AIP Conference Proceedings

RESEARCH ARTICLE | JUNE 14 2016

Red pepper (*Capsicum* spp.) fruit: A model for the study of secondary metabolite product distribution and its management FREE

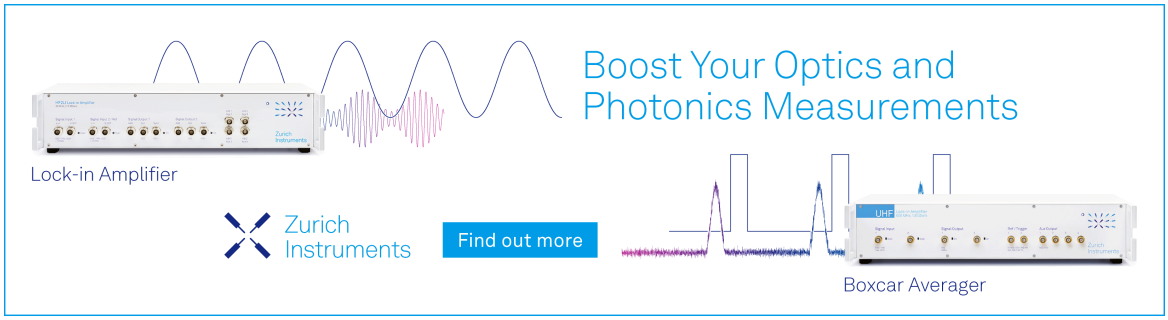
Laurentius Hartanto Nugroho

Check for updates

AIP Conf. Proc. 1744, 020034 (2016)
https://doi.org/10.1063/1.4953508

View Online
Export Citation

19 April 2024 18:19:55



AIP Publishing

Red Pepper (*Capsicum* spp.) Fruit: A Model for The Study of Secondary Metabolite Product Distribution and Its Management

Laurentius Hartanto Nugroho

Plant Structure and Development Laboratory, Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika selatan, Sekip Utara, Yogyakarta, 55281 Indonesia

Corresponding author: hartantonugroho2005@yahoo.co.id

Abstract. Red pepper (chili) is the plant with many benefits. The plant can be used as herbal medicines, food flavor and pet food. The plant is characteristic as a tropical plant. Therefore the plant is very potential as an export commodity. The usefulness of chili due to its secondary metabolite content called capsaicin. The compound is not distributed evenly in the fruit tissues. Moreover, the production of the compound in the plant tissues are affected by environment factors. Our anatomical research using paraffin method and light microscopy for the analyses found that chili fruit consist of pericarp, mesocarp, endocarp, septum, placenta, funiculus and seed. Our following research analyzed the capsaicin content in the various tissues of chili fruit using gas chromatography concluded that the compound is mostly accumulated in the fruit septum compared to other fruit tissues. Our study on the capsaicin content in various developing stage of chili fruit found that the highest capsaicin content was in the most ripened fruit. The time for fruit ripening varied based on the varieties. Fruit ripening mostly in between 35 d to 40 d after anthesis (DAA). Our other studies found that environment factors, namely growing medium and kind of fertilizer affected capsaicin content in the various stages of fruit development. Therefore, growing conditions and the selection of fruit tissues should be arranged based on the purposes of chili fruit utilization.

Keywords: Capsaicin, chili, red pepper, secondary metabolites.

19 April 2024 18:19:55

INTRODUCTION

Red peppers or chili (*Capsicum* spp.) is one of the seasonal crops commodity in the morph of shrubs. The plants can be cultivated in lowland or highland. Normally people used the fruit for various needs, it can be harvested several times during its life cycle. The fruit is elongated round shape with a tapered tip. Chili fruit has a high economic value because it is consumed in about one quarter people in the earth every day. The species of Capsicum peppers were distributed across the world in less than two hundred years after their discovery by Europeans in South and Central America and able to reproduce spontaneously [1]. Therefore, Capsicum has good prospects in the country and abroad. It became one of the export commodities.

