International Journal of Academic Research and Development
ISSN: 2455-4197
Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 3; Issue 2; March 2018; Page No. 375-383

Therapeutic effect of *Thespesia populnea* L. on opportunistic pathogens of HIV/AIDS patients

[1] Jancy Varghese, [2] S Rajamani, [3] Betty Daniel
[1] Research Student, Bharathiar University, Coimbatore, Tamil Nadu, India
[2, 3] Associate Professors, Department of Botany, St. Joseph's College, Bangalore, Karnataka, India

Abstract
Thespesia populnea L, also known as '*Portia tree*' is in the Family of Malvaceae. Here we try to evaluate the antimicrobial activity of these extracts against *Staphylococcus aureus* and *Streptococcus pyogenes* which are opportunistic pathogens in HIV/AIDS patients Dried powdered flower extracts derived by using Ethyl acetate and acetone. The extracts were subjected to Gas Chromatography and Mass Spectrophotoscopy (GCMS) analysis. A qualitative phytochemical analysis was performed for the detection of alkaloids, phenolic flavonoids, carbohydrates, aminoacids, proteins, anthroquinones, saponins, tannins, phylobotannins, glycosides, cardiac glycosides, anthocyanides, terpenoids, and steroids. These extracts were tested against *Staphylococcus aureus* and *Streptococcus pyogenes* and antimicrobial activity was estimated by turbidometry and well diffusion method. When a software application was used for docking of antimicrobial compounds, it was observed that the extracts showed antimicrobial activity against the microorganisms of our study. This result suggests that *T. populnea* L flower contains phytochemicals with medicinal properties.

Keywords: growth rate, diffusion method, inhibition zone, docking, drug-resistant, and antioxidant

Introduction
Plants have been a valuable source of natural products for maintaining human health over a long period of time. Especially in the last decade there have been intensive studies for natural therapies [1]. The use of plant compounds for pharmaceutical purposes has gradually increased. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs [2]. Plants contain a variety of compounds which can perform important biological functions. They are rich in a wide variety of secondary metabolites like terpenoides, tannins, alkaloids and flavonoids which are found to have antimicrobial properties [3]. Saponnins are naturally occurring plant glycosides, known to have antifungal activities; flavonoids are used for anti-cancerous activity, and whereas tannins show antimicrobial activity [4]. Some of the plants are being used in traditional systems of medicine for hundreds of years in many countries out of which, *T. populnea* L is considered to have high tannin content with therapeutic value [5]. For years *T.populnea* L is being used in the Indian ayurvedic system of medicine for treatment of diabetes mellitus [6, 7]. The barks and flowers possess astringent, hepato-protective and antioxidant activity. An ayurvedic preparation containing *T.populnea* L namely "*panchvalkala*" possesses free- radical scavenging activity [8].
In the indigenous system of medicine the paste of the fruits, leaves and roots of *T.populnea* is applied locally for their anti-inflammatory effects on swollen joint [9]. Aqueous extracts of the fruits of this plant are reported for its wound healing activity[10].The leaves are applied locally on and also for skin diseases, hepatitis, jaundice, ulcers, wounds, psoriasis, scabies, urinary tract infections, diabetes, cholera, cough, asthma and guinea worm infections[11]. It is an effective remedy for scabies, psoriasis, skin diseases, dysentery, piles, diabetes [12], cholesterol-lowering, anti-cholinesterase, anti-inflammatory, and antioxidant properties of *T. Populnea* L. [13]. The poultices prepared from fruits, flowers and leaves are also found to be useful in rheumatoid arthritis [14]. A polyherbal formulation containing *T.populnea* L as one of the ingredients was shown as a useful remedy for Alzheimer's disease [15]. The floral extract of *T. Populnea* L exhibited anti-steroidogenic activity in mouse ovary. The weight of the uterus and ovaries were reduced significantly and the cholesterol and ascorbic acid content in ovaries were significantly elevated due to the treatment with extract of *T.populnea* [16]. The present work aims at studying the antimicrobial activity of *T. populnea* L flower.
Previous phytochemical studies reveal the presence of carbohydrate, protein, tannins, phenol, flavonoids, terpenes, saponins and gums in the ethanolic extract of the bark [17, 18]. The decoction of leaves is anti-cough and anti-headache agent. Infusion of bark is a treatment for intestinal disorders [19]. However not much work has been done on flower extract of this plant. In recent years, infections caused by bacteria such as *S.aureus and S. pyogenes* have been recognized as an acute problem with HIV infected patients due to their intrinsic resistance to many antibiotic classes and its capacity to acquire practical resistance to all effective antibiotic. The objective of this study was to find alternatives for the treatment of opportunistic infections in HIV/AIDS patients. Considering bacterial evolution and the current increase of antibiotic resistance, the discovery of new natural compounds that can be used to treat infections with lower secondary effects than existing antibiotics is becoming crucial, in order to guarantee the health of future generations [20]. In this regard, flower of *T.populnea* L have proved to be particularly interesting sources of antimicrobial, antidiabetic and anticancer compounds.

