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Acute toxicity, antibacterial and antioxidant abilities of *Elephantopus mollis* H.B.K. and *Elephantopus scaber* L.

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ABSTRACT

Elephantopus mollis H.B.K. and Elephantopus scaber L. were collected at To mountain in An Giang province and assessed for extraction efficiency, resistance ability to common bacterial strains in humans and animals, antioxidation, and toxicity. The extraction efficiency of two species was 11-26% and 16-28%, respectively and depending on the part of the plant. The flowers of both species had better antioxidant results than other parts with EC_{50} at 32.2051 μg/mL and 59.9778 μg/mL, respectively, which was highly different from the rest of the plant ($p<0.05$). The leaves of both species had higher antibacterial properties than the other parts. For the six bacterial strains tested, both studied species had the strongest inhibiting ability for the growth of E. coli. (at a concentration of 200 mg/mL) different from that of Ampicillin ($p<0.05$). These two species were not toxic to Mus musculus at the dose of 8,000 mg/Kg.

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1 INTRODUCTION

Vietnam, a fast developing country (MOHA and UNFPA, 2015), has been had a strong expansion of non-communicable diseases, rich-poor gap issues, conservation of natural resources and the environment (Bui *et al*., 2016). Overuse of medicine and stimulant, long-term exposure to toxic chemicals and environmental pollution make the multiple cases of non-communicable diseases such as liver diseases, diabetes, cardiovascular and cancer more and more increasing (Budnik *et al.*, 2018). On the other hand, differences in economic conditions as well as health care system in urban and rural have led people to choose different medicines use. Low-income people in rural areas often choose folk remedies to treat high-cost illnesses such as cancer or diabetes (Campbell-Lendrum and Prüss-Ustün, 2018). Therefore, the use and development of natural medicine from the plants locally sourced are increasingly important (Do Huy Bich *et al*., 2006). Traditional herbal remedies used to prevent and treat diseases are almost common worldwide (Scartezzini and Speroni, 2000; Oliver, 2013; Gall *et al*., 2018). Generally traditional medical system

may indicate safety, but no efficacy of treatments, especially in herbal medicine where tradition is almost completely based on remedies containing active principles at very low and ultra-low concentrations. Even, many people have been relying on magical-energetic principles. Hence, clinical administration is the pharmacological activity that has been applied based on conventional laboratory techniques and clinical trials (Firenzuoli and Gori, 2007). In Vietnam, many valuable herbs mentioned in folk remedies or scientific documents have been studied at various levels (Pham Hoang Ho, 2003; Vo Van Chi, 2012). *Elephantopus* genus includes perennial tree species, common in hot and humid climates (La Dinh Moi *et al*., 2005). These are herbaceous species with many therapeutic uses in traditional remedies, especially liver diseases such as acute viral hepatitis and cirrhosis (Do Huy Bich *et al*., 2006; Vo Van Chi, 2012). In Vietnam, two species of *Elephantopus* have been recorded as *Elephantopus mollis* H.B.K. and *Elephantopus scaber L.* with relatively similar morphology, especially in the dry form (Pham Hoang Ho, 2003; Vo Van Chi, 2012). This may cause confusion for users when collecting or treating the disease (Do Tat Loi, 2004; Vo Van Chi, 2012). Studying the toxicity, antibacterial and antioxidant abilities of these two species locally is necessary to compare the pharmacological efficacy of the two species for considering their potential in clinical practice.

2 METHODOLOGY

2.1 Experimental materials

Experimental materials were *E. mollis* and *E. scaber* collected at To mountain - An Giang province at the time of flowering (Figure 1). The experimental material was determined according to the classification system of plants in the Vietnamese herb book (Pham Hoang Ho, 2003). Parts of the plant including the roots, stems, leaves, flowers, and the whole plant were dried and ground into powder. Twenty-five grams of medicinal powder was boiled in 100ºC water for 1 hour (1: 30 ratio) with three replicates. Base on the method of Truong *et al.* (2018), the extract was then collected, cooled, filtered and heated directly to a viscous form, followed by drying at 60°C to a constant mass to obtain the crude extract which was stored at 4°C for further experiment.

