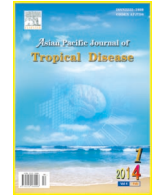




Contents lists available at ScienceDirect

## Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading

doi:10.1016/S2222-1808(14)60311-X

© 2014 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

# Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of *Zingiber officinale* Roscoe

Yuva Bellik\*

Faculty of Life and Nature Sciences, A. Mira University, Bejaia, 06000, Algeria

## PEER REVIEW

**Peer reviewer**

Dr. Conrad A. Omonhinmin, Biological Sciences, College of Science & Technology, Covenant University, Nigeria.

Tel: +234-805 651-2546

E-mail: conrad.omonhinmin@covenantuniversity.edu.ng

**Comments**

The study is fair as it confirms earlier reports on the potency of ginger extract as effective anti-oxidant and antimicrobial agents. The study indicates that ginger can be used in medicinal formulations.

Details on Page 43

## ABSTRACT

**Objective:** To compare *in vitro* antioxidant and antimicrobial activities of the essential oil and oleoresin of *Zingiber officinale* Roscoe.

**Methods:** The antioxidant activity was evaluated based on the ability of the ginger extracts to scavenge ABTS<sup>•+</sup> free radical. The antimicrobial activity was studied by the disc diffusion method and minimal inhibitory concentration was determined by using the agar incorporation method.

**Results:** Ginger extracts exerted significant antioxidant activity and dose-dependent effect. In general, oleoresin showed higher antioxidant activity [IC<sub>50</sub>=(1.820±0.034) mg/mL] when compared to the essential oil [IC<sub>50</sub>=(110.14±8.44) mg/mL]. In terms of antimicrobial activity, ginger compounds were more effective against *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*, and less effective against *Bacillus cereus*. *Aspergillus niger* was least, whereas, *Penicillium* spp. was higher sensitive to the ginger extracts; minimal inhibitory concentrations of the oleoresin and essential oil were 2 mg/mL and 869.2 mg/mL, respectively. Moreover, the studied extracts showed an important antifungal activity against *Candida albicans*.

**Conclusions:** The study confirms the wide application of ginger oleoresin and essential oil in the treatment of many bacterial and fungal diseases.

## KEYWORDS

*Zingiber officinale*, Essential oil, Oleoresin, Antioxidant and antimicrobial activities

## 1. Introduction

Several products based on traditional knowledge are important sources of income, food and health care for large parts of the populations throughout the world<sup>[1,2]</sup>. Herbs and spices, which are important parts of the human diet, have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative, antioxidative, antimicrobial and other various medicinal values<sup>[3–7]</sup>. Many scientific experiments have documented the antimicrobial and antioxidative properties of some spices, herbs and their components.

Ginger (*Zingiber officinale* Roscoe) has been cultivated for thousands of years as a spice and for medicinal purposes<sup>[8]</sup>. It is considered a safe herbal medicine with only few and insignificant adverse side effects. The distinct yellow, pungent, aromatic rhizome is the plant's organ that confers its value to the spice and the source of oleoresin and the essential oil. The oleoresin contains the phenolic pungent principles of ginger which represent 5%–8% of the dry weight<sup>[9]</sup>. The essential oil produced by *Zingiber officinale* rhizomes is pale yellow to light-amber and can be extracted with yields ranging approximately from 1.5% to 3.0% depending on the quality of the crop<sup>[10]</sup>. Both oil and oleoresin are used in various foods, beverages, such as soft

\*Corresponding author: Dr. Yuva Bellik, Faculty of Life and Nature Sciences, A. Mira University, Bejaia, 06000, Algeria.

Tel: +21334293442

Fax: +21334291727

E-mail: byouba@gmail.com

Foundation Project: Supported by Laboratory of Research on Local Animal Products, Ibn-Khaldoun University of Tiaret, Algeria, and CNEPRU (Grant No. 102320080002).

## Article history:

Received 7 Oct 2013

Received in revised form 9 Oct, 2nd revised form 12 Oct, 3rd revised form 17 Oct 2013

Accepted 28 Nov 2013

Available online 28 Feb 2014

drinks, and many types of medicinal products.

