Bulgarian Chemical Communications, Volume 51, Issue 3 (pp. 378 - 383) 2019 DOI: 10.34049/bcc.51.3.5049

Phytochemical screening and antimicrobial activity of extracts of *Cassia alata* L. leaves and seeds

P. T. Q. Le

Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, No. 12 Nguyen Van Bao, Ward 4, Go Vap district, Ho Chi Minh city, Vietnam

Received January 11, 2019; Revised March 25, 2019

The aim of the study is to determine the presence of some bioactive compounds in *Cassia alata* L. leaves and seeds extracts such as tannins, saponins, anthraquinones and flavonoids. Total polyphenol contents (TPC) of leaves and seeds extracts are 59.211 mg GAE/g DW and 1.816 mg GAE/g DW, respectively, while their antioxidant capacities (AC) are 8.14 μmol Fe/g DW and 2.75 μmol Fe/g DW, respectively. The antimicrobial activity is determined by the paper disc diffusion method combined with the minimum inhibitory concentration (MIC). Leaves extract inhibits *S. aureus* and *E. coli* at MIC of 400 mg/mL; *S. enteritidis* and *B. subtilis* at MIC of 800 mg/mL. Besides, seeds extract also inhibits *S. aureus* at MIC of 200 mg/mL; *E. coli*, *S. enteritidis* and *B. subtilis* at MIC of 400 mg/mL. However, leaves and seeds extracts of *C. alata* do not show any inhibitions on the growth of *A. niger*.

Keywords: Antibacterial activity, Antioxidant, *Cassia alata* L., Extract, MIC.

INTRODUCTION

Currently, there are many synthetic chemical compounds, which can inhibit the growth of microorganisms, that are widely used in food industry and medical technology. However, these compounds can affect consumer health and cause antimicrobial resistance. Therefore, it is an important challenge in chemical and medical field [1]. Until now, many studies have demonstrated that the roots, stems, leaves, and fruits of some plant species contain antimicrobial substances called phytoncides or plant antibiotics [2,3]. Therefore, some plant extracts can inhibit many microorganisms, for example, *Polygonum multiflorum* Thunb. root extract inhibited *S. aureus* and *S. enteritidis* [4]*;* extract of *Hyphaene thebaica* L. Mart. (Arecaceae) fruit inhibited *S. aureus* and *S. typhi* [5], etc.

In Vietnam, there are many herbal plants such at ginseng, dangshen, lingzhi mushroom, etc., that contain precious substances and are used in food industry or medicine [6]. Among them, *Cassia alata* L., which is planted as ornamental plant in Vietnam, contains various bioactive substances. According to the study of Meenupriya *et al*. [7], the authors identified phytochemical compounds such as alkaloids, flavonoids, saponins, tannins, coumarins, terpenoids, steroids, glycosides in *Cassia alata* L. leaves extract collected from India. The ethnomedical uses of *Cassia alata* L. have been recorded in many provinces in Vietnam. It can cure many diseases such as scabies, tokelau, etc. [6].

* To whom all correspondence should be sent:
E-mail: lephamtanquoc@iuh.edu.vn

There are many researches on the extraction of bioactive compounds and their antifungal activity from *Cassia alata* L. leaves extract [8] but until now there is no report on phytochemical screening and antimicrobial activities of leaves and seeds extracts of *Cassia alata* L. in Vietnam by the combination of minimum inhibitory concentration and the paper disc diffusion method. Therefore, the current research was undertaken to determine some bioactive compounds and antimicrobial activities of ethanol extracts of *Cassia alata* L. leaves and seeds.

EXPERIMENTAL

Plant collection

Cassia alata L. leaves and seeds were collected from Ho Chi Minh city (Vietnam), cleaned by tap water, then dried at 60°C until moisture is lower than 13%. After that, the samples were milled and sieved with a sieve diameter of 500 μm; packed under vacuum condition and stored at room temperature.

Organisms collection

A total of five tested microorganisms including: two gram-positive bacteria as *Bacillus subtilis* (ATCC 11774), *Staphylococcus aureus* (ATCC 25923), two gram-negative bacteria as *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076) and the fungus *Aspergillus niger* were used in this study to determine the antimicrobial activity of extracts. All microorganisms were kindly provided by the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh city.

