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Original Research Article

STANDARDIZATION OF SOME BIOACTIVES IN GINGER EXTRACT

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Abstract: The aim of the present study was to estimate the bioactives from some marketed preparations of ginger especially with reference to gingerol and to compare with active constituents present in crude drug and estimation of the gingerol contents with the help of different analytical techniques like TLC, IR and UV spectrphotometry also evaluation of antibacterial activity of the marketed ginger preparations and to correlate it with gingerol contents. From the study, it was observed that the range of gingerol is from 90.08% to 104.92%w/w., which was found to be fairly compliant with the I.P. specifications i.e.90-120% w/w. These differences in the range of gingerol content suggest that there must be different exogenous and endogenous factors affecting the quality and purity of the drug, it is very essential, being vital and important. Due to this reason the need was generated for the pharmacological, phytochemical evaluation and standardization of various formulations available in the market. The present study was extended for determination of antibacterial property by cup plate method by taking Ofloxacine as a Standard.The sample of Green Pharmacy indicated that there was high antibacterial property. Hence from the result it was clear that presence of high amount of gingerol in ethanol extract (Green Pharmacy)

Key words:-Ginger, gingerol, standardization, antibacterial

Introduction: The standardization procedure applicable only to herbal extracts. The ginger extract commonly used therapeutically. [1]The ginger extracts used in such preparations may be collected in various conditions and from different geographical regions. Therefore, we may expect a wide variation in the gingerol contents present in various marketed herbal drug preparations herbal drugs are used. The biological studies of the ginger extracts showed the high antimicrobial effects. This may be used in food industries as preserver of food product against spoilage by bacteria and fungi. The UV spectrophotometric procedure is a quick and

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reliable method for the quantitative monitoring of gingerol in very low concentrations in raw materials, processed powders and in herbal preparations containing *Zingiber officinale.* Ginger contains a number of pungent constituents and active ingredients. The major pungent compounds in ginger, from studies of lipophilic rhizome extracts, have yielded potentially active gingerols. The characteristic odor and flavour of ginger was caused by a mixture of gingerol and shogaol.[2,3,4,5]

Literature Survey: Shinde Sachin K *et al* 2012; reported estimation of gingerol from *Zingiber officinale* rhizome extract by UV spectroscopic method. [2] (Amrit Pal Singh 2003) studied medicinal properties of ginger and tries to analyze the current status of research in the pharmacological activities of ginger[3]. Mohammad Sharrif Moghaddasi 2012; studied various pharmacological activities by using ginger extract. [4]

Objective: The aim of the present study was to estimate the bioactives from some marketed preparations of ginger especially with reference to gingerol and to compare with active constituents present in crude drug. Estimation of the gingerol contents with the help of different analytical techniques like TLC, IR and UV spectrphotometry. Evaluation of antibacterial activity of the marketed ginger preparations and to correlate it with gingerol contents.

Experimental work

Table-1 Dosage form of 4 ginger preparations used in the present study

Marketed Components	Dosage Form	Description
Himalaya Sunthi	Capsule	Yellow (After removal of cap)
AvipattikarChurna	Coarse Powder	Light yellow
SunthichurnaRitesh	Coarse Powder	Creamish yellow
Sunthichurna Green Pharmacy	Coarse Powder	Light yellow

Extract prepared by soxhlation were used for TLC Studies.

Preparative Thin Layer Chromatography [6]

The seperation of phytochemical mixtures available in small quantities was carried out by preparative thin layer chromatography (PTLC). About 80 mg of the mixture to be seperated was dissolved in the minimum quantity of solvent and spotted on the PTLC plate at a distance of about 1cm from the base in the form of a band. The plate was kept in the solvent chamber containing about 100 ml of the selected solvent system. The solvent was allowed to run up to 75 % and then removed from the chamber, dried and viewed under UV light

GINGER

Synonym : Rhizomazingiberis , Zingibere

Biological source:

Ginger consists of dried rhizome of *Zingiber officinale* Roscoe, family Zingiberaceae.

