Recent Studies on Phytochemicals and Pharmacological Effects of *Eleutherine americana* Merr.

Muhamad Insanu*a*, Siti Kusmariyani*a*, Rika Hartati*a*

*aSchool of Pharmacy, Institut Teknologi Bandung, Jl Ganesha 10, Bandung 40132, Indonesia*

**Abstract**

*Eleutherine americana* Merr., a medicinal plant, which has been widely cultivated in South East Asia. Ethnobotanically, the plant is known for treating coronary abnormality. Other pharmacological investigations showed its potency as antimicrobial and anti-inflammatory agents, and also as inhibitors of α-glucosidase, HIV replication, and topoisomerase II. Phytochemically, three groups of compounds have been isolated i.e. naphthalene, naphtoquinone and anthraquinone. This review highlights recent data on biological activities and discusses strategies for developing the plant into a more valuable commodity.

**Keywords:** *Eleutherine americana*; Iridaceae; naphthalene; naphtoquinone; anthraquinone

**1. Introduction**

*Eleutherine Americana* Merr. also known as *E. bulbosa* (Miller) Urb., is a medicinal plant belonging to Iridaceae family. This plant grows in 600-2000 m above sea level. It arises in sulphuric areas. The plant is originated from tropical America1, but now is widely cultivated in Kalimantan island (Indonesia)2, Hainan island (South China)3-5, Thailand6, and South Africa1.
2. Traditional Uses

Traditionally *E. americana* is used ascarminative, together with galangal it can treat cold and nasal congestion in children\(^1\). In Dayak tribe, some people used the bulb for increasing breast-milk production as well as treatment of diabetes, breast cancer, stroke, hypertension and sexual disorder\(^2\). In other areas it is found to treat coronary disorder\(^3\), and used as diuretic, emetic, purgative\(^4\), prothrombin decreasing\(^5\), antifertilit\(^6\), antihipertension\(^7\), wound healing activity\(^8\).

3. Phytochemistry

There are three big groups of compounds which have been isolated from *E. Americana* i.e. naphthalene, anthraquinone and naphtoquinone. Other compounds were also reported from this plant including stigmasterol-3-O-β-D-glucopyranoside, kadsuric acid and stigmasterol (Table 1 and Fig. 1.). The compounds isolated from *E. americana* were hongconin; eleutherol; isoeleutherol; eleutherin; isoeleutherin; eleutherolin; eleutherinoside A & B; elecanacin; eleuthinone A; eleuthraquinone A&B; eleucainorol; erythrolaccin; \((-\)-3-[(acetyloxypropyl)]-2-hydroxy-8-methoxy-1,4-naphthoquinone; \(1,5\)-dihydroxy-3-methyl anthraquinone; \(3,4,8\)-trihydroxy-1-methyl-anthra-9,10-quinone-2-carboxylic acid methyl-ester; \(3,4\)-dihydro-1,3-dimethyl-1-H-naphthol (2,3) pyran-5,10,dione; \(4,8\)-dihydroxy-3,4-dimethoxy-1-methyl-anthraquinone-2-carboxylic acid methyl ester; \(3,2\)-acetoxy prophy 2-hydroxy-8-methoxy-1,4-naphthoquinone; \(1,2\)-dihydroxy-8-methoxy-3-methylantraquinone; \(9\)-hydroxy-8-methoxy-1-methyl-1,3-dihyronaphtho [2, 3-c] furan-4-O-β-D-glucopyranoside; \(1, 2\)-dihydroxy-8-methoxy-3-methyl-anthra-9,10-quinone; \(9\)-methoxy-1, 3-dimethyl-1H-naphtho [2, 3-c] pyran-5, 10-dione\(^3,4,5,6,13,14,2\).

