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IN VITRO SCREENING OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY AND *IN SILICO* ANALYSIS OF THREE SIDED *CISSUS QUADRANGULARIS*

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ABSTRACT

The present study is to screen the antibacterial and antioxidant activity of the plant three sided *Cissus quadrangularis* (Muppirandai) and to perform *in silico* analysis for the compounds identified through GC-MS analysis. Ethanolic extract of the plant was prepared by soxhlet extraction. Antibacterial activity was tested against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Salmonella typhi*, among which the extract was more effective against *Staphylococcus aureus*. Phytochemical analysis proved the presence of constituents such as flavonoids, carbohydrates, tannins, phenols, saponins, proteins and amino acids. Antioxidant potential of the plant was evaluated by DPPH assay. Nine compounds were identified through GC-MS analysis and were subjected to docking study, which was performed with LuxS receptor 1JVI using Autodock 4.2, Cygwin and Discovery studio. These compounds exhibited high negative binding energy and a maximum of three hydrogen bonds were formed between the ligand Cholestane-3, 6, 7-triol, (3à, 5à, 6à, 7à) and the receptor.

KEYWORDS: **Ethanol, phytochemical, GC-MS, ligand, receptor, docking.**

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INTRODUCTION

Many infectious diseases caused by bacteria, fungi and viruses pose a threat to human and animal health. The impact is severe in developing countries due to lack of availability of modern medicines and also resistance of microorganisms to the drugs. This led to the discovery of antimicrobial agents from plants, revealing their chemical structures and their useful bioactivities. Literature and ethnobotanical reports claim that plants can serve as a basis for modern drugs in pharmaceutical industry[1]. Medicinal plants are an effective and economic source of therapeutically important drugs and they also do not produce any side effects as that of antibiotics[2]. Research works are being performed to screen the bioactivities of plants with therapeutically potential secondary metabolites[3]. They are responsible for odor and flavor of the plants[2]. Polyphenolic compounds such as phenols, flavanoids and tannins have antioxidant and anticancer properties. Consumption of naturally available antioxidants leads to reduction in coronary heart diseases and cancer mortality[4]. The valuable compounds present in plants can be identified by analysis using Gas Chromatography – Mass Spectrometry (GC-MS)[5]. The discovery of novel drugs from natural sources is highly important because many isolated molecules are complex. Some of them are less potent, but can serve as pharmacophore, for chemical modification and for drug designing[6]. Molecular docking plays a major role in drug designing. The binding potential of the constituents present in plants can be determined by studying their interactions with the receptors. Obtaining new compounds by organic synthesis is a time consuming and expensive process and hence an alternative method is to screen the small molecule databases of novel compounds[7]. *Cissus quadrangularis* is a tendril climber plant belonging to the family Vitaceae[8]. This plant is found mostly in India, Srilanka, Malaya, Java, West Africa and Thailand. It is commonly known as 'bone setter' due to its ability to join bones[6]. It is locally known as Pirandai in Tamilnadu. The leaves of *Cissus* are lobed or simple, broadly ovate or reniform, serrate, cordate, dentate, and sometimes trifoliate and glabrous. The flowers are small, greenish white, bisexual, tetramerous and opposite to the leaves[8]. The fruits are globose or obovoid fleshy berries[9]. The whole plant including all parts is documented to be used for treatment purpose by ethnobotanists in traditional medicine system[8]. The stem acts as an antihelmintic, analgesic, digestive tonic and a dyspeptic[5]. *Cissus quadrangularis* is used against obesity, diabetes, metabolic syndrome and high cholesterol[9]. Powder of dried root heals bone injury[10]. The stem paste can cure muscular pains, burns and wounds and bites of poisonous insects. Powder of leaves and young shoots are administered to treat bowel infections related to indigestion[11]. Siddha literatures explain different types of *Cissus quadrangularis* (Pirandai). They include 'Olaippirandai' (two sided), 'Muppirandai' (three sided), 'Sadurappirandai' (four sided), 'Uruttuppirandai' (round), 'Teempirandai' (sweetish), 'Kalippirandai', and 'Pulippirandai' (acrid taste)[12]. Out of these, only the four sided plant is extensively studied. The plant selected for the study is three sided *Cissus quadrangularis* (Muppirandai) since it is underutilized. With this background information, the present study was done to screen the antibacterial activity by well diffusion and disc diffusion methods and to evaluate the antioxidant activity by DPPH assay and to perform *in silico* analysis for the compounds present in ethanolic extract of 'three sided *Cissus quadrangularis*'.