Ginger has been extensively studied for its biological activities. The antioxidative as free radical scavenging properties and antimicrobial activities of the ginger compounds have been well established<sup>[11–14]</sup>. On the other hand, comparative studies of oleoresin and essential oil performed concomitantly, under the same conditions, have not been reported so far. Thus, the overall objective of the present study was to compare the antioxidant activity and antimicrobial potency of both ginger essential oil and oleoresin.

## 2. Materials and methods

### 2.1. Plant materials

The air-dried roots of ginger, of Chinese origin, were purchased from the local spice store in Bejaia, Algeria. Mature and healthy rhizomes were ground using a mortar and a pestle. The essential oil was extracted directly from the mortar and pestle crushed tissues. For the extraction of the oleoresin, the tissues were further ground into a fine powder (500 µm particles) with an electric mill (Ika Labor Technik, Staufen, Germany).

### 2.2. Extraction of the essential oil and the oleoresin

Essential oil was extracted by hydrodistillation process. The extracted essential oil [(0.48±0.19)% w/w] was kept in air-tight sealed glass vials, covered with aluminum foil, and stored at 4 °C until further studies. Oleoresin compounds were extracted from dry ginger powder by the Soxhlet apparatus method. Briefly, the samples weighting about 10 g were packaged by filter paper, tied, and soaked in methanol at 70 °C for 4–8 h. The methanol extract was totally evaporated by rotary evaporator to yield the oleoresin [(10.23 ±1.02)% w/w]. Each weighed dry oleoresin sample was then reconstituted in 10 mL of methanol and was stored in the dark at low temperature (4 °C) until tested.

### 2.3. ABTS<sup>•+</sup> free radical scavenging activity

Antioxidant activity was measured by using radical cation decolorization assay<sup>[15]</sup>. This assay based on the inhibition by antioxidants of the absorbance of the free radical cation from ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt. ABTS was incubated with potassium persulfate in order to produce the free radical cation (ABTS<sup>•+</sup>). In brief, ABTS was dissolved in deionized water to make a 7 mmol/L concentration solution. ABTS<sup>•+</sup> was

produced by mixing ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and the mixture was allowed to stand in the dark at room temperature for 12–16 h before use. In our study, the ABTS<sup>•+</sup> solution was diluted with PBS, pH 7.4, to an absorbance of 0.70 (±0.02) at 734 nm. After addition of 2 mL of diluted ABTS<sup>•+</sup> to 20 µL of ginger oleoresin or essential oil in PBS, the absorbance reading was taken exactly 6 min after initial mixing. PBS blank were run in each assay. Ascorbic acid was used as positive control. All experiments were carried out in triplicate. Radical scavenging activity was expressed as the percentage of inhibition. IC<sub>50</sub> was the effective concentration at which 50% of ABTS<sup>•+</sup> was scavenged.

### 2.4. Antimicrobial study

#### 2.4.1. Microorganisms

The antimicrobial activity of the essential oil and oleoresin of ginger was evaluated against three strains of Gram-positive bacteria [*Bacillus subtilis* ATCC 33862 (*B. subtilis*), *Bacillus cereus* ATCC 11778 (*B. cereus*) and *Staphylococcus aureus* ATCC 25923 (Oxa S) (*S. aureus*)] and one strain of Gram-negative bacterium [*Escherichia coli* ATCC 29523 (*E. coli*)], and three fungi [*Aspergillus niger* ATCC 16404 (*A. niger*), *Candida albicans* (*C. albicans*), *Penicillium* spp]. The microbial strains were kindly provided by Pasteur Institut of Algiers.

#### 2.4.2. Disc-diffusion test

Ginger compounds were investigated by the disc diffusion using 6 mm filter discs<sup>[16]</sup>. Microorganisms were cultured at 37 °C for 4 h and prepared to turbidity equivalent to 0.5 McFarland Standard. The suspension was added to the top of agar. Sterile discs were impregnated with 3 µL of the oleoresin or essential oil and placed on the test plate. Plates were subsequently incubated at the appropriate temperature for 24 h and zones of inhibition were calculated by measuring the diameter in mm. In the case of fungus *C. albicans*, the test was performed in sterile Petri dishes containing potato dextrose agar medium (PDA). The oleoresin and essential oil were adsorbed on sterile paper disc and placed on the surface of the medium previously inoculated with a suspension of fungi. All Petri were incubated at 28 °C for 24 h. The zone of inhibition was determined by measuring the diameter of the clear zone around each disc.