Ethanol extraction

The dried samples were extracted by microwave-assisted extraction (MAE) with 50% aqueous ethanol, dried seeds/ethanol ratio of 1/20 (w/v), dried leaves/ethanol ratio 1:10 (w/v) and microwave power of 194 W for 5 min. After the extraction process, the extract was filtered by Whatman paper no. 4 and the solvent was evaporated under vacuum conditions in a water bath at 45°C for 30 min. After that, the residue was freeze-dried during 7 h at -20°C, <1 mbar, then the dry extract was stored at 4ºC until further experiments.

Phytochemical analysis

Identification of flavonoids. Ferric chloride test: Three drops of 5% $FeCl_3$ solution were added to the extract. The formation of greenish-black color indicated the presence of phenolic nucleus [9].

Identification of tannins. Gelatin test: Few drops of 10% gelatin solution were added to the extract. Formation of a precipitate indicated the presence of tannins [10].

Identification of anthraquinones. Borntrager's test: 2 mL of the extract were added to 5 mL of chloroform in a test tube and shaken for a few mins with an equal volume of 10% ammonia solution. The presence of free anthraquinones was evidenced by layering such as violet, pink or red [10].

Identification of saponins. Frothing test: The extract was placed in a test tube and added to 10 mL of distilled water; shook vigorously for 30 sec, then kept for 30 min and observed. The formation of foam indicated the presence of saponins [9].

Haemolysis test: Few drops of animal blood were added to the extract (dissolved in normal saline) by a syringe and mixed gently by inverting the tube and kept for 15 min. The settling down of red blood cells denoted the presence of saponins [11].

Determination of total polyphenol content (TPC) and antioxidant capacity (AC) of polyphenols by the phenanthroline method

The TPC of the extract was determined by the Folin Ciocalteu colorimetric method [12] with slight modifications. The results were calculated from a standard curve of gallic acid. TPC was expressed as mg of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

The AC of the extract was slightly modified and determined by the phenanthroline method [13]. The reaction between iron and 1,10-phenanthroline produces orange-red complex. AC was presented as μmol of Fe per gram of dry weight (μmol Fe/g DW).

Determination of antimicrobial activity and minimum inhibitory concentration (MIC) evaluation

The minimum inhibitory concentration (MIC) evaluation was performed by the paper disc diffusion method for antibiotic susceptibility testing according to the Kirby-Bauer test. The sterile paper discs of 6 mm diameter were prepared using various concentrations of dryness extract of leaves and seeds (50, 100, 200, 400 and 800 mg/mL); gentamicin (10 μg/disc) and ketoconazole (50 μg/disc) were used as positive controls to compare the antibacterial activity and antifungal activity, respectively; 5% dimethylsulfoxide (DMSO) was used as negative control. Firstly, 0.1 mL of bacteria suspension (0.5 McFarland standard, approximately 1.5×10^8 cfu/mL) and 0.1 mL of fungus suspension (approximately 0.4×10^4 – 5×10^4 cfu/mL) were spread on the surface of the Mueller-Hinton agar media for bacterial strains and potato dextrose agar media for fungal strains by a sterile hockey stick, respectively. Then, the sterile paper discs were impregnated with 20 μL of each of extracts. The dishes were incubated during 24 h at 37ºC for bacterial strains and 72 h at 30ºC for fungal strain. After that, the zones of inhibition were expressed in mm as the diameters of clear zones around the discs.

Data analysis

Experimental results were analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at ($p<0.05$) were determined by Fisher's least significant difference (LSD) procedure using Statgraphics software (Centurion XV). The values obtained were expressed in the form of a mean±standard deviation (SD).

RESULTS AND DISCUSSION

Identification of bioactive compounds

Phytochemical analysis of *Cassia alata* L. leaves and seeds was successfully performed; ethanol is a good solvent for the extraction of bioactive compounds of this plant. There are tannins, saponins and flavonoids in both *Cassia alata* L. leaves and seeds (Table 1). These results suggest that these parts of the plant can have an antibacterial capacity and antioxidant capacity because of the presence of these compounds. Tannins are polyphenols that are commonly found in plants such as *Cleome Ciliata* leaves [14] or

Polygonum multiflorum Thunb. roots [4], etc. Tannins can be used to treat various human diseases, including diarrhea, stomach ulcers, snake bites and wounds [15]. In addition, they are good antioxidants, anti-aging, anti-inflammatory, anti-cancer agents [16]. The presence of flavonoids in this material is similar to that in *Paullinia pinnata* Linn leaves [11] or *Polygonum multiflorum* Thunb. roots [4] and these bioactive compounds have a strong antioxidant capacity. Several previous studies indicated that secondary metabolites of phenolic compounds such as flavonoids are responsible for a variety of pharmacological activities [17]. Note that free anthraquinones are only present in seeds extract; they are an important bioactive ingredient which possesses anti-cancer, anti-bacterial, anti-inflammatory effects [18]. In addition, it can activate the nucleotide repair system in human cells [19].

