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Extraction of 6-gingerol from *Zingiber officinale* using three different solvents and its purification using thin layer chromatography

Meghana N Bhandari, Rajani C Dombale and Mayuri M Pagar

Abstract
In this growing age where life is as uncertain as the multi-drug resistance capacity of any known micro-organism, immense efforts are continually being taken each day to empower the already existing drug repository. In this similar scenario, one such unexplored domain is phytochemicals. Phytochemicals are non-nutrient, bioactive plant compounds that can cure major non-communicable yet risk posing human diseases as they have been shown to possess enormous potential to reduce oxidative damage to cells, show anti-inflammatory properties and trigger the apoptosis of damaged cells. In this paper, focus has mainly been given on ginger as the prime source for 6-gingerol, its extraction and subsequent purification. Some daily used products have also been evaluated for the presence of 6-gingerol and a comparative analysis has been made to suggest the best method for obtaining a maximum yield for the compound.

Keywords: multi-drug resistance, bioactive, anti-inflammatory, apoptosis

1. Introduction
Zingiber officinale or simply known as ginger, is a natural root which has been known to possess medicinal properties since the early ages. It is used as an important component in the Ayurvedic, Chinese and Tibb-Unani systems of medicine. Ginger has a number of pungent constituents and active ingredients, out of which the main ones are gingerols which can be converted to shogaols, zingerone and paradols as indicated by the studies of lipophilic rhizome extracts. Ginger is known to exhibit a variety of pharmacological effects including anti-pyretic, hypotensive effects, analgesic and anti-tussive effects, which can be clearly observed in its usage as a home remedy for treatment of toothache, diarrhea, constipation, cough and cold. 6-Gingerol [5-hydroxy-1-(4`-hydroxy- 3`-methoxyphenyl)-3-decanone] is known to have its ramifications on a number of biological pathways involved in apoptosis, cell cycle regulation, cytotoxic activity and inhibition of angiogenesis [1,2]. Thus due to its efficacy as well as regulation of multiple targets and safety for human use, it is being actively considered as a potential therapeutic agent for prevention and/ or treatment of various diseases.

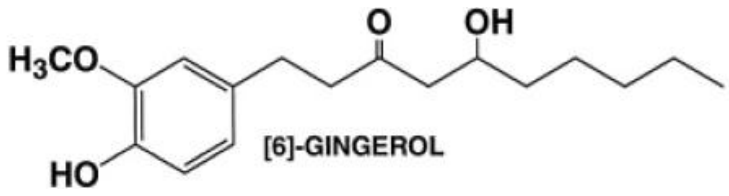

Fig 1: Chemical Structure of 6- Gingerol [2].

2. Materials and Methods
2.1 Materials
All of the solvents and chemicals used were of analytical grade (AR grade), a majority of which were sourced from our Department. Ethanol was obtained from a local chemical vendor in Pune, Maharashtra, India. Fresh ginger root and dried ginger (sunthi) were obtained randomly from a local market in Pune. The tea, cough syrup, toothpaste and Ayurvedic ointment were purchased from respective local stores.

2.2 Methods

2.2.1 Sample preparation

5g of dried ginger root (Sunthi) was grated and immersed in solutions of three different test solvents namely methanol, ethanol and dimethyl sulfoxide (DMSO), each with varying concentrations of 40 %, 60% and 80% respectively. These concentrations were prepared by taking the relevant volumes of the corresponding solvent and mixing it with distilled water to obtain a final volume of 100ml [3] This was done in an attempt to determine the optimum extraction solvent that could be used to obtain a maximum concentration of 6-gingerol.

2.2.2 Extraction of 6-gingerol

The prepared samples were kept for one hour in water bath, at a bath temperature of 80 ℃. After an hour, the samples were removed from the bath and filtered using Whatmann filter paper no. 1. The retentate was again mixed with 50ml of the test solvent of same concentration and kept back into the water bath for one hour. The corresponding samples obtained were re-filtered and the filtrates procured in both the cases were mixed [3]. These samples were used as crude extracts of 6-gingerol for further quantification.

