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Investigation of Antimicrobial, antioxidant activity of *Alpinia officinarum* (L.) Wild. and in silico approach on anti-breast cancer receptors through GC-MS analysis

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Abstract

Alpinia officinarum (L.) Wild. is perennial cultivated herb in the family Rubiaceae, it dry root contains antioxidant, analgesic, antiulcer, antidiarrhea, antidiabetic, anti-emetic, anti-inflammatory, and anticoagulation effects. In ancient time it has been utilized conventional method both ayurvedic and Chinese medicine. The ethanolic root extract gives significant in vitro radical scavenging activity of DPPH (77% in 100 □g/mL) and ABTS (62% in 100 □g/mL) assays against the ascorbic acid positive control. The antimicrobial activity recorded significant results while comparing with commercial antibiotics controls, the bacterial species of Escherichia coli and Staphylococcos aereus exhibited 2.2 ± 0.6 (mm) and 2.4 ± 0.7 (mm) in 5 mg/mL and fungal species mucor exhibits 2.8 ± 1.2 (mm) in 5 mg/mL concentration range, respectively. Different classes of primary metabolites like catechin, saponin, tannin and carbohydrates were present in all solvent extract of root. Around 30 types of volatile compounds were detected through GC-MS (Gas chromatography-mass spectrometry) analysis in ethanolic root extract. With that lead, specific plant compounds (such as Thymine, pyrogallol, galangin and 2-furanmethanol) were identified, of which some were responsible for the impact of cytotoxic effect in MCF-7 cells through molecular docking studies, the receptors such as Akt-1, HER-2, COX-2 and PI3K.

Keywords: *Alpinia officinarum*, antioxidant, antimicrobial activity, GC-MS and molecular docking.

1. Introduction

Bioactive compounds produced by plants are boon in treating several human diseases such as cancer, diabetes, inflammation, antimicrobial etc. There are more than 8000 bioactive compounds (phenolic and flavonoids), identified in plant parts such as leaf, root, stem, flower, seed and fruit. Biologically active phytochemicals are naturally present in plants, which benefit human health with their macro and micronutrients [1]. Plants with high medicinal property are free from microbial diseases (bacterial, fungal and viral disease) and through the articulate through rich colour, aroma, and flavour and by extruding toxic materials by their plant parts. Treating microbial infections is a hellacious task because of its increasing multi drug resistant activity [2]. Antimicrobial agents such as lactams and glycopeptides are categorized according to microbial cell wall synthesis mechanism. Plant's phytochemicals (flavonoids, phenolics acids, saponins, tannin, catachins and phenolics triterpenes etc) plays crucial role in exhibiting antioxidant property [3]. Generally, the medicinal plants possess antioxidant molecule, vitamins, chlorophyll and carotenoids which involves in ROS mechanism. In recent research, it is clearly proved that number of pathogenesis of diseases is associated with reactive oxygen species (ROS) in the plant and animal cells [4]. ROS is controlled by well organized systematic/enzymatic and non-systematic/non-enzymatic method in all the living organisms. ROS (reactive oxygen species) has dual role in biology, particularly during signalling reactions in cells. Recent investigation shows that ROS also play a vital role in several biological processes in human body cells such as cell proliferation, apoptosis/cell death (by oxidation/oxidative stress), inflammation and differentiations [5]. The tumor stimulating inflammation is one of the significant steps in cancer development in the body cells; it is so powerful that, it influences both chronic and acute inflammation process. Phytochemicals are known to control tumor formation effectively and also in alarming ROS for balancing cancer tissues in human body (e.g., control the cancer cell proliferation, cell cycle arrest, develop anticancer ability (immunity) and stimulate apoptosis) [6]. Biologically active plant chemicals are able to collapse cell division (cancer cells). Among the various cancer, breast cancer is one of the life threatening complications faced by women, hence it is mandatory for developing new and efficient breast cancer drugs [7]. In the present study we have investigated the effective antimicrobial properties from ethanolic root extract of *Alpinia officinarum*. Besides the anticancer property, antimicrobial and antioxidant effects was also performed during the study. Initially, preliminary phytochemical analysis was carried out with three different solvents to check various phytochemical classes. Among the solvent, the ethanolic root extract possess large number of phytochemicals (catachins, carbohydrates, saponins and tannins).

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and reagents used for these assays were purchased from reputed vendors (SRL, India and HiMedia, Mumbai, India) and were certified of investigative/ultra-pure grade.

2.2. Plant material collection.

In Trichy district, the *Alpinia officinarum* (L.) Wild rhizome was collected.

Preparation of *Alpinia officinarum* Rhizome:

The rhizomes of *Alpinia officinarum* were thoroughly cleaned and washed with tap water and distilled water to remove any dust particles. Subsequently, they were dried under shade for 15 days to reduce moisture content. Once dried, the rhizomes were ground using a grinding machine and packed into brown bottles for storage.