Apart from phenolic compounds, saponins were also detected in both *Cassia alata* L. leaves and seeds extracts. They also exist in other plants such as *Polygonum multiflorum* Thunb. roots [4], *Paullinia pinnata* Linn leaves [11]. Basically, both *Cassia alata* L. leaves and seeds extracts contain many bioactive compounds. This is advantageous for the application of these compounds in medical technology.

Determination of total polyphenol content (TPC) and antioxidant capacity (AC) of extracts

Table 2 shows that TPC of leaves extract is 32 times higher than that of seeds extract while AC of leaves extract is 3 times higher than that of seeds extract. TPC of leaves extract is also higher than that of *Polygonum multiflorum* Thunb. root extract [4]. At the same time, AC of leaves and seeds extracts is higher than that of some vegetable oils extracts [13] or fresh garlic clove, garlic capsule, and baked garlic extracts [20]. This indicates that *Cassia alata* L. leaves and seeds are potential materials, especially the leaves, to be used for medical applications or functional products.

Table 1. Phytochemical constituents of *Cassia alata* L. leaves and seeds extracts

No.	Phytoconstituents	*Cassia alata* L. leaves extract	*Cassia alata* L. seeds extract
1	Tannins		
	$FeCl_3$ test	+	+
2	Saponins		
	a. Frothing test	+	+
	b. Haemolysis test	+	+
3	Anthraquinones		
	Borntrager's test	-	+
4	Flavonoids		
	a. $FeCl_3$ test	+	+
	b. NaOH test	+	+

"+": Positive test
"-": Negative test

Table 2. TPC and AC of *Cassia alata* L. leaves and seeds extracts

Materials	*Cassia alata* L. seeds extract	*Cassia alata* L. leaves extract
TPC (mg GAE/g DW)	1.82 ± 0.17^a	59.21 ± 3.02^b
AC (µmol Fe/g DW)	2.75 ± 0.11^a	8.14 ± 0.59^b

Various lowercase letters in the same row denote significant difference ($p<0.05$)

Table 3. Moisture and yield of dry extracts of *Cassia alata* L. seeds and leaves

Physicochemical characteristic	*Cassia alata* L. seeds dry extract	*Cassia alata* L. leaves dry extract
Moisture (%)	3.18 ± 0.43^a	3.31 ± 0.51^a
Yield (%)	16.67 ± 2.8^b	8.16 ± 2.01^a

Various lowercase letters in the same row denote significant difference ($p<0.05$)

Identification of moisture and yield of dry extracts

Based on the results of this study, the moisture of extracts of *Cassia alata* L. leaves and seeds is 3.31% and 3.18%, respectively (Table 3). They are similar to the dry extract of *Polygonum multiflorum* Thunb. root (3.03%) [4]. The dry extract should be stored at 4ºC to avoid oxidation reaction and degradation of bioactive compounds.

The results suggest that the yield of recovery extracts from *Cassia alata* L. is quite high, extracts of leaves and seeds are 8.16% and 16.67%, respectively. These results are higher than those in the recent study of Võ *et al.* [21] (the dry extract from trunk and leaves of *Pouzolzia zeylanica* L. was nearly 2.96%) and the study of Đái *et al*. [22] (the extract from trunk and leaves of *Streptocaulon juventas* Merr. was 3.55%). This study proves that the obtained yields depend on various factors such as material, solvent, extraction methods, etc.

