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SYNERGISTIC EFFICACY OF THREE PLANT EXTRACTS, *BERGENIA CILIATA, ACORUS CALAMUS* AND *DIOSCOREA BULBIFERA* FOR ANTIMICROBIAL ACTIVITY

K. GOWRI SHANKAR *[1], ALBIN T FLEMING[1], R.VIDHYA[1], NAMRATA PRADHAN[1]

[1]Department of Advanced Zoology and Biotechnology, Loyola College, Chennai-600034, Tamil Nadu, India.

ABSTRACT

To evaluate the synergistic potency of three different plants namely rhizome of *Bergenia ciliata, Acorus calamus* and tuber of *Dioscorea bulbifera* in exhibiting the antibacterial activity, phytochemical evaluation and molecular docking analysis. 5mg/ml, Sample II and III acetone extract of plant mixture containing *A. calamus, B. ciliata* and *D. bulbifera* in the ratio 1:2:1 and 1:1:2 against *E. coli,* showed 12mm and 13mm, *P. aeruginosa* showed 13mm and 16mm and *B. subtilis* showed 23mm and 18mm zone of inhibition. To identify, the chemical compounds responsible for antimicrobial activity the acetone extract of sample II and III which was analysed using GC-MS and Molecular Docking. Compared to other metabolites alpha.-(N,N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone and Cis-Asarone are most potent metabolites which has lowest docking score against the receptors at various degrees. The results will be useful for further design of novel cancer and microbial inhibitors derived from synergistic plant extract.

KEY WORDS: **Preliminary Phytochemical Screening,Antimicrobial Activity,GC-MS Analysis, Synergy, Molecular Docking.**

K. GOWRI SHANKAR
Department of Advanced Zoology and Biotechnology, Loyola College, Chennai-600034, Tamil Nadu, India.

INTRODUCTION

Human beings have used plants for the treatment of diverse ailments for thousands of years[1,2] According to the World Health Organization, 80% populations still rely on traditional medicines for their psychological and physical health requirements[3], since they cannot afford the products of western pharmaceutical industries[4], together with their side effects and lack of healthcare facilities[5]. Rural areas of many developing countries still rely on traditional medicine for their primary health care needs and have found a place in day-to-day life[6]. People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses[7,8]. Herbal medicines that are extracted from different plant parts such as roots bark seeds, leaves and flowers contain a variety of naturally-occurring bio-chemicals, which contribute to the plant's medicinal benefits mostly against microorganisms[9]. The crude plant extracts of herbal plant in the form of decoction, tincture, infusion or herbal extract are traditionally used for the treatment of many diseases[10]. Chemical metabolites which have the ability to target two essential bacterial such as DNA Gyrase and DNA topoisomerase IV for antibacterial property. Gyrase controls DNA supercoiling and relieves topological stress arising from the translocation of transcription and replication complexes along DNA; topoisomerase IV is a decatenating enzyme that resolves interlinked daughter chromosomes following DNA replication of prokaryotes. Since both enzymes are required for cell growth and division[11]. Deaths from cancer worldwide are projected to continue to rise to over 13.1 million in 2030. Apoptosis or cell suicide is a highly regulated process that occurs in almost all living cells. It involves the activation of a series of molecular events, leading to cell death that is characterized by cellular, morphological and biochemical changes. These include cell shrinkage, chromatin condensation and nuclear fragmentation, membrane blebbing, caspases activation, and the formation of membrane bound vesicles termed as apoptotic bodies[1213]. Caspases are cysteine-aspartic proteases families which play an important role in apoptosis, necrosis and inflammation. Caspases are regulated at a post-translational level ensuring that they can be rapidly activated. The caspases implicated in apoptosis can be further divided into two functional subgroups based on their known or hypothetical roles in the process: initiator caspases (caspases-2, -8, -9, and -10) and effector caspases (caspases-3, -6, and -7). The caspases that have been well described are caspases-3, -6, -7, -8 and -9[14,15]. Bioinformatics and computational biology have the potential not only to hasten the drug discovery process and to decrease the costs, but also to change the advance of drug designing. This plant-based, traditional medicine system continues to play an essential role in health care, they play dual role in the development of new drugs: (1) they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; (2) a phytomedicine to be used for the treatment of diseases. Therefore, such plants should be investigated to better realize their properties, safety and usefulness. In this present study we used the rhizome of the plants *B. ciliata, A. calamus* and the tuber of *D. bulbifera*.The preliminary phytochemical testing of *B. ciliata* rhizome showed the presence of high amount of phenolics and tannins along with flavonoids. The amount of total phenolics was found to be 5.78% (w/w)[23]. Phytochemical test performed on rhizome extract of *Acorus calamus* showed the presence of phytochemicals such as carbohydrates, reducing sugars, proteins, amino acids, flavonoids, steroids, anthraquinone glycosides, alkaloids and tannins and absence of non-reducing polysaccharides and cardiac glycosides[42]. Similarly, *D. bulbifera* tuber extract was found to be rich in phenolic content. Total reducing sugar was also found to be comparatively high (up to 3.41 mg/mL), followed by starch. *Dioscorea bulbifera* is known to contain a saponin known as diosgenin, 26µg/mL in *D. bulbifera* tuber extract[43]. The aim of the study is to examine the synergistic efficacy of three plant extracts namely, *B. ciliata, A. calamus* and *D. bulbifera* for antimicrobial activity. GCMS analysis of high antimicrobial extracts was identified for chemical composition and to analyze them against the major apoptotic receptors caspase-3 (1QX3) and caspase-9 (1JXQ) as a target and to inhibit DNA topoisomerase IV (4EMV) as antibiotics using AutoDock Vina.

