International Journal of Agricultural Sciences and Natural Resources
2018; 5(2): 25-29
http://www.aascit.org/journal/ijasnr
ISSN: 2375-3773

AASCIT American Association for Science and Technology

Evaluation of Antibacterial, Antioxidant and Antiobese Activities of the Fruit Juice of Crabapple Mangrove *Sonneratia caseolaris* (Linn.)

Doan Van Thuoc[1, *], Nguyen Thi Ngoc Mai[1], Le Thi Viet Ha[2], Lai Duy Hung[3], Dang Huong Tra[3], Nguyen Kim Hung[3], Nguyen Phuc Hung[1]

[1]Faculty of Biology, Hanoi National University of Education, Hanoi, Vietnam
[2]Ngo Quyen High School, Haiphong, Vietnam
[3]Tran Phu High School, Haiphong, Vietnam

Email address

thuocdv@hnue.edu.vn (D. V. Thuoc), ngocmaispsinhhnue@gmail.com (N. T. N. Mai), lethivietha9896@gmail.com (Le T. V. Ha), duyhunglai@gmail.com (L. D. Hung), huongtradang2012@gmail.com (D. H. Tra), nguyenkimhung2k@gmail.com (N. K. Hung), hungnp@hnue.edu.vn (N. P. Hung)

[*]Corresponding author

Citation

Doan Van Thuoc, Nguyen Thi Ngoc Mai, Le Thi Viet Ha, Lai Duy Hung, Dang Huong Tra, Nguyen Kim Hung, Nguyen Phuc Hung. Evaluation of Antibacterial, Antioxidant and Antiobese Activities of the Fruit Juice of Crabapple Mangrove *Sonneratia caseolaris* (Linn.). *International Journal of Agricultural Sciences and Natural Resources.* Vol. 5, No. 2, 2018, pp. 25-29.

Received: April 8, 2018; **Accepted**: April 19, 2018; **Published**: May 31, 2018

Abstract: A study was carried out to investigate the antibacterial, antioxidant, and antiobese activities of fruit juice of the mangrove plant *Sonneratia caseolaris*. The fruit juice was prepared by three different methods: water extract of fruit flesh (J1), hot water extract of fruit flesh (J2), and hot water extract of whole fruit (J3). All three juice samples exhibited antibacterial and antioxidant activities, and sample J3 showed the highest activities compared with sample J1 and J2. Sample J3 was then chosen for an antiobese activity test. The results demonstrated that the crabapple mangrove fruit juice had an effect to prevent the increase in body weight of the mice. The study suggests fruit juice of *S. caseolaris* as a potential source of active compounds with antimicrobial, antioxidative, and antiobesity properties and can be used as natural antibacterial, antioxidative, and antiobesity agents in pharmaceutical and food industries.

Keywords: *Sonneratia caseolaris*, Fruit Juice, Antibacterial, Antioxidant, Antiobese

1. Introduction

Mangrove forests are special ecosystems situated at the inter-phase between land and sea of the tropical and subtropical areas. It is well known that mangrove plants were potential sources to provide high bioactive compounds such as polyphenols, flavonoids, anthocyanins, vitamins, antioxidants, and antibiotics. Beula *et al* [1] have reported that antiviral, antioxidant and toxicological activities were found in theextracts ofmangrove species in South East coast of India. In addition, antioxidant, antidiabetic, antiobese and antibacterial compounds have also presented in the extracts of different parts of mangrove trees such as *Sonneratia caseolaris, Sonneratia avatar, Sonneratia apetala*, *Sonneratia alba, Axonopus compressus, Rhizophora mucronata,* and *Avicennia marina,* [2-7].