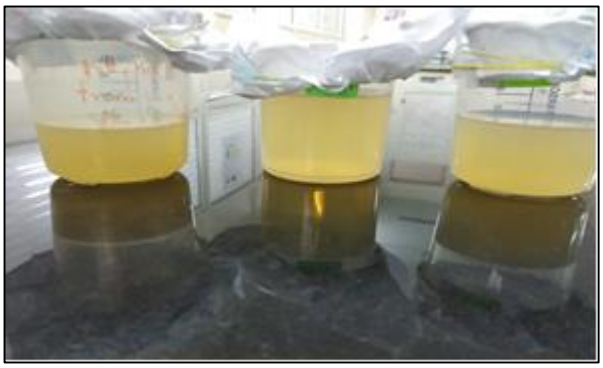

Fig 2: Crude extract

2.2.3 Estimation of 6-gingerol concentration

The concentration of 6-gingerol in the extract was calculated on the basis of Beer Lambert's Law by using the UV-Vis spectrophotometer (UV1800, Shimadzu). The wavelength in the absorption spectrum of 6-gingerol which gave maximum absorbance was found out to be 279nm (λmax). The molar absorptivity coefficient of 6-gingerol was known to be 2530 M^-1cm^-1. The dense solutions that were obtained after combining the two filtrates were diluted using the respective test solvent whilst keeping the same concentration and the dilution factor was calculated accordingly.

2.2.4 Spectrum analysis for compound

The absorption of a crude extract obtained using 80% methanol was monitored by measuring its absorption spectrum with the help of a UV-Vis spectrophotometer (UV1800, Shimadzu). The operational range of wavelength was varied from 260nm to 300nm. The absorption spectrum of the extracted sample was obtained in graphical form. The ideal peak should be obtained at 279-280nm [8]. The peak absorbance was obtained at 279nm which indicates that 6-gingerol was indeed present in the sample.

2.2.5 Study of other samples

a) **Wet Sample:** Was prepared by slicing five grams of fresh ginger and mixing it with methanol solution of three varying concentrations respectively (40%, 60%, 80%).
b) **Soaked Sample:** Was prepared by slicing five grams of fresh ginger and soaking it in methanol solution of three varying concentrations (40%, 60%, 80%) respectively for 72 hours.

   Such different types of studies were carried out to determine the optimum conditions for achieving a maximum 6-gingerol concentration.
c) **Real Sample:** Was prepared by mixing five grams or five milliliters of items used in daily life, in 80% methanol solution. The items used for analysis were honitus cough syrup, cough syrup, dantkanti toothpaste, Ayurvedic ointment, organic tea, meswak toothpaste, vicks-cough tablet and paracetamol syrup respectively.

2.2.6 Ultrasonication

In an attempt to further maximize the 6-gingerol concentration obtained in the crude extract, the dried, the wet and the soaked samples were ultrasonicated. Each of these samples was split up into equal portions, with each portion being kept into the ultrasonicator for 30 minutes, with pulse on 15 seconds and pulse off 5 seconds. The operational amplitude was varied in the range from 20% to 80%.

2.2.7 Aqueous two phase system

The crude extract obtained with dried ginger root in 80% methanol solution was used for aqueous two phase partitioning system (ATPS). The organic and aqueous layers of the system were formed by mixing together petroleum ether, ethyl acetate, methanol and water in the ratio 5:5:6.5:3.5 respectively [5, 9]. The system was kept undisturbed for an hour, after which it was seen forming two distinct layers. The two layers were neatly separated with the help of a micropipette. The absorbance of the two corresponding layers was monitored using UV-Vis spectrophotometer (UV1800, Shimadzu).

Fig 3: ATPS Blank

Fig 4: ATPS for sample

2.2.8 Thin layer chromatography

The purity of the crude extract, the ultrasonicated sample and aqueous two phase sample was checked using thin layer chromatography. The mobile phase for the system was prepared by mixing hexane and diethyl ether in the ratio 30:70 and forming a 20 milliliter system. The three different types of samples were loaded onto the TLC plate by taking 20 micro liters of each sample. The loaded samples were air dried for 30 minutes and the plate was immersed into the mobile phase system. The plate was left undisturbed for an hour and the corresponding spots observed were investigated to calculate retention factor for each.