MATERIALS AND METHODS

Plant materials

The rhizome of *B. ciliata*, *A. calamus* and tuber of *D. bulbifera* was used in this study were collected from various regions of West Bengal. Plants were identified by Prof. P Jayaraman, Ph.D (Director) at Plant Anatomy Research Centre, west tambaram, Chennai. The plant material was dried in shade.

Preparation of plant extracts

The dried and powdered plant materials were extracted with chloroform, acetone and methanol to afford corresponding extracts. Preparation of extracts from the plant samples are shown in Table 1. Solvents were evaporated under reduced pressure and stored at -4°C for further use and the weights of the extracts are in Table 2.

Phytochemical analysis

Preparation of aqueous extracts for screening phytochemical constituents based on Table 1 and carried out using standard methods[16-19].

Micro-organisms

Clinical isolates of the microorganisms were used along study are *E. coli, P. aeruginosa* and *B. subtilis* were obtained as a gift sample from Dr. S. Karthick Raja Namasivayam, Department of Biotechnology, Sathyabama university, Chennai, Tamil Nadu, India.

Determination of Antibacterial activity

The inoculum of microorganism was prepared from pure culture[20]. The antibacterial activity of plant extract was determined by agar well Diffusion Method of Muller Hinton Agar (MHA) medium[21]. The Muller Hinton Agar

medium is poured into the petriplates, after solidification the inoculums, were spread on the solid plates with sterile swab moistened with the bacterial suspension. The crude extracts were dissolved in Dimethyl sulphoxide (DMSO) and extracts were loaded on the 6 mm diameter well which was made using a sterile cork borer. The extracts were placed in 6mm diameter well with the different concentration (1.25, 2.5 and 5mg/mL). The standard 6mm diameter disc with piperacillin (100mcg/disc), spectinomycin (100mcg/disc) and gentamicin (120mcg/disc) was used as a positive control and Dimethyl sulphoxide as a negative control for antibacterial activity.

