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Original Article

The inhibitory and anticancer properties of *Annona squamosa* L. seed extracts

Propriedades inibitórias e anticancerígenas de extratos de sementes de *Annona squamosa* L.

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Abstract

Although *Annona squamosa* Linn. (Annonaceae) has been used in traditional medicine and is known to have several pharmacological properties, its impact on EGFR kinase has not been fully investigated. An assay (biochemical) was used to govern the potential of different *A. squamosa* seed extracts to scavenge free radicals in petroleum ether, acetone, ethanol, and methanol. We also tested *A. squamosa* leaf extracts for their ability to inhibit the growth of HEK 293, MCF7, and HepG2 cell lines. The PSE, ASE, ESE, and MSE all contained anti-cancer substances like anethole, cyclopentane, 1,1,3-trimethyl, and phosphonate oxide tributyl, according to phytochemical analysis. ESE extracts from *A. squamosa* seeds have been selected based on free radical generation probabilities, cytotoxicity studies, and phytochemical analysis. Subsequent insilico studies have been conducted, and the results have shown that interactions between compounds present in ESE extracts and the EGFR kinase are what give these compounds their inhibitory effects. Preliminary phytochemical and pharmacological activities were studied and reported. *A. squamosa* ESE extracts inhibited the growth of MCF7 cells, and a pharmacokinetic study showed that the compounds anethole, cyclopentane, 1,1,3-trimethyl, and phosphonium oxide tributyl had few undesirable side effects. These substances can be used to both prevent and treat cancer diseases.

Keywords: EGFR kinase, *Annona squamosa* Linn, MCF7, pharmacokinetic, phytochemicals.

Resumo

Embora a *Annona squamosa Linn.* (*Annonaceae*) tenha sido utilizada na medicina tradicional e seja conhecida por diversas propriedades farmacológicas, seu impacto na EGFR quinase ainda não foi totalmente investigado. Um ensaio bioquímico foi utilizado para controlar o potencial de diferentes extratos de sementes de *A. squamosa* para eliminar radicais livres em éter de petróleo, acetona, etanol e metanol. Extratos de folhas de *A. squamosa* também foram analisados em relação à sua capacidade de inibir o crescimento de linhagens celulares HEK 293, MCF7 e HepG2. O PSE, ASE, ESE e MSE continham substâncias anticancerígenas como anetol, ciclopentano, 1,1,3-trimetil e óxido de fosfonato tributil, de acordo com a análise fitoquímica. Extratos de ESE de sementes de *A. squamosa* foram selecionados com base em probabilidades de geração de radicais livres, estudos de citotoxicidade e análise fitoquímica. Estudos *in silico* subsequentes foram realizados e os resultados mostraram que as interações entre os compostos presentes nos extratos de ESE e a EGFR quinase são o que confere a esses compostos seus efeitos inibitórios. As atividades fitoquímicas e farmacológicas preliminares foram estudadas e relatadas. Os extratos de ESE de *A. squamosa* inibiram o crescimento de células MCF7, e um estudo farmacocinético mostrou que os compostos anetol, ciclopentano, 1,1,3-trimetil e óxido de fosfônio tributil tiveram poucos efeitos colaterais indesejáveis. Essas substâncias podem ser usadas para prevenir e tratar doenças cancerígenas.

Palavras-chave: EGFR quinase, *Annona squamosa Linn*, MCF7, farmacocinética, fitoquímicos.

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1. Introduction

Human diseases have been treated using natural remedies made from plants, animals, and minerals (Ji et al., 2009). Numerous drugs in international pharmacopoeias are discovered through the study of ethnopharmacology and conventional medicine (Leonti and Casu, 2013). Customary remedies can influence drug development and suggest potential research targets (Yuan et al., 2016). New, powerful technologies are revolutionising the screening of medicinal plants. Examining historical trends in medical and pharmaceutical developments makes it easier to understand the benefits of current drug development. Throughout the history of drug development, plants have played an important role in the discovery of novel bioactive compounds. Many compounds derived from plant species have been developed to treat a wide range of diseases. Many nutraceuticals are used in unregulated markets because they are thought to improve health and quality of life. Cancer-fighting plant-based medicines can be investigated. The pharmaceutical industry is focused on developing novel or indigenous methods of manufacturing existing drugs, as well as developing plant-based medications (Katanaev et al., 2019).

Based on this type of screening, some significant plant products used in chemotherapy are eventually advertised as drugs. According to reports, Annonaceae plants have been used in Indian medicine to treat conditions with symptoms similar to cancer. *A. squamosa* Linn. (Annonaceae), is popular, with the common name of "custard apple or sugar apple," while in Urdu it is called "Sharifa" (Chen et al., 2016). It comes from Central America and the West Indies. Additionally, the plant is grown for food all over South Asia, particularly in tropical areas. Antioxidant, antidiabetic, cytotoxic, genotoxic, antitumor, and anti-lice properties of *A. squamosa* Linn. (Wang et al., 2014) carbohydrates, Alkaloids, fixed oils, phenolics and tannins are all present in it. Terpenoids, flavonoids, and phenols, for example, have been shown to have antioxidant and anticancer activity in various plants. These compounds have been studied for their phytochemical and pharmacological properties to shed light on the mechanisms underlying cancer pathways (Othman et al., 2019). *A. squamosa* Linn contains a natural product that may inhibit EGFR overexpression, an essential protein for all types of cancer. This study used in-vitro and in-silico methods to assess the antioxidant and cytotoxic effects of fractions from this plant on human cancer cells.

2. Material and Methods

2.1. Chemicals, collection of samples (leaf and seed), and preparations of seed extracts

All the chemicals (petroleum ether, acetic anhydride, calcium sulfate, chloroform, methanol, sulphuric acid, ethyl acetate, hydrochloric acid) used were of analytical grade. The fully matured fresh leaves, fruits, and seeds of the fruit *A. squamosa* were collected seeds, dried and powdered (1.0 kg), were isolated with petroleum ether (PES) (B.P. 60-80 °C), acetone (B.P. 61-62 °C), ethanol (EES) (BP 77.1 °C), and others, respectively. The solvent in the flask evaporated when it was heated, shifted to the condenser as a liquid, and was later transformed into the chamber. It took several hours to complete the extraction process. Using a rotary evaporator, the extract has been concentrated (Kothari and Seshadri, 2010).

2.2. Phytochemical screening

Alkaloids are basic nitrogenous substances with definite activity (pharmacological and physiological). The presence of alkaloids, glycosides, tannins, phenols, flavonoids, and saponins in leaves extract of *A. squamosa* was examined. Mayer's reagent, when added in limited drops, causes a white-yellowish precipitate to form the solution (alkaloid) (Siddiqui and Ali, 1997). Best alkaloids are precipitated as a neutral or faintly solution (acidic) by Mayer's reagent. Alcohol extract vanished to dryness and the residue was heated in a water bath (boiling) with HCL (2%). Glycosides are substances that increase one or extra sugars when they are hydrolyzed (glycones). A few drops of ferric chloride and concentrated sulfuric acid are added to the extract's solution in glacial acetic acid, and mixture is then examined for a reddish-brown indicative of glycoside (Siddiqui and Ali, 1997). The colour of the extract is determined by adding 1.5 mL of extract solution, 1.0 mL of water, and 1-2 drops of ferric chloride solution. Green and black colors show the presence of tannins, while the appearance of bluish color indicates phenols (Iyengar, 1981). In this experiment, saponins were visible as a frothing appearance (creamy miss of small bubbles) After shaking 0.2 gm of the extract with 5 mL of distilled water, sapons were visible (Iyengar, 1981).

2.3. Free radical scavenging assay

There are several experimental limitations to the scavenging of free radical determinations (Kaur and Kapoor, 2001). 5p5-=6 The most commonly used methods for measuring free radical scavenging potentials involve the production of radical species, where the presence of free radical scavengers determines the disappearance of radicals (Cao et al., 1993). The ability of *A. squamosa* seed extracts to go through free radicals was determined using various appropriate assays. As a benchmark, L-ascorbic acid was used.

2.4. Scavenging assay

The activity (scavenging) of the numerous extracts and L-AA (L-ascorbic acid) was expected with the stable radical DPPH (diphenypicrylhydrazyl) scavenging assay method given by Miliauskas et al. (2004). The superoxide anion activity (scavenging) of samples (seeds of *A. squamosa* extract and ascorbic acid) was measured by Liu with minor modifications and is assayed by the reduction of NBT (nitroblue tetrazolium) (Liu et al., 1997). The reaction mixture was read at 560 nm after incubation at 25 °C for 5 minutes. The result was calculated as the percentage of superoxide anion scavenging activity.

