Optimization of 6-Gingerol Extraction Assisted by Microwave from Fresh Ginger Using Response Surface Methodology

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Abstract

The present study investigates optimum conditions for Microwave assisted extraction (MAE) of 6-gingerol. Ginger was dried using a cross flow dryer at 55 ± 2 ^{0}C for eight hours. Ginger powder was extracted at three different watts (400 W, 500 W, and 600 W), temperatures (50 ^{0}C, 60 ^{0}C, 70 ^{0}C) and time (10, 20, 30 min) for optimum yield. 6-gingerol content was found to be 21.15 ± 0.13 and 18.81 ± 0.15 mg/g in fresh ginger and dried ginger, respectively. Optimized condition obtained by RSM for 6-gingerol was 400 W, 70 ^{0}C at 10 min extraction time. The results of MAE are expressed by 2-D contour plot and response surface curve by keeping one variable constant which showed highest yield at 600 W, 70 ^{0}C for 30 min extraction time. Microwave assisted extracts exhibited higher antioxidant activity in comparison with conventional extracts.

Keywords: 6-gingerol, Response Surface Methodology, MAE, Antioxidant Activity, Polyphenols.

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1. Introduction

Ginger (*Zingiber Officinale Roscoe*) rhizomes have been broadly used as a spice in large part because it's a unique and desirable taste and aroma. It is perennial and belongs to Zingiberaceae family. It is a traditional medicine in China and is claimed to strengthen the body after blood loss and lower pulse rate [1]. Fresh ginger has 6-gingerol as a principal component and 8 & 10-gingerols occurs at lower concentrations [2] On heating or alkali treatment, gingerols get converted to a similar series of homologous shogaols [3] Many extraction techniques have been explored for the preparation of antioxidant-rich conserves which includes solvent extraction [4], soxhlet extraction, supercritical fluid extraction [5] and microwave-assisted extraction [6]. Between these methods, microwave assisted extraction is a reasonably new method used for the extraction of natural compounds [7]. Microwave assisted extraction (MAE) system for biologically active compounds has several benefits over other conventional extraction methods [8]. This process requires a short time, a lesser amount of solvents, provide greater extraction rates and superior quality products with lesser costs

Ginger rhizome possesses several chemical constituents; most of them show good antioxidant capacity due mainly to the presence of phenolic compounds [9]. The major pungent principle isolated from ginger oleoresin is 6-gingerol and has been found to have significant antioxidative activity. Inhibition of phospholipids peroxidation has been determined by an induced FeCl3-ascorbate system [10]. In ICR mice model, Gingerol inhibits phorbol ester-induced inflammation, epidermal ornithine decarboxylase activity, and skin tumor advancement. And also, by using lipopolysaccharide (LPS)-stimulated macrophages as a model of inflammation tested the anti-inflammatory activity of 6- gingerol [11] and they found that 6-gingerol inhibits reactive oxygen species (ROS) and inducible nitric oxide synthase (NOS) over the suppression of PKC-α and NF-kappaB pathways in lipopolysaccharide [12]. By blocking NF-κB and PKC signaling, 6-gingerol acted as an anti-inflammatory agent and hence can be used in the chemoprevention of cancer / inflammatory ailments. Ishiguro K et al., (2007) [13] has reported the action of 6-gingerol on two human pancreatic cancer cell lines i.e. BxPC-3 expressing mutated p53 and HPAC expressing wild-type (Wt) p^{53} [14] Metastasis of MDA-MB-231 human breast cancer cells were inhibited by 6-Gingerol [15]. In human epidermoid carcinoma A431 cells, 6-gingerol induces regulated mitochondrial cell death pathway by reactive oxygen species [16]. A study on chemical and bioactive properties of volatile oil and oleoresins of Spices was reported [17-18]. It was also known that some of the phenolic constituents of *Zingiber officinale* show antiallergic potential on RBL-2H3 cells [19]. Recently, evaluation of antioxidant activity of 6-gingerol and its optimized microwave –assisted extraction has been reported [20].