#### 2.4.3. Determination of minimum inhibitory concentration (MIC)

Prior to the experiment, the bacterial strains were inoculated onto the surface of nutrient agar media; the inoculum suspensions were obtained by taking five

colonies from 24 h cultures. The colonies were suspended in 5 mL of sterile saline (0.85% NaCl) and shaken for 15 seconds. The density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to  $1-5 \times 10^8$  CFU/mL). The inoculum suspensions of *C. albicans* were obtained by taking five colonies from 24 hour-old cultures grown on PDA. The colonies were suspended in 5 mL of sterile saline (0.85% NaCl). The inoculum suspensions were shaken for 15 seconds and the density was adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to  $1-5 \times 10^6$  CFU/mL) with sterile saline. Likewise, *A. niger* was cultured on PDA for 7 d at 25 °C. Spore suspensions were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland Standard turbidity (corresponding to  $0.4 \times 10^4-5 \times 10^4$  spore/mL). PDA was also the basic medium for *Penicillium* spp. culture.

Using the incorporation method described by Alzahrani *et al.*[17], a serial dilutions of ginger essential oil and oleoresin were prepared with DMSO (1/2, 1/5, 1/10, 1/50, 1/100, 1/1000). A total of 100 µL of each diluted extracts were incorporated into Mueller Hinton agar media for bacteria and PDA media for fungi. Afterward, 30 µL of the inoculum were incorporated into wells. The plates were incubated at 37 °C for 18–24 h for bacteria and at 25 °C for 48 h for fungi. MIC was determined as the lowest concentration of ginger extracts inhibiting the visible growth of each organism on the agar plate. All MIC values were expressed as concentration (mg/mL).

### 2.5. Statistical analysis

Data were expressed as mean±SD of three triplicates. Statistical analysis was performed with Statistica Software version 5.5 (Statsoft, France). Difference on statistical analysis of data were considered significant at  $P < 0.05$ .

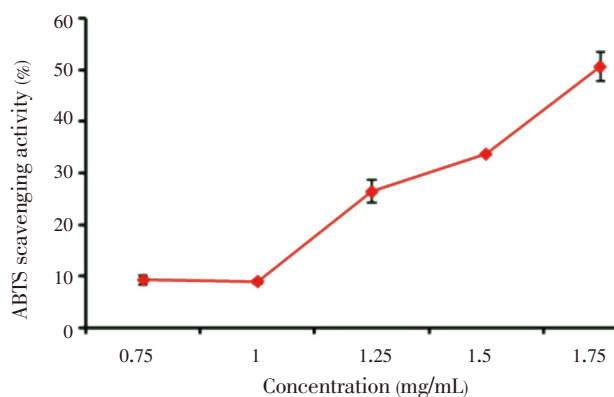
## 3. Result

### 3.1. Antioxidant activity estimated by ABTS assay

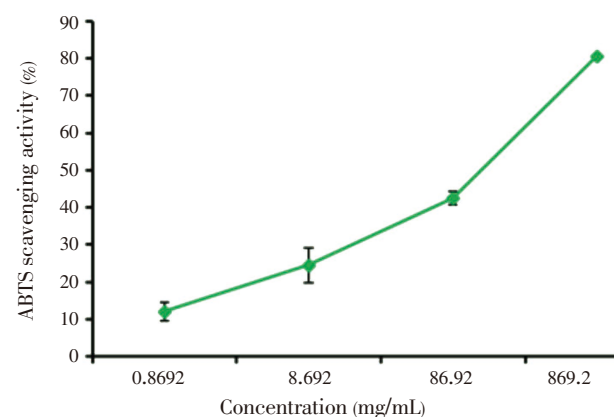
The ABTS assay is one of the most frequently used analytical strategies for antioxidant activity. The reliable method to determine radical scavenging capacities involves the measurement of the disappearance of free radicals, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic) acid radical (ABTS<sup>•+</sup>)[18].