In the beginning, chili fruit is used for flavoring various dishes. Researches showed that bioactive compound in chili fruit played a rule in pain relief, and weight reduction, had cardiovascular and gastrointestinal effects [2]. Other researchers reported that the plant fruit had antioxidant property [3] and hypoglycaemic activities [4], suppressed accumulation of intracellular triglyceride [5], induce humoral immune responses [6] beneficial effect on broiler growth performance and lipid peroxidase [7], biological control [8]. Moreover, Corson and Crews [9] reported that chili fruit had anticancer property.

The efficacy of chili is mostly determined by the content of secondary metabolites. The existence of a secondary metabolite group characterized by spicy taste of chili. Its one of the important characters that show chili quality. Spicy flavor is determined by capsaicinoid content in the fruit. One of it is capsaicin. Capsaicin *(8-methyl-N-vanillyl-6-nonenamide)* is an alkaloid class which is commonly found in the genus *Capsicum* [10].

Towards the sustainable use of biodiversity in a changing environment: From basic to applied research
AIP Conf. Proc. 1744, 020034-1–020034-7; doi: 10.1063/1.4953508
Published by AIP Publishing. 978-0-7354-1401-3/$30.00

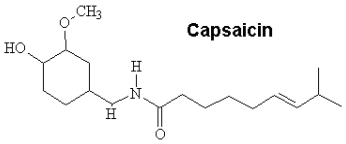


FIGURE 1. Chemical structure of capsaicin [11]

Systematically, chili is a member of Solanaceae tribe with many variations both at the level of species and varieties. Currently there are 12 known types of chili. However, the most widely cultivated in Indonesia are only a few types, namely red chili, cayenne, paprika, and chili ornamental [12]. Commonly, Indonesian traditional markets sell only three kinds of chili, these are big-red peppers (*Capsicum annuum* L. var. Abreviata Eingerhuth), curly-red peppers (*Capsicum annuum* L. var. Longum Sendt) and cayenne (*Capsicum frutescens* L.).

Chili Fruit Anatomy

Chili fruit comprise with three main parts, namely pericarp, ovarium and ovulum. Pericarp of chili consist of four distinct regions. Those are: (i) The outer part of pericarp called epidermis composed of sub-rectangular cells. The outer cell walls are evenly thickened and cuticularized. (ii) The mesocarp complied of rounded shape of parenchyma cells with cellulose walls. Most of them are yellowish oily dropleds oil and yellowish-red chromatopores. Same of the cells contain sandy calcium oxalate crystals [13]. Vascular bundles occur along toward the inner part of this region. (iii) A single layer of very big cells called giant cells. (iv) The inner epidermis which called indocarp consist of single layer cells with wavy walls. Beneath of the giant cells are cells with very thick and lignified wall. The area form a number of schlerenchyma cell islands. One island under each giant cells. The area between schlerencyma cell islands compose of thin parenchyma cells.

The structure which split the area in the inner part of pericarp called septum. The septum consist of inner epidermal and parenchima cells with vascular bundle along parenchyma cells. Therefore, there are two rooms in the inner part of pericarp. The room called ovarium. In the ovarium can be seen ovulum (seed) connected to the septum with placenta. The area between placenta and ovulum are called funiculus.

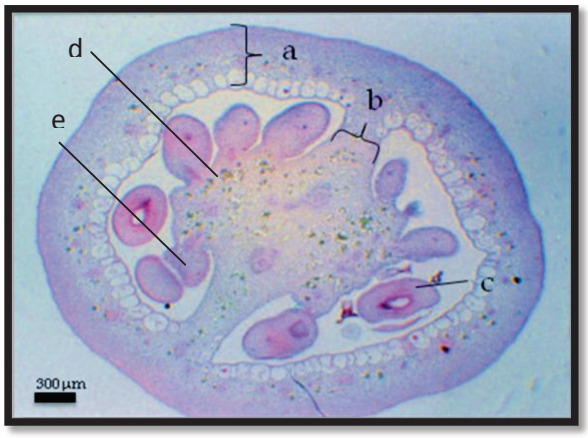


FIGURE 2. Cross section of cayenne (*Capsicum frutescens* L.) fruit observed under Nicon light microscope. a) pericarp, b) septum, c) seed, d) placenta, e) funiculus [14].

