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GC-MS Profiling of Capsaicinoids Present in *Capsicum chinense* Jacq. cv. (Naga King Chilli) and Evaluation of its Antifungal Activity

LEVONO* and MANISHA PRASAD

Department of Chemistry, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad-211 007, India

*Corresponding author: E-mail: alevkenn1@gmail.com

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Capsicum chinense Jacq. cv. (Naga King Chilli) of family Solanaceae mainly known for its pungency are cultivated in North-Eastern India. In this study, Capsaicin the major component of capsaicinoids was quantified using UV spectrophotometer. The Capsaicin content in dry chilli was found to be greater as compared to fresh attributing to the possibility of peroxidase reaction in fresh chilli with lapse of time. The compounds of capsaicinoids present in the dry and fresh chilli of *Capsicum chinense* was identified by gas chromatography-mass spectroscopy (GC-MS). Capsaicin was found to be present in both the dry and fresh chilli while dihydrocapsaicin was found to be present only in dry chilli. The extract of the dry and fresh chilli were further evaluated against plant pathogens *Fusarium oxysporum* and *Fusarium udum*. Dry chilli extract showed a better result, the reason of which may be due to higher concentration of capsaicinoids as reported by various researchers. In conclusion, *Capsicum chinense* a natural source of capsaicinoids and its effective role in antifungal activity could lead to important changes in disease control plan of pest, pathogens of plant borne disease and may offer new direction for greater research work on chilli peppers.

Keywords: *Capsicum chinense*, Antifungal activity, Capsaicinoids, *Fusarium oxysporum*, *Fusarium udum*.

INTRODUCTION

Capsicum chinense (Naga King Chilli) a prominent crop known for its high pungency and unique aroma are grown in North-eastern part of India [1]. Although Naga King Chilli has been cultivated and consumed for over hundreds of years, its saga of fierceness remain vague until 2006, where it earned the distinction of being the hottest chilli in the World [2].

The protoalkaloids capsaicinoids are responsible for the fiery sensation of the chilli [3].These secondary metabolites are reported to be present only in Capsicum species. Capsaicinoids contain components capsaicin, dihydrocapsaicin, norhydrocapsaicin, norcapsaicin, nornorcapsaicin and nonivamide.

Capsaicin, the major component of capsaicinoids responsible for 60-90 % of total pungency of the chilli are reported to be present in high content in *Capsicum chinense*. About 3-5 % capsaicin are reported to be present in this particular chilli as compare to other chilli variety in India which have content less than 1 % of capsaicin [4,5]. Capsaicin besides being a savoury ingredient in food, they are reported to have numeral applications.

Capsaicin are reported to trigger brain to produce endorphins, a natural pain killer. They are incorporated in number of medical appliances such as to fight against pain of arthritis, sprains, control of skin diseases like shingles, psoriasis, respiratory problems and bowel complains.Capsaicin also used in preparation of pepper spray comes handy in riot control and as self-defence kit for lonely women.Also capsaicin are reported to be antiinflammatory and antioxidants [6].

Researchers have reported capsaicinoids to be a good antifungal agent [7]. North-eastern India with favourable agro-climate wide range of horticulture crops are cultivated. However, due to incidence of the crop diseases, poor knowledge of farming and continuous use of traditional practice, number of crops are reported to be destroyed causing huge loss in the economy. Among the infectious plant pathogens, *Fusarium oxysporum* and *Fusarium udum* have been reported to cause diseases in number of plants in north-eastern India. Gingers a prominent cash crop cultivated over the hills of Sikkim are reported to be destroyed massively by *Fusarium oxysporum* [8,9]. Swer *et al.* [10] reported *Fusarium oxysporum* to be one of the most common pathogen isolated from soil in Meghalaya, India. *Fusarium oxysporum* are also reported to be destructive against tomato in Assam, Meghalaya and Sikkim states of India [11]. *Fusarium udum* are reported to cause most serious and destructive soil borne disease of pigeonpea cultivated at Senapati district and Sadar hill of Manipur [12].

