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DECIPHERING THE NOVEL PHYTOCONSTITUENTS OF *Cassia alata* FLOWERS AND ITS MOLECULAR MODELING AGAINST *Staphylococcus pneumoniae*

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ABSTRACT

Herbal medicine constructs the base of today's therapy as the plants provide an infinite source of nutraceuticals. Employing herbal medicines in managing the microbial infections is a trend of today's world. The search for new anti-infectives, especially antimicrobial drugs, is still in demand. In most of the researches, bark, root and leaves of plants are used as drug in the treatment of various therapies but flowers are not much studied. In this study, *Senna alata* flowers are selected for novel phytoconstituents and its anti bacterial activity that is supported by molecular modeling against *Staphylococcus pneumoniae*. When compared to other extracts studied, aqueous extract of *C.alata* flowers showed a high content of secondary metabolites and flavonoids, alkaloids, terpenoids. Aqueous extract of *C.alata* flowers by GC-MS analysis showed the detailed peak of 30 compounds, nearly 14 hit compounds are detected and the top two higher composition of compounds identified are camphene (RT=4.727) tocopherol, (RT=5.695) iosorbide dinitrate. In the antibacterial study of *C.alata* flowers, the zone of inhibition is close to the standard tetracycline and to scrutinize the possible mechanisms behind the antimicrobial activity of phytochemicals in *C.alata* flowers, molecular docking is carried out. Flowers of *C.alata* consist of high content of Camphene, a terpenoid that can easily diffuse across cell membranes to reduce the opportunity for bacteria to develop its resistance. The top compound called camphene is docked against the MurF1 protein of *Staphylococcus pneumoniae* that showed the binding affinity is strong with binding energies -8.8 Kcal/mol. Phenolic compound called isosorbide is predicted to exert strong inhibition against MurF1 protein *Staphylococcus pneumonia.*

Key words: Phytoconstituents, Senna *alata* flower, antibacterial activity, molecular modeling, S*taphylococcus pneumonia*

INTRODUCTON:

In a global trend, growing interest toward the medicinal plants for the presence of potentially useful bio-active materials, and this trend has been encouraged by the limitations in uses of synthetic compounds as antibiotics and antivirals. World Health organization (WHO) appraised that 80% of inhabitants in developing countries basically relies on herbal medicines **(**Hussein, El-Anssary, 2018). Recently, it was discovered that one-third of the commonly used drugs are obtained from natural sources and this has led to the documentation of about 40,000–70,000 medicinal plant species with outstanding therapeutic potentials **(**Rao et al.,1975**)**. Biological screening, separation of the phytochemicals, and clinical trials of the medicinal plants have advanced over the years unfolding the secrets of ancient herbal remedies (Bibi *et al*., 2017). Traditional medicine is effective in dealing with diseases caused by bacteria or oxidative stress (Baydoun *et al*., 2015; Singh et al., 2017). Drugs are generally more expensive and less accessible than traditional materials, and also because of the emergence of drug-resistant microbes. In developing countries, due to the cost of efficient antimicrobials, a large proportion of the population utilizes medicinal plants for the treatment of infectious diseases. Medicinal and aromatic plants and their derivatives represent an integral part of life. The antimicrobial properties of certain Indian medicinal plants have been reported (Ramchandra *et al*., 1993; Mehmood *et al.,* 1999; Perumal Samy & Ignacimuthu, 2000; Vaijayanthimala *et al*., 2000; Srinivasan *et al.,* 2001; Rajesh Dabur *et al.,* 2004). Antibiotics and the innovative antimicrobials, antibiotics and the chemotherapeutic agents have been of value in controlling many infections.

Senna alata(L.) Roxb is commonly known as a “Candle tree” has characteristics with tap-root, stems erect and woody (about 3-4 meters tall), and a leaf stalk length of 50-80 cm. The flowers are bright yellow and arranged in long-stemmed bunches that bloom from the base of bunches. The fruit shaped like a straight pod is up to 25 cm and seeds are blackish brown, triangular shape with a flattened shape (Angelina et al., 2021**)**. It becomes herbal plant to treat various diseases in many countries. Seeds and leaves have high potency as fungicides and medicine for eczema in India (Shiddamal layya *et al*., 2010). In the Indian system of medicine, namely Ayurveda, Siddha, and Unani, decoctions of the leaves, flowers, bark, and wood are used in skin diseases such as eczema, pruritus, itching, and

constipation (Rao *et al.*, 1975).The flower and leaves decoction used intreatment of ringworms, scabies, blotch, eczema, scabies and infections (Oladeji *et al*.,2016). C.alata has been characterized by various bioactive compounds, including alkaloids, phenolics, favonoids, tannin, steroids, and triterpenoids (Somchit *et al*., 2020; Fatmawati *et al*.,2003)

