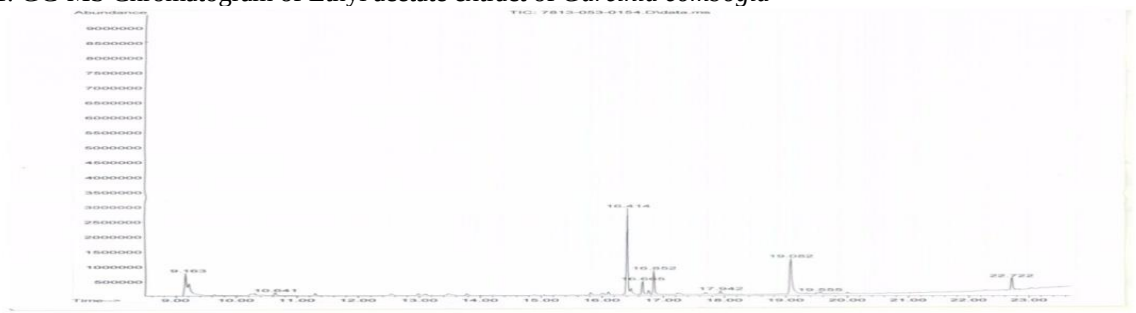

Fig. 2: GC-MS Chromatogram of Ethyl acetate extract of *Bauhinia variegata*

presence of many phytochemicals[13]. These phytocompounds possessed antioxidant potential on par with BHT[14]. Many of these phytocompounds had wide biological activities. Hence it was planned to investigate on the inhibitory effect of secondary metabolites by GC-MS on *Bauhinia variegata* and *Garcinia cambogia* RBP4 as the target protein using Argus software.

MATERIAL AND METHODS

The leaves of *Bauhinia variegata* and fruit rinds of *Garcinia cambogia* were collected from medicinal plant vendor. Care was taken to select healthy ones. The leaves and fruit rind were authenticated by Dr. P. Jayaraman, Director of National Institute of Herbal science, Plant Anatomy Research Center, Chennai. The specimens were used for the studies.

Preparation of extract: The leaves of *Bauhinia variegata* and fruit rinds of *Garcinia cambogia* was dried in the shade, finely powdered and passed through 80mesh sieve and stored in airtight container at room temperature (30±2˚C). About 500gm of the powder was taken in Soxhlet extractor and extracted with Ethyl acetate. The solvent was evaporated using rotary evaporator. The extracts were collected and subjected to freeze drying at -30˚C for 3 days (72 hrs) and lyophilized at temperature -40˚C. The dried extracts were stored in a glass bottle for further analysis.

Instrument and chromatographic conditions: GC-MS analysis were carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 ×0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5. EI was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C/min, then 5°C/min to 280°C/min, ending with a 9 min isothermal at 280°C.

Table 2: Gc-Ms An`Alysis Of Ethylacetate Extract Of *Bauhinia Variegata*

S.No.	RT	Compound	Molecular Formula	Molecular Weight	Peak Area%
1.	9.15	1,2,3 Propanetriol 2-Propanone Hydroperoxide	$C_6H_8O_6$	176.12gmol^{-1}	21.4
2.	10.63	Triacetin Glycerol 1,2 diacetate	$C_9H_{14}O_6$	218.20gmol^{-1}	2.24
3.	16.41	Bicycloheptane 1,19-Eicosadiene	$C_{17}H_{25}NO_2$	275.38gmol^{-1}	27.42
4.	16.66	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53gmol^{-1}	7.29
5.	16.85	Cyclohexene, 4-Propyl 1,4-Eicosadiene	C_6H_{10}	82.14gmol^{-1}	9.67
6.	17.94	Hexadecanoic acid, Ethyl ester	$C_{16}H_{32}O_2$	256.42gmol^{-1}	3.24
7.	19.07	Phytol	$C_{20}H_{40}O$	296.53gmol^{-1}	21.59
8.	19.55	2-Myristynoyl-glycinamide 3,5-Ethanoquinolin-10-one Silicic acid, Diethyl bis ester	$C_7H_{12}O_4$	160.17gmol^{-1}	2.50
9.	22.72	Phthalic acid	$C_6H_4(COOH)_2$	166.14gmol^{-1}	4.64

Injector temperature is 250° C. 2µl sample is injected. Turbo mass gold perkin Elmer detector was used. Running time of GC-MS was 36min.

MS programme: Library NIST version (2005) was used. The inlet temperature was maintained at 200˚C, source temperature was 200˚C. Mass spectra were taken at 70 eV, a scan interval of 0.5 and fragments from 40 to 550Da. Solvent delay 0-2min. Total MS running time was 36 min.

Identification of components: Interpretations of mass spectrum were conducted using database of National Institute Standard and Technique (NIST08s) WILEY 8 and FAME for more patterns. The spectrums of the unknown components were compared with the spectrum of the known components stored in the NIST08s WILEY-8 and FAME library. The Name, Molecular weight, Molecular formula and structure of the components of the test material were ascertained.