Material and Methods

Fresh flowers of *T. populnea* L were collected from different areas in Karnataka and Tamil Nadu. The collected flowers were cut into small pieces and shade dried for 15 days. Once completely dried, they were ground into coarse powder and stored in the bottles. This was used as sample to obtain crude extracts using ethyl acetate and acetone. An extract of measured amount, 30 grams was taken for extraction in Sauxlet apparatus. The solvents were measured 180ml for the extraction process. For each solvent Sauxlet apparatus was run for 3 days. The initial weight of the petriplate was determined. The crude extract obtained from extraction process was collected on to the petriplates and air dried for 2days. These air dried petriplates were weighed and the final weight of the petriplate was determined. The air dried sample was reconstituted with 10ml pure solvent from which the extract was obtained. The reconstituted extract was stored in the bottles until used for the assay.

Phytochemical analysis

Was done for qualitative phytochemical analysis to detect the active chemical compounds such as alkaloids, phenolic flavonoids, carbohydrates, aminoacids, proteins, saponins, tannins, phylobotannins, glycosides, cardiac glycosides, anthocyanides, terpenoids, and steroids by following standard procedures described by Sofawara (1993), Trease and Evans (1989), Harborne (1973) and Edeoga (2005), (11)

Gas Chromatography Mass Spectroscopy (GCMS) analysis

The crude extracts were submitted to GC-MS analyses and were found to contain a high number of metabolites. The extracts obtained from Sauxlet apparatus were also subjected to GCMS. The chromatogram of ethyl acetate extract of *T.populnea* L and, acetone showed three major peaks and two major peaks respectively. This has been identified after comparison of the mass spectra with NIST (National Institute of Standards and Technology) library, indicating the presence of three phytocomponents. Mass spectra of the separated components from the extracts were compared with the known components in the NIST database. The name, molecular weight and structure of the components of the test materials were determined[12].The compounds present in the extracts were analysed. The selected compounds were subjected to protein docking using auto doc 4.

Test organisms

S.aureus and *S.pyogenes* were isolated from clinical samples collected from the infected areas of HIV/AIDS patients from different rehabilitation centres and government hospitals in Karnataka and Tamil Nadu states, India with collection swabs provided by Hi*media*. All the tubes were filled with nutrient broth and kept for incubation at 37^{o} C for 18-24 hours. The isolated colonies were further identified with gram staining and other biochemical tests for each of the organism.

Determination of antimicrobial activity

The crude extracts were tested against *S.aureus* and *S.pyogenes*. Pure cultures of these micro-organisms were prepared a day before the experiment using nutrient broth. The extracts were serially diluted using nutrient broth. The dilutions were made up to $10^{-5.}$ From each serially diluted extracts, 1ml was pipetted into the microtubes using micropipettes. To each of these test tubes 100µl of the pure culture of microbes were added. These tubes were incubated at 37ºC. The incubation period was determined based on the doubling time of each of these microbes. Periodic analysis of microbial growth was carried out by measuring the turbidity of the culture using spectrophotometer readings at 650nm. The obtained values were depicted on the graphs.

Well diffusion assay

The selected bacterial cultures tc (*S.aureus* and *S.pyogenes*) were swabbed on sterile Muller Hinton agar using sterile cotton swabs. Agar wells were prepared with the help of sterilized cork borer of 10mm diameter. Using a micropipette, 100µl of different extracts was added to the wells in the plate and media as control. The plates were incubated in an upright position at 37^{0}C for 24 hours. The diameter of inhibition, using ampicillin as control was measured and recorded.

Spectrometry

S.aureus and *S.pyogenes* were grown in different dilutions of the ethyl acetate extract and acetone extracts. Nutrient broth and solvents were used as control.

Protein docking

A docking study of target proteins involved in antibacterial mechanisms was performed to extend the knowledge on standard antibiotics to herbal compounds which reported antibacterial activity. Docking studies were performed by the application of software Autodock4 on selected three compounds in order to evaluate their affinity to bacterial and viral proteins that are known targets for some antibiotics with different mechanism of action like: inhibitors of cell wall synthesis, inhibitors of protein synthesis, and inhibitors of nucleic acids synthesis. Docking was performed on antibacterial compound linoleic acid, antiviral compounds Capric acid or Deccanoic from Ethyl acetate extract and pentatriacontane from acetone extract. In the present work, the knowledge on target proteins of standard antibiotics was extended to antimicrobial plant compounds.

Results

For the pharmacological as well as pathological discovery of novel drugs, the essential information regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extracts [13].

Phytochemical analysis: Phytochemical analysis revealed the presence of various compounds in all five flower extracts. (Table 1).

Table 1: Phytochemical constituents of the plant extracts (Presence= +, Absence= ---)

Compounds	Extracts of *Thespesia populnea L*				
	Petroleum ether	**Acetone**	**Ethyl acetate**	**Ethanol**	**Aqueous**
1.Alkaloids	+	+	+	+	+
2.Phenolic Flavonoids	-	-	+	+	+
3.Flavanoids	+	+	+	+	+
4.Carbohydrates	+	+	+	+	+
5.Aminoacids and proteins	-	-	-	-	-
6.Anthroquinones	+	+	+	+	+
7. Saponins	-	-	-	-	+
8. Tanins	+	+	+	+	+
9.Phylobatannins	--	-	-	+	+
10.Glycosides	-	-	-	-	+
11.Cardiac glycosides	-	-	-	-	+
12. Anthocyanides	-	-	-	-	-
13.Terpenoids	-	-	-	-	+
14.Steroids	-	-	-	-	+

Gas Chromatography Mass Spectroscopy (GCMS) analysis

GCMS done on Ethyl acetate and acetone extracts gave 33 and 27 compounds. (Figure 1 and figure 2, Tables 2 and 4). There are 13 and 15 theraupeutically active compounds in ethyl acetate extract and acetone extract respectively (Tables 3 and 5).