Box and Wilson (1951) described Response Surface Methodology (RSM), as a tool for optimizing process parameters [21] It is a group of mathematical and statistical technique established on the fit of a polynomial equation to the experimental data that has been fruitfully used for emerging, improving and optimizing process [22]. RSM has been used to model and optimize biological techniques [23- 24] including extraction methods. The objective is to instantly tune the level of these variables to achieve the best condition. To visualize the shape of the response surface, the response can be represented graphically either in three-dimensional space or as counter-plots. Application of RSM in the optimization of investigative methods and, integrated predominantly, its advantages to conventional one variable a- period optimization [25]. For example, group of enormous evidence from a few experiments and the probability of assessing the effect of the variables on the response and recently determination of capsaicinoids in peppers, curcuminoids from turmeric by Ultrasonic, Supercritical carbon dioxide and microwave assisted extraction techniques were reported[26-27] Among these, MAE is the simplest and the most economical technique for extraction of many plant derived compounds The enhancement of product recovery by microwave is generally attributed to its heating effect, which occurs due to the dipole rotation of the solvent in the microwave field. This causes the solvent temperature to rise, which then increases the solubility of the compound of interest. Specifically, solvent heating by microwave occurs when molecules of the polar solvent could not align themselves quickly enough to the high frequency electric field of microwave. This discrepancy causes the solvent molecules to dissipate the absorbed energy in the form of heat [6]

Though there are several studies on the biological properties of 6-gingerol, their extraction or isolation efficiency by Microwave technique using RSM on temperature and oleoresin yield have not been studied. Hence, in the present work RSM is designed to investigate the influence of temperature, extraction time, microwave radiations and quantities of extraction along with 6-gingerol have been carried out.

2. Materials and Methods

2-1. Preparation of Sample

Ginger was purchased from Devaraja market, Mysore, Karnataka, India. The rhizomes were broken intopieces to expose the crevices and then washed in running water to remove the adhering mud. Again, the cleaned rhizomes were scraped with a knife to remove dirt as well as spoiled portion. Ginger rhizomes were soaked in potassiummetabisulphate solution (1 g/L) for 12 h and washed thoroughly with water and followed by air drying [28]

2-2. Drying of Ginger

Fresh ginger was sliced (Size in 30 mm.) using a slicing machine and uniformly spread in perforated trays. The material was dehydrated in a cross-flow drier at 55± 2 ^{0}C for eight hours. The dried ginger slices were ground using Comminuting Mill (CADMILL) from Cadmach Machinery Co. Pvt. Ltd.

2-3. Determination of Moisture and Bulk density

Moisture was estimated using Toluene Co-distillation Method [29] by refluxing about 10 g of sample at the boiling point for a minimum of 2 hours. This method involves the reflux distillation of ground ginger with toluene, which is having a greater boiling point (110.6 ^{0}C), and a lower specific gravity than water and volume measured it. Dry-bulk density is well-defined as the mass (weight) of the dry solids divided by the total amount of the wet sample, [30]

Bulk density (ℓ) = m/v

2-4. Particle Size Analysis of Ginger Powder

The particle size of the Ginger powder has been analyzed by the SALD-2300, Laser Diffraction Particle Size Analyser A general purpose analysis model was used with particle refractive and absorption indices of 1.52 and 0.1, respectively. Unlike dry or sieve analysis, the reported size is possibly for partially hydrated samples, but all the particles were probably detected within the range (0–2 mm) of the mastersize [31]

2-5. Experimental design

Extraction yield of experiments were confirm using central composite experimental design, [31]. Box-Benhnken Design combined with quadratic response model of three levels with two factorials were performed to optimize the extract methodology. 27 experiments were planned and conducted in duplicate with the ranges of the independent variables i.e. watts (400-600 W), temperatures (50-70 ^{0}C) and Times (10-30 min). Design Expert software (Version 6.0) was used for experimental design and the statistical analysis. Determine the statistical significance in present model. Analysis of variance (ANOVA) and response surface analysis were used. The suitable model was projected through the ANOVA ($P < 0.05$) and regression analysis (R^2). Response surface plot was used to demonstrate the relationship between the response and independent variables. Initially, to predict the response variables, a second-order polynomial model was used and Table1. Shows the regression coefficient of polynomial functions of response surface of total extract yield, which is used for predicting the yield by using formula.

$$Y= a_0+a_1(mw) + a_2(T)x\ a_3\ (t)+ a_4(mw)^2 +a_5(T)^2+a_6(t)^2 +a_7(mw)(T)+a_8(mw)t+ a_9(T)(t)$$

Where, a_1, a_2, a_3, a_4, a_5, a_6, a_7, a_8 and a_9 = Coefficients of model.