Capsicum chinense being a natural source of capsaicinoids and capsaicinoids been reported to have numeral applications

open doors for exploring its potential and beneficial properties. Many experts also believe that capsaicinoids are used as a medicine and has a great future that scientists are just beginning to discover.

EXPERIMENTAL

Plant material *Capsicum chinense* was collected from Kohima, India. The dried chilli was dried using sun dried method. The sun dried method was selected as they are the most preferable method practice by the traditional native people of north-eastern, India. The dried chillies were packed in airtight condition and stored until further used.

Identification of chilli species: The chilli species of *Capsicum chinense* was identified by Taxonomist of Department of Horticulture, SHUATS, Allahabad, India.

Extraction procedure: The extraction of both the fresh and dried chilli was done using cold maceration method as described by Dubey *et al*. [3]. The fruits of both the dried and fresh chilli were taken separately and washed thoroughly using distilled water to removed dust or any other unwanted particles adhering to the surface of chilli. 10 grams of chilli was weighed, added to 100 mL of acetone and was subjected to continuous shaking for 3 h. The filtrate obtained was distilled and crude was collected.

Quantification of total capsaicin content: Capsaicin content was determined by spectrophotometric method [3]. 1 mg of crude extract of both dried and fresh chilli was redissolved in 5 mL of 0.4 % NaOH and 3 mL of 3 % phosphomolybdic acid and allowed to stand for 1 h. The solution was filtered and centrifuged at 5000 rpm for 10 to 15 min. The absorbance of blue colour solution was read at 650 nm against blank reference solution in UV spectrophotometer. The standard capsaicin was obtained from Naturite Agro Product, Hyderabad, India. The content of capsaicin both in dried and fresh chilli *Capsicum chinense* was calculated from the linear regression equation obtained from standard capsaicin calibration curve and expressed in µg/mg.

Gas chromatography-mass spectroscopy (GC-MS): The GC-MS analysis of both the dried chilli extract and fresh chilli extract was performed at SAIF, Chandigarh. The gas chromatography-mass spectroscopy analysis was performed as described by Pino *et al*. [13]. GC-MS analysis of extracts was performed using Thermo Scientific TSQ 8000. GC-MS equipped with fused silica capillary column of HP-5 (30 m × 0.25 mm × 0.25 µm) was used for separation of compounds. The temperature of the oven was held from 50 ºC for 2 min and then increased to 280 ºC at 4 ºC per minute. The carrier gas (helium) flow rate was 1 mL/min. The flame ionization detector was operated in electron impact mode of 70 eV at 230 ºC. Detection was performed in the scan mode between 30 and 400 Da. The test materials were ascertained by comparing the retention time and mass spectra with the known compounds using automated library search on the NIST (National Institute of Standard and Technology) MS search programme.

Antifungal activity: The antifungal activity was evaluated using Food Poisoning Technique [14]. 0.1526 g of the extract of dried and fresh chilli was weighed and dissolved in 62.5 mL of 10 % of DMSO. 15 mL of solution was mixed with 85 mL of PDA. The plates were left for 15 min to solidify. Then 4 mm diameter of fungal colony punched with borer was placed onto plates containing media with extract in aseptic condition. The plates were incubated for 3-5 days at 28 ºC. The medium without any treatment serve as control. Test fungi were inoculated and percentage inhibition of mycelial growth was determined. After three days colony diameter was measured in millimetre. For each treatment three replicates were maintained. Percentage inhibition was calculated as follows:

$$\text{Percentage inhibition (I, \%)} = \frac{C - T}{C} \times 100$$

where, C = colony diameter in control, T = colony diameter in treatment.

Statistical analysis: The statistical analysis was performed using one way ANOVA-Analysis of variance following complete randomized design.