Ginger contains about 1-2% of volatile oil and 5-8 % of resionous matter, starch and mucilage. The pungent nature of ginger is due to gingerol, an oily liquid consisting of homologous phenols.

Chemicals: Petroleum ether(60-80^0), Ethanol, Methanol, Chloroform, Benzene, Acetone, Ethyl acetate, n-hexane, n-Butanol, diethyl ether, Acetic acid, Aluminum chloride, Sulphuric acid, Hydrochloric acid, Ferric chloride, Sodium hydroxide, Potassium acetate, Sodium carbonate,Nutrient agar media, Silica Gel- 254 . Ginger preparations were procured from retail market.

Infrared Spectroscopy: The infrared spectra of isolated compound obtained from preparative TLC of extract were recorded using SHIMADZU-FTIR 8400 spectrophotometer using potassium bromide pellet technique.In that, Curcumin was used as a standard, because of structural similarity between curcumin and gingerol.

Estimation of Total Gingerol Content

Standard Preparation- Curcumin[7]

Accurately 10 mg of cucumin was dissolved in methanol and then diluted to get the concentration of 20,40,60,80 &100 µg/ml.

Curcumin was used to make standard calibration curve**.**

Sample Preparation-(isolated compound from various ginger prepartions)

0.007 g of isolated compound of gingerol was dissolved in specified amount of methanol and filtered. & absorbance was taken at 427 nm by UV spectrophotometer. From the absorbance, total gingerol content was estimated.

Evaluation of Antimicrobial Activities ofthe Various Ginger Extracts:

Procedure for cup-plate method: [8, 9]

Stock solution:

The ethanol extract was washed with n-hexane. The n-hexane fraction was then taken up for determining the antimicrobial activity. The test compounds (5 mg) were dissolved in methanol (10 mL), to produce 500µg/mL. Further dilutions were made with methanol to produce 6.25, 12.5, 25, 50, µg/mL. Similarly, the dilutions were prepared for standard drug i.e., Ofloxacin in a concentration of 500 µg/mL.

Result and Discussion

Preliminary Table-2 Phytochemical Screening of Various Extracts of Crude Ginger

Sr. no.	Constituents	Petroleum ether extract	Chloroform extract	Ethanol extract
1.	Carbohydrates	-	-	-
2.	Flavanoids	-	-	+
3.	Alkaloids	-	-	-
4.	Tannins	-	-	+
5.	Phytosterols	++	+	++
6.	Glycosides	-	-	-
7.	Saponins	-	-	+
8.	Proteins	-	-	-
9	Diterpenes	++	+	-

Remarks: + (Present) **;** – (Absent)

From the result of Preliminary phytochemical screening, it was observed that ethanolic extract of ginger showed presence of flavonoid, saponin. Petroleum ether, Chloroform and Ethanolic extracts showed presence of Phytosterols. Petroleum ether and Chloroform extracts showed presence of diterpenes.

Refractive index of Ethanolicginger extracts 1.4981

From the result it was observed that refractive index of ethanolic extract is nearer to the refractive index of gingerol that is 1.511.

Table-3 Physicochemical Parameters of Various Ginger Preparations

Parameters	**A**	**B**	**C**	**D**	**E**	**I.P.STD.**
Ash Values						
Total ash	10.23%	9.64%	7.26%	7.28%	8.13%	NMT 6%
Acid insoluble ash	1.92%	1.37%	1.28%	1.20%	1.56%	NMT 2%
Water soluble ash	3.98%	3.41%	4.33%	2.36%	3.45%	NMT 1.7%
Extractive Values						
Alcohol soluble extractive	8.97	7.56	6.20	6.54%	8.30%	NMT 4.5%
Water soluble extractive	9.10%	8.69%	9.54%	11.27%	9.85%	NLT 10%

Note:- A-Ginger Powder, B-Sunthi Churna(Green Pharmacy), C-Sunthi Capsule (Himalaya), D-Avipattikar Churna (Dhootapapeshwar), E- Sunthi Churna (Ritesh Pharmaceuticals).