T= Temperature in ^{0}C

t = Time in min

Y= yield in gram (g)

2-6. Preparation of Ginger extract

Conventional Extraction Method: Dehydrated ginger slices were ground and passed through 30 mesh (500 µm) to get a powder, which was used as the raw material for extracts. Dehydrated Ginger powder (100 g) was loaded into a glass column and extracted with ethanol at the ratio of 1:10. Ethanol was added and permitted to stand for two hours contact time and eluted. The extract was distilled using rota vapor at 50 ^{0}C under reduced pressure (40 milli bar) and kept at 4^{0}C.

Total extract content (W) = W2-W1

W1 = weight of empty dried round bottom flask

W2 = weight of round bottom flask with desolventized ginger extract.

Microwave-assisted extraction (MAE): Dehydrated Ginger powder (10 g) was taken into Round bottom (RB) Flask and extracted with ethanol as a solvent at the proportion of 1:10. Ethanol (95% pure) as a solvent has been used and three different watts (400 W, 500 W, and 600 W), temperatures (50 ^{0}C, 60 ^{0}C, 70^{0}C) and time (10 min, 20 min, 30 min) were set for the extraction. The extract obtained was distilled using rotavapor at 50°C under reduced pressure (40 milli bar) and stored at 4^{0}C for further studies

2-7. Estimation of 6-gingerol by TLC

Thin-layer chromatography was used to detect the 6-gingerol, its Rf value calculated, and quantitative study was carried out by UV-spectrophotometer (UV-1800) method. 6-gingerol was separated on TLC silica plates and estimated colorimetrically by reaction with Folin-Denis reagent (BIS: 7826-1975).

Preparation of sample: A 0.05 g of ginger oleoresin was weighed and dissolved in 0.5 ml of diethyl ether. TLC chamber was filled with hexane and diethyl ether with the ratio of 1:1.5 (40:60) which has been thoroughly saturated with the solvent vapors. Ten µl of sample was spotted to the 20 X 20 cm silica gel TLC plates (Merck, Germany) after dividing the plate into six equal parts. The plate was exposed for 5 to 10 min to drive off the solvent and sprayed with Folin- Denis reagent. Two major blue spots developed. The spot with the higher Rf (0.54 to 0.71) being waxes and coloring matter and spot with the lower Rf value (0.26 to 0.29) being 6-gingerol. The area representing 6-gingerol was scooped with a small stainless steel spatula by encircling the spot as well as 0.1 to 0.2 cm away from the actual spot. 3 ml distilled water was added and shaken well in a test tube. 1 ml Folin-Denis reagent was pipetted into the tube and mixed well. After 3 to 4 min, 1 ml saturated NaCO3 was added mixed thoroughly for 5 min and kept for 1 hour. A reagent blank was prepared with approximately the same amount of all reagents without a sample to serve as a blank. The optical density (OD) was read in a UV-spectrophotometer (UV-1800) Shimadzu at 725 nm. The amounts of 6-gingerol were calculated against the standard graph derived by using vanillin at different concentration. (AOAC)

% of [6]-gingerol content = (X × 100) /Q

Where,

X= the graph reading for gingerol in 1 ml solution in µg and

Q= the quantity of extract in spot

2-8. Qualitative analysis of 6-gingerol by HPLC

50 mg ginger extracts were dissolved in 2ml of acetone (AR). 10µl was injected into the HPLC using a reversed phase C-18 column, having particle size 3µm and 4.6x250 mm. 2485 dual wavelength detector was used with Water: Methanol: Acetonitrile (52:5:43) as mobile phase [31]. The HPLC parameter was as follows flow rate – 2.0ml/min. Consisting model 515 pumps the eluting compound for the detection at the maximum wavelength 280nm. [32]