RESULTS AND DISCUSSION

Quantification of capsaicin: Capsaicin was quantified using phosphomolybdic acid reduction for capsaicin method. The phenolic group in capsaicin reduces the phosphomolybdic acid to lower acids of molybdenum (Molybdenum blue). The different concentration of capsaicin solution gives blue colour and the intensities of the colour are directly proportional to the concentration of capsaicin [15].

Using linear regression equation obtained from standard capsaicin curve, capsaicin content was found to be 130.2 µg/mg in dry chilli and 103.8 µg/mg in fresh chilli. In Schoville heat units scale, the dried chilli showed the pungency level of 2083200 SHU, while the fresh chilli showed 1660800 SHU pungency level. The capsaicin content was found lower in fresh chilli. Lesser amount of capsaicin in fresh chilli extract may be due to peroxidase reaction. In chilli, the phenolic group present are oxidized by the peroxidase enzyme to phenoxy radical, which participates in reaction where polymers are the product [16]. As fresh sample are fragile and tend to deteriorate faster than dried sample, this peroxidase reaction may have occurred at the interval between the time of collection and time of experimental work or during the time of experimental work of extraction as cold maceration method was used where no thermal application was applied. According to Bernal *et al.* [17] capsaicin and peroxidase enzyme are co-localized in the placental and outermost epidermal cell layer of the pepper fruit which enhances the peroxidation reaction to take place.

Higher content of capsaicin in dried chilli extract may be due to application of heat as it was sun dried right after the collection. The application of heat may have inactivated the enzyme responsible for peroxidase reaction in dried chilli. This finding are similar to Paz *et al*. [18] where they reported that capsaicin content was found higher in thermally treated peppers attributing to several factors such as dehydration of food matrix, improved extractability of this compound by cell disruption during thermal process and inactivation of capsaicin destroying enzyme such as peroxidases.

GC-MS profiling of capsaicinoids both in dry and fresh chilli *Capsicum chinense*: GC-MS analysis revealed the presence of capsaicinoids both in fresh and dry chilli extract. Capsaicin

was found to be present in both the fresh and dried chilli extract while dihydrocapsaicin was found to be present only in dried chilli extract (Table-1).

TABLE-1
COMPOUND OF CAPSAICINOIDS DETECTED IN FRESH AND DRY *Capsicum chinense*

Compound of capsaicinoids in fresh chilli	Compounds of capsaicinoids in dry chilli
Capsaicin	Capsaicin Dihydrocapsaicin

Capsaicin (m.f. $C_{18}H_{27}NO_3$; m.w. 305) was identified through the mass splitting spectra (Figs. 1 and 2). The peaks observed for capsaicin at *m/z* 262 is due to loss of (-C_3H_7) and at *m/z* 196 due to loss of (-C_5H_8). The base peak is observed at *m/z* 137.1 due to cleavage of -$NHCOCH_2$ from the terminal carbon of the ring. For dihydrocapsaicin (m.f. $C_{18}H_{29}NO_3$; m.w. 307), the peaks were observed at *m/z* 264 which is due to loss of (-C_3H_7) and at *m/z* 196 due to loss of (-C_5H_8). The base peak was observed at *m/z* 137.1 due to cleavage of (-$NHCOCH_2$) from the terminal carbon of the ring (Fig. 3).

In both the capsaicin and dihydrocapsaicin, the base peak at *m/z* 137.1 are observed due to fragment ion that corresponds to that of 2-methoxy-4-methylphenol moiety. The fragmentation of capsaicin and dihydrocapsaicin shows that the nitrogen present in capsaicinoids is not a part of heterocyclic ring, proving it as protoalkaloids. The fragmentations of the compound are shown in Fig. 4.