4. Pharmacological Properties

4.1. Antimicrobial

*E. americana* was previously reported to show good antibacterial activity. Ethanol extract of *E. Americana* inhibited several Gram positive bacteria like *Staphylococcus aureus* ATCC 23235 and 27664, *Streptococcus mutans* and *S. pyogenes* with their Minimum Inhibitory Concentrations (MICs) were ranged between 120-125 \(\mu\)g/mL while their Minimum Bactericidal Concentrations (MBCs) were ranged between 250-1000 \(\mu\)g/mL. The MIC/MBC were 250/250, 125/500, 250/500, respectively\(^2\). It also inhibited the growth of methycillin resistant *Staphyllococcus aureus* (MRSA) with their MICs were ranged between 62.5-1000 \(\mu\)g/mL. It was proved that giving the extract for 12h with 2 MIC and 4 MIC enlarged the cells and increased the thickness of cell membranes. It will denature the cytoplasm and disrupt the membrane and causing leakage in that of *S. aureus*. The growth curve of bacteria that was given the extract of 4 MIC showed a 5 log reduction relative to the control\(^16\). It also inhibited the formation of biofilm in *S. pyogenes* at concentration of 1/32 to \(\frac{1}{2}\) MIC\(^17\). This extract was also good for inhibiting *Campilobacter* species (including *Campylobacter coli* MUMT 18630, *C. fetus* ATCC 27374, *C. jejuni* ATCC 81176, *C. lari* ATCC 43675, and *C. upsaliensis* DMST 19055) with the Inhibition zones ranged from 10 to 37 mm. The MICs were ranged from 31.25 to 500 \(\mu\)g/mL while the minimum bactericidal concentrations ranged were from 31.25 to 1,000 \(\mu\)g/mL\(^18\).

Nowadays, the *E. americana* extract is used for food preservative\(^2\). The investigation on the effect of ethanol extract was done for home made salad *in vitro* and *in vivo*. It showed good stability in response to heat and pH treatments. No significant differences in the appearance, color and taste\(^19\). The effect of Ethanolic extract of *E. americana* against lipase, protease enzymes related to the production of enterotoxin by *S. aureus* were observed. The result showed inhibition to both enzymes which delay the production of enterotoxin in food by *S. aureus*\(^20\). After it was partially purified, the fractions consist of eleutherol, eleutherin, isoeleutherin, hongconin, two anthraquinones, and elecanacin. Their MICs against MRSA were ranged from 31.25 to 1000 \(\mu\)g/mL\(^21\). Not only active against bacteria, crude ethanolic extract was also active against several fungi like *Aspergillus niger*, *Rhizopus* spp. and *Pénicillium* spp\(^22\). From the explanation above, it was concluded that this plant possessed good
antimicrobial activity due to the content of naphtoquinones. It was believed that they affected electron transport in the cell component.