2.3. Extraction Process:

Extraction was performed by taking 20 g of powdered *Alpinia officinarum* rhizomes in a 500 ml beaker containing 400 ml of deionized water. The beaker was covered with aluminum foil to shield it from light. The mixture was then shaken using a mechanical shaker for 90 minutes and warmed at 50°C for 1 hour on a magnetic stirrer. After cooling to room temperature overnight, the solution was filtered through Whatman No.1 filter paper to obtain a clear solution, which was stored at 4°C for future experiments.

2.4. Green Synthesis of Copper Nanoparticles (Cu NPs):

A 1 mM aqueous solution of copper sulfate ($CuSO_4 \cdot 2H_2O$) was prepared and stored in brown bottles. Then, 100 ml of plant leaf extract was mixed with 400 ml of the 1 mM copper sulfate solution (1:4 ratio) dropwise with continuous stirring. The mixture was incubated at room temperature for 24 hours, and the color change was monitored periodically (after 30 and 60 minutes). The change from blue to dark green color visually indicated the formation of copper nanoparticles (Cu NPs). Subsequently, the solution was centrifuged for 15 minutes at 10,000 rpm, and the obtained Cu NPs supernatant was filtered using Whatman filter paper No.1 to remove impurities. The nanoparticles were then dried, ground, and prepared for further analysis.

2.5. Antimicrobial activity

The bacterial cultures were tested for the investigation of antimicrobial activity such as -Escherichia coli (MTCC584), Pseudomonas aeruginosa (MTCC1034), Staphylococcus aureus (MTCC9542). The fungal cultures utilized were - Candida albicans (ATCC20), Mucor (ITCC20) and Aspergillus niger (ATCC902). Nutrient agar was used to inoculation for microbial strains through well diffusion method [8], after that plates were incubated at 37 ◦C for 18-24 hrs. The concentration range of A. officinarum ethanolic root extract at 1,2,3,4 and 5 mg/mL was assessed. Ampicillin was used as positive controls.

2.6. DPPH radical scavenging activity

This assay was performed by following the well-liked protocol [9]. DPPH methanolic solution, 1 mL of 0.1 mM (it shows violet in color) was taken and mixed with same volume of the root extract at 10-100 µg/mL range. L-ascorbic acid was act as standard and the tested sample were placed in the dark room temperature for 20 minutes. There after violet was turned pearl yellow in color and read at 520 nm by UV-visible spectrophotometer. The percentage of inhibition was calculated by following formula,

Inhibition % = (Ac-As/Ac) × 100

Where, Ac - absorbance of the control and As - absorbance. of the sample.

2.7. ABTS assay

The ABTS solution was become 7 mM conc. using double distilled water. For radical generation the ABTS solution was mixed with 2.45 mM conc. of potassium persulfate in equal volume (1:1 ratio). And this mixed solution was kept in dark at room temperature for 12-16 hrs. After this reacted solution was produce more radical cations (ABTS•+), then it was adjust OD value to 0.7 at 734 nm by decolorize (dilution) with double distilled water. The different concentration range of root extract was added with newly prepared ABTS•+ solution. L-Ascorbic acid was acted as standard drug. The OD value was measured at 734 nm [10]. Radical scavenging activity (%) was calculated as per the formula used for DPPH assay.

2.8. Qualitative phytochemical analysis

Qualitative screening of A. officinarum ethanolic root extract for phytochemical analysis was performed according to Brindha et al. (1982) with various solvents (aqueous, methanol and ethanol). The root extraction has been performed using Soxhlet apparatus [11].

2.9. GC-MS profiling of the ethanolic leaf extracts of A. officinarum

The phytochemical evaluation of ethanolic root extracts of A. officinarum was carried out using GC-MS (Gas Chromatography Mass Spectrophotometer - Perkin Elmer Clarus 500, Connecticut, USA) offered with flame ionization detector, capillary column (30 m length × 0.25 mm ID coated with 5% phenyl 95% dimethylpolysiloxane) with a film thickness of 0.25 µm [12].

2.10. Molecular docking studies

The docking study was executed to analyse the molecular interactions between the 3D model of (PDB ID: 3CQW, 3PP0, 5F1A and 5NGB) and phytochemical compounds using MGL tools (AutoDock 4.2) [13]. The best output analysed using PyMoL, a molecular visualization tool to identify the interactions between the protein receptors & ligands. The 2D poses of the best hits of each of the compounds were generated using Accelrys Discovery Studio Visualizer 2.5 [14]. ChimeraX 1.14 [15] was used for visualizing proteins and for checking the binding sites after completion of docking runs.

3. Results and Discussions

3.1. Antimicrobial activity of A. officinarum ethanolic root extract

Herbal ailments consider one of the crucial fields of traditional medicine in India especially in rural areas. Thus, chemotherapy is used by a large scale of Indian population for the curing of human disease. To support the appropriate use of plant medicine and to validate their effective sources of new finding, as well as it is necessary to study medicinal plants, which have ancient character in a more strengthen way [16]. Antimicrobials of plant origin have enormous therapeutic potential. Herbal medicines are contributing effective in the curing of infections and disease, but the synthetic drugs may cause side effect and also responsible for the microbial resistant [17]. The A. officinarum ethanolic root extract have significant antimicrobial activity against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus shows 2.1 ± 1.2 (mm), 1.1 ± 1.3 (mm) and 2.4 ± 0.7 (mm) at 5mg/mL concentration range. Fungal strains Mucor and Candida albicans possess 2.4 ± 1.3 (mm) and 1.6 ± 1.1 (mm) zone of inhibition in the 5mg/mL concentration, respectively. But commercial antibiotic disc ampicillin did not effect against both bacterial and fungal cultures. Crude extract contains effective antimicrobial activity because of its synergetic activity of mixed compounds.

