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PHYTOCHEMICAL SCREENING OF *IN VITRO* AND *IN VIVO* GROWN PLANTLETS OF *BACOPA MONNIERI* L

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ABSTRACT

Different types of extracts of *in vivo* and *in vitro* grown plantlets of *Bacopa monnieri* L. were qualitatively evaluated to identify the chemical constituents like saponins, alkaloids, resins, Quinones, tannins, phenols, flavonoids, coumarins, proteins and amino acids. Wild and fresh plantlets were collected and different parts of these plantlets were cultured on MS (Murashige and Skoog) medium supplemented with BAP (benzyle amino purine) + AdS (adenine sulphate)+citric acid to get micropropagated plantlets. One and half months old micropropagated plantlets as well as wild or field grown plantlets were taken for tap water washing, air drying, grinding and homogenizing to get fine powder. Cold extraction or solvent extraction methodology was followed to prepare extracts from plantlets. During current investigation methanol, ethanol and water were used as nonpolar and polar solvents. As chemical extraction, mainly depends on the type of solvents with varying polarity, it was investigated that saponins were highly present in water extract, 30% methanol and 30% ethanol extract, while saponins were not found in absolute methanol and ethanol**.** Standard procedures were used to identify the different constituents present in both the samples.

KEYWORDS: Ethanol, Extract, Methanol, Micropropagation, Reagents, Saponins, Water

INTRODUCTION

Bacopa monnieri L. Pennell family Scrophulariaceae, commonly known as Water Hyssop, Brahmi, Jal Brahmi and Nir-Brahmi, is a reputed drug of Ayurveda. It is used in traditional medicine for various nervous disorders (The Ayurvedic Pharmacopoeia of India1999). Traditionally, it was used as a brain tonic to enhance memory development, learning and concentration (Mukherjee DG and Dey CD 1966). Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome and gastric ulcers also supports the Ayurvedic uses of Bacopa (Rajani M, Srivastava et al. 2004). Compounds responsible for the Pharmacological effects of Bacopa include alkaloids, saponins and sterols. The main constituents responsible for Bacopa's cognitive effects are some saponins, which include Bacosides A and Bacoside B. These are complex mixtures of dammarane type of triterpenoidal saponins (Mahato SB, et al. 2000). The bacosides aid in repair of damaged neurons by enhancing kinase activity and ultimately nerve impulse transmission (Singh H.K, Dhawan BN 1997).

Loss of cholinergic neuronal activity in the hippocampus is the primary feature of Alzheimer's disease. Bacosides appear to have antioxidant activity in the hippocampus frontal cortex and striatum (Bhattacharya S et al. 2000) and possesses anti inflammatory activity. Bacopa also gives anticancer effects, possibly due to inhibition of DNA replication in cancer cell lines (Elangovan et al- 1995) A recent *in vitro* study also demonstrated Bacopa extract's specific anti-microbial activity against– *Helicobacter pylori* a bacteria associated with chronic gastric ulcers (Goel R.K et al. 2003)

Therapeutic doses of Bacopa are not associated with any side effects and has been used safely in Ayurvedic medicine for several hundred years (Martis G et al. 1992)

Keeping in view the tremendous medicinal importance of this plant *in vitro,* grown plantlets of *Bacopa monnieri* were previously regenerated in our laboratory (Mehta A et al. 2008; Mehta A 2017), and then current investigation has been carried out. Plant Tissue Culture is one of the strategies for *ex vitro* biodiversity conservation as well as for the manipulation of the production of chemical constituents/secondary metabolites in the cultured plantlets/callus tissue. Time for time medicinal plants should be evaluated for the presence of phytochemical substances as changing climate condition always affect the presence/absence of these phytochemicals. Moreover, extraction and pharmacological screening are the primary steps for isolation, characterization of bioactive compound, toxicological evaluation and clinical evaluation.

During current investigation, efforts have been made to develop simple and rapid method for determination of various chemical substances from polar and non-polar solvent extracts of shade dried and finely powered *in vitro* and *in vivo* grown plantlets of *Bacopa monnieri.*

MATERIALS AND METHODS

Collection of Plant Materials

Wild and fresh plantlets were collected from the wetland of nearby areas of Ranchi district in the month of September, and taxonomic identification of these plantlets were carried out in the University Department of Botany, Ranchi University in the laboratory of Prof. Kunul Kandir, a Plant Taxonomist. These fields grown *in vivo* plantlets are referred as sample1 (S1) and one and half month old micro propagated plantlets (Figure 2) are referred as sample2 (S2), which were previously regenerated on MS medium supplemented with BAP (Benzyle amino purine) + AdS (adenine sulphate) + citric acid (Mehta A 2017).

