WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 3, 166-176. Research Article ISSN 2277– 7105

CHEMICAL ANALYSIS OF TRAIT, PUNJAB'S *ZINGIBER OFFICINALE* RHIZOME AS A CRUDE DRUG

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Article Received on
15 Dec 2014,

Revised on 16 Jan 2014,
Accepted on 07 Feb 2015

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ABSTRACT

Crude drugs, particularly phytomedicines, are world's mostly used alternative medicines that are also now being used for various bioactive compounds' extraction to design new safe and effective drugs. *Zingiber officinale* (ZO) rhizome is also one of such crude drug having great therapeutic importance for various life threatening ailments. Although, studies related to its chemistry have been conducted before but due to different protocols and different geographical areas' varieties, there is lack of congruency among the results. Moreover, previous data mostly report chemical analysis of fresh ZO rhizome and lack detailed information about its crude drug form that is rich in 6-gingerol, a major potent bioactive compound, in addition to other valuable constituents. Keeping in view of WHO recommended strategies and guidelines of 2002-05 and 2014-2013 for phytomedicines, it is also mandatory to study variable chemistry of ZO of different geographical areas before to declare its global safe usage as a medicine or in new drugs' formulations. In our study, the chemistry of Trait, Punjab, Pakistan's ZO crude drug and its methanolic extract was studied. Low moisture content (4.07±0.06%) but high carbohydrate

(56.92%), fat (10.55±0.01%), fiber (18.24±0.02%) and 6-gingerol (6.50%) contents were found in our sample in comparison to previous studies. Yet, our findings were mostly congruent with few Indian studies. Lastly our findings may be useful if Trait, Punjab, Pakistan's ZO specie is used as a crude drug or in new drugs' compositions.

KEYWORDS: *Zingiber officinale*, crude drug, 6-gingerol, HPLC, proximate analysis, phytochemistry.

INTRODUCTION

'Crude drugs' are naturally occurring, unprocessed substances that are mostly derived from plants or animals and are used as medicine or in medicines' composition. If crude drugs are derived from plants then are termed as phytomedicine; a sub category of traditional medicines. They are used by 80% world's population in the form of their crude extracts or bioactive compounds having least or no side effects and low price rates as compared to western drugs.[1] Their phytotherapeutical importance have been highly reckoned in current two decades, particularly after the world health organization's recommended strategies and guidelines of 2002-05 and 2014-2013 for their global research, formulation, regulation and safe and effective usage at low prices to improve public heath, especially in poor income areas of world.[2, 3]

Zingiber officinale (ZO), Zingiberaceae family, is a commonly used spice and phytomedicine having therapeutic potential to treat pain, indigestion, vomiting, cold, diabetes, dyslipidemia, coronary artery diseases, cancer and inflammatory diseases due its unique chemical composition having important bioactive compounds called gingerols in addition to other valuable constituents.[4-8]

Although, studies related to chemical composition of ZO have been conducted before, though there are many discrepancies among their results due to different protocols used for chemical analyses, extract preparation and storage; and also due to different ZO varieties cultivated in different geographical areas having variable soil chemistry.[9-11] Furthermore, these studies mostly present chemical composition of fresh rhizomes and are lacking in information about comprehensive chemical analysis of their crude drugs and crude extracts; raw materials mostly used for the isolation of bioactive compounds (mainly gingerols) to reveal their mystic therapeutic potentials.[12]

Thus, there is need of the time that studies must be conducted worldwide to assess the variable chemical composition of ZO crude drug and its extracts before to declare its safe and effective usage as phytomedicine or in new drugs' formulations. Our study to investigate the chemistry of Trait, Punjab's ZO as crude drug and its methanolic extract, is also a leading step in this regards.