Antibacterial activity and minimum inhibitory concentration (MIC) evaluation

DMSO is a sulfur-containing organic compound with the formula $(CH_3)_2SO$, a colorless liquid that can be mixed with a wide range of organic solvents as well as with water. It is used as a negative control for various anti-microorganism tests of herbal plant extracts such as *Polygonum multiflorum* Thunb. root [4], the fruit pulp of wood apple [23], etc. In addition, gentamycin and ketoconazole are also positive controls for various antibacterial and antifungal tests [4,24]. The results show that these positive controls have high anti-microorganism capacities (zone of inhibitions ≥14 mm for fungus and ≥18 mm for bacteria) (Tables 4, 5, 6). Gentamycin binds quickly to the ribosome of bacteria, inhibits protein synthesis process and reduces the accuracy of the information RNA which leads to wrong combination of amino acids in the polypeptide chain of bacteria [25]. Furthermore, ketoconazole can inhibit the uptake of precursors of RNA and DNA, inhibits synthesis of peroxidative and oxidative enzymes and increases membrane permeability, which inhibits the growth of fungi [26].

Tables 4 and 5 show that dry seeds and leaves extracts of *Cassia alata* L. have antimicrobial activity against *S. aureus*, *E. coli*, *S. enteritidis* and *B. subtilis*. For seeds extract of *Cassia alata* L., *S. aureus* is inhibited at MIC of 200 mg/mL while the rest of bacteria are inhibited at MIC of 400 mg/mL. At the same time, leaves extract of *Cassia alata* L. can inhibit *S. aureus*, *E. coli* at MIC of 400 mg/mL and *S. enteritidis*, *B. subtilis* at MIC of 800 mg/mL. All inhibition zones are broader than 10 mm and the antimicrobial effect increases with the increase in extract concentration. However, seeds and leaves extract of *Cassia alata* L. did not inhibit *A. niger* (Table 6); this result is similar with that in the study of Khan *et al.* [8] who reported that all parts of *Cassia alata* L. did not inhibit *A. niger*. In general, the antibacterial capacity of the extract of *Cassia alata* L. seeds is better than that of *Cassia alata* L. leaves mainly because it has antibacterial capacity at lower MIC.

Antibacterial capacity depends on the bioactive compounds in the extract, especially tannins, anthraquinones, flavonoids and saponins. For tannins, there are many antimicrobial mechanisms, such as alteration of the microorganism metabolism through the inhibition of oxidative phosphorylation, inhibition of enzyme activity by formation of complex with substrates of bacteria, etc. [27,28].

Some flavonoids have been shown to have strong antibacterial activity such as apigenin, galangin, flavon and flavonol glycosides, isoflavones, flavanones and chalcones [29]. Lipophilic flavonoids can also break down microbial membranes [30]. Mori *et al.* [31] suggested that the B ring of flavonoids could interleave or form hydrogen bonds with the overlap of bases in the nucleic acid molecule and further lead to inhibition of DNA and RNA synthesis of bacteria. Another study demonstrated the inhibition of quercetin, apigenin, and 3,6,7,3',4'-pentahydroxyflavone against DNA replication of *E. coli* [32].

The presence of anthraquinones in *Cassia alata* L. seeds extract can improve the antibacterial capacity. They have been used as a colorant in food, medicine, cosmetics, hair dyes and textiles. Currently, more than 300 naturally occurring anthraquinones are discovered. Some anthraquinones are found in many traditional Chinese medicine herbs such as *Rheum*, *Cassia*, *Aloe* and *Polygonum* [33]. Anthraquinones can inhibit respiration of some bacteria [34], interfere in redox of enzyme NADH dehydrogenase in bacteria [35]. Apart from the above phenolic compounds, saponins are also detected in *Cassia alata* L. seeds and leaves extracts. Various biological effects of saponins are reported including hemolytic and antibacterial activities [36]. The antimicrobial activity of saponins depends on the structure of aglycone of saponins. The efficiency of glucose utilization of microorganisms is quickly reduced by the presence of saponins, which in turn affects the development of microbes, reduces the activity of key enzymes in physiological metabolism, suppresses the synthesis of relevant proteins, and finally executes the antibacterial effect [37].