Flowers are locally credited for the treatment of syphilis and diabetes and are effective against skin diseases and are used as edema laxative and vermifuge. A decoction combined with zingiber officinale, is used as a treatment for anthelmintic. It is cooked and used as a remedy for intestinal worm the leaf contains the purgative anthraquinone and also show some antimicrobial activity (Angelina et al., 2021). *C.alata* flowers contain a group of phytochemicals like saponin, alkaloid, steroid, flavonoid, tannin, phenol and carbohydrate and are well known for their laxative and pharmacological effects on humans and animals (Rahman *et al*.,2015). The Infectious Disease Society of America (IDSA) suggests that Pneumococcal disease is an infection caused by *Staphylococcus pneumoniae* that can affect many different systems in our body. It may result in conditions with sinusitis and it can also lead to pneumonia, blood infection (sepsis), bacterial meningitis and may be life-threatening at any age. The resistant of bacteria towards antimicrobial agents, increase in treatment costs and the adverse effects of synthetic drugs have necessitated the development of alternative, safe, efficient, and cost-effective natural medicines from plants. Previous studies have also shown the efficacy of the leave extracts and phytochemicals from *C.alata* against some clinical isolates of MDR bacteria (Hazni *et al.*, 2008) which attracted researchers' attention to explore the full potential of the plant as antibacterial agents. However, to the best of our knowledge, no studies have been conducted correlating the antimicrobial effect of flower extracts of *Senna alata* with the chemical constituents. Hence, in this study, the chemical compositions and antimicrobial properties of flower extracts from *C.alata* on molecular modeling against *Staphylococcus pneumonia* is focused.

2. Methods and materials:

2.1 Qualitative screening of phytochemicals

All the qualitative screening of phytoconstituents of the extracts of *Senna alata flowers* were performed as per the described procedure (Trivedi *et al.,* 1969; Sethuraman *et al*., 2018).

2.1.1 Wagner's test

To 4 ml extract, 3 drops of Wagners reagent was added and left undisturbed for 5 minutes. The reddish-brown precipitate is the indicator of presence of alkaloid.

2.1.2 Sodium hydroxide test

To dissolve 0.2 g of the extract, a cold dilute solution of sodium hydroxide and diluted hydrogen chloride was utilized and yellow colour is the indicator of presence of flavonoids.

2.1.3Copper acetate test

To 5 ml extract, 12 drops of Cu(OAc) solution were carefully added and incubated. The formation of beryl green colour is the indicator of presence of terpenes.

2.1.4 Salkowski Test

To 5 ml extract, 2.5 ml $CHCl_3$ and 2.5 ml conc. H_2SO_4 were added and carefully mixed. The red fluorescence of the chloroform layer and the greenish yellow fluorescence of the acid layer demonstrate the steroids availability in the sample.

2.1.5 Foam test

To 3 ml of the extract, 2 ml water was added and agitated rapidly for roughly 10 minutes, stable foam appearance is the indicator of saponin presence.

2.1.6 Ferric chloride test

To the 2 ml of collected filtrate 3 drops of a 10% ferric chloride solution was added. A greenish blue or violet colour is the indicator of presence of phenolics.

2.1.7 Lead acetate test:

4 ml of Pb solution were mixed well with 4 ml of extract and the presence of white precipitate is indicates the presence of tannins and phenols.

2.1.8 Borntrager's test

2 ml of extracts was boiled and filtered them with mild sulphuric acid. The filtrate was then completely combined with chloroform and shaken. Ammonia was gradually added after the organic layer was separated. The ammoniacal layer changes

color from pink to red when anthraquinone glycosides are present.

2.1.9 Fluorescence test

Add 1N sodium hydroxide solution was mixed with a 2 ml of extract and the fluorescence of bluish green is indicates the presence of coumarin glycosides.

