www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2017; 6(2): 179-182

www.thepharmajournal.com
Received: 29-12-2016
Accepted: 30-01-2017

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Isolation and standardization of gingerol from ginger rhizome by using TLC, HPLC, and identification tests

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Abstract
Many drugs commonly used today are herbal origin. About 25% of prescription drug in the US contains atleast one active ingredient derived from plant material. Herbal medicine is the oldest form of healthcare known to mankind. Herbs has been used by all cultures throughout history. Herbal medicine are also in great demand in developed world for primary healthcare because of their efficacy, safety and lesser side effects. The aim of present study is to isolate and standardize gingerol obtained from Zingiber officinale rhizome. Zingiber officinale rhizomes were extracted with ethanol (95%) by simple maceration process. This extract was studied phytochemically and gingerol is isolated by using TLC and HPLC technique. The isolated compound was found to be oleoresins. Gingerol constituents responsible to reduce effects such as emesis and nausea.

Keywords: Herbal medicine, Zingiber officinale, gingerol, emesis, nausea

1. Introduction
Zingiber officinale belonging to family Zingiberaceae is an ancient Indian medicine used in several disorders. Zingiber officinale commonly called as 'Adrak' in Hindi and Urdu, 'Sunthi' in Marathi. It has various vernacular names such as Ginger, Srngaveram, Adrak, Sunthi. *Zingiber officinale* is possibly native to India and China. It is now widely grown as a commercial crop in south and Southeast Asia, tropical Africa (especially Sierra Leone and Nigeria), Latin America, the Caribbean (especially Jamaica) and Australia. Ginger has a distinctive thickened, branched rhizome (underground stem) which sometimes looks somewhat like a swollen hand. The rhizome has a brown corky outer layer (usually removed before use) and a pale yellow centre with a spicy lemon-like scent. Shoots (pseudostems), up to 1.2 m tall, arise annually from buds on the rhizome. Ginger is a well-known tropical herbs whose root is used in both Traditional Chinese Medicine and Western Herbal Medicine. It has a long history of medicinal used dating back 2500 years in China and India for conditions such as headaches, nausea, rheumatism, and colds, has been used since ancient times for a variety of condition, including fevers, and digestive problems, and as an appetite stimulant.

2. Materials and Methods
2.1 Chemical and Reagents
FTIR Spectrophotometer (Perkin elmers), UV Spectrophotometer (SHIMADZU 1800), HPLC Waters 1650, all chemicals and solvents used were of A.R. Grade and IPA was obtained from Alkem Labs. Ltd. Mumbai.

2.2 Plant material
The ginger rhizome were purchased from local market of Pune. The material were cleaned & dried under shade & then placed in oven at 20-40 ^{0}C. The dried rhizomes were weighed and stored in desicator.

2.3 Isolation of gingerol from ginger
Dry ginger was crushed to a coarse powder and extracted with95% ethanol by simple maceration process. Solvent was evaporated by distillation to obtain thick pasty mass. The thick pasty mass was suspended in water. The Ginger resin precipitates in water which was removed by filtration and the residue obtained was dried under vacuum.

2.4. Standardization of gingerol from ginger
The gingerol, active constituent of ginger rhizome extract was standardized by various methods specified in the compendias. The various tests such as TLC. HPLC, Identification test are performed to identify the gingerol present in extract.

2.4.1 TLC Method
2.4.1.1 Preparation of plates
Prepare a suspension of coating substance and spread a uniform layer of suspension, 0.25 to 0.30 mm thick, on flat glass plate of 20 cm long. Dried in air and heat at 100 to 105^0 for atleast 1 hr. store the plates protected from moisture, dry the plates at time of use if necessary.

2.4.1.2 Mobile phase
Hexane: Diethyl ether (30:70)

2.4.1.3 Test solution
Reflux 1 g of the coarsely powdered substance under examination with 25 ml of methanol for 15 minutes, cool and filter. Wash the residue with 10 ml of methanol. Combine all the filtrates and concentrate to 10 ml.

