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RESEARCH ARTICLE

Evaluation of Phytochemicals and Anticancer Potential of *C. maxima*: An *In-silico* Molecular Docking Approach

C. S. Vidhya[1, 2], M. Loganathan[3], S. Bhuvana[4], N. Baskaran[5] and R. Meenatchi[1*]

[1]Department of Primary Processing Storage and Handling, NIFTEM - Thanjavur, (Formerly IIFPT), Thanjavur – 613005, Tamil Nadu, India; meena@iifpt.edu.in
[2]Affiliated to Bharathidasan University, Tiruchirappalli – 620 024, India
[3]Department of Academics and Human Resource Development, NIFTEM - Thanjavur, (Formerly IIFPT), Thanjavur – 613005, Tamil Nadu, India
[4]Central Instrumentation Lab, NIFTEM – Thanjavur, (formerly IIFPT), Thanjavur – 613005, Tamil Nadu, India
[5]Department of Food Processing Business Incubation Centre, NIFTEM - Thanjavur, (Formerly IIFPT), Thanjavur – 613005, Tamil Nadu, India

Abstract

Cucurbita maxima belongs to the *Cucurbitaceae* family and has many traditional medicinal properties claimed that are used in food. The various parts of the *Cucurbita maxima* are reported to have versatile activities such as antimicrobial, antioxidant, anti-insecticidal, anti-inflammatory and anticancer properties. The current study was designed to evaluate the phytoconstituent profiles of *C. maxima* pulp, peel and seed extracts (aqueous and methanol) extracted at different temperatures (40 °C, 50 °C, 60 °C) and time (1 and 2 hr). For these extracts, qualitative and quantitative determination were performed, and the aqueous seed extract of *C. maxima* at 50 °C for 2 hours had higher phytoconstituents, which was further taken for the GCMS analysis. Furthermore, the top hit compounds from the GCMS such as Guanosine (CAS), 8,11,14-Eicosatrienoic acid, Farnesol and 13-Tetradecenal were docked against p53 Y220S mutant (6SI2), and Fibroblast growth factor receptor 1 protein (FGFR1) (4V05). The results revealed that Guanosine with p53 and 8,11,14-Eicosatrienoic acid with FGFR1 have good binding affinities of -7.2 and -6.3 kcal/mol respectively. Conclusively, the top compounds from the aqueous seed extract of *C. maxima* extracted at 50 °C for 2 hours have significant breast cancer activity and it has to be further taken to *in vitro* and *in vivo* studies in the future.

Keywords: Anticancer, *Cucurbita maxima,* FGFR1, Molecular Docking, p53

Abbreviations

C. maxima	-	*Cucurbita maxima*
Pe	-	Peel
Pu	-	Pulp
Se	-	Seed
A-40(1)	-	Aqueous extract at 40 °C for (1hr)
A-40(2)	-	Aqueous extract at 40 °C for (2hr)
A-50(1)	-	Aqueous extract at 50 °C for (1hr)
A-50(2)	-	Aqueous extract at 50 °C for (2hr)

*Author for correspondence

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A-60(1) - Aqueous extract at 60 °C for (1hr)
A-60(2) - Aqueous extract at 60 °C for (2hr)
M-40(1) - Methanolic extract at 40 °C for (1hr)
M-40(2) - Methanolic extract at 40 °C for (2hr)
M-50(1) - Methanolic extract at 50 °C for (1hr)
M-50(2) - Methanolic extract at 50 °C for (2hr)
M-60(1) - Methanolic extract at 60 °C for (1hr)
M-60(2) - Methanolic extract at 60 °C for (2hr)
FGFR1 - Fibroblast Growth Factor Receptor 1 protein

1. Introduction

Cancer has become a life-threatening disease and breast cancer is the most frequent type of cancer with a nearly 14% incidence rate of all cancers with a high mortality rate[1,2]. Radiotherapy and chemotherapy are the current treatments, which are successful but have side effects such as anemia, shortness of breath and a rapid heartbeat[3,4]. In India, women in urban and rural areas have been diagnosed with breast cancer and the incidence rate is constantly increasing every year[5]. Early detection and diagnosis of breast cancer in India is challenging due to improper awareness among women. Even after the chemotherapy and radiotherapy, the survival rate is poor in breast cancer pathogenesis[6,7]. General surgeons treat the bulk of breast cancer patients in poor nations. At the community level, ineffective surgical care of breast cancer is prevalent.

1.1 Repercussions

Surgical oncology and breast surgery are two surgical subspecialties that deal with breast cancer[8]. The Reactive Oxygen Species (ROS) triggers the various signal transduction pathways that leads the cancer cell growth, survival, proliferation and metastasis[9,10]. It alters the MAPK pathway, PI3K, mTOR and other signalling pathways and leads to the cancer pathogenesis[11,12].

Due to the drawbacks of the existing therapies, we are in need of alternative therapies such as alternative and herbal medicine from natural sources like plants[13]. Many plants have been reported to have versatile pharmacological activities such as antimicrobial, antioxidant, anti-insecticidal, anti-inflammatory and anticancer properties due to the presence of their phytoconstituents like alkaloids, flavonoids and polyphenols[14-16]. *Cucurbita maxima* belongs to the *Cucurbitaceae* family and is known to have significant medicinal properties and spread over 800 species and 130 genera[17]. *C. maxima* has been shown to have significant pharmacological activities, such as an antimicrobial and an antioxidant in nature[18,19]. However, the exact phytoconstituents responsible for this activity remain unknown. The discovery of natural chemopreventive drugs that target the molecular pathways of carcinogenesis has been made possible by understanding the process of molecular carcinogenesis[13,20].