GC-MS analysis

Gas Chromatography - Mass Spectrometry has become one of the most important analytical tool. The use of it has spread to a large number of areas such as molecular physics, chemistry, biology and medicine. One of the important analytical tool is shimadzu GCMS QP 2010 Plus. A Volume of 1mL of clear extract was injected into GCMS (shimadzu GCMS QP 2010 Plus) with an oven programming of 100ºC (1min), 240ºC (1 min) and 280ºC (3min). The components were separated using helium (1ml/min) as carrier gas. The GC initial injector temperature was maintained at 260ºC and interface temperature was maintained at 280ºC. The split ratio was set as 6. The equilibrium time was set at 3min. the column flow rate was adjusted to 1.9mL/min and column inlet pressure of 136.6 kPa. The Start Time 2.50min and End Time 33.0 min and the Start mass scan was set to 50.00 m/z and End at 650.0m/z with a Scan Speed of 2000. Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC – MS compounds present in the plant extracts were identified.

Docking methods

The 3-D crystal structure of the targeted apoptotic cancer receptor caspase-3 (ID: 1QX3) and caspase-9 (ID: 1JXQ) and microbial protein DNA topoisomerase IV (ID: 4EMV) was retrieved from the protein data bank (PDB) (www.rcsb.org/pdb). Structural and active site studies of the protein were done by using CASTP (Computed Atlas of Surface Topography of Proteins) and PyMOL molecular visualization software. All the chemical metabolites were screened against the proteins. The PubChem and ChemSpider database was used for retrieving the structure of ligand molecules. The selected chemical structures were generated from SMILES notation (Simplified Molecular Input Line Entry Specification) by using the Chem3D pro 12.0.2.1076. The molecular docking was performed using AutoDock Vina is an open source program for doing molecular docking[22]. The ligand and target proteins were geometrically optimized and docked using docking program AutoDock Vina.

RESULT AND DISCUSSION

With the phenomenal increase in the demand for herbal medicine in the last two decades, a need has been felt for ensuring the quality, safety and efficacy of the herbal drugs. Table 1 shows Sample preparation for extraction. Dry weight and percentage of plant extracts are shown in Table 2.Phytochemical evaluation is one of the tools for quality assessment, which includes preliminary phytochemical screening[23]. *A. calamus* was found devoid of tannins but since tannins are present in *B. ciliata* and *D. bulbifera*, all the four combination samples detected positive for tannins. The crude water extract showed the presence of tannins in *B. ciliata*[24]. Quinones are absent in *A. calamus* and *D. bulbifera* but strongly present in *B. ciliata* due to which Quinones was found to be there in all four combination samples namely Sample I, II, III and IV. Flavonoids are present in all samples except *B. ciliata* shown in Table 3.Aqueous extract on rhizome of Acorus calamus was subjected to preliminary analysis. Phytochemical test revealed the presence of coumarins, flavonoids and alkaloid[25]. When test was carried out for the detection of Anthocyanins and Beta-cyanins, all the samples were found to possess Beta-cyanins except for *B. ciliata* which possessed none. Saponins, Alkaloids and Coumarins were present in all the samples tested, be it the combination of all the three plants or the individual plants. The presence of alkaloids by phytochemical analysis of hydro-alcoholic extract of the tubers of *D. bulbifera* was revealed[26]. The aqueous extract of *A. calamus* was prepared and subjected to phytochemical analysis. It revealed the presence of alkaloids, flavonoids, saponins[27]. On the basis of phytochemical analysis of *A. calamus* rhizome using water as solvent[28] were found to be presence of alkaloid, flavonoids and saponins and were positive in rhizome extract of *A. calamus.* When the different samples and their combinations were tested for anti microbial activity against *Escherichia coli,* maximum zone of growth inhibition was observed in Sample I acetone solution, where *B. ciliata*, *A. calamus* and *D. bulbifera* were combined, *B. ciliata* being in higher concentration than the other two plants. This zone of growth inhibition was 16mm. Thus it was seen that the combination of all three with *B. ciliata* being in higher concentration produced a greater zone of growth inhibition than when they were tested separately. This point out that synergistic action produced greater activity against *E. coli* by increasing the activity of *B. ciliata* by the other two plants as *B. ciliata* is in higher concentration in Sample I are shown in Table 4.However, the methanolic extract of rhizome of *Acorus calamus* exhibited inhibitory activity against *E. coli* with inhibition zones of 6.8 mm[29]. Among the three plants, maximum activity against *E. coli* was witnessed for *B. ciliata* especially the acetone extract among the all the extracts taken. Sample I, which contains all the plants with *B. ciliata* being in higher concentration than the other two plants, was proved to be the best combination as it produced activity against *E. coli* with chloroform, acetone as well as methanol extracts, that too for all extract concentrations unlike other combinations are shown in Table 4. It was evaluated the antibacterial activity of *B. ciliata* extracts by cup diffusion method using *E. coli* .The rhizome extract showed activity against tested microorganism[30].On the other hand, it was seen that when acetone extract of *A. calamus* was tested against *P. aeruginosa* there was no activity found but activity was found in acetone extract of Sample II where *A. calamus* is present in higher concentration than the other two plants. The zone of growth inhibition obtained