Crabapple mangrove *Sonneratia caseolaris* (Linn.) is a species belonging to family Sonneratiaceae. It is a common tree in coastal estuaries in Vietnam and locally known as *Bần chua* (in Vietnamese). This plant is a medium-sized evergreen tree normally attending up to a height of 15 - 20 m. *S. caseolaris* usually blooms in April and May, fruit ripening in October and November, and each tree can reach to 350 fruits with an average of 10 - 15 fruits per kilogram [8, 9]. Recently, some studies have reported on the pharmacologically important biological activities of this species growing in Thailand and Malaysia [10-13]. However, most of the findings have focused on antimicrobial and antioxidative properties of the crabapple mangrove but not antiobese activity. Moreover, the differences in natural conditions such as temperature and edaphic parameters between Vietnam and the above mentioned countries may affect chemical

constituents, thus biological activities of this species. The present study was conducted to evaluate antioxidant, antibacterial and antiobese activities of fruit juice of *S. caseolaris* growing in the coastal estuary in Vietnam.

2. Materials and Methods

2.1. Sample Collection and Juice Preparation

Raw crabapple mangrove fruits were collected in Vinhquang commune, Tienlang district, Haiphong province, Vietnam. The fruits were washed with water, stalk removed and then used to prepare different types of juice following a method described by Baba *et al* [14] with slight modification. Juice 1 (J1) - water extract of fruit flesh, the fruits were peeled and blended with distilled water (1:2, w/v), the fruit juice was then filtered through sieve (60 mesh) to remove seeds and other residues. Juice 2 (J2) - hot water extract of fruit flesh, the fruit juice was prepared similar to J1, but boiling water was used instead of distilled water. Juice 3 (J3) – hot water extract of whole fruit, the whole fruits were blended and boiled with water for 3 minutes, the fruit juice was then filtered through sieve (60 mesh) to remove seeds and other residues.

2.2. Antibacterial Activity Determination

The antibacterial activity of the three fruit juices was tested against four bacterial strains (*Bacillus subtilis*, *Escherichia coli*, *Vibrio cholerae*, and *Salmonella typhimurium*). The bacterial strains were provided by the Department of Biotechnology and Microbiology, Hanoi National University of Education, Vietnam. One hundred microliters of each type of the fruit juice were used for antibacterial assay using an agar well diffusion method described by Murray *et al* [15]. For the negative control, 100 µL of distilled water were used. The Petri dishes were incubated at 4ºC for 6 hours for diffusing, and then transferred to the condition of 37ºC for 24 hours. The inhibition zones around the wells were measured in millimeters.

2.3. Antioxidant Activity Determination

The antioxidant activity of each type of the fruit juice was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity as described by Ibeh *et al* [5]. A solution of 0.1 mMDPPH in methanol was prepared. Then, 900 µl of this solution were mixed with 100 µl of diluted fruit juice. Ascorbic acid at dilution series (0.5; 0.25; 0.125; and 0.0625 mM) was used for the standard curve. After incubation at room temperature for 30 min in dark condition, the amount of DPPH remaining was determined by measuring absorbance at 517 nm using a spectrometer (Biotek, USA). The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.

2.4. Antiobese Activity Determination

Thirty male Swiss mice (*Mus musculus*) (4-week old and 24 g body weight) provided by National Institute of Hygiene and Epidemiology (Vietnam) were used to test antiobese activity of the fruit juice J3. The mice were randomly divided in three groups (10 mice per group). They were reared in plastic cages and fed with different diets purchased from Testdiet company (USA) for five weeks. One group named BD was given a basal diet (58Y2 containing 10% energy originated from fat) with distilled water supply. The other two groups named HD and HJ were provided a high fat diet (58Y1 containing 60% energy originated from fat) with distilled water and fruit juice J3 supply, respectively. Feed and water was given to experimental mice *ad libitum*. Body weight of the mice was monitored every week.