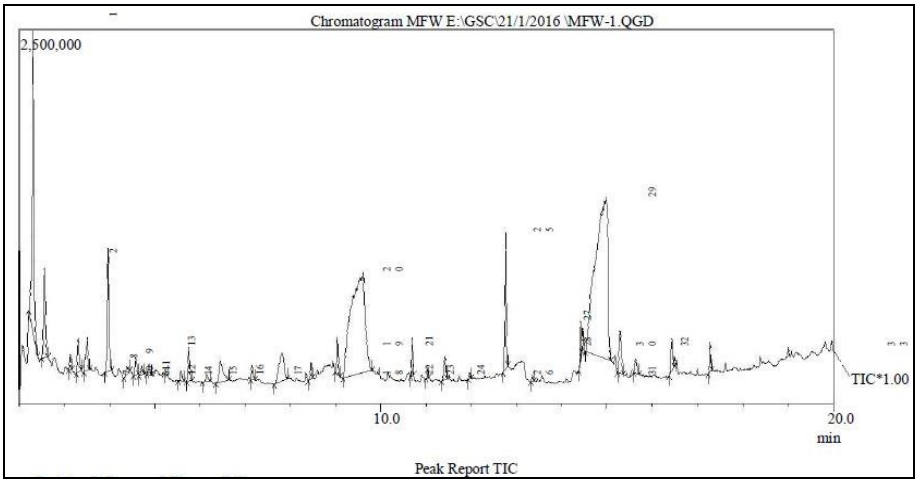


Fig 1: GCMS Chromatogram of Ethyl acetate flower extract of T.populnea L

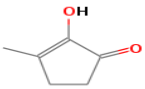
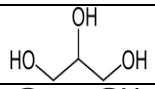
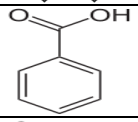
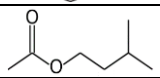
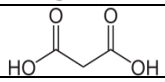
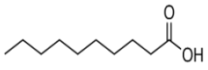
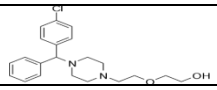
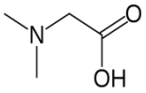
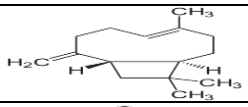
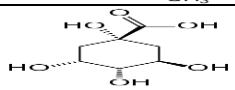
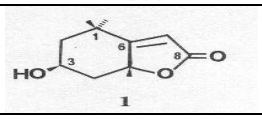
Table 2: Compounds present in ethyl acetate extracts of *Thespesia populnea* flower

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	2.302	2.208	2.408	5814446	9.89	2-cyclopenten-1-one,2-hydroxy
2	2.555	2.492	2.633	1904590	3.24	Glycerin
3	3.127	3.092	3.167	210048	0.36	Benzoic acid,2-butoxy-methyl ester
4	3.299	3.258	3.367	660083	1.12	1,3,5-Triazine-2,4,6-triamine (CAS) 2,4,6-Triamino-s-tria
5	3.499	3.425	3.558	759895	1.29	1-Butanol, 3-methyl-, acetate (CAS) Isoamyl acetate
6	3.960	3.892	4.042	1987461	3.38	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (C
7	4.378	4.317	4.450	199306	0.34	
8	4.569	4.525	4.642	339022	0.58	4-Vinyl-tetrahydro-4-pyranol
9	4.709	4.650	4.767	181179	0.31	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
10	4.873	4.817	4.933	229537	0.39	Propanedioic acid, phenyl-
11	5.237	5.208	5.283	79512	0.14	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (C
12	5.575	5.517	5.642	206318	0.35	Tetradecanoic acid
13	5.748	5.700	5.825	531723	0.90	Ethanone, 1-(2-hydroxy-5-methylphenyl)- (CAS) 1-Hydro
14	6.137	6.050	6.217	194795	0.33	Decanoic acid (CAS) Capric acid
15	6.445	6.350	6.642	954174	1.62	1,2,4-Benzenetriol (CAS) 1,3,4-Benzenetriol
16	7.151	7.117	7.217	207501	0.35	2-Hydroxy-4-methylbenzaldehyde
17	7.812	7.642	7.933	1554287	2.64	Ethanone,1-92-hydroxy-5-methylphenyl)
18	8.458	8.400	8.492	185226	0.32	N,N-Dimethyl glycined
19	9.040	8.983	9.100	448292	0.76	(-)-Caryophyllene oxide
20	9.614	9.167	9.808	14884487	25.32	Quinic acid
21	10.695	10.658	10.733	444541	0.76	Tetradecanoic acid
22	11.036	10.992	11.067	129049	0.22	(-)-Loliolide

23	11.424	11.350	11.475	445091	0.76 Benzoic acid, 4-hydroxy-3,5-dimethoxy- (CAS) Syringic a
24	11.972	11.942	12.008	79207	0.13 2,5-Cyclohexadiene-1,4-dione, 2,3,5-trimethyl-6-(3-methy
25	12.766	12.708	12.817	1754365	2.98 Hexadecanoic acid (CAS) Palmitic acid
26	13.360	13.325	13.400	85250	0.15 1-NAPHTHALENECARBONITRILE, 8-AMINO-
27	14.428	14.383	14.450	526340	0.90 9,12-Octadecadienoic acid, methyl ester, (E,E)-
28	14.466	14.450	14.483	174861	0.30 Docos-13-enoic acid
29	14.981	14.533	15.100	21364354	36.35 Maltose
30	15.295	15.242	15.375	973529	1.66 6-O-Acetyl-1- 4-bromophenyl sulfonyl -.beta.-d-glucosid
31	15.649	15.600	15.725	294391	0.50 4-BENZYLOXY-6-HYDROXYMETHYL-TETRAHYDR
32	16.445	16.392	16.492	585980	1.00 (R)-2-PHENYLPROPIONIC ACID
33	17.267	17.225	17.308	385797	0.66 Octadecatrienoic acid
				58774637	100.00

