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Phytochemical, proximate and elemental constituents of *Aspilia africana* (Wild sunflower) flowers

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Abstract
In recent times, medicinal plants have gained global acceptance and are now being used in the management of diseases and infections. Several plants and plant parts have been found to possess a myriad of phytocompounds that makes them therapeutically relevant. This study was designed to investigate the phytochemical, proximate and elemental constituents of *Aspilia africana* flowers. The results obtained revealed several therapeutically relevant phytochemicals including tannins, saponins, cardiac glycosides, anthraquinones, flavonoids, alkaloids, carotenoids, steroids, anthocyanins, phlobatannins, phenolics and terpenoids in varied quantities in its crude state, most of which are retained though in smaller quantities in its aqueous extract. The proximate analysis showed the presence of carbohydrates (53.47%), proteins (17.53%), fats (2.37%), ash (9.17%), crude fibre (9.10%) and moisture (8.47%) in the dry crude state while the aqueous extract revealed lower percentages of proximate compounds with the exception of fat and crude fibre which were completely lacking. The macro and micro elements found in the samples used for this study includes magnesium, sodium, potassium, phosphorous, calcium, iron, zinc, copper, while lead and cadmium were absent. This shows that *A. africana* flowers have medicinal values and can be used in the treatment and amelioration of diseases and symptoms.

Keywords: Medicinal plants, phytochemistry, proximate content, elemental content, *Aspilia africana*, therapeutic relevance.

Introduction

Medicinal plants refer to any plant part containing substances that are relevant for therapeutic purposes or are precursors for chemo- pharmaceutical synthesis. The medicinal importance of plants has been attributed to secondary metabolites like alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids etc. This is because they activate, catalyse or initiate some curative reactions in humans

The enormity and versatility of plant complex material synthesis cannot be over emphasized. However, some of these materials have no immediate known metabolic function. In a narrower sense, the term phytochemical is often used to represent the numerous active metabolic compounds found in plants that are also called secondary metabolite. Plant secondary metabolites contain phytotoxins which protect them against attack from diseases, pathogen and deprivation of nutrients. These active principles in plants are species specific and though they may not be actively involved in the immediate metabolic needs of the plants, they readily play a role in helping the plants interact with their immediate environment, thus increasing their overall survivability (Kennedy and Wightman, 2011) [16].

They are also responsible for the development of traits for colors of plants as well as the way they smell eg blueberries and garlic respectively. Phytochemical or secondary metabolites are not necessarily essential nutrients; rather they are chemical substances with biological significance (Brown *et al.,* 2001; Pusztai, 2004) [9, 25]. They possess a number of bioactive principles responsible for their protective functions for human consumers. Plant secondary metabolites serve as a source of prophylaxis and treatment in a myriad of abnormal conditions, some of which include cardiovascular and neurological disorders, antitumor, antimicrobial activities etc. Scientists estimate that there exist hundreds of thousands of different phytochemicals having ameliorative effects on diseases of various forms (Liu, 2004) [17], including tannins, saponins, anthraquinones, cardiac glycosides, flavonoids etc. *Aspilia africana* (Asteraceae) is a common herbaceous plant widely known for its ethno-medicinal values. It is a perennial plant with long history of Agicultural uses as Farmers usually graze their cattle, sheep, and feed their rabbits and hares with it in most African countries especially in West Africa. It is also known as the iodine or haemorrhage plant as it is commonly used to stop bleeding even from large arteries. This study was carried out to elucidate the various phytochemicals of *Aspilia africana* flowers, as wells as its proximate and elemental make up, in a bid to determine whether or not it is therapeutically relevant.

Materials and Methods

Reagents/Chemicals

All reagents used were of analytical grade and products of British Drug House (BDH) England, E. Merck, Darmstadt, Germany and Aldrich Chemical Company

Collection and Identification of Plants

The fresh flowers of *Aspilia africana* were collected between September and November 2016 from Iguomo village, near Okada, Benin City, Edo State. It was identified and authentication was done at the Forest Research Institute of Nigeria, Ibadan by Mr. A. O. Akintola and a herbarium specimen with herbarium no. FHI- 110561 was deposited for future references.

