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RESEARCH ARTICLE

# Evaluation of Antimicrobial Property of *Thespesia Populnea* Root Extracts against Genitourinary Tract Infectious Pathogens

**Ch. Suvarna Lakshmi[1], A. Uma[1*], M. Lakshminarasu[2], B. Venkanna[1]**

[1]*Centre for Innovative Research, IST, Jawaharlal Nehru Technological University Hyderabad (JNTUH), Kukatpally-500085, Hyderabad, A.P, India.*

[2]*Centre for Biotechnology, IST, Jawaharlal Nehru Technological University Hyderabad (JNTUH), Kukatpally-500085, Hyderabad, A.P, India.*

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## ABSTRACT

The therapeutic properties of plants used to cure diseases of human beings or animals dates back to earlier times of human history. *Thespesia populnea* belongs to malvaceae family found in coastal and tropical regions of India. The various parts of the plant including leaves, flowers, fruits, bark and root possess splendid medicinal properties such as anti-microbial, anti-oxidant, anti-inflammatory, anti-fertility, hepatoprotective, anti-steriodegenic and purgative activities. The present study is emphasized on the extraction, isolation and, characterization of phytochemical constituents exists in *Thespesia populnea* root system. The extracts prepared by the employment of various organic solvents such as n-hexane, methanol, ethyl acetate and water were analyzed for their antimicrobial properties against various pathogens involved in genitourinary tract infections. Amongst all the extracts, methanolic-extract exhibited significant antimicrobial activity. Furthermore, the phytochemical analysis displayed that the root system of *Thespesia populnea* comprises of alkaloids, terpenoids, glycosides, saponins, polysteroids and tannins. The crude methanol extract was further studied for chemical elucidaton by GC-MS technique. The prevailing compounds identified from the methanol root extracts of *thespesia populnea* were tetratetra acontane, octadecane, 3-ethyl-5-(2-ethylbutyl), hexadecanoic acid, methyl ester, heptadecane, 9-hexyl-, spiro[isobenzofuron-1(3H),9′-(9H)xanthene]-3-one, 17-pentatriacontene, glycerol-1-palmitate, brequinar, astaxanthin, betulin, 7,8-epoxylanostan-11-ol, 3-acetoxy etc showed antimicrobial activity against GUTI.

## KEYWORDS

Thespesiapopulnea, Root, Extracts, Antimicrobial Property, Genitourinarytract Infections, GC-MS Analysis

## INTRODUCTION

Globally, plant extract based drugs play a predominant role in health care needs of humans. Medicinal plants possess natural chemicals which are bioactive for treating various deadly diseases[1].

***Address for Correspondence:**
**Dr. A. Uma**
Jawaharlal Nehru Technological University,
Hyderabad, India.
**E-Mail Id**: vedavathi1@jntuh.ac.in

Plants synthesize various secondary metabolites including flavonoids, tannins, terpenoids, alkaloids to cope with abiotic stresses and they are medically active[2]. All over the world ~10% of plant flora used to treat various diseases. Without any scientific knowledge, 67% of rural area people utilizing medicinal flora as traditional medicines[2]. These bioactive components are popularly known as

phytoconstituents or phytochemicals which play a critical role in combating various microbial infestations. Future threat of public health is of antimicrobial resistance due to severe exploitation of synthetic antibiotics. The synergism assay conducted on bacteria using well-known antibiotics such as ampicillin, oxytetracycline, chloramphenicol and majority of the bacteria showed resistance to the employed antibiotics[3]. Large pharmaceutical companies are hesitant to develop novel antibiotic drugs due to the emerging of antibiotic resistant microbes[4]. The development of novel antibiotics is majorly associated by combining synthetic drugs with naturally bioactive constituents[3].