Ash values are helpful in determining the quality and purity of a crude drug especially in the powdered form. On incineration crude drugs normally leave an ash usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.

Extractive value is used for evaluation of crude drug. It gives an idea about the nature of chemical constituents present in crude drug and is also useful for estimation of chemical constituents, soluble in particular solvent used for extraction.

From the above result it was observed that the Physicochemical parameters of all marketed samples are within the Pharmacopoeial limit except sample Avipattikar Churna (Dhootapapeshwar). The Water soluble extractive value of Avipattikar Churna sample is 11.27%, All samples comply with I.P. standard whare as AvipattikakarChurna which have water soluble extractive value less than 10%.

Table 4-Refractive indices of ethanolic extracts of various marketed preparations

The refractive index of ethanolic extract of ginger rhizome was found to be 1.498 and that of marketed samples are1.490, 1.479, 1.485, 1.476 which is nearer to that of gingerol.

Extract	H	D	G	R
R.I.	1.49(	1.47(	1.48(	1.47(

Note- R.I. -Refractive index, H-Sunthi Capsule (Himalaya), D-Avipattikar Churna (Dhootapapeshwar) G-Sunthi Churna (Green Pharmacy), R-Sunthi Churna (Ritesh Pharmaceuticals)

Table 5 -Phytochemical Screening for Pet. Ether extract

	H	***D***	***G***	***R***
Carbohydrates	-	-	-	-
Flavonoids	-	-	-	-
Alkaloids	-	-	-	-
Tannins	-	-	-	-
Phytosterols	++	++	++	++
Glycoside	-	-	-	-
Saponins	-	-	-	-
Proteins	-	-	-	-
Diterpenes	++	++	++	++

Table-6 Phytochemical Screening For Chloroform extract

	H	***D***	***G***	***R***
Carbohydrates	-	-	-	-
Flavonoids	-	-	-	-
Alkaloids	-	-	-	-
Tannins	-	-	-	-
Phytosterols	+	+	+	+
Glycosides	-	-	-	-
Saponins	-	-	-	-
Proteins	-	-	-	-
Diterpenes	+	+	+	+

Table-7 Phytochemical Screening For Ethanol extract

	H	***D***	***G***	***R***
Carbohydrates	-	-	-	-
Flavonoids	+	+	+	+
Alkaloids	-	-	-	-
Tannins	+	+	+	+
Phytosterols	+	+	+	+
Glycosides	+	+	+	+
Saponins	-	-	-	-
Proteins	-	-	-	-
Diterpenes	-	-	-	-

Note: - H-Sunthi Capsule (Himalaya), D-Avipattikar Churna (Dhootapapeshwar) G-Sunthi Churna (Green Pharmacy), R-Sunthi Churna (Ritesh Pharmaceuticals)

Table -8 Result of TLC

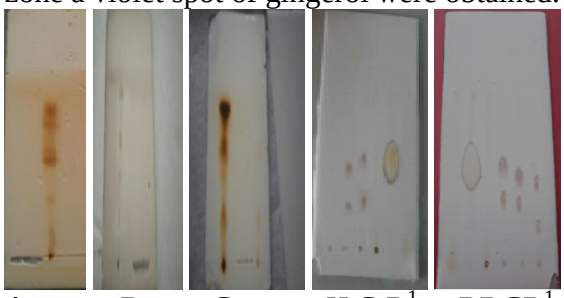
Ginger Extract	**Solvent system**	**Detecting reagent**		**Color of spot**	**Rf value**
		Iodine vapour	**Vanillin sulphuric acid**		
A	Chloroform -ethyl acetate (8:2)	3	3	Dark Brown	0.55, 0.65, 0.73
B	Chloroform -ethyl acetate (8:2)	3	3	Dark Red	0.33, 0.45,0.69
C	n-hexane- diethyl ether (3:7)	3	3	Dark Red	0.57,0.68,0.75
D	n-hexane- diethyl ether (4:6)	2	2	Violet	0.46,0.57
R	n-hexane- diethyl ether (4:6)	2	2	Violet	0.42,0.58
G	n-hexane- diethyl ether (4:6)	2	2	Violet	0.56,0.60
H	n-hexane- diethyl ether (4:6)	2	2	Violet	0.58,0.56
C	n-hexane- diethyl ether (4:6)	2	2	Violet	0.48,0.59
R^1	n-hexane- diethyl ether (4:6)	1	1	Violet	0.61