Methods

Extraction of Plant material

The fresh flowers were destalked, pooled, thoroughly rinsed with distilled water and left to drain at room temperature, air-dried, pulverized and stored in air tight containers for subsequent use. The powdered plant materials were macerated with distilled water for 24 hours. The mixture was then filtered using Whatman's (No.l) filter paper and then lyophilized to give the crude aqueous extracts.

Phytochemical Screening

The dry crude and aqueous extract of *Aspilia africana* flowers were tested for the presence of, tannins, anthraquinones, steroids, alkaloids terpenoids, cyanogenic glycosides, phlobatannins, saponins, anthocyanins, flavonoids, and cardiac glycosides, using standard procedures for detecting phytocompounds in line with the methods of Harbone, (1989) and Sofowara, (1993).

Proximate analysis

The determination of crude fat, protein, carbohydrate, ash, moisture and crude fibre was carried out by methods of AOAC (1996).

Elemental analysis

Estimation of micro-mineral and macro-mineral contents was carried out by Atomic Absorption Spectrophotometry (Perkin-Elmer, 1997) [24] and Flame Emission Photometry (Isaac and Kerber, 1972) [15] respectively.

Statistical Analysis

All statistical analysis of data obtained in the course of this study were carried out using the IBM Statistical Package for Social Sciences (SPSS version 20). The results are expressed as mean ± SEM. Statistical significance was determined by analysis of variance (ANOVA). Significant differences between groups were determined in ANOVA using Duncan post-hoc test at $p<0.05$

Results

Qualitative phytochemical content of dry crude and aqueous extract of *Aspilia africana* flowers.

The qualitatively revealed, saponins, tannins, anthraquinones, cardiac glycosides, steroids, alkaloids, flavonoids and carotenoids were found in both dry crude and aqueous flower extract of *Aspilia africana*. Phenolics and phlobatannins were detected exclusively in the aqueous extract and crude plant material respectively. Cyanogenic glycosides, terpenoids and anthocyanins were not detected in both the crude and the aqueous extract as shown in table 1.

Table 1: Phytochemical composition of dry crude and aqueous flower extract of *Aspilia africana*.

Parameter	Dry crude flower	Aqueous flower extract
Saponins	+++	++
Tannins	++	++
Anthraquinones	+++	+
Terpenoids	-	-
Cardiac glycosides	+	+
Steroids	++	+
Alkaloids	+++	++
Phenolics	_	+
Flavonoids	+	+++
Anthocyanins	_	_
Phlobatannins	+	_
Carotenoids	+++	+++

Key: - = Absent, + = Trace, ++ = moderately present, +++ = abundantly present.

Quantitative Phytochemical Constituents of dry crude and aqueous extracts of *Aspilia africana* flowers.

The plant materials were screened quantitatively for secondary metabolites and the results expressed in $mgl00g^{-1}$ of plant sample are represented in the table 2. The results show that all the secondary metabolites were found to be higher in the crude dry flower when compared with the aqueous flower extract.

Table 2: Quantative Phytochemical content of the dry crude and aqueous extract of *Aspilia africana* flowers.

Parameter (mg/100g)	Crude	Aqueous
Alkaloids	1175.00 ± 7.63^a	721.66 ± 6.00^b
Tannins	336.66 ± 1.66^a	158.33 ± 6.00^b
Anthraquinone	448.33 ± 4.40^a	193.33 ± 4.40^b
Terpinoids	73.33 ± 4.40^a	13.33 ± 1.66^b
Flavonoids	956.66 ± 6.00^a	226.66 ± 6.00^b
Phenolics	11.83 ± 0.44^a	4.40 ± 0.32^b
Saponins	511.67 ± 3.33^a	190.00 ± 2.89^b
Cardiac glycosides	17.77 ± 0.15^a	5.17 ± 0.17^b
Carotenoids	1363.33 ± 7.26^a	200.00 ± 2.89^b
Cyanogenic glyccosides	0.00 ± 0.00	0.00 ± 0.00
Steroids	190.00 ± 5.00^a	51.67 ± 3.33^b
Anthocyanins	73.33 ± 1.67^a	23.33 ± 1.67^b
Phlobatannins	34.00 ± 1.00^a	21.67 ± 1.67^b

Values represent mean ± SEM of triplicate determinations. The values on the same row that are followed by different superscript letter differ significantly. ($p<0.05$).