Table 3: Bioactive compounds identified from GC-MS analysis of ethyl acetate flower extract of *T.populnea L*

S. No.	Compound Name	Structure	Function
1	2-cyclopenten 1- one,2-hydroxy		Toxic
2	Glycerin		Eye Disorders, Cerebral Edema, Vasodialator, Constipation, Vehicle for Other Medications
3	Benzoic acid		Constituent of Whitfield's ointment which is used for the treatment of fungal skin, benzoic acid is also a major ingredient in both tincture of benzoin and Friar's balsam,
4	Isoamyl acetate		Used to test the effectiveness of respirators or gas masks
5	Propanedioic acid		surgical adhesive, Heart Disease, Fibromyalgia, Skin-Care Benefits
6	Tetradecanoic acid		Effective at causing the liver to synthesize cholesterol
7	Decanoic acid (capric acid)		Has strong antiviral and antimicrobial properties
8	1,2,4-Benzenetriol		Used to maintain healthy acid pH levels in the vagina, to prevent bacteria from growing and causing odor.
9	Hydroxy-4-methylbenzaldehyde		Additives in cigarettes.
10	N,N-dimethyl glycine		It is athletic performance enhancer, immunostimulant, and a treatment for autism, epilepsy, or mitochondrial disease.
11	Caryophyllene oxide		It is an antifungal against dermatophytes
12	Quinic acid		It is used in the medication for the treatment of influenza A and B strains called Tamiflu has been successfully developed and launched into the market.
13	(-)-Loliolide		Anti- repellent

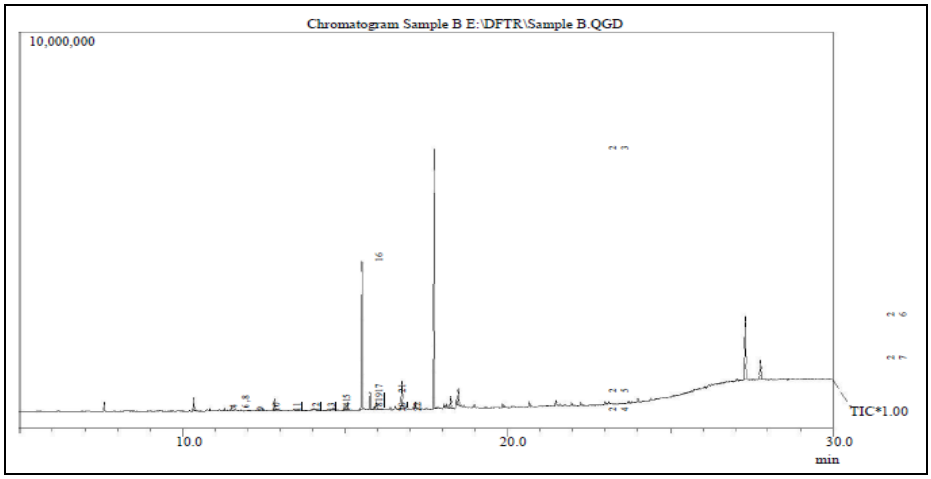


Fig 2: GCMS Chromatogram of acetone extract flower extract of *T. populnea L*

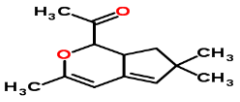
Table 4

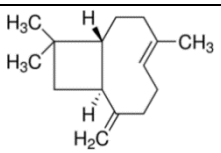
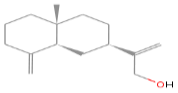
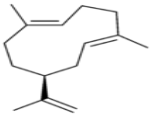
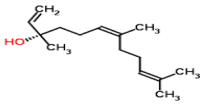
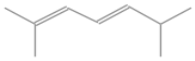
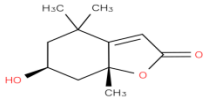
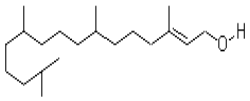
Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	10.332	10.292	10.367	498476	1.79	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta{c}pyran-1-yl)
2	10.399	10.375	10.433	53655	0.19	. Dodecanoic acid
3	10.841	10.808	10.875	84253	0.30	trans-Caryophyllene
4	11.283	11.242	11.325	115892	0.42	(+)-.BETA.-COSTOL
5	11.384	11.350	11.417	45100	0.16	
6	11.500	11.458	11.558	219446	0.79	GERMACRENE-D
7	11.703	11.683	11.742	40043	0.14	
8	11.806	11.775	11.825	32526	0.12	(+)-.BETA.-COSTOL
9	12.468	12.425	12.508	93823	0.34	d-Nerolidol
10	12.819	12.792	12.850	448207	1.61	Tetradecanoic acid
11	13.420	13.375	13.458	69891	0.25	2,4-Heptadiene, 2,6-dimethyl-
12	14.038	13.983	14.067	76592	0.28	
13	14.639	14.600	14.683	120767	0.43	Tetradecanoic acid (CAS) Myristic acid
14	14.978	14.933	15.008	206303	0.74	(-)-Loliolide
15	15.064	15.033	15.092	316421	1.14	Nonadecane (CAS) n-Nonadecane
16	15.502	15.458	15.550	5904313	21.20	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phyt
17	15.760	15.717	15.800	666274	2.39	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phyt
18	15.899	15.850	15.925	140867	0.51	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (CAS) Isobutyl
19	15.953	15.917	15.992	297734	1.07	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
20	16.548	16.517	16.608	171084	0.61	cis,cis,cis-7,10,13-Hexadecatrienal
21	16.734	16.675	16.808	1267785	4.55	Hexadecanoic acid (CAS) Palmitic acid
22	17.165	17.133	17.217	301059	1.08	Falcarinol
23	17.726	17.675	17.783	10838254	38.91	(5-Methylhepta-1,3-dienyl)benzene
24	18.235	18.192	18.292	544520	1.96	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS) Phyt
25	18.482	18.433	18.533	769591	2.76	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl lino
26	27.291	27.233	27.342	3518009	12.63	Stigmasta-5,22-dien-3-ol, (3.beta.,22E)- (CAS) Stigmasterol
27	27.767	27.717	27.808	1010310	3.63	.n-Hexadecanoic acid
				27851195	100.00	