*Thespesia populnea* belongs to malvaceae family, a traditional plant used to cure various diseases including skin, liver, urethritis and gonorrhea diseases, cutaneous infections for instance scabies, eczema, ringworm, psoriasis and guinea worm had been treated by applying paste of roots, leaves and fruits. Plant infusions used in treatment of dysentery, cholera, hemorrhoids[5], and this plant grows well in coastal forests and tropical regions and is highly resistant to drought conditions[6]. The bark, leaves, flowers, roots, and fruits of the plant consist of radiant medicinal ingredients such as gossypol, quinones (thespesone, mansonone-D, H) for anti-inflammatory effect. It can also be employed as astringent, hepatoprotective, antioxidant and antifertility drug[7,8].

Genitourinary tract infections (GUTI) are the most prevalent and serious infections for many adult women and female children and is also associated with amniotic fluid infection, clinical chorioamniontis, premature rupture of membranes (PROM), preterm delivery, low birth weight and postpartum endometritis, bacteriuria (Asympotatic bacteria) will develop acutepyelonephritis. Due to lack of bladder defense mechanisms and unable to identify anatomical abnormality causes birth defects and threat to new born and foetus leading to maternal infections. As the diagnosis of accurate incidence is difficult this condition is considered benign[9]. No established scientific reports observed on *T.populnea* root against genitourinary tract infections. The present investigation is aimed to extract and characterize bioactive constituents present in the root system of *T.populnea.*

## MATERIALS AND METHOD

### Collection of Plant Material

The roots of *Thespesia populnea* were procured from Pragathi Resorts, Hyderabad, India. Dr. K. Guravareddy Scientist of Regional Agriculture Research Centre (RARC) identified and authenticated the plants in the regions of Guntur, Andhra pradesh, India. After brought to the laboratory they were washed thoroughly under running tap water followed by sterile distilled water washes and subjected to air-drying at room temperature, under shade. Afterwards the dried material was grounded to fine powder using laboratory blender. Thereafter, the powdered material was stored in ziplock bags at 4$^{o}$C for further experiments.

### Extraction

Extraction was carried out using Serial Exhaustive Extraction process (SEEP)[10]. The polarity of the solvents increased successively (n-hexane > ethyl acetate > methanol > water) to obtain a wide range of active components.100gm of plant root powder was filled in a thimble and was extracted successively with 1L of respective solvent. The extracts were filtered and the resultant filtrates were concentrated under reduced pressure at 40°Cusing a Rotary Evaporator. Thereafter, all the extracts were preserved at 4°C in air-tight bottles for further use.

### Preliminary Phytochemical Screening

Initially, the extracts were subjected to qualitative and quantitative analysis for various phytochemical constituents including alkaloids, carbohydrates, steroids, proteins, phenols, tannins, flavonoids, glycosides, gums, saponins and terpenes.

### Qualitative Analysis of Plant Extracts

Different qualitative chemical tests were performed for establishing the chemical profile

of consequence n-hexane, ethyl acetate, methanol and aqueous extracts. The following tests were performed to detect various phytoconstituents present in extracts[10].

**Detection of Alkaloids**

50 mg of solvent free extract was stirred with few mL of dilute hydrochloric acid and filtered. To a few milliliter of filtrate, a drop or two of Mayer's reagent (1.36g of mercuric chloride and 5g of potassium iodide in 100mL distilled water) was added along the sides of the test tube. The formation of white or creamy precipitate indicates the test as positive.

**Detection of Carbohydrates and Glycosides**

100mg of the extract was dissolved in 5 mL of water and the filtrate was collected. To 2 mL of filtrate, two drops of alcoholic $\alpha$-naphthol solution was added, the mixture was subjected to vigorous shaking and 1 mL of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. A violet ring indicates the presence of carbohydrates.

**Detection of Saponins**

50 mg of extract was diluted with distilled water and made up to 20 mL. Then the suspension was shaken in a graduated cylinder for 15 minutes. The formation of 2 cm layer foam indicates the presence of saponins.