3. Results and Discussion

The dried ginger (Sunthi) powder was tested in three different solvents and subsequent concentration of 6-gingerol was calculated. The results can be tabulated as follows:

a) Dried Sample Study

Table 1: Methanol study of dried sample

Sample number	Solvent concentration	Absorbance (279 nm)	Concentration (µg/ml)	Yield (mg per gm Sunthi)
1.	40%	0.2051	23.8645	0.715
2.	60%	0.4505	52.4182	1.572
3.	80%	0.6975	81.1581	2.434

Table 2: Ethanol study of dried ginger

Sample number	Solvent concentration	Absorbance (279 nm)	Concentration (µg/ml)	Yield (per gm Sunthi)
1.	40%	0.1081	12.5780	377.341
2.	60%	0.1950	22.6893	680.681
3.	80%	0.3120	36.3029	1089.08

Table 3: DMSO study of dried ginger

Sample number	Solvent concentration	Absorbance (279 nm)	Concentration (µg/ml)	Yield (µg per gm sunthi)
1.	40%	0.0733	8.5288	255.866
2.	60%	0.1840	21.4094	642.283
3.	80%	0.1396	16.2432	487.297

Thus an overall comparative analysis was made to determine the optimum solvent.

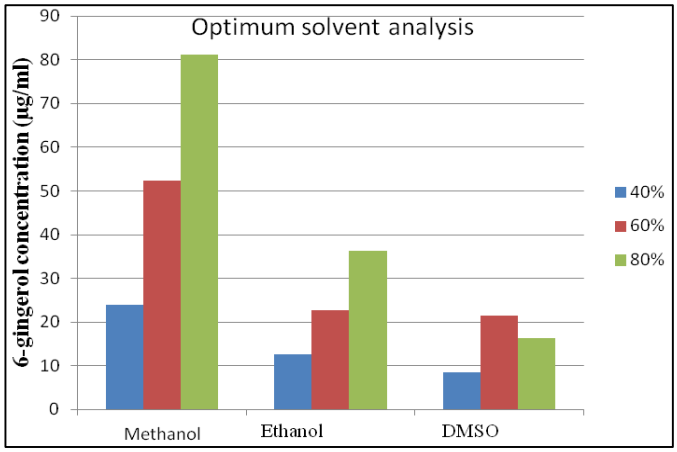

Fig 5: Optimum solvent analysis

The spectrum analysis for the compound yielded the following results:

Table 4: Absorption spectrum (260- 300 nm)

Sr. No.	Wavelength (nm)	Absorbance
1	260	1.211
2	261	1.222
3	262	1.237
4	263	1.256
5	264	1.279
6	265	1.305
7	266	1.336
8	267	1.367
9	268	1.402
10	269	1.439
11	270	1.476
12	271	1.527
13	272	1.557
14	273	1.598
15	274	1.637
16	275	1.671
17	276	1.705
18	277	1.733
19	278	1.752
20	279	1.765
21	280	1.764
22	281	1.763
23	282	1.744
24	283	1.717
25	284	1.676
26	285	1.630
27	286	1.581
28	287	1.526

29	288	1.455
30	289	1.363
31	290	1.259
32	291	1.147
33	292	1.043
34	293	0.952
35	294	0.876
36	295	0.816
37	296	0.771
38	297	0.738
39	298	0.711
40	299	0.690
41	300	0.676