MATERIALS AND METHODS

All chemicals used were of analytical grade obtained from Himedia Laboratories Private Limited, India.

Collection of plant sample

Fresh plants were collected from Kolli hills, Namakkal District, Tamilnadu. The stem of the plant was cleaned by washing in water and was air dried under shade for few days. The dried plant sample was then ground to a coarse powder by using a domestic electric grinder.

The sample was then stored in an air tight container[13].

Preparation of extracts

10g of plant powder was weighed and extracted with 100ml of ethanol using soxhlet apparatus. The extract was then concentrated by allowing the solvent to evaporate. After 2-3 days, completely concentrated extract was obtained. The concentrated ethanol extract was collected in eppendorfs and stored in refrigerator[13].

In vitro antibacterial activity of three sided Cissus quadrangularis In vitro antibacterial assay by disc diffusion method

Disc diffusion assay was performed based on a standard method described by Kirby- Bauer (1966). Bacterial culture was spread onto the plate containing Muller Hinton (MH) agar medium by spread plate technique. A stock of 0.1g of plant extracts in 1ml distilled water was prepared. From the stock, 10µl was taken which has a concentration of 100µg/10µl/disc. Sterile water and standard antibiotic tetracycline disc were used for negative control and positive control respectively. Analysis was done in triplicates. The zone of inhibition was calculated by measuring the diameter of inhibition around the discs[1].

In vitro antibacterial assay by well diffusion method

Bacterial culture was spread onto the plate containing Muller Hinton (MH) agar medium by spread plate technique. Different concentrations (250µg, 500µg, 750µg, 1000µg) of plant extract were added into the wells. Sterile water and erythromycin were used as negative control and positive control respectively. Analysis was done in triplicates. The zone of inhibition was calculated by measuring the diameter of inhibition around the wells[10].

Phytochemical analysis

Qualitative phytochemical tests were performed with the ethanol extract of the plant to predict the phytochemical constituents present in the plant extract[3].

In vitro antioxidant activity

In vitro antioxidant activity was determined by DPPH assay, described by Blois *et al.*, 1958. The extract was dissolved in methanol to a concentration of 1mg/ml. 100µl of 0.1% DPPH was added to different concentrations (25µg, 50µg, 75µg) of plant extract. Analysis was done in triplicates. Methanol was used as blank. 0.1% DPPH in methanol was used as control. 100µl of 0.1% DPPH in methanol was added to different concentrations of 0.16% BHT and was used as standard[14]. Percentage of inhibition was calculated by:

% Inhibition = [Absorbance of control – Absorbance of test sample / Absorbance of Control] × 100

Sample analysis by GC-MS

Analysis of sample to identify the compounds by GC-MS was performed at IIT, Chennai and the model of the instrument is JEOL GCMATE II GC-MS.

Docking

Docking study was performed for the compounds obtained through GC-MS analysis. The structures of the ligands were drawn using ChemSketch 12.01 (Freeware version, ACD labs) in order to obtain the MOL format. The MOL files were converted to PDB format using Open Babel tool. The receptor taken for docking was a quorum sensing receptor (PDB ID: 1JVI). PDB structure of the receptor was obtained through Protein Data Bank (PDB) database. Selection of receptor depends on their role in bacterial life cycle[15]. Initially, heteroatoms were removed from the receptor. Then hydrogen bonds and charges were added to the receptor using Autodock 4.2. Blind docking was carried out by increasing the grid box size so that the whole protein was accommodated. PDBQT files of target and ligand, gpf and dpf files were retrieved using Autodock 4.2. Molecular docking was done using software known as Cygwin. The final docked complex was thus formed using cygwin. Results with 10 different conformations were obtained. The conformation with a minimum binding energy was selected. The docked

complex was analyzed with Discovery studio visualizer. The ligand binding patterns were well understood by this tool and the complex was then saved as an image file[16]. .