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh rhizomes of ZO were collected from Trait (Murree Hills, Punjab, Pakistan) and its specie was verified by Dr. Muhammad Ajaib (Herbarium Section, The Department of Botany, Government College University, Lahore). After washing the sample with distilled water, its light outer skin was peeled off and later it was cut into small pieces. These pieces were dried at 25 ^{o}C for 15 days under the shade to use them as crude drugs. The dried pieces were then ground into fine powders by using electric mill for 5-10 minutes and later these powders were stored in air-tight glass as stock samples in a refrigerator until required for analyses. All the analytical work of this study was performed at Postgraduate Biochemistry Research Lab, The Institute of Biochemistry and Biotechnology, UVAS; and Pharmaceutical Chemistry Lab, The Institute of Pharmaceutical Sciences, UVAS, Lahore, Pakistan.

Proximate Analysis

The proximate analysis was performed to estimate the contents of moisture, crude fiber (CF), ash, crude fat, crude protein (CP) and nitrogen free extract (NFE) in ZO crude drug by following the respective methods described in AOCC (2003).[13]

Mineral Analysis

As per conditions described in AOCC (2003)[13] for mineral analysis, iron (Fe), magnesium (Mg) and zinc (Zn) contents were estimated by using atomic absorption spectrophotometer (Z-8230 Polarized Zeeman); sodium (Na) and potassium (K) quantities were determined by using flame photometer (AFP-100) while phosphorous (P) content was estimated by spectrophotometer (V-1100).

Extract Preparation

The ZO methanolic extract (ZOME) was prepared by using soxhlet apparatus. ZO dried powder (50g) was uniformly packed in a filter paper, placed into a thimble and was extracted with 250mL methanol taken in the receiver. The extraction process continued for 24 hours

until the solvent in siphon tube became colorless. The methanolic extract was concentrated under reduced pressure (23-26 mm Hg) at 35 ^{o}C by using rotary evaporator until the golden brown viscous materials was obtained. The extract was further kept in hot air oven at 30 ^{o}C until all the solvent was evaporated. The final extract was kept in dark glass container at -4 ^{o}C until use.[14]

Phytochemical Analysis of ZO Crude Drug's Methanolic Extract

ZOME was analyzed for the presence of different phytoconstituents like carbohydrates, amino acids, proteins, glycosides, flavonoids, glycosides, steroids and alkaloids by using the following methods[15]

Test for carbohydrates- ZOME (1%) was mixed with equal volume (1+1 mL) of Fehling A and B solution, heated for few minutes and observed for green, yellow or red precipitate for carbohydrates' presence.

Test for Proteins- Biuret test: 1 mL (1%) of ZOEM was mixed drop wise with Biuret reagent. By shaking after addition of each extract, appearance of violet color was noted.

Test for Amino Acids- Ninhydrin solution 2 mL was added to 1mL (1%) of ZOEM in test tube; and then test tube was heated in water bath for 2-3 minutes to observe purple coloration.

Test for Steroids- Salkowski test was performed to observe red coloration by taking 2 mL of extract, 2mL of chloroform and 2mL of concentrated H_2SO_4.

Test for Glycosides- Keller-Kilani test was performed to confirm presence of glycosides (green to blue color) by taking 2 mL of ZOEM, 1 mL of glacial acidic acid and 5-6 drops of $FeCl_3$ (10%) and 5-6 drops of concentrated H_2SO_4.

Test for Flavonoids- HCl 0.5 mL (10%) and few pieces of Mg metal were mixed in 1 mL of each extract. A reddish color was observed for the presence of flavonoids.

Test for Alkaloids- ZOEM was mixed with ammonia and then extracted with chloroform solution. To this, HCl acid was added; and the acid layer was assessed by Mayer, Hager and Wanger's tests for the presence of alkaloids.

HPLC Analysis of 6-Gingerol

The HPLC analysis of ZO methanolic extract was carried out by using Shimadzu-20-A system, Japan with PDA-UV-20A detector. For chromatographic separations, a Merck (Germany) C-18 HPLC-packed column (4.6 mm x ID x 250 mm) with 5 µm particle size, type porous spherical was used and methanol was used as mobile phase solvent. Main operating parameters of HPLC were 1.0 mL/min column flow rate, 5 µL injection volume, 48 min chromatographic run time and UV detection at 230 nm.[16] The 6-gingerol content (%) in ZOME was determined by following the external standard method in which peak areas along with retention time were compared with analytical standard (6-gingerol, Carbosynth Limited, UK).