The current study was performed to evaluate the phytoconstituents profile of various parts of *C. maxima* (peel, pulp and seed) of various extracts (aqueous and methanol) at different temperatures (40 °C, 50 °C, 60 °C) and time (1 and 2 hr). The high phytochemical-rich extract was subjected to GCMS study and the top compounds were docked against p53 Y220S mutant (6SI2) and Fibroblast Growth Factor Receptor 1 protein (FGFR1) (4V05) through *In silico* molecular modeling studies to determine their binding affinities as prescribed by the previous studies[21].

2. Material and Methods

2.1 Requirements

All the analytical grade chemicals were purchased from Himedia, India. All the glassware was thoroughly sterilized before use.

2.2 Sample Collection, Processing and Extraction

The fresh fruit of *Cucurbita maxima* was purchased from the local market in Thanjavur. Then it was authenticated by Dr. Raveendran, Department of Biotechnology, TNAU, Coimbatore. The vegetables

were washed and separated into different parts: peel, pulp and seeds. Then the parts of *C. maxima* were shade dried. The dried pumpkin was milled with an electric milling machine (Almtech Enterprise C/15 Industrial Estate). The milled pumpkin flour was stored in an airtight packaging container for further analysis[22]. Sun-drying was not performed because of the denaturing effect of heat-sensitive phytoconstituents. Pulp, peel and the seed of *C. maxima* were macerated separately using distilled water and methanol. Further, these macerated extracts were kept at different times (1 hr and 2 hr) in the water bath of varying temperatures (40 °C, 50 °C, 60 °C).

2.3 Qualitative Screening of Phytochemicals

All the qualitative screening of phytoconstituents of *C. maxima* (peel, pulp and seed) of various extracts (aqueous and methanol) at different temperatures (40 °C, 50 °C, 60 °C) and time (1 and 2 hr) were performed as per the described procedure[23].

2.3.1 Wagner's Test

To 5 mL of extract, 4 drops of Wagner's reagent were added and left undisturbed for 10 minutes. The reddish-brown precipitate is an indicator of alkaloid presence.

2.3.2 Sodium Hydroxide Test

To dissolve 0.4 g of the extract, a cold dilute solution of sodium hydroxide and diluted hydrogen chloride were utilized. The absence of the yellow color is the indicator of flavonoids presence.

2.3.3 Copper Acetate Test

To 3 mL of extract, 8 drops of $Cu(OAc)_2$ solution were carefully added and incubated. The formation of a beryl green color is an indicator of terpene presence.

2.3.4 Salkowski Test

To 3 mL of extract, 1.5 mL of $CHCl_3$ and 1.5 mL of conc. H_2SO_4 were added and carefully mixed. The red fluorescence of the chloroform layer and the greenish yellow fluorescence of the acid layer demonstrate the steroids availability in the sample.

2.3.5 Foam Test

To 5 mL of the extract, 4 mL of water was added and agitated rapidly for roughly 10 minutes; a stable foam appearance is the indicator of saponin presence.

2.3.6 Ferric Chloride Test

For 5 minutes, a mixture of 1 mL extract and 8 mL D. H_2O was cooked. To the 4 mL of collected filtrate, 5 drops of a 10% ferric chloride solution was added. A greenish blue or violet color is the indicator of phenolics presence.

2.3.7 Lead Acetate Test

3 mL of $Pb(C_2H_3O_2)_2$ solution were thoroughly mixed well with 2 mL of extract. The presence of white precipitate is an indicator of tannin and phenol presence.

2.3.8 Borntrager's Test

4 mL of extracts were boiled and filtered with mild sulphuric acid. The filtrate was then completely combined with chloroform and shaken. Ammonia was gradually added after the organic layer was separated. The ammoniacal layer changes color from pink to red when anthraquinone glycosides are present.

2.3.9 Fluorescence Test

3 mL of extract was mixed with a 1 N sodium hydroxide solution. The fluorescence of bluish green is the indicator of coumarin glycosides presence.

2.3.10 Kellar Killani's Test

To dissolve a few mL of extracts in water, a mixture of glacial acetic acid, ferric chloride and strong sulfuric acid was utilized. The formation of a brown ring at the junction indicates the presence of cardiac glycosides.

2.3.11 Spot Test

4 mL of extract was sandwiched by Whatman paper and squeezed for around 3-5 minutes. The residual oil on paper is an indicator of fixed oil's presence.

2.4 Total Phytoconstituents Quantification

The total alkaloid content, total flavonoid content, total phenolic content, total saponin content and total tannin content of phytoconstituents of the *C. maxima* (peel, pulp and seed) of various extracts (aqueous and methanol) at different temperature (40°C, 50°C, 60°C) and time (1 and 2 hr) were performed as per the described procedure[23].

2.4.1 Total Alkaloids Content

3 mg/mL equivalent of extract was dissolved in DMSO, followed by the addition of 2 mL of 1N HCl, and then filtered. In addition to this, 5 mL bromo-cresol green dye solution and 5 mL of phosphate buffer was also added to it. The reaction mixture is then violently shaken with 3-4 mL of chloroform before being collected and the volume made up with $CHCl_3$. Atropine was used as a standard with concentrations 20-100 mg/ml. Finally, the absorbance was taken at 470 nm.