| No | Name of compound                                   | Group of compound | Activity                                                                 | References                          |
|----|-----------------------------------------------------|-------------------|--------------------------------------------------------------------------|-------------------------------------|
| 1  | Eleutherol                                          | Naphtalene        | Increasing coronary flow in guinea pig                                   | [5]                                 |
| 2  | Eleutherin                                          | Naphtoquinone     | Increasing coronary flow in guinea pig                                   | [4, 5, 26]                          |
|    |                                                     |                   | *Pyricularia oryzae*, MMDC = 30 μg/mL                                    |                                     |
|    |                                                     |                   | Inhibit proliferation of K562, IC₅₀ = 49 μg/mL                            |                                     |
|    |                                                     |                   | Inhibit human topoisomerase II IC₅₀ = 50 μg/mL                           |                                     |
|    |                                                     |                   | Inhibit RAW 264.7 lipopolysacharide-activated mouse macrophage cell with IC₅₀ = 11.4 μM |                                     |
| 3  | Isoeleutherin                                       | Naphtoquinone     | Increasing coronary flow in guinea pig                                   | [4, 5, 14, 26, 27]                  |
|    |                                                     |                   | *Pyricularia oryzae*, MMDC = 30 μg/mL                                    |                                     |
|    |                                                     |                   | Inhibit proliferation of K562, IC₅₀ = 33 μg/mL                            |                                     |
|    |                                                     |                   | Inhibit HIV replication in Hg lymphocytes , IC₅₀ = 8.5 μg/mL             |                                     |
|    |                                                     |                   | Inhibit RAW 264.7 lipopolysacharide-activated mouse macrophage cell with IC₅₀ = 7.7 μM |                                     |
| 4  | 4,8-dihydroxy-3-methoxy-1-methyl anthraquinone-2-carboxylic acid methyl ester | Anthraquinone    | *Pyricularia oryzae*, MMDC = 55 μg/mL                                    | [5, 26]                             |
| 5  | 8-hydroxy-3,4-dimethoxy-1-methylantra quinone-2-carboxylic acid methyl ester | Anthraquinone    | *Pyricularia oryzae*, MMDC = 170 μg/mL                                   | [5, 26]                             |
|    |                                                     |                   | Inhibit proliferation of K562, IC₅₀ = 266 μg/mL                           |                                     |
| 6  | 3,4,8-trimethoxy-1-methylantra quinone-2-carboxylic acid methyl ester | Anthraquinone    |                                                                         | [5]                                 |
| 7  | Hongconin                                           | Naphtalene        | *Pyricularia oryzae*, MMDC = 130 μg/mL                                   | [3, 14, 26]                         |
|    |                                                     |                   | Inhibit proliferation of K562, IC₅₀ = 174 μg/mL                           |                                     |
|    |                                                     |                   | Inhibit RAW 264.7 lipopolysacharide-activated mouse macrophage cell with IC₅₀ = 19.8 μM |                                     |
| 8  | Elecanacin                                          | Naphtoquinone     |                                                                         | [4]                                 |
| 9  | Isoeleutherol                                       | Naphtalene        | Inhibit HIV replication IC₅₀ = 100 μg/mL                                 | [4]                                 |
| 10 | (-)-3-[2-(acetyloxy)propyl]-2-hydroxy-8-methoxy-1,4-naphtoquinone |             |                                                                         | [14]                                |
| 11 | Eleutherinol                                        | Naphtoquinone     | Inhibit RAW 264.7 lipopolysacharide-activated mouse macrophage cell with IC₅₀ = 34.4 μM  | [14]                                |
| 12 | 1,5-dihydroxy-3-methylnanthraquinone                | Naphtoquinone     |                                                                         | [14]                                |
| 13 | Dihydroeleutherin                                   | Naphtoquinone     | *Pyricularia oryzae* MMDC = 60 μg/mL,                                   | [14, 26]                            |
|    |                                                     |                   | Inhibit RAW 264.7 lipopolysacharide-activated mouse macrophage cell with IC₅₀ = 21.7 μM |                                     |
| 14 | 2,5-dimethyl-10-hydroxynaphopyrone 8-O-β-            | Naphtalene        |                                                                         | [28]                                |
| Compound | Structure | Activity
|----------|-----------|----------|
| Eleuthoside A | Naphtalene | β-glucosidase inhibitor with IC₅₀ = 0.5 mM [2] |
| Eleuthoside B | | |
| Eleuthinone A | Naphtoquinone | |
| Eleuthraquinone A | Anthraquinone | |
| Eleuthraquinone B | Anthraquinone | |
| Eleucanarol | Naphtalene | |
| 1,2-dihydroxy-8-methoxy-3-methylanthraquinone | Anthraquinone | |
| Eleutherinoside A | Naphtalene | |
| Eleutherinoside B | | |
| 1,3,6-trihydroxy-8-methyl-anthraquinone | | Pyricularia oryzae, MMDC = 150 μg/mL, Inhibit proliferation of K562, IC₅₀ = 154 μg/mL [26] |
| β-sitosterol | | |
| Kadsuric acid | | |
| 9,9′-dihydroxy-8,8′-dimethoxy-1′-dimethyl-1H, 1H′-[4,4′]-bis[naphtal[2,3-c][furan]-1-3,3′-dione | Weak activity against the growth of Pyricularia oryzae, MMDC = 145.8 μg/mL [26] |
| 6,8-dihydroxy-3,4-dimethoxy-1-methyl-anthraquin-one-2-carboxylic acid methyl ester | Weak activity against the growth of Pyricularia oryzae, MMDC = 50.2 μg/mL, Inhibit the proliferation of K562 (human erythroleukemia with IC₅₀ = 49.1 μg/mL) [26] |
| 2-acetyl-3,6,8-trihydroxy-1-methyl anthraquinone | Anthraquinone | Weak activity against the growth of Pyricularia oryzae, MMDC = 124.8 μg/mL, Inhibit proliferation of K562, IC₅₀ = 35 μg/mL [26] |
| 4-hydroxy-eleutherin | | |
| 2,5-dimethyl-10-hydroxynaphthopyrone 8-O-β-glucopyranoside | | |
| Eleuthoside C | | |
| 9,10-dihydro-8-hydroxy-3,4-dimethoxy-9,10-dioxo-2-anthracencarboxylic acid methyl ester | | |
| Erythrolaccin | | |

### 4.2. Antiinflammatory

*In vitro* and *in vivo* antiinflammatory evaluation of four naphthalene derivatives have been conducted. Based on *in vitro* result, isoeleutherin inhibited iNOS expression and NO overproduction [24], which was strengthened by *in vivo* result i.e. Eleutherine and isoeleutherine possessed antiinflammatory and antihypnociceptive activity due to inhibition of carrageenan–induced paw oedema. The *in vivo* results had correlation with the synthesis and production of kines, cytokine, chemokines, prostanoids, or sympathetic amines [11].
Fig. 1. Chemical compounds isolated from *Eleutherine americana*
4.3. Antivirus

_E. Americana_ had a potential to be developed as antiviral agent, because isoeleutherine and isoeleutherol inhibited HIV replication as shown by inhibition of HIV replication with their IC$_{50}$ of 8.5 μg/mL and 100 μg/mL respectively$^4$.