2.4.1.4 Reference solution
Reflux 0.5 g of coarsely powdered *sunthi RS* with 5 ml *methanol* for 15 minutes, cool and filter. Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm.

2.4.2 HPLC Method
Reflux about 3 g of the coarsely powdered substance under examination with 100 ml of methanolon a water–bath for 15 minutes cool and filter. Reflux the residue further with methanol till the last extract turns colourless, cool and filter. Combine all the filtrates and concentrate to 50.0 ml.
Reference solution:
A 0.1 per cent w/v solution of *6-gingerol RS* in methanol.
Chromatographic system
- a stainless steel column 25 cm x 4.6 mm packed with octadecylsilane bonded to porous silica (5 µm),
- mobile phase: 55 volumes of *acetonitrile* and 45 volumes of *water,*
- flow rate. 1.3 ml per minute,
- spectrophotometer set at 278 nm,
- a 20 µl loop injector.

2.4.3 UV Method development
For the quick estimation of this extract there is no reported UV-Visible method, so simple UV spectroscopic method was developed. Calibration curve of rhizome extract was prepared in methanol at maximum wavelength of 281.40nm.

3. Pre-formulation study
It needed to ensure the development of stable, effective and safe dosage form.

3.1 Solubility analysis
Solubility is made by adding solute in small incremental amount to fixed volume of solvents such as distilled water, ethanol, chloroform, acetone and ether. Then it is examined for undissolved particles.

3.2. Confirmation of drug
3.2.1 UV-VIS Spectrophotometric method
Methanol was selected for preparation of calibration curve. 100mg of crude extract was dissolved in methanol and diluted upto 100ml to get concentration of 1000ppm which is treated as stock solution. This stock solution was diluted further to get different concentrations. Resultant solutions were scanned for λmax in the range of 200-400 nm using UV-spectrophotometer.

3.2.2 IR spectrum interpretation
Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. A small quantity (5 mg) of the extract was dispersed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The sample was scanned from 4000 - 400 $^{cm-1}$.
Determination of purity:
The purity of extract was determined by HPLC analysis.

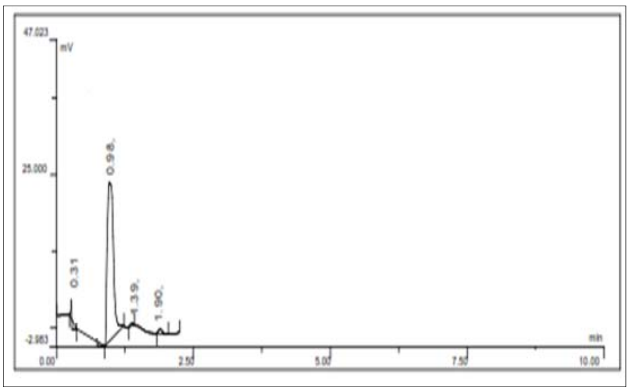
3.3 Calibration curve of gingerol
In Methanol Stock solution of ginger extract were pipette out in to series of 10ml volumetric flasks and diluted with methanol to get final concentration of 20-100mcg/ml. The absorbance of resultant solution was measured at 281.40nm.

4. Result and Discussion
4.1 Standardization of gingerol from *Zingiber officinale* rhizome extract
4.1.1 TLC
Gingerol is analysed for retention factor. TLC plate showed result illustrated in figure 10 of TLC chromatogram. Clear spot observed from ethanolic extract when visualized by eye, however under UV lamp in long wavelength 365nm the spot colour were fluorescent blue.

Table 1: Rf Values for ethanolic extract of Zingiber officinale by TLC

Solution	Solvent Front Height (cm)	No. of spots	Spot height(cm)	Rf Value
Reference Solution	5.5	1	5.4	0.98
Test Solution	6.2	1	6.0	0.97

4.1.2 HPLC
The standard curve for the concentration Vs peak height was drawn and line of equation was originated. From the line of equation of standard drug y= 0.2228x + 37.658 and based on the calculations and findings, it was consequently found that 100 mg of extract would be consist of 18.276 mg i.e. 18.276% of active content.



