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Variability for Antioxidant Activities and Quality Traits of Two Ginger (*Zingiber officinale* Roscoe) Clones Growing in Ethiopia

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ABSTRACT

Ginger rhizomes growing in two different geographical locations, namely Tepi in Southern Regional States and Chilga woreda of Gonder in the North-Western regions of Ethiopia, were extracted by a typical reflux apparatus to see the variability in content of oleoresin, essential oil and gingerol of the two main ginger production areas. In the study, the oleoresin content of the ginger samples from Tepi and Chilga showed 8.0 and 6.5% (w/w), which were statistically different at $P < 0.01$. Gingerol content within oleoresin was 11% for the Tepi clone and 7.0% for the Chilga clone. The maximum essential oil content (1.0%) was recorded for the Tepi clone and it was only 0.4% for the Chilga clone. Similarly, the antioxidant activities with respect to the free radical 2,2-diphenyl-1-picryl hydrazyl, were also evaluated and showed radical scavenging activity with an IC_{50} value of 1.81 and 2.84 µg/mL, for Tepi and Chilga clones, respectively.

Keywords: antioxidant, DPPH, oleoresin, *Zingiber officinale*

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe, Zingiberacae) is native to tropical South East Asia and is grown commercially in most tropical regions (Abeysekera *et al.* 2005). The main ginger-growing countries are India, China, Jamaica, Taiwan, Sierra Leone, Nigeria, Fiji, Mauritius, Indonesia, Brazil, Costa Rica, Ghana, Malaysia, Bangladesh, Philippines, Sri Lanka, Thailand, Trinidad, Uganda, Hawaii, Guatemala and Many Pacific Ocean Islands (Peter 2007). In Ethiopia, it has long been used as a flavouring agent, carminative and stimulant and is now the most important rhizomatous spice for the local market. Ginger prominently grows in northern and southern highland areas of Ethiopia (Roukens and Worku 2005).

Ginger is widely used around the world in foods as a spice. Characterized by its typical flavour and aroma, ginger is noted for its richness in oleoresin, essential oil and fiber content. It is commercially available in various forms such as green ginger, dry ginger, ginger powder, ginger oil, ginger oleoresin and preserved ginger (Kizhakkayil and Sasikumar 2009). Ginger is known to be effective as an appetite enhancer, an improver of the digestive system, and an anti-cold remedy (Wresdiyati *et al.* 2007). The underground rhizome of this plant species is valued throughout the world as a spice or flavouring agent for its two major classes of constituents, vis. essential oils and oleoresins (Tyler *et al.* 1988; Balladin *et al*. 1998). The more volatile essential oil (EO) consists of monoterpenes and sesquiterpenes, which contribute to the characteristic flavour of ginger, and oleoresin, which is responsible for the pungent flavour, which is also a source of antioxidants (Halvorsen *et al*. 2002; Stoilova *et al*. 2007; Sanwal *et al*. 2010). The principal compounds responsible for the pungency of ginger are gingerols and shogaols. Gingerols are a homologous series of phenolic ketones present in the rhizomes of ginger. They have been shown not only to exhibit a number of pharmacological effects including inhibition of prostaglandin biosynthesis, anti-hepatotoxicity, cardiotonic, and anti-platelet, but also have antioxidative effects (Cho *et al*. 2001).

The content of the active principle as well as the yields of ginger oleoresin containing gingerols and other bioactive compounds are not uniform and can vary significantly between cultivars and regions in which ginger is growing (Ratnambal *et al*. 1987; Kizhakkayil and Sasikumar 2009; Sanwal *et al*. 2010). However, there is no report on ginger oleoresin yield and assessment of their antioxidant activity in major growing areas of Ethiopia.

The present study was thus undertaken to explore the variability in EO and oleoresin yields, and antioxidant activities of two ginger clones growing in two different geographical locations of Ethiopia.

MATERIALS AND METHODS

Plant material

Rhizomes of two ginger cultivars growing in two different geographical locations, namely Tepi in Southern Regional States and Chilga Woreda of Gonder, were brought to the National Aromatic and Medicinal Plants Laboratory of the Ethiopian Institute of Agricultural Research, and left to dry under shade until moisture content fell to within a range of 7-12%.

