ECLIPTA PROSTRATA: PHARMACOLOGY, MOLECULAR GENETIC PROPERTIES, AND CULTIVATION

VO TRUNG AU[1,2*], MUMUNI A. MUSAH WALA[4], DAM SAO MAI[3]

[1] *Faculty of Commerce and Tourism, Industrial university of Ho Chi Minh city*

[2] *Office of Science Management and International Affairs, Industrial university of Ho Chi Minh city*

[3] *Vice Principal, Industrial University of Ho Chi Minh City, Ho Chi Minh City, Vietnam*

[4] *GAP personnel/ Melitta Hazeldine House Overdale, Telford TF3 4JL, United Kingdom*

[*] *Corresponding author: votrungau@iuh.edu.vn*

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ABSTRACT. *Eclipta prostrata* (L.), (Asteraceae), commonly known as *Cỏ Mực* or *Nhọ Nồi* in Vietnamese, has been used as a traditional medicine in many countries especially in tropical and sub-tropical regions. Some important pharmacological properties of this plant are analgesic, antioxidant, anti- inflammation, HIV1 (Human immunodeficiency virus 1), anti- tumor, antibacterial, anti- termite etc. Previous reviews indicated the photochemical and pharmaceutical properties of *Eclipta* spp. while others were on its tissue cultivation. However, many details about the botanical, experimental or research on *E. prostrata* are limited. Researches on *Eclipta* spp. mostly focus on the pharmaceutical properties while the biodiversity and biological cultivation is still not marked. Current status of bioactivities, pharmacological profile, biotechnology application, molecular approach, and chloroplast whole genome sequence and their agronomic properties of *E. prostrata* are covered in this review.

Keywords: *Eclipta prostrata, medicinal plant, phytochemical, cultivation, chloroplast whole genome.*

1. INTRODUCTION

Medicinal plants have immense significance and simply available source of therapy in the primary healthcare system of humans. According to World Health Organization (WHO), 80% of the world's population are using traditional medicines which involve the use of their constituents or plant extracts as tea and functional foods [1]. More than 50,000 plants species are used in traditional medicines (TMs) worldwide and Asian medicines are not exceptional. These plants contain the products of herbal medicines, ethnos medicines, essential oils, cosmetics and secondary products such as flavonoids, alkaloids, phenolic compounds and tannins [2,3]. The scholarly TM systems and folklore medicine are the two main ways of using Asia medicinal plants. However, both are neglected and undocumented but folklore medicine is still thriving in many Asian countries [4,5].

A lot of studies on the biological properties with therapeutic potential have been recorded worldwide and it was observed that over 7,000 medical compounds used in drugs in modern pharmacopoeia are derived from plants [6]. Vietnam has identified 14,000 species of flora, 10,500 species of highly valuable plants, and among which approximately 3,780 species have medicinal properties [7]. *Eclipta prostra**ta*** is a native and popular plant in Asia especially in India, China and Vietnam, mainly used as folk medicine. This plant belongs to therophyte herb of the family Asteraceae and recognized to possess some pharmacological features in the treatment of several disease, including liver disease, blood lipid development, hair dyeing, snakebite envenomation, cancer, anti- HIV, loose teeth, diabetes type II, anti-inflammatory, dizziness and haemoptysis [8-12]. More recently, 3,5-diCQA found in *E. prostrata* was closed to an antiviral compound named remdesivir, that could treat Covid-19 by binding to Mpro Protein (serine -type protease) [13].

Recently, several scientific publications concerning the pharmaceutical potentials and the potency of *E. prostrata* in treating illness has been proven. However, research on the agronomic, full genome sequence and biological impact on the plant has been ignored.

2. MATERIALS AND METHODS

To gather pertinent information from literature articles published before January 2022, a variety of scientific databases including Google Scholar, Web of Science, PubMed, and Scifinder were employed. In addition, local books such as "Cây thuốc và vị thuốc Việt Nam" and "Từ điền cây thuốc Việt Nam" were consulted. The search was conducted using keywords such as "*Eclipta prostrata*", "cultivations", "phytochemistry", "medicinal plant", and "chloroplast genome", with no time limitations. The validity of plant taxonomy was also verified by utilizing "The Plant List" database. Chloroplast genome map of *E. prostrata* was generated using OGDRAW (http://ogdraw.mpimp-golm.mpg.de/).