Proximate Composition of *Aspilia africana* crude and aqueous flower extract.

Proximate compositions of the crude and aqueous flower extract of *Aspilia africana* are shown below. The crude flowers were found to have higher concentration of protein, ash, crude fibre, fat and carbohydrate when compared with the aqueous extract. The aqueous extract on the other hand had higher amount of moisture than the crude dry flower.

Table 3: Proximate compositions of crude and aqueous flower extract of *Aspilia africana*.

Parameter (%)	Dry crude flower	Aqueous extract
Moisture content	8.47 ± 0.09^a	95.30 ± 0.12^b
Protein	17.53 ± 17.02^a	0.57 ± 0.033^b
Fat	2.37 ± 0.03^a	0.00 ± 0.00^b
Ash	9.17 ± 0.09^a	0.10 ± 0.00^b
Crude fibre	9.10 ± 0.58^a	0.00 ± 0.00^b
Carbohydrate	53.47 ± 0.47^a	4.03 ± 0.15^b

Values represent mean ± SE of triplicate determinations. The values on the same row that are followed by different superscript letter differ significantly (p<0.05).

Macro-mineral content of crude and aqueous extract of *Aspilia afriana* flower.

The levels of magnesium (mg), sodium (Na), potassium (K), phosphorus (P) and calcium (Ca) are shown below. The results show that sodium has a very concentration in the crude plant material, followed by phosphorus and calcium. Whilst potassium and magnesium are present in very low concentrations in the crude plant material, all the macro-minerals are present in trace concentrations in the aqueous extract of *Aspilia Africana* flowers.

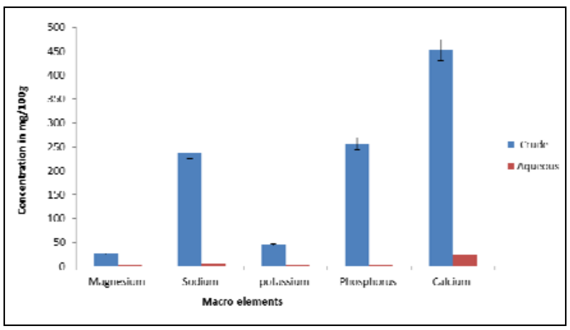

Fig 1: Macro-elemental compositions of *Aspilia africana* crude and aqueous flower extract. Values are Mean ± SEM.

Micro Mineral Composition of *A. africana* crude and aqueous flower extract.

Figure 2 shows the micro mineral content of the crude and aqueous flower extract of *A. africana*. The result shows that Iron (Fe) is the most abundant micro-mineral as it has high concentrations in both crude and aqueous extract. The concentration of copper (Cu) and zinc (Zn) are also higher in the crude while cadmium (Cd) and lead (Pb) are lacking in both crude and aqueous extract of *A. africana* flowers

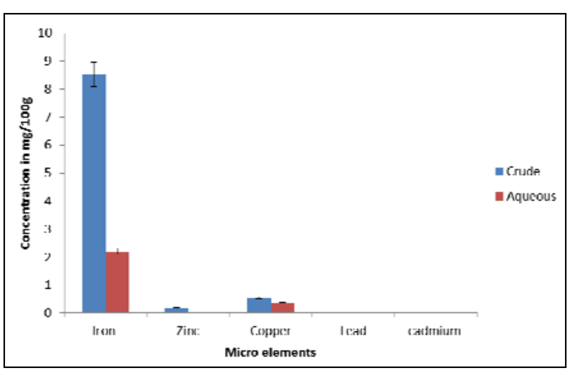

Fig 2: Micro mineral composition of crude and aqueous extract of *Aspilia africana* flowers