2.4.2 Total Flavonoid Content

The $AlCl_3$ assay was performed to quantify the total flavonoid content. After mixing 3 mL of the extract with 7 mL of distilled water and 0.4 mL of a 5% $NaNO_2$ solution the mixture was kept undisturbed. Approximately after 8 min, 0.4 mL of 10% $AlCl_3$ and 5 mL of 1.5M NaOH solutions were added and made up to 10 mL of total volume. Quercetin was used as a standard with concentrations of 20-100 mg/ml. Finally, the absorbance was taken at 510 nm.

2.4.3 Total Saponin Content

To 15g of extract, 125 mL of 15% ethanol was added and heated for 2 hours at 65°C with stirring. The filtered sample was extracted with 300 mL of 15% ethanol and the mixture was heated to evaporate the excess solvent. To the 40 mL of extracted sample, 20 mL of $(C_2H_5)_2O$ was added and only the aqueous layer was collected and fractioned by n-butanol. Then the whole fraction was washed thrice with 20 mL of 5% NaOH. Diosgenin was used as a standard with concentrations of 20-100 mg/ml. Finally, the absorbance was taken at 550 nm.

2.4.4 Total Tannins Content

Folin-Ciocalteu reagent was used to quantify the total tannin content of the extracts. To 5 mL of extract, 4 mL of water, 0.6 mL of Folin-Ciocalteu, and 2 mL of 25% Na_2CO_3 solution were added, and the total volume was made up to 12 mL and kept inditurned for 30 min. Gallic acid was used as a standard with concentrations of 20-100 mg/ml. Finally, the absorbance was taken at 725 nm.

2.4.5 Total Phenolics Content

Folin-Ciocalteu reagent was used to quantify the total phenolic content of the extracts. To 3 mL of extract, 8 mL of water and 1.8 mL of Folin-Ciocalteu were added and kept undisturbed for some time. After 8 min, 6.5 mL of 10% sodium carbonate was added and made upto the 25 mL of total column and again incubate for 2 hr at 37°C. Gallic acid was used as a standard with concentrations 20-100 mg/ml. Finally, the absorbance was taken at 550 nm.

2.5 Quantification of Phytochemicals through GC-MS

The GCMS-QP2010 SE was used to quantify the phytoconstituents present in the *Glycine max* seed extracts. The RTX-5 MS capillary column of 0.25m diameter and 25m length was used to identify and quantify chemical components. Working conditions for the GC were kept between 45°C and 290°C, with a rise of 5°C every minute. The oven temperature and injection port temperature were set at 115°C and 295°C, respectively. He was used as a carrier gas (mobile phase) with a 1.5 mL/min flow rate. The ionizer temperature was set at 235°C and the interface temperatures at 290°C. The detector voltage was set to 0.10 kV and the solvent cut-off period was set to 5 minutes. The mass range of 20-300 m/z was set and the compounds were compared with Wiley library[22].

2.6 Molecular Docking

From the GC-MS studies, top hit compounds were taken for the molecular modeling study and docked against cancer molecular targets as prescribed

Table 1. Qualitative screening of *C. maxima peel*

Phytochemical Analysis	*C. maxima* peel											
	Methanol Extraction						Aqueous extraction					
	Temperature						Temperature					
	40 °C		50 °C		60 °C		40 °C		50 °C		60 °C	
	Time Duration						Time Duration					
	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr
Alkaloids	+	+	-	-	+	+	+	+	-	+	++	++
Flavonoids	+	+	-	+	+	+	+	++	+	+	-	+
Terpenoids	+	+	+	+	++	+	-	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	+	+	+	-	+
Saponin	-	+	+	+	-	+	+	-	-	+	+	+
Tannin	+	+	-	+	-	+	-	-	-	+	-	-
Tannins and Phenolics	+	+	+	+	+	+	+	+	+	+	+	+
Anthraquinone glycosides	-	+	+	+	++	+	+	+	-	-	-	+
Coumarin glycosides	-	+	+	+	+	+	+	+	+	+	-	+
Cardiac glycosides	-	+	+	+	+	+	+	+	-	+	-	+
Fats and oils	-	++	-	-	-	-	-	-	-	+	-	-

Note: (+) = Present; (++) = relatively high quantity present; (-) = Not detected

earlier[24-26]. The 3D structures of Quercetin (5280343), 8,11,14-Eicosatrienoic acid (5280581), Farnesol (445070) and 13-Tetradecenal (522841) were retrieved from PubChem and the 3D structures of p53 Y220S mutant (6SI2) and Fibroblast Growth Factor Receptor 1 protein (FGFR1) (4V05) were retrieved from the protein data bank respectively. The *in silico* molecular docking was performed in Autodock and all the parameters were set to default. The binding affinities of the compounds against the molecular targets were predicted.

3. Results and Discussion

3.1 Qualitative Screening of Phytoconstituents

The qualitative screening of phytochemicals present in the *C. maxima* peel extracts at varying times and temperatures were given in Table 1. This shows the abundance of phytoconstituents present in the peal of the *C. maxima,* on those fats and oils were absent

Table 2. Qualitative screening of *C. maxima* pulp

Phytochemical Analysis	*C. maxima* pulp											
	Methanol Extraction						Aqueous Extraction					
	Temperature						Temperature					
	40 °C		50 °C		60 °C		40 °C		50 °C		60 °C	
	Time Duration						Time Duration					
	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1hr	2 hr
Alkaloids	+	+	-	+	+	+	+	+	++	+	+	+
Flavonoids	-	-	-	+	+	+	-	+	+	++	-	+
Terpenoids	+	+	-	+	+	+	+	+	+	+	+	+
Steroids	-	+	-	-	-	+	-	+	-	-	-	+
Saponin	-	-	-	+	-	+	-	-	-	+	-	-
Tannin	-	-	+	+	-	-	-	+	-	+	-	-
Tannins and Phenolics	+	++	+	+	+	+	+	+	+	+	+	+
Anthraquinone glycosides	+	+	-	+	+	+	++	+	++	+	+	+
Coumarin glycosides	+	++	+	+	+	+	+	++	+	++	-	-
Cardiac glycosides	+	+	-	+	-	+	+	+	+	+	+	++
Fats and oils	-	-	-	+	-	-	-	+	-	-	+	++

Note: (+) = Present; (++) = relatively high quantity present; (-) = Not detected

in most of the extracts. Compared to others A-50(1) and A-50(2) peel extracts of the *C. maxima* contains more alkaloids contents and M-60 (1) peal extracts of the *C. maxima* contains more terpenoids. Tannins and phenolics were present in all the above extracts of peel.