The Ruch-described methods were used to regulate the scavenging ability of H_2O_2 using these methods (Ruch et al., 1989). At 230 nm, H_2O_2 concentration was measured spectrophotometrically. The 50 mM phosphate buffer

devoid of H_2O_2 acted as a blank and calculating scavenging ability. In parallel, the nitrite detection method is used to study the interaction of nitric oxide with various extracts of *A. squamosa* seeds (Green et al., 1982). The reaction of NO with oxygen produced nitrites, which were then broken down into carbon monoxide and carbon dioxide. A mixture of plant extracts and water acted as a control, while the positive control was ascorbic acid.

2.5. Reducing power assay

Samples of *A. squamosa* seeds were prepared using potassium ferricyanide and buffer (phosphate) and were incubated at 50 °C in an H2O bath for 20 minutes. After termination, it was analyzed at 700nm using a spectrophotometer (Shimadzu, Japan) (Oyaizu, 1986).

2.6. Cell proliferation study

Cell cytotoxicity was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay (Mosmann, 1983). This assay was resolute using a minor variation of the mode from our previously published article (Ansari et al., 2016). In a 96-well plate, accelerating progression MCF-7, HepG-2, and HEK293 cells were seeded at a density of about 5 $X10^3$ cells per well. On the following day, cells were treated with dose-dependent concentrations of extract (5-80 g for cancer cell lines and 5 g to 200 g for HEK 293). Coumarin (positive control) was used for both cytotoxicity and activity studies. After 48hours, removed the supernatant. Then add 20 µl of MTT solution (5 mg/mL in PBS) to each well, and incubated at 37 °C for 4 hours. After four hours, the supernatant was drained from each well. Using an enzyme-linked immunoabsorbent assay (ELISA) reader machine (BioRad), MTT had been mixed and read at 570 nm. All tested compounds' IC_{50} values were assessed. The selectivity index is the ratio of activity of made compounds and standard drugs going on usual cell line HEK293 to that of calculated (IC_{50}) cell lines (HepG-2 and MCF-7). The ideal range for choosing cancer treatment drugs was thought to be valued greater than three (Fang et al., 2006).

2.7. Examining phytochemicals

Mass spectrometry and Gas chromatography (GC-MS) recognize seeds' petroleum ether, acetone, ethanol, and methanol extracts. The sample was added while in splitless mode. The chemical makeup of each extract was known by contrasting the peak retention times generated by the chromatographic process (Singh and Kumar, 2013; van Den Dool and Kratz, 1963).

2.8. Preparation of Ligands, protein and molecular docking

The process was carried out by the University of Bristol using the ChemBioDraw Office 12.0 software and Maestro 10.5 application module ligands (Singh and Bast, 2014). Bristol have extracted the structure of the kinase domain of the EGFR from a protein database (PDB ID:1M17) (Friesner et al., 2006). We confirmed natural compounds imitative from *A. squamosa* seed extracts contrary to the kinase (EGFR) in this learning (Friesner et al., 2004; Tripathi et al., 2013). Each of the chosen protein molecules was docked with a natural compound using GLIDE-XP. Following the preparation of ligands and proteins, a receptor grid file was created. Based on the optimal energy value and kinds of interfaces for each target, the best-fit compounds were selected.

2.9. Prime MM-GBSA and ADME/T properties studies

For a single ligand and a single receptor, the Prime MM-GBSA approach determines the ligand binding energies and ligand strain energies. It incorporates a non-polar solvation term, an SGB polar solvation model, and OPLSAA molecular mechanics (GNP). Using Maestro 10.5's QikProp application, the ADME/T characteristics of the best-docked compounds were predicted (pharmacokinetics, biochemical, structural, physicochemical, and toxicity belongings) (Lu et al., 2004; Jorgensen and Duffy, 2002).

2.10. Boiled-egg plot and biological activity spectrum

A BOILED-Egg plot be responsible for reassuring upkeep and a exclusive statistical plot on the way to provision the two estimates: gastrointestinal immersion and brain penetration. In the cartesian plane, if our compounds are found in the yolk area, the likelihood of Blood Brain Barrier (BBB) is greater than before. The regions are not mutually exclusive (Jain et al., 2019). A compound's complex pharmacological effects are denoted by way of its best-docked substance (BAS), and the situation structural characteristics determine its essential belongings. The SMILES string of compounds (natural) was uploaded to PASS online, which then predicted the pharmacological effects and announcement using biological entities (Lagunin et al., 2000).

2.11. FTIR (Fourier Transform Infrared Spectrophotometer)

One of the most reliable methods for identifying chemical bonds (functional groups) in compounds is FTIR (Hari and Nair, 2018). By analyzing the infrared absorption spectrum, the chemical bonds in a molecule can be identified. For the FTIR analysis, dried powders of various solvent extracts of each plant material were used. A Shimadzu FTIR spectroscope with a read range of 400 to 4000 cm^{-1} and a tenacity of 4 $cm^{-}1$ was loaded with each plant powdered (extract) sample.

2.12. The XRD evaluation

Dried ripe seed samples were powdered excellently for the XRD investigation. The mineral phases were examined using a diffractometer, generator, and a graphic representation plotter from Philips. Cu K radiation at 30 kV and 20 mA was used by BRUKER to create the D8 Advanced XRD pattern (Fernandes and Sellappan, 2019).

2.13. EDS: Energy Dispersive X-ray Spectroscopy

Powdered samples (extract) were clean and finished a 120um filter. The samples (extract) were prepared, dried, and powdered in a blender. Samples were used for further exploration (Fernandes and Sellappan, 2019).

3. Results

3.1. Preparation of extract and phytochemical screening

A. squamosa seed extract was prepared by evaporating the various fractions under reduced pressure. The different types of phytoconstituents of the *A. Squamosa* seeds were analyzed Table 1. Phenolic, and flavonoid constituents were present in ethanol and methanol seed extracts compared to other extracts (Figure 1A).

3.2. Assay for the removal of free radicals

The radical go-through potentials from *A. squamosa* (extracts) seeds were sedate by way of DPPH assay, and they revealed notable activities (antioxidant) (Figure 1B). Extracts of ESE, MSE extracts, and L-ascorbic acid showed significant inhibition of radicals (DPPH) now a drench reliant on the way. From 10 to 100 μgml^{-1}, various extracts free radical activity (scavenging) abruptly increased. The value (EC_{50}) deliberate intended for ESE and MSE extract were 9.00 ±0.26μgml^{-1} and 9.80 ±0.13μgml^{-1} (P <0.05) correspondingly for instance equated with 11.70 ±0.16μgml^{-1} for ascorbic acid (Table 2). The outcomes shown in Figure 1C compare the standards and extracts of *A. squamosa* in terms of their ability to scavenge superoxide radicals. The superoxide radical activity (scavenging) of ESE extract (83.75±0.10%) and MSE extract (78.51±0.29%) were less (P <0.05) of L-ascorbic acid (89.78±0.11%), by the side of the highest concern. of 400µg/mL. The significant alterations of (P <0.05) were too perceived among the values (EC_{50}) acquired from *A. squamosa* (extract) and standards (Table 2).

The free radical doing activity (scavenging) of *A. squamosa* (extracts) was assessed by way of the H_2O_2 (hydrogen peroxide) method. The peak activity was eminent of ESE and MSE (extract) the mentioned compound at high concentrations. Nitric oxide free radical activity (scavenging) was illustrious in place of the ESE and MSE extracts of *A. squamosa* at 400 µg/mL was evident, while the standard ascorbic acid revealed a lesser amount of inhibition on the same conc. (Figure 1E). Significant differences (P <0.005) were also perceived among the values (EC_{50}) gotten for the *A. squamosa* (extracts) and standards (Table 2).

3.3. Reducing power assay

The reducing influences of five diverse *A. squamosa* (extracts) are revealed in Figure 2, The ESE and MSE extract has more reducing power than ascorbic acid.

Table 1. Phytochemical analysis of extracts of *A. squamosa* seeds.

S. No	Phytochemical	PSE	ASE	ESE	MSE
1	**Glycosides**	+	-	-	-
2	**Tannins**	++	-	+	-
3	**Phenols**	-	-	++	+++
4	**Flavonoids**	-	_	+++	++
5	**Alkaloids**	+	++	+	+
6	**Saponins**	+	++	+	-

PSE (Petroleum ether seed extract), ASE (Acetone seed extract), ESE (Ethanol seed extract), MSE (Methanol seed extract).

3.4. Effectiveness of A. squamosa seed extracts against cancer

We have executed an assay (MTT) to assess the activity (cytotoxicity) of *A. squamosa* seed extracts. Figure 2A compares the cell viabilities of extracts subjected to various treatments using coumarin as a reference chemical (compound). We bring into being that PSE and ASE (extracts) are nontoxic check-out 100 µg conc., however ESE and MSE (extracts) are non-hazardous up to 200 µg conc. to the cells (HEK293). The values (IC_{50}) of extracts PSE, ASE, ESE, and MSE against HEK293 cells were 92.72µg, 148.62µg, 240.05µg, and 152.32µg correspondingly by way related to the standard (Table 3).