Figures 1, 2, and 3 show the scavenging effect increased significantly ( $P < 0.05$ ) with increase in concentrations of ginger extracts (oleoresin and essential oil) and standard substance (ascorbic acid). Others, the same plant was recorded by researchers with similar results, which

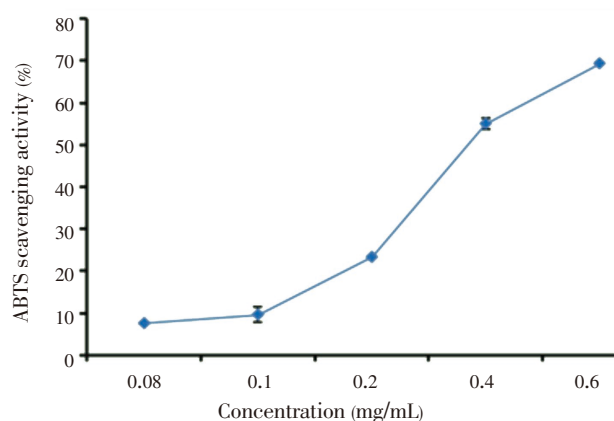
demonstrated that scavenging activity was concentration dependent[13].



**Figure 1.** ABTS<sup>•+</sup> scavenging activity against increasing concentrations of oleoresin.



**Figure 2.** ABTS<sup>•+</sup> scavenging activity against increasing concentrations of essential oil.



**Figure 3.** ABTS<sup>•+</sup> scavenging activity of different concentrations of ascorbic acid.

For concentrations of ascorbic acid ranging from 0.08 to 0.6 mg/mL, the scavenging activity obtained evolved from (7.54±0.25)% to (69.32±0.01)%. The scavenger effect of the studied extracts varies from (9.17±0.94)% to (50.58±2.86)% for oleoresin with concentrations ranging from 0.75 to 1.75 mg/mL, and from (12.09±2.44)% to (80.53±0.00)% for essential oil

with concentrations ranging from 0.869 to 869.2 mg/mL. This scavenging activity was significantly affected by the extract concentration with a strong coefficient of correlation ( $r=0.94$  for oleoresin and  $r=0.96$  for essential oil). Moreover, the required concentrations for the neutralization of 50% of the concentration of the ABTS were  $(1.820\pm0.034)$  mg/mL for the oleoresin and  $(110.14\pm8.44)$  mg/mL for essential oil.

### 3.2. Antimicrobial activity

The bacteria and fungi used in this study are associated with various forms of diseases<sup>[19]</sup>. Results of antimicrobial susceptibility revealed that *E. coli* was the most susceptible to the action of oleoresin with an inhibition zone diameter of 40 mm followed by *B. subtilis* (31 mm) and *S. aureus* (20 mm), whereas essential oil was more active on *S. aureus* (30 mm). *C. albicans* showed higher sensibility toward both ginger extracts. Takahashi *et al.*, Sasidharan and Menon have also reported the inhibition of *C. albicans* by ginger oleoresin and essential oil<sup>[11,14]</sup>.

The antimicrobial activities of ginger oleoresin and essential oil obtained by the incorporation method are shown in Table 1. Both ginger extracts exhibited different degrees of antibacterial activity. The strongest antibacterial effect of the essential oil was observed against *S. aureus*, followed by *B. subtilis* and *E. coli*. Conversely, oleoresin exerted the most effect on *E. coli* followed by *B. subtilis* and *S. aureus*.

**Table 1**

Antimicrobial activity of ginger essential oil and oleoresin against the tested strains using incorporation method.

Microorganisms	Minimal Inhibitory Concentration (mg/mL)	
	Oleoresin	Essential oil
Bacteria <i>S. aureus</i>	50	8.69
<i>B. subtilis</i>	20	86.92
<i>E. coli</i>	10	173.84
Fungi <i>Penicillium</i> spp.	2	869.20
<i>A. niger</i>	–	–

–: inactive.