3.2. Antioxidant activity of A. officinarum ethanolic root extract

Antioxidants are involved in the field of medicine to prevent from the harmful effect of oxidation and are also concerned in the nutrient supplements to reduce the action of oxidative stress [18]. Most of the antioxidant is produce by the phytocompounds which belongs to the flavonoids, alkaloids and phenolic compounds as well as vitamins and carotenoids from the fruits and vegetables [19]. The mechanism of antioxidants molecules is responsible for the structural activity relationship of the atoms. Since some secondary metabolites relatively enormous in plant materials may play important role of therapeutic outcomes in the treatment of disease [20]. In this present investigation of A.

officinarum ethanolic root extract inhibits considerable antioxidant effect against DPPH and ABTS free radicals. The plant ethanolic root extract exhibited 43-77% of free radical scavenge in 10-100 μg/mL concentration against DPPH radicals, L-ascorbic acid used as standard drugs it shows 76-90% of inhibition. Against ABTS radicals cations, A. officinarum shows considerable inhibition 30-62% in the concentration range 10-100 □g/mL. L-ascorbic yields 67-87% of inhibitions.

3.3. GC-MS analysis

In this section, the present investigation aimed to analyse the phytocompounds of the qualitative and quantitative root extract of A. officinarum. Aqueous, ethanol and methanolic extract shows the presence of catechin, flavonoids, saponin, tannin and carbohydrates (Table 2). These types of phytocompounds are well known to consist number of biological activities together with antimicrobial, antioxidant, anti-inflammatory, antiplasmodial and anticancer activities [21]. Through GC-MS analysis the ethanolic root extract of A. officinarum possess around 30 types of phytochemical composition (Table 3). In this extract contains large amount of Gingerenone A (14.16%), it has antiproliferation, antiviral and antioxidant activity [22]. 5-Hydroxymethylfurfural have occupied 11.13% in the root extract it possesses antioxidant activity, these compounds may be responsible for the inhibition of 43-77% DPPH radicals and 30-62% of ABTS radicals cations.

3.4. Akt-1, HER-2, COX-2 and PI3K

PI3K is an important player in several cancers and is a downstream effector of receptor tyrosine kinases such as insulin receptor and HER2, which transduce growth factor signalling [23]. PI3K catalyzes the production of phosphatidylinositol-3,4,5-triphosphate (PIP3), which in turn, activates Akt (protein kinase B) and other kinases. This protein has a catalytic domain (p110) and a regulatory domain (p85). The PI3K/Akt/COX-2 pathway is commonly dysregulated in almost all human cancers and hence, the proteins of this pathway are prime targets of anticancer therapeutic regimes [24]. PI3K-Akt-COX-2 pathway is responsible for cellular longevity, cellular proliferation through nutrient uptake (as well as anabolism) and finally, cell survival through inhibition of apoptosis [25]. Often, inhibitors of this pathway decrease cellular proliferation and increase cell death. Since cancer cells achieve immortality, many therapeutic molecules are directed at activation of apoptosis. In this work, we have found many A. officinarum phytochemicals to powerfully bind to PI3K/HER-2/COX-2/Akt-1 (with binding energies in the array of -6 kcal/mol to -5 kcal/mol). Once again, the compounds FML, GLN and PGL were the top order compounds by desirable quality of their encouraging binding energies and below Ki values. Control compounds such as Dactolisib, Taselisib, Paracetamol, aspirin, lapatinib, neratinib, afatinib and pyrotinib were found to bind strong to the protein, as suggested by the poor weakly to the protein, as suggested by the poor ΔG values obtained in the docking results (Figure 4, Figure 5, figure 6 and figure 7 and Table 4). The kinase domain of human p110 is located between residues ~696 to 1068 [26]. In PI3K p110α, the key residue involved in phosphoryl group transfer reaction is Lys802. Residues involved in substrate stabilization in PI3K which line the binding pocket are 941-KKKKFGYKRER-951 [27]. The drug XL765 was found to dock at the site of natural ligand LXX in the kinase domain of p110 and some common residues were found in interactions of both the inhibitor XL765 and the natural ligand [28]. The residues found to be involved in XL765 binding were Lys890 and Met953; also, the residues Lys802, Met804, Ile831, Val882, Ala885, Met953 and Ile963 were found to interact with the compound through strong non-bonded interactions.