Proliferation assays by cumulative conc.of extracts PSE, ASE, ESE, MSE, and coumarin as reference compounds are shown in Figure 2B. Extracts of ESE impede the proliferation of MCF7 cells almost tierce periods more than the extra extracts. Assay (proliferation) with concentrations (increasing) of extracts PSE, ASE, ESE, MSE, and coumarin as reference compounds against HepG2 is shown in Figure 2C. PSE, ASE, and MSE (extracts) show higher IC_{50} values as related to the reference compound (Table 3), which suggests that extracts ESE are dynamic active against HepG2 cells (cancerous) as compared to other extracts. The ESE and MSE extracts presented decent selectivity against cell lines (MCF7) with an SI worth of more than 3 as associated to other extracts (Table 3). The PSE and ASE (extracts) were less selective and more active against HepG2 cell lines.

3.5. Constituents of extracts' phytochemicals

Table 4 lists the five identifiable PES constituents, AES contain 13 which are listed in Table 5, 9 EES have 9 presented in Table 6, and MSE showed 12 in Table 7 that are being perceived by analysis (GC-MS). The R.T (retention time), top zone, and percentage area of these compounds are enumerated in Tables 4, 5, 6, and 7. Additionally, ASE and ESE extracts contained many phytochemicals. Consider the 1,2,3-propanetriyl ester of 9-octadecenoic acid.

3.6. EGFR kinase and extract compound' molecular docking

As indicated in Table 8, we used different drugs in XP mode to inhibit the EGFR kinase with low G values. The importance of lipophylactic, hydrogen-connecting, p-p stacking, and cation-p interactions as key contributors to the active site was underlined. The molecular docking process has the highest free energy value (G score) in comparison to those receptor molecules. The best Gscore compounds, with values (13,74, -12,75, and -10,73 kcal/mol), according to EGFR kinase molecular docking against substances ESE, were anethole, cyclopentane, 1,1,3-tromethyl, and phosphorus oxide tributyl. The work attempts to summarise many interconnections, including hydrogen and electrostatic connections, and depict protein-ligand interactions. The best G-score for the active EGFR kinase site in CID22311 has led to the discovery of the most potent and attractively bound anethole (Table 8). Three hydrogen bonds were made by the substance anethole with the EGFR kinase residues (Leu A: 764, Thr A: 766, and Ala A: 719) (Figure 3A). Anethole interacts with additional residues

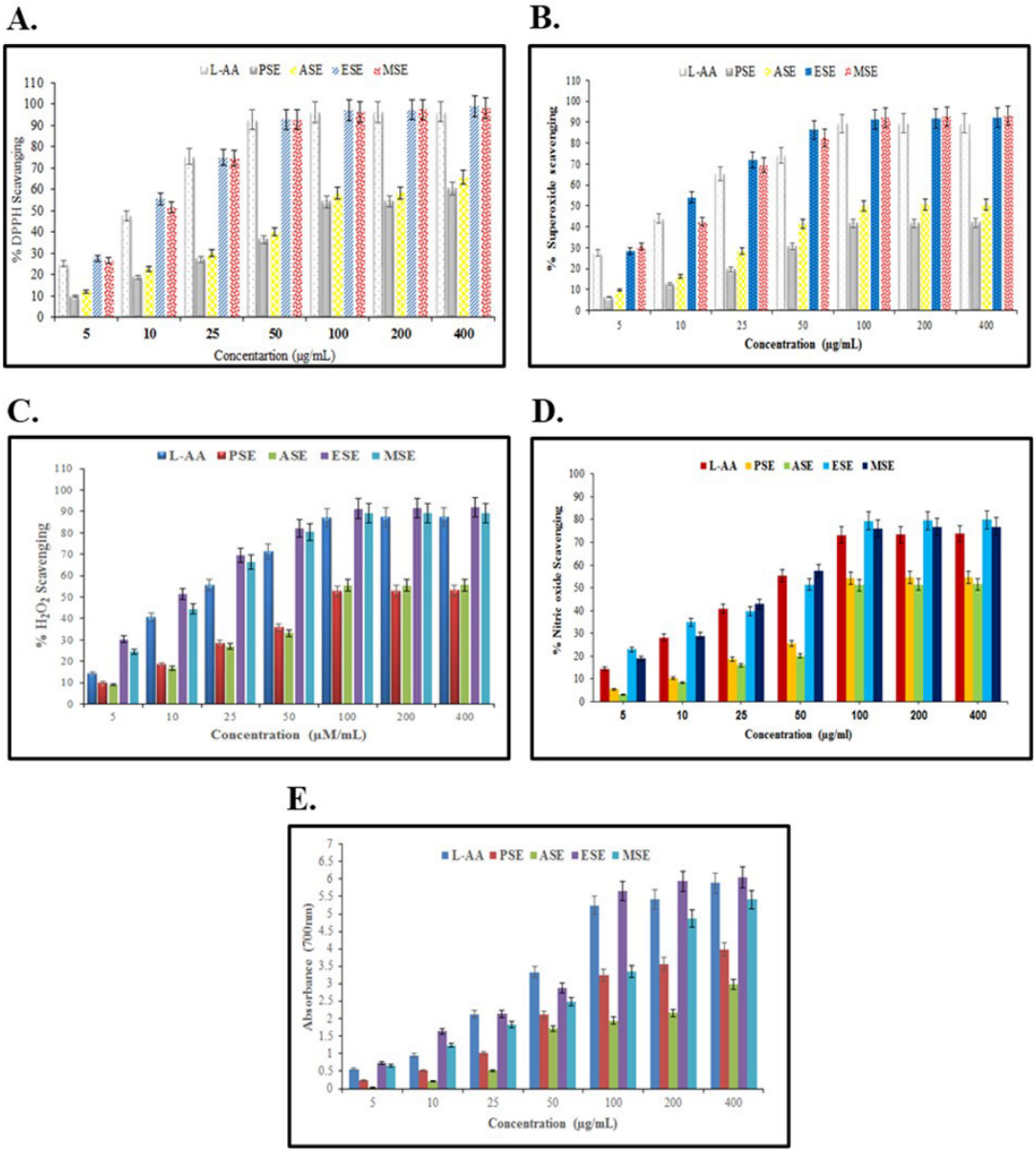

Figure 1. (A) DPPH scavenging activity of *A. squamosa* seed extracts (B) Superoxide scavenging activity of *A. squamosa* seed extracts (C) H_2O_2 scavenging activity of *A. squamosa* seed extracts (D) Nitric oxide scavenging activity of *A. squamosa* seed extracts (E) Reducing potential of the *A. squamosa* seed extracts.

Table 2. Scavenging activity of *A. squamosa* seeds extracts.

Type of Extract	(DPPH) EC_{50} (µg/mL)±S.D	Superoxide EC_{50} (µg/mL)±S.D	Hydrogen peroxide EC_{50} (µg/mL)±S.D	Nitric oxide EC_{50} (µg/mL)±S.D
PSE	87.50 ±0.24	>400.00±0.17	92.50 ±0.21	88.50 ±0.14
ASE	93.20 ±0.10	99.90 ±0.22	98.10 ±0.42	93.10 ±0.28
ESE	9.00 ±0.26	9.40 ±0.13	10.80 ±0.32	47.50 ±0.31
MSE	9.80 ±0.13	22.11 ±0.32	14.50 ±0.21	39.50 ±0.11
L-AA	11.70 ±0.16	13.00 ±0.13	23.50 ±0.21	42.50±0.14

Values are expressed as mean ± standard deviation (n = 3). L-Ascorbic acid (L-AA) was used as a standard. EC_{50} value is defined as the amount of antioxidants necessary to decrease the radical concentration by 50%. PSE (Petroleum ether seed extract), ASE (Acetone seed extract), ESE (Ethanol seed extract), MSE (Methanol seed extract)

Table 3. IC_{50} (µg) and selectivity index (SI) values of *A. squamosa* seeds extracts on HEK293, MCF7, and HepG2 cell lines.