Metabolite Content of Chili Fruit

Pungent flavor from red pepper derived from its secondary metabolite, known as capsaicin which is a component of capsaicinoid. Barbero *et al*. [15] showed that the five major capsaicinoids: nordihydrocapsaicin, capsaicin, dihydrocapsaicin, homocapsaicin and homodihydrocapsaicin present in the red pepper (*Capsicum annuum* L.) during fruit ripening. Ninety percent pungent taste came from capsaicin and dihydrocapsaicin. Other than spicy compound, chili also contain protein, fat, carbohydrate, mineral (calcium, phosphor, iron), vitamin (A, B_1, B_2, B_3 / niacin, C), flavonoid, row fibber and other compound which is useful as a drug (oleoresin, flavonoid, essential oil, and carotenoid) [16, 1]. Jarret *et al*. [17] found 4 principal fatty acids, namely were palmitic 12.9 %, stearic 3.4 %, oleic 6.7 % and linoleic 76.0 % in five varieties of *Capsicum* spp. Linoleic acid was the principal fatty acid in all samples.

19 April 2024 18:19:55

Capsaicin Content in the Development Stage of Chili Fruit

During fruit ripening, capsaicin content increased until maximum production. Then capsaicin content change and degrade into other secondary metabolites [10]. Most of the peroxydase activities occur at the placenta and pericarp epidermis. Through gel permeation chromatography, it is known that major oxidative product is 5, 5'-dicapsaicin and 4'-*O*-5-dicapsaicinether. Peroxydase activity increased when capsaicinoid concentration decreased [18]. It is assumed that peroxydase catalyse capsaicinoid oxidation. Thus, capsaicin content of red pepper need to be investigated at certain stage of fruit development.

In the experiment, two varieties of chili species used as models, those are white and green cayenne. Each cayenne species as much as 2 g were extracted with acid-bases protocols. The extracts were then analyzed using gas chromatography combined with mass spectrometry [Agilent GC 6890N 5975B MSD]. The capillary column was Agilent 19091S-433 model, HP-5MS 5 % Phenyl Methyl Siloxane. Capsaicin was purchased from Sigma. The results were shown in the following Fig. 3 and Table 1.

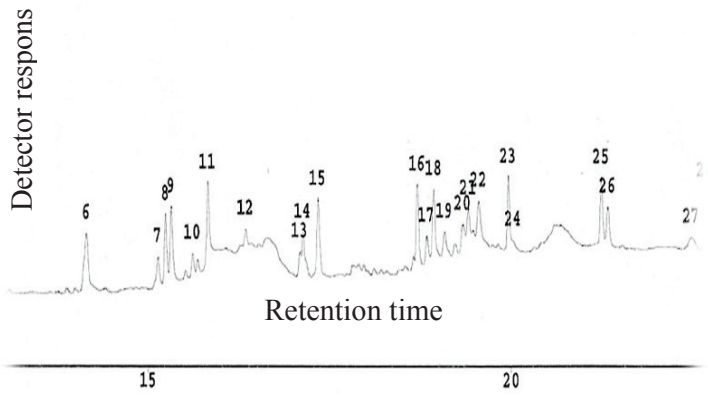


FIGURE 3. GC-chromatograms of chile fruit 14 d after anthesis. Peak number 25 with retention time 21.234 minutes identified as capsaicin.

TABLE 1. Relative capsaicin content (%) in white and green cayanne in various fruit development stage.