3. BOTANICAL DESCRIPTION AND DISTRIBUTION

E. prostrata is widely distributed in tropical and subtropical regions including 86 countries and is commonly found in Asia, North America and some countries in Africa, Europe, Oceana and Central America and the Caribbean [14-16]. It strives well in moist soil, hilly regions, pH from 4-8 and a wide range of temperature from 20-38 celsius and can be harvested after 2-3 months [17].

In Vietnam, *E. prostrata* was recorded by Holm et al. [14] and described by Pham [18] though the plant has been used for several generations. It has simple leaves arrangement, opposite, ovate of oblong-lanceolate, 2-10 cm long, 1-3 cm wide, apex acute or blunt. The flowers grow from the axis of the leaf in clusters, small in shape with a white color. The head of flowers is approximate 1cm in diameter. Ray flowers margina has l2-3 mm long, pistillate, fertile, corolla white, and ligulate. Disk flowers numerous has 1.5-2 mm long, central, perfect, fertile, corolla whitish, and tubular. It has five stamens, separated filaments; anthers are merged to form a tube around the style. An eclipta plant can produce 17,000 seeds in one growing season [19]. The life span of *E. prostrata* starts from summer with high potential mass percentage and have an optimum growth condition than other weathers. During the life cycle, *E. prostrata* can have the flower of emergency after five weeks and anchene in the next two weeks [20].

4. TRADITIONAL USES FROM MEDICINAL VALUES

In Vietnam, *E. prostrata* (Cỏ Mực, or Nhọ Nồi) is a common plant in commercial medicinal market. The treatment dose usually involves 30-50g fresh or 12-20g dry and is consumed in the form of extracted juice.

It has been used to treat many diseases such as: internal and external bleeding, hemorrhage, epistaxis, hemorrhoids, menorrhagia, bloody stool and urination, vomiting and bleeding under the skin, hemoptysis, furry tongue, measles, asthma, sore throat, burns and skin fungus. In addition, in India, this plant is known as an anthelmintic, hair rejuvenator, digestive, liver stimulant, excellent appetite stimulant, anti-inflammatory and analgesic [21]. In Korea, the plant is called genus Eclipta and is an important raw material for pharmaceutical and food industries [22]. In Nepal, the mixture of Eclipta prostrata and an aromatic (essential) oil is used to treat catarrhal inflammation and jaundice, and the leaves are used to treat scorpion stings [23]. In China, the leaves are used for blood spitting, hematuria, tooth loss, tinnitus (ringing in the ears), dizziness, and uterine bleeding [10].

5. PHYTOCHEMICAL COMPONENTS

Previous reports found out that *E. prostrata* contains a lot of natural compounds including flavonoids, alkaloids, triterpenoids saponins, phenolic, essentials oil, thiophenes and steroid [9-10, 24-25]. Moreover, Dhandapan et al 2008 was recorded that *E. prostrata* has a high content of tannin (11.86%), saponin (1.7%), alkaloid (0.34%) and flavonoid (0.87%) respectively [26]. Fang et al 2015 have been identified six phenolic acids, six flavonoids' glycosides and one coumarin in *Eclipta prostrata* with HPLC, DAD, ESI, MS/MS technique [27]. Eight group of natural compounds contents in *E. prostrata* is shown in Table 1.

Table 1: the natural compounds contents in *Eclipta prostrata* [26, 27, 88].