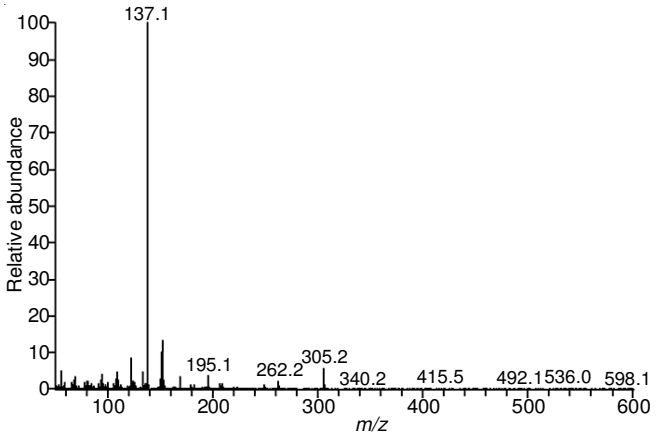


Fig. 1. Mass spectra of capsaicin (dry chilli)

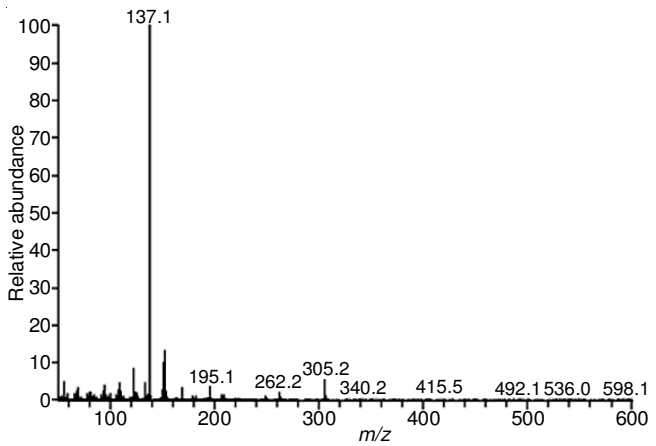


Fig. 2. Mass spectra of capsaicin (fresh chilli)

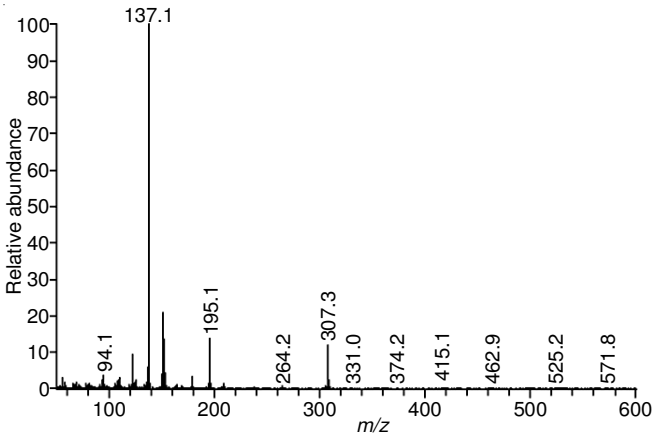
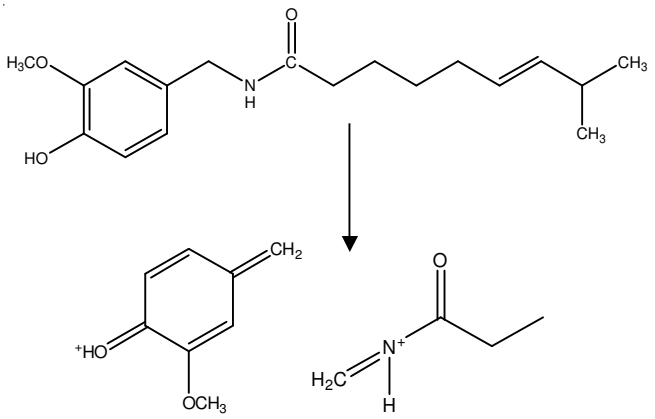