Table 4. Antibacterial activities of dry extract of *Cassia alata* L. seeds

Bacterial strains	Zone of inhibition (mm)						
	Gentamycin (10 µg/disc)	DMSO 5%	Concentrations of *Cassia alata* L. seeds dry extract (mg/mL)				
			800	400	200	100	50
S. aureus	27.3 ± 0.5^{Cd}	-	16.7 ± 0.5^{Cc}	14.7 ± 0.5^{Cb}	12.7 ± 0.5^{a}	-	-
E. coli	19.7 ± 0.5^{Bb}	-	10.0 ± 0.8^{Aa}	9.7 ± 0.5^{Aa}	-	-	-
S. enteritidis	18.5 ± 0.4^{Ac}	-	12.3 ± 0.5^{Bb}	11 ± 0^{Ba}	-	-	-
B. subtilis	20.3 ± 0.5^{Bc}	-	11 ± 0^{Ab}	9.7 ± 0.5^{Aa}	-	-	-

Various lowercase letters in the same row denote significant difference ($p<0.05$).
Various uppercase letters in the same column denote significant difference ($p<0.05$).
"-": Negative test.

Table 5. Antibacterial activities of dry extract of *Cassia alata* L. leaves

Bacterial strains	Zone of inhibition (mm)						
	Gentamycin (10 µg/disc)	DMSO 5%	Concentrations of *Cassia alata* L. leaves dry extract (mg/mL)				
			800	400	200	100	50
S. aureus	27 ± 1.4^{Cb}	-	15.3 ± 0.5^{Ba}	14.7 ± 1.7^{Ba}	-	-	-
E. coli	20 ± 1.4^{Bb}	-	11 ± 0.8^{Aa}	10.3 ± 0.5^{Aa}	-	-	-
S. enteritidis	18.3 ± 0.5^{Ab}	-	12.3 ± 1.7^{Aa}	-	-	-	-
B. subtilis	20 ± 0.5^{Bb}	-	14.7 ± 0.5^{Ba}	-	-	-	-

Various lowercase letters in the same row denote significant difference ($p<0.05$).
Various uppercase letters in the same column denote significant difference ($p<0.05$).
"-": Negative test

Table 6. Antifungal activities of dry extract of *Cassia alata* L. leaves and seeds

Bacterial strains	Ketoconazole (50 µg/disc)	DMSO (5%)	Concentrations of *Cassia alata* L. leaves and seeds dry extract (mg/mL)				
			800	400	200	100	50
Zone of inhibition of *Cassia alata* L. seeds dry extract (mm)							
A. niger	15.7 ± 1.2^{A}	-	-	-	-	-	-
Zone of inhibition of *Cassia alata* L. leaves dry extract (mm)							
A. niger	14 ± 1.6^{A}	-	-	-	-	-	-

Various uppercase letters in the same column denote significant difference ($p<0.05$).
"-": Negative test

CONCLUSION

The results show that the extracts of *Cassia alata* L. leaves and seeds contain some bioactive compounds such as tannins, flavonoids, anthraquinones and saponins. TPC and AC of leaves extract are higher than those of seeds extract. The dry extracts of seeds and leaves can inhibit some bacteria but they can not inhibit *A. niger*.

Acknowledgement: *The author wishes to thank Vo Thi Dung, Nguyen Thi Tuyet Nhung, Nguyen Tran Ky Duyen and Duong Thi Men for their supports in this study.*