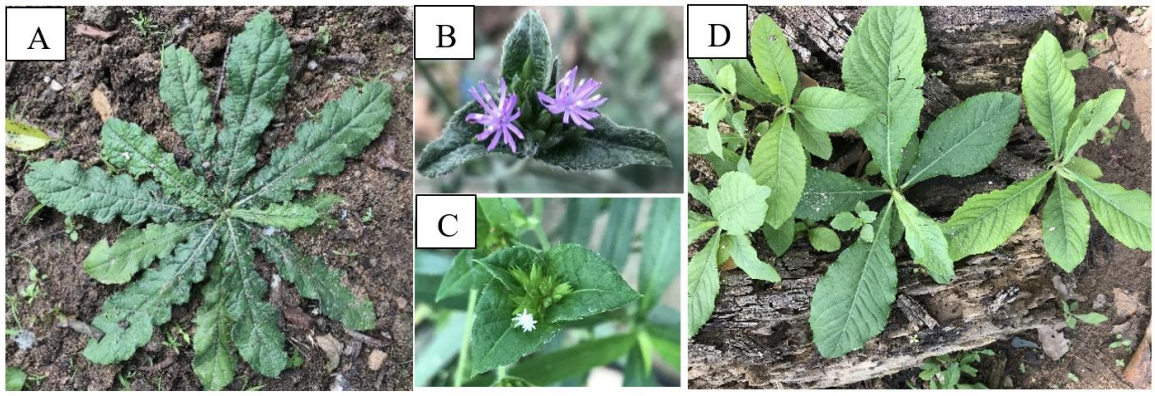

Fig. 1: Morphology of *E. scaber* (A and B) and *E. mollis* (C and D)

2.2 Antioxidant assay

The capability of root, stems, leaves, flowers and the whole plant extracts of the two species to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the method of Aquino *et al.* (2001) with some modifications. The reaction mixture consisted of 40 µL DPPH (1,000 µg/mL) and 960 µL extract. The reaction mixture was incubated in the dark at room temperature for 20 minutes. Absorbance was then measured at 515 nm with UV-VIS color spectrophotometer (Labomed, USA). Percentage inhibits free radicals compared to Vitamin E (Sigma Aldrich). EC_{50} value is calculated based on linear equation of Vitamin E and extracts.

2.3 Antibacterial assay

The antibacterial activity of the extract was determined based on the formation of aseptic ring around the well with the extract by the method of Parkavi *et al.* (2012) and Nguyen Thi Ai Lan *et al.* (2019) with some modifications. The bacterial strains used in the experiment were: *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Salmonella spp.* and *Escherichia coli*. Ampicillin at a dose of 0.001 mg/mL was used as positive control. Each bacterium was tested with three replicates corresponding to extracts of each plant organ of the two herbs. These were prepared in five concentrations of

10, 50, 100, 150, and 200 mg/mL. The samples were incubated for 24 hours at room temperature.

2.4 Acute toxicity experiment

Toxicity of the two species extract was tested based on OECD 420 model for 96 hours according to the regulations of the Vietnamese Ministry of Health (2015) on *Mus musculus* white mouse (23.6 ± 2.1 g/individual). Each experimental concentration was assigned to 10 individuals with a starting dose of 5 mg/kg and ended at 8,000 mg/kg.

2.5 Statistics

The empirical data is managed and processed by IBM SPSS Statistic 22.0 (IBM, USA). Interaction between plant parts and concentration treatments in assessing antibacterial and antioxidant capacity was assessed by comparing two-factor variance method by Duncan post-hoc at 95% confidence.

3 RESULTS AND DISCUSSION

3.1 Extraction efficiency

E. mollis and *E. scaber* had high extraction efficiency. From 25 g of medicinal powder from different parts, leaves of *E. mollis* showed the highest extraction efficiency, which was statistically different from the rest of the plant ($p<0.05$). Meanwhile, *E. scaber* stems and leaves were both capable of achieving high extraction efficiency.

The extraction performance of the two species was significantly higher (*E. mollis*: 11-32%, *E. scaber:* 15-28%) in comparison with some other species such as *Caesalpinia sappan* L. (6.8%) (Nguyen Tan Dat and Ba Tiep Nguyen, 2016), *Streptocaulon juventas* Merr. (3.35%) (Dai Thi Xuan Trang *et al*., 2015) or *Helicteres hirsuta* L. with the highest efficiency of 9.96% (Tran Van Tien and Thi Mai Huong Vo, 2017).