Varieties	Days after antheses (DAA)			
	14	21	28	35
White cayenne	2.08[a]	76.79[bc]	100.00[c]	59.73[bc]
Green cayenne	18.17[a]	20,99[ab]	26.02[ab]	74.98[c]

*Notes: 1. The relative capsaicin content were calculated by setting capsaicin content of 28 DAA in white cayenne to be 100 %.
2. The value followed by the same character showed there were no significant differences among each other based on DMRT analyzes at 5 % significance.

From the above results, it can be known that the time of ripening between two varieties were different. The capsaicin content increased as the chili reached maturity. This results is in agreement with those of Tundis *et al*. [4]. However, optimum capsaicin content was not always in the optimum stage fruit ripening. Barbero *et al*. [15] showed that a sharp decrease in the total capsaicinoid content (32 %), followed by a gradual decrease until 80 d of ripening. Yaldiz *et al*. [19] added that the capsaicin contents of different Capsicum species and lines were affected by harvest time and drying parameters.

Localization Studies of Capsaicin in Chili Fruit

Capsaicin content was unequal among the part of chili fruit [20]. Claus [21] states that the capsaicin in chili mostly accumulated in the septum, while Lewis [22] stated that capsaicin was most numerous in the pericarp and a little in the seeds. Capsaicin is spread unevenly in the fruit epidermal and is found in high concentrations in placental tissue. Ethno-botanical practice showed that discarding the seeds were done to reduce the chili spicy. The practice indicates that the spicy taste of chili fruit come from the seeds.

19 April 2024 18:19:55

Information above shows that the distribution of capsaicin in the chili fruit is still a hot topic to be discussed. Research was carried out to solve the problem. The research used big-red chili (*Capsicum annuum* L. var. abreviata Eingerhuth) and big-curly chili (*Capsicum annuum* L. var. longum Sendt) as models.

The chili fruit in the age of (14, 21, 28 and 35) DAA were harvested. Pericarp, septum and seed were separated. Two gram of each part of chili in every state of development were grounded and extracted with acid-base method. The extracts were then analyzed using gas chromatography combined with mass spectrometry. The type of equipment was the same as the previous research. The result was shown in the Table 2

TABLE 2. Relative capsaicin content (%) in big-red and big-curly chilies at different part of chili fruit in various fruit development stage.

DAA	Capsaicin content (%) in big-red chili			Capsaicin content (%) in big-curly chili		
	pericarp	Septum	Seed	pericarp	Septum	Seed
14	0	0	0	0	0	0
21	0.31	0	0	0	45.24	0.97
28	0.37	0.86	1.86	1.96	58.57	16.76
35	1.99	6.98	0.99	1.16	100.00	8.88

*Note: 1. The relative capsaicin content were calculated by setting capsaicin content of big-curly chili septum to be 100 %.

The result shows that the highest capsaicin content was at the fruit septum in the various development stage for both kind of varieties.

Capsaicin Content in Chili Grown in the Sand Beach Media

Land scarcity is a challenge for scientist to look for another alternative cropping land. One of the solution is using marginal land for cropping. The existence of extra salt in the soil could reduce plant growth by reducing water potential or nutrition absorption. Barrileaux and Grace [23], found that *Sapium sebiferum* grown at media using land from closer distance to the beach showed slower grow. It was indicated that planting distance from beach influenced the vegetative growth. Thus, the impact of different distance coastal land from beach on red pepper was needed to have a further discussion. The used of red pepper as plant model because the national market demand on it tend to increase year by year.

The research design was Completely Randomized Design. Five different levels of salinity medium were used. They were A. 15.20 dS · m^{-1}, B. 5.70 dS · m^{-1}, C.2.10 dS · m^{-1}, and D. 2.85 dS · m^{-1} obtained from Pandansimo Beach, Bantul and E. 3.25 dS · m^{-1} obtained from Sleman, DIY as a control. Seedling being transferred to the polybag after it had four leaves. Fruit development was determined every week, started at the first day after anthesis (DAA) and completed at 35 DAA. Capsaicin content was determined at 14 DAA and 35 DAA, performed with Gas Chromatography.