HPLC chromatogram of *Ginger extract*

Table 2: Standard calibration curve of gingerol by HPLC

Sr. No.	Conc. ug/ml of standard solution	Peak height %
1	20	40.59
2	40	48.0
3	60	51.69
4	80	55.96
5	100	58.89

Table3

Sr. No.	Conc. of test solution	Peak height %
1	Unknown	41.73

4.1.3 UV Method

Extract was found to obey Beer-Lambert's law in the concentration range of 20-100 µg/ml with regression coefficient (r2) values 0.9995. The regression equations were calculated as y = 0.0097x + 0.0132 for methanol.

Table 4: Different validation parameters of the calibration

Parameters	Results
Linearity correlation coefficient	0.9995
y- intercept	0.0132
Slope	0.0097
Range	20-100 µg/ml
LOD	4.5 µg/ml
LOQ	13.6 µg/ml

4. Pre-formulation study

4.1 Determination of Solubility

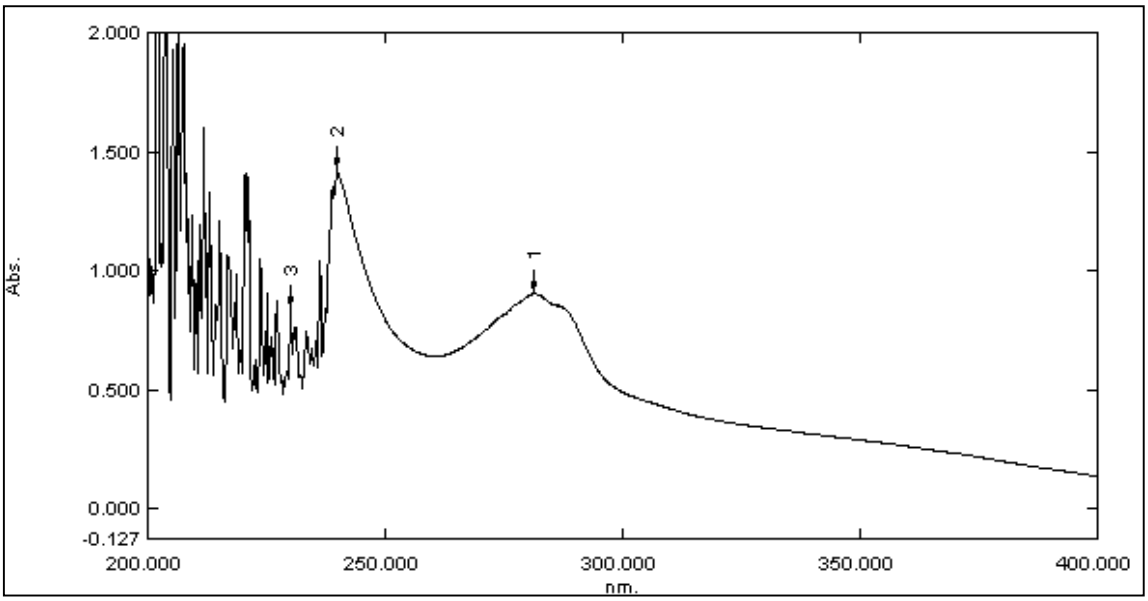
Table 5: Solubility profile of the ginger extract

Solvent	Solubility behaviour
Water	Insoluble
Acetone	Insoluble
Ethanol	Soluble
Methanol	Soluble
Chloroform	Soluble

4.2 Conformation of Drug

4.2.1 UV Spectroscopy

After studying the UV- spectra of ginger rhizome extract, it was found that it shows maximum absorbance at 281.4 nm. So absorbance at 281.4 nm was considered as λ_{max} for Ginger extract.