3. Results and Discussion

3.1. Antibacterial Activity of Fruit Juice

Crabapple mangrove fruit juice was used as a kind of drink in many Asia countries [14]. It will be interesting if the juice containing bioactive compounds that can inhibit the growth of some gastrointestinal infections such as *E. coli*, *V. cholerae*, and *S. typhimurium*. In order to screening of antibacterial activity in three different extracts of crabapple mangrove fruit, the agar well diffusion assay was employed. The results showed that all three fruit juice samples could inhibit four tested bacterial strains including both Gram-negative (*E. coli*, *V. cholerae*, and *S. typhimurium*) and Gram-positive (*B. subtilis*) (Table 1). Among the three extracts, sample J3 containing whole fruit showed the highest antibacterial activity as compared with J1 and J2 samples containing only the fruit flesh.

In the previous studies, Yompakdee *et al* [11] reported that the methanol extract samples of different parts of the crabapple mangrove tree such as leaves, flower, and fruit could inhibit the growth of 11 bacterial strains including *E. coli*, *Pseudomonas aeruginosa*, *B. subtilis*, *Staphylococcus aureus*. Simlai *et al* [16] also reported the methanol and water extracts of bark tissue of *S. caseolaris* exhibited antibacterial activity against *B. subtillis* and *Proteus vulgaris*. More recently, the ability of *S. caseolaris* fruit methanol extract to inhibit the growth of microbes such as *E. coli*, *S. aureus*, and *Candida albicans* was also reported [17]. The antibacterial activity could be due to the presence of flavonoids, phenolics, tannins, and alkanoids in extracted samples [11, 16]. The highest antibacterial activity of sample J3 in the present study may relate to the amount of extracted active compounds such as tannin and phenol in the skin of the crabapple mangrove fruit.

Table 1. *Antibacterial activity of three juice samples of S. caseolaris fruit.*

Bacteria	Inhibition zone diameter (mm)			
	J1	J2	J3	Control
E. coli	19	18	21	-
V. cholerae	32	33	35	-
S. typhimurium	25	26	27	-
B. subtillis	24	27	29	-

3.2. Antioxidant Activity of Fruit Extracts

Antioxidant activity of the fruit juice of *S. caseolaris* was examined using DPPH reducing power assay. The results of reducing power assay showed that all three extract samples exhibited antioxidant activity and the reducing rate increased with increasing concentration of extracts. Among all three samples, sample J1 and J2 exhibited significantly high IC_{50} values compared with that of sample E3, however, there was no significant difference between sample J1 and J2 (Table 2). The lowest IC_{50} value of sample J3 demonstrated that the whole fruit extract sample exhibited the highest antioxidant activity. Sample J1 and J2 (fruit flesh extract) also showed the antioxidant activity, but with about 5.5 folds lower than that of sample J3.

Table 2. *IC50 values obtained from DPPH assay of three juice samples of S. caseolaris fruit.*

Sample	IC_{50} (mg/ml)*
J1	41.8
J2	40
J3	7.3

*mg of fruit was used for preparing fruit juice

The antioxidant activity of the extract samples from various parts of some *Sonneratia* species such as *S. caseolaris* and *S. alba* was also reported in the previous studies [13, 16, 18]. The antioxidant activity could be due to the presence of flavonoids such as luteolin and 7-O-β-glocoside which were detected from many parts of *S. caseolaris*including skin and flesh of ripe fruit [2, 13].

3.3. Antiobese Activity of Fruit Juice

Based on the above results, with the exhibition of the highest antibacterial and antioxidant activities, juice sample J3 was chosen for antiobese activity test. Figure 1 showed the changes in body weight of the mice during five weeks of experiment. After five weeks, the average body weight of mice in HD group, BD group, and HJ groupwere 62.1, 54.3, and 53.1 g, respectively. The mice fed with a HD diet (58Y1 containing 60% energy from fat) and supplied with distilled water resulted in significant increases in body weight compared to those fed with a BD diet (58Y2 containing 10% energy originated from fat) and supplied with distilled water. It is well known that diet energy is one of the most important factors responsible for the increase in body weight. Therefore, the greater body weight of the mice fed with the high fat diet compared to those fed with the basal diet should be attributable to the higher dietary energy. The significant differences in body weight of the mice between these two groups after two weeks feeding 58Y1 and 58Y2 diets indicated that the high energy diet used in the present study could induce abnormally high body weight in the mice.