0 260 290 320 350 380 410 440 470

Table 5: Bioactive compounds identified from GC-MS analysis of acetone flower extract of *T.populnea L*

S. No.	Compound Name	Structure	Function
01	1-(3,6,6-Trimethyl-1,6,7,7a-tetrahydrocyclopenta{c}pyran-1-yl)		Antifungal

02	Dodeconoic acid		It is used as a pharmaceuticals drugs like nandrolone, fluphenazine, bromperidol, and haloperidol
03	Trans-caryophyllene		Used in food flavouring food flavoring
04	(+)- Beta-costol		Beta-Costol is found in herbs and spices. Beta-Costol is a constituent of the essential oil of Costus
05	Germacrene-d		It has antimicrobial and insecticidal properties
06	D-nerolidol		It is used as a flavoring agent and in perfumery. It is also currently under testing as a skin penetration enhancer for the transdermal delivery of therapeutic drugs.
07	Tetradecanoic acid		Myristic acid acts as a lipid anchor in biomembranes.
08	2,4 Hepatadiene 2,6-dimethyl		It has a pleasant smell and is used in cosmetics for its fragrance.
09	(-)- Loliolide		Antioxidant activity and cell protective effect
10	Nonadecane (cas)n- nonadecane		The egg parasitoid *Trissolcus basalis* uses n-nonadecane, a cuticular hydrocarbon from its stink bug host Nezara viridula, to discriminate between female and male hosts
11	2- Hexadecen-1-ol 3,7,11,15-tetramethyl- [r-(cas) phy		It is used in the fragrance industry It is used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents.

Determination of antimicrobial activity

Well diffusion study done with all five extracts of *T.populnea* L. flower showed inhibition of both *S.aureus* and *S.pyogenes.* (Figures 3and 4). Zone inhibition was measured shown by all 7 strains each of both the organisms. Antibiotic Ampicillin was used as control. (Figure 5)

Well diffusion method to check the effects of five extracts of the flower of *T.populnea* L

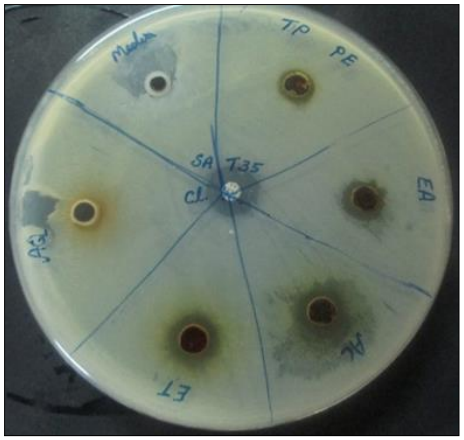


Fig 3

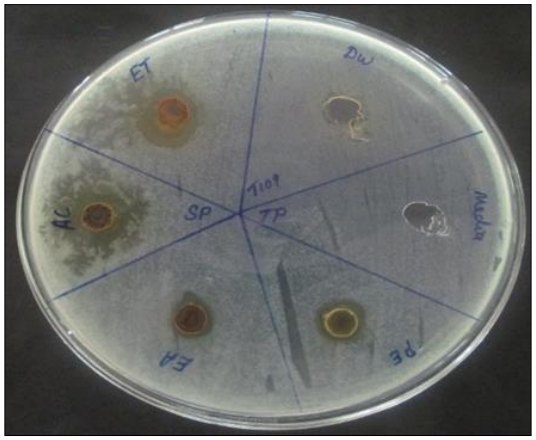


Fig 4

SA-*Staphylococcus aureus*, ***SP***-*Streptococcus pyogenes*, ***PE***-*Petroleum ether*, ***EA***-*Ethyl acetate*, ***AC***-*Acetone*, ***ET***-*Ethanol*, ***AQ***-*Aqueous*, ***CL***-*Control- Ampicillin*