RESULTS AND DISCUSSIONS

Proximate Composition ZO Crude Drug

After analyzing the organoleptic properties of Trait, Punjab's ZO crude drug (Table 1) that were the same as described by Sharma et al. 2013[17], its proximate composition as tabulated in table 2, shows that its protein content (8.87±0.02%) is almost the same as determined in previous Pakistani study[18] (8.43±0.32%) while all other constituents except moisture are considerably high. One possible reason of these differences is that they reported their findings of fresh ZO rhizome as compared to our ZO crude drug's findings. Moreover, in their study ginger was purchased from local market where according our survey, ZO is mostly imported from Burma, Thailand and China. Therefore, market ZO can't be considered as a 'local specie' representative.

Table 1. Organoleptic Properties of Z. Officinal

Property	***Z. Officinale***
Color	Golden Brown
Taste	Pungent
Order	Pungent

Table 2. Proximate Composition (%) of *Z. Officinale*

Constituents	***Z. Officinale***
Moisture	4.07±0.06
Crude Fiber	18.24±0.02
Ash	6.72±0.02
Crude Fat	10.55±0.01
Crude Protein	8.87±0.02
Nitrogen Free Extract	51.63±0.02

Values are means ± standard deviations of triplicate estimations, *Significant at p<0.05

The low moisture level (4.07±0.06%) of our sample as compared to previous studies [17, 19, 20] may be advantageous in most probability of having maximum amount of 6-shugaol; a dried ginger's more potent bioactive compound than 6-gingerol in anticancerous, antioxidative, anti-inflammatory, antifouling and antibacterial effects and is formed by 6-gingerol's dehydration.[21-23] However, further studies are required to confirm the high percentage of 6-shugaol in Trait, Punjab's ZO crude drug.

Almost, the same carbohydrate content of ZO were found in our study as determined in an Indian study (56.92%)[17] however this level is comparatively low as found in (71.3%) Nigerian study (71.3%).[20] The high fiber content (18.24±0.02%) found in our study as compared to earlier studies[17, 19, 20] also suggests that that our ZO has high serum cholesterol lowering potential if used as a therapeutic food regularly, particularly by dyslipidemic patients. Similarly, high ash content (6.72±0.02%) determined in our study as compared to low ash contents estimated in two previous studies[18, 20] indicates that Trait, Punjab's ZO has also high mineral contents. Yet, the ash content is almost the same as determined is in Indian and Nigerian studies.[17, 19] Moderately high fat content (10.55±0.01%) in our studied sample in comparison with Nigerian findings in dried ZO rhizome[19] also highlights its essential oils' medicinal importance.[24]

Mineral Contents of ZO Crude Drug

Although, Ca content in our study (240.10±0.17 mg/100g) is less than that of the Indian ZO crude drug's finding[17] but it has almost the same level as reported in Nigerian drug.[19] The presence of high Fe level (174.77±0.66 mg/100g) in our ZO crude drug in comparison with previous findings, indicates its most probable medicinal importance if used by iron deficient population yet this iron content was less than that of the Nigerian sample.[19]

Moreover, Mg and P levels (5.03±0.06 mg/100g and 3.50±0.00 mg/100g) in our sample are less than the previous findings[17, 19] but its comparatively high Zn (149.50±0.10 mg/100g), Na (320.17±0.29 mg/100g) and K (534.10±1.00 mg/100g) contents advocate its therapeutic significance to treat aforesaid minerals' deficiency caused ailments.

Table 3. Mineral Composition (mg/100g) of *Z. Officinale* and *C. Longa* Crude Drugs

Mineral	*Z. Officinale*
Ca	240.10±0.17
Mg	5.03±0.06
Fe	174.77±0.66
Zn	149.50±0.10
P	3.50±0.00
Na	320.17±0.29
K	534.10±1.00

Values are means ± standard deviations of triplicate estimations.