Group	Name of chemical inside	Chemical formula
Thiopenes	Polyacetylenic thiopenes	
Flavonoids	Apigenin	$C_{15}H_{10}O_5$
	Luteolin	$C_{15}H_{10}O_6$
	Luteolin-7-glucoside	$C_{21}H_{20}O_{11}$
Steroids	Diosgenin	$C_{27}H_{42}O_3$
	Tigogenin	$C_{27}H_{44}O_3$
	Lanosterol	$C_{30}H_{50}O$
Miscellaneous	1-Nonacosanol	$C_{29}H_{60}O$
	Stearic acid	$C_{18}H_{36}O_2$
	Lacceroic acid	C32H64O2
	dihydroxybenzoic acid	C7H6O4
Triterpenes	Ecliptasaponin A, B, C (1) & D	C36H58O9
	Oleanolic acid	$C_{30}H_{48}O_3$
	α- amyrin	$C_{30}H_{50}O$
	β- amyrin	$C_{30}H_{50}O$
	Ursolic acid	$C_{30}H_{48}O_3$
Coumarins	Wedelolactone	$C_{16}H_{10}O_7$
	Dimethylewedelolactone- 7-glucoside	$C_{21}H_{18}O_{12}$
	Wedelolactone	$C_{16}H_{10}O_7$
	Demethylwedelolactone	$C_{15}H_8O_7$
	Isodemethylwedelolactone	$C_{15}H_8O_7$
Alkaloids	Ecliptine	
	Nicotine	$C_{10}H_{14}N_2$
	Steroidal	
	Alkaloids	

6. PHARMACOLOGICALLY ACTIVITIES

E. prostrata is play an important role in traditional treatment due to contains valuable natural compounds, including coumestans, demethylwedelolactone, tannin, weldelolactone and flavonoids (Tab 1). In that wedelolactone is found to possess strong hepatoprotective or anti-hepatotoxicity, anti- tumor, anti-inflammatory and snake venom [28- 31]. Wedelolactone and demethylwedelolactone belong to the phenolic compounds group from this plant have been shown to possess various biological properties including anti-

inflammatory, anti-carcinogenic and anti- atherosclerotic, which can be related to antioxidant activity [32,33]. Triterpenoids isolated from this plant displayed anti-proliferative and antimicrobial potentials [34,35], and is effective for 28 strains of Negative and Positive bacteria [36]. Moreover, in the treatment of diseases the different parts of the EP plant also have important functions (Tab 2).

Table 2: Pharmacologically activities of *Eclipta prostrata*

No	Medicinal value	Part used, extracted contents	References
1	UVB protect	Aerial part	[37]
2	infective *Trichophyton* and *Microsporum* spp.	Aerial part	[38]
3	Antibacterial activity	Leaves	[39]
4	Antidiabetic principle	Whole plant	[40]
5	Malaria	Leaves	[41]
6	HIV -1	Aerial part	[42]
7	Ovarian cancer cell	Aerial part	[43]
8	Hepatotoxicity	Leaves	[44]
9	Lipid lowering	whole plant or leaves	[45]
10	Anti venom	Aerial part	[46,47]
11	Invitro: Inhibit cell migration and in vivo: exhibits anti angiogenic	Aerial part	[48]
12	Immunomodulatory activity	Whole plant	[49]
13	Anti-proliferative in HBCs	Aerial part	[34]
14	Anti-inflammation	whole plant	[50,11]
15	Osteoprotective	Triterpenoid	[51]
16	Induces apoptosis	α- erthienylmethanol	[52]
17	Inhibition of ticks and fluke in veterinary	Leaves	[53]
18	Inhibition of Japanese encephalitis vector, *Culex tritaeniorhynchus*	Leaves	[54]
19	Against fish nodavirus	Leaves (Dasyscyphin C)	[55]
20	Antimicrobial activity	Leaves (saponin)	[56]
21	Induces autophagic and apoptotic cell death in human ovarian cancer cells	Eclalbasaponin II	[57]
22	anti-termitic activity	Leaves	[58]
23	Antitumor activity	Eclalbasaponin I	[9]
24	Herbicidal activity,	2-phenyliminothiazolidine	[59]
25	Against Haemonchus contortus	Leaves	[60]
26	Antioxidant activity	Leaves	[61]
27	Osteoporosis prevention	Whole plan	[62]
28	Promoting hair growth (Anagen)	Aerial part	[63]
29	Induces apoptosis in human endometrial cancer cell	Aerial part	[52]

7. CULTIVATION STUDIES

According to history, 80 percent and 20 percent of valuable quantities of medicinal plants were obtained from the forest and the non-forest regions respectively [64]. In contrast, nowadays, the advantages of using medicinal plants outweigh industrial drugs and this is deemed to change the scenario of obtaining these plants with over 80 percent cultivated and less than 20 percent from the forest [65]. Thus, scientists are studying on domestication of wild plant but it requires a great deal of research and, in particular, high value medicinal plant need more time and money.