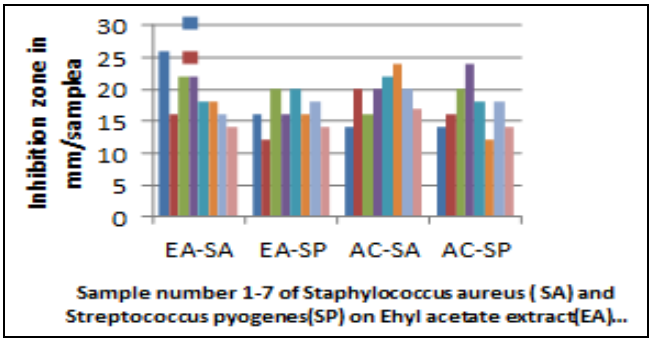


Fig 5: Zone of inhibition showed on plant extracts as sensitivity assay

Spectrometry

The growth rate of *S.aureus* and *S.pyogenes* showed decrease in gowth rate during the dubling time of incubation in both the extracts. The growth was more inhibited in the first, second and third dilutions for both the organisms. (Figures 6 to 9).

Graphs depicting the growth rate of S.aureus and S.pyogenes in different dilution of Ethyl acetate flower extract (Fig. 7-9).

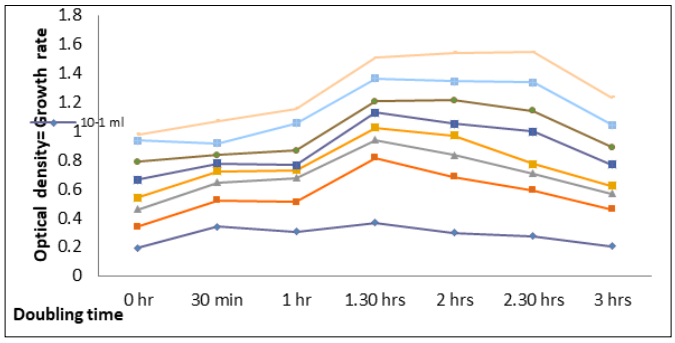


Fig 6: *S.pyogenes* in Ethyl acetate extract

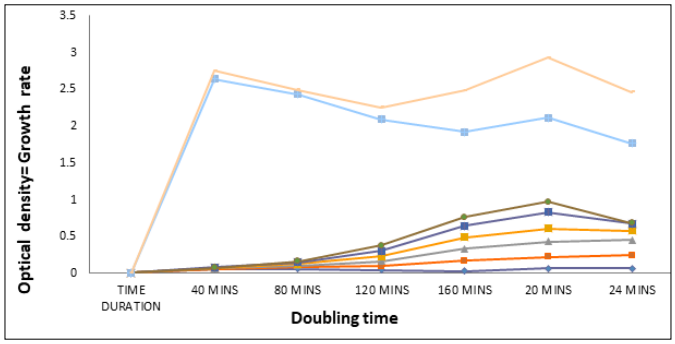


Fig 7: *S. aureus* in Ethyl acetate extract

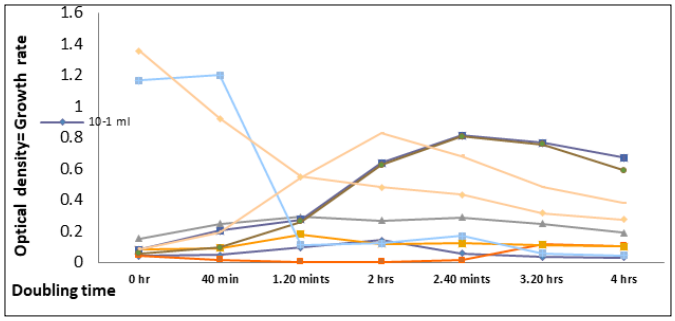


Fig 8: *S.pyogenes* in Acetone extract

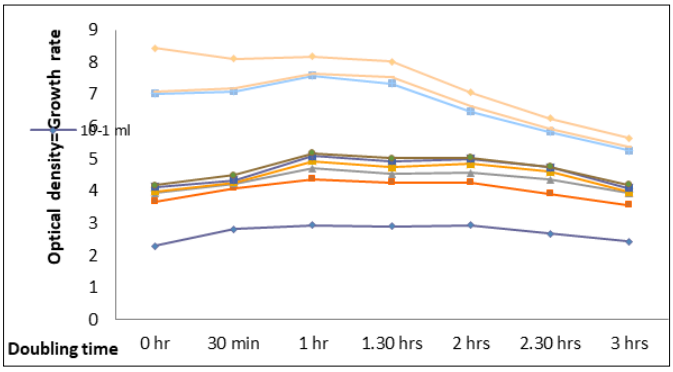


Fig 9: *S. aureus* in Acetone extract

Docking of compounds

The minimum energy binding shows maximum affinity of selected plant compound to the bacterial and viral proteins. Overall, it seems that for the selected compounds (namely, linoleic acid, capric acid and pentatriacontane) the main mechanism of action is inhibition of cell wall synthesis. The proteins selected for this study are, Carotenoid dehydrosqualene synthase (PDB ID:3ACX), Figure), an enzyme which catalyses the biosynthesis, for S. *aureus.* In the host, targeting this protein inhibits the progression of infections by *S.aureus.*

Docking of the selected compounds of antibacterial and anti-HIV effects with microbial proteins:

