

GC-MS analysis and Molecular docking of Quercetin compounds of *Phytolacca octandra* with the target protein of infection causing *Staphylococcus aureus*

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Abstract

Phytolacca octandra is a perennial usually about 1m high herb, dense and erect in full sun. As only few reports were available on the studies about the bioactive compounds and various activities in the *Phytolacca octandra*, the present study focuses on the bio active compounds attributed to antibacterial activity in the plant extracts by Gas Chromatography - Mass Spectrometry (GCMS) and molecular docking methods. Antibacterial activity of *Phytolacca octandra* showed maximum inhibitory zones of 21mm, 18mm, 19mm, 19mm and 20mm against respective organisms for 25mg/ml of acetone extracts. The outcome of *Phytolacca octandra* extracts that was exposed to GC-MS analysis, showed the presence of 20 more compounds. The most identified compounds to have anti-oxidant activity are Dodecane, Octadecane and Octacosane. The other major compounds present in extract are Cyclohexen-oxopropyl, 1,2-Benzenedicarboxylic acid. The overall docking energies of the target protein, rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase with quercetin with the number of hydrogen bonds were presented in the study; The docking report revealed -8.01Kcal/Mol binding energies and 8 hydrogen bonding between the *Phytolacca octandra* compound, quercetin and the target binding protein, rhlG of infection causing pathogen *Staphylococcus aureus*.

Keywords: *Phytolacca octandra*, Antibacterial activity, Bio-active compounds, Gas Chromatography - Mass spectrometry (GCMS), Molecular docking

INTRODUCTION

The world's oldest pharmacological and therapeutic writings came from India and China. Pharmacognosy also is vastly updated by the incorporation of phytochemical

methods. The characters more often studied in chemotaxonomy are secondary metabolites of pharmaceutical significance such as alkaloids, glycosides, flavonoids (Kokate et al., 2004). Modern aspects of pharmacognosy include not only the crude drugs but also their natural derivatives.

Such plants due to the presence of bio active compounds possess various activities which can obtain a vital role in pharmaceutical industry (Daniel, 1991). Likewise, Members of the family Phytolaccaceae consist of various plants containing significant medicinal values.

The family Phytolaccaceae consists of 17 genera and 125 species. *Phytolacca* is the largest genus of the family with 35 species. Phytolaccaceae is commonly known as Pokeweed family. The name Phytolaccaceae Lindl are conserved over Petiveriaceae Link. *Phytolacca octandra* is a native of tropical America (Lawrence, 1974). *Phytolacca octandra* is a perennial herb, usually about 1m high with a similar spread, which may be sprawling and open in shade or more compact, dense and erect in full sun. Stems are smooth and green or reddish. It has been introduced long ago to Kodaikanal and Nilgiri Hills in India. Now it seems to be restricted to Kodaikanal, Ooty and Munnar in South India, as a common and abundant wayside weed (Sivarajan VV and Indu, 1987).

Phytolacca species have been found to have some important medicinal uses as well. The tincture of *Phytolacca decandra* L. was reported as a remedy for cancer in America and widely used in treating some form of chronic rheumatic complaints (Sauer 1950). The roots are reported to be emetic and cathartic. Analysis of aqueous extracts of *Phytolacca americana* L. showed the presence of seven phenolic compounds namely, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, m-hydroxybenzoic acid, coumaric acid and cinnamic acid (Wu, 2007). The use of *Phytolacca* as a narcotic and medicine has attracted the attention of phyto-chemists, for the last several

decades and a formidable number of bioactive compounds have been detected (Steinmetz 1960).

The phytotoxic effects of the species, *Phytolacca esculenta* Van, *Phytolacca insularis* L. and *Phytolacca americana* L. were different, even though the levels of total phenolic compounds were similar. They can be distinguished by their allelopathic potential and morphologies (Yong et al., 2005). Strong anti-inflammatory saponins were isolated from callus mass derived from stems and roots of *Phytolacca americana* L. Triterpenoids saponins such as Phytolaccoside A, B and D were also reported (Hung and Hyun 1985).

In our previous studies phytochemical compounds and anti-oxidant properties of this plant extract was presented extensively (Vithya et al., 2000). Since only few reports were available on the studies about the bioactive compounds and various activities in the *Phytolacca octandra* In this present study we focused on the significant bio active compounds present in the plant extracts by Gas Chromatography - Mass Spectrometry (GCMS) and antibacterial activity, and Molecular docking of target protein with extracted compounds from plants of *Phytolacca octandra*.