Recently, many researchers are concerned about *E. prostrata* because it is known to have various pharmacological properties and was traditionally used in treatments but lacks adequate scientific research on agronomic characteristics, optimal cultivation conditions and environmental effects.

From 2008, Bhagirath and David [17] showed the influence of environmental factor on the germination of *E. prostrata* seed in tropical environment. Results indicated that the seed germination is favored by long day light, greater in the soil surface below 0.5 cm deep, temperature in light/dark about 30/20 Celsius, pH parameter from 4 to 10, corresponding 87 to 93% of seed germinated. In addition, the effect of salinity stress on germination is obtained at different concentrations of NaCl thus 0 mM to 250 mM recorded germination rate from 83% to 0%, respectively.

Kalita and Singh [65] demonstrated that plant growth and yield was better with wider spacing viz 25 x25 cm and 30x 30 cm and F5- $N_{60}P_{40}K_{20}$ + 5 t FYM/ha fertilizer supplementation. The average total fresh and dried biomass of *E. prostrata* after harvest during the two years' work was approximated to be 187 q/ha and 28 q/ha, respectively.

On the other hand, some scientists are concerned with the aim to change or reduce the use of chemical or organic fertilizer supplements during the cultivation of *E. prostrata*. Previous studies on the function of microorganism in agricultural fields which can exist in synergy with plants roots and increase nutrient element, especially arbuscular mycorrhizal fungi are a crucial candidate which can associate with more than 80% of terrestrial plants [66]. Research of medicinal plants have shown that they can be a non- mycorrhizal, due to the presence of various secondary metabolites which inhibits mycelium penetration into the host plants [67]. Fortunately, arbuscular mycorrhizal fungi (AMF) association was achieved on *E. prostrata*. Sundar et al. (2010) [68] showed the association between 21 AM fungi species found in the root and soil of three medicinal plants and rate of root colonization in *E. prostrata* was 64% high.

The nutrients acquisition of the plants increased the active surface area of the root system. Subsequently, protein and amino acid content were higher in AMF plant compared to non- AMF plant [69]. The growth of AMF inoculated with *E. prostrata* under water stress, showed positive results with high biomass and root length, against stress more than in the control treatment [70].

Sinha and Raghuwanshi [71] elucidated that drought stress tolerant was observed on the growth of *E. prostrata* by the influence of AMF and helper bacteria. The result showed a higher content of proline and phenolic of 48% and approximately 120% respectively in the AMF treated plants. In addition, 143.7 % higher dried biomass, an increase of 42 % chlorophyll a and b and secondary metabolism was observed in AMF treated plants compared to non-AMF plant. This showed the roles and mutualistic association with *E. prostrata* which can help plant against drought stress and maintain its integrity.

In 2019, Vo et al. [72] indicated that the highest impact of mycorrhizal inoculation and sand-peat (growth substrate) 60:40% of weight composition on the biomass, and individual polyphenol concentration of *Eclipta prostrata*. And through the HPLC technique to determine some secondary metabolites of *E. prostrata*, inoculated found that the treatment with mycorrhizal fungi has significant differences in polyphenol contents between the treatments. One year later, Duc et al. [73] showed that salt tress affected direct to growth of EP. However, the polyphenol compound from EP changed, wedelolactone high in group of plants cultivated with arbuscular mycorrhizal fungi after 8 weeks.