2-9.1 Polyphenols

The Samples (ginger extract) were analysed for total polyphenols by Folin-Ciocalteu method, Different concentration of the extract was mixed with FC reagent and sodium carbonate solution [33] After allowing it to stand for 60 mins at 27 ^{0}C for that observance was measured at 765nm using an UV Spectrophotometer using gallic acid as a standard and the concentration was calculated. The results were articulated as gallic acid equivalents per gram of extract.

2-9.2 Antioxidant activity

According to Kubra & Rao (2012) [33], Radical scavenging activity (RSA) has been done for the ginger extract with slight modifications using the 2, 2′-diphenyl-1-picryl-hydrazyl radical (DPPH) [34]. Various concentrations of the extracts (each one mL) were taken in different test tubes and made up to 1mL by using distilled water. Add the 4mL methanolic solution of DPPH (0.1 mM) was added to those tubes, shaken vigorously and allowed to stand at 27 ^{0}C for 20 min in the dark. OD of the samples was measured at 517 nm against control. RSA was calculated using the formula,

RSA% = Control OD- Sample OD/Control OD X 100

3. Results and Discussion

3-1. Moisture, Bulk Density and Particle Size

Toluene co-distillation method was used to estimate moisture content of both fresh and dried powdered ginger samples (AACC, no 44–19, 1995) The moisture content of fresh ginger was 83.8± 0.5%. In dehydrated ginger (powder), the moisture content was 8.0± 0.5%. The experiments were conducted in duplicates, and average values are given. The bulk density of ginger powders increased and their porosity decreased with decreasing particle size. [31] Bulk density (ℓ) = m/v (Where, ℓ = Bulk density, m = mass, v = volume). Ginger powder bulk density was 0.45 g/cm3, and average particle size has been estimated as 221 µm.

3-2. Conventional Extraction method

Ground ginger is extracted with ethanol and batch counter-current extraction is done by cold percolation on a bed of material packed into a glass column, gives yield 12.8% of oleoresin which consists 7.8% of 6- gingerol.

3-3. MAE (Microwave Assisted Extraction)

Ethanol (95% pure, MERCK, Germany) was used as a solvent for MAE. Experiments were carried out by varying conditions like wattage (400, 500, 600 watts), temperature (50, 60, 70 ^{0}C) and time (10, 20, 30 minutes). The extracts obtained were de-solventized and oleoresins collected. Table 2 shows percentage yield, and prediction values by MAE. Although highest oleoresin yield (26%) was obtained at 600 watts, 70 ^{0}C at 30 min. But maximum 6-gingerol (16.8%) content was obtained at 400 watts, 70 ^{0}C, at 10 min. With increasing temperature and time, the 6-gingerol content decreased and shogalos increased. Similar results were

reported by Gallo M et al., (2010) according to them moist heat treatment at higher temperatures (120 or 130 ^{0}C) and for a preset time gave better results for obtaining high quantity of bioactive components of shogaols.

3-4. Estimation of 6-gingerol by TLC & HPLC

6-gingerol content was analyzed by Thin-layer chromatography (TLC) and quantified. The Rf value obtained by TLC. Extract yield, prediction, difference in MAE and 6-gingerol content in extracts is shown in Table 2. From the results (Table 2) the optimized conditions were found to be 400 Wattage, 70^{0}C with 10 minutes. In Figure 1B and 1C shows HPLC Profile of 6-gingerol Standard and optimized MAE sample. HPLC profile confirms the presence of 6-gingerol, and its analogues such as 8-gingerol, 10-gingerol and 6-shogoal.