REFERENCES

1. C. K. Amadou, *Afric. Health J.*, **2**, 20 (1998).
2. D. Portz, E. Koch, A.J. Slusarenko, *Eur. J. Plant Pathol.*, **122**, 197 (2008).
3. H. A. Mabrouk, E. M. H. Labib, M. A. Zaki, *Egypt. J. Aquat. Res.*, **37**, 199 (2011).
4. L. P. T. Quoc, N. V. Muoi, *Carpathian J. Food Sci. Technol.*, **10**, 137 (2018).
5. W. Aboshora, Z. Lianfu, M. Dahir, M. Qingran, S. Qingrui, L. Jing, N. Q. M. Al-Haj, A. Ammar, *Trop. J. Pharm. Res.*, **13**, 2057 (2014).
6. T. L. Đỗ, Herbal Plants and their Medicine in Vietnam, Medicine Press, Ha Noi, 2004.
7. J. Meenupriya, A. S. Vinisha, P. Priya, *World J. Pharm. Sci.*, **2**, 1723 (2014).
8. M. R. Khan, M. Kihara, A.D. Omoloso, *Fitoterapia*, **75**, 561 (2001).
9. A. Sofowora, in: Textbook of Pharmacognosy, W. C. Evans (eds.), Spectrum Books Limited, Ibadan, 1993, p. 50.
10. W. C. Evans, Trease and Evans Pharmacognosy, W.B Sauders Press, London, 2002.
11. A. Z. Yusuf, A. Zakir, Z. Shemau, M. Abdullahi, S. A. Halima, *J. Pharmacogn. Phytother.*, **6**, 10 (2014).
12. A. Siddiqua, K. B. Premakumari, R. Sultana, S. Vithya, *Int. J. Chem. Tech. Res.*, **2**, 205 (2010).
13. A. S. Szydłowska-Czerniak, C. Dianoczki, K. Recseg, G. Karlovits, E. Szłyk, *Talanta*, **76**, 899 (2008).
14. S. C. Umerie, N. H. Okorie, S. C. Ezea, A. N. Okpalaononuju, *Int. J. Curr. Res. Rev.*, **4**, 5 (2012).
15. L. M. S. Perera, D. Ruedas, B. C. Gomez, *J. Ethnopharmacol.*, **77**, 1 (2001).
16. M. Atanassova, V. Christova-Bagdassarian, *J. Univ. Chem. Technol. Metall.*, **44**, 413 (2009).
17. A. K. Pandey, *Natl. Acad. Sci. Lett.*, **30**, 383 (2007).
18. G. Srinivas, R. J. Anto, P. Srinivas, S. Vidhyalakshmi, V. P. Senan, D. Karunagaran, *Eur. J. Pharmacol.*, **473**, 117 (2003).
19. L. C. Chang, H. M. Sheu, Y. S. Huang, T. R. Tsai, K. W. Kuo, *Biochem. Pharmacol.*, **58**, 49 (1999).
20. J. Songsungkan, S. Chanthai, *Int. Food Res. J.*, **21**, 2377 (2014).
21. T. T. A. Võ, C. L. Trần, T. T. T. Trần, P. Q. Đỗ, *Can Tho Univ. J. Sci.*, **52**, 29 (2017).
22. T. X. T. Đái, H. B. N. Lâm, T. T. A. Võ, *Can Tho Univ. J. Sci.*, **40**, 1 (2015).
23. A. Senthilkumar, V. Venkatesalu, *Ind. Crops Prod.*, **46**, 66 (2013).
24. M. Masibo, Q. He, *Malays. J. Microbiol.*, **5**, 73 (2009).
25. T. R. Zembower, G. A. Noskin, M. J. Postelnick, C. Nguyen, L. R. Peterson, *Int. J. Antimicrob. Agents*, **10**, 95 (1998).
26. J. H. Van-Tyle, *Pharmacotherapy*, **4**, 343 (1984).
27. A. Scalbert, *Phytochemistry*, **30**, 3875 (1991).
28. M. A. Rebecca, E. L. Ishii-Iwamoto, R. Grespan, R. K. N. Cuman, S. M. Caparroz-Assef, J. C. P. Mello, C. A. Bersani-Amado, *J. Ethnopharmacol.*, **83**, 101 (2002).
29. T. P. T. Cushnie, A. J. Lamb, *Int. J. Antimicrob. Agents*, **26**, 343 (2005).
30. D. E. Moerman, *J. Ethnopharmacol.*, **52**, 1 (1996).
31. A. Mori, C. Nishino, N. Enoki, S. Tawata, *Phytochemistry*, **26**, 2231 (1987).
32. K. A. Ohemeng, C. F. Schwender, K. P. Fu, J. F. Barrett, *Bioorg. Med. Chem. Lett.*, **3**, 225 (1993).
33. Y. W. Wu, J. Ouyang, X. H. Xiao, W. Y. Gao, Y. Liu, *Chi. J. Chem.*, **24**, 45 (2006).
34. C. H. Chen, T. T. Li, H. L. Su, C. I. Wang, *Acta Biochim. Biophys. Sin.*, **3**, 426 (1963).
35. Y. Zhang, Q. H. Chen, *Acta Biochim. Biophys. Sin.*, **18**, 239 (1986).
36. E. Karimi, H. Z. Jaafar, S. Ahmad, *Molecule*s, **16**, 4438 (2011).
37. Yu, Z. H., X. Z. Ding, L. Q. Xiz, X. Q. Xiao, Z. P. Cao, S. Xu, S. Liu, X. M. Liu, *Food Sci.*, **34**, 75 (2013).