The qualitative screening of phytochemicals present in the *C. maxima* pulp extracts at varying times and temperatures was given in Table 2. This demonstrates the abundance of phytoconstituents present in the pulp of *C. maxima,* on which M-50(1) lacks most of the phytoconstituents. Alkaloids, flavonoids and glycosides are highly present in aqueous extracts of *C. maxima* pulp. All types of glycosides were present in most of the extracts.

The qualitative screening of phytochemicals present in the *C. maxima* seed extracts at varying times and temperatures was given in Table 3. This shows the abundance of phytoconstituents present in the seed of *C. maxima.* On comparing with peel and pulp, all extracts of *C. maxima* seeds having a rich amount of phytoconstituents which was indicated with double plus. *C. maxima* extract from the peel, seed and pulp has a lower fat and oil concentration. A-50(2) was rich in phytochemicals when compared to all the extracts of pulp, peel and seeds.

The quantitative phytochemical determination of methanolic and aqueous extracts of *C. maxima* peel was given in Table 4 and Table 5, respectively. Total flavonoid

Table 3. Qualitative screening of *C. maxima* seed

Phytochemical Analysis	*C. maxima* seed											
	Methanol Extraction						Aqueous Extraction					
	Temperature						Temperature					
	40 ℃		50 ℃		60 ℃		40 ℃		50 ℃		60 ℃	
	Time Duration						Time Duration					
	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr
Alkaloids	+	+	+	+	-	+	+	+	+	++	+	+
Flavonoids	-	+	+	+	-	+	-	+	++	++	+	+
Terpenoids	+	+	+	+	+	+	-	+	++	++	+	+
Steroids	-	+	-	-	+	+	-	-	+	+	-	+
Saponin	-	+	-	-	-	+	+	+	-	-	-	+
Tannin	-	-	-	-	-	+	+	+	-	-	-	-
Tannins and Phenolics	+	+	-	+	+	+	+	+	+	++	+	-
Anthraquinone glycosides	-	+	-	+	+	+	+	+	++	++	+	+
Coumarin glycosides	-	+	-	-	-	+	-	+	+	++	-	-
Cardiac glycosides	-	+	+	+	-	-	-	-	+	+	+	+
Fats and oils	+	++	-	+	-	-	-	-	-	+	-	-

Note: (+) = Present; (++) = relatively high quantity present; (-) = Not detected

Table 4. Quantitative phytochemical analysis of a methanolic peel extract

Phytochemical Analysis	peel (mg/Equivalent g values)					
	Temperature					
	40 °C		50 °C		60 °C	
	Time Duration					
	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr
Total Alkaloids	77±1.25	81±2.75	65±1.75	60±2.25	90±0.25	82±0.75
Total Flavonoids	140±0.50	148±2.25	150±2.75	144±3.50	180±0.50	168±2.25
Total Phenols	101±1.75	98±2.0	78±2.25	85±3.0	125±0.50	116±1.50
Total Saponins	25±0.50	22±0.75	18±0.75	16±1.75	32±0.25	28±0.75
Total Tannins	22±0.25	24±1.25	20±0.50	23±1.25	15±0.75	16±0.75

Table 5. Quantitative phytochemical analysis of aqueous peel extract

Phytochemical Analysis	peel (mg/Equivalent g values)					
	Temperature					
	40 °C		50 °C		60 °C	
	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr
	Time Duration					
Total Alkaloids	66±1.25	72±2.25	64±1.75	58±1.75	60±1.75	55±1.50
Total Flavonoids	111±1.50	128±2.50	143±2.75	130±3.50	130±2.75	126±2.0
Total Phenols	98±1.50	102±2.0	101±3.25	120±2.25	113±1.75	101±1.75
Total Saponins	35±1.25	38±1.75	33±1.50	35±1.50	29±0.50	32±2.25
Total Tannins	24±0.75	29±0.75	32±0.75	25±0.5	18±0.50	19±0.75

Table 6. Quantitative phytochemical analysis of a methanolic extract of *C. maxima* pulp

Phytochemical Analysis	pulp (mg/Equivalent g values)					
	Temperature					
	40 °C		50 °C		60 °C	
	Time Duration					
	1 hr	2hr	1hr	2hr	1hr	2hr
Total Alkaloids	90±2.25	92±2.0	82±2.50	83±2.25	89±1.75	94±1.75
Total Flavonoids	152±2.75	158±2.75	170±3.75	175±2.75	165±2.75	160±3.0
Total Phenols	84±1.75	88±1.50	90±2.50	92±1.75	95±2.0	94±2.75
Total Saponins	32±1.50	30±0.25	26±1.50	20±1.75	22±0.50	20±0.50
Total Tannins	18±0.75	19±0.75	20±0.75	24±0.75	24±0.25	25±0.50