8. MOLECULAR GENETICS, AND PHYLOGENY TREE

8.1. *Genome size, chloroplast genome sequence and phylogenetic tree of E. prostrata*

E. prostrata belongs to the eudicots plant group and genome close to *Helianthus annuus* plant (sunflower has 17 chromosomes). However, the rate genomic and genetic studies for understanding the function and breeding of this plant. *E. prostrata* has total 22 alleles (2n= 22) [74]. The *Eclipta* plant genome size of approximately 4.27 x109 bps was determined by DNA extraction from the fresh young leaves and flow cytometric calculations using Pisum sativum as the standard [75]. Moreover, genome size represents the genomic library and an additional parameter for species-specific phenology [76,77]. The major organelles in plants have a gene sequence, such as chloroplasts, mitochondria, and nuclei. Consequently, the chloroplast plays a key role in the active metabolic centres of photosynthesis, in cellular responses to signals, and in response via retrograde signalling. The production synthesis of amino acids, nucleotides, fatty acids, phytohormones, vitamins, and a variety of metabolites, as well as the assimilation of sulphur and nitrogen. Plant responses to heat, drought, salt, light, pH, and pathogens occur in the chloroplast organism [78,79]. Thus, the complete genome sequences were discussed with all scientists and more than 800 complete genome sequences were made available in the National Centre for Biotechnology Information (NCBI) organelle genome database, including 300 crops and 80 trees [79]. The chloroplast genome of the plant E. prostrata was 151,757 bps long and consisted of a large single copy sequence of 83,285 bps, a small single copy region of 18,346 bps, and a pair of inverted repeats of 25,063 bps. In addition, the genome housed 80 protein-coding sequences, 30 tRNA genes, and 4 rRNA genes.

8.2. *Phylogenetic tree of E. prostrata plant*

Studies showed the difference in morphology between *E. prostrata*, by having cypselas smooth and fringed and *E. alba* with cypselas tuberculate along the ribs or over whole surface [80]. This may be explained by new found from Kim et al., 2017 [81] about comparing the chloroplast genome sequence, which differs at raw data base pair 1,473,824,997 (*E. prostrata*) vs 1,027,482,009 (*E. alba*). In addition, Cp genome average coverage also recorded a difference between *E. alba* and *E. prostrata* plant with 89.3 (bp) and 317.7 (bp), respectively (Tab 3). Phylogenetic analysis was done using 10 chloroplast genomes of 10 species belonging to Ateraceae family. *E. prostrata* and *E. alba* are in the same group indicating that they have very narrow genetic diversity. In addition, *Helianthus annuus* plant is also in tribe group with *E. alba* and *E. prostrata* plant.

Table 3. Summary of NGS data chloroplast genome of *E. alba* and *E. prostrata* [89]

Collected species	Raw data bases (bp)	Cp genome coverage (bp)	Cp length (bp)	Genbank accessions	LSC length (bp)	IR length (bp)	SSC length (bp)
E. alba	1,027,482,009	89.31	151,733	MF993496	83,300	25,075	18,283
E. prostrata[z]	1,473,824,997	317.69	151,757	KU361242	83,285	25,063	18,346

[z)] reported by Park et al., (2016) [82]

In the Table 3 showed the difference in cp genome and raw data base between *Eclipta prostrata* and *Elipta alba*, it may be as a result of the difference in primary and secondary metabolism in the plant synthesis.