Processing of Plant Materials

S1 and S2 plantlets were washed under running tap water and cut into small pieces to facilitate drying. Air drying and shade drying was preferred over oven drying during the current investigation (Figure 3). Shade dried and air-dried plant material was then homogenized and ground using an electric blender to obtain powder (Figure 4, 5). The powder was further passed through a 2mm sieve to obtain finer particles. The powdered samples were stored in clean and dry airtight- bottles for further investigations.

Preparation of Extracts (Extraction)

Presently, cold extraction or solvent extraction method (cold maceration) was followed for the production of crude extract from both the plant sample 1 and 2. During present investigation solvents used were absolute methanol, 30% methanol, absolute ethanol, 30% ethanol and water. Powdered sample was dissolved in each above mentioned solvents in 10:1 ratio in conical flasks, plugged with cotton wool and then kept on a rotary shaker at 190-220 RPM for agitation for 24 hours. After 24 hours of continuous agitation samples were filtered and fill rate of each solvent was used as crude extracts.

Screening of Saponins

Saponins are the main bioactive compounds of *Bacopa monnieri,* therefore, first of all primary screening of saponins was carried out before going through the other phytochemical screening. 1 ml of crude extracts from each solvent was taken and 5ml of distilled water was added and shaken vigorously. Formation of persistent foam was observed for 10-15 min which confirms the presence of saponins. (Table1, Figure 1)

Procedure of Phytochemical Screening of Secondary Metabolites from 3 Types of Extracts on Sample1 and Sample2:

The presence of different metabolites was qualitatively screened (Figure 6, 7) by using the standard procedures to identify the constituents (Harborne, J.B 1998; Sazada S et al. 2009;Wagner et al.1996;Solohokara et al.2015;Solomon C U et al.2013; Yadav M et al.2014)

- **Test for Alkaloids (Wagner's Test):** 1ml of plant extract was taken and 3-5 drops of Wagner's reagent were added and observe for the formation of a reddish brown precipitate or coloration.
- **Test for Flavonoids (Alkaline reagent Test):** 1ml of extract was taken and treated with 3-5 drops of 20% NaOH solution. Formation of intense yellow color was observed, which became colorless on the addition of 0.5ml dilute HCl indicates the presence of flavonoids.
- **Test for Phenols (Ferric Chloride Test):** 1ml of extract was taken 5-6 drops of 5% aqueous ferric chloride solution were added and observed in the formation of deep blue or black color for positive results.
- **Test for Amino Acid and Proteins (1% Ninhydrin Solution in Acetone):** 1ml of extract was taken and 2-5 drops of Ninhydrin solution were added and kept it in a boiling water bath for 1-2 min and observed in the formation of purple color for positive results.
- **Test for Saponins (Foam Test):** 1ml of extract was taken and 5ml of distilled water was added and shaken vigorously. Formation of persistence foam was observed for 10-15 min that confirms the presence of saponins.
- **Test for Tannins (Braymer's Test):** 1ml of extract was taken and treated with 1ml of 10% alcohol ferric chloride solution and observed for formation of blue or greenish color for positive results.
- **Test for Terpenoids (Salkowski's Test):** 1ml of extract was taken and 0.5 ml of chloroform was added along with 3-5 drops of conc. H2SO4. The reddish brown precipitate was observed, which was produced immediately.
- **Test for Quinones:** 1ml of extract was taken and treated with 0.5ml of conc. HCl and observed for formation of yellow precipitate or coloration for positive results.
- **Test for Resins:** 1 ml of extract was taken and 5ml of distilled water was added and observed for the turbidity.
- **Test for Coumarins:** 1 ml of extract was taken and 1.5ml of 10% NaOH was added and then observed in the formation of yellow color which indicates the presence of coumarins.