Table 7. Quantitative phytochemical analysis of an aqueous extract of *C. maxima* pulp

Phytochemical Analysis	pulp (mg/Equivalent g values)					
	Temperature					
	40 °C		50 °C		60 °C	
	Time Duration					
	1 hr	2 hr	1 hr	2 hr	1 hr	2 hr
Total Alkaloids	88±1.75	89±2.25	80±2.25	74±2.75	70±1.75	64±2.75
Total Flavonoids	140±2.50	155±2.50	156±2.0	150±3.0	152±2.0	154±0.75
Total Phenols	80±1.50	72±1.75	50±1.75	52±1.75	68±1.50	76±1.50
Total Saponins	18±0.75	19±0.50	20±1.50	18±2.0	19±1.75	22±0.75
Total Tannins	25±0.25	28±0.25	32±1.75	18±1.75	26±0.50	28±0.75

Table 8. Quantitative phytochemical analysis of methanolic extract of *C. maxima* seed

Phytochemical Analysis	***C. maxima* seed (mg/Equivalent g values)**					
	Temperature					
	40°C		**50°C**		**60°C**	
	1hr	**2hr**	**1hr**	**2hr**	**1hr**	**2hr**
	Time Duration					
Total Alkaloids	92±0.75	85±1.25	98±0.75	107±1.75	112±1.25	120±0.75
Total Flavonoids	145±2.75	152±1.75	158±2.25	150±2025	178±1.75	169±1.75
Total Phenols	112±2.50	120±1.50	105±1.50	106±1.75	155±2.25	162±1.50
Total Saponins	38±1.25	40±0.75	36±1.25	34±1.25	30±0.75	38±1.25
Total Tannins	22±1.75	25±1.25	20±0.75	24±0.75	30±1.25	32±0.50

Table 9. Quantitative phytochemical analysis of an aqueous extract of *C. maxima* seeds

Phytochemical Analysis	**seed (mg/Equivalent g values)**					
	Temperatures					
	40°C		**50°C**		**60°C**	
	Time Durations					
	1hr	**2hrs**	**1hr**	**2hrs**	**1hr**	**2hrs**
Total Alkaloids	105±1.50	118±3.25	112±0.75	124±0.25	120±2.50	128±3.75
Total Flavonoids	218±1.25	240±0.75	310±0.25	358±0.50	265±0.50	270±0.25
Total Phenols	110±1.75	124±2.50	198±0.75	285±0.50	158±1.50	147±2.5
Total Saponins	35±0.75	44±2.25	44±0.25	48±0.75	52±2.5	50±0.75
Total Tannins	22±0.75	28±1.50	15±0.75	12±0.50	25±2.25	29±1.25

content is higher in M-60(1) and M-60(2) compared to aqueous extracts A 60(1) and A 60(2). Saponin and tannin content are lower in both methanol and aqueous extracts of *C. maxima* peel. Total phenol content was higher in the aqueous extract of *C. maxima* peel compared to the methanol extract of *C. maxima* peel. Total alkaloid content is slightly increased in the methanolic extract of *C. maxima* peel. The quantitative determination of the aqueous extract of *C. maxima* peel was determined and all the standard gram equivalent values of alkaloids, flavonoids, total phenols, total saponins and tannins were shown as mean value ± SD in triplicates as shown in Table 5[36] Total alkaloid content was higher in aqueous seed extract (A-40(2)), flavonoid content was higher in the A-50(1) saponin was lesser in A-60(1) extract and higher in A-40(2) extract.

The quantitative phytochemical determination of methanolic and aqueous extracts of *C. maxima* pulp was