Fig. 3. Mass spectra of dihydrocapsaicin



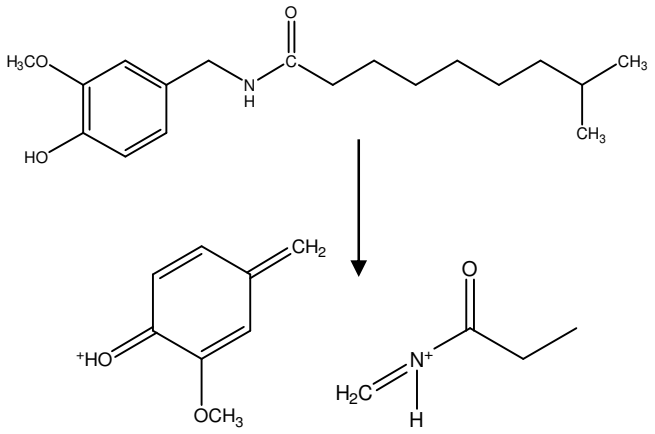


Fig. 4. Fragment moieties of capsaicin and dihydrocapsaicin

Along with capsaicinoids, some other bioactive compounds were also identified as presented in Table-2. A total of 11 compounds were identified among them 9 compounds were found to be present in fresh chilli and 3 compounds in dry chilli.

From the GC-MS analysis, it was found that number of bioactive compounds in dried chilli degraded as compared to fresh chilli, the reason may be attributed to application of heat. Toontom *et al.* [19] explained that the effects of drying on volatile chilli compounds could be distinguished as: the compounds may decreased, disappeared or increased or new compounds may be formed due to application of heat. It was found that one compound namely 4-hydroxy-4methyl-2-pentanone

TABLE-2
GC-MS ANALYSIS OF DRIED AND FRESH CHILLI EXTRACT OF *Capsicum chinense*

Name of compounds	Retention time	m.w.	m.f.
Dried chilli			
Capsaicin	68.20	305.2	$(C_{18}H_{27}NO_3)$
Dihydrocapsaicin	68.71	307.3	$(C_{18}H_{29}NO_3)$
4-hydroxy-4-methyl-2-Pentanone	6.20	116	$(C_6H_{12}O_2)$
Fresh chilli			
Capsaicin	51.76	305.1	$C_{18}H_{27}NO_3$
Tetratriacontane	48.12	478	$C_{34}H_{70}$
Docosane,11-butyl	48.20	366	$C_{26}H_{54}$
Tritetracontane	56.37	604	$C_{43}H_{88}$
Octadecane,3-ethyl-5-(2-ethylbutyl)	60.45	366	$C_{26}H_{54}$
Lycoxanthin	65.11	552	$C_{40}H_{56}O$
Astaxanthin	68.32	596	$C_{40}H_{52}O_4$
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)	68.77	430	$C_{27}H_{42}O_4$
Bezenepropanoic acid, 3,5-bis(1,1,- dimethyl ethyl)-4-hydroxy-octadecyl ester	52.99	530	$C_{35}H_{62}O_3$

appeared in the dried chilli corresponding to flavouring agent [20].

From the compounds identified, some were reported to have therapeutic potentials by different researches. 3-Ethyl-5-(2-ethyl-butyl)octadecane was reported to have antifungal property [21]. 2-(3-Acetoxy-4,4,14-trimethyl androst-8-en-17-yl) propanoic acid was reported to be an antibacterial agent [22]. Moreover, this compound was also reported to be a potential protein tyrosine phosphatase 1B (PTP 1B) inhibitor and considered to be a potential therapeutic agent for the treatment of type-II diabetes [23].

Two carotenoids lycoxanthin and astaxanthin were identified in the fresh chilli extract which attributes to red colour of the chilli. Moreover, 11-butyl docosane, tritetracontane and tetratriacontane are also identified.

Antifungal activity: The acetone extract of both dried and fresh chilli was evaluated against the plant pathogens *Fusarium oxysporum* and *Fusarium udum*. The percentage inhibition of fresh chilli extract against *Fusarium oxysporum* and *Fusarium udum* was found to be 50.76 and 56.25 %, respectively. While the percentage inhibition of dried chilli extract against *Fusarium oxysporum* and *Fusarium udum* was 58.98 and 60.41% (Table-3).