**Detection of Proteins and Amino Acids**

100 mg extract was dissolved in 10 mL of distilled water and filtered through Whatmann No.1 filter paper. Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) was added to 2 mL of aqueous filtrate. A characteristic blue colour appearance indicates the presence of amino acids and whereas the appearance of purple colour indicates the presence of protein.

**Detection of Phenolic Compounds**

50 mg of extract was dissolved in 5 mL of distilled water and few drops of neutral 5% (%w/v) ferric chloride solution were added. Formation of deep blue or black colour indicates the presence of phenolic compounds.

**Detection of Tannins**

50 mg of extract was dissolved in water and, heated on a water bath for1 hr followed by treating with 10%(w/v) ferric chloride. Formation of blue or dark greenish grey colour indicates the presence of tannins.

**Detection of Terpenoids (Salkowski test)**

0.2 g of the extract of the plant sample was mixed with 2 mL of chloroform followed by the addition of concentrated $H_2SO_4$ (3mL). A reddish brown coloration in the interface indicates positive results for the presence of terpenoids.

**Detection of Flavanoids**

The extract was treated with sulphuric acid and observed for the formation of orange colour which indicates the presence of terpenoids.

**Detection of Sterols**

1 mL of extract was treated with chloroform and acetic anhydride followed by adding few drops of $H_2SO_4$ and the formation of dark pink or red colour indicates the presence of sterols.

**Detection of Anthraquinones**

About 50 mg of methanolic extract was heated with 10% (w/v) ferric chloride solution and 1 mL of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. The formation of pink or deep red coloration of aqueous layer indicates the presence of anthraquinones[11].

**Detection of Reducing Sugars (Fehling's Test)**

The aqueous ethanol extract (0.5 g in 5 mL of water) was added to boiling Fehling's solution (A and B) in a test tube and the colour reaction was observed.

**Quantitative Estimation of Phytoconstituents**

***Estimation of Total Phenolic Content***

TPC was determined spectrophotometrically using UV visible spectrophotometer (UV-2450, Shimadzu) by Foline Ciocalteau reagent using

gallic acid (GA) as the standard, according to the method described by Singleton *et al*. The method is based on the redox reaction and the absorbance was measured at 725 nm against a reagent blank.

### *Estimation of Total Flavonoids Content*

A standard solution of rutin (20-100µg/mL) was prepared and added to 10mL of solution containing1.5mL of 95% ethanol, 0.1mL of 10% aluminium chloride, 0.1mL of 1M potassium acetate and distilled water in a volumetric flask. Test solution prepared with same dilution, well mixed and absorbance was taken at 358nm. Calibration curve was prepared for estimation of flavonoids.

### *Microbial Cultures*

Pure cultures of *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus foecalis, Gardenerella vaginalis, Candida albicans,* were provided and maintained at the Department of Microbiology of Osmania Medical College, Hyderabad, India. Thereafter, the MH slants of all the strains were stored at 4ºC.

### *Anti-microbial Screening Studies*

According to National Committee of Clinical Laboratory Standard, anti-microbial activity was determined by Well Diffusion Method[12,13]. The crude extracts were properly dissolved in Dimethylsulfoxide (DMSO) and properly measured at different concentrations (10 mg/mL, 20 mg/mL, 30 mg/mL and 40 mg/mL).

### *Minimum Inhibitory (MI) and Bactericidal (MB) Concentrations*

MIC and MBC of compounds were determined by broth dilution methods[11]. 2.4% MH media (Muller Hinton) media was used for culturing the microorganisms. The prepared broths containing microorganisms in test tubes along with testing compounds were incubated at 37°C for overnight. 2.4 g of sterilized MH media was weighed and dissolved in 100mL of distilled $H_2O$, then autoclaved. The media was cooled to 45°C; and distributed into the sterilized fraction tubes( fraction volume 0.5mL). For each tube the same volume of culture was added (0.5mL), and then mixed the first tube with test sample of about in concentration of 2 mg/mL. Later 0.5 mL of pure broth was added and made the final volume of the tube to 3 mL with broth.