Table 1: Extraction performance of parts of *E. mollis* and *E. scaber*

Parts of the plant	N	Ratio±SD (%)
E. mollis root	3	13.0±1.1[a]
E. mollis stem	3	11.1±0.8[a]
E. mollis leaves	3	25.8±1.2[cd]
E. mollis flower	3	18.5±0.6[b]
E. mollis	3	32.3±0.5[e]
E. scaber root	3	24.1±1.4[c]
E. scaber stem	3	28.5±0.8[d]
E. scaber leaves	3	27.7±0.6[d]
E. scaber flower	3	15.8±0.8[b]
E. scaber	3	18.4±1.4[b]

Means ratio ± SD have the same letter are not significantly different (Duncan, $p>0.05$).

3.2 Antioxidation

E. mollis flowers had the best antioxidant capacity, in which, at a concentration of 70 µg/mL, the extract was able to eliminate 92% of free radicals (Figure 2). The flowers of *E. scaber* also showed high antioxidant capacity, in which, at a concentration of 70 µg/mL, the flower of *E. scaber* was also able to reduce 61% free radicals. *E. scaber* root showed the weakest resistance, at a dose of 700 µg/mL, that could only reduce 53% of free radicals.

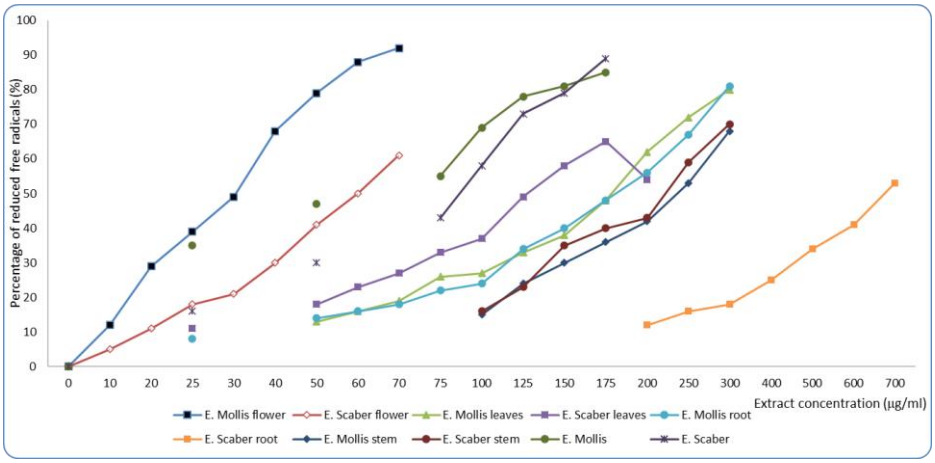

Fig. 2: Antioxidant ability of *E. mollis* and *E. scaber*

When analyzing EC_{50} value, flowers of the two species both showed high antioxidant ability, at 32.2 µg/mL, 50% of free radicals can be removed (Table 2). The antioxidant capacity of *E. mollis* flower was 2.62 times lower than that of Vitamin E but higher than that of other herbal plants which are considered to have high antioxidant capacity such as *Streptocaulon juventas* (EC_{50} 349.35 µg/mL) (Dai Thi

Xuan Trang *et al*., 2015), *Morinda citrifolia* (EC_{50} 1025.2 μg/mL) (Dai Thi Xuan Trang *et al*., 2012), or *Solanum hainanense* (EC_{50} 1734 μg/mL) (Nguyen and Eun, 2011).

Table 2: Antioxidant activity of *E. mollis* and *E. scaber*

Parts of plant	n	EC_{50} (μg/mL)	R^2
E. mollis flowers	72	32.2051	0.9721
E. mollis leaves	63	183.5357	0.9690
E. mollis stem	63	219.2174	0.9678
E. mollis root	72	185.8889	0.9797
E. mollis	72	153.3182	0.8776
E. scaber flowers	72	59.9778	0.9555
E. scaber leaves	63	132.0789	0.9759
E. scaber stem	63	218.0800	0.9443
E. scaber root	72	751.1429	0.9309
E. scaber	72	105.2353	0.9675
Vitamin E	72	12.3349	0.9695

Compared with other medicinal plants in the same family or genus, *E. mollis* showed higher antioxidant capacity than *Silybum marianum* (EC_{50} 39 μg/mL) (Mhamdi *et al*., 2016) and *E. tomentosus* (EC_{50} 887 μg/mL) (Yam *et al*., 2008). The antioxidant capacity of *E. scaber* is lower than *S. marianum* but higher than other species listed above, especially flowers of *E. scaber*.