Table 1. Antimicrobial activity of A. officinarum ethanolic root extract

Antibiotic (μg/disc)	**zones are represented as radius (mm)**						
	Conc. of the root extract (mg/mL)	***Escherichia coli***	***Pseudomonas aeruginosa***	***Staphylococcus aureus***	***Mucor***	***Aspergillus niger***	***Candida albicans***
-	1	1.2 ± 2.1	0.3 ± 1.1	1.5 ± 1.2	1.8 ± 1.0	-	1.1 ± 1.8
-	2	1.5 ± 0.5	0.5 ± 1.6	1.8 ± 0.9	1.9 ± 1.4	-	1.3 ± 0.8
-	3	1.6 ± 0.4	0.8 ± 0.8	1.9 ± 2.0	2.1 ± 2.4	-	1.2 ± 0.6
-	4	1.8 ± 1.1	0.9 ± 0.6	2.2 ± 1.5	2.2 ± 0.2	-	1.4 ± 1.0
-	5	2.1 ± 1.2	1.1 ± 1.3	2.4 ± 0.7	2.4 ± 1.3	-	1.6 ± 1.1
Ampicillin (10 μg)	-	-	-	-	-	-	-

Table 2. Preliminary phytochemical screening of A. officinarum with various solvents

Name of the Test	Aqueous	Methanol	Ethanol
Sugar	+	+	+
Catechin	+	+	+
Flavonoids	-	-	-
Saponin	+	+	+
Tannins	+	+	+
Amino acid	-	-	+
Carbohydrates	+	+	+

Table 3. GC-MS analysis of A. officinarum ethanolic root extract

Peak	RT	Area %	Compound name	Molecular weight	Molecular formula	Medicinal properties
1	4.089	0.27	2-FURANMETHANOL	98.10g/mol.	C5H6O2	No significant result
2	4.493	0.22	THREO-1,2-DIMETHYL-2-METHOXYCARBONYLAMINOETHYLVINYL SULFIDE	-	-	No significant result
3	4.75	0.53	1-Butanol, 2-amino-3-methyl-, (.+/-.)-	103.16g/mol	C5H13NO	No significant result
4	5.355	0.39	1-PYRROLIDINECARBOXAMIDE, N-1,3-HEXADIENYL-	-	-	No significant result
5	6.492	9.83	PROPANENITRILE, 3-(METHYLTHIO)-	101.17g/mol.	C4H7NS	No significant result
6	8.734	1.04	Thymine	126.11g/mol.	C5H6N2O2	Vitamin B1 deficiancy
7	10.042	1.03	BENZOIC ACID, 2,6-BIS(TRIMETHYLSILOXY)-, TRIMETHYLSILYL ESTER	-	-	No significant result
8	10.288	3.63	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.12g/mol.	C6H8O4	Antioxidant properties
9	12.188	11.13	5-Hydroxymethylfurfural	126.11g/mol.	C6H6O3	Antioxidant properties
10	12.57	0.56	DISULFIDE, PROPYL 1-(PROPYLTHIO)ETHYL	-	-	No significant result
11	13.45	1.23	PENT-4-ENOIC ACID	100.12g/mol.	C5H8O2	No significant result
12	13.975	5.99	Cyclohexasiloxane, dodecamethyl-	444.92g/mol.	C12H36O6SI6	Blood-handling equipment, as a blood defoaming agent, as protective barriers,

						lubricants and as surface treatment of wound dressings
13	15.609	1.87	Pyrogallol	126.11g/ mol.	C6H6O3	Topical antipsoriatic and antioxidant activity
14	16.952	4.49	1-PROPANOL, 2,2-DIMETHYL-	88.16g.mol.	C5H12O	No significant result
15	17.595	1.8	1,3-DIPHENYL-1-((TRIMETHYLSILYL)OXY)-1(Z)-HEPTENE	-	-	No significant result
16	20.01	3.18	Diethyl phthalate	194.18g/ mol.	C10H10O4	Antioxidant activity
17	20.58	3.9	n-Butyl nitrite	103.12g/ mol.	C4H9NO2	
18	20.84	0.68	1-(3,4-DITRIMETHYLSILOXYPHENYL)-2-ISOPROPYLAMINOETHANOL	-	-	No significant result
19	21.646	1.09	ACETAMIDE, N-[2-(ACETYLOXY)-2-(4-CHLOROPHENYL)ETHENYL]-	-	-	No significant result
20	22.015	0.4	4-HYDROXY-4-ISOPROPYL-5-METHYL-2-HEXYNYL ACETATE	212.28g/ mol.	C12H20O3	No significant result
21	22.188	1.06	3,9-DIOXA-2,8-DISILAUNDEC-6-ENE, 4-(2-FURANYL)-2,2,8,8,10-PENTAMETHYL-, (E)-	-	-	No significant result
22	23.644	0.25	(SS)- or (RR)-2,3-hexanediol	118.17g/ mol.	C6H14O2	No significant result
23	31.889	8.78	Tolazoline	160.22g/ mol.	C10H12N2	Gastrointestinal bleeding, peripheral vascular disease, raynaud's disease etc.
24	33.374	14.88	3-Heptanone, 5-hydroxy-1,7-diphenyl-	282.4g/mol.	C19H22O2	No significant result
25	36.206	1.08	3,3-DIMETHYL-1-HYDROXY-1-PHENYL-2-BUTANONE	-	-	No significant result
26	37.255	0.39	N-ALLYLOXYMETHYLACRYLAMIDE	-	-	No significant result
27	37.331	14.16	Gingerenone A	356.4g/mol.	C21H24O5	Antiproliferation, antiviral activity and antioxidant activity
28	37.565	5.29	Galangin	270.24g/ mol.	C15H10O5	Antimutagenic activity and Antioxidant properties
29	39.038	0.1	10,11-DIHYDRO-5(3(TRIFLUOROACETYLMETHYLAMINO)P	-	-	No significant result

			ROPYL)-5H-DIBENZ(B,F)AZEPINE			
30	39.625	0.75	METHANESULFONIC ACID, TRIFLUORO-, 1,3-PROPANEDIYL ESTER	-	-	No significant result

Figure 1. Zone of inhibition was obtained both bacterial and fungal strains comparison commercial of antibiotic disc.