S.No	Type of Extract	HEK293	MCF7		HepG2	
		IC_{50}(µg)±S.D.	IC_{50}(µg)±S.D.	SI	IC_{50}(µg)±S.D.	SI
1	PSE	92.72±0.44	91.62±1.43	1.27	61.92±0.16	1.49
2	ASE	148.62±1.14	79.11±2.29	1.11	73.94±2.21	2.01
3	ESE	240.05±0.39	15.45±1.22	12.75	32.18±2.04	7.45
4	MSE	152.32±0.97	54.19±1.27	16.29	60.92±1.03	2.50
5	Coumarin	132.38±0.23	24.81±1.29	7.87	23.79±2.27	5.56

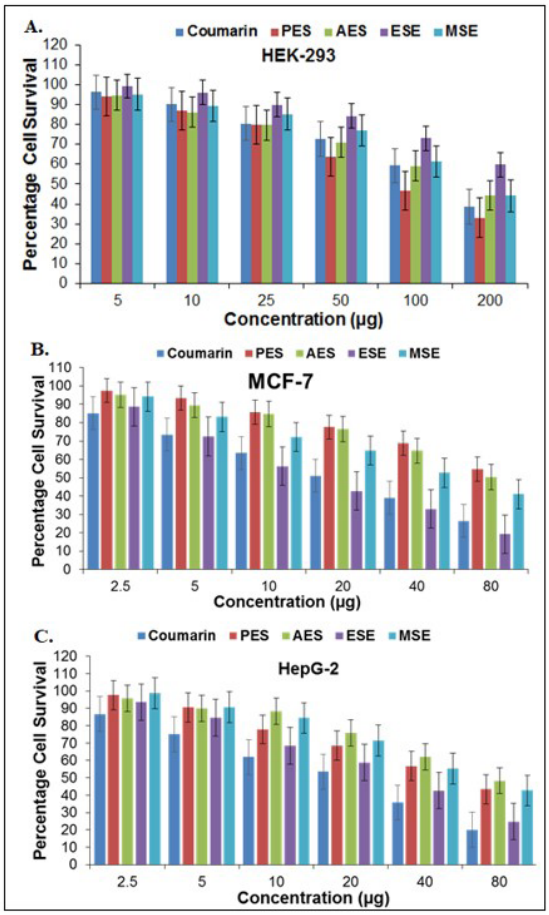

Figure 2. Cytotoxicity assay of (A). Extracts PSE, ASE, ESE and MSE on H,EK293 cells. (B) Anti-proliferation assay of extracts PSE, ASE, ESE and MSE on M,CF7 cells and (C). Extracts PSE, ASE, ESE and MSE on H,epG2 cells. The mean SD of the data, which reflect testing of experiments, is displayed. The positive control utilised is coumarin. [PSE (petroleum ether seed extract),ASE (acetone seed extract), ESE (ethanol seed extract), MSE (Methanol seed extract)].*p < 0.02; **p < 0.05; ***p < 0.002 related by the control.

in the EGFR kinase interaction site (Val A:702). Figure 3B depicts the interactions between the residues (Val A:702, Lys A:721, Met A:742, Leu A:764 and Leu A:820) in the EGFR kinase binding site and the chemical cyclopentane, 1,1,3-trimethyl. It has been discovered that the phosphonate oxide tributyl molecule interacts with the amino acid residues of EGFR kinase that are involved in hydrogen bonds, such as Thr A:830 in Figure 3C. Asp:831, Leu:820, Val:702, Lys:721, Leu:764, and Ala:719 are the binding sites shown in Figure 3D. An effective EGFR kinase inhibitor can include anethole, cyclopentane, 1,1,3-trimethyl, and phosphonium oxide. When compared to other extract compound, ESE extract has demonstrated a higher G score for EGFR Kinase.

3.7. Compounds in extracts that interact with EGFR kinase were studied for binding energy

Comparing ESE extract to other compound extracts, the binding energy of ESE extract to ligand proteins shows a strong bonding connection and accurate ligand-protein binding. We calculated ligand-binding energies using the Prime MM-GBSA method in order to assess the clarity of docking studies.

3.8. Assessment of ADME and toxicity

We evaluated the pharmacokinetics and pharmacodynamics of lead natural substances using the Maestro 10.5 Qikprop application. Anethole, clopentane, 1,1,3-trimethyl, and phosphonium oxide tributyl produced the highest G-scores. The noteworthy feature of these compounds is their excellent Lipinski's Rule of Five-compliant QPlogPo/w, QPlogHERGK+ channels, QPlogBB, QPlogKP, and QPlogKhsa values (Table 9). The characteristics picked have been found to affect metabolism, cell permeability, and bioavailability for anti-oxidant application.

3.9. Boiled-egg plot and biological activity predictions

Table 10 provides a summary of the plot's findings. This result justifies the yellow zone of the BOILED-Egg plot including all three chemicals (Figure 4A). The virtual medication with anethole, cyclopentane, 1,1,3-trimethyl, and phosphonium oxide tributyl, which is almost in the middle of the yellow region, has the lowest value for TPSA. The plot's grey and white areas don't contain any of the compounds (Figure 4B). Pharmacological effects, particular toxicities, and modes of action are all included in the biological activity spectrum (BAS) of a substance. The Pa (active compounds) and Pi (inactive compounds) values range from 0 to 1, respectively. The findings of the PASS prediction showed that the anticancer activity of the chosen compounds is indicated by the highest Pa value over the Pi value (Table 11). EGFR kinase activity may be

Table 4. Active components in PSE (Ethyl acetate leaves extract) with their retention time (RT, min), molecular weight (MW), and area percentage.

S.No	RT	Peak Area	Name of compound	Area%
1	5.742	6597387	1,2-Propanediol, 3-methoxy-	18.47
2	9.644	10743663	Ethanol, 2,2'-oxybis-	30.08
3	13.484	9979031	2,3-Butanediol, 1,4-dimethoxy-	27.94
4	16.991	5847028	2,3-Butanediol, 1,4-dimethoxy-	16.37
5	21.306	2549801	2,3-Butanediol, 1,4-dimethoxy-	7.14

Table 5. Active components in ASE (Ethyl acetate leaves extract) with their retention time (RT, min), molecular weight (MW), and area percentage.

S.No	RT	Peak Area	Name of compound	Area%
1	17.011	903945	Unknown	1.17
2	18.901	518542	Oleic Acid	0.67
3	19.635	3283870	6-Octadecenoic acid	4.27
4	21.412	973987	Triarachine	1.27
5	23.393	4135487	9-Octadecenoic acid, 1,2,3-propanetriyl ester	5.38
6	23.644	547625	Unknown	0.71
7	24.196	543486	Unknown	0.71
8	24.315	4624614	Unknown	6.01
9	24.530	12026740	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	15.63
10	27.720	19861082	Unknown	25.82
11	27.760	14995102	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	19.49
12	27.815	11162748	Unknown	14.51
13	28.376	3355363	Unknown	4.36

Table 6. Active components in ESE (Ethyl acetate leaves extract) with their retention time (RT, min), molecular weight (MW) and area percentage.

S.No	RT	Peak Area	Name of compound	Area%
1	14.033	6273097	Anethole	46.40
2	16.553	233024	Cycloheptasiloxane, tetradecamethyl-	1.72
3	25.534	753100	Triarachine	5.57
4	28.633	5312461	9-Octadecenoic acid, 1,2,3-propanetriyl ester,	39.29
5	29.140	948836	Octacosyl trifluoroacetate	7.02
6	15.290	58462	Phosphine, tributyl-	13.06
7	17.695	20122	Cyclopentane, 1,1,3-trimethyl-	9.45
8	17.951	16111	Nonane, 1-iodo-	3.60
9	25.175	227397	Phosphine oxide, tributyl-	50.81

choked by these drugs, according to docking studies, which may prevent cancer infection.

3.10. FTIR spectral, XRD, and EDS investigation

The absorption peaks from the ESE extract of *A. squamosa* are displayed below (Figure 5A and Table 12). Strong absorption at 3279.44 cm-1 in the I.R. spectrum (cm-1, KBr) suggested the presence of a hydroxyl group, but mild absorption at 2922.10 cm^{-1} indicated C-H stretching. The absorption attributed the C-O stretching of the ester group at 1245.60 cm^{-1}, whereas the absorption indicated the presence of the olefinic (>C=C, stretching) group at 1605.60 cm^{-1}. Absorption at 1050 cm^{-1} proved the existence of the -C-O-C connection. The 821.20 cm^{-1} and 769.87 cm^{-1} peaks were identified as C-H waging vibrations. The surface morphology and crystalline makeup of the ESE

Table 7. Active components in MSE (Ethyl acetate leaves extract) with their retention time (RT, min), molecular weight (MW) and area percentage.

S.No	RT	Peak Area	Name of compound	Area%
1	11.670	361892	Cyclopentasiloxane, decamethyl-	4.40
2	14.275	597695	Cyclohexasiloxane, dodecamethyl-	7.27
3	16.554	427692	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	5.20
4	21.515	360572	Hexadecanoic acid, methyl ester	4.38
5	21.992	450272	n-Hexadecanoic acid	5.48
6	23.193	477649	9,12-Octadecadienoic acid (Z,Z)-	5.81
7	23.237	990112	9-Octadecenoic acid (Z)-, methyl ester	12.04
8	23.465	275184	Heneicosanoic acid, methyl ester	3.35
9	28.615	779100	Ethyl iso-allocholate	9.47
10	29.150	339272	Unknown	4.13
11	29.801	730688	Ethyl iso-allocholate	8.89
12	30.544	2433564	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	29.59

Table 8. Lowest binding energy for the ligand-EGFR kinase (PDB ID: 1M17) interaction, along with scores for various interaction types, as detected by GLIDE.