Discussion

Phytochemical analysis is of pivotal importance in identifying new and potent sources of therapeutically and industrially valuable phytocompounds in plants. It is an important tool in the determination of the bioactive components of medicinal plants, hence the fundamental scientific basis of their impact on health management. Qualitative and quantitative phytochemical analysis was carried out on the dry flower sample and the aqueous extract of *Aspilia africana* flowers. The qualitative and quantitative analysis of the phytochemical composition showed that the dry flower sample and the aqueous extract of *Aspilia africana* flowers possessed useful secondary metabolites of biological and medicinal interest. The result obtained in this study is in line with the works of Abii and Onuoha, (2011), Okwu and Josiah 2006 and at variance with the works of Uduak and Emana, (2013) [34, 35] who reported the complete absence of alkaloids and anthraquinones. There seem to be variation in the qualitative detectability of phytochemicals which may due to the processing procedure used on the extracts which may have resulted in the absence, increased or reduced presence of some phytochemicals. The notable differences between this study and previous works could be as a result of differences in reagents used and methods employed in the analysis, the time of the year when the plant materials were collected and geographical location as well as time lag between collection of plant materials and time of extraction. It is usually advisable to collect plant materials and extract almost immediately to forestall the physiological changes that may occur over time but this is usually not practiced especially as most Researchers take to drying their plants (which takes variable time) before extraction. However, the presence of these secondary metabolites in the flower of *Aspilia africana* is an indication of its potency as a medicinal plant material. Alkaloids have been used as a stimulant of the central nervous system, topical anaesthetic in ophthalmology, pain relievers, analgesic, and anti-spasmodic (Stray, 1998; Okwu, 2004; Osuagwu *et al.,* 2007; Samali *et al.,* 2012) [32, 22, 23, 27]. The phenolics present in the plant extract are a clear indication that the extract may have anti-microbial properties. This result is in line with reports of Agbor *et al*. (2012) [6], according to them, the methanol, ethanol and chloroform extracts of *Aspilia africana* leaves inhibited an array of microorganisms. The high saponin contents of *Aspilia Africana* leaf justify its use to treat wounds and stop bleeding. It has been reported that saponins have the ability to precipitate and coagulate red blood cells. They characteristically able to form foams in aqueous solutions, bring about hemolytic activity, have bitterness properties as well as cause cholesterol binding (Sodipo *et al.,* 2000; Okwu, 2004) [29, 22]. They have been found to be potent in strengthening cardiac muscle contractions. However, various studies have shown that though saponins are not toxic, they have the ability to can trigger physiologic responses that are more or less adverse when consumed especially in animals. They bring about cytotoxic effects as well as inhibition of growth in a number of cells and can be said to have anticancer or tumour inhibiting activity and anti-inflammatory properties (Akindahunsi and Salawu, 2005) [7]. Tannins have been reported as having astringent activities which helps to hasten wound healing and treat inflammations. This may be the reason why *Aspilia africana* is renowned for wound healing in line with the findings of Dimo *et al*. (2002) [12]. Flavonoids are antioxidants which have free radical scavenging ability, and are therefore able to prevent oxidative damage in cells

(Salah *et al.,* 1995; Okwu, 2004; Del-Rio *et al.,* 1997) [26, 22, 11]. As antioxidants, they have anti-inflammatory potentials (Okwu, 2004) [22]. This in part may be the reason behind the use of *Aspilia africana* in the treatment wounds, burns and ulcers in ethno-medicine. Cardiac glycosides are also important as they have been reported to be cardio-active and used in the treatment of heart conditions (Trease and Evans, 2002) [33].

The elemental content of plants play crucial roles in enhancing the activities of plants against different diseases due to definite correlation between mineral content in the human body with some disease conditions (Ceyik *et al.,* 2003). Again, more than 27% of known enzymes require minerals for their activity as may be seen with antioxidant enzymes (Abdulkadir *et al.,* 2011) [1]. This result is in line with the works of Okwu and Josiah, (2006) who reported the presence of the above macroelements in the leaves of *Aspilia africana*, with calcium as the most predominant. The differences in the concentrations of these elements between studies may be as a result of differences in the parts used, location and time of harvest. Inorganic elements are known for playing very important roles in the physiological processes that are involved in the maintenance of human health. The high concentration of calcium in the flowers of *Aspilia africana* suggests that this flower sample can produce a significant proportion of calcium and other essential minerals if consumed appropriately. Normal extracellular calcium concentrations have been reported to play vital roles in blood coagulation and other metabolic and maintenance functions (Okaka and Okaka, 2001) [19, 20]. So that the high calcium concentration of *Aspilia africana* flowers may the reason behind its effectiveness in wound healing and blood coagulation. These elements also act as inorganic cofactors in metabolic processes hence their absence can lead to impaired metabolism (Iheanacho and Udebuani, 2009) [14]. Since calcium helps in bone formation and blood coagulation, calcium and phosphorus deficiency may contribute to bone loss, bowl leggedness, knock knees, thoracic deformities and curved spine. Potassium is an intracellular cation and with sodium it controls the electric potential of the body's nerve pressure (Adeyeye and Aye, 2005) [4]. Sodium functions to regulate the amount of water retained in the body at any given time, and its passage to and from the cells which is relevant in various functions of the body. Inadequate Na^+, K^+ and $Mg^{2+,}$ which are mostly required in living cells, may lead to inadequate electrolyte balance in blood and poor enzymatic activity (Alli, 2009) [8]. Magnesium which was present in a relatively small concentration in *Aspilia Africana* flower have been reported to have the ability to bleeding disorders, growth retardation, congenital abnormalities, impaired spermatogenesis amongst other functions. Its deficiency causes muscular cramps, rigidity and spasm (Abii and Onuoha, 2011).