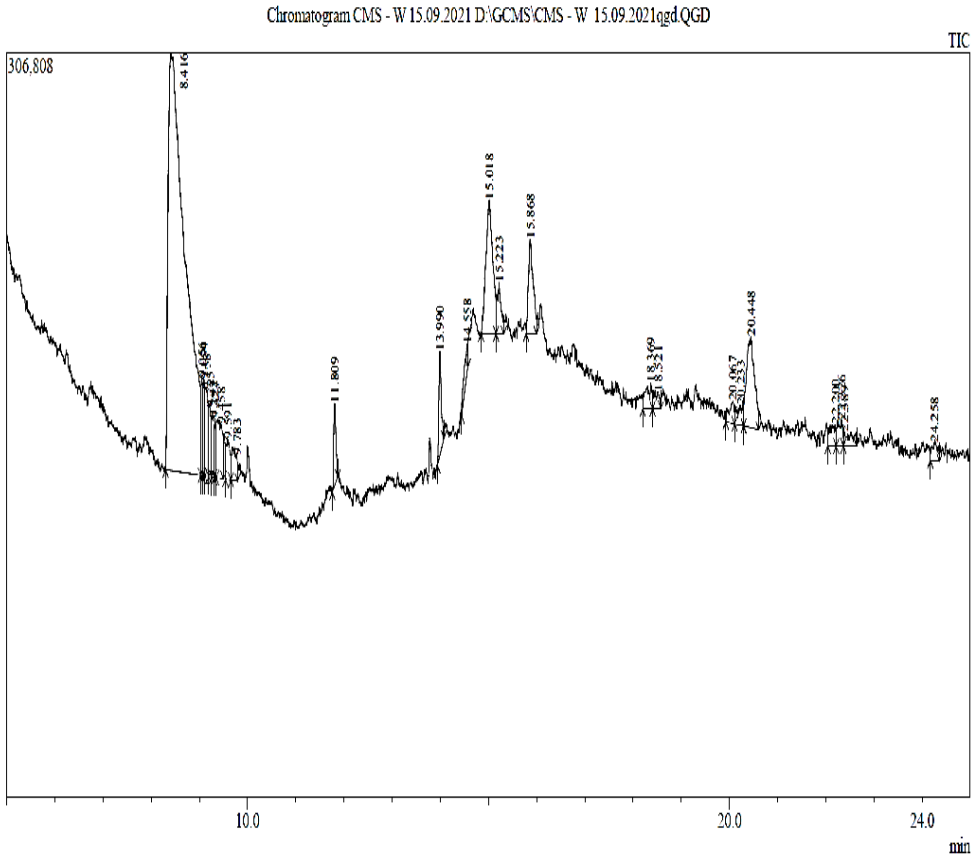

Figure 1. GC-MS Chromatogram of *C. maxima* aqueous seed extracted at 50°C for 2hr.

given in Table 6 and Table 7, respectively. Total alkaloid content was higher in methanolic extract of *C. maxima* pulp M-60(2), flavonoid content was higher in the M-50(2) saponin was lesser in M-50(2) and M 60(2) extract and higher in M-40(1) extract. Total alkaloid content was higher in aqueous seed extract A-40(2), flavonoid content was higher in the A-50(1) saponin was lesser in A-40(1) and A50(2) extract and higher in A-60(2) extract.

The quantitative phytochemical determination of methanolic and aqueous extracts of *C. maxima* seed was given in Table 8 and Table 9, respectively. Total alkaloid content was higher in methanolic seed extract M-60(2), flavonoid content was higher in the M-60(1) saponin was lesser in M-60(1) extracts and higher in M-40(2) extracts. Total alkaloid content was higher (128±3.75) in the aqueous seed extract A-60(2), flavonoid content was lesser in the A-40(1) and saponin was lesser in A-40(1) extracts and higher in A-60(1) aqueous extracts. Comparatively, A-50(2) extract shows high phenolic, and flavonoid content(40), and low tannin content compared to the other extracts. Seed extract of A-50(2) was taken for in vitro studies illustrated.

Comparatively, the aqueous seed extracted at 50°C for 2 hours had higher phytoconstituents and this particular extract was taken further in the GCMS analysis.

3.2 GCMS Analysis

The GCMS analysis of the C. maxima aqueous seed extracted at 50°C for 2hr reported nearly 25 compounds based on their different Retention Time (RT) and peak area percentage. The first hit that was identified at a Retention Time (RT) of 8.41 min was Quercetin, whereas N-Methyl-4-(2-thiophenecarboxamido) phthali was the last hit with a 24.258 min RT as given

Table 10. GCMS peak report

Peak No.	RT	Area%	Height%	Compound name
1	8.416	56.79	23.91	Quercetin
2	9.056	1.28	5.51	2-Butene-1,4-diol, diformate
3	9.094	1.29	5.50	dl-Proline
4	9.158	2.07	4.87	N-(P-Anisidino methyl)-4-methylph
5	9.225	1.29	3.88	2-Propanamine, N-(1-methylethyl)- (CAS) N-I
6	9.292	1.09	3.33	1,2,4-Butanetriol (CAS) Triol 124
7	9.327	0.73	3.35	Allyl fluoroformate
8	9.458	2.63	3.09	4,5-Octanediol, 2,7-dimethyl-
9	9.591	1.28	2.29	1,3-Dioxolane, 4-methyl-
10	9.783	1.24	1.44	L-Leucine (CAS) N-Leucine
11	11.809	1.44	4.70	Tetradecanoic acid
12	13.990	2.06	5.95	9-Octadecenoic acid (Z)- (CAS) Oleic acid
13	14.558	1.06	1.61	Cyclopentanol, 3-methyl-
14	15.018	7.35	7.66	8,11,14-Eicosatrienoic acid
15	15.223	1.62	2.94	1-Tetradecanol (CAS) Alfol 14
16	15.868	3.58	5.40	13-Tetradecenal
17	18.369	1.19	1.48	Pyrido[2,3-d] pyrimidin-4(3H)-one, 3- [2-(1-pi
18	18.521	0.71	0.99	2(3H)-Furanone, dihydro-3-methylene-
19	20.067	0.85	1.25	3(2H)-Furanone, 5-methyl-2-octyl-
20	20.233	0.94	1.10	3-Cyanopropionamide
21	20.448	5.87	5.22	Farnesol
22	22.200	0.99	1.22	Cyclopentane carboxamide, N-(4-fluorophenyl
23	22.326	0.96	1.47	Xanthosine (CAS) Xanthine riboside
24	22.389	0.86	0.81	Octadec-9-en-1-al dimethyl acetal
25	24.258	0.84	1.03	N-Methyl-4-(2-thiophenecarboxamido) phthali

Table 11. Mean binding energy of Ligand and Receptors

Receptors	Ligand	Mean binding energy (Kcal/mol)
P53 Y220S mutant	Quercetin	-6.3
	8,11,14-Eicosatrienoic acid	-4
	Farnesol	-4.7
	13-Tetradecenal	-3.8
FGFR1	Quercetin	-5.8
	8,11,14-Eicosatrienoic acid	-7.2
	Farnesol	-7.8
	13-Tetradecenal	-5.7