Extracts	Compounds	Binding Energy MM-GBSA (kcal/mol)	G-Score	Lipophilic E vdw	H-bond	Electro	Protein ligands interaction
PES	1,2-Propanediol, 3-methoxy-	-82.7177	-8.813	-6.21	-1.57	-0.57	Arg C:4, Thr C:5, Asp A: 292, Glu A:234,
	Ethanol, 2,2'-oxybis-	-78.2194	-8.711	-4.19	-2.02	-0.53	Asn A:279, Glu A:278, Glu A:234, Glu A:228
	2,3-Butanediol, 1,4-dimethoxy-	-72.2345	-8.221	-5.83	-1.07	-0.58	Glu A:234, Asn A:274, Glu A:278, Asp A:292
	Known Inhibitors						
	CID22311	-43.1365	-8.99	-3.93	-0.97	-0.53	Glu A:228, Ala A:230 and Asp A:274
AES	Oleic Acid	-89.7111	-9.60	-4.18	-2.26	-0.5	Gly A:89, Ser A:94, Lys A:111, Ser A:160, Tyr A:161, Ala A:162, Glu A:166, Glu A:209
	6-Octadecenoic acid	-76.2194	-8.42	-4.79	-0.7	-0.2	Gly A:90, Ala A:162, Glu A:166, Asp A:205, Glu A:209, Asp A:223
	Triarachine	-72.2345	-7.05	-5.59	-0.7	-0.25	Leu A:88, Val A:96, Lys A:111, Tyr A:161, Ala A:162, Gly A:165, and Glu A:166
	E,E,Z-1,3,12-Nonadecatriene-5,14-diol						
	Known Inhibitors						
	CID22311	-39.1365	-8.99	-2.66	-2.11	-0.21	Lys A:111, Lys A:163, Thr A:222, and Asp A:223

Table 8. Continued...

Extracts	Compounds	Binding Energy MM-GBSA (kcal/mol)	G-Score	Lipophilic E vdw	H-bond	Electro	Protein ligands interaction
ESE	Anethole	-94.1167	-13.74	-5.92	-0.62	-0.14	Gly A:89, Ser A:94, Ser A:160, Tyr A:161, Ala A:162, Glu A:209 and Asp A:223
	Cycloheptasiloxane, tetradecamethyl-	-91.1104	-10.94	-5.28	-0.62	-0.31	Val A:882, Ala A:885, and Lys A:890
	Triarachine	-75.4340	-8.75	-4.85	-0.67	-0.34	Val A:882, Ala A:885, Thr A:886, and Lys A:890
	9-Octadecenoic acid, 1,2,3-propanetriyl ester,						
	Octacosyl trifluoroacetate						
	Phosphine, tributyl-						
	Cyclopentane, 1,1,3-trimethyl-		-12.75				
	Nonane, 1-iodo-						
	Phosphine oxide, tributyl-		10.73				
	Known Inhibitors						
	CID22311	-59.5311	-8.99	-2.11	-2.66	-0.91	Met A:804, Ser A:806, Lys A:833, Glu A:880, Val A:882, and Asp A:964
MSE	Cyclopentasiloxane, decamethyl-	-88.1143	-9.52	-4.95	-0.33	-0.12	Gln A:85, Val A:86, Ile A:87 and Ser B:2035
	Cyclohexasiloxane, dodecamethyl-	-81.1121	-8.79	-4.17	-0.18	-0.11	Thr A:57, Tyr A:113 and Asp B:2102
	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane	-73.0111	-7.50	-3.91	-0.34	-0.13	Arg A:73, and Asp B:2102,
	Hexadecanoic acid, methyl ester						
	n-Hexadecanoic acid						
	9,12-Octadecadienoic acid (Z,Z)-						
	9-Octadecenoic acid (Z)-, methyl ester						
	Heneicosanoic acid, methyl ester						
	Ethyl iso-allocholate						
	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester						
	Known Inhibitors						
	CID22311	-35.0213	-8.99	-1.86	-0.27	-0.11	Tyr A:57, Arg A:73, Gln A:85, Tyr A:113 and Ser A:118

Table 9. Evaluation of drug-like properties of the lead molecules by Qikprop Maestro 10.5 molecular docking suite.

Molecule	QPlog Po/w (-2.0 to 6.5)	QPlog HERG (acceptable range: above -5.0)	QPPCaco (nm/s) <25-poor >500-great	QPlog BB (-3 to 1.2)	QPP MDCK (nm/s)	Q Plog Kp (-8.0 to -0.1)
Anethole	3.09	-8.158	50.367	-0.543	23.953	-5.767
Cyclopentane, 1,1,3-trimethyl	3.198	-8.015	50.441	-0.628	23.987	-5.864
Phosphine oxide, tributyl	0.887	-4.804	100.053	-1.541	41.088	-4.179

Predicted IC50 value for blockage of HERG K+ channels; (acceptable range above -5.0) Molecule STOCK, InterBioScreen's library (IBS), Q P log Poct; was predicted partition coefficient of octanol/gas, (8.0 to 35.0); QPP Caco, predicted apparent Caco-2 cell permeability in nm/s. Caco-2 cells is a model for the gut blood barrier (nm/s) <25—poor, >500—great. Q P log BB, predicted brain/blood partition coefficient; QPP MDCK, predicted apparent MDCK cell permeability in nm/s. MDCK cells are considered to be a good mimic for the blood–brain barrier; (nm/s) <25—poor, >500—great; Q P log KP, Predicted skin permeability; Q P log Khsa Prediction of binding to human serum albumin; (acceptable range -1.5 to 1.5).

Table 10. Boiled egg parameters

Molecule	MW	TPSA	XLOGP3	MLOGP	GI absorption	BBB permeant
Anethole	148.20	9.23	3.30	2.67	High	Yes
Cyclopentane, 1,1,3-trimethyl	112.21	0.00	3.61	3.81	High	Yes
Phosphine oxide, tributyl	218.32	26.88	3.13	3.27	High	Yes

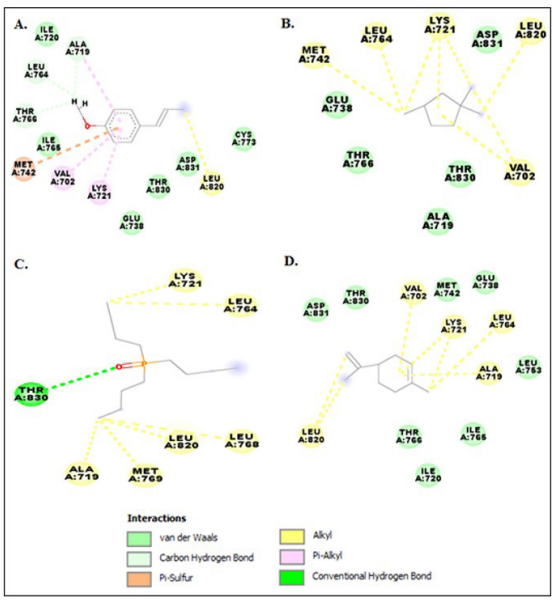

Figure 3. Drugs' molecular interactions with the EGFR kinase: (A) Anethole; (B) Cyclopentane, 1,1,3-trimethyl; (C) Phosphine oxide, tributyl; (D) CID22311.

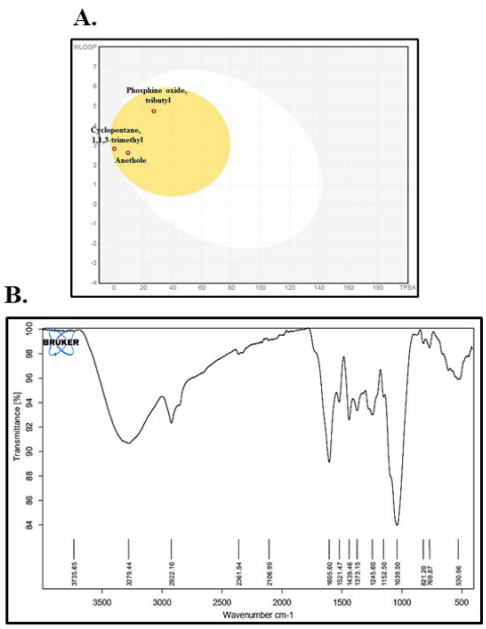

Figure 4. (A) Boiled-egg Plot; (B) FTIR study of *A. squamosa* seed in ethanol extract.

extract were determined using X-ray diffraction (XRD) investigation in the 35–70° at 2 angle range. The XRD pattern of the ESE extract is depicted in Figure 5B, with high-intensity peaks at about 38°C that correspond to (110) Bragg reflections. These peak positions exactly match the lattice parameters of the extract's face centre cubic (fcc) structure. Phytoconstituents such tannins, flavonoids, glycosides, carbohydrates, lipids, and fixed oil are found in plants, according to qualitative phytochemical screening. The percentage of elements discovered in *A. squamosa* ESE as determined by Energy Dispersive X-ray Spectroscopy (EDS) is displayed in Table 13, Figure 5, and SEM pictures (Figures 5C), respectively. Using EDS spectroscopy, the ESE of *A. squamosa* was found to contain carbon, hydrogen,

Table 11. Biological activity spectrum of compounds (Pa – Active; Pi – Inactive).