In almost all the metabolic processes in living organisms, iron is implicated as a key element which forms an integral part of most enzymes and proteins, acting as a catalytic centre in most enzymatic activities, and playing major roles in the process of oxygen binding in haemoglobin. (Okaka and Okaka, 2001) [19, 20].

Zinc also provides a protective mechanism against virus and its deficiency leads to the weakening of the immune system diarrhea, mental depression and slows down wound healing (Strausel and Saltman, 2000) [31]. Copper has an intrinsic ability to destroy a wide range of disease causing pathogens (Feder, 2008) [13]. *Aspilia africana* flower can therefore be recommended for inclusion in the diets of patients with iron deficiency anaemia. The mineral composition values obtained in this study probably reflects the physiological state of the flowers when they were harvested, which was in the dry season.

Proximate analysis is an analysis carried out scientifically to partition the right amounts of both nutrients and non-nutrients (hazardous substances) within an organic material into categories based on common chemical properties. It is employed by many researchers for the purpose of studying things like animal feed, bio-fuels and coal. On the other hand, food, supplements and plants products for human consumption are tested to ascertain their nutrient levels (Sadika *et al.,* 2006).

The analysis of the dry crude flowers of *Aspilia africana* revealed 8.47% moisture content, 17.53% Protein, 2.37% fat, 9.17% crude ash, 9.10% crude fibre and 53.4% carbohydrate. These results are comparable to the findings of Uduak and Umana, (2013) [34, 35] who reported moisture content of 15.7%, protein content of 7.87%, fat content of 3.86%, ash content of 6.10%, crude fibre of 12.30% and carbohydrate content of 75.97% in the stem of *Aspilia africana.* The aqueous extract revealed 95.30% moisture content, 0.57% protein, 4.03% carbohydrate, 0.10% ash and 0.00% fat and crude fibre.

The percentage moisture content of the *Aspilia africana* in the aqueous extract was much higher (95.30%) than in the dry flower dry flower which was 8.47%. This result is in agreement with the findings of Uduak and Ikoedem (2013) [34, 35] who reported the moisture content of 8.5% for *Aspilia africana* petals. Reports by Moyosore *et al*., (2013) stated a lower level (67.2 ± 0.1%) of the moisture content in the aqueous extract of *Tapinanthus bangwensis.* Due to the high values of the moisture content in the aqueous extract of *Aspilia Africana* flowers (95.30%), that of *Ipomoea batattas* (82.21%) will be considered relatively low (Antia *et al.,* 2006). Generally, high moisture content is tantamount to high susceptibility to microbes and may trigger enzyme activity (Adejumo and Awosanya, 2005) [3]. On the other hand low moisture content indicates less chances of microbial degradation of drugs during storage. The general requirement for moisture content in a crude drug is not more than 14% (British Pharmacopoeia, 1980), thus the values obtained from this study was within the accepted range for the dry flower but not the aqueous extract and this is in agreement with Musa *et al.* (2005) [18]; Schuna, (2010) [28].