### *Thin Layer Chromatography (TLC)*

Thin layer chromatography was performed using Petroleum ether: Ethyl acetate (9:1) (MP-A) and ethyl acetate: toulene: formic acid (4.5:2.5:0.75) (MP-B) as mobile solvent systems. Anisaldehyde-$H_2SO_4$ (AS reagent) and ethanolic-$FeCl_3$ were used as detecting reagents, respectively and the obtained $R_f$ values were analysed[14].

### *GC-MS Analysis*

A Perkin Elmer Clarus-500 gas chromatography coupled with a Perkin Elmer Clarus-500 mass spectrometer used for analysis. A fused silica capillary column of 30m×0.32mm was used for separation of compound mixture at temperature 50ºC (10 min) to 350ºC (5 min) with a ramp of 20ºC $min^{-1}$. Electron energy of 70eV mass selective detector was operated in the electron impact (EP). Helium gas acts as a carrier gas in the column at constant flow rate.

## RESULTS

### Phytochemical analysis

The preliminary phytochemical studies showed the presence of flavonoids, phenols, steroids, carbohydrates, reducing sugars, anthrocyamidines, saponins, tannins and glycosides in methanol extract of T*hespesia populnea* root.

In n-Hexane extract, steroids, reducing sugars, tannins and glycosides were observed. Ethyl extracts analysis revealed the presence of flavonoids, steroids, carbohydrates, reducing sugars and tannins. While in aqueous extract alkaloids, flavonoids, phenols, steroids, carbohydrates, reducing sugars, saponins, glycosides and tannins were identified (Table 1). The amounts of flavonoids and phenols were estimated from the calibration curve (Fig. 1) and

they were analyzed to be 16 ug/mL and 25 ug/mL, respectively.

Table 1**:** Phytochemical Constituents Present in the Root of *Thespesia Populnea*

| Phytochemical compounds | Solvent extracts | | | |
|---|---|---|---|---|
| | Hexane | Ethyl acetate | Methanol | Water |
| Alkaloids | - | - | - | + |
| Flavonoids | - | + | + | + |
| Phenols | - | - | + | + |
| Steroids | + | + | + | + |
| Carbohydrates | - | + | + | + |
| Protein | - | - | - | - |
| Reducing sugars | + | + | + | + |
| Anthrocyamidines | - | - | + | - |
| Anthroquinones | - | - | - | - |
| Saponins | - | - | + | + |
| Tannins | + | + | + | + |
| Glycosides | + | - | + | + |

(-) Refers As Absent & (+) Refers As Present

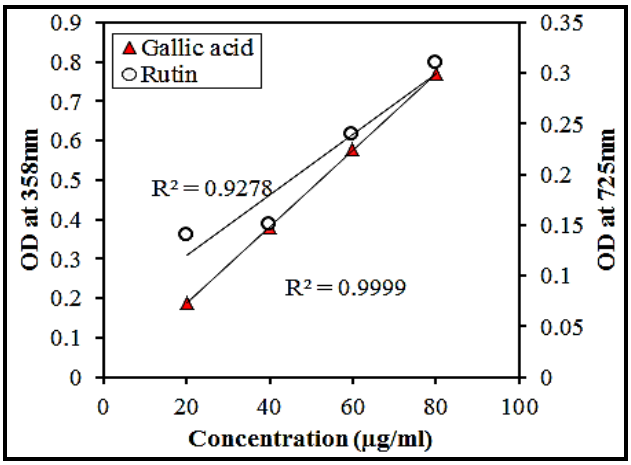



Figure 1: Calibration Plot For Total Phenols and Flavonoids; Standards Used Were Gallic Acid and Rutin, Respectively

## Antimicrobial Screenings

### *Zone of Inhibitions*

The antimicrobial activity of n-Hexane, ethyl acetate, aqueous and methanol extracts of *T.populnea* root against the tested pathogens of genitourinary tract infections (*Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus fecalis, Gardenerella vaginalis, Candida albicans)*. The results displayed that the methanolic extract of root exhibited highest activity (tables 2 to 4).