TABLE 3. The relative content of capsaicin (%) in 14 d and 35 d after anthesis (DAA) of red pepper fruit planted in the medium with various salt concentrations.

Medium A		Medium B		Medium C		Medium D		Medium E	
14 DAA	35 DAA	14 DAA	35 DAA	14 DAA	35 DAA	14 DAA	35 DAA	14 DAA	35 DAA
-	-	-	-	25	59	77	100	18	33

*Notes: 1. Medium A. 15.20 dS · m^{-1}; B. 5.7 dS · m^{-1}; C. 1.1 dS · m^{-1} and E. 3.25 dS · m^{-1}.
2. The percentage was calculated based on the highest total capsaicin content which was brownish-green fruits on the planting medium C (1.1 dS · m^{-1}) which is defined as the capsaicin content of 100 %.
3. – mean the plant died before producing fruit.

Capsaicin content of the green fruit (14 DAA) was lower than the mature one (35 DAA) at all medium. The highest capsaicin content at 14 DAA and 35 DAA obtained from plant grown at medium D. It mean the highest content of capsaicin was produced by the red pepper grown in medium with lower salt content compared to those of normal soil. It was predicted that salt content effected the time of fruit ripening.

Capsaicin Content in Chili Grown with Fermented Cow Urine as An Organic Fertilizer

Environmental factors affect the content of secondary metabolites. One of the environment factors is nutrients. Results of Johnson and Decoteau [24] study showed that an increase in growth, yield, and the content of capsaicin in plants Jalapeno Pepper (*C.annum* L.) when the plants were grown on sand medium with nitrogen applications. Hendrison [25] added that trigonelin alkaloid content in the seeds of fenugreek (*Trigonella*

19 April 2024 18:19:55

foenum- graecum L.) varies depending on the pH, nitrogen fertilization and soil fertility. One effort to support the availability of nutrients in the environment is fertilizing with organic fertilizer. The use of organic fertilizer in agriculture will not damage the environment. It can also cope with high prices and limited amount of factory fertilizer, and to prevent an increase in soil pollution [26].

Fertilization using fermented cow urine have a positive impact on plant growth and production. Naswir [27] proved that the increase of tomato growth and production after the plants were given with nutrition from fermented cow urine.Based on previous facts, research on the effect of fermented cow urine on the content of capsaicin on big-red chilies (C. *annum* L. var abrieviata Eingerhuth) and curly-red chili (*C. annum* L. var. Longum Sendt) should be carried out.

This study used a factorial design. The first factor was the dose of fertilizer cow urine, which were 0 mL (P0), 5 mL (P1), 10 mL (P2), 15 mL (P3), and 20 mL (P4) which was poured into the planting medium (polybag). Fertilization was done 5 times started in the plant age (35, 40, 45, 50, and 55) d after transplanting. The second factor was chili varieties, namely were big-red (CB) and curly-red chilies (CK).

The fruits 40 DAA were harvested and cleaned, as much as 2 g of fresh fruits were grounded and extracted using a method which was the same as previous method. The capsaicin analyses were done using gas chromatography. The compound was identified by comparing the retention time with injected standard capsaicin purchased from sigma.

TABLE 4. The relative content of capsaicin (%) in big-red and curl-red chilies at the age 40 DAA treated with fermented cow urine fertilizer.

Big-red chili					Curly-red chili				
0 mL	**5 mL**	**10 mL**	**15 mL**	**20 mL**	**0 mL**	**5 mL**	**10 mL**	**15 mL**	**20 mL**
44	44	52	74	87	59	60	69	100	87

*Notes: 1. The highest capsaicin content of the sample is converted to 100 %.
2. LSD value: 6.57

TABLE 5. Number of compound and peak area total of 40 DAA big-red and curly-red chili after treated with various concentration of fermented cow urine.