were 11mm, 12mm and 13mm for 1.25mg/ml,2.5mg/ml and 5mg/ml concentration of extract are shown in Table 4. This shows how the presence of all three plants produced increased activity probably because of induction of *A. calamus* to produce activity as *A. calamus* is in higher concentration than the other two plants in Sample II. The methanolic extract of *A. calamus* showed the inhibitory action against *P. aeruginosa*[31]. The antimicrobial activity of methanolic extract of bark samples of mangrove species *Avicennia officinalis* (L.), *Sonneratia alba* (Smith), and *Rhizophora mucronata* (Pior) against *Pseudomonas aurginosa* showed zone of inhibition (mm) 16.33±0.57, 34.66±0.70 and 36.66±0.57[41].The evaluations antibacterial activity of *A. calamus* rhizomes in-vitro shows different concentrations of petroleum ether extract (50-2000µg) were tested against bacteria. Among the four types of bacteria tested, high inhibition zone was observed on *P. aeruginosa* 1.62cm followed by *S. aureus* 1.66cm. *E. coli* and *B. subtilis* showed smaller zone of inhibition 1.34cm and 1.04cm respectively[32]. The antibacterial activity of the petroleum ether, chloroform, methanol and aqueous extracts of rhizome of *A. calamus* by the agar well-diffusion method had a maximum activity was seen in chloroform extract with maximum zone of inhibition as 25mm for *P. aeruginosa*[33].Thus the overall observations show that the acetone extracts of the combination of all the three different plants produce good results against *B. subtilis*. Even though it did not show any activity when tested individually except for *B. ciliata*, when combined it displayed activity at all combinations be it Sample I, II or III. Among the three different plant concentrations, *B. ciliata* showed maximum activity against *B. subtilis* as it showed activity in acetone and methanol extracts in all concentrations. The extracts that showed the most effectual result for antibacterial studies were acetone extracts of Sample II and III. These two extracts were then subjected to GCMS analysis to find the compounds present in them. GCMS analysed that the compound present in acetone extract of Sample II is alpha.-(N,N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone [2-(Dimethylamino)-1-(3-hydroxy-4-methoxyphenyl] shown in Figure 1. There were six compounds found in acetone extract of sample III by GCMS analysis shown in Figure 2 which are Asarone [(E)-2,4,5-Trimethoxypropenylbenzene], Azaron [Benzene, 1,2,4-trimethoxy-5-(1-propenyl)] , Benzene, 1,2,4-trimethoxy-5-(1-propenyl) [cis-Asarone] , 1-Hydroxysulfonyl-3,4,4-trimethyl-2-azetidinone , Myristic acid [n-Tetradecanoic acid] and Linoleic acid ethyl ester [Ethyl linoleate]. Asarone was the compound found in majority by the GCMS analysis. The rhizome extract of *A. calamus* was analyzed by GC-MS and the compounds identified were β-asarone, cis-asarone, or cis-1, 2,4-trimethoxy-5-(1-propenyl)-benzene[34].The rhizome and leaf oils of *A. calamus* was analyzed[35] by GC and GC–MS; 29 and 30 constituents were identified from the rhizome and leaf oils, respectively, comprising 99.7% of each of the oils β-Asarone (83.2%) and α-asarone (9.7%) were the major constituents in the rhizome oil, while β-asarone (85.6%) and linalool (4.7%) were the major constituents in the leaf oil. The GCMS analysis conducted for crude ethanol extract of *A. calamus* rhizomes. It was found that one purified fraction contained β-Asarone as a major component according to GCMS analysis. This fraction showed antibacterial property[36]. Thus, this indicates that these compounds present in the acetone extracts of sample II and III, analysed by GCMS analysis, maybe responsible for the effective synergistic antibacterial activity observed. The goal of ligand-protein docking is to predict the predominant binding model(s) of a ligand with a protein of known three dimensional structures[37]. The binding affinity of the receptors with the ligands was measured by kcal/mol. The docking scores for alpha.-(N,N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone ligand binding to cellular receptors like caspase-3 with -6.4 kcal/mol and caspase-9 with -4.4 kcal/mol as a target. And to inhibit DNA topoisomerase IV with -4.6 kcal/mol for alpha.-(N,N-Dimethylamino)-3'hydroxy4'methoxyacetophenone are shown in Table 5 and Figure 3. The docking score of Cis-Asarone from Sample III Acetone extract binds to 1JXQ, 1QX3 and 4EMV receptors with a binding energy of -6.1, -4.8 and -5.2 Kcal/mol are shown in Table 7 and Figure 4. In Table 7 and in Figure 5 (A) shows the 4EMV receptor binds to Hydroxysulfonyl-3,4,4-trimethyl-2-azetidinone with -4.9 Kcal/mol, in Figure 5 (B) and (C) 1JXQ receptor binds to Myristic acid and linoleic acid ethyl ester with -5.3 Kcal/mol to the amino acids LYS 396, ILE 154, ALA 149, ALA 152 and -6.0 Kcal/mol to the amino acid LEU 155. The results obtained from this study would be useful in both understanding the inhibitory mode as well as in rapidly and accurately predicting the activities of new inhibitors on the basis of docking scores[38-40].

