

RESEARCH ARTICLE

Chemical composition and antifungal activity of the crude extracts isolated from *Zingiber officinale* against *Macrophomina phaseolina*

■ SUMATI CHOUDHARY, ANIL KUMAR DWIVEDI AND POOJA SINGH

SUMMARY

Fungitoxic property of extracts of rhizome of *Zingiber officinale* against *M. phaseolina* were tested at different concentrations of 5 per cent, 10 per cent and 20 per cent under *in vitro* conditions using poisoned food techniques. Extracts of rhizome of *Zingiber officinale* showed antifungal activity against the pathogen and with an increase in the concentration of extracts, a progressive decrease in mycelial growth was observed. The essential active compound of plant material is the fraction isolated by means of several methods such as TLC, column chromatography and HPLC and is chemically defined as terpenoids namely monoterpene. The identification of active component was done by comparing the retention time (R_t) and chromatographic peaks of sample of *Zingiber officinale* rhizomes with their respective active component zingerberene and camphene. The HPLC fingerprint profile of the sample of *Zingiber officinale* rhizomes showed major peaks at the retention time of 5.32 and 2.83 min, respectively, whereas, the pure standard of zingerberene and camphene showed major peaks at the retention time 5.35 min and 2.87 min, respectively.

Key Words : *Zingiber officinale*, Antifungal, *Macrophomina phaseolina*, Zingerberene, Camphene

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Ginger (*Zingiber officinale*) a member of Zingiberaceae family is a well known spice used in the daily diet in many Asian countries (Demin

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and Yingying, 2010). It is a rhizomatous plant grown throughout South-eastern Asia, China and in the parts of Japan, Austria, Latin America, Jamaica and Africa. It has been used as a spice and medicine in India and China since ancient times. It was known in Germany and France in the ninth century and in England in the 10th century for its medicinal properties (Sasidharan and Nirmala, 2010).

Over three quarters of the world population still rely on plants and plant extracts for health care (Sasidharan and Nirmala, 2010). Ginger compounds are active against specific type of diarrhea which is leading to cause death

in infants in developing countries. Moreover, it has been found that ginger is effective in treating nausea caused by sea sickness, morning sickness and chemotherapy, though it was found superior over a placebo for post operative nausea (Sebiomo *et al.*, 2011). In addition, it has been reported that the main ingredients of ginger like volatile oil, gingerol, zingerone, shogaol, camphene work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic and anti-tumor (Lee *et al.*, 1986; Penna *et al.*, 2003; Kadnur and Goyal, 2005; Islam and Choi, 2008; Kim *et al.*, 2008; Isa *et al.*, 2008; Wang *et al.*, 2009 and Shim *et al.*, 2011).

Moreover, it is consumed worldwide as flavouring agent which is used extensively in food, beverage and confectionary industries in the products such as marmalade, pickles, chutney, ginger beer, ginger wine, liquors and other bakery products (Wang *et al.*, 2011). In south India, ginger is used in the production of candy called injimurappa meaning ginger candy in Tamil (Sebiomo *et al.*, 2011).

Currently, there is growing interest to detect natural compounds, characteristics and activities like plant extracts of herb and spices for the preservation of foods, flavour characteristics and sometimes show antioxidant activity as well as antimicrobial activity (Shim *et al.*, 2011; Wang *et al.*, 2009 and Tahereh and Mahsa, 2010). This gives the motivation for our present study to focus on ginger.

The aim of this study was to determine the chemical composition and antimicrobial activity for different extracts of ginger by investigating its effect on inhibition of biological activity of fungi.

MATERIAL AND METHODS

Preparation of plant extracts:

Rhizomes were collected from *Zingiber officinale* plants growing in University Campus, Gorakhpur University. Collected rhizomes of *Zingiber officinale* (Adarakh) were washed thoroughly in running tap water and then ground in distilled water (100g/100ml). The macerate was filtered through double layered cheese cloth and centrifuged at 3,500 rpm for 20 minute. The supernatant was collected and served as plant extracts.

In vitro evaluation of plant extracts against *M. phaseolina*:

Inhibitory effect of plant extract was assessed using poisoned food technique (Singh and Tripathi, 1999).

