Plant Science Today (2015) 2(1): 24-28
doi:10.14719/pst.2015.2.1.103

RESEARCH COMMUNICATION

RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from *in vitro* cultures of *Zingiber officinale* Roscoe

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Abstract

Relation between 6-gingerol content and antioxidant activity in *in vitro* grown cultures of ginger was studied. Reverse phase HPLC analysis revealed that rhizome derived callus culture and micropropagated plants produced lowest amount of 6-gingerol compare to conventionally grown plants. The antioxidant activity of extracts was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay and Ferric Reducing power assay (FRAP) and correlated with the content of total phenolics and total flavonoids in the extracts. Strong correlation was found between antioxidant activity, total phenolics and 6-gingerol content.

Keywords: HPLC; Ginger; 6-gingerol; antioxidant activity; callus

Received: 15 November 2014
Accepted revised version: 19 December 2014
Published online: 1 January 2015

Publisher: Horizon e-Publishing Group

CITATION
Pawar, N., S. Pai, M. Nimbalkar, and G. Dixit. 2015. RP-HPLC analysis of phenolic antioxidant compound 6-gingerol from *in vitro* cultures of *Zingiber officinale* Roscoe. *Plant Science Today* 2: 24-28. doi: 10.14719/pst.2015.2.1.103

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Introduction

The rhizome of ginger (*Zingiber officinale,* Rosc.) Zingiberaceae has long served culinary and medicinal uses (Afjal *et al*., 2001). It has been used as a spice for over 2000 years (Bartley and Jacobs, 2000). Its roots and the obtained extracts contain polyphenol compounds (6-gingerol and its derivatives), which have a high antioxidant activity (Chen *et al*., 1986; Herrmann, 1994). Two major groups of compounds including gingerol-related compounds and diarylheptanoids have been reported as bioactive components from this plant (Koo *et al*., 2001; Masuda *et al*., 2004). Gingerols and shogaols give ginger its pungency and consist of a homologous series of aldols each containing a phenolic group. Its major pungent constituent, [6]-gingerol has been reported to exhibit antioxidative activity against linoleic acid autoxidation and peroxidation of phospholipid liposomes and to scavenge trichloromethylperoxyl- and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Aeschbach *et al*., 1994; Sekiwa *et al*., 2000; Pawar *et al*., 2011). In addition to these antioxidative effects, our recent study (Ippoushi *et al*., 2003; Ippoushi *et al*., 2005) revealed that [6]-gingerol inhibits nitric oxide synthesis in activated J774.1 macrophages and prevents oxidation and nitration reactions induced by peroxynitrite (Radi *et al*., 2001), a strong reactive nitrogen species. The purpose of this study was to assess the antioxidant activities and to determine 6- gingerol content from the different *in vitro* ginger extract.

Material and methods

Plant material sterilization and inoculation of explant

Rhizome shoot explant was washed with solution of laboline and rinsed thoroughly with distilled water. After the treatment of 0.1% $HgCl_2$ for 6 minutes, the explants were given three to four washes of sterilized double distilled water and blotted well on a sterilized tissue paper. The rhizome explant cut to appropriate size and cultured aseptically on MS medium

(Murashige and Skoog, 1962) supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and NAA for callus induction and IAA and BAP for shoot multiplication. The cultures were maintained on similar media compositions and sub-cultured after every four weeks for shoot multiplication. Plants were removed for hardening after 6 subculture and transplanted in open field. After a period of 45 days, established callus was taken up for quantification of 6-gingerol.

Extract Preparation

Rhizome of conventionally grown plant and *in vitro* grown plant was removed from field and washed with tap water and then rinsed with double distilled water. Rhizomes along with calli were dried under oven for 60°C and pulverized separately. The one gram of powder of rhizome/calli from each treatment was dissolved in 25 ml methanol and sonicated for 30 minutes. The mixture was centrifuged at 10,000 rpm for 10 minutes and the supernatant was filtered through Whatmans filter paper no.1. All these extracts were kept at 4°C and for assay diluted extracts were used.

Quantification of Total Phenolic Content (TPC)

Total phenolic content was quantified using modified Folin – Ciocalteu method described by (Wolfe, Wu, and Liu, 2003). The assay mixture was prepared using 0.125 ml different concentrations of standard Tannic acid with 0.250 ml of Folin Ciocalteu reagent, 1.25 ml of distilled water and incubated for 10 min in dark. After 10 min, 1 ml 7% aq. sodium carbonate and 1 ml of distilled water is added and the reaction mixture is incubated in dark for 90 min at 37°C. The absorbance of blue colour was read at 760 nm using distilled water instead of std. tannic acid in the reaction mixture as blank on double beam spectrophotometer. Similarly, extracts prepared were also quantified and the results were compared to the standard curve of above standards and expressed as mg/Tannic or equivalent per gram dry powder for the samples under study.