3-5. Polyphenol and Antioxidant Activity

The total polyphenol content of ginger extracts, obtained from solvent percolation method and microwave extraction was found to be 445mg/100g and 843 mg/100g respectively, as gallic acid equivalents and shown in Figure 2 A. An earlier study [32] has shown that, microwave extraction resulted in four times higher bioactive compounds in comparison with sonication. These phenolic and flavonoid compounds present in plant extract were responsible for antioxidant activity [35-37]., DPPH radical scavenging activity was in the range of 85-90 % at 40 ppm (Figure 2B).

3-6. Response surface analysis

Optimization of the experimental conditions for achieving maximum 6-gingerol content and antioxidant activity in ginger extracts RSM was successfully implemented. Two-dimensional (2D) response surface curves and contour plots were represented to show the effects of the independent variables and their interaction. Figure 3 A, shows effect of Temperature (T), microwave power (MW) on yield at a constant time (20 min), as a result oil yield increases with increased T and MW. Whereas Figure 3B and 3C shows effect of temperature and time on yield at MW (500W) and effect of time and microwave power on yield at 50 ^{0}C, respectively. Here it indicates that, increased yield was observed at the process variables, i.e. time and temperature. Figure 4 represents the effect of temperature and microwave on 6-gingerol and showed highest yield (16%) of 6-gingerol up to 60 ^{0}C at 400 MW and decreases with increasing temperature and microwave power. Box-Behnken experimental design was used for the determination of optimal temperature, time and quantity of aqueous extracts with antioxidant activity from ginger [21].

In conclusion, MAE at 600 W, 70 ^{0}C for 30 min extraction time resulted in highest yield (26%), whereas 6-gingerol content was higher at 400 W, 70°C for 10 min extraction time. Experimental results showed good agreement with the polynomial regression models, while RSM has been applied for MAE, which is expressed by 2-D contour plots and response surface curve keeping one variable constant. Microwave extracts of ginger exhibited higher antioxidant activity (90%) followed by extracts obtained from conventional method (85%) in comparison with BHA.

Conflict of Interest

Vedashree M declares that she has no conflict of interest. Madhava Naidu M declares that he has no conflict of interest. This article does not contain any studies with human or animal subjects.

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Figure Captions

Figure 1 A. HPLC Profile of [6]-gingerol Standard

B. HPLC profile of ginger extract from Microwave extraction

Figure 2 A. Polyphenol content in ginger extracts

B. Antioxidant activity of ginger extracts

Figure 3 Two-dimensional (2D) response surface curves and contour plots

Figure 4 Three-dimensional (3D) response surface curve of 6-gingerol

Table1. Regression Coefficient of Polynomial Functions of Response Surface of Total Extract Yield, Which Is Used For Predicting The Yield.

	Coefficients
Intercept	0
(MW) a_1	0.025987632
(Temp) a_2	0.307184449
(Time) a_3	-0.382786549
(MM) a_4	-2.72E-05
(TT) a_5	-0.002965266
(tt) a_6	-0.005304899
(MT) a_7	1.28E-05
(Mt) a_8	0.000820932
(Tt) a_9	0.005376181

$$\mathbf{Y= a_0+a_1(mw) + a_2(T)x\ a_3\ (t)+ a_4(mw)^2 +a_5(T)^2+a_6(t)^2 +a_7(mw)(T)+a_8(mw)t+ a_9(T)(t)}$$

Where, a_1, a_2, a_3, a_4, a_5, a_6, a_7, a_8 and a_9 = Coefficients of model.

T= Temperature in ◦C

t = Time in min

Y= yield in gram (g)

Table 2. Extract yield, prediction, difference in MAE and 6-gingerol content in extracts