Extraction of ginger oleoresin

Dried and powdered rhizomes (100 g) were extracted by refluxing in a water bath. They were soaked in a round-bottom flask containing ethanol as the solvent. The flask was then heated constantly for 3 h and the temperature of the water bath was controlled so as not to exceed 40°C. Once the extraction was over, the extracts were filtered and evaporated using a Rota-Evaporator (Bűchi Rota Vapor R-205, Switzerland) to obtain a dark viscous oleoresin. After the extraction process was complete, the yield was calculated gravimetrically on a dry weight basis as the ratio of weight of oleoresin (Bisrat *et al*. 2009).

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Research Note

Essential oil

EOs were extracted from air-dried ginger samples after 4 h distillation by hydrodistillation in a Clevenger-type apparatus. The EO obtained thus was weighed, and its content was calculated as the weight (g) of EO per weight (g) of dried rhizomes as described by Abeysekera *et al.* (2005).

Isolation of total gingerols by Preparative Thin Layer Chromatography (PTLC)

The ethanol extract/oleoresin of rhizomes of the ginger clones was dissolved in 2 ml of ethyl acetate and applied, in the form of bands, on homemade silica gel plates to quantify total gingerols comprising of 6-, 8- and 10-gingerol. During the process, a solvent system consisting of hexane and ethyl acetate (40: 10, v/v) was used as the mobile phase. The three required bands, total gingerols with R_f values ranging from 0.50 to 0.80, were scraped off and collected into a beaker to which 2 ml of acetone was added in a recovering process and finally filtered and concentrated under reduced pressure (Bisrat *et al.* 2009).

Scavenging effect on 2,2-diphenyl-1-picryl hydrazyl radical (DPPH)

The hydrogen-donating ability of the oleoresins was examined following the method described by Blois (1958) in the presence of DPPH stable radical. The oleoresin samples and the control (vitamin C) were dissolved in methanol to prepare a solution equivalent to 10, 5, 2.5, and 1.25 µg dried sample/ml solution. In this assay, 50 µl of each concentration of sample solution were added to 5 ml of 0.004% DPPH solution and incubated for 30 min at 37°C. After incubation, the absorbance of DPPH against the blank was measured at 517 nm using a Jenway Model 6500 spctrophotometer as was used by Bua-in and Paisooksantivatana (2009). The percentage inhibition of radical scavenging activity was then calculated by the following equation (Blois 1958):

$$\% \text{ Inhibition} = [(A_c - A_s)/A_c] \times 100$$

where A_c is the absorbance of the control (0.004% MeOH solution of DPPH without test sample) and A_s is the absorbance of the test sample.

Oleoresin concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as percentage inhibition against test sample concentration. Tests were carried out in triplicate.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2007 and were based on three replicates for the oleoresin content and their radical scavenging activities. Data are expressed as means ± standard deviation (SD). Statistical analysis was performed with a Student's *t*-test. Differences were considered to be statistically significant when $P < 0.01$

RESULTS AND DISCUSSION

Essential oil content

EO content of ginger rhizomes of Chilga and Tepi types, calculated on a dry weight basis, were 6.5 and 8%, respectively (**Table 1**). Similarly, the oleoresin of Tepi type gave 1.0% EO content, which is 60% higher than the Chilga type. Budavari (1996) reported ginger EO yield of 1.0-3.3% by steam distillation. In addition, Kizhakkayil and Sasikumar (2009) indicated that the EO yield of 46 ginger germplasms ranged from 0.9 to 4%. A wide range of EO yields (0.8-4.4%) was reported by Connell (1970) from ginger grown in Australia, India, Africa and Jamaica.

Oleoresin content

The percentage oleoresin was generally maximum for Tepi type (11.0%) and minimum (7%) for Chilga type (**Table 1**).