Table 1
Sample preparation for extraction

Samples	*Bergenia ciliata* (rhizome) (gm)	*Acorus calamus* (rhizome) (gm)	*Dioscorea bulbifera* (tuber) (gm)	Total (gm)
Sample I	10	5	5	20
Sample II	5	10	5	20
Sample III	5	5	10	20
Sample IV	5	5	5	15
Sample V P1	20	-	-	20
Sample V P2	-	20	-	20
Sample V P3	-	-	20	20

Table 2
Dry weight and percentage of plant extracts

Solvents (150mL)	Dry weight and percentage of plant extracts (gm)						
					Sample V		
	Sample I	Sample II	Sample III	Sample IV	Bergenia ciliata (rhizome) (P1)	Acorus calamus (rhizome) (P2)	Dioscorea bulbifera (tuber) (P3)
Chloroform	1.337 (6.68%)	1.175 (5.87%)	0.91 (4.55%)	0.864 (5.76%)	0.143 (0.71%)	0.696 (3.48%)	0.15 (0.75%)
Acetone	1.054 (5.27%)	2.287 (11.43%)	1.72 (8.6%)	2.591 (17.27%)	2.238 (11.19%)	0.776 (3.88%)	2.84 (14.2%)
Methanol	2.391 (11.95%)	1.887 (9.43%)	5.689 (28.44%)	6.847 (45.64%)	4.326 (21.63%)	2.911 (14.55%)	0.888 (4.44%)