Quantitative determination of total flavonoid contents

Total flavonoid contents in all the above extracts were determined by using a method given by (Luximan – Ramma *et al*., 2002). 1% plant extract (1.5 ml) was taken for the determination of total flavonoids. To this, 1.5 ml of 2% aluminium chloride in methanol was added. The reaction mixture was incubated for 10 minutes at room temperature. The OD was measured at 368 nm against 2% $AlCl_3$, as blank. The OD measurements were compared to standard curve of Quercetin (a standard flavonoid) concentrations and expressed as milligrams of Quercetin equivalent per gram dry weight of ginger.

Antioxidant activity: DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay

The antioxidant activities were determined as the measure of radical scavenging using DPPH assay as determined by (Brand-Williams *et al*., 1995). Three ml of a methanolic solution of DPPH (25ppm) was mixed with 20 µl of different concentration of standard Ascorbic acid and the mixture was incubated for 30 min in dark. The absorbance at 515 nm was measured using methanol as blank. The inhibition percentage of DPPH (% DPPH) was calculated and the results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC) as per method described by (Gil *et al*., 2000).

Antioxidant activity: Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing/antioxidant power (FRAP) assay was used to measure the total antioxidant power ginger extracts. In the FRAP assay, reductants (antioxidants) in the sample reduce Fe^{3+}/tripyridyltriazine complex, present in stoichiometric excess, to the blue colored ferrous form, with an increase in absorbance at 593 nm. The ΔA is proportional to the combined (total) ferric reducing/antioxidant power (FRAP value) of the antioxidants in the sample. Antioxidant activity assays were performed by the method described by (Benzie and Strain, 1996). The results were expressed as

Table 1. Showing activities of DPPH and FRAP in mM and content of Phenolic, Flavonoid and 6-gingerol in %

Particulars	6-gingerol	DPPH	FRAP	Total phenols	Total Flavonoids
Conventional rhizome	0.165	1.493	2.732	1.578	1.422
Callus	0.056	0.720	1.680	0.648	0.753
Micropropagated rhizome	0.078	0.845	1.901	0.790	0.772