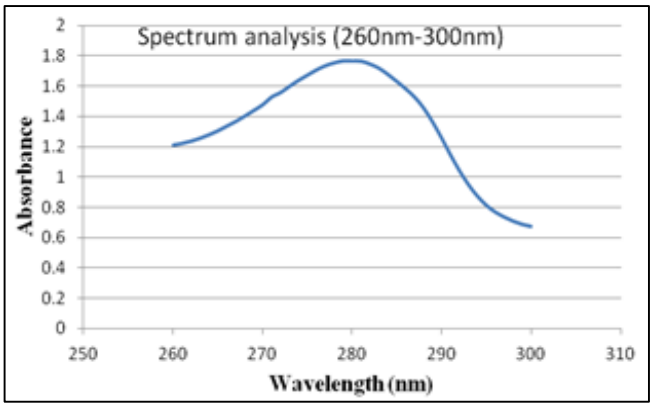

Fig 6: Absorbance spectrum of 6-gingerol

b) Wet Sample Study
Solvent: Methanol

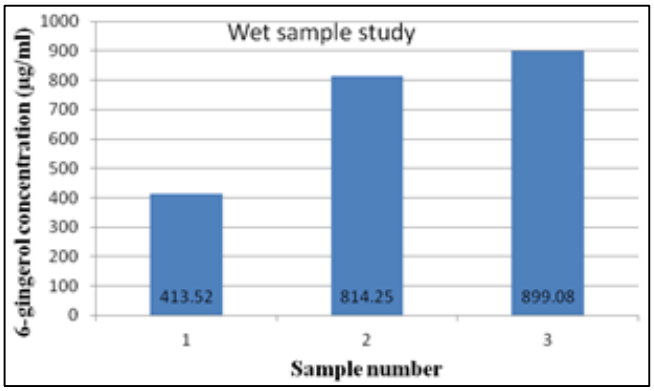

Fig 7: Wet ginger analysis

c) Soaked sample study
Solvent: Methanol (sample soaked for 72 hours)

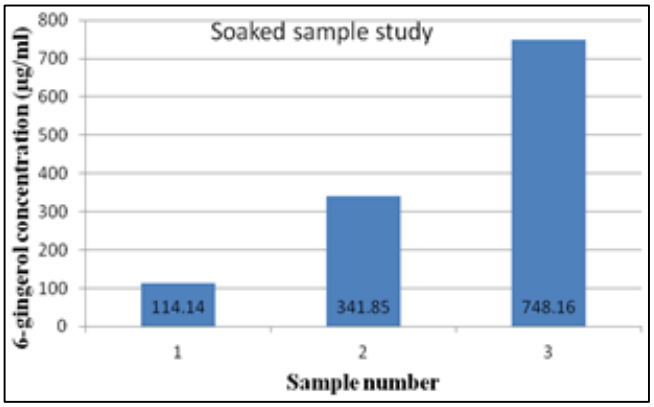

Fig 8: Soaked ginger analysis

Real Sample Study
Solvent: 80% Methanol

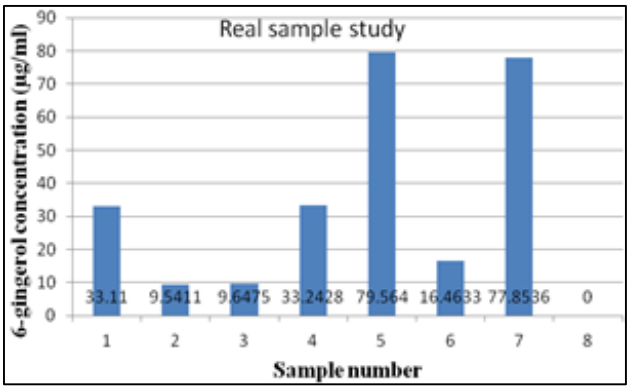

Fig 9: Real sample analysis

Ultrasonication
a) Dried Sample: (sunthi in 80% methanol)

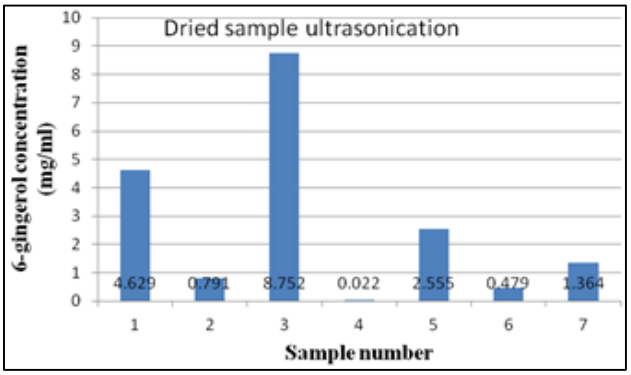

Fig 10: Dried ginger ultrasonication

b) Wet Sample: (fresh ginger in 80% methanol)