Molecule	Pa	Pi	Activity
Anethole	0.991	0.039	Anticancer
Cyclopentane, 1,1,3-trimethyl	0.912	0.022	Anticancer
Phosphine oxide, tributyl	0.821	0.038	Anticancer

Table 12. FTIR peak values and functional groups in ethanol extract of *A. squamosa* seed.

SL.NO	Wavenumber (cm^{-1})	Frequency ranges (cm^{-1})	Functional Groups
1	3279.44	3000-3500	alcohol OH stretch
2	2922.10	2500-3000	carboxylic acid O.H. stretch, -C-H stretch
3	1605.60	1500-2000	C=C alkene, C=O aldehyde, C=O anhydride
4	1245.60	1200-1500	C-O-C stretch, C=C aromatic, CH_2 bend, CH_3 bend
5	1039.50	1000-1200	C-F, C-OH stretch, C-O-C stretch
6	821.20	750-1000	C-Cl, C-Br, Esters, amines
7	530.96	500-750	alkyl halide

Table 13. Atom percentage of elements of ESE of *A. squamosa*.

Element	Weight (%)	Atomic (%)
C	225.20	76.59
O	91.25	23.30
Si	0.17	0.02
Cl	0.09	0.01
K	0.11	0.01
Ca	0.16	0.02
Cu	0.29	0.02
Zn	0.26	0.02
Br	0.26	0.01
Zr L	0.03	0.00
Total	**317.81**	

phosphorus, chlorine, potassium, calcium, platinum, sulphur, and iron. Carbon and hydrogen serve as the skeleton of these plants.

4. Discussion

Plants through a extensive history of practice in old-style medicine denote a enormous resource for pharmaceutical sciences to discover and investigate new remedies (Gurib-Fakim, 2006). *A. squamosa*, for example, has long been used in traditional (old-style) medicine in India, China, and the Middle East, but its chemical and pharmacologic depiction lags in arrears that of added species in the genus, by way of *A. reticulata* and *A. muricata*. Many research have examined the biological functions of various *A. squamosa* components, but very few have examined the seed (Kumar et al., 2021). This study aimed to look into the activities (biological) of *A. squamosa* seeds and see if they could be used as complementary medicine or a new remedy for diseases like cancer.

Herbal remedies are prepared in a variety of ways in traditional medicine, including short infusions and longer decoctions. Processing medicinal plants play several essential roles in their therapeutic use. These include lowering toxicity and side effects, improving biological effects, preserving active ingredients, and making administration more manageable (Negi et al., 2018). Typically, medicinal plants' therapeutic value is attributed to their antioxidant capabilities (Goyal et al., 2012). *A. squamosa* contains antioxidant components such gallic acid, ellagic acid, methyl gallate, catechin, and other phenolics, according to the biochemical study (Gohar et al., 2013). The ESE and MSE extracts of *A. squamosa* possess the highest conc. of phenolic and flavonoid compounds. Highest phytochemical content increased the total antioxidant potential. Quantitative estimation proved that the extracts possess the highest capacity for scavenging and reducing free radicals. Succinate dehydrogenase, an enzyme found in metabolically active cells (dividing), converts the yellow tetrazolium salt MTT to formazan (Quesnelle et al., 2007; Yue et al., 2012). A study has shown that extracts of ESE impede the spread of MCF7 cells nearly 3 times more than extracts of PSE, ASE, and MSE. Figure 3C depicts a cell proliferation assay using HepG2 as the test subject and increasing conc. of the extracts PSE, ASE, ESE, MSE, and coumarin as the control substances. PSE, ASE, and MSE (extracts) show higher IC_{50} values as related to the reference compound (Table 3), It implies that as compared to other extracts, ESE extracts are more effective against HepG2 cancer cells.

The selectivity index (SI) value for the efficiency of particular *C. lanceolatus* extracts against malignant cells has been computed. Extracts with a high SI value (>3) suppress cancer cells specifically. Extracts having a SI value of three are regarded as hazardous to normal cells. The PSE, ASE,

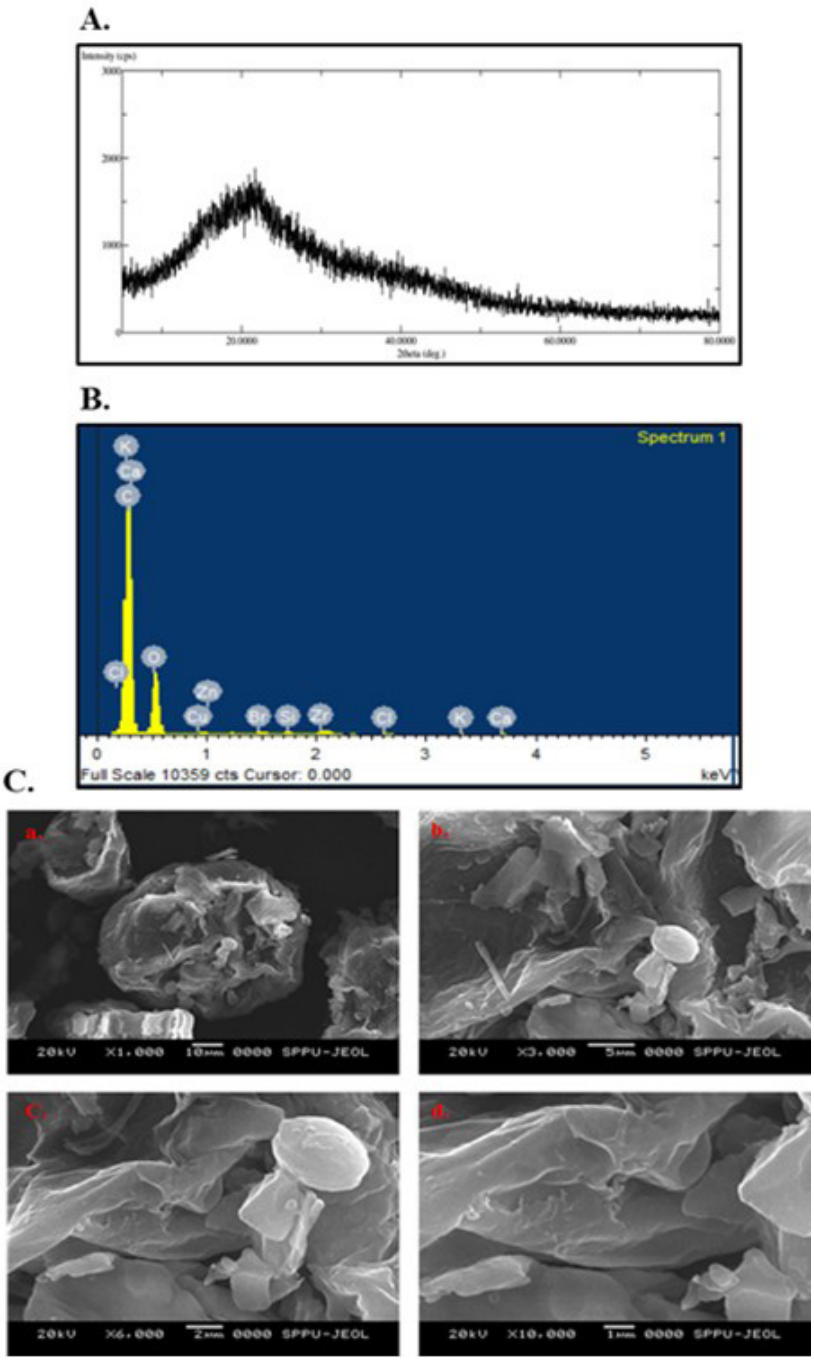

Figure 5. (A) XRD spectra of *A. squamosa* presentation projecting extract image. (B) ESE of *A. squamosa* powder measured by energy dispersive X-ray spectroscopy (C) ESE of *A. squamosa* powder as seen using scanning electron microscopy.

ESE, and MSE extract is primarily made up of fatty acids and esters, ethers, and backbone chemicals, according to the GC/MS investigation. Mass spectra of compounds with a similarity index (S.I.) of more than 70% were reported by the NIST library. Both normal and hyperproliferative cells utilise the EGFR for cell growth, differentiation, and motility. Therefore, it is crucial to comprehend how the EGFR signalling pathway is controlled in order to forecast diseases and target EGFR for treatment (Rajendran et al., 2012). This in silico study aimed to investigate whether the ethanolic extract of *A. squamosa* L. seed could exert its anticancer impact in ovarian cancer through reducing the binding energy of EGFR kinase with natural compounds.