2.1.10 Kellar Killani's test:

To dissolve a few ml of extracts in water, a mixture of Glacial acetic acid, ferric chloride, and strong sulphuric acid was utilized. The formation of a brown ring at the junction indicates the presence of cardiac glycosides.

2.1.11 Spot test

2 ml of extract was sandwiched by Whatman paper and squeezed for around 2-3 minutes. The residual oil on paper indicates the presence of fixed oils.

2.2 Quantitative analysis of phytochemicals

The total alkaloid content, total flavonoid content, total phenolic content, total saponin content, and total tannin content of *Senna alata* flower extracts were performed as per the described procedure (Sethuraman *et al*., 2018).

2.2.1 Estimation of Total alkaloids

2 mg/ml equivalent of extract was dissolved in DMSO followed by the addition of 1 ml of 1N HCl, then filtered. In addition to this 4 ml bromo-cresol green dye solution and 4 ml phosphate buffer was also added to it. The reaction mixture is then violently shaken with 1-5 ml of chloroform before being collected and the volume was made up with $CHCL_3$. Atropine was used as a standard with concentrations 20-100 mg/ml and the absorbance was taken at 470 nm.

2.2.2 Estimation of Total flavonoid

The $AlCl_3$ assay was performed to quantify the total flavonoid content. After mixing 2 ml of the extract with 8 ml of distilled water and 0.6 ml of 5% $NaNO_2$ solution the mixture was kept undisturbed. Approximately after 8 min, 0.6 ml of 10% $AlCl_3$ and 4 ml of 1.5M NaOH solutions were added and make up to 10 ml of total volume. Quercetin was used as a standard with concentrations 20-100 mg/ml and the absorbance was taken at 510 nm.

2.2.3 Estimation of Total Saponin

To 20g of extract, 150 ml of 15% ethanol was added and heated for 3 hr at 58-60° C with stirring. The filtered sample was extracted with 350 ml of 15% ethanol and the mixture was heated to evaporate the excess solvent. To the 50 ml of extracted

sample, 25 ml of $(C_2H_5)_2O$ was added and only the aqueous layer was collected and fractioned by n-butanol. Then the whole fraction was washed thrice with 25 ml of 4 % NaOH. Diosgenin was used as a standard with concentrations 20-100 mg/ml. Finally, the absorbance was taken at 550 nm.

2.2.4 Estimation of Total Tannins

Folin-Ciocalteu reagent was used to quantify the total tannin content of the extracts. To 3 ml extract, 5 ml water, 0.4 ml Folin-Ciocalteu, and 1.5 ml of 25% Na_2CO_3 solution was added and the total volume was made up to 10 ml and kept undisturbed for 30 min. Gallic acid was used as a standard with concentrations 20-100 mg/ml and the absorbance was taken at 725 nm.

2.2.5 Estimation of Total phenolics

Folin-Ciocalteu reagent was used to quantify the total phenolic content of the extracts. To 2 ml extract, 7 ml water and 1.5 ml Folin-Ciocalteu was added and kept undisturbed for some time. After 8 min, 6.5 ml of 10% sodium carbonate was added and the made upto the 30 ml of total column and again incubate for 1 hr at 37° C. Gallic acid was used as a standard with concentrations 20-100 mg/ml and the absorbance was taken at 550 nm.

2.3 GC-MS analysis of the *C.alata* flower

GC-MS-QP2010 SE was used to quantify the phyto constituents present in the *C.alata* flower extracts. The RTX-5 MS capillary column of 0.25m diameter and 25m length was used to identify and quantify chemical components. Working conditions for the GC were kept between 45°C and 290°C, with a rise of 5°C every minute. Oven temperature and injection port temperature was set at 115°C and 295°C, respectively. He was used as a carrier gas (mobile phase) with 1.5 ml/min flow rate. The ionizer temperature was set at 235°C and the interface temperatures 290°C. The detector voltage was set to 0.10 kV and the solvent cut-off period was set to 5 minutes and the mass range of 20-300 m/z was set and the compounds were compared with Wiley library (Al-Huqail et al., 2018).