Table 3
Phytochemical analysis of different plant samples from aqueous extract

Test for Phytochemicals	Sample I	Sample II	Sample III	Sample IV	Sample V (P1) (*Bergenia ciliata*)	Sample V (P2)(*Acorus calamus*)	Sample V (P3) (*Dioscorea bulbifera*)
Tannins	++	+	+	+	+	-	+
Saponins	+	+	+	+	++	+	+
Quinones	+	+	+	+	+	-	-
Flavonoids	+	+	+	+	-	+	++
Alkaloids	+	+	+	+	+	+	+
Glycosides	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-
Terpenoids							
Phenols	-	-	-	-	-	-	-
Coumarins	+	+	+	+	++	-	+
Steroids	+	+	+	+	+	+	++
Anthocyanin	-	-	-	-	-	-	-
Beta-cyanin	-	-	-	-	-	-	-
	+	+	+	+	-	+	+

"+++" abundant, "++" moderately present, "+" present, "-" Absent

Table 4
Antibacterial activity of Chloroform, Acetone and Methanolic extracts.

Solvents	Concentration (mg/mL)	Zone of Inhibition (mm)																					
		Escherichia coli							*Pseudomonas aeruginosa*							*Bacillus subtilis*						
		Sample I	Sample II	Sample III	Sample IV	Sample V			Sample I	Sample II	Sample III	Sample IV	Sample V			Sample I	Sample II	Sample III	Sample IV	Sample V		
						P1	P2	P3					P1	P2	P3					P1	P2	P3
Chloroform	1.25	9	-	-	-	-	-	-	-	9	-	-	9	9	-	-	9	-	8	-	-	-
	2.5	10	-	-	-	9	-	-	-	10	9	-	10	10	9	8	10	-	9	-	-	-
	5	12	-	-	-	10	-	9	-	11	10	9	12	12	11	9	12	-	11	-	-	-
Acetone	1.25	-	13	11	11	11	-	-	11	12	11	12	12	-	-	11	12	12	-	12	-	-
	2.5	11	15	12	13	13	-	9	12	14	15	14	16	-	-	13	16	16	-	14	-	-
	5	12	16	13	15	14	9	10	13	15	16	16	18	-	-	15	23	18	-	16	-	-
Methanol	1.25	11	-	11	10	-	9	-	11	10	12	11	12	9	8	11	10	-	-	14	-	-
	2.5	12	9	13	11	-	10	9	15	11	13	14	13	10	9	14	18	-	-	16	-	-
	5	13	10	14	12	-	11	11	17	12	14	16	14	11	10	16	21	-	-	18	-	-

"-" no zone of inhibition

Table 5
Microorganism Positive Control analysis.

Antibiotics	Zone of growth inhibition (mm)		
	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtilis*
Pipercillin	13	11	-
Gentamycin	20	37	36
Spectinomycin	13	21	-

"-" no zone of inhibition

TABLE 6
GC-MS and Molecular Docking analysis of Sample II Acetone extract.

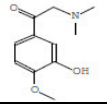
Peak	Compound Name	Chemical formula	Molecular weight	Retention time	Peak Area %	Chemical structure	1JXQ			1QX3			4EMV		
							Binding energy kcal/mol	Hydrogen bonds	Amino acid residues	Binding energy kcal/mol	Hydrogen bonds	Amino acid residues	Binding energy kcal/mol	Hydrogen bonds	Amino acid residues
1.	alpha.-(N,N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone	$C_{11}H_{15}NO_3$	209	10.183	100.00		-6.4	5	TRP 360, ARG 372	-4.4	3	TRP 214, ARG 207	-4.6	3	ASN 51, ASP 78

TABLE 7
GC-MS and Molecular Docking analysis of Sample III Acetone Extract.