Constitutive STAT3 (signal transducers and activators of transcription 3) activation is associated with the overexpression of EGFR in epithelial malignancies,

which imparts resistance to chemo-induced apoptosis (Quesnelle et al., 2007). In fact, this protein (STAT3) is frequently phosphorylated and inappropriately activated by the action of EGFR and other tyrosine kinases in solid ovarian cancers (Yue et al., 2012). The key finding is that Bcl-2, Bcl-xL, and P.I. 3-kinase/Akt activity are among the antiapoptotic processes that accumulate when STAT3 is activated at tyr-705 (Prenzel et al., 2001). We investigated the manner of binding interactions in the EGFR kinase active site in the extract of ESE and discovered that natural chemicals in the extract have a stronger attraction to the EGFR. In order to assess molecular docking, the post-scoring method MM/GBSA was used to compute the binding energy of a hypothetically screened molecule to the ESE receptor (Table 8). According to the binding free energy study, it had a greater affinity with the receptor than the present medication.

The majority of medications fail clinical trials as a result of cellular toxicity and pharmacokinetic characteristics. It has been determined how to achieve cystic bioavailability for EGFR kinase inhibitors by evaluating the in silico pharmacokinetic characteristics of a few different drugs (Tables 9). The physicochemical characteristics of a drug molecule, such as its aqueous solubility (lags), lipophilicity (Clg P), polar surface area (M.W), and molecular weight (D.W), directly affect its absorption and bioavailability (Muchmore et al., 2010; McQuaid, 2007). A number of malignancies and sarcomas are treated with platinum, a chemotherapeutic medication with a platinum base. In vitro, platinum complexes cause DNA crosslinking and binding that lead to apoptosis. It can aid in restoring vital components that the sickness had insufficient amounts, enabling the recovery of the illness.

5. Conclusion

Natural products collectively with vegetation, animals, and minerals were the concept for treating human diseases. The have aTheyke a observe of ethnopharmacology and traditional treatment primes frequent drugs to global pharmacopeia. Old-style (Traditional) capsules can reason drug format and viable targets for scientific investigation. The feasibility of this look at for inhibi EGFR kinase in ovarian most cancers turned into investigatinvestigatingf molecular docking and pharmacokinetics techniques. AnticancerAn anticancerned into anticipated to the scientific importance level. The occurrence of flavonoids in ESE *A. squamosa* turned into located in the direction of accountable for the hobby. Plant derived chemical compounds take an extended records of withinside the remedy of human diseases. Ordinary (natural) merchandise described for almost 1/2 of of all new chcal entities delivered withinside the final decades. More studies is wanted to areolate the energetic phytoconstituents gift withinside the extract and experiments at the drug'sonecuperation motion on ovarian most cancers apacity aspect effects. The studies into the Mode of motion ought to pave the manner for growing a brand new anti-most cancers remedy regimen.

6. Abbreviations

A. squamosa: *Annona squamosa,* **PES:** Petroleum ether, **L-AA:** L-ascorbic acid, **DPPH**: Diphenypicrylhydrazyl, **NBT:** Nitroblue tetrazolium, **ELISA:** Enzyme-linked immunoabsorbent assay, **FTIR:** Fourier Transform Infrared Spectrophotometer, **XRD:** X-ray diffraction, **BAS:** Biological activity spectrum, **SI:** selectivity index, **EDS:** Energy Dispersive X-ray Spectroscopy, **FCC:** Face centre cubic, $\mathbf{H_2O_2}$:Hydrogen peroxide.

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References

ANSARI, M.F., SIDDIQUI, S.M., AHMAD, K., AVECILLA, F., DHARAVATH, S., GOURINATH, S. and AZAM, A., 2016. Synthesis, antiamoebic and molecular docking studies of furan-thiazolidinone hybrids. *European Journal of Medicinal Chemistry*, vol. 124, pp. 393-406. PMid:27597415.

CAO, G., ALESSIO, H.M. and CUTLER, R.G., 1993. Oxygen-radical absorbance capacity assay for antioxidants. *Free Radical Biology & Medicine*, vol. 14, no. 3, pp. 303-311. http://dx.doi.org/10.1016/0891-5849(93)90027-R. PMid:8458588.

CHEN, Y., CHEN, Y., SHI, Y., MA, C., WANG, X., LI, Y., MIAO, Y., CHEN, J. and LI, X., 2016. Antitumor activity of Annona squamosa seed oil. *Journal of Ethnopharmacology*, vol. 193, pp. 362-367.; published online 2016. http://dx.doi.org/10.1016/j.jep.2016.08.036. PMid:27566205.

FANG, S.Y., TSENG, C.C., YANG, Y.L., LEE, E.J., CHEN, H.Y., BHARDWAJ, A. and CHEN, T.Y., 2006. Nitric oxide scavenger carboxy-PTIO reduces infarct volume following permanent focal ischemia. *Acta Anaesthesiologica Taiwanica*, vol. 44, no. 3, pp. 141-146. PMid:17037001.

FERNANDES, M.C. and SELLAPPAN, K., 2019. Elemental composition and X-ray diffraction studies of Strobilanthes species. *Indian Journal of Biochemistry & Biophysics*, vol. 56, no. 2, pp. 144-149.

FRIESNER, R.A., BANKS, J.L., MURPHY, R.B., HALGREN, T.A., KLICIC, J.J., MAINZ, D.T., REPASKY, M.P., KNOLL, E.H., SHELLEY, M., PERRY, J.K., SHAW, D.E., FRANCIS, P. and SHENKIN, P.S., 2004. Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *Journal of Medicinal Chemistry*, vol. 47, no. 7, pp. 1739-1749. http://dx.doi.org/10.1021/jm0306430. PMid:15027865.

FRIESNER, R.A., MURPHY, R.B., REPASKY, M.P., FRYE, L.L., GREENWOOD, J.R., HALGREN, T.A., SANSCHAGRIN, P.C. and MAINZ, D.T., 2006. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes. *Journal of Medicinal Chemistry*, vol. 49, no. 21, pp. 6177-6196. http://dx.doi.org/10.1021/jm051256o. PMid:17034125.

GOHAR, A., MAATOOQ, G., GADARA, S. and ABOELMAATY, W., 2013. One new pyrroline compound from *Callistemon viminalis* (Sol. Ex Gaertner) G. Don Ex Loudon. *Natural Product Research*, vol.

27, no. 13, pp. 1179-1185. http://dx.doi.org/10.1080/14786419.2012.718771. PMid:22967219.

GOYAL, P.K., JAIN, R., JAIN, S. and SHARMA, A., 2012. A Review on biological and phytochemical investigation of plant genus Callistimon. *Asian Pacific Journal of Tropical Biomedicine*, vol. 2, no. 3, pp. S1906-S1909. http://dx.doi.org/10.1016/S2221-1691(12)60519-X.

GREEN, L.C., WAGNER, D.A., GLOGOWSKI, J., SKIPPER, P.L., WISHNOK, J.S. and TANNENBAUM, S.R., 1982. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Analytical Biochemistry*, vol. 126, no. 1, pp. 131-138. http://dx.doi.org/10.1016/0003-2697(82)90118-X. PMid:7181105.

GURIB-FAKIM, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, vol. 27, no. 1, pp. 1-93. http://dx.doi.org/10.1016/j.mam.2005.07.008. PMid:16105678.

HARI, N. and NAIR, V.P., 2018. FTIR spectroscopic analysis of leaf extract in hexane in Jasminum azoricum L. *Recent Research in Science and Technology*., vol. 4, no. 8, pp. 170-172.

IYENGAR, M.A. Study of crude drugs. Manipal College of Pharmaceutical Sciences; 1981.

JAIN, D., UDHWANI, T., SHARMA, S., GANDHE, A., REDDY, P.B., NAYARISSERI, A. and SINGH, S.K., 2019. Design of novel JAK3 Inhibitors towards Rheumatoid Arthritis using molecular docking analysis. *Bioinformation*, vol. 15, no. 2, pp. 68-78. http://dx.doi.org/10.6026/97320630015068. PMid:31435152.

JI, H.-F., LI, X.-J. and ZHANG, H.-Y., 2009. Natural products and drug discovery. Can thousands of years of ancient medical knowledge lead us to new and powerful drug combinations in the fight against cancer and dementia? *EMBO Reports*, vol. 10, no. 3, pp. 194-200. http://dx.doi.org/10.1038/embor.2009.12. PMid:19229284.

JORGENSEN, W.L. and DUFFY, E.M., 2002. Prediction of drug solubility from structure. *Advanced Drug Delivery Reviews*, vol. 54, no. 3, pp. 355-366. http://dx.doi.org/10.1016/S0169-409X(02)00008-X. PMid:11922952.