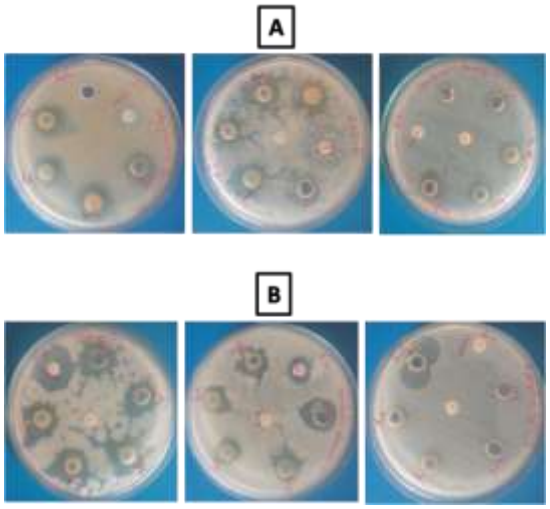
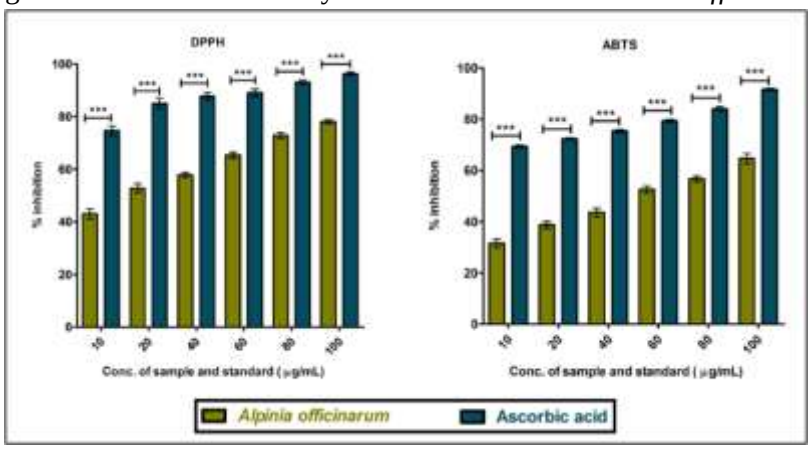


Figure 2. Antioxidant efficacy of ethanolic root extract of *A. officinarum*



Values denotes mean ± standard deviation (n =3). The mean difference is significant at the levels of *p < 0.05, **P < 0.01 and ***P < 0.001 Vs. standard. ns – non significant.

Figure 3. Active site binding interaction of breast cancer protein receptors (Akt-1, HER-2, COX-2 and PI3K) and plant compounds through surface view.

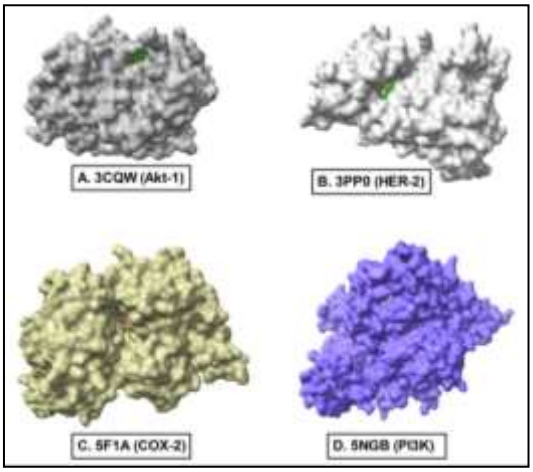
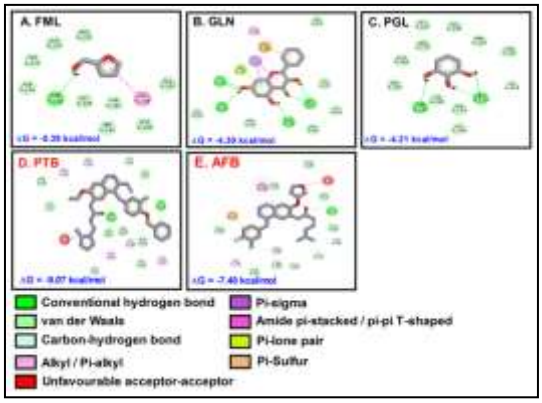
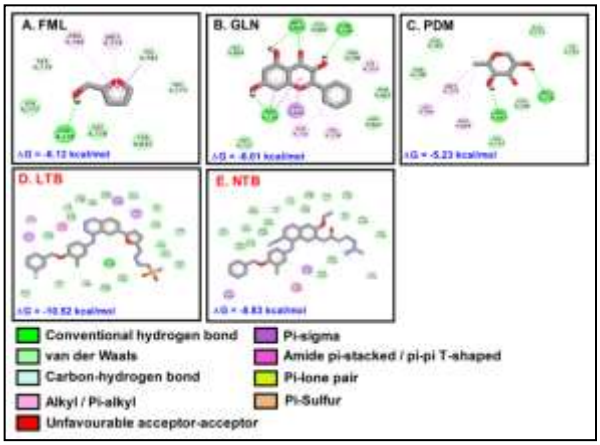