2.4 Antibacterial activity of the *C.alata* flower extract

The antibacterial activity of the flower extract was determined by the paper disc method and respective nutrient agar medium was prepared and autoclaved at 121˚C for 30-45 min to sterilize the contaminating microorganisms. Then it was allowed to cool down at room temperature and when it reaches nearly 50˚C, the media was poured in the

culture plates and allowed for further solidification. Then the overnight grown *streptococcus pneumonia* culture was added to the plates by pour plate method and incubated at 50˚C for 12-24 hrs. The extract along with the standard and control were tested in the plate and zone of inhibition was measured (Sundaramoorthy *et al*., 2014).

2.5 Molecular modeling of *C.alata*

The three-dimensional structures of Multidrug Efflux Pump MepR (PDB ID: 4LLL) were retrieved from Protein Data Bank (PDB) and the excess chains, bound molecules and ions were removed, and the protein was prepared for docking. The three-dimensional structures of Oleanolic acid (PubChem ID: 10494) were retrieved from PubChem and the energy minimization of the ligand was performed and the ligand was prepared for the docking (Mekala *et al*., 2022).

Results and discussion:

The results of qualitative analysis of *C.alata* flowers revealed the presence of flavonoid, alkaloid, terpenoid, phenol, tannin, anthraquinone, steroid, protein and amino acid **(Table 1)**. Moreover, aqueous extract showed a high number of secondary metabolites compared with other extracts and are reported to have many biological and therapeutic properties. Most of the known functions of alkaloids are related to protection and serve specific function in biologically active at low doses. Alkaloid, polyphenol, flavonoid and saponin have high pharmaceutical value and are rich of antioxidant, which are able to against free radicals and reduce blood fat (DEA,2010). Most of the researches revealed antimicrobial activity of plants is due to their bioactive compounds such as alkaloids flavonoids, tannins, phenolic acids (Rao *et al*.,1975).

In this study quantitative determination of *C.alata* flower is found that out of three extracts, aqueous flowers extract of *C.alata* recorded higher concentration of alkaloid, phenols and flavonoids. The alkaloid content of flower extract was similar to the values reported for *C.alata* flower (6.50 mg/100g) by Abdulwaliyu et al.,(2018). The phenol content of the flowers of *C.alata* is 18.40±0.65% and are reported to show multiple activities like antioxidant, anticarcinogenic, anti-inflammatory**.** The tannin content is reported to be 4.15±1.45% and is a polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins **(**Vijayarekha and Sengottaiyan, 2016**)**. Flavonoids have been used against the cancer causing tumors and it inhibits the promotion of growth and progression of tumor**.** The content of saponin in the flower was found to be 2.25±2.25% and

saponins are naturally occurring surface – active glycosides and cause the leakage of proteins and degradation of cell wall enzymes from the cell.

Most of the studies have been done in leaves of Senna in the previous works and not much work is carried in flowers. Hence flowers of *C.alata* are selected for GC-MS analysis and it showed the detailed peak of 30 compounds (Fig 3 and Table 3). Nearly, 14 hit compounds were detected from 3.00 to 27.064 retention times and the top two higher composition compounds identified are Camphene (RT=4.727) Tocopherol and (RT=5.695) ,iosorbide dinitrate. This is supported by study on methonalic leaf extract of *C.alata* by FTIR and GCMS analysis that also recorded bioactive compounds (Kavipriya and Chandran, 2018; Oladeji et *al*., 2016; *Oladeji et al.,* 2020).

Antibacterial activity of the *C.alata* flower extract is also studied against *Staphylococcus pneumonia.* The zone of inhibition against *S.pneumoniae* produced by the aqueous flower extract of *C.alata* along with the control and standard are given in **Fig 4** and **Table 4** respectively. Flower sample produce the zone of inhibition of about 12 mm and it is close to the standard tetracycline used in the test. The antimicrobial potential of flower extracts of *C.alata* might be attributed to the presence of phytocompounds such as Camphene which is proved by molecular docking studies. Phytochemicals such as terpenoids that can easily diffuse across cell membranes to induce biological reactions (Naveed, 2013) and it reduces the opportunity for bacteria to develop resistance as the bacteria can be targeted via several mechanisms. Besides, the compounds also confer synergetic effects when used in combination with less effective antibiotics (Pajohi et al, 2011; Kanedi and Mohammad,2016). The compounds exerts the antimicrobial activity by various mechanisms such as depressing the nuclear or ribosomal enzyme synthesis, the membrane structure alteration and affecting the metabolic activity of the microbial cell, as well as inhibiting the secretion of their toxins (Redo *et al.*, 1989; Ultee and Smid, 2001; Porras *et al.*,2021). Flowers of *C.alata* consist of Camphene, a terpenoids that can easily diffuse across cell membranes to reduce the opportunity of bacteria to develop resistance (Bonifácio *et al.*, 2014). Previous studies also revealed that the presence of certain phytochemicals in the extract that could inhibit the growth of *S.pneumonia* (Timothy *et al.*, 2012).