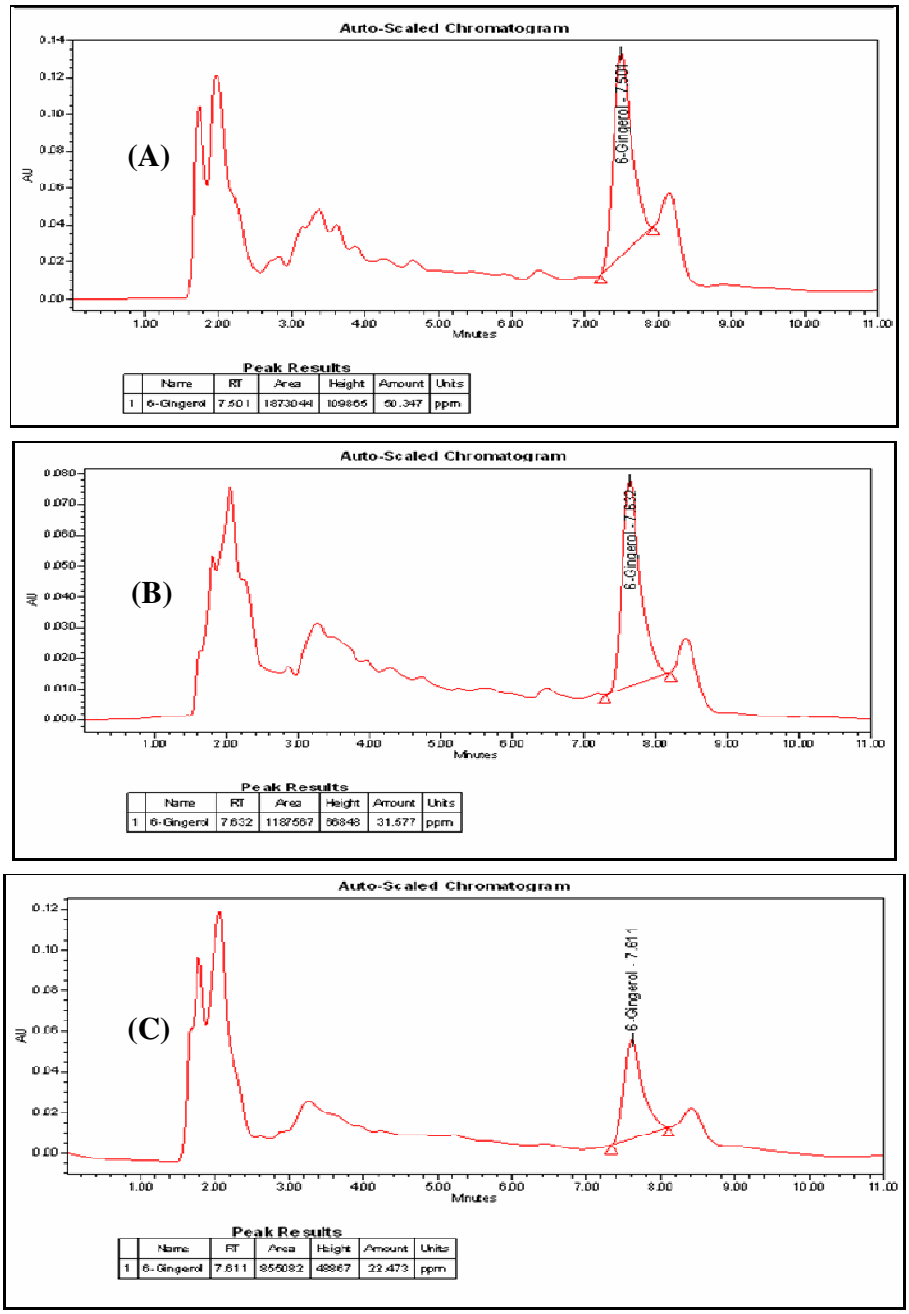

Fig. 1. Chromatograms: A: Conventional rhizome, B: Micropropagated rhizome and C: Callus

ascorbic acid equivalent antioxidant capacity (AEAC).

Results and Discussions

HPLC analysis for 6- Gingerol content

Using HPLC analysis, callus, micropropagated rhizome and conventionally grown rhizome were investigated for 6-gingerol content (Fig. 1A to 1C). Which revealed that concentration of 6-gingerol was three fold more (0.16%) in conventionally grown ginger rhizome than callus (0.056) and about half (0.078) in micropropagated plant rhizome (Table 1). Results are in accordance with tissue cultural studies in *Camptotheca acuminata* by (Sakato and Misawa, 1974) which yielded very less amount Camptothecin from the suspension cultures. Similarly, (Wiedenfield *et al*., 1997) in callus cultures of *C. accuminata* and (Roja, 2008) in Shoot cultures of *Ophiorrhiza rugosa* has reported low amount of Camptothecin. The *in vitro* grown rhizome has lowest 6- gingerol content because, developing normal size of rhizome comparable to that

of mother plants requires three year (Nirmal Babu, 1997).

Antioxidant activities, total phenolic and total flavonoid content

The highest phenolics content found to be in conventionally grown rhizome sample (1.57gm/100gm of dry weight) compared to callus (0.648gm/100gm of dry weight) and micropropagated grown ginger rhizome (0.79gm/100gm of dry weight). Similar results were obtained for total flavonoid content which was expressed in Quercetin equivalent. Highest content (1.44gm/100gm of dry weight) of flavonoid was in conventionally grown rhizome sample followed by micropropagated rhizome sample (0.77gm/100gm of dry weight) and lowest was in callus sample (0.75gm/100gm of dry weight). The conventionally propagated plants exhibited highest antioxidant activity compared to *in vitro* cultures. The highest DPPH and FRAP activity was recorded in conventionally grown plant while in micropropagated plant it was 845.34 and 1901.09 µM Ascorbic acid eq. respectively and lowest activity was recorded in callus sample. Present results matches with (Grzegorczyk *et al*., 2007) work on *Salvia officinalis* L. Correlation between all the traits was above R^2=0.90. Strong correlation was found between Phenolic and DPPH (R^2 = 0.999) and Gingerol and FRAP (R^2 = 0.999). The trend of increase or decrease of all activities and content was similar for conventionally grown rhizome, micropropagated rhizome and callus.

Conclusion

As expected, the extracts of callus and micropropagated plants of ginger proved to be less active in all methods tested. It is accepted that the dedifferentiation of plant tissues during establishment of callus and cell cultures is often connected with reduction of content of secondary metabolites. According to our knowledge, there are no detailed data regarding the composition of phenolic compounds in ginger cultures were present. So this preliminary study contributes new knowledge of the composition of phenolic compounds by HPLC analysis. Further studies necessary to explain the differences observed.

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