Phytochemicals in ZO Crude Drug's Methanolic Extract

All the phytochemicals except steroids, which were found in our study (shown in table 4) were the same as determined in previous studies.[25, 26] Yet, our finding confirms the steroids' finding in ZO ethanolic extract by Sharma et al.[17]

Table 4: Phytochemical analyses of Ginger and Turmeric extracts

S. No.	Test	Ginger Extract
1	Alkaloids	Present
2	Amino Acids	Present
3	Carbohydrates	Present
4	Proteins	Present
5	Steroids	Present
6	Glycosides	Present
7	Flavonoids	Present

6-gingerol Content in ZOME

Among the gingerols, the 6-gingerol is main bioactive compound present in ZO, however, its amount varies in literature due to different processing protocols, different ZO varieties, and types of cultivars and ZO of different geographical areas.[11, 27] The estimated 6-gingerol content (6.50%) in our study by HPLC analysis (Fig. 1) in methanolic extract of ZO Trait, Punjab's sample was almost the same as that of the Dehli (Indian) samples estimated by Mishra et al.[28] In Korean study,[16] 6-gingerol content at differ temperatures of drying and extraction was estimated, however, the optimal level of their study was 2.50% less than our finding.

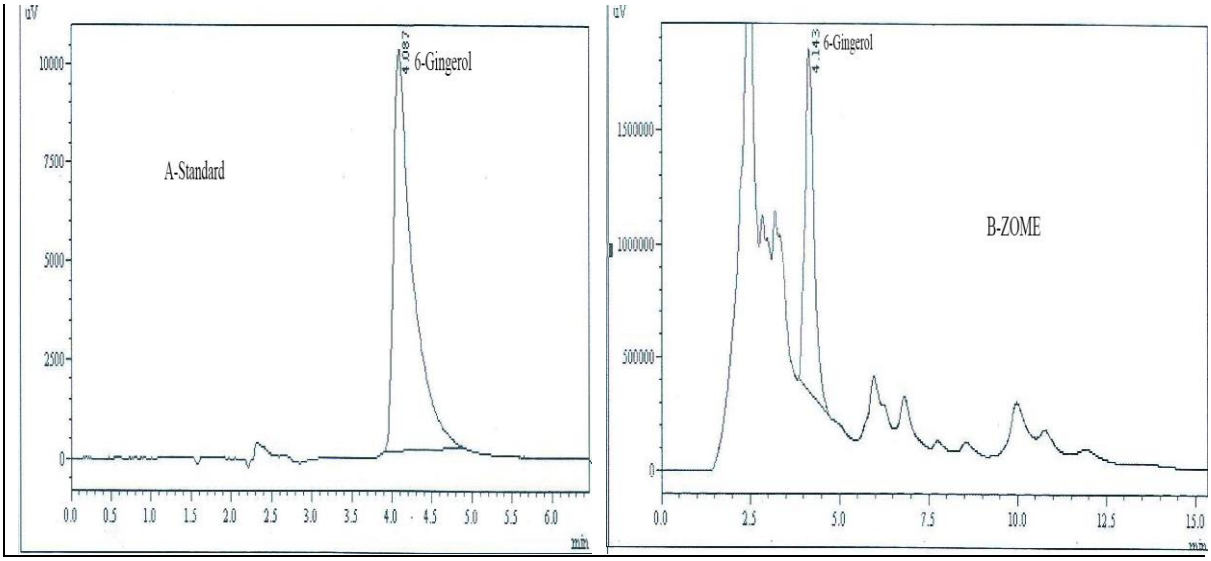

Fig. 1 (A) HPLC Chromatogram of 6-Gingerol Standard. (B) HPLC Chromatogram of ZOME. Retention time of ZOME containg 6-gingerol is almost the same as compared to 6-Gingerol standard.

The presence of high 6-gingerol contents in our sample is advantageous for its optimal extraction to be used in new effective drugs' formulations for many life threatening diseases as it has strong antidiabetic,[29, 30] antidyslipidemic,[31] anticancerous,[22, 32] antibacterial[33] antioxidative[33, 34] anti-inflammatory and analgesic[35] therapeutic potential.