The dried chilli extract was shown to have better inhibitory effect than the fresh chilli extract. The reason may be attributed to higher concentration of capsaicin (major component of capsaicinoids) which was found higher in dried chilli extract than in fresh chilli extract by UV spectrophotometric study. This is supported by fact that *Fusarium* fungus are the primary cause of pre-dispersal of chilli seed mortality and experimentally demonstrated that capsaicinoids protect chilli from the *Fusarium* [24]. Tewksbury *et al*. [24] also demonstrated that more pungent chilli with higher concentration of capsaicinoids have more resistance to fungus than the chilli with lower concentration of capsaicinoids. Another factor responsible for less antifungal activity of fresh chilli extract may also be due to an antagonistic effect. In fresh chilli due to the presence of number of bioactive compounds, antagonistic effect might have occur thereby affecting the inhibition [25].

TABLE-3
ANTI-FUNGAL ACTIVITY OF DRY AND FRESH CHILLI EXTRACTS OF *Capsicum chinense* AGAINST *Fusarium oxysporum* AND *Fusarium udum*

Fungal strains	Fungal strain No.	Inhibition for fresh chilli (%)	Inhibition for dry chilli (%)
Fusaruim oxysporum	Fo47	50.76	58.98
Fusarium udum	Fo01	56.25	60.41

Statistical analysis: The statistical analysis showed that the dried and fresh chilli extract against *Fusarium oxysporum* and *Fusarium udum* were significant since the F_{cal} value and p value at 5 % is greater than F_{tab} value, thereby rejecting the null hypothesis and accepting the alternate hypothesis that there is significant antifungal activity on plant pathogens on treatment with extract of *Capsicum chinense* in both the cases (Table-4). Thus, *Capsicum chinense* extract has significant potential against the control of plant pathogens *Fusarium oxysporum* and *Fusarium udum*.

Conclusion

From this study, it was found that Capsaicin, major component of capsaicinoids reported to be present only in genus capsicum and was found to be present in high amount. Present findings also showed that extract of *Capsicum chinense* have effective potential in control of plant pathogens where higher concentration of capsaicinoids have better control suggesting that isolation of capsaicinoids from this chilli can lead to important change in prevention of crops-borne diseases.

REFERENCES

1. M.K. Meghvansi, S. Siddiqui, M.K. Khan, V.K. Gupta, M.G. Vairale, H.K. Gogoi and L. Singh, *J. Ethnopharmacol.*, **132**, 1 (2010); https://doi.org/10.1016/j.jep.2010.08.034.
2. Guinness Book of World Records, Hottest Spice (2006). www.guinnessworldrecords.com.

TABLE-4
ANOVA FOR *Fusarium oxysporum* AND *Fusarium udum*

Source	d. f.	S.S.	M.S.S.	F. cal.	F. tab. 5 %	Result	S. Ed. (±)	C.D. at 5 %
Fusarium udum								
Treatment	1	12.53	12.53	52.67802	7.71	S	0.398	0.844
Error	4	0.95	0.24	-	-	-	-	-
Total	5		-	-	-	-	-	-
Fusarium udum								
Treatment	1	0.17	0.17	8	7.71	S	0.118	0.250
Error	4	0.08	0.02	-	-	-	-	-
Total	5		-	-	-	-	-	-