Protein which is the major compound containing nitrogen in any food sample is used as an index of protein termed "crude protein" as distinct from true protein (Okon, 2005) [21]. In this study the composition of crude protein 17.53% present in the dry flower compared favorably with that of *Tapinanthus bangwensis* (15.32 ± 0.44) but was more than the normal levels recorded for most medicinal plants (Abolaji *et al.,* 2007; Odoemena and Ekpo, 2005). Uduak and Ikoedem, (2013) [2, 34, 35], reported a crude protein content of 11.81% for *Aspilia africana* petals. This indicates that *Aspilia africana* may possess relevant nutritive values as proteins which are vital for the synthesis of body tissues and regulatory substances, eg enzymes and hormones were present (Vaughan and Judd, 2003) [36]. The crude protein presented in the aqueous extract of this study was much lower (0.57%), implying that the dry flower plant material in *Aspilia Africana* is a better source of protein. Variation in the content of components of the same class may be related to genetic origin, geographical location, source, handling, solvent of extraction, extraction time and cultivation conditions. In addition, analytical techniques employed may also be responsible for the slight variations in the final results obtained (Loaksonen *et al.,* 2003).

The crude fat (2.37%) in this study was low compared to that of *Aspilia africana* petals (6.5%), *Amaranthus hybridus* (4.80%), *Talinum triangulare* (5.90%), *Calchorus africanum* (4.20%), *Baseila alba* (8.71%) (Akindahunsi and Salawu, 2005; Uduak and Ikoedem 2013) [34, 35, 7]. No crude fat was detected in the aqueous extract of *Aspilia africana* which implies that it has very low fat content. Dietary fat functions to make food more palatable by the absorbance and retaining of flavors (Antia *et al.,* 2006). Excess fat consumption is implicated in most diseases eg, cardiovascular disorders, cancer etc. therefore diets with energy provision of 1.2% calories has been reported to be sufficient for consumption in humans (Kris-Etherton *et al.,* 2002).

The total ash content which is the measurement of the amount of residual inorganic substances not volatilized after an organic matter has been ignited by heat and burnt away. Ash may be derived from the plant tissue itself (i.e. physiological ash) or from the extraneous matter, especially sand and soil adhering to the leaf surface (i.e. non-physiological ash), and these kinds of ash are determined together, therefore it is referred to as total ash (African Pharmacopoeia, 1986). High ash value represents the level of contaminations, adulteration, substitution or improper handling in the course of processing (Chandel *et al.,* 2011) [10]. In this study, ash content of 9.17% was found in the dry flower and 0.10% in the aqueous extract; which was reasonably low, indicating that there was either little or no contamination when stored and/or low mineral content preserved in the leaves of the plant or a consequence to volatilization due to the high temperature applied. This result compared favorably with that of Uduak and Ikoedem, (2013) [34, 35] which highlighted a lower total ash value of 5.16%. This is similar to earlier reports on *Tapinanthus bangwensis* (Moyosore *et al.,* 2013) which gave a value of 3.5±0.1% but in contrast to some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* (20.05%), *Occimum gratissimum* (8.0%) and *Herbiscus esculentus* (8.0%) (Akindahunsi and Salawu, 2005) [7].

The crude fibre content of 9.10% and 0.00% were recorded for dry flower and aqueous extract respectively. This is at variance with the findings of Uduak and Ikoedem, (2013) [34, 35] who recorded a total ash content of 22% for *Aspilia africana* petals. Fibre determination is the criteria for judging purity of herbal drugs (Agarwal, 2005) [5]. The implication of this result therefore is that the aqueous extract used in this study is devoid of impurities.

The total carbohydrate content obtained from this study was 53.43% for the dry flower and 4.03% for the aqueous extract. Uduak and Ikoedem (2013) [34, 35] reported total carbohydrate content of 59.69% which is in line with the findings of the present study. However the carbohydrate content of the aqueous extract reduced drastically to 4.03%, which goes to show that the dry flower flower is a better source of energy relative to the aqueous extract.

Conclusion

The present study has revealed the presence of several therapeutically relevant phytocompounds in the flowers of *Aspilia africana,* which validates its use as a medicinal plant part, nutritionally safe for consumption with several useful mineral elements. Again, the phytochemicals (quantitative), mineral elements and proximate compounds were generally more in the crude sample than in the aqueous extract, which is an indication that the water used for the aqueous extraction diluted the crude sample. We therefore recommend further studies using alcohols and other solvents for extraction.

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