CONCLUSION

Overall, our study comprehensively reported the general chemistry of Trait, Punjab's ZO crude drug and its methanolic extract; that may be useful if this specie is utilized in new drugs' compositions. Yet, our findings are not sufficient to represent ZO of Pakistan's locality instead more studies must be conducted on different samples from other areas of Pakistan to make any consensus.

ACKNOWLEDGEMENTS

We are grateful to **Dr. Waseem Shahzad** (Director, Institute of Biochemistry & Biotechnology, UVAS, Lahore, Pakistan) and **Dr. Farzana Chowdhary** (Director, Institute of Pharmaceutical Sciences, UVAS, Lahore, Pakistan) for providing us lab facilities to complete this study.

REFERENCES

1. Craig WJ. Health-promoting properties of common herbs. Am J Clin Nutr, 1999; 70 (3 Suppl). S491-499.

2. World Health Organization. WHO traditional medicine strategy 2014-2023.WHO Geneva, 2013; 1-76.
3. World Health Organization. WHO traditional medicine strategy 2002-2005. WHO Geneva, 2002; 1-74.
4. Rackova L, Cupakova M, Tazky A, Tazky A, Micova J, Emil Kolek E, Kostalova D. Redox properties of ginger extracts: Perspectives of use of Z. officinale Rosc. as antidiabetic agent. Interdiscip Toxicol, 2013; 6 (1): 26-33.
5. Paul P, Islam K, Mustari A, Khan MZI. Hypolipidemic effect of ginger extract in vanaspati fed rats. Bangl J Vet Med, 2012; 10(1 & 2): 93-96.
6. Govindarajan V. 1982. Ginger-chemistry technology and quality evaluation: Part-1. Crit Rev Food Sci Nutr, 1982; 17: 1-96.
7. Govindarajan V. 1982. Ginger-chemistry technology and quality evaluation: Part-2. Crit Rev Food Sci Nutr, 1982; 17: 189-258.
8. Connell D, Sutherland MA. Re-examination of gingerol, shogaol and zingerone: The pungent principles of Ginger (Zingiber officinale Roscoe) Australian J Chem, 1969; 22(5):1033-43.
9. Jolad SD, Lantz RC, Chen GJ, Bates RB, Timmermann BN. Commercially processed dry ginger (Zingiber officinale): composition and effects on LPS-stimulated PGE2 production. Phytochem J, 2005; 66(13): 1614-1635.
10. Jolad SD, Lantz RC, Solyom AM, Chen GJ, Bates RB, Timmermann BN. Fresh organically grown ginger (Zingiber officinale): composition and effects on LPS-induced PGE2 production. Phytochem J, 2004; 65(13): 1937-1954.
11. Vernin G, Parkanya C. Chemistry of ginger. In: Babu K (ed.). Ginger: The genus zingiber, Boca Raton, FL, USA; CRC Press: 2004, pp. 87-180.
12. Lahlou M. The Success of Natural Products in Drug Discovery. Pharmacology & Pharmacy, 2013; 4(3A): 17-31.
13. Association of official analytical chemists. Official methods of analysis of the association of official's analytical chemists. 17th ed., Arlington, Virginia; AOAC: 2003.
14. Atta AH, Elkoly TA, Mouneir SM, Kamel G, Alwabel NA, Zaher S. Hepatoprotective effect of methanol extracts of Zingiber officinale and Cichorium intybus. Indian J Pharm Sci, 2010; 72(5): 564-70.
15. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J of Phytol, 2011; 3(12): 10-14.