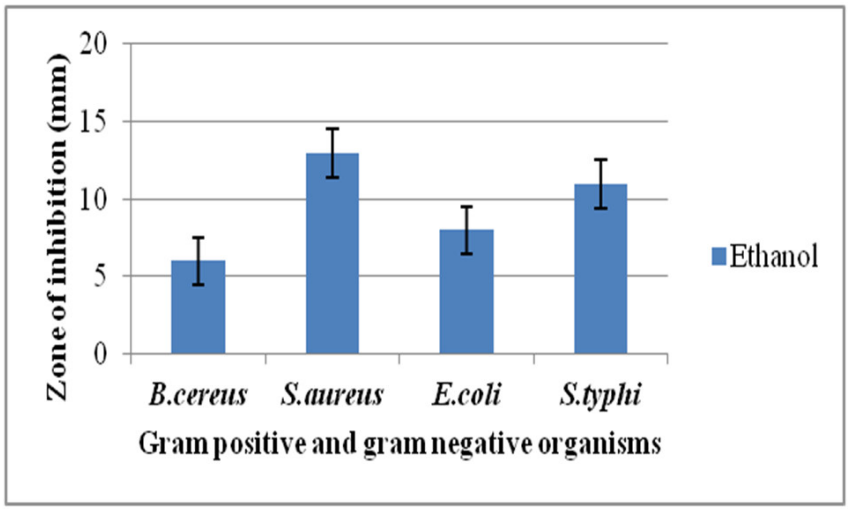
RESULTS AND DISCUSSION

In vitro antibacterial activity of three sided Cissus quadrangularis

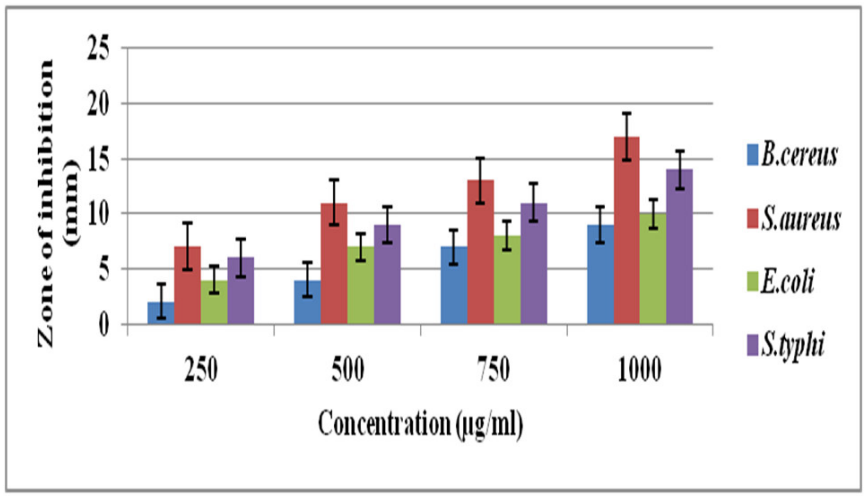
In both disc diffusion (Graph 1) and well diffusion (Graph 2) assays, the ethanolic extract of the plant exhibited higher antibacterial activity against *Staphylococcus aureus*. There was a minimum antibacterial potential against *Bacillus cereus*. In disc diffusion assay, the maximum zone of inhibition formed was 13mm against *Staphylococcus aureus* while that for well diffusion assay was 17mm. The zone of inhibition of extract for positive control (tetracycline) against *Bacillus cereus* was 19mm, *Staphylococus aureus* was 25mm, *Escherichia coli* was 20mm and *Salmonella typhi* was 22mm. No zone of inhibition was observed in negative control (sterile water). These results correlate with the previous researches which suggest that the ethanolic extract was very much active against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*[17]. The plant extract inhibited the growth of *Escherichia coli* and *Salmonella typhi*[10]. The results revealed that the antibacterial activity of ethanolic extract of the plant was effective against both gram positive and gram negative organisms.