4.4. Antihypertension

Based on other reports, eleutherol, eleutherine and isoeleutherine were potential as antihypertension. They acted as vasodepressants and were able to increase coronary flow in isolated guinea pig heart$^{3,5}$. 
4.5. Antidiabetic

The effect of eleutherinoside A against maltase was tested. The result showed a strong inhibition effect at concentration of 0.5 mM\(^2\).

4.6. Antidermatophyte and antimelanogenesis

Eleutherine isolated from n-hexane fraction of \(E.\) americana showed potential activity against \(T.\) mentagrophytes. Concentration of eleutherine at 10, 20, 40, 60 and 80 \(\mu\)g/disc showed 7, 8, 13, 16, 17 of inhibition zones respectively. At concentration of 5 ppm, it inhibited melanin formation. It was concluded that eleutherine could be used as antidermatophyte and antimelanogenesis\(^25\).

4.7. Cytotoxic activity

Cytotoxic effect of 6,8-dihydroxy-3,4-dimethoxy-1-methyl-anthraquin-one-2-carboxylic acid methyl ester was tested. It inhibited the proliferation of human erythroleukemia cancer cell line K562 with the IC\(_{50}\)value of 49.1 \(\mu\)g/mL\(^26\).

5. Conclusion

\(E.\) americana Merr. has a great potential and value to be cultivated as an economical crop. It contains secondary metabolites with interesting biological activities. As discussed, compounds isolated and elucidated from this plant mostly came from three big groups: i.e. anthraquinones, naphtoquinones and naphtalenes. Some experiments have been conducted in the past for validating pharmacological uses especially for antimicrobial, antihypertensive, antidiabetic, anti-inflammatory and antiviral activities. Recent progress on \(E.\) americana studies indicates this plant is valuable.

References

1. Ifesan BOT, Joycharat N, Voravuthikunchai, SP. The mode of antistaphylococcal action of \(E.\) americana. \(FEMS\) Immunol Med Mic. 2009;\textit{57}, 193-201.
2. Ieyama T, Gunawan-Puteri MDPT, Kawabata J. a-Glucosidase inhibitors from the bulb of \(E.\) americana. Food Chem 2011;\textit{128}, 308-11.
3. Chen Z, Huang H, Wang C. Hongconin, a new naphthalene derivative from Hong-Cong, the rhizome of \(E.\) americana Merr. et Heyne (Iridaceae). \textit{Chem Pharm Bull} 1986;\textit{34}, 2743-6.
4. Hara H, Maruyama N, Yamashita S, Hayashi Y, Lee KH, Bastow KF, \textit{et al.} Elecanacin, a novel new naphthoquinone from the bulb of \(E.\) americana. \textit{Chem Pharm Bull} 1997;\textit{45}, 1714-6.
5. Komura H, Mizukawa K, Minakata H. New anthraquinones from \(E.\) americana. \textit{Chem Pharm Bull} 1983;\textit{31}, 4206-8.
6. Mahabusarakam W, Hemtasin C, Chakthong S, Voravuthikunchai SP, Olawumi IB. Naphthoquinones, anthraquinones and naphtalenes derivatives from the bulbs of \(E.\) americana. \textit{Planta Med} 2010;\textit{76}, 345-9.
7. Saralamp P, Chuakul W., Temsiririrkkul RC T. \textit{Medicinal Plants in Thailand} Amarin Printing and Publishing. Bangkok; 1996.
8. Johnson T. \textit{CRC Ethnobotany Desk Reference}. CRC Press, New York; 1999.
9. Bianchi C, Ceriotti G. Chemical and pharmacological investigations of constituents of \(E.\) bulbosa (Miller) Urb. (Iridaceae). \textit{J Pharm Sci-US} 1975;\textit{64}, 1305-8.
10. Weniger B, Haag-Berrurier M, Anton R. Plants of Haiti used as antifertility agents. \textit{J Ethnopharmacol} 1982;\textit{6}, 67-84.
11. Tessela P, Monache FD, Quintão N, da. Silva G, Rocha L, Lucena G, \textit{et al.} A new naphthoquinone isolated from the bulbs of \(C.\) paludosa and pharmacological activity of two main constituents. \textit{Planta Med} 2011;\textit{77}, 1035-43.
12. Villegas LF, Fernandez ID, Maldonado H, Torres R, Zavaleta A, Vaisberg AJ, \textit{et al.} Evaluation of the wound-healing activity of selected traditional medicinal plants from Peru. \textit{J Ethnopharmacol} 1997;\textit{55}, 193-200.
13. Liu XJ. Chemical constituents of \(E.\) americana. \textit{Chinese Trad Herb Drugs} 2012;\textit{43}, 223-5.
14. Han AR, Min HY, Nam JW, Lee NY, Wiryawan A, Suprapto W, \textit{et al.} Identification of a new naphthalene and its derivatives from the bulb of \(E.\) americana with inhibitory activity on lipopolysaccharide-induced nitric oxide production. \textit{Chem Pharm Bull} 2008;\textit{56}, 1314-6.
15. Limsuwan S, Subhadhirasakul S, Voravuthikunchai SP. Medicinal plants with significant activity against important pathogenic bacteria. *Pharm Biol* 2009;47, 683-9.