MATERIALS AND METHODS

Collection of plant materials and preparation of extract: *Phytolacca octandra* plant

Phytolacca octandra herbal leaf powder was commercially procured from a local supplier at Coimbatore, Tamil Nadu, India. About 50g of *Phytolacca octandra* herbal leaf powder was extracted with 125mL of acetone separately using Soxhlet

extraction apparatus for 3 days. The extract was evaporated to dryness using rotary flash evaporator. Similarly, petroleum ether and aqueous extracts were also prepared. From the resultant herbal extract, 5 different concentrations (5mg/l, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml) were prepared to evaluate the antibacterial and antifungal efficacy. All the five concentrates of herbal extract was stored in the refrigerator prior to use.

Antimicrobial activity of the plant extract using agar-well diffusion method

Bacterial cultures used

Two bacterial cultures, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* which have the ability to cause hospital-acquired infections were commercially procured from a diagnostic laboratory at Coimbatore, Tamil Nadu, India.

The antibacterial activity of *Phytolacca octandra* leaves extracts were evaluated against the significant organisms (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae*) by well diffusion method. About 0.1% inoculum suspensions of five bacterial cultures were swabbed uniformly over each agar plate surface. Under sterile conditions, 6mm wells were cut on the agar surface of each Nutrient Agar (NA) plates.

About 50µl each of *Phytolacca octandra* leaves extracts, in 5% dimethyl sulfoxide (DMSO) were loaded into the well and the plates were incubated at 37°C for 24 - 48h. The antibacterial activity was evaluated in terms of zone of inhibition

around the wells of each extract in all the inoculated NA plates. The inhibition clear zones were measured and recorded in millimeter.

GC-MS analysis of the extract to identify the antimicrobial compound

The present study characterized the chemical profile of *Phytolacca octandra* using GC-MS. The consequences concerning to GC-MS investigation led to the recognition of lot of compounds from the GC fractions of the column purified extract of *Phytolacca octandra*. These compounds were acknowledged through mass spectrum attached with Gas chromatography. The active principles with their retention time (RT), molecular formula (MF), and molecular weight (MW) are graphically illustrated in the present research. The GC chromatogram shows the relative concentration of various compounds getting eluted as a function of retention time. The heights of the peak point out the relative concentration of the presented components. The mass spectrometer analyzed the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are figure print of that compound which is identified from the National Institute of Standards and Technology (NIST) data library.

Molecular docking of target protein with extracted compounds from plants

Quercetin is the strong antibacterial compound reported to be present in the extracts of *Phytolacca octandra*. In the present study, the molecular docking of Quercetin against the target protein of infection causing pathogen *Staphylococcus aureus* was investigated using bioinformatics tools.

RESULTS AND DISCUSSION

Antimicrobial activity of the plant extract using agar-well diffusion method

The antibacterial activity of *Phytolacca octandra* extracts against five test bacteria, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* was evaluated by agar diffusion method. Five different concentrations of acetonic extracts of *Phytolacca octandra* was used to determine the antibacterial activity against each organism. The results obtained in this study indicated that *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* tested against the acetone extract of *Phytolacca octandra* showed maximum inhibitory zones of 21mm, 18mm, 19mm, 19mm and 20mm for 25mg/ml concentration respectively. The acetoneic extract of subsequent concentrations 20mg/ml and 15mg/ml also showed promising inhibitory zones during the analysis against all the test organisms. Inhibitory zones measuring 17mm, 14mm, 15mm, 17mm and 15mm were obtained for 20mg/ml of plant extracts against the respective organisms as mentioned above. Similarly, for 15mg/ml of plant extracts, 13mm, 12mm, 12mm, 11mm and 13mm of inhibitory zones were obtained. Interestingly, for the other two concentrations viz., 10mg/ml and 5mg/ml no inhibitory zones were observed against any test organism (Table-1).

Antibacterial inhibitory zones obtained during the analysis revealed that the pharmacological active compounds present in the herbal extracts disrupt the cell membrane of the microbes through the

physical and ionic phenomenon. The compounds present in the extract thus inhibited growth of test organisms by using an electrochemical mode of action to penetrate and disrupt their cell walls. When the cell walls are penetrated, leakage of metabolites occurs and other cell functions are disabled, thereby preventing the organism from duplication (Rosas-Pinon et al., 2012). The significant activity exerted in this present research was due to the fact that the natural bioactive compounds responsible for the antibacterial activity are mostly extracted in the solvents and these active compounds may be able to penetrate the thick cell walls through general diffusion channels formed by the bacterial porins present therein, and affect the bacterial enzymes that are responsible for survival and virulence of organisms resulting in cellular lysis (Shan et al., 2007).