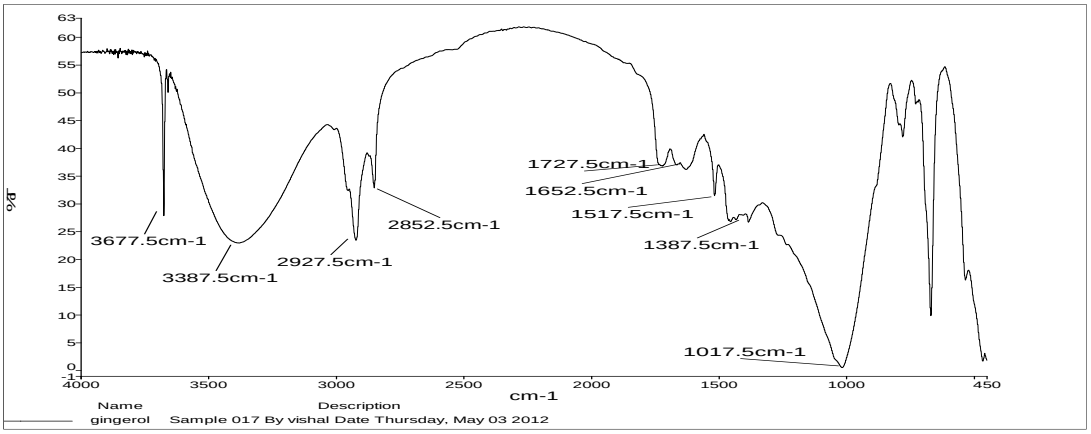
UV Spectra of Ginger extract

4.1.2 IR spectrum interpretation

The identity of drug was confirmed by comparing IR spectrum of drug with reported spectrum of Gingerol. The characteristic absorption bands in the infrared absorption spectrum of gingerol and a summary of the description of the characteristic IR bands for gingerol are described.

Table 6: Interpretation of Ginger Extract

Sr. No.	Functional Group	Theoretical Peaks (cm^{-1})	Practical Peaks (cm^{-1})
1	C-H(stretch)	2700-3300	2927.5, 2852.5
2	C-O (stretch)	900-1300	1017.5
3	N-H (stretch)	3100-3500	3387.5
4	O-H (stretch)	3000-3700	3677.5
5	C=O (stretch)	1600-1900	1652.5
6	C=C (stretch)	1475-1610	1517.5



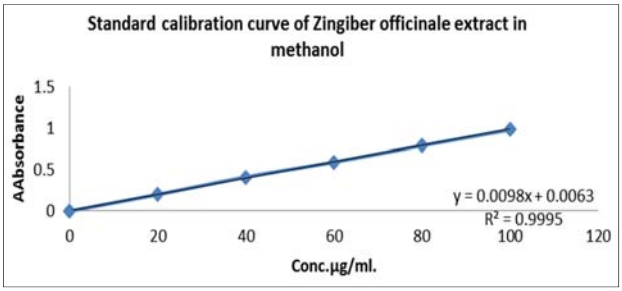
FTIR spectra of *Zingiber officinale* extract

4.1.3 Determination of Purity

The purity of Ginger extract was done by using HPLC analysis method depending on retention time (Rt Value). Pure gingerol shows the Retention time at 1.0 min. The analytical graph of HPLC scanning shows presence of various peaks at different retention time. Out of that the peak of gingerol appears at 0.98. Depending upon that it was found that the extract containing gingerol is 98 % pure.

4.1.4 Standard Calibration Curve

4.1.4.1 Standard Calibration Curve of Gingerol in methanol



λ_{max} value for Ginger extract was found to be 281.4 nm from UV spectra.

Table7: Hence, this wavelength was selected for preparation of calibration curve for ginger extract. Calibration curve data of Ginger extract in methanol solvent

Medium	Equation	R^2
Methanol	y= 0.0097x + 0.0132	0.9995

5. Conclusion

However, further spectral analysis of isolated compound such as ^{13}C-NMR and mass spectroscopy could not be taken. As far as our knowledge goes this bioactive compound is novel. Zingiber officinale provides deeper insights into indigenous method of application and effectiveness of plant derivatives in treating different ailments. Therefore, the structural ilucidation, nomenclature, validation and pharmacological screening of isolated gingerol will require for proving their clinical reliability, safety and efficacy.

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