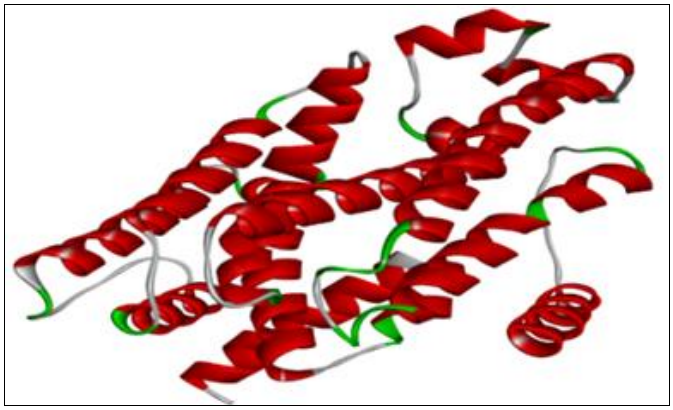


Fig 10: *Selected protein of S.pyogenes*- Cysteine protease

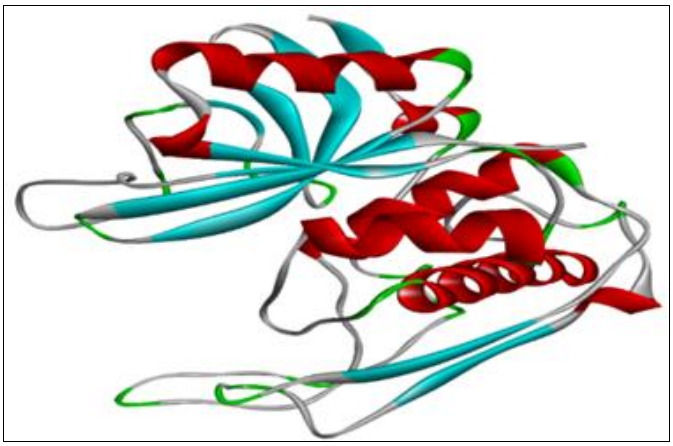


Fig 11: Selected protein of *S.aureus*: Carotenoid dehydrosqualene synthase

Table 6: Binding Energy Score

S.aureus: Carotenoid dehydrosqualene synthase				*S.pyogenes:*Cysteine protease(PDB ID:4RKX)		
Rank	**Sub-Rank**	**Run**	**Binding Energy(kcal/mol)**	**Sub-Rank**	**Run**	**Binding Energy(kcal/mol)**
1	1	10	-6	1	1	-4.77
2	1	9	-5.95	2	11	-3.69
3	1	13	-5.7	1	10	-4.31
4	1	5	-5.34	1	7	-4.19
5	1	3	-5	1	13	-3.83
6	1	4	-4.73	1	2	-4.1
7	1	15	-4.68	1	12	-3.95
8	1	8	-4.59	1	15	-3.93
8	2	11	-4.2	1	4	-3.91
9	1	14	-4.55	1	9	-3.83
10	1	12	-4.5	1	14	-3.72
11	1	1	-4.25	1	3	-3.56
12	1	2	-4.22	1	8	-3.23
13	1	7	-4.15	1	6	-2.98
14	1	6	-3.72	1	5	-2.87

Table 7: Antimicrobial Test: (Best binding energy)

Organism	**Protein-Name**	**PDB-ID**	**Binding Energy**
S. aureus	Carotenoid dehydrosqualene synthase	3ACX	-6
S.pyogenes	Cysteine protease	4RKX	-4.77

Discussion

In the present study we identified the antimicrobial activity of flower extracts of *T.populnea* L. The study demonstrated that this plant can be effective as modern medicine to arrest some of the microbial opportunistic infections found in HIV/AIDS patients. By measuring their growth rate, both extracts were found be very effective on both organisms which cause opportunistic infection in HIV patients. It was revealed that when the concentration of extracts were higher, rate of inhibition of organisms were higher. The reduction was more prominent in the first dilutions of both ethyl acetate and acetone. However with increase in the incubation period, growth rate dropped down in different dilutions of the ethyl acetate extract and the growth was seen to be inhibited more in the first dilution. Flower extracts of *T.populnea* L are potential sources of novel antimicrobial compounds especially against bacterial and viral pathogens found in HIV/AIDS patients. Our study conducted *in vitro* showed that the plant extracts inhibited bacterial growth, but their effectiveness varied. The inhibition produced by various solvent extracts depends upon the type of bioactive compounds present in them. The medicinal plants selected are the source of primary and secondary metabolites. Both metabolites, specifically secondary metabolites are found to be responsible for antimicrobial, anti-viral and anti-cancerous activity. The phytochemical studies and crystallographic studies on selected compounds showed promising results which might be helpful in pharmaceutical industries for ailments and discovering promising drugs for serving humanity especially most target group HIV/AIDS. Results revealed that the ethyl acetate extract of *T.populnea* L was effective against all the strains of the three microbes.

Conclusion

T.populnea L is widely used in traditional medicine to combat and cure various ailments, thus appear to be rich in both primary and secondary metabolites. The anti-inflammatory, astringent, antioxidant and antimicrobial activities can be attributed to these metabolites. These properties of various extracts from *T.populnea* L have been of great interest in research and in food industry because of their possible uses as natural additives emerged from a growing tendency to replace synthetic antioxidants and antimicrobials with natural ones. Our results revealed the importance of plant extracts to control microbes which are becoming threat to human health. The uses of plants to heal diseases including infectious one has been extensively applied. Data from our results reveal the great potential of few plants for therapeutic treatment. The observed activity may be due to the presence of potent phyto-constituents in the flower extracts. More research pertaining to the use of plants as therapeutic agents should be emphasized. Therefore, further studies needed to be conducted to search for new compounds. Exploitation of these pharmacological properties involves further investigation and identification of these active compounds is necessary.