Regarding the antifungal activity, ginger oleoresin and essential oil were effective in inhibiting *Penicillium* spp. at the concentrations of 2 mg/mL and 869.2 mg/mL, respectively, and inactive against *A. niger*. There are several reports on the inhibitory effect of ginger on the growth of *A. niger*<sup>[13,14,20]</sup>. However, in the present study, *A. niger* was found to be completely resistant towards the tested samples *i.e.* essential oil and oleoresin.

## 4. Discussion

In the present study, the antioxidant activity is observed

at low concentrations for oleoresin when compared to the essential oil. This confirms the result presented above where a superiority of the oleoresin was observed, which is in agreement with our previous findings<sup>[12]</sup>. However, ginger extracts showed less antioxidant activity than ascorbic acid [ $IC_{50}=(0.420\pm0.009)$  mg/mL].

In fact, the results gathered in the present work showed that ginger oleoresin exerted significantly higher antioxidant as well as antimicrobial activities when compared to the essential oil which disagree the results reported by Singh *et al.* where it was shown that essential oil was more effective than oleoresins regarding antioxidant and antimicrobial activities<sup>[13]</sup>. Indeed, Singh *et al.* studied Indian ginger whereas the rhizome investigated in this study was from Chinese origin<sup>[13]</sup>; thus, it is worth noting that the botanical origin of plant material is much important when dealing with biological properties of phytochemicals, thus, these divergences undoubtedly would be linked to the difference of plant source. Moreover, the marked antioxidant and antimicrobial activity of essential oil and oleoresin from spices and herbs is believed to be due to phenolic compounds. It is well-known that ginger essential oil and oleoresin contain considerable amounts of phenolic compounds (eugenol, shogaols, zingerone, gingerdiols, gingerols, *etc.*)<sup>[21,22]</sup>, which are responsible for the observed antimicrobial and antioxidant potency. However, it is likely that the overall efficiency of essential oil and oleoresin result from the synergistic action of all constituents<sup>[13]</sup>.

The results showed that the essential oil and oleoresin of *Zingiber officinale* exhibited significant antioxidant and antimicrobial activities. It can be concluded that ginger essential oil and oleoresin could be a promising alternative antioxidants having significant activity. Since, they have exhibited moderate to significant antimicrobial properties, hence, they can be used in the treatment of many bacterial and fungal diseases as well as a naturally food additives and preservatives which considered in new applications of food technology.

### Conflict of interest statement

The author declares that there is no conflict of interest.

### Comments

#### Background

*Zingiber officinale*, with ginger efficacy as an indigenous medicinal plant is widespread. The essential oils and oleoresin extracts are believed to be particularly effective

as anti-microbial agents. Though previous study on ginger of Indian origin has recorded considerable anti-microbial effects, the present study seeks to quantify these effects in ginger of Chinese origin.

### Research frontiers

This study is to ascertain the antioxidant and anti-microbial effect of ginger essential oils and oleoresin against ascorbic acid (vitamin C).

### Related reports

Earlier work by Singh *et al.*, 2008, recorded similar anti-oxidant and anti-microbial effects of essential oils, which were more potent than that of oleoresin.

### Innovations & breakthroughs

The paper provides information on the efficacy of ginger essential oils and oleoresin as anti-oxidant and anti-microbial agents.

### Applications

The result of this study confirms ginger as a formidable component of indigenous food and medicinal formulations and its continued applications.

### Peer review

The study is fair as it confirms earlier reports on the potency of ginger extract as effective anti-oxidant and anti-microbial agents. The study indicates that ginger can be used in medicinal formulations.