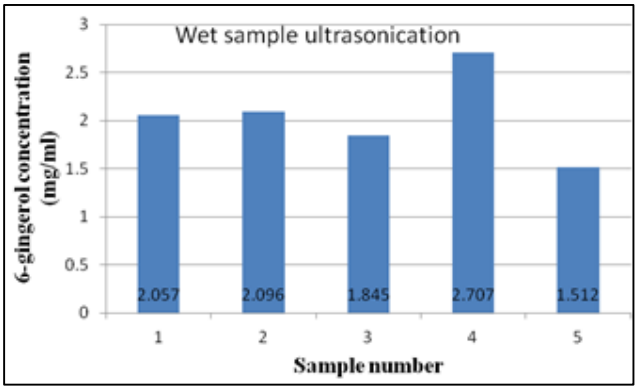

Fig 11: Wet ginger ultrasonication

c) Soaked sample: (fresh ginger soaked in 80% methanol for 72 hours)

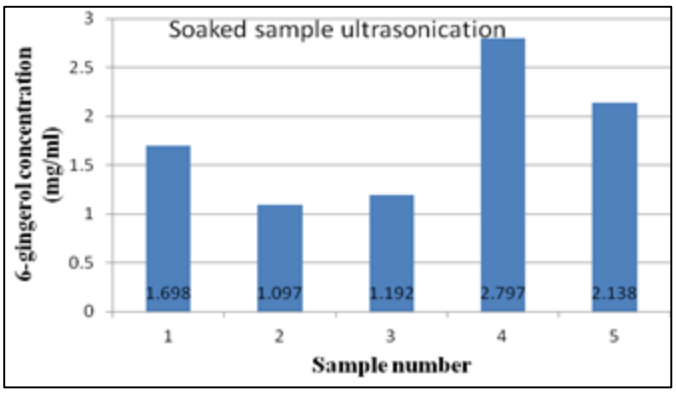

Fig 12: Soaked ginger ultrasonication

A comparative study was carried out to observe the effect of ultrasonication technique on the crude extract.. As reported previously in one of the papers the concentration of 6-gingerol obtained from crude extract using HPLC technique was 17mg/ml. In retrospect, its concentration and yield have both shown a significant surge after ultrasonication technique thus making ultrasonication a positive test for optimisation.