To scrutinize the possible mechanisms behind the antimicrobial activity of phyto chemicals in *C.alata* flowers, molecular docking studies is carried out. Molecular docking results of active compounds of MurF1 protein from *S.pneumonia* shows the binding affinities of various components and are estimated by Autodock vina version (v.4.2.6) and Pymol 2.8 software is used to dock 35 active ingredients with the key target MurF1. It is inferred that the isosorbide dinitrate has the lowest binding affinity of -8.8 kcal /mol. The top compound called Camphene is docked against the MurF1 protein of *S.pneumoniae* showed that the binding affinity is strong with binding energies -8.8 Kcal/ mol respectively. Phenolic compound called isosorbide is predicted to exert strong inhibition against MurF1 protein of *S.pneumoniae*. This finding indicates that phenolic compounds, including chlorogenic acid (**P01**), are able to express fungicidal effects alone and in combination with the azoles. This can be an efficient approach to oppose and prevent azole resistance in some fungi strains.

Conclusion:

In this study, quantitative flowers extract analysis of *C.alata* showed higher concentration of alkaloids, phenols and flavonoids that is supported by GC-MS analysis to reveal the presence of 30 bio active compounds. The flower extracts of *C.alata* exhibited strong antimicrobial activities against the *S.pneumoniae* and it is close to tetracycline that might be attributed to the camphene. Camphene is docked against the MurF1 protein of *S.pneumoniae* which showed that the binding affinity is stronger with binding energies -8.8 Kcal/ mol respectively. Phenolic compound called isosorbide is predicted to exert strong inhibition against MurF1 protein of *S.pneumoniae*. Hence, it is concluded that flowers of *C.alata* drawing the attention for developing a lead to new herbal antimicrobial drug.

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Table1. Qualitative analysis of flower extract of S*enna alata*

S. no	**Phytoconstituents**	**Aqueous extract**	**Ethanol Extract**	**Methanol extract**
1	Alkaloids	+	+	-
2	Flavonoids	+	+	+
3	Terpenoids	+	-	-
4	Phenol	+	+	+
5	Tannins	-	+	+
6	Quinones	+	+	+
7	Saponins	+	+	+
8	Reducing sugar	-	-	-
9	Anthraquinones	+	+	+
10	Steroids	-	-	-
11	Glycoside	+	-	-
12	Protein andFree amino acid	-	-	-