NOTE:- A-Pet ether crude ginger extract, B-Chloroform crude ginger extract, C-Ethanol crude ginger extract, H- Ethanol extract of Himalaya, D- Ethanol extract of Dhootapapeshwar, R- Ethanol extract of Ritesh Pharmaceutical, G - Ethanol extract of Green Pharmacy. R^1-Reference solution (Resorcinol)

Discussion: Various developing systems were tried to get the proper resolution of the TLC chromatograms for the marketed preparations and crude ginger extracts the satisfactory resolution was obtained in the solvent system as shown in the Table No. 7.

For Pet. Ether extract showed 3 spots having Rf value 0.55, 0.65, 0.73. For Ethanol extract of ginger; showed 3 spots having Rf value 0.68, 0.57, 0.75. For Chloroform extract showed 3 spots having Rf value 0.33, 0.45, 0.69. For ethanolic extract of Dhootapapeshwar showed two spots having Rf value 0.46, 0.57 and For ethanolic extract of Ritesh Pharmaceutical showed two spots having Rf value 0.42, 0.58. For ethanolic extract of Green Pharmacy showed two spots having Rf value 0.56, 0.60, For Ethanol extract of Himalaya showed two spots having Rf value 0.56, 0.58 and for ethanolic extract of crude ginger showed two spots having Rf value 0.48, 0.59.

In the TLC analysis the B.P. procedure was used in which resorcinol was taken as a reference standard. The n-hexane- diethyl ether 4:6 was taken as a developing system. The chromatogram showed intense violet zone of resorcinol having Rf value of 0.61. Below this zone a violet spot of gingerol were obtained.



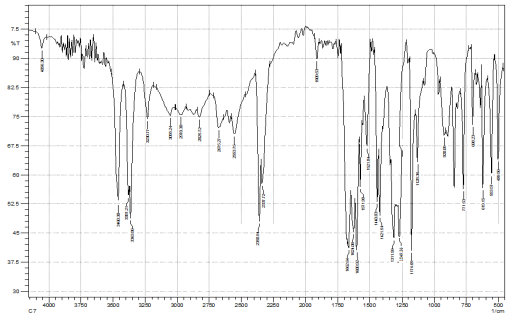
A B C H G R^1 RDCR^1

Table 9-Physical Characteristics of Isolated Compound for crude ginger :-

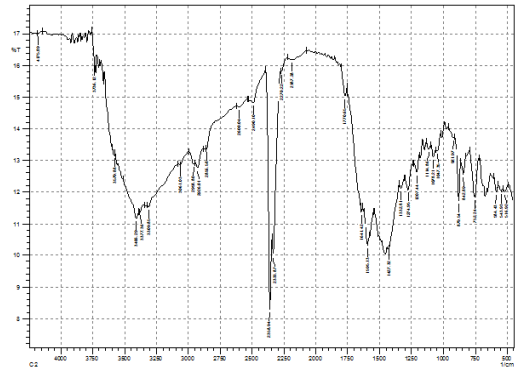
Nature	fine powder
Colour	creamish white
Rf value	0.54
Melting point	35-37^{0}C

Figure 2 shows a yellowish band having Rf value of 0.54 in the solvent system n-hexane:diethyl(4:6) for gingerol isolated from the ethanol extract of crude ginger.