16. Ifesan BOT, Hamtasin C, Mahabusarakam W, Voravuthikunchai SP. Inhibitory effect of *Eleutherine americana* Merr. extract on *Staphylococcus aureus* isolated from food. *J Food Sci* 2009;74, M31-M6.

17. Limsuwan S, Voravuthikunchai SP. *Boesenbergia pandurata* (Roxb.) Schltr., *Eleutherine americana* Merr. and *Rhodomyrtus tomentosa* (Aiton) Hassk. as antibiofilm producing and antiquorum sensing in *Streptococcus pyogenes*. *FEMS Immunol Med Mic* 2008; 53, 429-36.

18. Sirirak T, SP, Voravuthikunchai. *Eleutherine americana*: A candidate for the control of Campylobacter species. *Poultry Science* 2011; 90, 791-6.

19. Ifesan BOT, Siripongvutikorn S, Voravuthikunchai SP. Application of *Eleutherine americana* Crude Extract in Homemade Salad Dressing. *J Food Protect* 2009; 72, 650-5.

20. Ifesan BOT, Voravuthikunchai SP. Effect of *Eleutherine americana* Merr. Extract on Enzymatic Activity and Enterotoxin Production of *Staphylococcus aureus* in Broth and Cooked Pork. *Foodborne Pathog Dis* 2009; 6, 699-704.

21. Ifesan BOT, Hamtasin C, Mahabusarakam W, Voravuthikunchai SP. Assessment of Antistaphylococcal Activity of Partially Purified Fractions and Pure Compounds from *Eleutherine americana*. *J Food Protect* 2009; 72, 354-9.

22. Ifesan BOT, Ibrahim D, Voravuthikunchai SP. Antimicrobial activity of crude ethanolic extract from *Eleutherine americana*. *J Food, Agric Environ* 2010; 8, 1233-6.

23. Holmes H, Currie DJ, Maltman JR, Silver RF, Lough CE, Lesky GJ. Evidence for mode of chemical action of 1,4-naphtoquinones in bacteriostasis. *Chemotherapy* 1964; 9, 241-7.

24. Song SH, Min HY, Han AR, Nam JW, Seo EK, Seoung WP, et al. Suppression of inducible nitric oxide synthase by (-)-isoeleutherin from the bulbs of *Eleutherine americana* through the regulation of NF-κB activity. *Int Immunopharmacol* 2009; 9, 298-302.

25. Kusuma IW, Arung ET, Rosamah E, Purwatiningsih S, Kaspradini H, Syafrizal, et al. Antidermatophyte and antimelanogenesis compound from *Eleutherine americana* grown in Indonesia. *J Nat Med* 2010; 64, 223-6.

26. Xu J, Qiu F, Duan W, Qu G, Wang N, Yao X. New bioactive constituents from *Eleutherine americana*. *Front Chem China*. 2006;1, 320-3.

27. Hara H, Maruyama N, Yamashita S, Hayashi Y, Lee KH, Bastow KF, et al. Elecanacin, a novel new naphthoquinone from the bulb of *Eleutherine americana*. *Chem Pharm Bull* 1997; 45, 1714-6.

28. Paramapojn, S., M. Ganzera, W. Gritsanapan, H. Stuppner. Analysis of naphthoquinone derivatives in the Asian medicinal plant *Eleutherine americana* by RP-HPLC and LC-MS. *J Pharm Biomed* 2008; 47, 990-3.

29. Liu XJ, Yan XQ, Wang NL. Studies on chemical constituents of *Eleutherine americana*. *J Chin Med Mat* 2009; 32, 55-8.