MW	Temp (◦C)	Time(t)	Extract Yield (%)	Predicted Yield, (%)	6-gingerol content In extract (%)	Predicted 6-gingerol %
400	50	10	13.91 (±0.12)	15.9	01.24 (±0.60)	01.75
	50	20	11.52 (±0.61)	16.4	03.20 (±0.21)	05.77
	50	30	19.53 (±1.20)	15.9	03.59 (±0.20)	03.54
400	60	10	18.25 (±0.24)	16.3	14.80 (±0.06)	13.30
	60	20	17.81 (±0.91)	17.4	11.94 (±0.73)	12.52
	60	30	18.10 (±0.63)	17.4	14.28 (±0.20)	12.80
400	70	10	14.75 (±0.18)	16.1	16.80(±0.14)	16.18
	70	20	17.35 (±0.60)	17.7	14.08 (±0.05)	14.25
	70	30	17.81 (±0.56)	18.3	09.97 (±0.09)	09.73
500	50	10	15.01 (±0.63)	16.9	02.54 (±0.49)	01.66
	50	20	22.51 (±0.35)	18.3	13.02 (±0.02)	06.62
	50	30	16.53 (±0.42)	18.6	06.30 (±0.14)	05.32
500	60	10	17.82 (±0.28)	17.3	05.50 (±0.49)	10.20
	60	20	21.51 (±0.42)	19.2	12.94 (±0.07)	14.01
	60	30	17.35 (±0.46)	20.1	15.64 (±0.11)	11.56
500	70	10	21.25 (±0.53)	17.1	05.22 (±0.21)	06.40
	70	20	19.75 (±0.54)	19.6	06.81 (±0.14)	09.07
	70	30	20.02 (±0.28)	21.0	02.40 (±0.24)	05.48
600	50	10	17.12 (±0.65)	17.4	02.62 (±0.35)	04.55
	50	20	21.03 (±0.17)	19.6	13.64 (±0.25)	10.44
	50	30	19.52 (±0.21)	20.7	03.62 (±0.27)	10.07
600	60	10	20.25 (±0.54)	17.8	13.14 (±0.67)	10.07
	60	20	20.05 (±0.69)	20.6	12.71 (±0.19)	14.81
	60	30	22.35 (±1.42)	22.2	15.33 (±0.79)	13.30
600	70	10	13.65 (±0.47)	17.7	01.90 (±0.28)	03.26
	70	20	18.62 (±0.49)	20.9	09.71 (±0.35)	06.85
	70	30	26.03 (±0.56)	23.1	04.91 (±0.14)	04.20

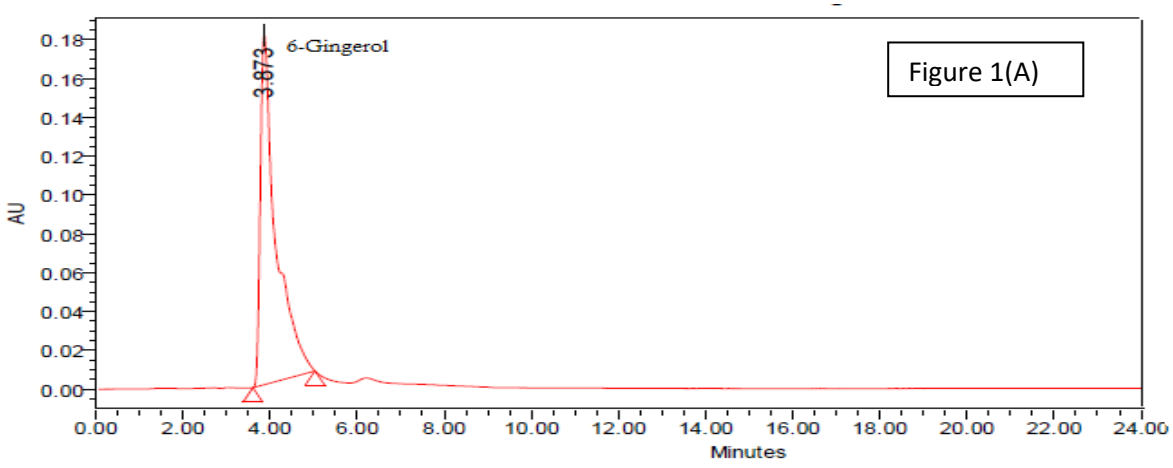

Figure 1(A)