## References

- [1] Ali-Shtayeh MS, Jamous RM, Al-Shafie' JH, Elgharabah WA, Kherfan FA, Qarariah KH, et al. Traditional knowledge of wild edible plants used in Palestine (Northern West Bank): A comparative study. *J Ethnobiol Ethnomed* 2008; **4**: 13.
- [2] Nissen N, Evans S. Exploring the practice and use of Western herbal medicine: perspectives from the social science literature. *J Herb Med* 2012; **2**(1): 6–15.
- [3] Souza EL, Stamford TL, Lima EO, Trajano VN, Filho JM. Antimicrobial effectiveness of spices: an approach for use in food conservation systems. *Braz Arch Biol Tech* 2005; **48**(4): 549–558.
- [4] Leopoldini M, Russo N, Toscano M. The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chem* 2011; **125**(2): 288–306.
- [5] Zaika LL. Spices and herbs: their antimicrobial activity and its determination. *J Food Safety* 1988; **9**(2): 97–118.
- [6] Bellik Y, Boukraâ L, Alzahrani HA, Bakhotmah BA, Abdellah F, Hammoudi SM, et al. Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: an update. *Molecules* 2013; **18**(1): 322–353.
- [7] Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Plant profile, phytochemistry and pharmacology of *Asparagus racemosus* (Shatavari): a review. *Asian Pac J Trop Dis* 2013; **3**(3): 242–251.
- [8] Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. *Food Chem Toxicol* 2008; **46**(2): 409–420.
- [9] Zarate R, Yeoman MM. Changes in the amounts of gingerol and derivatives during a culture cycle of ginger, *Zingiber officinale*. *Plant Sci* 1996; **121**(1): 115–122.
- [10] Rani K. Cyclisation of farnesyl pyrophosphate into sesquiterpenoids in ginger rhizomes (*Zingiber officinale*). *Fitoterapia* 1999; **70**(6): 568–574.
- [11] Takahashi M, Inouye S, Abe S. Anti-*Candida* and radical scavenging activities of essential oils and oleoresins of *Zingiber officinale* Roscoe and essential oils of other plants belonging to the family Zingiberaceae. *Drug Discov Ther* 2011; **5**(5): 238–245.
- [12] Bellik Y, Benabdesselam F, Ayad A, Dahmani Z, Boukraa L, Nemmar A, et al. Antioxidant activity of the essential oil and oleoresin of *Zingiber officinale* Roscoe as affected by chemical environment. *Int J Food Prop* 2013; **16**(6): 1304–1313.
- [13] Singh G, Kapoor IP, Singh P, de Heluani CS, de Lampasona MP, Catalan CA. Chemistry, antioxidant and antimicrobial investigations on essential oil and oleoresins of *Zingiber officinale*. *Food Chem Toxicol* 2008; **46**(10): 3295–3302.
- [14] Sasidharan I, Menon AN. Comparative chemical composition and antimicrobial activity fresh & dry ginger oils (*Zingiber Officinale* Roscoe). *Int J Curr Pharm Res* 2010; **2**(4): 40–43.
- [15] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; **26**(9–10): 1231–1237.
- [16] Bauer AW, Kirby WM, Sherris JC, Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. *Am J Clin Pathol* 1966; **45**(4): 493–496.
- [17] Alzahrani HA, Alsabehi R, Boukraâ L, Abdellah F, Bellik Y, Bakhotmah BA. Antibacterial and antioxidant potency of floral honeys from different botanical and geographical origins. *Molecules* 2012; **17**(9): 10540–10549.
- [18] Miller NJ, Rice-Evans CA. Factors influencing the antioxidant activity determined by the ABTS<sup>•+</sup> radical cation assay. *Free Radic Res* 1997; **26**(3): 195–199.
- [19] Nester EW, Anderson DG, Roberts Jr CE, Pearsall NN, Nester MT. *Microbiology: a human perspective*. 4th ed. New York: McGraw-Hill Higher Education; 2004.
- [20] Tagoe DN, Nyarko HD, Akpaka R. A comparison of the antifungal properties of onion (*Allium cepa*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) against *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum*. *Res J Med Plant* 2011; **5**(3): 281–287.
- [21] Pawar N, Pai S, Nimbalkar M, Dixit G. RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from different ginger cultivars. *Food Chem* 2011; **126**(3): 1330–1336.
- [22] Ding SH, An KJ, Zhao CP, Li Y, Guo YH, Wang ZF. Effect of drying methods on volatiles of Chinese ginger (*Zingiber officinale* Roscoe). *Food Bioprod Process* 2012; **90**(3): 515–524.