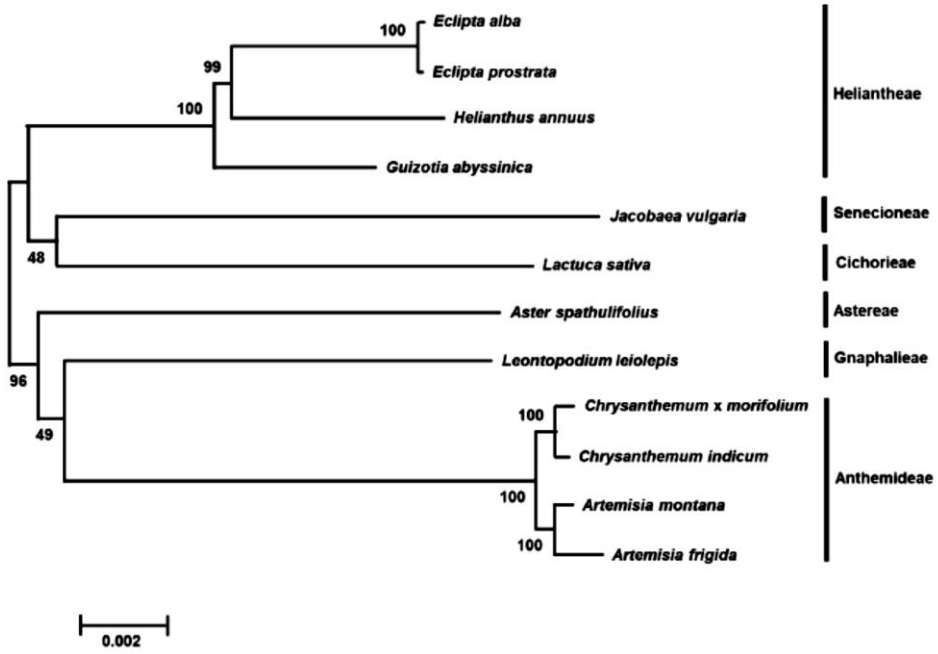

Figure 2: Phylogeny of *Eclipta prostrata* and *Eclipta. alba*

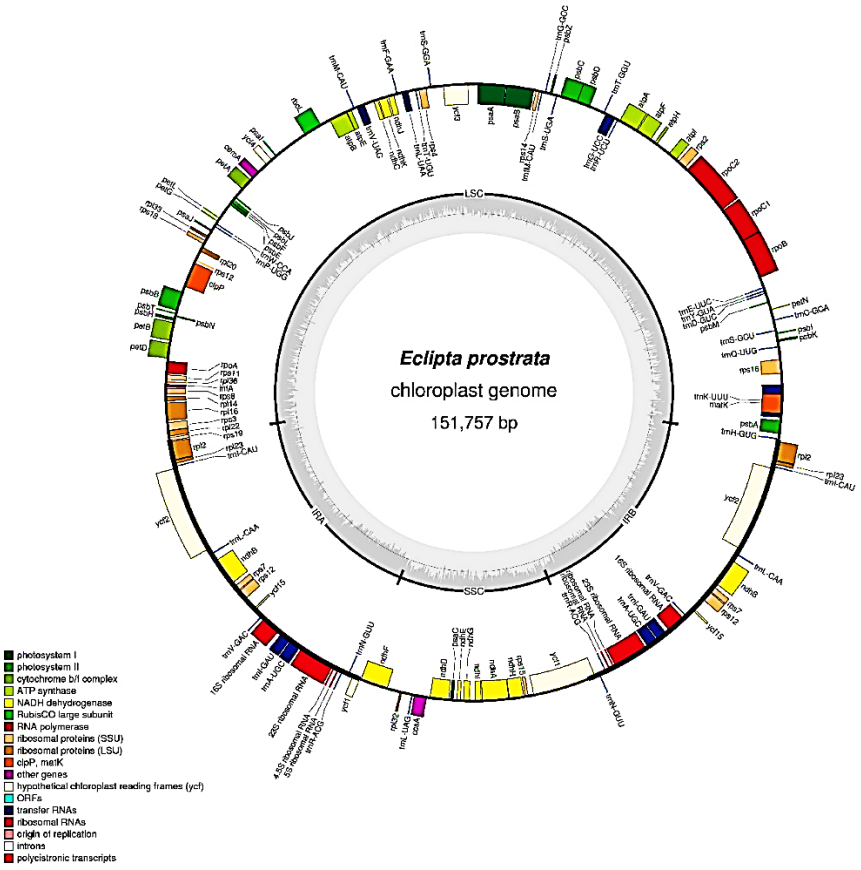

Figure 1**.** Chloroplast genome map of *E. prostrata* was generated using OGDRAW (http://ogdraw.mpimp-golm.mpg.de/). Genes transcribed clockwise and counterclockwise are indicated on the outside and inside of the large circle, respectively.