Figure 4. Protein receptor-ligand interactions between Akt-1 (3CQW) and phytocompounds obtained from A. officinarum.



Binding of FML (2-furanmethanol), GLN (galangin) and PGL (pyrogallol) was stabilized by hydrogen bonding of the ligands to Akt-1. Interestingly, the control drug pyrotinib shows higher - ΔG than plant compounds used for treating breast cancer.

Figure 5. Protein receptor-ligand interactions between HER-2 (3PP0) and phytocompounds obtained from root extract A. officinarum.



Binding of FML (2-furanmethanol), GLN (galangin) and PDM - 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl) was stabilized by hydrogen bonding of the ligands to HER-2. Interestingly, the control drug lapatinib shows higher - ΔG (-10.52) than phytochemicals.

Figure 6. Binding interaction between ligand (such as - 2-furanmethanol, GLN - galangin and TME - thymine) and breast cancer protein receptor (COX-2) along with control commercially available control drugs.

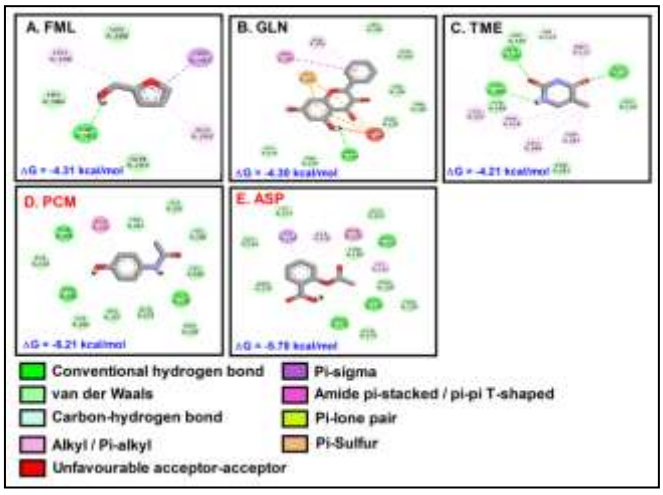
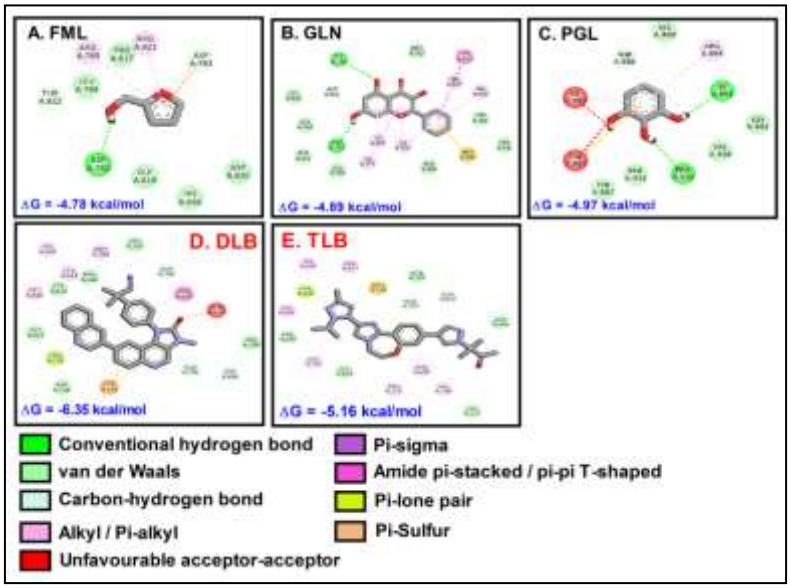


Figure 7. Protein receptor-ligand interactions between PI3K (5NGB) and phytocompounds obtained from root extract A. officinarum.



Binding of FML - 2-furanmethanol, GLN - galangin, PGL – pyrogallol was stabilized by hydrogen bonding of the ligands to PI3K. Interestingly, the control drug DLB (dactolisib) shows higher - ΔG (-10.52) than phytochemicals.