GC-MS analysis of the extract to identify the antimicrobial compound

The outcome of *Phytolacca octandra* extracts that was exposed to GC-MS analysis, showed the presence of 20 more compounds. In Table-2, the significant compounds obtained based on their retention peak (Fig. 1) was presented.

Dodecane, Octadecane, Octacosane are the major compounds responsible for the anti-oxidant activity of the plant extract. The spectrum sketch of GC-MS deep rooted the presence of two major antibacterial components like 2-lauro-1,3-didecain (29.13%), and 1,2-Benzene dicarboxylic acid (23.3%). The other significant compounds are 3-Allyl-5,6-dihydro-1,4,2-dioxazine (24.46%), Propynal (3.94%), 5-Heptadecene Octadecanoic acid (1.77%),

Hexadecanoic acid (1.4%), Tri-O-methylphorbol 12-acetate (1.85%), Octacosane (1.36%), Pentatriacontane (3.03%), Dodecane, Tetradecane, Hexadecane, 2,6,10,14-tetramethyl, Dodecane, 2,6,11-trimethyl, Tridecane, 2-deutero-1,4,5,8-tetraazaphenanthrene, L-(-)-Fucose, benzyloxime, N-(phenyl-2-pyridinylmethylene, trans,cis-2,3-Dimethyl-4-(iodomethyl)-butyrolactone, Octadecanedioic acid, Nonadecane, Triacontane, carbomethoxy-1,2,3,4-tetrahydronaphthalene and 2-(1-Phenanthryl) benzaldehyde. The most identified compound to have antimicrobial property are Cyclohexen-oxopropyl, 1,2-Benzenedicarboxylic acid (23.3%), 5-Heptadecene Octadecanoic acid (1.77%), Hexadecanoic acid (1.4%), Octadecanedioic acid and 2-(1-Phenanthryl) benzaldehyde. All these screened compounds were found to be the derivative of phenol compounds. Hence the antimicrobial properties were found to contain in all these screened compounds.

GC-MS is the best technique to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitrogen compound (Subbaiyan et al., 2014). Gas chromatography has been the method of choice for analysis of volatile oils for many years. The constituents of volatile oils are identified using a combination of different GC techniques, including GC in combination with mass spectrometry. GC-MS is the most powerful technique used to identify the components present in the oils.

Molecular Docking of Quercetin with target protein of *Staphylococcus aureus*

Quercetin is the strong antibacterial compound reported to be present in the

extracts of *Phytolacca octandra*. In the present study, the molecular docking of Quercetin against the target protein of infection causing pathogen *Staphylococcus aureus* was investigated.

Proteins used for the docking studies are Rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase enzyme and its transcriptional activator proteins are rhlG. In silico molecular docking of quercetin and rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase coding gene rhlG, the docking energy in terms of binding energies was investigated based on the number of hydrogen bonds formed during the docking process. The overall docking energies of the target protein, rhlG with quercetin with the number of hydrogen bonds were described below in detail.

Docking analysis of rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase coding rhlG with Quercetin

The structure of Quercetin was retrieved from Pubchem database, drawn using ACD chemsketch was optimized and saved in MDL MOL format and converted to PDB format using Open babel molecular converter (Fig. 2). In Table-3, the properties of quercetin was presented.

Proteins used for the docking study, rhlG is involved in the rhamnolipid biopathway, also termed as rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase biosynthesis pathway. The structure of rhlG from organism *Staphylococcus aureus* was retrieved from PDB database and its three dimensional structure was visualized using RASMOL (Fig. 3).

Docking of rhlG and Quercetin and visualizing hydrogen interactions between them using Acceryls Discovery Studio Visualizer was presented in Table-4 and Fig. 4.

The overall docking energies of the target protein, rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase with quercetin with the number of hydrogen bonds were presented (Table-5). The docking report revealed – 8.01Kcal/Mol binding energies and 8 hydrogen bonding between the *Phytolacca octandra* compound, quercetin and the target binding protein, rhlG of infection causing pathogen *Staphylococcus aureus*.