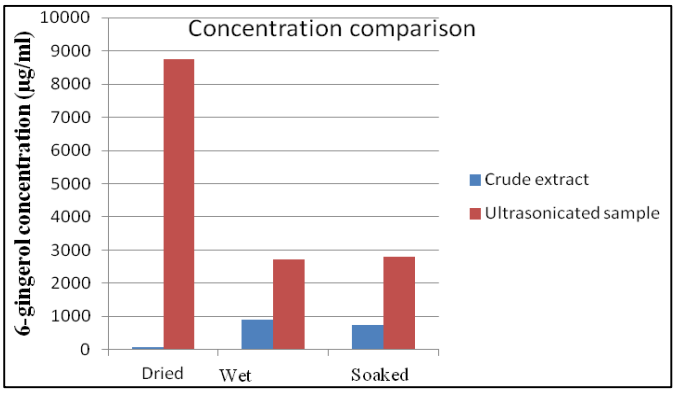

Fig 13: Concentration comparison

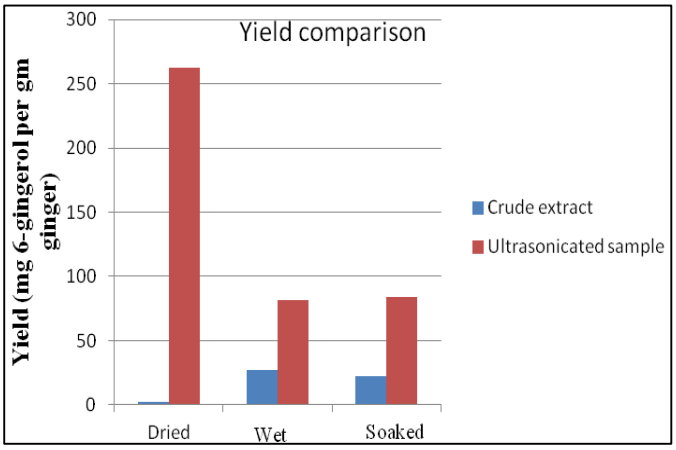

Fig 14: Yield comparison

Aqueous two Phase System: The concentration of 6-gingerol in both the layers was calculated:

Table 5: Aqueous two phase system

Upper Layer	**Blank**	**Sample**	**Dilution factor**	**Concentration (µg/ml)**	**Yield (per gm sunthi)**
	0	-0.0941	1:10	nil	nil
Lower Layer	Blank	Sample	Dilution factor	Concentration (mg/ml)	Yield (per gm sunthi)
	0	0.4966	1:100	5.778	173.34

Thin layer chromatography was performed to validate the previous results and the corresponding retention factor for each sample was calculated.

Table 6: Retention factor calculation

	Crude extract	**Aqueous two phase system (lower phase taken)**	**Ultrasonicated sample**
Spot distance (cm)	4.5	4.5	6
Solvent distance (cm)	6.5	6	6.8
Rf value	0.692	0.75	0.882

Table 7: Concentration and yield comparison of crude extract and ultrasonicated sample

	Crude extract (µg/ml)	**Yield (mg 6-gingerol per gm ginger)**	**Ultrasonicated sample (µg/ml)**	**Yield (mg 6-gingerol per gm ginger)**	**Fold change for an increase in yield**
Dried Sample	81.1581	2.434	8752	262.56	107.87
Wet Sample	899.08	26.972	2707	81.21	3.01
Soaked Sample	748.16	22.444	2797	83.91	3.738

The Rf value in our investigation showed a significant increase after ultrasonication and ATPS were performed. It was done with an aim to determine the purity of each sample and the sample with the highest concentration. The results of the study can be tabulated as follows:

Table 8: Comparative study for crude extract, ultrasonicated sample and aqueous two phase system

	Concentration of 6-gingerol (mg/ml)	**Yield (mg 6-gingerol per gm ginger)**	**Retention factor (Rf value)**
Crude extract	0.0811	2.434	0.692
Ultrasonicated Sample	8.752	262.56	0.882
Aqueous two phase system (lower phase)	5.778	173.34	0.750

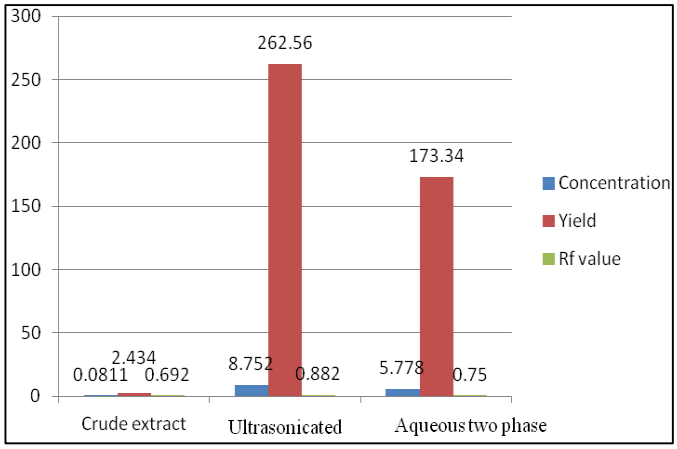

Fig 15: Comparative study for crude extract, ultrasonicated sample and aqueous two phase system

4. Acknowledgement
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5. Conclusion
In the present investigation, it can be concluded that dried ginger root is the best source for extraction of 6-gingerol and that ultra-sonication technique is useful to maximize the concentration of 6-gingerol in the crude extract.

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