	Varieties	**Fermented cow urine concentration**				
		0	**5**	**10**	**15**	**20**
Number of compound	Big-red	34	38	37	48	47
	Curly-red	42	43	45	46	46
Peak area total	Big-red	1 390 749	1 300 165	1 449 695	1 993 211	2 412 427
	Curly-red	1 669 255	1 663 210	1 917 501	2 106 118	1 896 599

The content of capsaicin in curly-red chili peppers higher than those of big-red chili when the chilies were grown in the soil without fertilizer treatment. Cow urine fertilizer application was able to increase the content of capsaicin in both kinds of chilies. The qualitative and quantitative alkaloid content of curly-red chili peppers was higher than those of big-red chili peppers after treated with fermented cow urine.

Capsaicin Content in Chili Grown in Hydroponic Medium

Capsaicin content from red pepper vary depend on the variety, season, growth situation (media), and ripening stage [28]. Normally farmer use soil as chili growing media. One alternative in planting chili for the land efficiency is hydroponic techniques. Hydroponics is also known as a farming without soil and its application not only use water but other media such as sand, gravel, coconut husk and rice husk could be used [29]. In the hydroponic techniques, fertilizer or nutrients absolutely required because media in hydroponic techniques is only functioned as supporting for the growth of a plant. This is very different to soil media because it functioned both as the support the growth of a plant also contains a variety of useful nutrients for plant growth. Based on the facts, it is necessary to know the content of capsaicin in the chili when it was grown in the variety of hydroponic media.

In this study, cayenne were grown in four kinds of hydroponic media, namely sand, rice husk, coconut husk, and sawdust. Soil media was used as a control. NPK fertilizer with four levels of concentration were (0, 5, 10, and 15) mL · L^{-1} of water supplied by 100 mL · d^{-1} per polybag. The concentration of fertilizer were increased twice after 14 d growing.

Old cayenne seedling 4 d were transferred to treatment medium. The NPK fertilizer treatment were applied in the same time with the removal of the seedling to the treatment medium. The experimental design used was Randomized Complete Block Design (RCBD) [30]. The fruit, with 35 DAA were harvested for the capsaicin analyses. The capsaicin were analyzed using Thin Layer Chromatography followed by Thin Layer

19 April 2024 18:19:55

Chromatography Scanner (TLC Scanner) for capsaicin quantification. Before harvesting, the number of fruit in each plant were calculated. The results shown in following Table.

TABLE 6. Relative capsaicin content (%) in chili fruit grown in various growth media and fertilizer.

Soil				Sand				Hull of rice				Coconut husk				Sowdust			
K0	**K5**	**K10**	**K15**	**K0**	**K5**	**K10**	**K15**	**K0**	**K5**	**K10**	**K15**	**K0**	**K5**	**K10**	**K15**	**K0**	**K5**	**K10**	**K15**
74.5	100	89.4	93.6	21.3	29.8	14.9	17.0	-	-	-	-	-	-	-	-	-	-	-	-

*Notes: 1. K0, K5, K10, K15 and K20 mean NPK concentration of (0, 5, 10, 15, 20) ML· L^{-1} water respectively.
2. The relative capsaicin content were calculated by setting capsaicin content in the K5 treated chili grown in soil to be 100 %.
3. – means the plant died before producing fruit.
4. LSD (0.01): 16.33

TABLE 7. The number of fruit in each plant of three months old cayenne plant.