Table 1 Essential oil and oleoresin content of gingers. N = 6

Cultivar	Essential oil content (w/w)	Oleoresin content (w/w)	Total gingerol content in oleoresin (w/w)	Volatile oil content in oleoresin (w/w)
Chilga type	6.5	7.0	6.0	0.4
Tepi type	8.0	11.0	9.0	1.0

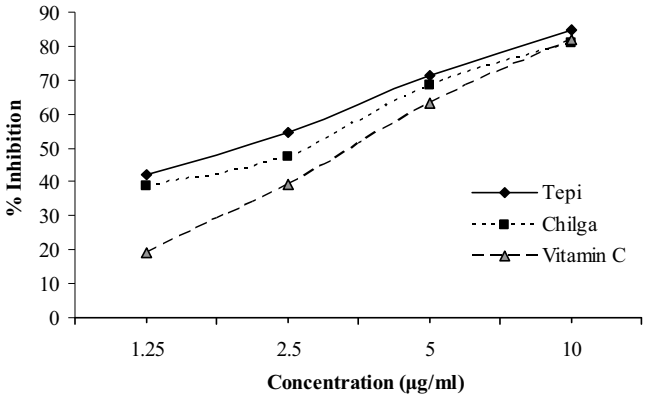

Fig. 1 Radical scavenging activities of ginger oleoresin from the two locations. N = 6

Both ginger types are within the range of international oleoresin content as confirmed by earlier reports (Ratnambal *et al*. 1987; Abeysekera *et al*. 2005; Vernin and Parkanyi 2005).

Gingerol in ginger oleoresin

The pungent principle in ginger is gingerol and the estimation of gingerol presented in **Table 1** shows that it varies from 6.0% in Chilga type to 9.0% in Tepi type. Ratnambal *et al.* (1987) reported a wide variation in gingerol content within oleoresin in 14 ginger cultivars and the range of variation was observed to be 9.1% ('Vengara') to 28.05% ('Jugijan').

Antioxidant activity

It is universally well known that plant extracts exhibit antioxidant activity as they contain polyphenol compounds possessing the capacity of donating hydrogen atoms or electrons and thereby scavenging free radicals (Stoilova *et al*. 2007). The DPPH method has been widely applied for estimating the antioxidant activity of various natural products (Molyneux 2004). This method has the advantage of being a stable, easy and rapid way to study the antioxidant activity of food items (Bua-in and Paisooksantivatana 2009). When DPPH reacts with an antioxidant compound, the colour changes from deep violet to light yellow and the absorbance at 517 nm on UV/visible light spectrophotometer decreases (Blois 1958).

The ability of ginger oleoresin to act as donors of hydrogen atoms was therefore tested by DPPH analysis. In this study, the oleoresin of ginger rhizomes from Tepi showed better radical scavenging activity with an IC_{50} value of 1.81 µg/mL than that of Chilga with an IC_{50} of 2.84 µg/mL. Both extracts showed, on the other hand, better radical scavenging activity than Vitamin C, the standard used for the experiment (IC_{50} = 4.5 µg/mL). The oleoresin of ginger for both locations showed radical-scavenging activity in a concentration-dependent manner, where the significant effect in inhibiting DPPH reached as much as 88% for Chilga and 82% for Tepi at 10 µg/ml (**Fig. 1**). IC_{50} values of ginger oleoresin from Tepi and Chilga were statistically significant ($P \leq 0.01$) and had high regression coefficients of R^2 = 0.942 and 0.936, respectively.

The higher the yield of ginger solvent extract or oleoresin yield indicates that there exists a higher yield of poly-

phenolic compounds in the extract, which in turn possesses higher antioxidant activities. The composition of these polyphenolic compounds, on the other hand, varies from one location to the other based on various soil and climatic factors of the given area. As can be seen, the higher antioxidant activity of the variety of ginger from Tepi is directly proportional to its oleoresin yield depicting that there exists a high concentration of polyphenolic compounds obtained from ginger growing from this particular location.

CONCLUSION

It is difficult to estimate the antioxidant activity of an individual phytochemical dietary compound. Thus, determination of the antioxidant activity on the total solvent extract or the oleoresin allows a more realistic evaluation of potential health effects of foods. So, the antioxidant activity in ginger extract was determined by the DPPH free radical assay. The results suggest that the content of ginger oleoresin can vary from one geographical location to the other depending on various edaphic and climatic factors. Similarly, ginger oleoresin is an excellent source of antioxidants providing protection against a wide range of chronic diseases. From this study, it was noted that the content of ginger oleoresin, EO and total gingerols from the Tepi area were higher than that of the Chilga area accompanied by higher radical scavenging activity. The result indicates the possibility of identifying a geographical location that best suits the commercial production of ginger in Ethiopia.