Graph 1

Antibacterial activity of ethanol extract by disc diffusion method



Graph 2
Antibacterial activity of ethanol extract by well diffusion method



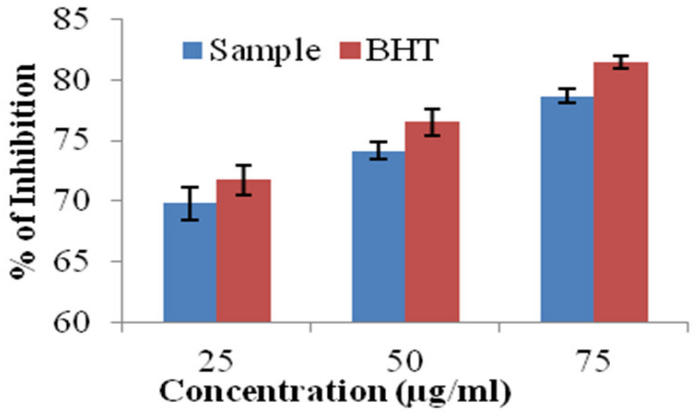
Phytochemical analysis

The phytochemical constituents present in the plant were determined by various phytochemical tests. The results revealed the presence of compounds such as flavanoids, carbohydrates, tannins, phenols, saponins, proteins and amino acids. Glycosides, alkaloids and gums and mucilages were absent in the ethanol extract of the plant. The active phytochemical constituents reported to be present in the plant *Cissus quadrangularis* were tannins, saponins, flavanoids, terpenoids and caratenoids[10]. The qualitative phytochemical analysis revealed the presence of compounds such as glycosides, tannins, saponins and flavanoids[3]. These active phytochemical constituents estimated by qualitative phytochemical analysis might be responsible for the antibacterial activity exhibited by the plant.

In vitro antioxidant activity

A maximum inhibition of 78.62% (Graph 3) was obtained at 75µg/ml concentration of plant extract revealing a higher antioxidant activity of the plant. 69.79% inhibition was achieved for a lower concentration of 25µg/ml. A previous research work explained that the ethanol extract of the plant *Cissus quadrangularis* produced 73% scavenging activity for 400µg/ml concentration of extract[4]. 30% inhibition had occurred with the plant extract *Cissus quadrangularis* by DPPH assay, exhibiting its antioxidant efficacy[18]. This study demonstrated that the ethanolic extract of three sided *Cissus quadrangularis* possess high antioxidant potential.

Graph 3
Antioxidant activity of ethanol extract by DPPH assay



Sample analysis by GC-MS

In GC-MS analysis (Figure 1) nine compounds (Table 1 and Figure 2) were identified to be present in the plant three sided *Cissus quadrangularis*. They include 2-2 (carboxyvinyl) pyridine, trans; 16-octadecenoic acid, methyl ester; Estra-1,3,5(10)-trien-17à-ol; Methyl 9,12-epithio-9,11-octadecanoate; Strychane, 1-acetyl-20à-hydroxy-16-methylene; Pentacosane; Cholestane-3,6,7-triol, (3à,5à,6à,7à); Heptacosane; and Octacosane. The compounds were identified with the database of National Institute Standard and Technology (NIST).

Figure 1
Analysis of Ethanol extract of three sided Cissus quadrangularis by GC-MS