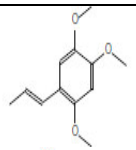
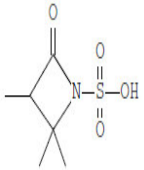
Peak	Compound Name	Chemical formula	Molecular weight	Retention time	Peak Area%	Chemical structure	1JXQ			1QX3			4EMV		
							Binding energy kcal/mol	Hydrogen bonds	Amino acid residues	Binding energy kcal/mol	Hydrogen bonds	Amino acid residues	Binding energy kcal/mol	Hydrogen bonds	Amino acid residues
1.	Cis-Asarone	$C_{12}H_{16}O_3$	208	5.058	89.05		-6.1	3	ALA 149, LYS 396, ILE 154	-4.8	2	SER 251, TYR 204	-5.2	2	THR 172, ASP 78
2.	Hydroxysulfonyl-3,4,4-trimethyl-2-azetidinone	$C_6H_{11}NO_4S$	193	5.900	1.20		-5.8	6	GLY 267, GLY 275, SER 325, LYS 278, ASP 326	-4.3	4	MET 39, TYR 37, HIS 277	-4.9	5	ASP 23, LYS 154, GLY 152
3.	Myristic acid	$C_{14}H_{28}O_2$	228	7.283	1.71		-5.3	4	LYS 396, ILE 154, ALA 149, ALA 152 LEU 155	-4.3	4	PHE 250, GLU 248, TRP 214, ASN 208	-3.8	2	ASN 51, ASP 78
4.	Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	8.192	5.14		-6.0	3		-4.2	4	ASN 208, ARG 207, SER 209	-	-	-

Figure 1
GC-MS chromatogram of Sample II Acetone extract

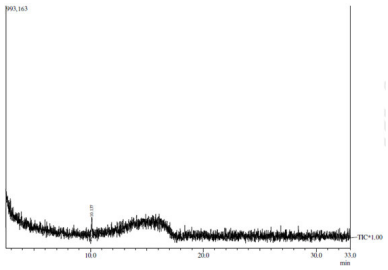


Figure 2
GC-MS chromatogram of Sample III Acetone extract

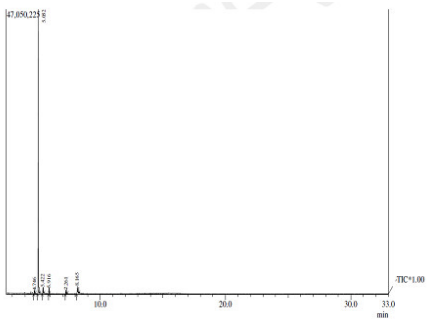
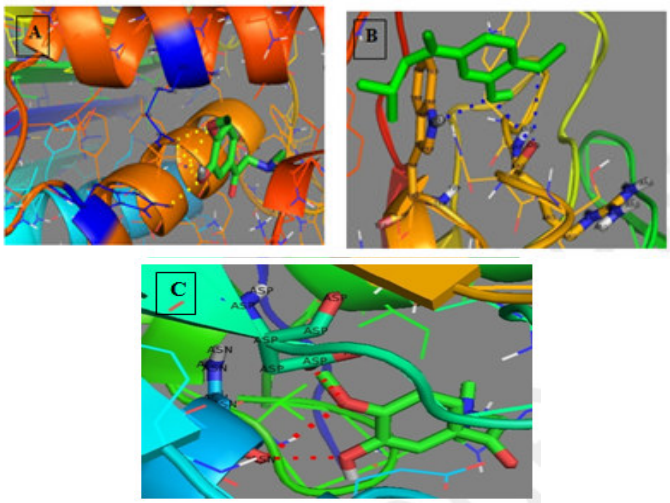
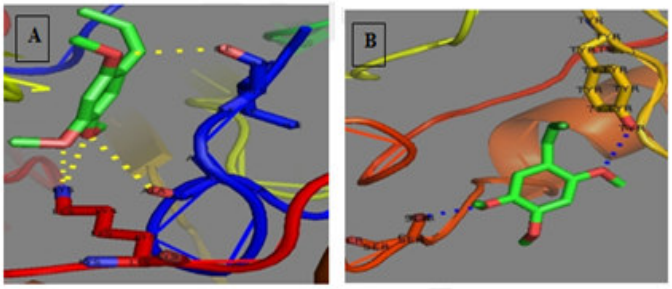