Soil				Sand				Hull of rice				Coconut husk				Sowdust			
K0	**K5**	**K10**	**K15**	**K0**	**K5**	**K10**	**K15**	**K0**	**K5**	**K10**	**K15**	**K0**	**K5**	**K10**	**K15**	**K0**	**K5**	**K10**	**K15**
20	25	31	30	7	33	58	72	-	-	-	-	-	-	-	-	-	-	-	-

*Note: – means the plant died before producing fruit. LSD (0.01):6.53

Cayenne was grown on sand media capable of producing more fruit than those of soil media but cayenne was grown on sand media produced lower capsaicin than those of soil media. People who want to uses cayenne for a garnish on the dish meal purposes, sand media is the most appropriate medium for the cultivation of cayenne. However, if the cayenne was utilized for its hot spicy, it is recommended to grow cayenne using soil media. Moreover, [31] reduced the pungency of Capsicum without losing its biological activity by fermentation method with bacteria isolated from traditional Korean foods. By comparing the capsaicin-degrading capacities of the isolated strains, the capsaicin and dihydrocapsaicin contents of *Bacillus subtilis* were found to be much less than the other. After 48 h-fermentation, the ferments showed sharp decreases in capsaicin and dihydrocapsaicin from initial contents of 103.0 mg · 100 g^{-1} and 16.7 mg · 100 g^{-1} to 12.2 mg · 100 g^{-1} and 7.6 mg · 100 g^{-1} , respectively.

CONCLUSION

Chili is seasonal plant which have many benefits for the human. It is useful for herbal medicines, cooking spices, and pet food. Due to its many functions and its character as a tropical plant, the plant fruit is very potential as an export commodity.

Utilization of chili due to its secondary metabolites content in the fruit namely capsicinoid. A kind of compound from that group is capsaicin which is mostly responsible for hot taste. Capsicinoid is an alkaloid which is very stable in the cooking processes. The compounds are not distributed evenly in the fruit. Septum is the fruit part which contain the highest capsicinoid compared to other parts. Because capsaicin is secondary metabolite, the compound content in the tissue could be affected by environment factors. Therefore the cultivation and harvesting management could be designed based on the purposes.

Our researches have proved that utilization of chili for a garnish on the dish meal, the plant should be grown in the sand medium and prepared without fruit septum. However, if the chili is utilized for herbal medicines or pet food, the plant should be grown in soil medium with the increment of N from organic fertilizer. Moreover, the chili should be processed with its fruit septum.

ACKNOWLEDGEMENTS

Deep gratitude were expressed to our research tim: Prof. Dr. Issirep Sumardi, Dr. Tri Rini Nuringtyas M.Sc., Dr. Ratna Susandarini M.Sc., Purin Chandra Purnama M.Sc., Andi Aisah M.Sc., Utaminingsih M.Sc., Sri Widiyaningsih S.Si. and Rani Anggraeni Herawan S.Si.