Appropriate amount of the extracts were separately mixed with molten PDA to get final concentration of 5, 10 and 20 per cent of the extracts. The medium was then poured aseptically in to sterile Petri plates. Mycelial discs of 5 mm dia from 7 day old actively growing culture of *M. phaseolina* were transferred to the centre of the solidified surface of the medium in individual plate. Control plates were also maintained where the culture discs were grown under the same conditions on PDA without extracts. Petri plates were incubated at 28±2°C. Each treatment was replicated thrice. Observations were taken when growth in control covered entire Petri plate. The per cent inhibition in mycelial growth over control was calculated.

$$I = \frac{C - T}{C} \times 100$$

where, I = Per cent inhibition
C = Colony diameter in control
T = Colony diameter in treatment

Isolation and identification of bioactive compound:

Column chromatography:

Preparation of crude extracts:

50 g of rhizomes of *Zingiber officinale* were weighed. 50 ml ethanol was added in dried and crushed rhizomes of *Zingiber officinale* and leaved it over night. 30 ml filterates from the well crushed paste were transferred in to a separate conical flask through funnel. The ethanolic extract of rhizomes of *Zingiber officinale*, were subjected to silica gel (60-120 mesh, Qualigens glass, Precious Electrochemindus Private Limited, Mumbai, India) chromatography through 5 x 45 cm column. Five milliliters fractions of 60 elutents were eluted with 99.9 per cent ethanol for each column preparations. Ethanol was evaporated under vacuum and the remaining solids obtained from all the 5 ml elutents were used for the determination of fungicidal activity.

Pure compounds:

Camphene and Zingerone were purchased from Sigma Chemical Co. USA.

Thin layer chromatography:

Thin layer chromatography (TLC) was performed by the method of Jaiswal and Singh (2008) to identify the active fungicidal component present in the rhizomes of *Zingiber officinale*. TLC was done on 20 x 20 cm pre-coated silica gel (Precious Electrochemicals Private

Limited, Mumbai, India) using benzene/ethyl acetate (9:1, v:v) as the mobile phase. Spots of column purified fractions of rhizomes of *Zingiber officinale* along with their respective components were applied on TLC plates with a micropipette. Further, the TLC plates were developed by I₂ vapour. Copies of chromatogram were made by tracing the plates immediately and retardation factor (R_f) were calculated.

High performance liquid chromatography:

The identification of active components presents in the rhizomes of *Zingiber officinale* were done by high performance liquid chromatography .

Sample preparation:

The samples of rhizomes of *Zingiber officinale* were prepared by weighing 50 mg each, of their column extract and then dissolving each of them separately in 20 ml of acetonitrile. The samples were then properly vortexed to ensure proper dissolution. Prior to sample injection the solutions were filtered through a Millipore filter (0.22 µm) to remove any undissolved particle.

Preparation of standard solution:

Pure standard solution of zingiberene and camphene were obtained by weighing 10 mg of each and then dissolving each of them in 20 ml acetonitrile. Proper dissolution of pure compounds were achieved by vortexing the mixture and the solution thus obtained were filtered through Millipore filter (0.22 µm).

Instrumentation:

The HPLC system was equipped with two LC-10 AT VP pumps, a Cecil CE 4201 UV-variable detector and a Microlitre®# 702 (Hamilton-Bonaduz, Schweiz) syringe with a loop size of 20 µL. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a reverse phase Luna µ C₁₈ Phenomenex column (250 mm x 4.6 mm) at 27°C. Acetonitrile (HPLC grade) was used as mobile phase solvent under a pressure of 260-270 kgf cm⁻² and run time of 15 min. The analysis was carried out at a flow rate of 1 mL min⁻¹, with column effluent being monitored at 260 nm. Data acquisition was done with Power Stream™ software.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized

under following heads :

Antifungal activity of extracts:

Fungitoxic property of extracts against *M. phaseolina* were tested at different concentrations of 5 per cent, 10 per cent and 20 per cent under *in vitro* conditions using poisoned food techniques. Extracts of rhizome of *Zingiber officinale* showed antifungal activity against the pathogen and with an increase in the concentration of extracts, a progressive decrease in mycelial growth was observed.

Chemical composition of the extracts:

Elutents which were collected through column chromatography were tested for fungicidal activity. After toxicity test it was found that in the rhizomes of *Zingiber officinale* eluent nos. 22-30 were highly toxic against the *M. phaseolina*.

The thin layer chromatography analysis demonstrated that the R_f values of zingiberene (0.9) and camphene (0.79) were equivalent to the R_f values of column-purified fractions of rhizome of *Zingiber officinale* (0.9,0.79).

The identification of active component was done by comparing the retention time (R_t) and chromatographic peaks of sample of *Zingiber officinale* rhizomes with their respective active component zingiberene and camphene (Fig.1-4). The HPLC fingerprint profile of the sample of *Zingiber officinale* rhizomes showed major peaks at the retention time of 5.32 and 2.83 min, respectively, whereas, the pure standard of zingiberene and camphene showed major peaks at the retention time 5.35 min and 2.87 min, respectively. Chromatogram from the HPLC analysis of the *Zingiber officinale* extracts are present in Fig. 1 to 4.