Table 2: Quantitative determination of phytoconstituents in *C.alata* flowers

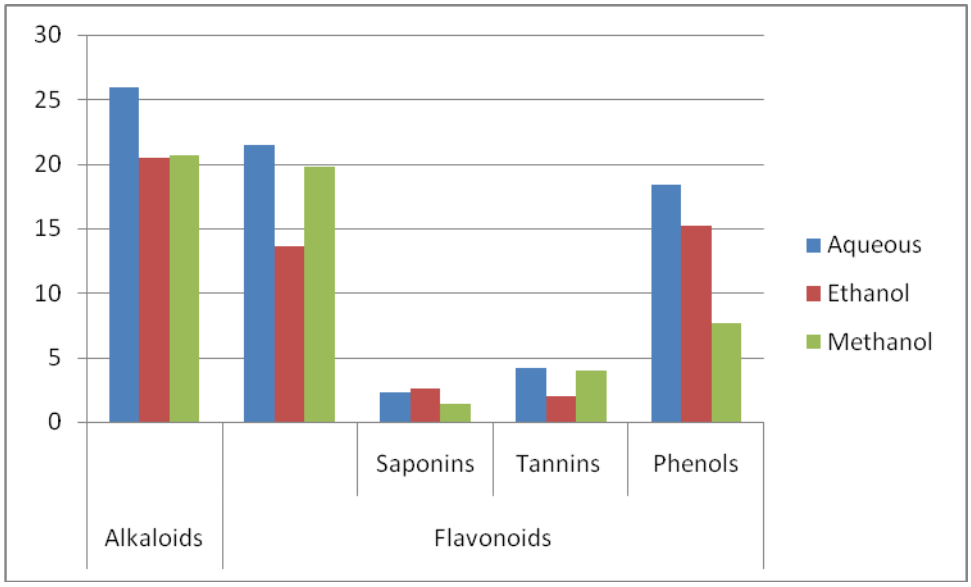

Fig 3: Spectral graph of GC-MS for *C.alata* flower extract

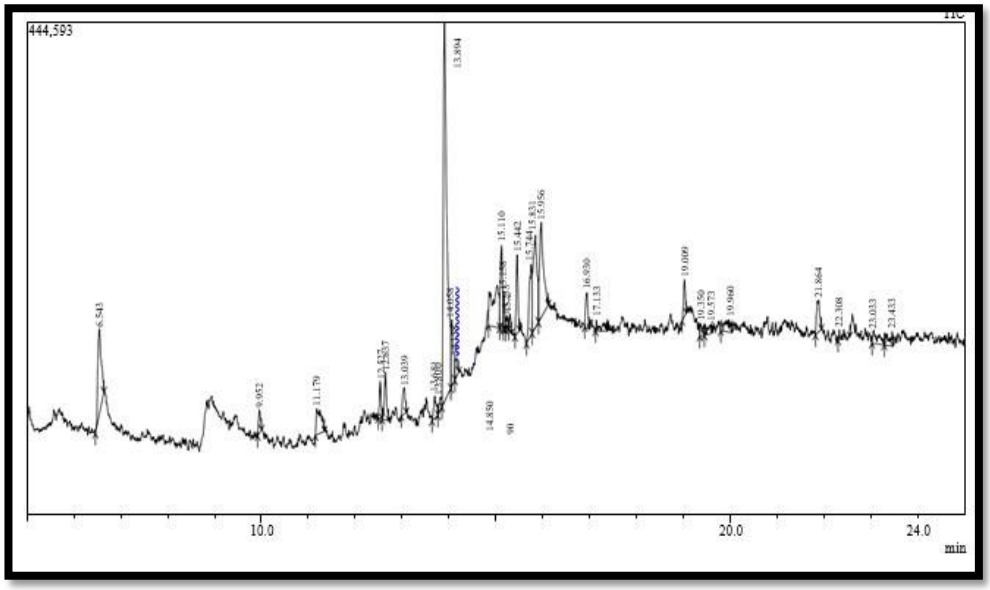

Table 3: Components identified in GC-MS of *C.alata* flower extract

Peak #	R.Time	Area	Area %	Height	Height %	A/H	Name
1	9.383	4229681	1.26	1354308	1.54	3.12	2-Pentanone,
2	9.458	2661466	0.80	651853	0.74	4.08	2(4H)-Benzofuranone,
3	9.955	4324574	1.29	1365722	1.56	3.17	1,2Benzenedicarboxylicacid
4	11.083	3788390	1.13	335913	0.38	11.28	lyxitol,1-o-nonyl
5	11.350	1992023	0.60	734244	0.84	2.71	5,7-octadien-2-on,3-acetyl
6	11.738	17521121	5.23	5065417	5.78	3.46	Isosorbide Dinitrate
7	12.040	4273994	1.28	1226491	1.40	3.48	Tetradecanoicacid
8	12.378	2142043	0.64	538825	0.61	3.98	Camphene
9	12.483	4231039	1.26	1387188	1.58	3.05	2-Cyclohexen-1-one,4-hydroxy
10	12.545	12483388	3.73	3904141	4.45	3.20	Kaempferol
11	12.647	12525982	3.74	3968918	4.53	3.16	2-Undecanone,6,10-dimethyl-
12	12.821	4392730	1.31	1004230	1.15	4.37	Pentadecanoicacid
13	13.046	8403846	2.51	1937974	2.21	4.34	Phthalicacid, Butylundecylester
14	13.485	6669014	1.99	2312672	2.64	2.88	Pentadecanoicacid,14-methyl ester
15	13.732	1764905	0.53	483611	0.55	3.65	13-Methylpentadec-14-ene-1,13-diol
16	13.944	78093811	23.33	15389820	17.55	5.07	n-Hexadecanoic acid
17	14.067	4654418	1.39	1342289	1.53	3.47	Di butyl phthalate
18	14.177	17112078	5.11	7008409	7.99	2.44	Hexa decanoicacid, ethylester(CAS)Ethylpa
19	14.915	2669734	0.80	721912	0.82	3.70	Hepta decanoicacid
20	15.126	4885147	1.46	1322601	1.51	3.69	1-Hexadecanol
21	15.175	2435118	0.73	988224	1.13	2.46	Sulfurousacid,hexylnonyl ester
22	15.356	3118191	0.93	1198753	1.37	2.60	Cyclopentanol,2,4,4-trimethyl
23	15.458	20963765	6.26	7032045	8.02	2.98	Phytol
24	15.764	30192196	9.02	6989276	7.97	4.32	9-Hexadecenoic acid
25	15.829	23503797	7.02	5520841	6.30	4.26	9,12,15-Octadecatrienoicacid,(Z,Z,Z)-
26	15.977	27639374	8.26	7143765	8.15	3.87	Octadecanoicacid
27	16.065	10954948	3.27	3271373	3.73	3.35	ethyllinoleolate
28	16.259	6823095	2.04	1113073	1.27	6.13	Octadecanoicacid,ethylester(CAS) Ethylste
29	18.732	7272319	2.17	1748400	1.99	4.16	4,8,12,16-Tetramethyl