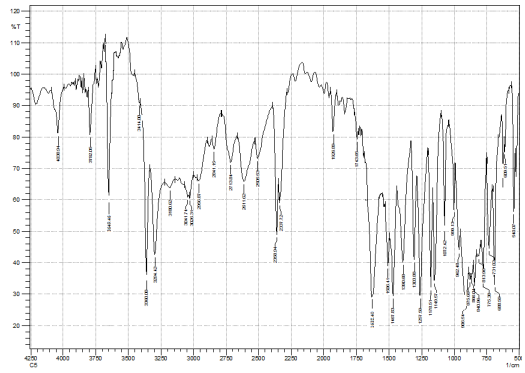
Results of IR Spectra-IR Spectra of Curcumin



IR Spectra of IA



IR Spectra of IB



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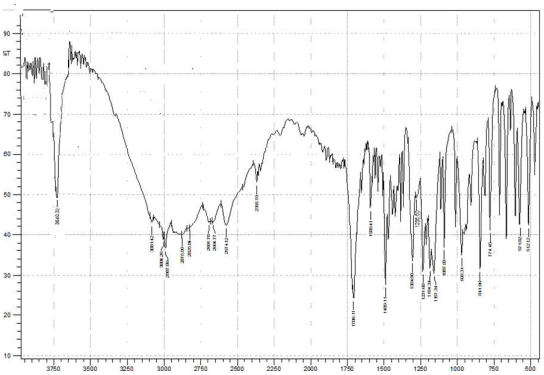

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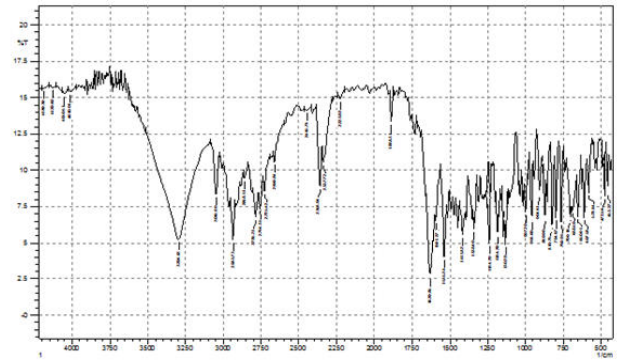

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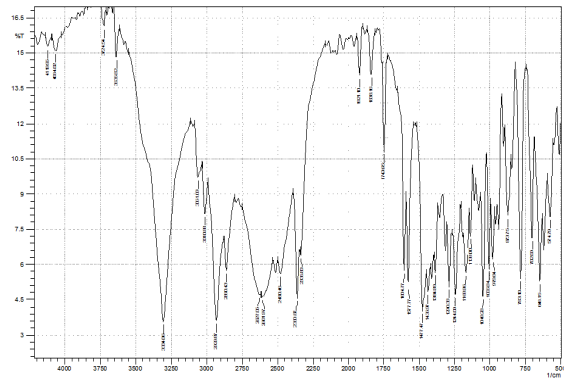
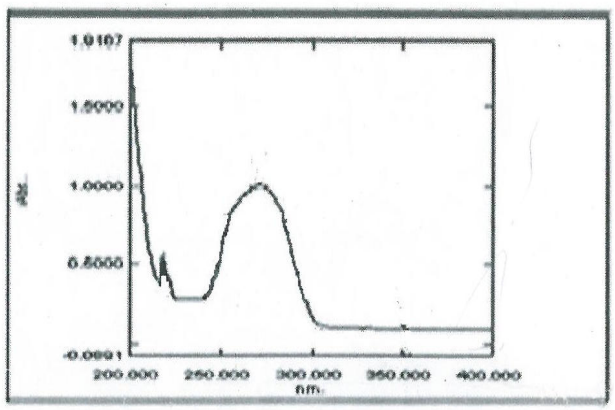


IA:-Isolated gingerol from ethanolic extract of Himalaya.
IB: - Isolated gingerol from ethanolic extract of Dhootapapeshwar.
IC:-Isolated gingerol from ethanolic extract of Green Pharmacy.
ID: - Isolated gingerol from ethanolic extract of Ritesh pharmaceuticals.
IE: - Isolated gingerol from ethanolic extract of crude ginger.
From the above results, it was observed that, all the characteristics functional group frequencies for the isolated gingerol samples were found to be matching with the absorption frequencies of the standard (curcumin).