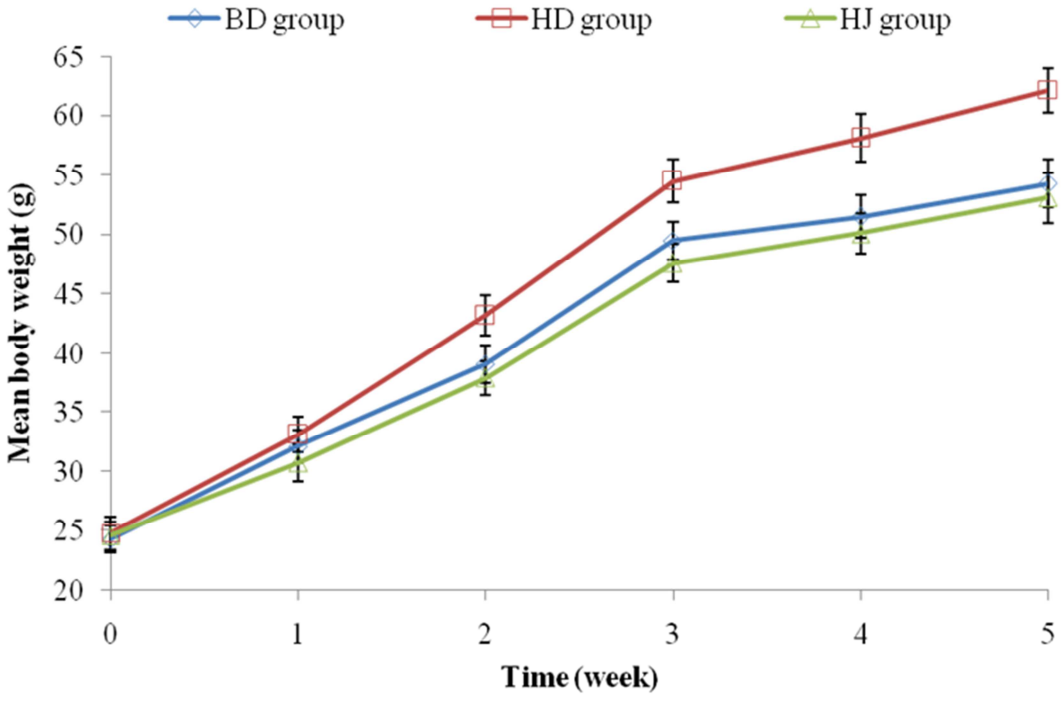

Figure 1. *Mean body weight of the mice during 5 weeks of experiment.*

Though feeding a high energy diet and supplying with distilled water produced significant increases in body weight of the mice, however, the body weight of the mice markedly reduced to a normal range when supplying the crabapple mangrove fruit juice J3 (Figure 1). In the present study, the amount of water and juice J3 consumed by the experimental mice was similar among the three groups and the food intake of HJ-fed mice was even greater than that of HD-fed mice (data not shown), thus indicating that the crabapple mangrove fruit juice J3 had an effect to prevent the increase in body weight of the mice.