In silico analysis: In the present study the nature of interactions, binding mode and selectivity of Retinol binding protein 4 with the GC-MS compounds having anti-lipidemic activity were studied. Docking was carried out with Argus lab 4.0.1.

Preparation of Ligands: Based on the GC-MS analysis, compounds were considered to be possible inhibitors of the Retinol binding protein. Anti-lipidemic activity containing compounds like Octadecanoic acid, Tertradecanoic acid, Hexadecanoic acid, phytol and Trans-Geranylgeraniol were subjected to *in silico* analysis. The activity of compounds was identified by Dukes phytochemical website. The CID files of the Ligands were downloaded from PUBCHEM.

Preparation of ligand structure: Ligand structures were constructed using chem sketch software (http://WWW.acdlabs.com), three dimentional optimizations were done and saved in mol file using Hartre-fock calculation method by Argus 4.0.1 software geomentry optimization of the ligand was performed.

Active Site Analysis*:* Pocket Finder, an online tool which uses hydrophobic probes, were used to predict possible binding sites. Energetically favorable probes sites were clustered and then ranked according to the sum of interaction energies.

Argus lab 4.0.1: Argus lab 4.0.1 is a molecular and drug docking software (Thompson & A.Mark). It is a very useful, highly-featured and easy-to-use molecular modeling, graphic and drug design programmes that were used to carry out docking studies.

Ligands from *Garcinia cambogia* are hexadecanoic acid, Octa decanoic acid, Tetradecanoic acid and Trans-geranylgeraniol and from *Bauhinia variegata* compounds like hexadecanoic acid and phytol were docked with retinol binding protein4 (RBP4) using this software.

RESULTS

GC-MS analysis of *Garcinia cambogia* and *Bauhinia variegata* illustrate compounds present in the Ethyl acetate extract. (Figure 1 & Figure 2) The active principle, molecular weight (M.W), concentration (%) molecular formula (MF) and retention time (RT) is presented in Table 1 and Table 2. *Garcinia cambogia* showed 31 compounds and *Bauhinia variegata* showed 9 compounds. The biological activities of these compounds were retrieved from Dukes phytochemical website. The compounds that have anti-hyperlipidemic activity were choosen as ligands. Figure 3-7 demonstrate the interaction between RBP4 and the ligands. Retinol binding protein4 plays a key role in combating obesity.

Compounds selected for molecular docking analysis are octadecanoic acid, n-hexadecanoic acid, Tetradecanoic acid, phytol and Trans-geranylgeraniol and docked with retinol binding protein4. Interaction energy in calories is shown in Table 3.

These studies clearly highlight that *Garcinia cambogia* compounds gave significant results. Tetradecanoic acid has interaction energy of -9.08kcal/mol. This showed

Table 3: *In silico* analysis of ligands with retinol binding protein4

Compound Name	Activity	Interaction Energy (Kcal/mol)	Interaction In site	Distance Å
Octadecanoic acid	Anti-inflammatory, Antiandrogenic, cancer preventive, dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge	-9.88	TYR-133(O-H…O)	2.7
			GLN-117(N-H…O)	2.5
n-hexadecanoic acid	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic, antieczemic, antiacne, alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary	-9.64	TYR-133(O-H…O)	2.7
			GLN-117(N-H…O)	2.5
Tetradecanoic acid	Antioxidant, cancer preventive, nematicide, hypocholesterolemic, lubricant	-9.08	TYR-133(O-H…O)	2.5
			GLN-117(N-H…O)	2.6
Trans-Geranylgeraniol	Antifungal, Antinoceptive, antilipimic, antitumor.	-16.45	-	-
Phytol				

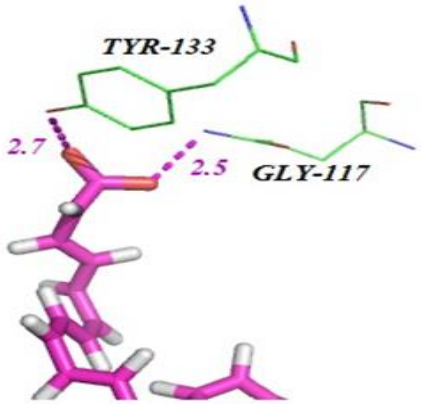

Fig. 3: Interaction of Octadecanoic acid of Garcinia cambogia with Retinol binding protein

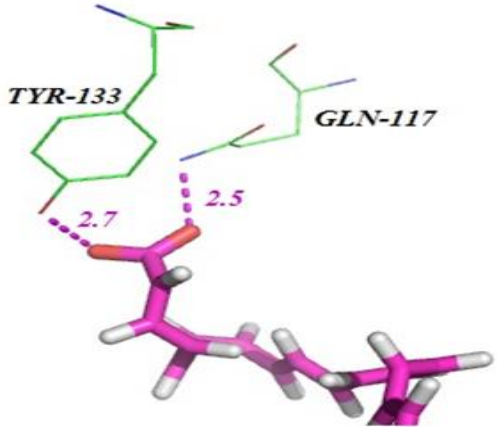

Fig. 4: Interaction of n-hexadecanoic acid of Garcinia cambogia and Bauhinia variegata with Retinol binding protein4