Based on the above docking energy results, the target proteins in the test organism causing infection for molecular docking approach was thus investigated using *in silico* studies. Gram-Positive *Staphylococcus aureus* was selected as the test organism. Bacterial reduction percentage obtained during the study also attributed that the compounds such as alkaloid, tannin, flavonoid, phenol, steroid, saponin and glycoside in *Phytolacca octandra* extract are the responsible bioactive agents. The antibacterial activity of such phytochemicals was reported by earlier studies and revealed that they inhibited the growth of microbes in many ways such as by inhibiting protein synthesis, interfering with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting metabolic pathway, interfering with cell wall synthesis or by preventing utilization of available nutrients by the microorganisms (Sheets et al., 2012). However these extracts do not affect the non-pathogenic bacteria which may be due to hindrance of penetration through the outer cell wall and

absence of specific enzyme in the bacteria. This mode of action of the plant extract against the specific bacteria may be due to its secondary mode of action against the bacterial enzymes instead of acting on the cell wall of the bacteria (Deris et al., 2014). The pathway responsible for the pathogenicity of organism, rhamnolipid biosynthesis pathway is studied. Inhibition of rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase enzyme in rhamnolipid pathway restricts to take up the organic matter which leads to bacterial death. Rhamnolipid reductase enzyme inhibitor inactivate the lipid (rhamnolipid precursor pathway) in the periplasmic space so that the antibacterial compounds in *Phytolacca octandra* can reach its target called antibiotic binding protein, and interrupt the biosynthesis of cell wall causing the death of the bacteria. The target for designing the drug against the *S. aureus* causing infection was thus identified using *in silico* studies.

CONCLUSION

The present study is corroborate the significance of antibacterial activity and bioactive compounds attributing to the activity present in the *Phytolacca octandra* plant extracts. *Phytolacca octandra* plant extracts exhibited different levels of antioxidant activity in all the models studied. Antibacterial activity of *Phytolacca octandra* showed maximum inhibitory zones of against respective organisms for the acetone extracts. GC-MS analysis revealed presence of 20 more compounds that are significantly attributing to antibacterial and anti-oxidant or anti-cancer activity. Quercetin is found to be the strong antibacterial compound reported to be present in the extracts of *Phytolacca octandra* in the present study.

Molecular docking of Quercetin against the target protein (Rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase enzyme) of infection causing *Staphylococcus aureus* showed overall docking energies of -8.01 Kcal/Mol binding energies and 8 hydrogen bonds. The docking indicated that the

pharmacological applications of the plant extracts and its bioactive compounds would be a promising concept in near future.

CONFLICT OF INTEREST

Authors declare no Conflict of interest in the present study.

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Table-1: Qualitative analysis of Antibacterial activity of *Phytolacca octandra* acetone extracts

S. No	Test Organisms	Zone of Inhibition (in mm)				
		5mg/ml	10mg/ml	15mg/ml	20mg/ml	25mg/ml
1	<i>Staphylococcus aureus</i>	-	-	13.0	17.0	21.0
2	<i>Escherichia coli</i>	-	-	12.0	14.0	18.0
3	<i>Pseudomonas aeruginosa</i>	-	-	12.0	15.0	19.0
4	<i>Enterobacter aerogenes</i>	-	-	11.0	17.0	19.0
5	<i>Klebsiella pneumoniae</i>	-	-	13.0	15.0	20.0

Table-2: Chemical nature and activity of phyto-components identified in *Phytolacca octandra* extracts by GC-MS analysis

S. No.	Name of the compound	Molecular formula	Chemical nature	Retention time	Peak area (%)	Activity
1	Dodecane	C ₁₂ H ₂₆	n-dodecane	7.86	0.27	Antimicrobial Antioxidant
2	Octadecane	C ₁₈ H ₃₈	n-octadecane	13.54	0.52	Antimicrobial Antioxidant
3	Cyclohexen-oxopropyl	C ₁₈ H ₁₉ NO	quinoline	18.01	1.07	Antibacterial
4	Eicosane	C ₂₀ H ₄₂	icosane	22.30	0.51	Antibacterial
5	Benzenedicarboxylic acid	C ₁₆ H ₂₂ O ₄	ethyl hexyl phthalate	31.49	23.30	Antibacterial
6	2-lauro-1,3-didecain	C ₃₅ H ₆₆ O ₆	didecain	34.53	29.13	Antibacterial
7	Octacosane	C ₂₈ H ₅₈	n-Octacosane	38.50	3.03	Antimicrobial Antioxidant