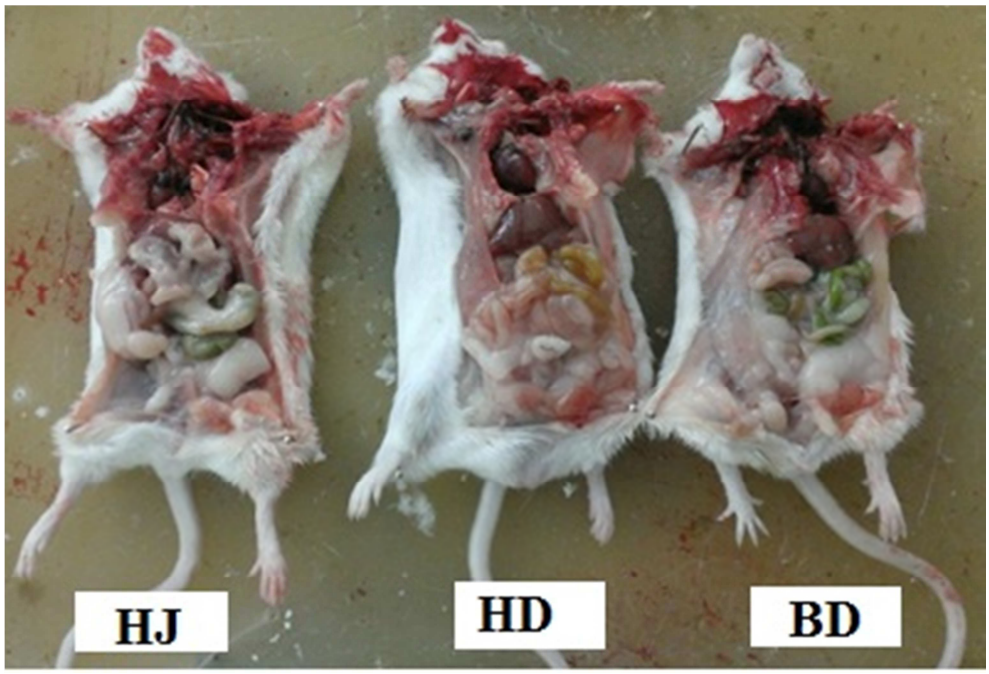

Figure 2. *Experimental mice dissected at the end of week 5.*

Dissection of the experimental mice at the end of week 5 using the juice showed no abnormalities in viscera except higher adipose tissues presented in HD-fed mice (Figure 2). This result suggests that oral administration of the fruit juice J3 for 5 weeks did not induce abnormal changes in appearance of viscera of the mice. However, a further study is necessary to evaluate if the juice has any toxic effects on mice when administrating for a longer time.

It has been reported that many plant species showed the antiobese effects. These species are mainly belong to the family Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae and Araliaceae. The antiobese effects such as body weight reduction, decrease in the levels of triglycerides, total cholesterol, and low density lipoprotein cholesterol with simultaneous increase in high density lipoprotein cholesterol were observed in the animals treated with the extracts from these plant families [19, 20]. Han *et al* [21] found that oolong tea prevented the obesity and fatty liver induced by a high-fat diet. A water extract of oolong tea enhanced noradrenaline-induced lipolysis, and the active substance was identified as caffeine. Flavonoids of *Nelumbo nucifera* showed a mild inhibitory effect on both adipocyte differentiation and pancreatic lipase activity [22]. Recently, the extract of *Rhizophora mucronata*, a mangrove plant, was reported to significantly reduce food intake, body weight, organ weight in obese mice. The extract of this species was rich in phenols, flavonoids, saponins, glycosides, terpenoids and tannins [6]. In addition, phenolic and flavonoid compounds were also found in the extract of *S. caseolaris* fruit [2, 13] and another mangrove species *S. apetala* [3]. In the present study, we reported preliminarily the antiobese effect of the crabapple mangrove fruit juice growing in the coastal estuary in Vietnam. A further study should be conducted to examine compounds responsible for this significant effect of the fruit.

4. Conclusions

In the current study, our results showed that the fruit extracts of *S. caseolaris* have antibacterial, antioxidant, and antiobese activities. Among three fruit extraction methods, the hot water extract of whole fruit exhibited the highest antibacterial and antioxidant activities. This extract also showed the effect to prevent the increase in body weight of mice. The results of this study are preliminary data, and further research will be focused on the isolation and identification of active compounds in *S. caseolaris* fruit, and also the biological effects of such compounds.

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