19 April 2024 18:19:55

REFERENCES

1. S. H. Smith, J. of *Ethnopharmacology* **167**. 64–77 (2015).
2. S. K. Sharma, A. S. Vij and M. Sharma. European Journal of Pharmacology **720**, 55–62 (2013).
3. M. A. Loizzo, M. Pugliese, F. Bonesi, F. Menichini and R. Tundis. LWT - Food Science and Technology **64**, 623–631 (2015).
4. R. Tundis, F. Menichini, M. Bonesi, F. Conforti, G. Statti, F. Menichini and M. R. Loizzo. LWT Food Science and Technology **53**, 370–377 (2013).
5. Z. Y. Feng, C. Hai-ning, C. Xiao-man, W. Zun-chen, S. Sheng-rong and U. N. Das. 2014. Nutrition **30**, 319–325
6. M. N. Yamaguchi, H. Yahagi, F. Kato, Takano and T. Ohta. Journal of Functional Foods **2**, 255–262 (2010).
7. K. C. Thiamhirunsopit. S. Phisalaphongb, S. Boonkirdb and S. Kijparkorn. Animal Feed Science and Technology **192**, 90–100 (2014).
8. S. J. Ahn, F. R. Badenes-Pérez and D. G. Heckel. Journal of Insect Physiology **57**, 1212–1219 (2011).
9. T. W. Corson and C. M. Crews. Cell **130**, 769-774 (2007).
10. M. A. Bernal and A. R. Barcelo. J. Agric. Food Chem. **44**, 3085–3089 (1996).
11. D. A. Krishna, *Capsicum The Genus of Capsicum*. (Taylor and Francis Group, London and New York. 2003).
12. Tjahyadi, C*abai [Chili]*, (Kanisius, Yogyakarta, 1991) [Bahasa Indonesia].
13. H. W. Youngken, *Texbook of Pharmacognosy*. (McGraw-Hill Company, New York, 1950)
14. R. A. Herawan, "Anatomi dan profil alkaloid buah beberapa aksesi cabai rawit (*Capsicum frutescens* L.), [Anatomy and fruit alkaloid profiles some accession cayenne (*Capsicum frutescens* L.)]" Undergraduate thesis. Universitas Gadjah Mada. 2010 [Bahasa Indonesia].
15. G. F. Barbero, A. G. Ruiz, A. Liazid, M. Palma, J. C. Vera and C. G. Barroso. Food Chemistry **153**, 200–206 (2014).
16. W. Y. Zang, Li and P. A. Wan. European Journal for Clinical Pharmacology **46**, 517–522 (1994).
17. R. L. Jarret, I. J. Levy, T. L. Potter and S. C. Cermak. Journal of Food Composition and Analysis **30**, 102–108 (2013)
18. M. Contreras-Padilla and E. M. Yahia, J. Agric. Food Chem. **46**, 2075–2079 (1998).
19. G. Yaldiz, M. Ozguven and N. Sekerogluc. Industrial Crops and Products **32**, 434–438 (2010).
20. J. W. Pursglove, E. G. Brown, C. L. Green and S. R. J. Robbins. *Spices*. Vol. 1. (Longman, London, 1981)
21. E. P. Claus, *Gathercoal and Wirth Pharmacognosy*. (Lea Febiger. Philadelphia, 1956)
22. J. S. Lewis, *Spices and Herb for The Food Industry.* (Food Trade Press, Orpinton England, 1984)
23. T. C. Barrilleaux and J. B. Grace. J. Bot. **87**, 1099–1106 (2000).
24. C. D. Johnson and D. R. Decoteau. Hort Science **7**, 1119–1123 (1996).
25. M. Hendrison, *Pengaruh pemupukan nitrogen pada pH tanah berbeda terhadap hasil dan kadar alkaloid biji kelebet* (Trigonella foenum-graceum L.) *di dua lokasi dengan ketinggian dan musim tanah berbeda* [Effect of nitrogen fertilization on different soil pH on the result and alkaloid content kelebet seeds (*Trigonella foenum-graceum* L.) in two locations with altitude and soil different season].[Bandung] [Intenet cited 2008 Februari 28], Available from: www.lib.itb.ac.id. [Bahasa Indonesia]
26. A. Rosmarkam dan N. S.
27. Naswir. *Pemanfaatan urin sapi yang difermentasi sebagai nutrisi tanaman* [Utilization of fermented cow urine as plant nutrition], 2003, [Bogor], [Intenet cited 2008 Februari 28], Available from: http://tumontou.net/Pemanfaatan Urine Sapi yang Difermentasikan sebagai Nutrisi Tanaman. html/ [Bahasa Indonesia].
28. J. S. Pruthi, *Spices and Condiments: Chemistry, Microbiology Technology. Advances in Food Research.* (Academic Press Inc. London, 1980).
29. L. Morgan, *Hydroponic Lettuce Production*. (Casper Publication, New South Wales, 1999).
30. K. A. Gomez and A. A. Gomez. *Statistical Procedures for Agricultural Research*. (John Wiley & Sons. Singapore, 1983).
31. S. M. Lee, H. S. Lee, J. Lee, K. W. Yuc, K. M. Kim, K. S. Rae, *et al.* Enzyme and Microbial Technology **47**, 200–205, (2010).

19 April 2024 18:19:55