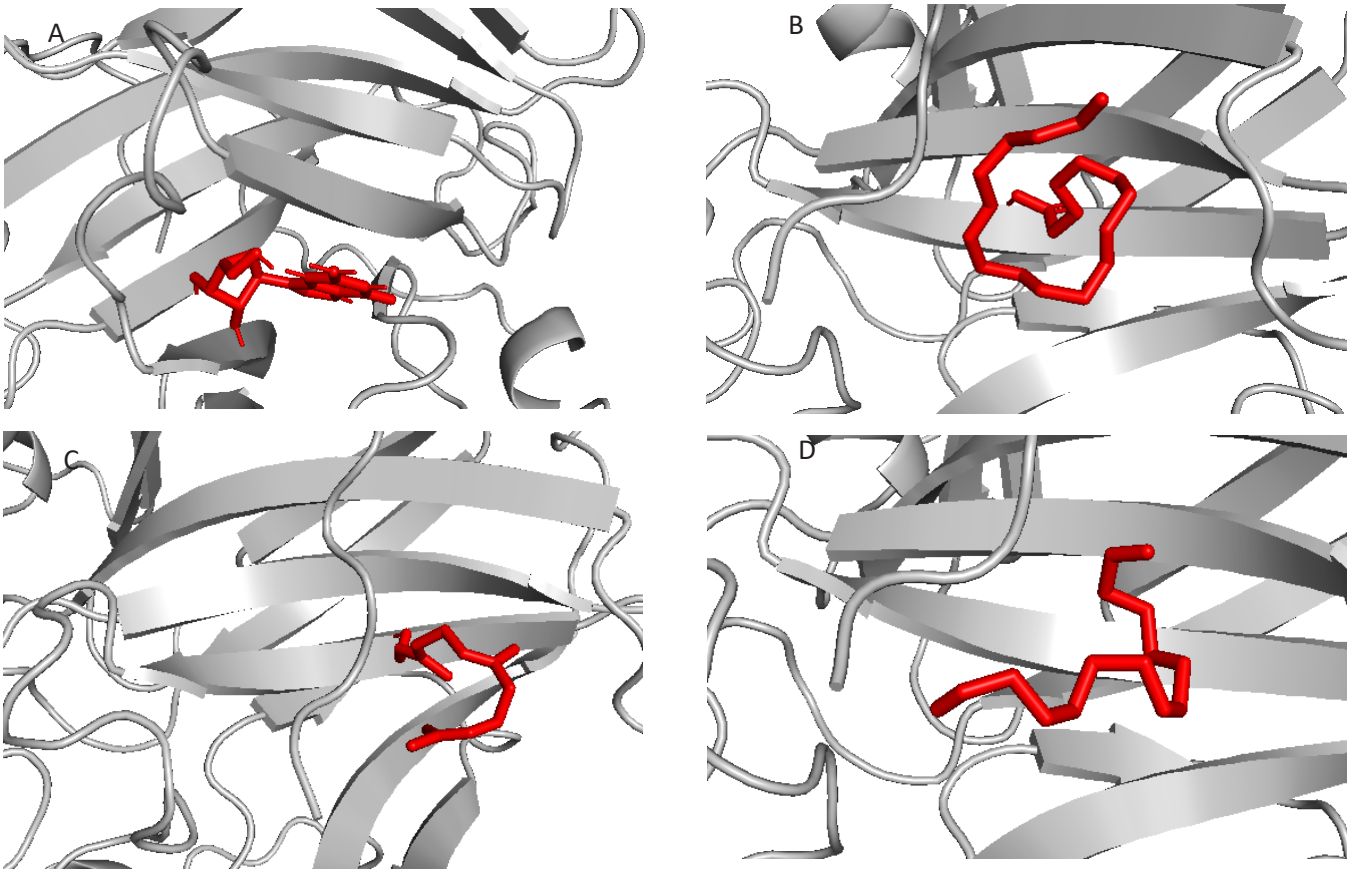

Figure 2. Compounds interaction with P53 Y220S mutant.