3. R.K. Dubey, V. Singh, G. Upadhyay, A.K. Pandey and D. Prakash, *Food Chem.*, **188**, 119 (2015); https://doi.org/10.1016/j.foodchem.2015.04.088.
4. Science Tech Entrepreneur (2007). http://assamagribusiness.nic.in/bhut%20jalakia.pdf.
5. K. Sanatombi and G.J. Sharma, *Not. Bot. Hort. Agrobot. Cluj-Napoca*, **36**, 89 (2008); https://doi.org/10.15835/nbha362345.
6. R.S. Silva, J. Azevedo, M.J. Pereira, P. Valentao and B.P. Andrade, *Food Chem. Toxicol.*, **53**, 240 (2013); https://doi.org/10.1016/j.fct.2012.11.036.
7. N. Gurnani, M. Gupta, D. Mehta and B.K. Mehta, *J. Taibah Univ. Sci.*, **10**, 462 (2016); https://doi.org/10.1016/j.jtusci.2015.06.011.
8. P.P. Rajan, S.R. Gupta, Y.R. Sarma and G.V.H. Jackson, *Indian Phytopathol.*, **55**, 173 (2012).
9. K. Khatso and N.T. Ao, *Int. J. Bio-Resour. Stress Manag.*, **4**, 317 (2013).
10. H. Swer, M.S. Dkhar and H. Kayang, *J. Org. Syst.*, **6**, 3 (2011).
11. V. Pandey, H.K. Pandey, D. Dayal, U.C. Joshi, T. Pant and Z. Ahmed, *Hort. Sci. (Prague)*, **36**, 26 (2009).
12. G.K.N. Chhetry and T.R. Devi, *J. Agric. Veterinary Sci.*, **7**, 01 (2014).
13. J. Pino, M. Gonzalez, L. Ceballos, A. Centurionyah, J. Trujilloaguirre, L. Latourneriemoreno and E. Sauriduch, *Food Chem.*, **104**, 1682 (2007); https://doi.org/10.1016/j.foodchem.2006.12.067.
14. R.C. Mohanty, P. Ray and S. Rath, *J. Microbiol.*, **1**, 27 (2012).
15. N.J. Amruthraj, J.P.P. Raj and L.A. Lebel, *Int. J. Pharm. Sci. Rev. Res.*, **28**, 247 (2014).
16. J. Diaz, F. Pomar, A. Bernal and F. Merino, *Phytochem. Rev.*, **3**, 141 (2004); https://doi.org/10.1023/B:PHYT.0000047801.41574.6e.
17. M.A. Bernal, A.A. Calderon, M.A. Pedreno, R. Munoz, A. Ros Barcelo and F. Merino de Caceres, *J. Agric. Food Chem.*, **41**, 1041 (1993); https://doi.org/10.1021/jf00031a004.
18. J.J. Ornelas-Paz, J.M. Martínez-Burrola, S. Ruiz-Cruz, V. Santana-Rodríguez, V. Ibarra-Junquera, G.I. Olivas and J.D. Pérez-Martínez, *Food Chem.*, **119**, 1619 (2010); https://doi.org/10.1016/j.foodchem.2009.09.054.
19. N. Toontom, M. Meenune, W. Posri and S. Lertsiri, *Int. Food Res. J.*, **19**, 1023 (2012).
20. http://www.thegoodscentscompany.com.
21. M.N. Abucacker and P.K. Devi, *Eur. J. Pharm. Med. Res.*, **2**, 1779 (2015).
22. J. Albinjose, E. Jasmine, T. Selvankumar and K.P. Srinivasakumar, *Int. J. Multidiscipl. Res. Develop.*, **2**, 88 (2015).
23. M. Venkatachalam, G. Singaravelu, K. Govindaraju and J.S. Ahn, *Curr. Sci.*, **105**, 827 (2013).
24. J.J. Tewksbury, K.M. Reagan, N.J. Machnicki, T.A. Carlo, D. Haak, A.L.C. Penaloza and D.J. Levey, *Proc. Natl. Acad. Sci. USA*, **105**, 11808 (2008); https://doi.org/10.1073/pnas.0802691105.
25. H.H.F. Koolen, F.M.A. da Silva, F.C. Gozzo, A.Q.L. de Souza and A.D.L. de Souza, *Food Res. Int.*, **51**, 467 (2013); https://doi.org/10.1016/j.foodres.2013.01.039.