RESULTS AND ILLUSTRATIONS

Table1: Screening of Saponins of Sample 1

Extract Type	Test For Saponin
Water extract	+++ve
Ethanol extract	-ve
Methanol extract	-ve
30% ethanol extract	+++ve
30% Methanol	+++ve

+++ = Highly present, -ve = Absent

Figure 1: Preliminary Screening of Saponin

Table 2: Phytochemical Screening Results

Sl No	Phytochemical Test	Sample 1 (S 1)			Sample 2(S 2)		
		Aqueous Extract	30% Ethanolic Extract	30% Methanolic Extract	Aqueous Extract	30% Ethanolic Extract	30% Methanolic Extract
1	Alkaloid (wagners test)	++ve	++ve	++ve	++ve	++ve	++ve
2	Saponin test(Foam test)	+++ve	+++ve	+++ve	+++ve	+++ve	++ve
3	Resins(Turbidometry)	-ve	-ve	-ve	-ve	-ve	-ve
4	Quinones	-ve	-ve	-ve	-ve	-ve	-ve
5	Tannin (10% alcoholic ferric chloride test)	++ve	++ve	++ve	+ve	++ve	++ve
6	Test for Phenol(aquous ferric chloride test)	+ve	+ve	++ve	+ve	++ve	+ve
7	Flavonoid (Alkaline reagent test)	+ve	++ve	++ve	-ve	+ve	+ve
8	Test for Coumarins	+ve	++ve	++ve	-ve	+ve	++ve
9	Test for Aminoacids and proteins	-ve	-ve	-ve	-ve	-ve	-ve

Figure 2

Figure 3

Figure 4

Figure 5

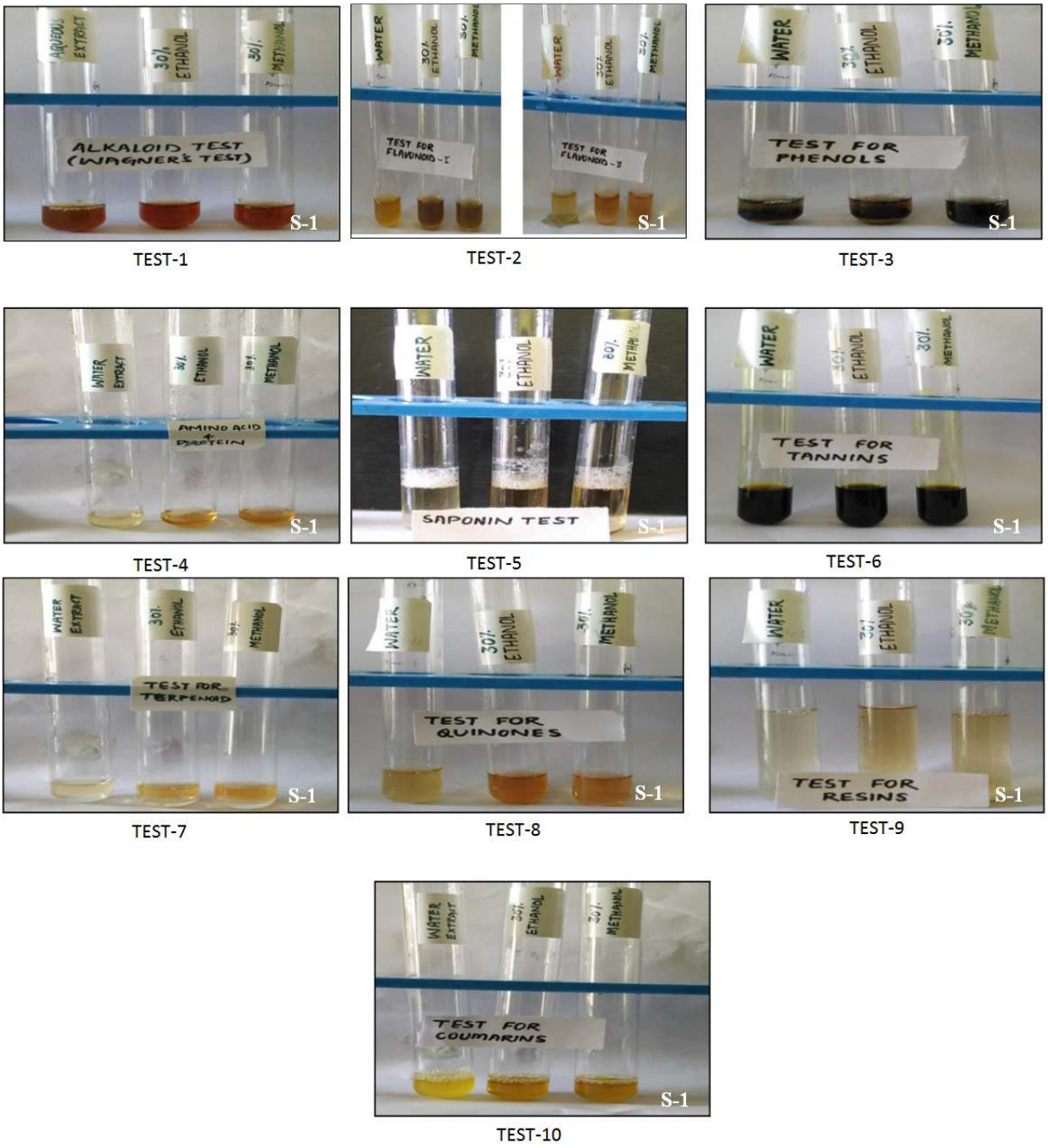
ALKALOID TEST (WAGNER'S TEST)
S-1
TEST-1
TEST FOR FLAVONOID - I
TEST FOR FLAVONOID - II
S-1
TEST-2
TEST FOR PHENOLS
S-1
TEST-3
AMINO ACID & PROTEIN
S-1
TEST-4
SAPONIN TEST
S-1
TEST-5
TEST FOR TANNINS
S-1
TEST-6
TEST FOR TERPENOID
S-1
TEST-7
TEST FOR QUINONES
S-1
TEST-8
TEST FOR RESINS
S-1
TEST-9
TEST FOR COUMARINS
S-1
TEST-10