Acknowledgment

Our sincere thanks are to the management of St. Joseph's College, Bangalore, for providing all laboratory facilities.

References

1. Ranjith Ani Shetty, Satya Swapna, B. Aishwarya, K. Chaitanya Sravanthi. Evaluation of antibacterial activity potential of extracts of *Ricinus communis*, *Zingiber officinalis* and *Punica granatum* in a Polyherbal Extract, 2012; (5):1385-1388.
2. Raid Al Akeel, Yazeed Al-Sheikh, Ayesha Mateen, Rabbani Syed, K. Janardhan, Gupta VC. Evaluation of antibacterial activity of crude protein extracts from seeds of six different medical plants against standard bacterial strains, Saudi Journal of Biological Sciences, NCBI, Elsivier. 2014; 21(2):147-151.

3. Sharda Vaidya, Arti Nair, Lakshmi Girish. Phytochemical screening of the leaf extracts of few selected plants of Ulhasnagar, International Journal of Botany and Research (IJBR), 2015, 7-16 ISSN(P): 2277-4815; ISSN(E): 2319-4456, 5(4).
4. Rajak Neha, Mutahir Arisha, Rai Sumit Kumar, Qualitative phytochemical analysis of medicinal plants in Garhwal Region, Journal of Biological and scientific opinion, 2015; 3(4).
5. Belhekar SN, Chaudhari PD, Saryawanshi JS, Mali KK, Pandhare RB. Antidiabetic and antihyperlipidemic effects of *Thespesia populnea* L fruit pulp extracts on alloxan-induced diabetic rats, Indian Journal of Pharmaceutical sciences, 2013; 75(2).
6. Vasudevan M, Gunnam KK, Parle M. Antinociceptive and anti-inflammatory effects of Thespesia populnea L bark extract, international journal of advances in pharmacy biology and chemistry. J Ethnopharmacol. 2007; 109:264-270.
7. Vasundaramma, Kamakshamma. Antihyperglycemic activity of *Thespesia populnea* L seeds methanol extract on stz induced albino rats. International journal of institutional pharmacy and life sciences, 2015; 5(2).
8. Denish Mika**,** Chandrasekaran Guruvayoorappan. The effect of *Thespesia populnea* on cisplatin induced nephrotoxicity, Journal of cancer research and therapeutic, 2013; 9:50-53.
9. Elakkiya S, Ananthi T. Studies on anti-inflammatory activity of *Thespesia populnea* Linn. on the drug induced male albino rats Journal of Chemical and Pharmaceutical Research. 2011; 3(5):473-477.
10. Siju EN, Rajalakshmi GR, Vipin VS, Jolly Samu, Arun Shirwaikar, Antioxidant Activity of *Thespesia populnea* Linn Fruits. International journal of pharmacy & life sciences, 2014; 3213-3216.
11. Savithramma M, Linga Rao, Bhumi G. Phytochemical screening of *Thespesia populnea*L. Soland and *Tridax procumbens* LN. Journal of Chemical and Pharmaceutical Research. 2011; 3(5):28-34.
12. Mitter CS. Investigation of Ethanol Soluble Bioactive Compounds in Tender Fruits of *Thespesia Populnea* L International Journal of Science and Research (IJSR). 2014; 3(11):497-499.
13. Mani Vasudevan, Milind Parle Memory-Enhancing Activity of *Thespesia populnea* L in Rats, Pharmaceutical Biology. 2007; 45(4):267-273.
14. Nagappa AN, Cheriyan B. wound healing activity of the aqueous extract of *Thespesia populnea* L fruit Fitoterapia. 2001; 72(5):503-506.
15. Patil PS, Venkatanarayanan R, Argade PD, Shinde PR. Assessment of pharmacognostic and phytochemical standards of *Thespesia populnea*Lroot. Asian pacific journal of tropical biomedicine, 2012; 1212-s1216.
16. Muhammad Asif, Syed Baqir Shyum Naqvi, Muhammad Akram evaluation of analgesic effects of *Thespesia populnea* L World journal of pharmaceutical research. 2014; 3(9):28-37.
17. Parthasarathy R, Ilavarasan R, Karrunakaran CM. Antidiabetic activity of *Thespesia Populnea*L bark and leaf extract against streptozotocin induced diabetic rats, International Journal of Pharm Tech Research. 2009; 1(4):1069-1072.
18. Navaneethakrishnan S, Suresh Kumar P, Shaji G, Satyanarayana T, Monic Josephine Nithila S, Priyanka B. Anti-Hyperglycemic Activity of *Thespesia populnea L* Bark Extracts in Alloxan Induced Diabetic Rats. International Journal of Chemical and Pharmaceutical Sciences, 2012; 3(1).
19. Parthasarathy R, Ilavarasan R, Karrunakaran CM. Antidiabetic activity of *Thespesia populnea* L bark and leaf extract against streptozotocin induced diabetic rats. International Journal of Pharm Tech Research. 2009; 1(4):1069-1072.
20. Archana Moon, Aqueel Khan, Bharath Wadher. Antibacterial potential of leaves of *Thespesia populnea L* and corresponding callus against drug resistant isolates. Indian Journal of Natural Products and Resources, 2010; (4):444-449.