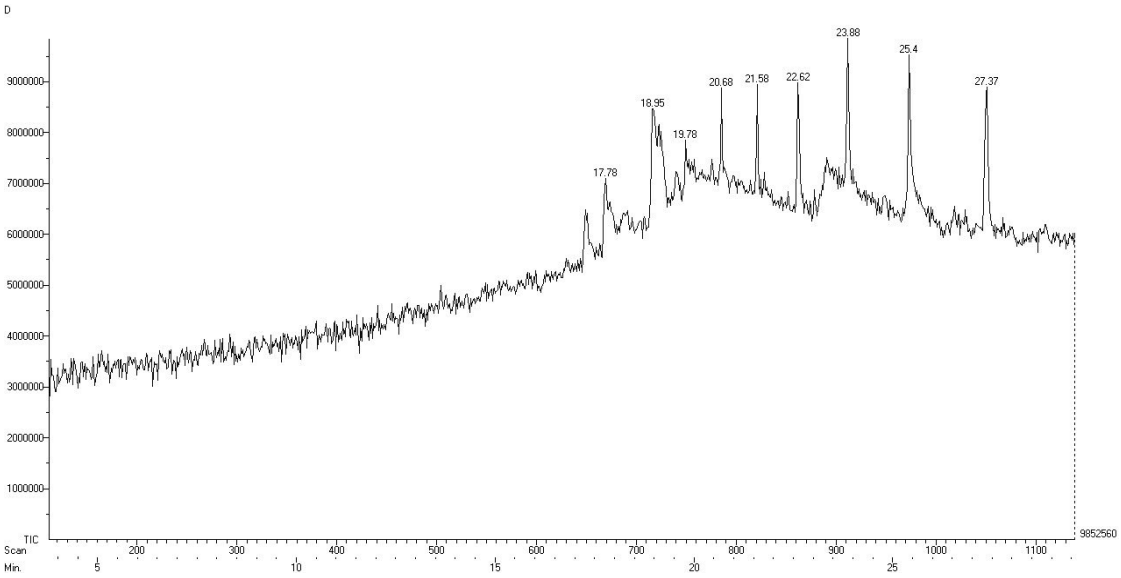
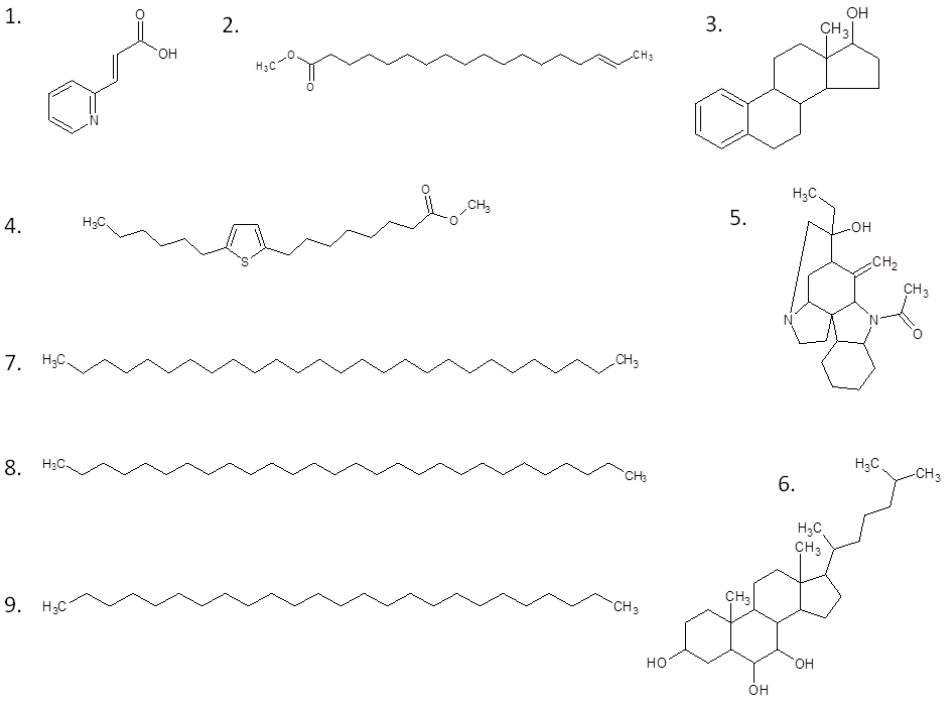