Table-3: Properties of Quercetin

Property Name	Property Value
Molecular Weight	302.238 g/mol
Molecular formula	C ₁₅ H ₁₀ O ₇
Hydrogen Bond Donor Count	5
Hydrogen Bond Acceptor Count	7
Rotatable Bond Count	1
XLogP3-AA	1.5

Table-4: Interactions between rhIG and Quercetin

rhIG		Quercetin	Distance (Å)	Docking Score (K _{CAL} /MOL)
Aminoacid	Atom	Atom		
GLU348	OE2	H1	2.42	-8.01
GLU348	OE2	H2	1.56	
HIS318	N	O	2.65	
HIS318	N	OXT	2.43	
ASN319	N	OXT	3.20	
PRO316	O	HXT	2.38	
GLU321	OE2	H	2.41	
GLU321	OE2	H	1.76	
CYS315	N	O	3.37	

Table-5: Overall docking energies of the target protein

Docking of Quercetin with target protein of <i>Staphylococcus aureus</i>	Docking energy	No. of Hydrogen bonds
rhIG coding rhamnolipids biosynthesis 3-oxoacyl-[acyl-carrier-protein] reductase	-8.01	8

Fig. 1: GC-MS analysis of *Phytolacca octandra*

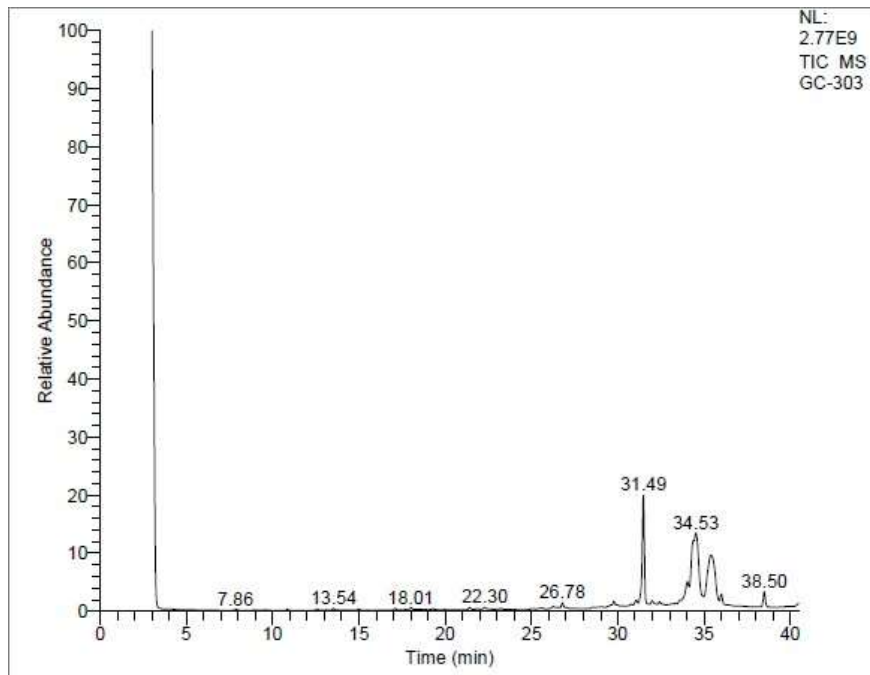


Fig. 2: Structure of Quercetin

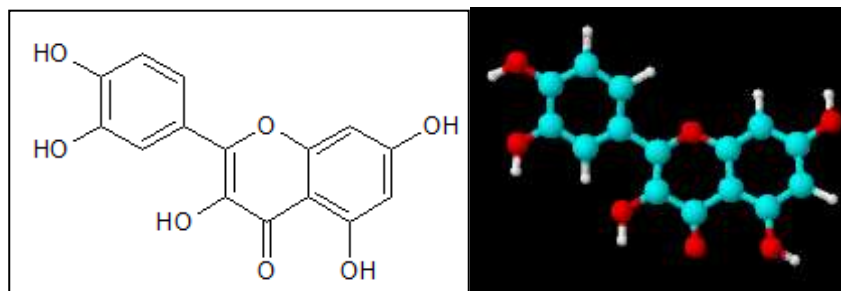


Fig. 3: Three Dimensional structure of rhIG



Fig. 4: Molecular Interaction between rhIG and Quercetin using Acceryls Discovery Studio Visualizer