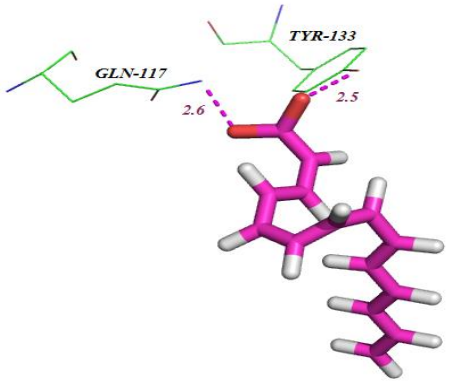

Fig. 5: Interaction of Tetradecanoic acid of Garcinia cambogia with Retinol binding protein4

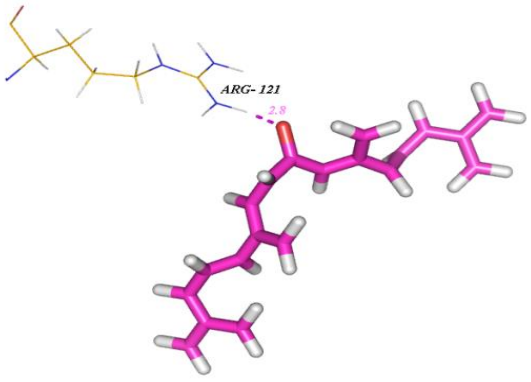

Fig. 6: Interation of Trans-geranyl geraniol of Garcinia cambogia with Retinol binding protein4

minimized energy and two interaction with two different amino acid like TYR-133(O-H…O) and GLN -117(N-H…O), followed by Hexadecanoic acid (-9.64Kcal/mol) and this shows interaction with TYR-133(O-H…O). Then Octadecanoic acid has -9.88Kcal/mol and shows interaction with TYR-133(O-H…O) and GLN-117(N-H…O), Trans-geranylgeraniol shows -16.45Kcal/mol.

Hexadecanoic acid and Phytol compounds have potent anti-hyperlipidemic activity. So, these ligands of *Bauhinia variegata* were choosen for *in silico* analysis. Docking with Retinol binding protein 4 shows Phytol has -3.78Kcal/mol interaction energy and similarly hexadecanoic acid has -9.88Kcal/mol. But phytol did not show any interaction between the protein and ligand, but Hexadecanoic acid has the interaction site at TYR-133(O-H…O).

When compared with these compounds Octadecanoic acid, n-hexadecanoic acid and tetradecanoic acid reveal better interaction with retinol binding protein 4 when compared to Phytol and Trans-geranylgeraniol. From these results obtained *Garcinia cambogia* showed better inhibitory activity with RBP4 than *Bauhinia variegate*.

DISCUSSION

The reliability of medicinal plants for its usage is evaluated by correlating the phytochemical compounds with their biological activity. The mass spectra analysis of the compounds eluted at different times were used to identify the nature and structure of the compounds. The compounds of *Garcinia cambogia* like tetra decanoic acid, hexadecanoic acid and octadecanoic acid shows better interaction with RBP4. Hexadecanoic acid has anti-bacterial, anti-fungal, anti-inflammatory, hypo-cholesterolemic, chemo preventive, hepatoprotective, nematicide, insectifuge, anti-histaminic, anti-eczemic, anti-acne, alpha reductase inhibitor, anti-androgenic, antiarthritic and anticoronary activity[15 16]. Octadecanoic acid and tetradecanoic acid have potential anti-bacterial and anti-fungal activity[17]. Octadecanoic acid has protective effects against cholestasis related liver damage[18]. Phytol is used in the fragrance industry and in cosmetics, shampoos, soaps, household cleaners, and detergents[19]. Compounds like tetradecanoic acid, hexadecanoic acid and octadecanoic acid are having hypo-cholesterolemic activity and therefore these compounds may act as potent drugs for the treatment of obesity.

CONCLUSION

Garcinia cambogia and *Bauhinia variegata* are used in ayurvedic medicine. Medicinal property of plant extract is due to presence of secondary metabolites identified by GC-MS analysis. The compounds present in the ethyl acetate extracts of *Garcinia cambogia* and *Bauhinia variegata* like, octadecanoic acid, hexadecanoic acid, tetradecanoic acid, phytol and transgeranyl geraniol were docked with Retinol binding protein 4. *Garcinia cambogia* compounds have better inhibitory activity against Retinol binding protein 4 than compounds from *Bauhinia variegata*. Hence these compounds may act as potent drugs to treat obesity.

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