Table 1
List of compounds identified in ethanol extract by GC-MS

S.NO	RETENTIONTIME	COMPOUND	MOLECULARFORMULA	MOLECULARWEIGHT	PEAK%
1	17.78	2-2(carboxyvinyl) pyridine, trans	$C_8H_7NO_2$	149.146	76.1
2	18.95	16-octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.487	15.8
3	19.78	Estra-1,3,5(10)-trien17à-ol	$C_{18}H_{24}O$	256.382	12.5
4	20.68	Methyl 9,12-epithio-9,11-octadecanoate	$C_{19}H_{32}O_2S$	324.521	20.6
5	21.58	Strychane, 1-acetyl-20à-hydroxy-16-methylene	$C_{21}H_{32}N_2O_2$	344.49	27
6	23.88	Cholestane-3,6,7 triol,(3à,5à,6à,7à)	$C_{27}H_{48}O_3$	420.668	30.7
7	25.4	Heptacosane	$C_{27}H_{56}$	380.733	34.8
8	27.37	Octacosane	$C_{28}H_{58}$	394.76	31.5
9	22.62	Pentacosane	$C_{25}H_{52}$	352.68	31.4

Figure 2
Structures of the nine compounds obtained through GC-MS analysis



Docking

Docking study explained that the ligands bind to the receptor with a good binding energy. The hydrogen bonds formed refers to the strength of binding between the ligand and the receptor[7]. Table 2 shows the binding energy of ligand with the receptor in kcal/mol, number of hydrogen bonds formed and the amino acid. The compounds identified from the ethanolic plant extract might affect the bacterial life cycle

receptor ultimately inhibiting the growth of bacteria. Compounds exhibiting higher negative binding energy contribute to the maximum antibacterial activity of the extract[15]. The compounds Estra-1,3,5(10)-trien-17à-ol and Strychane,1-acetyl-20à-hydroxy-16-methylene formed one hydrogen bond each with the receptor. 2-2 (carboxyvinyl) pyridine, trans, 16-octadecenoic acid, methyl ester and Methyl 9,12-epithio-9,11-octadecanoate formed two hydrogen bonds each with the receptor. Highest number of hydrogen bond of three was formed between the ligand Cholestane-3, 6, 7-triol, (3à, 5à, 6à, 7à) and the receptor.

Table 2
Docking analysis of ligands against the bacterial life cycle receptor

S.N	LIGAND	BINDING ENERGY (kcal/mol)	NO. OF HYDROGEN BOND	AMINOACID
1	2-2 (carboxyvinyl) pyridine, trans	-3.58	2	Gln85:N
2	16-octadecenoic acid, methyl ester	-3.42	2	Tyr89:OH
3	Estra-1,3,5(10)-trien-17à-ol	-5.2	1	Gly83:N
4	Methyl 9,12-epithio-9,11-octadecanoate	-3.7	2	Lys35:N
5	Strychane,1-acetyl-20à-hydroxy-16-methylene	-5.42	1	Met82: O
6	Cholestane-3,6,7-triol, (3à,5à,6à,7à)	-6.58	3	Lys35:N Gly83:N Gly83:N
7	Heptacosane	425.58	-	-
8	Octacosane	429.79	-	-
9	Pentacosane	172.1	-	-

CONCLUSION

Plants are a rich source of drugs. They are used in primary health care for treating human ailments. Advantages in using plants as drugs are that they are safe, cheaper and more reliable than the synthetic products. Hence plants can be used as effective pharmacological agents. In the present study antibacterial and antioxidant activity was screened and also the compounds present in the ethanolic extract of three sided *Cissus quadrangularis* were analyzed through GC-MS analysis and docking. The antibacterial activity tested by disc diffusion method showed that the ethanol extract was effective against the two gram positive and two gram negative organisms. Antibacterial activity by well diffusion method showed that 1000µg/ml concentration of ethanol extract was highly effective. With regard to the bacterial strains used, the plant was much effective against *Staphylococcus aureus*. Phytochemical constituents such as flavanoids, carbohydrates, tannins, phenols and saponins were all proved to be present in the plant by qualitative phytochemical analysis. A higher percentage of inhibition achieved by DPPH assay for the ethanol extract revealed that it could act as a good antioxidant agent. GC-MS analysis detected the presence nine compounds in the ethanol extract that may contribute to the antibacterial activity of the plant. Through docking study, high negative binding energy was obtained on binding of the ligand with the receptor. *In vivo* studies can also be performed to evaluate the efficacy of the plant for treating diseases in animals and human beings.

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