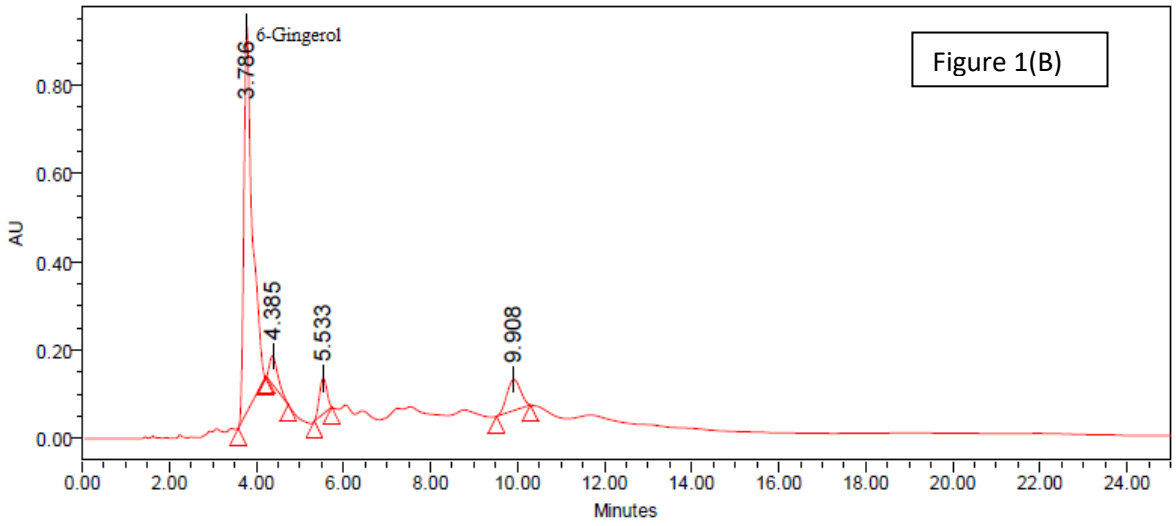

Figure 1 (A): HPLC Profile of [6]-gingerol Standard **(B):** HPLC profile of ginger extract from Microwave extraction