Figure 6

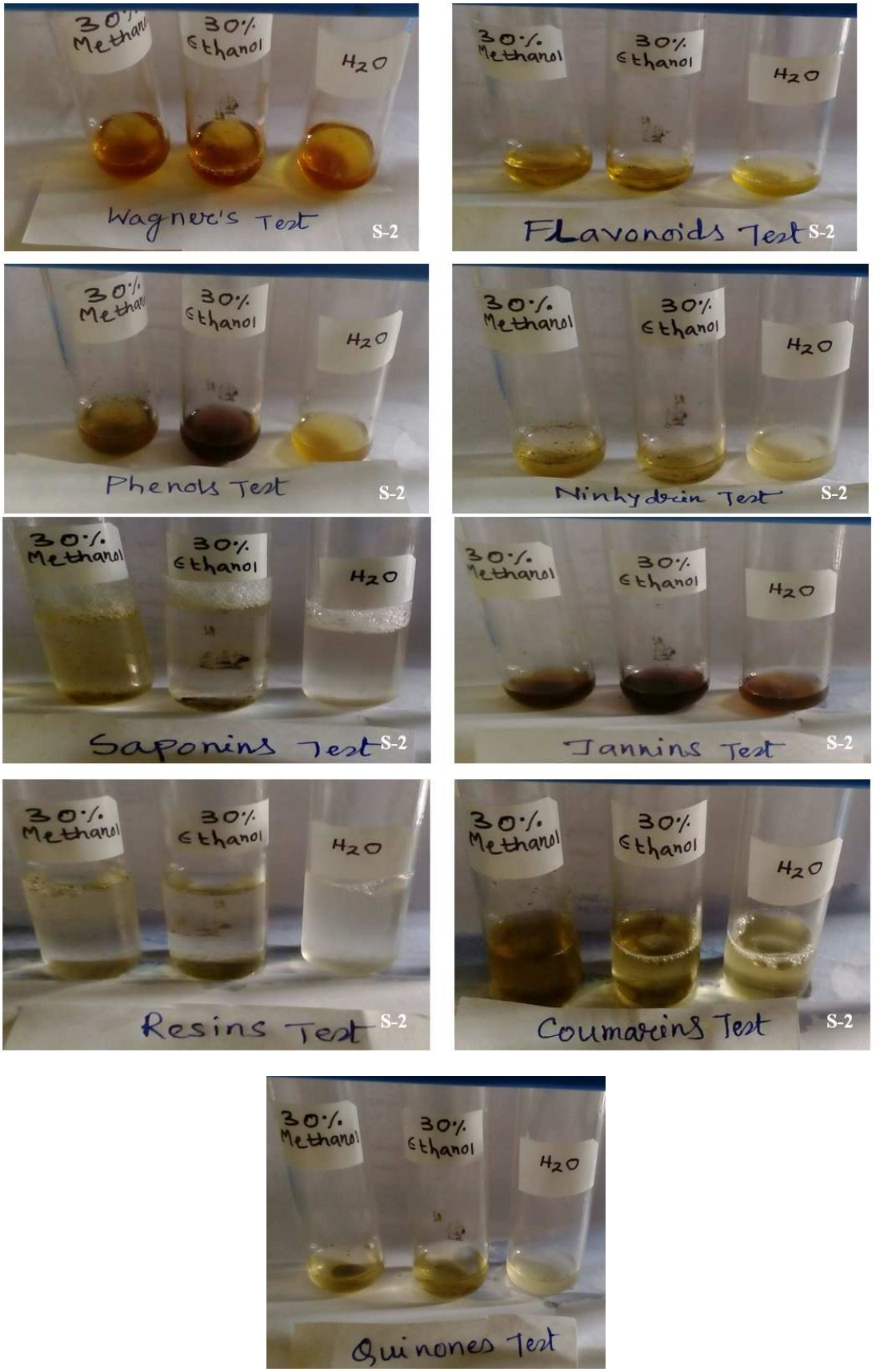
30% Methanol
30% Ethanol
H2O
Wagner's Test
S-2
Flavonoids Test
Phenols Test
Ninhydrin Test
Saponins Test
Jannins Test
Resins Test
Coumarins Test
Quinones Test