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REFERENCES

Abeysekera WKSM, Illeperuma CK, Amunugoda PNRJ, Wijeratnam SW (2005) Composition of ginger Clones dried at different temperatures for oil and oleoresin contents. *Sri Lankan Journal of Agricultural Sciences* **42**, 34-42

Balladin DA, Headley O, Chang-Yen I, McGaw DR (1998) High pressure liquid chromatographic analysis of the main pungent principles of solar dried West Indian ginger (*Zingiber officinale* Roscoe). *Renewable Energy* **13 (4)**, 531-536

Daniel MB, Solomon MA, Wossen MK (2009) *Laboratory Manual for Plant Products Analysis* (Vol 23 (1)), Ethiopian Institute of Agricultural Research, Addis Ababa, 42 pp

Blois MS (1958) Antioxidant determinations by the use of free radical. *Nature* **181**, 1199-1200

Bua-in S, Paisooksantivatana Y (2009) Essential oil and antioxidant activity of Cassumunar ginger (Zingiberaceae: *Zingiber montanum* (Koenig) Link ex Dietr.) collected from various parts of Thailand. *Kasetsart Journal (Natural Science)* **43**, 467-475

Budavari S (1996) *The Merk Index: An Encyclopedia of Chemicals, Drugs and Biologicals* (12th Edn), Merck and Dohme Corp., Whitehouse Station, N.J., USA, 1175 pp

Cho KJ, Kim JW, Choi I, Kim J, Hwang Y (2001) Isolation, identification and determination of antioxidant in ginger (*Zingiber officinale*) Rhizome. *Agricultural Chemistry and Biotechnology* **44 (1)**, 12-15

Connell DS (1970) The chemistry of the essential oil and oleoresin of ginger (*Zingiber officinale*, Roscoe). *Flavour Industry* **1**, 677-693

Halvorsen BL, Holte K, Myhstad MCW (2002) A systematic screening of total antioxidants in dietary plants. *Journal of Nutrition* **132**, 461-471

Kizhakkayil J, Sasikumar B (2009) Variability for quality traits in a global germplasm collection of ginger (*Zingiber officinale* R.). *Current Trends in Biotechnology and Pharmacy* **3 (3)**, 254-259

Molyneux P (2004) The use of the stable free radical diphenylpicrylhadrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal Science and Technology* **26 (2)**, 211-219

Peter KV (2007) *Spices, Horticultural Science Series - 5*, New India Publishing Agencies, New Delhi, pp 67-68

Ratnambal MJ, Gopalam A, Nair MK (1987) Quality evaluation in ginger (*Zingiber officinale* Rosc.) in relation to maturity. *Journal of Plantation Crops* **15 (2)**, 108-117

Roukens O, Worku T (2005) OLEORESINS, Export Potential of Ethiopian. Ethiopian Investment Promotion Department, Addis Ababa, Ethiopia

Sanwal SK, Rai N, Singh J, Buragohain J (2010) Antioxidant phytochemicals and gingerol content in diploid and tetraploid clones of ginger (*Zingiber officinale* Roscoe). *Scientia Horticulturae* **124**, 280-285

Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S (2007) Antioxidant activity of a ginger extract (*Zingiber officinale*). *Food Chemistry* **102**, 764-770

Tyler VE, Brady LR, Robbers JE (1988) *Pharmacognosy* (9th Edn), Lea and Febiger, Philadelphia, 150 pp

Vernin G, Parkanyi C (2005) Chemistry of ginger. In: Ravindran PN, Nirmal BK (Eds) *Ginger: The Genus Zingiber*, CRC Press. Boca Raton, FL, pp 87-180

Wresdiyati T, Astawan M, Muchtadi D, Nurdiana Y (2007) Antioxidant activity of ginger (*Zingiber officinale*) oleoresin on the profile of superoxide dismutase (SOD) in the kidney of rats under stress condition. *Jurnal Teknologi dan Industri Pangan* **8 (2)**, 118-125