3.3 Antibacterial ability

According to statistical analysis, the higher the extract concentration was, the greater the antibacterial activity was (Duncan, $p<0.05$). Leaf extract of both research species showed high antibacterial capacity against Gram-positive bacteria (*Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes*) that was better than the positive control, Ampicillin. For *Salmonella spp*., a gram-negative one, both species showed very good resistance, statistically significant difference compared to Ampicillin (Duncan, $p<0.05$). As for *E. coli*, Ampicillin showed the highest resistance ($p<0.05$). However, resistance to *E. coli* of *E. mollis* and *E. scaber* is highest compared to resistance to other bacteria, with antibacterial rings of up to 28.3±1.5 mm and 27.0±1.0 mm, respectively (Table 3).

Table 3: Antibacterial ability of two species of Elephantopus

Parts of plant	Antimicrobial ring diameter (mm)					
	B. cereus	***B. subtilis***	***S. aureus***	***L. monocytogenes***	***E. coli***	***Salmonella***
E. mollis						
Neg-control	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}
Root	9.7±0.6^{c}	11.3±0.6^{c}	8.7±1.5^{b}	9.7±0.6^{b}	15.0±1.7^{b}	12.3±0.6^{c}
Stem	8.3±0.6^{b}	9.0±1.7^{b}	8.3±1.5^{b}	8.3±0.6^{b}	15.0±0.0^{b}	11.3±0.6^{c}
Leaf	19.3±1.2^{f}	24.0±1.0^{f}	19.7±0.6^{e}	23.0±1.0^{e}	28.3±1.5^{e}	23.3±1.2^{f}
Flower	13.0±1.0^{d}	16.3±2.1^{d}	14.7±0.6^{c}	16.7±0.6^{c}	26.7±0.6^{d}	16.3±0.6^{d}
Whole plant	15.3±0.6^{e}	19.3±0.6^{e}	16.7±1.2^{d}	19.0±1.0^{d}	25.3±0.6^{c}	19.0±1.0^{e}
Pos-control	7.7±0.6^{b}	10.0±1.1b^{c}	14.7±1.2^{c}	24.5±2.2^{e}	32.4±1.1^{f}	9.7±1.0^{b}
E. scaber						
Neg-control	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}	6.0±0.0^{a}
Root	7.3±0.6^{b}	8.0±0.0^{b}	6.3±0.6^{a}	7.3±0.6^{b}	9.3±0.6^{b}	10.0±0.0^{b}
Stem	7.7±0.6^{b}	11.7±0.6^{c}	8.3±1.2^{b}	9.7±1.2^{c}	12.3±0.6^{c}	9.7±0.6^{b}
Leaf	16.7±1.2^{e}	18.7±1.5^{e}	18.3±1.2^{e}	17.7±0.6^{e}	27.0±1.0^{f}	21.0±2.0^{e}
Flower	10.3±1.2^{d}	14.0±2.6^{d}	11.3±0.6^{c}	18.0±1.0^{e}	22.3±1.5^{e}	17.7±2.5^{d}
Whole plant	9.0±0.0^{c}	11.0±1.0^{c}	10.7±0.6^{c}	11.7±0.6^{d}	15.3±0.6^{d}	12.0±1.7^{c}
Pos-control	7.3±0.6^{b}	10.2±1.1^{c}	13.3±1.0^{d}	22.9±1.0^{f}	31.9±1.0^{g}	9.6±0.8^{b}

The mean±standard deviations with the same letter in the same column (corresponding to each species) are not significantly different (Duncan, p> 0.05).

3.4 Possibility of acute toxicity

Experimental mice were poisoned with a starting dose of 5 mg/kg and gradually increased to 50, 300, 2,000, 4,000 and 8,000 mg/kg. The results showed that at the experimental treatment up to 8,000 mg/kg, equivalent to 200 mg/mouse (25 g), the mice still did not die. The Median lethal dose (LD_{50}) was unidentified. Comparing with Vietnamese Ministry of Health's reference to clinical drug trials (Ministry of Health, 2015), the two experimental herb species were non-toxic to tested mice and has the potential to undertake clinical studies in humans.

4 CONCLUSIONS

The two researched herb species had high extraction efficiency and were not toxic to tested mice. These two species showed high antioxidant capacity, especially in the flowers of both *E. mollis* and *E. scaber*. The results showed that the leaves of the two species had a better inhibition of the growth of bacterial strains than other parts. *E. coli* was the strain most strongly inhibited by the extract of the two studied species. These species are rhizomes and live for many years, using parts such as leaves and flowers for medicinal purposes are good for application and conservation.

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