Figure 7

DISCUSSIONS

Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made significant contributions towards human health. Natural products, such as plant as plant extracts, either as pure compound or as standardized extracts, provide ultimate opportunities for new drug discoveries, because of the unmatched availability of chemical diversity (Cosa P et al. 2006). Plants used in traditional medicine to contain a wide range of substance that can be used to treat chronic as well as infectious diseases (Durai Pandiyan V et al. 2006)

Extraction is the crucial first step in the analysis of medicinal plants to assure that potential active constituents are not lost, distorted or destroyed during pre-washing, drying, grinding and dissolving with the solvents. The selection of solvent system/type is another crucial step as the yield of chemical extraction, largely depends on the type of solvent with varying polarities, extraction time and temperature, sample to solvent ratio as well as on chemical composition and physical characteristics of the sample.

Since the crude extract is a mixture of so many compounds, at a time only one or two or more compounds may dissolve in one solvent, but at the same time another compound of the same mixture may not dissolve and requires another solvent. Therefore, processing of a crude source material for further analysis as well as the choice of solvent for sample reconstitution can have a significant bearing on the overall success of natural product isolation. The source material eg. Dried powered plant will initially need to be treated in such a way as to ensure that the compound of interest is efficiently liberated into the solution.

Currently, in all three types of extracts used all main secondary metabolites eg. Alkaloids, flavonoids, phenols, saponins, tannins and coumarins were found. Water was observed to be the most suitable solvent for dissolving saponins, which are the main bioactive compounds of *Bacopa monnieri,* though 30% methanol and 30% ethanol were equally good solvent in this regard. It was also observed that in case of sample 2, 30% methanol and 30% ethanol were comparatively more suitable solvents for flavonoid and coumarins.

Alkaloids are a large and diverse group of compounds, usually have profound physiological actions in humans with nervous system and they are very much reactive, even at a small dose. Many of the alkaloids, as isolated compounds or their semi synthetic derivatives are widely used in the pharmaceutical medicines. (Marini Bettolo 1986). Tannins are known to inhibit pathogenic fungi. The flavonoids and phenolic compounds have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anti carcinogenic etc (T P Lalitha et al. 2012)

CONCLUSIONS

Present study, carried out with the evaluation process for the presence of different phytochemicals present in *in vitro* and *in vivo* grown plantlets of *Bacopa monnieri* clearly indicates that micropropagated plantlets can be equally useful for constant supply of raw materials for secondary metabolite production. This will reduce the pressure on the natural habitat of this valuable medicinal plant species.

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