KATANAEV, V.L., DI FALCO, S. and KHOTIMCHENKO, Y., 2019. The Anticancer drug discovery potential of marine invertebrates from Russian Pacific. *Marine Drugs*, vol. 17, no. 8, pp. 474. http://dx.doi.org/10.3390/md17080474. PMid:31426365.

KAUR, C. and KAPOOR, H.C., 2001. Antioxidants in fruits and vegetables–the millennium's health. *International Journal of Food Science & Technology*, vol. 36, no. 7, pp. 703-725. http://dx.doi.org/10.1046/j.1365-2621.2001.00513.x.

KOTHARI, V. and SESHADRI, S., 2010. Antioxidant activity of seed extracts of Annona squamosa and Carica papaya. *Nutrition & Food Science*, vol. 40, no. 4, pp. 403-408. http://dx.doi.org/10.1108/00346651011062050.

KUMAR, M., CHANGAN, S., TOMAR, M., PRAJAPATI, U., SAURABH, V., HASAN, M., SASI, M., MAHESHWARI, C., SINGH, S., DHUMAL, S., RADHA., THAKUR, M., PUNIA, S., SATANKAR, V., AMAROWICZ, R. and MEKHEMAR, M., 2021. Custard apple (*Annona squamosa* L.) leaves: nutritional composition, phytochemical profile, and health-promoting biological activities. *Biomolecules*, vol. 11, no. 5, pp. 614. http://dx.doi.org/10.3390/biom11050614. PMid:33919068.

LAGUNIN, A., STEPANCHIKOVA, A., FILIMONOV, D. and POROIKOV, V., 2000. PASS: prediction of activity spectra for biologically active substances. *Bioinformatics (Oxford, England)*, vol. 16, no. 8, pp. 747-748. http://dx.doi.org/10.1093/bioinformatics/16.8.747. PMid:11099264.

LEONTI, M. and CASU, L., 2013. Traditional medicines and globalization: current and future perspectives in ethnopharmacology. *Frontiers in Pharmacology*, vol. 4, pp. 92. http://dx.doi.org/10.3389/fphar.2013.00092. PMid:23898296.

LIU, F., OOI, V. and CHANG, S., 1997. Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences*, vol. 60, no. 10, pp. 763-771. http://dx.doi.org/10.1016/S0024-3205(97)00004-0. PMid:9064481.

LU, J.J., CRIMIN, K., GOODWIN, J.T., CRIVORI, P., ORRENIUS, C., XING, L., TANDLER, P.J., VIDMAR, T.J., AMORE, B.M., WILSON, A.G., STOUTEN, P.F. and BURTON, P.S., 2004. Influence of molecular flexibility and polar surface area metrics on oral bioavailability in the rat. *Journal of Medicinal Chemistry*, vol. 47, no. 24, pp. 6104-6107. http://dx.doi.org/10.1021/jm0306529. PMid:15537364.

MCQUAID, K.R., 2007. Drugs are used in the treatment of gastrointestinal diseases. In: B.G. KATZUNG, ed. *Basic and clinical pharmacology*. New York: McGraw-Hill Education, pp. 1098-1099.

MILIAUSKAS, G., VENSKUTONIS, P. and VAN BEEK, T., 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, vol. 85, no. 2, pp. 231-237. http://dx.doi.org/10.1016/j.foodchem.2003.05.007.

MOSMANN, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55-63. http://dx.doi.org/10.1016/0022-1759(83)90303-4. PMid:6606682.

MUCHMORE, S.W., EDMUNDS, J.J., STEWART, K.D. and HAJDUK, P.J., 2010. Cheminformatic tools for medicinal chemists. *Journal of Medicinal Chemistry*, vol. 53, no. 13, pp. 4830-4841. http://dx.doi.org/10.1021/jm100164z. PMid:20329801.

NEGI, V.S., KEWLANI, P., PATHAK, R., BHATT, D., BHATT, I.D., RAWAL, R.S., SUNDRIYAL, R.C. and NANDI, S.K., 2018. Criteria and indicators for promoting cultivation and conservation of Medicinal and Aromatic Plants in Western Himalaya, India. *Ecological Indicators*, vol. 93, pp. 434-446. http://dx.doi.org/10.1016/j.ecolind.2018.03.032.

OTHMAN, L., SLEIMAN, A. and ABDEL-MASSIH, R.M., 2019. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Frontiers in Microbiology*, vol. 10, pp. 911. http://dx.doi.org/10.3389/fmicb.2019.00911. PMid:31156565.

OYAIZU, M., 1986. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*., vol. 44, no. 6, pp. 307-315. http://dx.doi.org/10.5264/eiyogakuzashi.44.307.

PRENZEL, N., FISCHER, O., STREIT, S., HART, S. and ULLRICH, A., 2001. The epidermal growth factor receptor family is a central element for cellular signal transduction and diversification. *Endocrine-Related Cancer*, vol. 8, no. 1, pp. 11-31. http://dx.doi.org/10.1677/erc.0.0080011. PMid:11350724.

QUESNELLE, K.M., BOEHM, A.L. and GRANDIS, J.R., 2007. STAT-mediated EGFR signaling in cancer. *Journal of Cellular Biochemistry*, vol. 102, no. 2, pp. 311-319. http://dx.doi.org/10.1002/jcb.21475. PMid:17661350.

RAJENDRAN, P., LI, F., SHANMUGAM, M.K., KANNAIYAN, R., GOH, J.N., WONG, K.F., WANG, W., KHIN, E., TERGAONKAR, V., KUMAR, A.P., LUK, J.M. and SETHI, G., 2012. Celastrol suppresses growth and induces apoptosis of human hepatocellular carcinoma through the modulation of STAT3/JAK2 signaling cascade in vitro and in vivo. *Cancer Prevention Research* , vol. 5, no. 4, pp. 631-643. http://dx.doi.org/10.1158/1940-6207.CAPR-11-0420. PMid:22369852.

RUCH, R.J., CHENG, S. and KLAUNIG, J.E., 1989. Prevention of cytotoxicity and inhibition of intercellular communication

by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, vol. 10, no. 6, pp. 1003-1008. http://dx.doi.org/10.1093/carcin/10.6.1003. PMid:2470525.

SIDDIQUI, A.A. and ALI, M., 1997. *Practical pharmaceutical chemistry*. New Delhi: CBS Publishers and Distributors.

SINGH, G. and KUMAR, P., 2013. Extraction, gas chromatography-mass spectrometry analysis and screening of fruits of Terminalia chebula Retz. for its antimicrobial potential. *Pharmacognosy Research*, vol. 5, no. 3, pp. 162-168. http://dx.doi.org/10.4103/0974-8490.112421. PMid:23901211.

SINGH, P. and BAST, F., 2014. In silico molecular docking study of natural compounds on wild and mutated epidermal growth factor receptor. *Medicinal Chemistry Research*, vol. 23, no. 12, pp. 5074-5085. http://dx.doi.org/10.1007/s00044-014-1090-1.

TRIPATHI, S.K., MUTTINENI, R. and SINGH, S.K., 2013. Extra precision docking, free energy calculation, and molec,ular dynamics simulation studies of CDK2 inhibitors. *Journal of Theoretical Biology*, vol. 334, pp. 87-100. http://dx.doi.org/10.1016/j.jtbi.2013.05.014. PMid:23727278.

VAN DEN DOOL, H. and KRATZ, P.D., 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of Chromatography. A*, vol. 11, pp. 463-471. http://dx.doi.org/10.1016/S0021-9673(01)80947-X. PMid:14062605.

WANG, D.S., RIZWANI, G.H., GUO, H., AHMED, M., AHMED, M., HASSAN, S.Z., HASSAN, A., CHEN, Z.S. and XU, R.H., 2014. Annona squamosa Linn: cytotoxic activity found in leaf extract against human tumor cell lines. *Pakistan Journal of Pharmaceutical Sciences*, vol. 27, no. 5, pp. 1559-1563. PMid:25176251.

YUAN, H., MA, Q., YE, L. and PIAO, G., 2016. The traditional medicine and modern medicine from natural products. *Molecules*, vol. 21, no. 5, pp. 559. http://dx.doi.org/10.3390/molecules21050559. PMid:27136524.

YUE, P., ZHANG, X., PALADINO, D., SENGUPTA, B., AHMAD, S., HOLLOWAY, R.W., INGERSOLL, S.B. and TURKSON, J., 2012. Hyperactive EGF receptor, Jaks, and Stat3 signaling promote enhanced colony-forming ability, motility, and migration of cisplatin-resistant ovarian cancer cells. *Oncogene*, vol. 31, no. 18, pp. 2309-2322. http://dx.doi.org/10.1038/onc.2011.409. PMid:21909139.