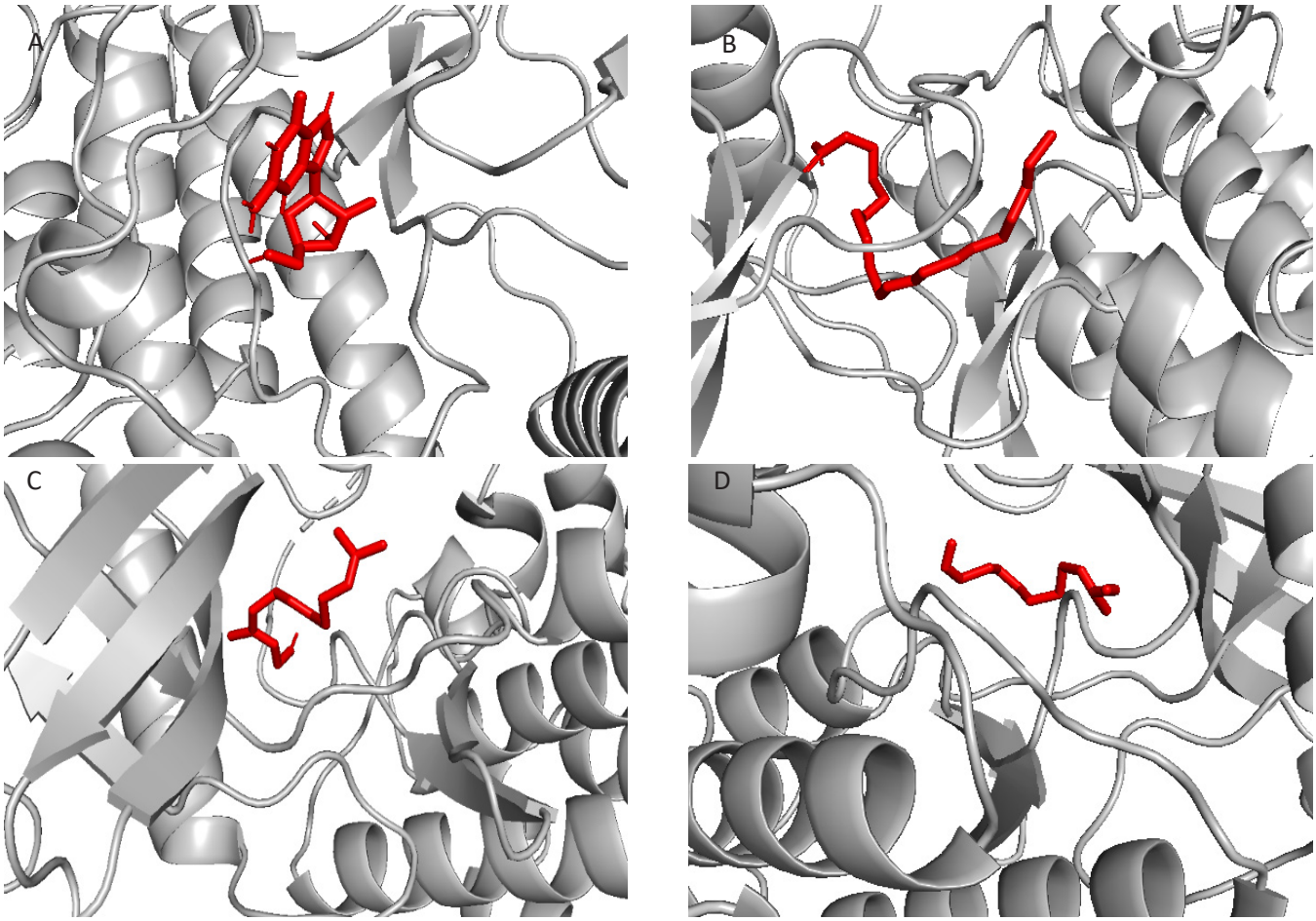

Figure 3. Compounds interaction with FGFR1.

in Figure 1 and Table 10. The compounds Quercetin (8.41), 8,11,14-Eicosatrienoic acid (15.018), Farnesol (20.448), 13-Tetradecenal (15.868).

3.3 Molecular Docking

From the GC/MS analysis, the top four highly present phytoconstituents in the aqueous extract of *C. maxima* seed were taken for the molecular modeling studies. These compounds were docked against two breast cancer receptors, namely P53 Y220S mutant (6SI2) and FGFR1 (4V05) respectively. The binding affinities of these two compounds against these two receptors were evaluated and their binding energies (Kcal/mol) values were given in Table 11, and the binding interaction of compounds with P53 Y220S mutant and FGFR1 were given in Figure 2 and Figure 3 (A-Quercetin, B-8,11,14-Eicosatrienoic acid, C-Farnesol, and D-13-Tetradecenal) respectively. Comparatively, Quercetin showed high binding affinities against the P53 Y220S mutant and 8,11,14-Eicosatrienoic acid showed high binding affinities against FGFR1.

4. Discussion

Plants have aroused the interest of researchers interested in exploring their phytochemical and pharmacological capabilities as a rich source of phytoconstituents[27-29]. *C. maxima* is a vegetable widely spread in Asia, North America and Mexico, and is widely used to treat various ailments like tumors, urolithiasis, heart difficulties, sterility, hypertension, skin disorders, and hair growth[30]. Significant biological activity is a function of versatile phytoconstituents present in it. *C. maxima* extracts have many active pharmaceutical ingredients

which will possess significant biological activities. The extraction of compounds from natural sources are correlated with the temperature, time, solvent polarity and pH conditions[31,32]. The study results revealed that the *Cucurbita maxima* seed extracted with distilled water at 50°C for 2hr is rich in phytoconstituents such as alkaloids and polyphenols.

Nowadays, plant based phytoconstituents are seeking more attraction due to their potent biological activities against various pathogenesis such as cancer. The plant extracts were reported to scavenge the reactive oxygen species and thus reduces the antioxidant stress inside the cell12,[33]. The plant-based polyphenols have the ability to modulate intracellular ROS levels inside the cancer cells. The modulation of ROS levels is correlated with the apoptotic mechanism in the cell, which can regulate the gene expression of pro-apoptotic and anti-apoptotic proteins[34].

Furthermore, the *in silico* molecular modeling studies interpret the binding mechanism of the compounds against molecular targets. P53 is an tumor suppressor gene, when it gets mutated its lacks its function and leads to the growth of the tumor cell, whereas the Fibroblast growth factor receptor 1 protein involves in the intracellular signal transduction for the growth of the tumor cell[35]. The inhibition of these molecular targets could way path to the inhibition of breast cancer. The binding affinities of compounds such as Quercetin, 8,11,14-Eicosatrienoic acid, Farnesol, and 13-Tetradecenal with the p53 Y220S mutant, and Fibroblast Growth Factor Receptor 1 protein (FGFR1) were evaluated.

5. Conclusion

From the versatile temperature and time-dependent extraction approach of various parts of *C. maxima* revealed that the aqueous seed extract of *C. maxima* extracted at 50°C for 2hr is rich in phytoconstituents which was further quantified by GCMS analysis. The molecular docking of the top 4 hit compounds from the GCMS data showed significant binding affinities and higher negative binding energies against mutant P53 and FGFR1. The overall data suggests that the aqueous seed extract of *C. maxima* extracted at 50°C for 2hr has strong anticancer potential and it could be further taken for *in vitro* and *in vivo* studies.

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