The present study design to obtain preliminary information on the *in vitro* antifungal activity of *Zingiber officinale* rhizomes on *M. Phaseolina*, the poison food technique was preferred to be used in this study, and crude extract of *Zingiber officinale* were analyzed by HPLC.

The results show that the extraction of ginger has an antifungal activity against *M. phaseolina*. This may be caused as a result of the presence of zingiberene and camphene as active ingredient within ginger. Since many studies indicated that the antifungal potency of ginger mainly caused by the presence of mono and

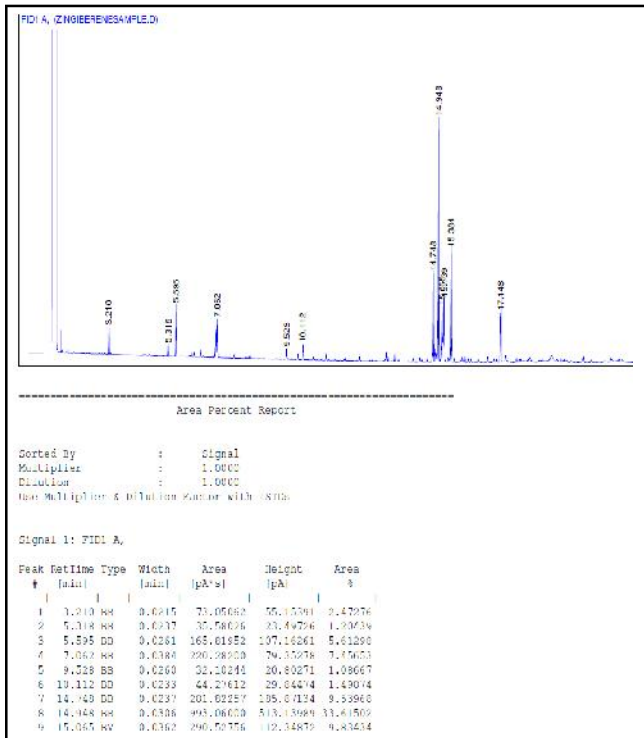


Fig. 1 : HPLC chromatogram of sample of zingiberene

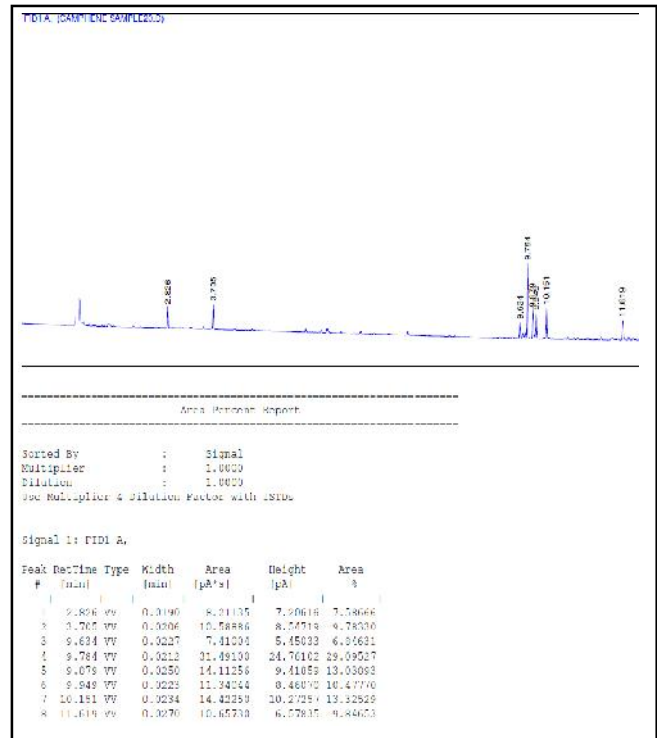


Fig. 3 : HPLC chromatogram of sample of camphene

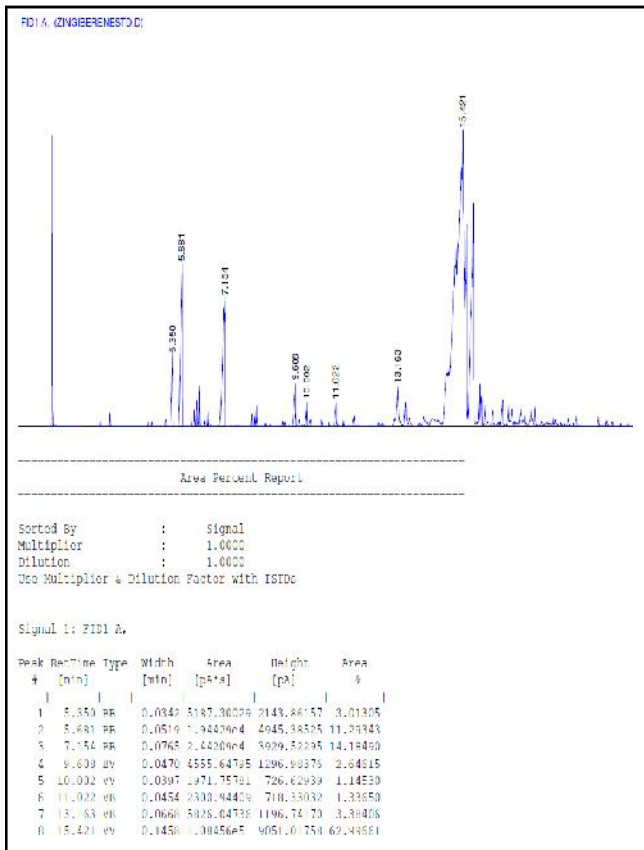


Fig. 2 : HPLC chromatogram of standard of zingiberene

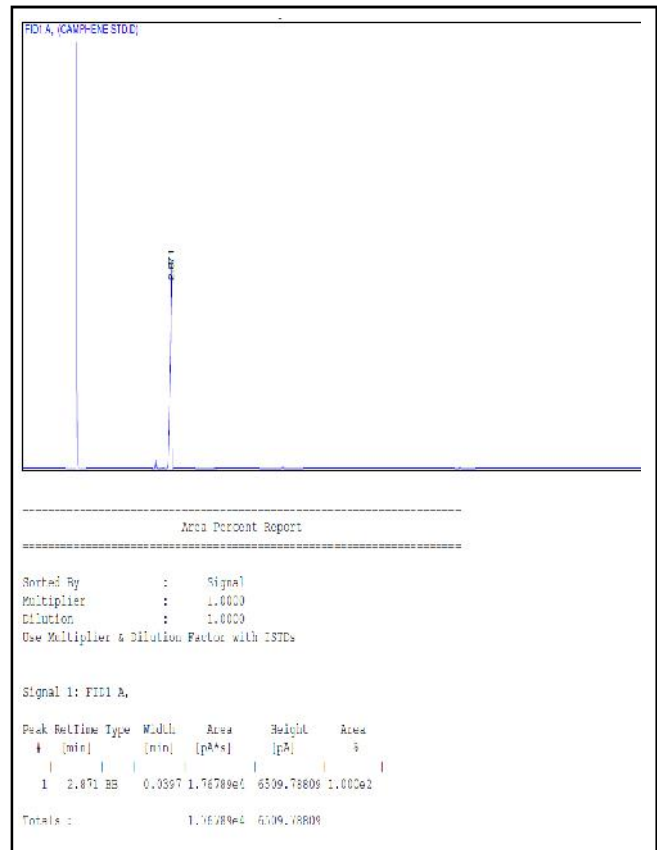


Fig. 4 : HPLC chromatogram of standard of camphene

sesquiterpenes hydrocarbons (camphene, zingiberene) (Michielin *et al.*, 2009; Elaissi *et al.*, 2011; Hossain *et al.*, 2011 and Singh *et al.*, 2008), which are primarily isolated from the rhizome of zinger.

Bajpai and his colleague also support our explanation that as a result of the presence of mono and sesquiterpenoids within plant extract, which consider main cause for their antifungal mode of action. Since these compounds have different ways of effect since these compounds not only attack cell walls and cell membranes *i.e.*, affecting their permeability and release of intracellular constituents (e.g. ribose, Na glutamate) but they also interfere with membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity). Thus, these compounds might have several invasive targets which could lead to the inhibition of fungal pathogen (Bajpai *et al.*, 2009).

In addition, the results of antifungal activity assays showed that the extract of *Zingiber officinale* had inhibitory effects on the growth of *M. phaseolina*. The present results are in agreements with previous study in literature mentioned above. The inhibitory effect observed may be a result of mono and sesquiterpene which is reported to have a wide range of antifungal activity. Since the main mechanism by which will produce its anti-fungal action through the disruption of fungal membrane integrity.

Conclusion :

From the obtained results of the present study we can indicate that ginger extracts have exhibited wide spectrum of antimicrobial properties. Therefore, they can be used for preserving various food stuffs against microbial spoilage and it can be incorporated in to medications for topical antifungal therapy.

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