From the above results it was observed that the τ max values of all the samples were found be complying with the value for the standard sample of gingerol i.e.280-284 nm. The τ max values for all the samples were found are present between 280-286 nm.

UV absorbance of the alcoholic extracts of the marketed preparations of ginger at 280 nm

Marketed Preparations	Absorbance at 280 nm
Himalaya	**0.988**
Dhootapapeshwar	**0.901**
Green Pharmacy	**1.031**
Ritesh	**0.887**
Crude ginger	**1.001**

From the absorbance values in the calibration curve, the total gingerol contents in various marketed preparations and crude ginger extract was determined.

Marketed Preparations	% Gingerol contents
Himalaya	**100.49**
Dhootapapeshwar	**92.55**
Green Pharmacy	**104.92**
Ritesh	**90.08**
Crude Ginger	**101.83**

The Gingerol contents of marketed samples were found to be in range from 90.08% to 104.92% The Gingerol contents were found to be 104.92, 100.49%, 101.83% for Green Pharmacy, Himalaya and Crude Ginger respectively. A relatively lower values i.e. 92.55% & 90.08% were observed for DhootapapeshwarandRitesh Pharmaceuticals respectively.

From the above results it was observed that, all the samples were found to be complying with I.P. specifications (The official limit of gingerol content is NLT 90% & NMT 120% of the stated amount of total gingerol).

Results of Antimicrobial activity of various ginger extracts

Table No.10: Antimicrobial activity data for Ginger extract (H-C) and Ofloxacin against *E. coli.*

Ethanolic Extract	The zones of inhibition (in mm)			
	E. coli			
	6.25µg/ml	**12.5µg/ml**	**25 µg/ml**	**50 µg/ml**
H	11	14	15	20
D	13	18	22	25
G	16	19	24	26
R	12	14	15	17
C	15	17	20	23
Std.-Ofloxacin	18	21	26	31

Table No. 11. Antimicrobial activity data for compounds (G-C) and Ofloxacin against *S.aureus*.

Ethanolic Extracts	The zones of inhibition (in mm)			
	S.aureus			
	6.25µg/ml	**12.5 µg/ml**	**25 µg/ml**	**50 µg/ml**
H	12	14	15	18
D	11	13	17	19
G	15	19	25	20
R	10	11	12	18
C	11	12	14	19
Std.Ofloxacin	16	18	26	31

Various ginger extracts, **G-C** were screened for the antimicrobial activity against different bacteria by using agar diffusion method and the results are shown in Table No.8 and 9.Ofloxacin was used as a standard compound. From the data, it was observed that antimicrobial activity is directly proportional to concentration. An increase in the concentration of solution resulted in an increase in the zones of inhibition. Among the entire ethanolic extracts sample of **Green Pharmacy** showed highest zone of inhibition against *S. aureus* and *E. coli* and

hence was found to be more effective than the other extracts. Ethanol extract of **Crude ginger, Himalaya, Dhootapapeshwar, Ritesh Pharmaceuticals** also showed remarkable activity when compared with **Green Pharmacy** in *E.coli* and ethanol extract of **Crude ginger, Himalaya, Dhootapapeshwar** also showed remarkable activity when compared with **Green Pharmacy** in *S.aureus.*

The result have indicated that the highest zone of inhibition were found in Green Pharmacy because of its high gingerol contents as compare to other samples.

Conclusion

From the study, it was observed that the range of gingerol was from 90.08% to 104.92%w/w., which was found to be fairly compliant with the I.P. specifications i.e.90-120% w/w. These differences in the range of gingerol content suggest that there must be different exogenous and endogenous factors affecting the quality and purity of the drug, it was very essential, being vital and important. The biological studies of the ginger extracts showed the high antimicrobial effects. This may be used in food industries as preserver of food product against spoilage by bacteria and fungi. The UV spectrophotometric procedure is a quick and reliable method for the quantitative monitoring of gingerol in very low concentrations in raw materials, processed powders and in herbal preparations containing *Zingiber officinale.*

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