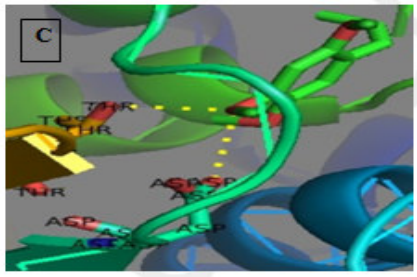
Figure 3
Molecular Docking analyses of alpha.-(N, N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone from Sample II Acetone extract to receptors



In Figure 3: (A) 1JXQ receptor binds to alpha.-(N,N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone with -6.4 Kcal/mol, (B) 1QX3 receptor binds to alpha.-(N,N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone with -4.4 Kcal/mol, and (C) 4EMV receptor binds to alpha.-(N,N-Dimethylamino)-3'-hydroxy-4'-methoxyacetophenone with -4.6 Kcal/mol.

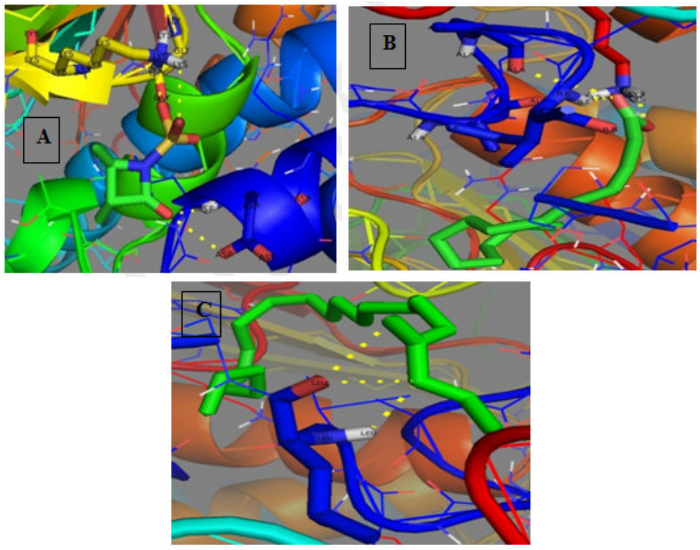
Figure 4
Molecular Docking analyses of Cis-Asarone from Sample III Acetone extract binds to receptors





In Figure 4: (A), (B) and (C) Cis-Asarone from Sample III Acetone extract binds to 1JXQ, 1QX3 and 4EMV receptors with a binding energy of -6.1, -4.8 and -5.2 Kcal/mol

Figure 5
Molecular Docking analyses of compounds from Sample III Acetone extract binds to receptors.



In Figure 5: (A) 4EMV receptor binds to Hydroxysulfonyl-3,4,4-trimethyl-2-azetidinone with -4.9 Kcal/mol, (B) and (C) 1JXQ receptor binds to Myristic acid and linoleic acid ethyl ester with -5.3 and -6.0 Kcal/mol.

CONCLUSION

Preliminary phytochemical analysis of aqueous extracts of three plant samples, *B. ciliata, A. calamus* and *D. bulbifera* estimated the presence of tannins, saponins, quinones, flavonoids, alkaloids, phenols, coumarins and beta cyanins. Antibacterial estimation proved that synergistic effects of the three plant extracts are more effective than individual plant samples for antibacterial activity. The current study supports the combination extracts with the concentration of acetone extracts of sample II and III that produced most effectual result for antibacterial activity against *E. coli, P. aeruginosa* and *B. subtilis* when compared with the individual samples. The GCMS and Molecular docking study shows the potential binding activity of the compounds present in the acetone extract of sample II and III against three receptors. In future compound isolation can be carried out to find the structural changes to the Asarone and its synergistic activity with other compounds. Thus this work could be extended to *In vitro* & *In* vivo studies which would serve as an important discovery in the field of anticancer drug design because these functional group compounds binds to caspases receptor.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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