16. Ok S, Jeong WS. Optimization of extraction conditions for the 6-Shogaol-rich extract from ginger (Zingiber officinale Roscoe). Prev Nutr Food Sci, 2012; 17: 16-171.
17. Sharma PK, Mansoori S, Rathor N, Kulshrestha A, Dakad AK., Evaluation of Zingiber officinale and Curcuma Longa rhizome as crude drug from their ethanolic extract. In Res J Pharm, 2013; 4 (12): 74-76.
18. Tanveer Saira, Shahzad Aamir, Ahmed Waqas. 2014. Compositional and mineral profiling of Zingiber officinale. Pak J Food Sci, 2014; 24(1): 21-26.
19. Latona DF, Oyeleke GO, Olayiwola OA. Chemical analysis of ginger root. IOSR-JAC, 2012; 1(1): 47-49.
20. Fapohunda SO, Mmom JU and Fakeye F. Proximate analyses, phytochemical screening and antibacterial potentials of bitter cola, cinnamon, ginger and banana peel. Academ Arena, 2012; 4(8): 8-15.
21. Dugasani S, Pichika MR, Nadarajah VD, Balijepalli MK, Tandra S, Korlakunta JN. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. J Ethnopharmacol, 2009; 127: 515-520.
22. Pan MH, Hsieh MC, Kuo JM, Lai CS, Wu H, Sang S, Ho CT. 6-Shogaol induces apoptosis in human colorectal carcinoma cells via ROS production, caspase activation, and GADD 153 expression. Mol Nutr Food Res, 2008; 52: 527-537.
23. Mahady GB, Pendland SL, Yun GS, Lu ZZ, Stoia A. Ginger (Zingiber officinale Roscoe) and the gingerols inhibit the growth of Cag A+ strains of Helicobacter pylori. Anticancer Res, 2003; 23: 3699-3702.
24. Bhargava S, Dhabhai K, Batra A, Sharma A, Malhotra B. Zingiber Officinale: Chemical and phytochemical screening and evaluation of its antimicrobial activities. J Chem Pharm Res, 2012, 4(1): 360-364.
25. Jeena K, Liju VB, Kuttan R. Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. Indian J Physiol Pharmacol, 2013; 57(1): 51-62.
26. Kaushik P, Goyal P. Evaluation of various crude extracts of Zingiber officinale rhizome for potential antibacterial activity: A study in vitro. AIM, 2011; 1: 7-12.
27. Puengphian C, Sirichote A. [6]-gingerol content and bioactive properties of ginger (Zingiber officinale Roscoe) extracts from supercritical CO2 extraction. As J Food Ag-Ind, 2008; 1(01): 29-36.
28. Mishra AP, Saklani S, Chandra S. Estimation of gingerol content in different brand samples of ginger powder and their anti-oxidant activity: A comparative study. Recent Research in Science and Technology. 2013; 5(1): 54-59.

29. Li Y, Tran VH, Koolaji N, Duke C, Roufogalis BD. (S)-[6]-Gingerol enhances glucose uptake in L6 myotubes by activation of AMPK in response to [Ca2+]i. J Pharm Pharm Sci, 2013; 16(2): 304-12.
30. Son MJ, Miura Y, Yagasaki K. Mechanisms for antidiabetic effect of gingerol in cultured cells and obese diabetic model mice. Cytotechnology, 2014 May 4. [Epub ahead of print].
31. Singh AB, Akanksha, Singh N, Maurya R, Srivastava AK. Anti-hyperglycemic, lipid lowering and anti-oxidant properties of [6]-gingerol in db/db mice. Int J Med Med Sci. 2009; 1(12): 536-544.
32. Jeong CH, Bode AM, Pugliese A, Cho YY, Kim HG, Shim JH, Jeon YJ, Li H, Jiang H, Dong Z. 2009. [6]-Gingerol suppresses colon cancer growth by targeting leukotriene A4 hydrolase. Cancer Res, 2009; 69: 5584-5591.
33. Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of levels of Etlingera species (Zingiberaceae) in peninsular Malaysia. Food Chemistry, 2007; 104: 1586-1593.
34. Kim HW, Murakami A, Abe M, Ozawa Y, Morimitsu Y, Williams MV, Ohigashi H, Suppressive effects of mioga ginger and ginger constituents on reactive oxygen and nitrogen species generation, and the expression of inducible pro-inflammatory genes in macrophages. Antioxid Redox Signal, 2005; 7: 1621-1629.
35. Young HY, Luo YL, Cheng HY, Hsieh WC, Liao JC, Peng WH. Analgesic and anti-inflammatory activities of [6]-gingerol. J Ethnopharmacol, 2005; 96: 207-210.