4. Conclusions

Based on the results, it has been accomplished that the ethanolic root extract of A. officinarum possess the significant antioxidant activity against both DPPH and ABTS free radicals. Moreover, the plant root extract confirmed modest antimicrobial activity both bacterial and fungal species. The preliminary qualitative phytochemical composition of the plant root extract was determined using preliminary screening protocols and the A. officinarum possessed saponins, flavonoids, catechins, tannins, and sugars. These compounds are responsible for the antioxidant and antimicrobial activities of the plant root extract. In silico study based on molecular docking analysis expose that FML (2-furanmethanol), GLN (galangin), PGL (pyrogallol) serve as Akt-1, HER-2, COX-2 and PI3K breast cancer receptors. These compounds may be control the breast cancer signaling pathways. In future studies could involve bioactivity, assimilatory and bioavailability studies that will encourage the efficacy of these phytochemicals as scaffolds for molecular dynamics and QSAR studies can concluded the conventional applications of A. officinarum. Therefore, the present preliminary investigation short out the traditional medicinal plant's antimicrobial and antioxidant profiles by mixture of in vitro and in silico techniques.

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APPENDICES

Table 1. Docking studies of *A. officinarum* phytochemicals

Protein Targets	Ligands	Binding energy	Ligand efficiency	inhib_constant (μM)	IE	VDE	EE
3CQW (Akt-1)	2-Furanmethanol	-5.39	-0.6	111.59	-6.29	-6.11	-0.17
	Galangin	-5.45	-5.9	92.18	-6.99	-7.34	-0.27
	Thymine	-5.52	-0.61	89.91	-5.52	-5.41	-0.11
	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	-4.87	-0.50	125.78	-5.89	-5.33	0.12
	Pyrogallol	-4.1	-0.54	212.17	-4.89	-4.75	-0.11
	Pyrotinib	-9.07	-0.45	0.024	-10.48	-10.28	0.0
	Afatinib	-7.48	-0.64	4.21	-8.26	-8.11	-0.0
3PP0 (HER-2)	2-Furanmethanol	-6.12	-0.61	98.12	-6.96	-6.88	-0.2
	Galangin	-6.01	-0.62	104.02	-6.77	-6.60	-0.22
	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	-5.23	-0.42	189.13	-5.88	-5.46	0.1
	Pyrogallol	-4.89	-0.61	2.56.78	-4.99	-4.85	-0.2
	Lapatinib	-10.52	-0.55	0.002	-11.56	-11.22	-0.1
	Neratinib	-8.83	-0.42	0.894	-9.87	-9.61	-0.2
5F1A (COX-2)	2-Furanmethanol	-4.31	-0.52	249.37	-5.62	-5.23	-0.25
	Galangin	-4.30	-0.50	256.37	-5.23	-5.10	-0.1
	Thymine	-4.21	-0.46	298.16	-5.23	-5.07	-0.2
	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	-3.95	-0.61	459.78	-4.69	-4.45	-1.0
	Paracetamol	-6.21	-0.62	15.26	-7.29	-7.11	-0.1
	Aspirin	-5.78	-0.45	18.24	-7.89	-7.56	-0.0
(PI3K)	2-Furanmethanol	-4.78	-0.48	313.55	-5.38	-5.31	-0.07
	Galangin	-4.89	-0.51	465.08	-5.44	-5.3	-0.14
	Pyrogallol	-4.97	-0.55	228.72	-4.97	-4.92	-0.05

5NGB	Dactolisib	-6.35	-0.48	23.18	-7.28	-7.13	-0.2
	Taselisib	-5.16	-0.55	20.78	-7.77	-7.16	-0.1

Table 2. Types of interactions and interacting residues of 3CQW (Akt-1) protein receptor involved in molecular docking of *A. officinarum* compounds and commercial controls drugs.

Types of interaction	**3CQW (Akt-1)**						
	2-Furanmethanol	**Galangin**	**Thymine**	**4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl**	**Pyrogallol**	**Pyrotinib**	**Afatinib**
Conventional hydrogen bond/Carbon hydrogen bond/Pi-donor hydrogen bond	Asp331	His207, Leu210, Leu202 and Leu213	Ala317, Thr312, Val330 and Gly311	Leu213, Leu202, Tyr474 and Arg206	Leu275, Thr312 and Gly334	Glu234, Phe161, Thr160, Gly157 and Gly162	Lys179, Lys158, Asp292, Glu234, Asp439 and Gly157
Van der waals	Arg273, Leu275, Tyr272, Asp274, Gly334, Tyr315, Trp333, Leu316 and Ala317	Ser478, Tyr474, Thr211, Lys289 and Gln203	Cys310, Val320, Asp331, Trp333, Gly334, Leu275, Arg275 and Asp274	Leu210, His207, Ser205, Thr211, Gln203, Ala476 and Lys214	Ala317, Val330, Trp333, Asp331, Arg273, Leu316, Gly311, Tyr315, Asp274 and Lys276	Lys163, Lys179, Lys158, Phe442, Gly159, Phe438, Tyr437, Asp439, Phe236, Asp292, Glu278 and Leu295	Tyr229, Ala230, Met227, Thr211, Thr291, Leu156, Phe438, Phe237, Phe236, Tyr437 and Gly159
Pi-lone pair/Pi-anion/ Halogen bond	Arg206 and Ser205	-	-	-	-	Asn279 and Asp274	Met281
Pi-sigma/ Alkyl/Pi – alkyl/Unfavourable acceptor-Acceptor	Ala282 and Ala478	-	Leu316 and Tyr315	Ala212	-	Leu156, Val164 and Phe237	Val164, Phe442 and Ala177

Table 3. Types of interactions and interacting residues of 3PP0 (HER-2) receptor docking with *A. officinarum* compounds and commercial controls drugs.