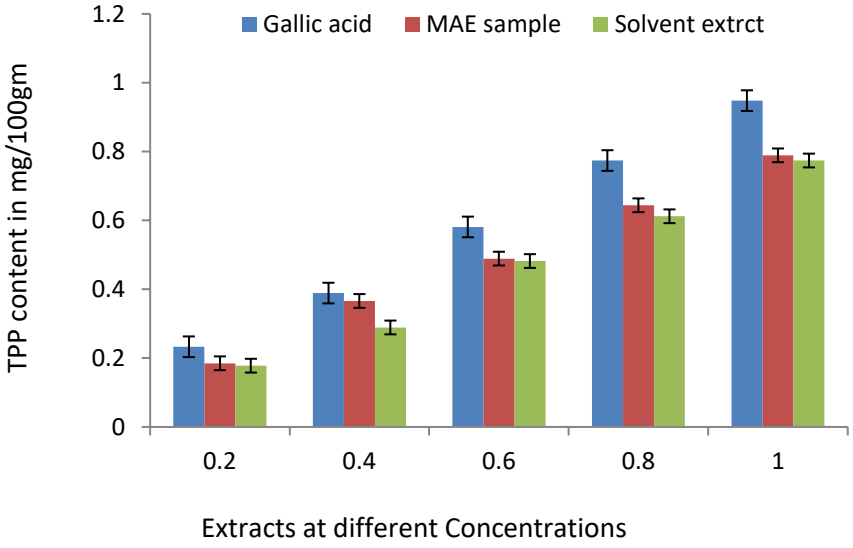

Figure 2 A. Polyphenol content in ginger extracts

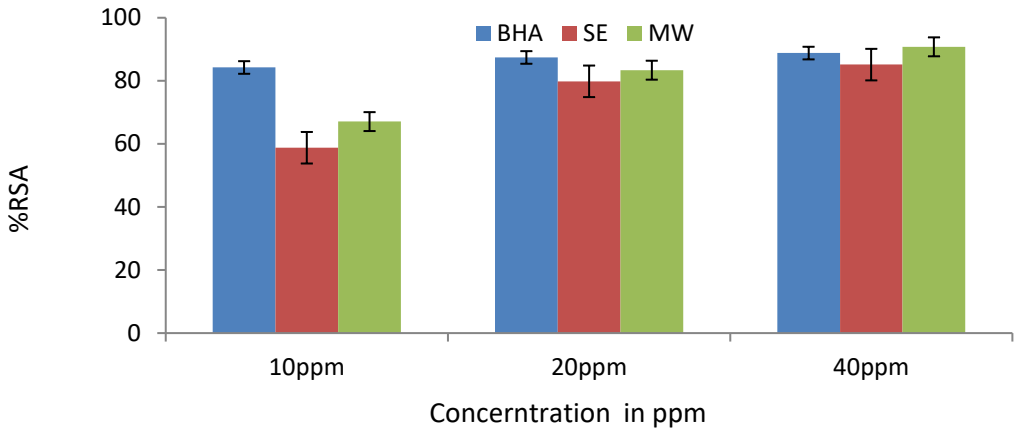

Figure 2 B. Antioxidant activity of ginger extracts

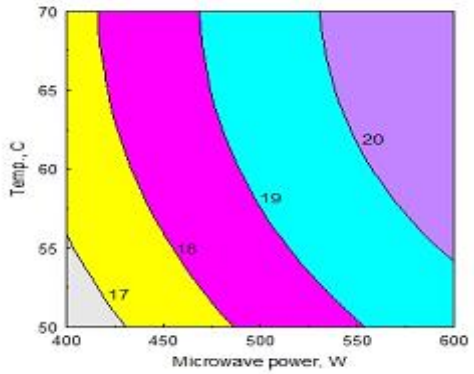

Figure 3, A Shows effect at Temperature (T), Microwave power (MW) on Yield at a constant time 20 min, Oil Yield increased as both T, MW increased**.**

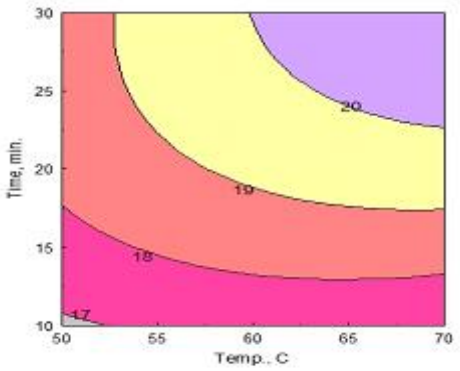

Figure 3 B, Effect of temperature and time on yield at a constant MW at 500W

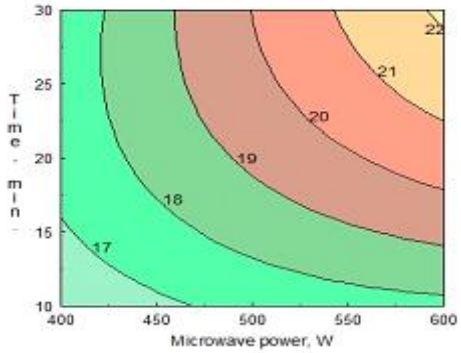

Figure 3 C, Effect of Time and Microwave power on yield at a constant temperature at 50^0 C. both times indicate increased yield at the process variables, Time, the temperature increased.

Figure 3- Two-dimensional (2D) response surface curves and contour plots

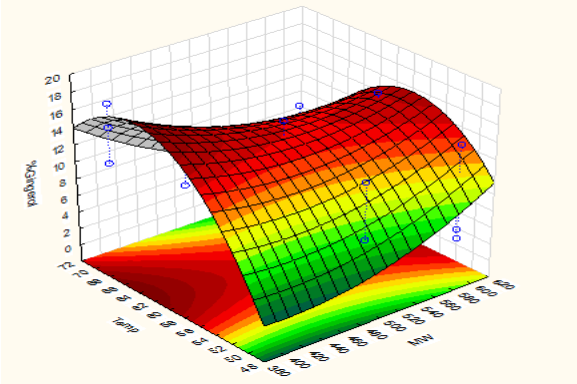

Figure 4- Three-dimensional (3D) response surface curve of 6-gingerol