							heptadecanolide
30	22.628	3040446	0.91	611511	0.70	4.97	Di-n-octylphthalate

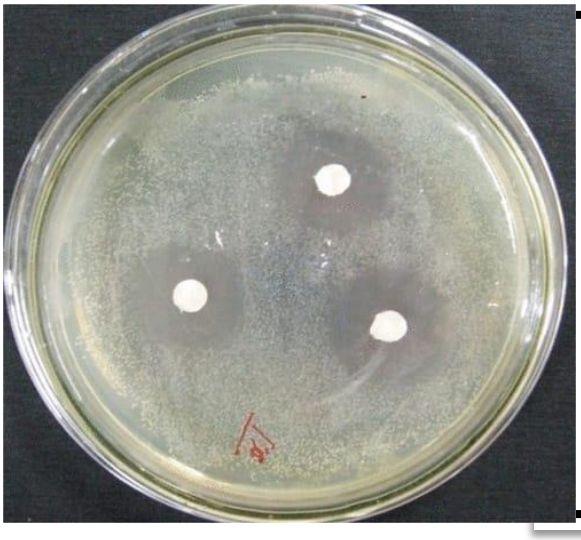

Fig4: Antibacterial activity of aqueous flower extract of *C.alata* against *S. pneumoniae*

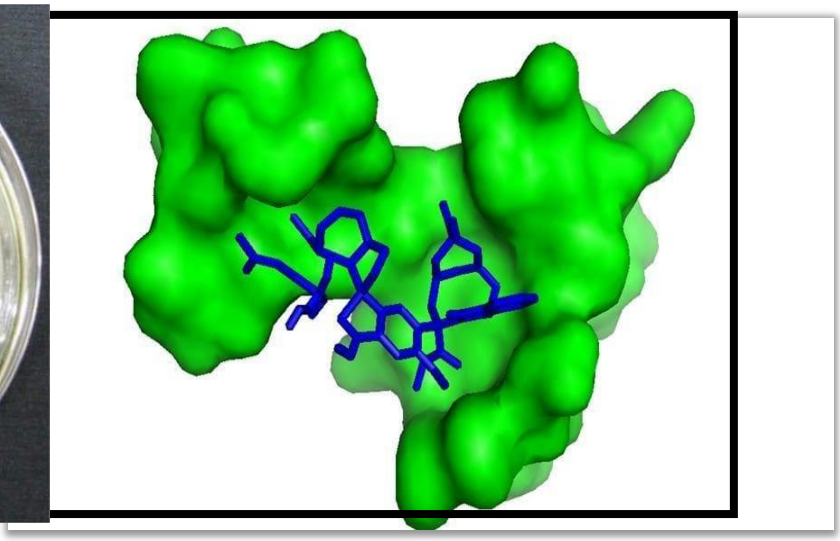

Fig 5:Molecular docking between phenolic compound and *S.pneumoniae*