Types of interaction	**3PP0 (HER-2)**						
	2-Furanmethanol	**Galangin**	**Thymine**	**4H-pyran-4-one, 2,3-dihydro-**	**Pyrogallol**	**Lapatinib**	**Neratinib**

				3,5-dihydroxy-6-methyl			
Conventional hydrogen bond/Carbon hydrogen bond/Pi-donor hydrogen bond	Gly776	Met801, Gln799 and Leu726	Ser779 and Met774	Glu770 and Asp863	Asp863 and Glu770	Leu414, Tyr281, Ser441 and His468	Thr5 and Ala276
Van der waals	Ser779, Val782, Val777, Val773Gly778 and Tyr835	Gly804, Thr798, Leu800, Thr862, Asp863 and Gly727	Pro780, Gly778, Val777, Gly776, Leu785 and Val773	Ala771, Ile767, Ser783, Thr798, Leu796 and Lys753	Phe864, Lys753, Gly865 and Ile767	Gln2, Thr2, Asn466, Gln84, Asp8, Gly417, Ile413, Thr7, Gly36, Gly6, Gly411, Arg412, Asn280, Gln35 and Phe269	Gly417, Arg412, Leu414, Asn280, Gly411, Ser441, Gly442, Cys4, His468, Asn466, Thr1, Pro467, Val3, Phe269, Val274, Pro278 and Tyr279
Pi-lone pair/Pi-anion/ Halogen bond		-	-	-	-	-	-
Pi-sigma/ Alkyl/Pi – alkyl/Unfavourable acceptor-Acceptor	Arg784 and Met774	Leu852, Lys753, Ala751 and Val734	Arg784, Leu836, Val782 and Tyr835	Met774, Leu785 and Phe864	Met774, Leu785, Leu796 and Ala771	Leu201, Thr5, Pro278, Val3 and Cys4	Ile413, Tyr281 and Leu291

Table 4. Types of interactions and interacting residues of 5F1A (COX-2) receptor involved in molecular docking of *A. officinarum* compounds and commercial drugs.

Types of interaction	**5F1A (COX-2)**					
	2-Furanmethanol	**Galangin**	**Thymine**	**4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl**	**Paracetamol**	**Aspirin**
Conventional hydrogen bond/Carbon hydrogen bond/Pi-donor hydrogen bond	Tyr385	Asn382	Ser530, Ala527, Tyr385 and Val523	Trp387 and Thr206	Thr206, Tyr385 and Ala199	Asp125, Ser126 and Asn375
Van der waals	His388, His386 and Gln203	Leu390, Gln203, Tyr385, Thr206, Phe210, His214 and Thr212	Val349, Tyr348, Phe381 and Gly526	His386, His207, Gln203 and Phe210	Trp387, Leu391, His388, Leu390, Gln203, Phe200, His207, His386 and Phe210	Ile377, Ala151, Thr149, Pro128, Thr129, Gln374, Arg376 and

						Gly533
Pi-lone pair/Pi-anion/cation/ Halogen bond		His207	-	-	-	-
Pi-sigma/ Alkyl/Pi – alkyl/Unfavourable acceptor-Acceptor	Try387, Leu390 and Ala202	His386, Trp387 and Ala202	Met522, Leu352, Phe518, Leu384 and Trp387	Ala202, Tyr385 and Leu390	Ala202	Ile124, Phe529, Ala378 and Lys582

Table 5. Types of interactions and interacting residues of 5NGB (PI3K) receptor involved in docking of *A. officinarum* compounds and controls drugs.

Types of interaction	**5NGB (Pi3K)**				
	2-Furanmethanol	**Galangin**	**Pyrogallol**	**Dactolisib**	**Taselisib**
Conventional hydrogen bond/Carbon hydrogen bond/Pi-donor hydrogen bond	Asp782, Asp783 and Thr822	Lys779, Asp787 and Asp911	Ile893, Thr886 and Pro931	Gln795, Gln792 and Asp60	Gln795 and Gln610
Van der waals	Pro817, Leu784, Gly819, His656 and Asp820	Met752, Cys815, Leu784, Phe912, Leu791, Phe908, Ser831 and Trp760	His909, Gly892, Val930, Phe932 and Tyr887	Asp736, Gly834, Cys815, Phe646 and Leu791	Gln792, Asp606, Phe646 and Gly814
Pi-lone pair/Pi-anion/cation/ Halogen bond	-	Met900	-	Leu735 and Lys642	Met788 and Cys815
Pi-sigma/ Alkyl/Pi – alkyl/Unfavourable acceptor-Acceptor	Arg785 and Arg821	Tyr813, Val827, Val828, Ile910, Ile825 and Ile777	Ile983 and Cys883	Leu816, Leu613, Phe609 and Gln610	Pro817, His650, Leu816, Leu735, Pro173, Phe609 and Val799