9. BIOTECHNOLOGY IN ECLIPTA PROSTRATA

The advance in molecular genetics and biotechnology in the field of medicinal plant are discussed with focusing on improve field-grown including slow growth cycles, fluctuation in quantity, low yield, and disease. There is increasing demand for plant raw material at the global level from pharmaceutical industry and dietary supplements; biotechnology has an answer for their conservation and improvement. Biotechnology plays a crucial role in multiplication and genetic achievement of medicinal plant by using technique in vitro propagation and genetic transformation. In the previous studies, there are several reports on in vitro plant propagation of *Eclipta* plant [83- 86]. Recently, Maciel et al 2021 [87] found that after 21st day hairy root C19 cultivated under Jasmonates (JA) and MeJA the result showed that wedelolactone, demethylwedelolactone and especially 3,5-diCQA are three major compounds found in root extraction. In that 3,5- diCQA can play a crucial activity against Sar-CoV 2 disease [87].

10. CONCLUSION

Eclipta prostrata is an important plant with high potential of medicinal compounds. In this brief review, the existing sufficient research on the biological properties, molecular genetics, agriculture properties and the second metabolism pathway of this plant in the molecular process. Moreover, the difference between *E. alba* and *E. prostrata* is obvious from morphology to molecular and especially in the chloroplast genome. This finding will help marketing business which can recognize *E. alba* and *E. prostrata* by specific marker [81].

In addition, *E. prostrata* can be cultivated on the field under ambient conditions and introduction of microorganisms into the rhizosphere can have a positive effect on the growth of the plant and enable it to produce more constitutive medicine for human use. Finally, further investigations are required for a better understanding and utilization of this plant.

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CÂY NHỌ NỒI (CỎ MỰC): DƯỢC LÝ HỌC, ĐẶC TÍNH DI TRUYỀN PHÂN TỬ VÀ NGHIÊN CỨU ỨNG DỤNG TRỒNG TRỌT

VÕ TRUNG ÂU[1,2], MUMUNI A. MUSAH WALA[3], ĐÀM SAO MAI[1]

[1] *Khoa Thương mại và Du lịch, Trường Đại học Công Nghiệp Thành phố Hồ Chí Minh*

[2] *Viện Nghiên cứu Ứng dụng và Đổi mới Sáng tạo trong Doanh nghiệp*

[3] *Viện GAP/ Melitta Hazeldine House Overdale, Telford TF3 4JL, Vương quốc Anh*

**Tác giả liên hệ: votrungau@iuh.edu.vn*

TÓM TẮT. *Eclipta prostrata*, họ Cúc (Asteraceae), thường được gọi là Cỏ mực hoặc Nhọ nồi, đã được sử dụng làm thuốc y học cổ truyền ở nhiều nước, đặc biệt là ở các vùng nhiệt đới và cận nhiệt đới. Các đặc tính dược lý quan trọng nhất của loại cây này là giảm đau, chống oxy hóa, chống viêm nhiễm, diệt virus HIV 1(virus làm suy giảm hệ miễn dịch ở người), chống khối u, chống vi khuẩn, chống mối mọt, v.v. Các nghiên cứu trước đây chỉ ra các đặc tính quang hóa và dược phẩm của cỏ mực trong khi nhữngnhà khoa học khác đã thuần hóa và nuôi cấy mô trong phòng thí nghiệm . Tuy nhiên, nhiều chi tiết về thực vật, thí nghiệm hoặc nghiên cứu về cây cỏ mực vẫn chưa được rõ ràng. Các nghiên cứu về cây cỏ mực chủ yếu tập trung vào các đặc tính dược phẩm trong khi sự đa dạng sinh học và canh tác sinh học vẫn chưa được được nghiên cứu. Hiện trạng về hoạt tính sinh học, đặc tính dược lý, ứng dụng công nghệ sinh học, phương pháp tiếp cận phân tử, và trình tự bộ gen toàn bộ lục lạp và các đặc tính nông học của cây Cỏ mực được đề cập trong bài tổng quan này.

Từ khóa: *Cỏ mực, cây dược liệu, dược chất, nuôi trồng, bộ gen lục lạp*

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