Table 2: Antimicrobial Activity of n-Hexane Extract of Root

| Microorganisms | Diameter of zone of inhibition (mm) at different concentration levels | | | | Standard drug (µg/ml) |
|---|---|---|---|---|---|
| | n-Hexane extract | | | | |
| | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | |
| *E.coli* | 19±0.2 | 22±0.3 | 23±0.1 | 25±0.2 | 32±0.2 |
| *E.foecalis* | 19±0.1 | 20±0.2 | 21±0.4 | 24±0.1 | 28±0.1 |
| *P.aeruginosa* | 14±0.4 | 20±0.2 | 21±0.1 | 22±0.1 | 35±0.4 |
| *S.aureus* | 15±0.2 | 18±0.1 | 18±0.1 | 21±0.4 | 35±0.1 |
| *S.epidermidis* | 16±0.3 | 18±0.2 | 19±0.2 | 20±0.1 | 35±0.4 |
| *K.pneumonia* | 16±0.1 | 17±0.1 | 18±0.3 | 19±0.2 | 32±0.3 |
| *G. vaginalis* | 9±0.2 | 11±0.1 | 12±0.3 | 15±0.2 | 18±0.3 |
| *C. albicans* | 8±0.1 | 11±0.3 | 14±0.2 | 16±0.1 | 30±0.1 |

**Note:** Standard drug used: ciprofloxacin for G (+Ve) and G (-Ve) bacteria at concentration (5 µg/ml); Flucanozole for *Candida albicans* (5 µg/ml), Metronidazole 20 µg/ml for *Gardenerella vaginalis*. Data are means (n=3) ± standard deviation of three replicates.

Table 3: Antimicrobial Activity of Methanol Extract of Root

| Microorganisms | Diameter of zone of inhibition (mm) at different concentration levels | | | | Standard drug (µg/ml) |
|---|---|---|---|---|---|
| | n-hexane extract | | | | |
| | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | |
| *E. coli* | 20±0.4 | 21±0.2 | 26±0.2 | 28±0.1 | 32±0.2 |
| *E. foecalis* | 20±0.2 | 20±0.1 | 20±0.3 | 23±0.1 | 28±0.1 |
| *P. aeruginosa* | 15±0.3 | 18±0.2 | 24±0.1 | 25±0.2 | 35±0.4 |
| *S. aureus* | 15±0.2 | 18±0.1 | 20±0.3 | 21±0.1 | 35±0.1 |
| *S. epidermidis* | 15±0.1 | 17±0.4 | 20±0.2 | 20±0.3 | 35±0.4 |
| *K. pneumonia* | 17±0.2 | 18±0.1 | 19±0.1 | 23±0.2 | 32±0.3 |
| *G. vaginalis* | 6±0.3 | 15±0.2 | 15±0.1 | 16±0.2 | 18±0.3 |
| *C. albicans* | 10±0.2 | 11±0.3 | 14±0.1 | 19±0.1 | 30±0.1 |

**Note:** Standard drug used: ciproflaxcin G(+Ve) and G(-Ve) bacteria of concentration (5 µg/ml); Flucanozole for *Candida albicans* (5 µg /mL), metronidazole 20 µg/mL for *Gardenerella vaginalis*. Data are means (n=3) ± standard deviation of three replicates.

Table 4: Antimicrobial Activity of Ethyl Acetate Extract of Root

| Microorganisms | Diameter of zone of inhibition (mm) at different concentration levels | | | | Standard drug (µg/ml) |
|---|---|---|---|---|---|
| | n-hexane extract | | | | |
| | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | |
| *E. coli* | 21±0.3 | 22±0.2 | 22±0.1 | 23±0.2 | 32±0.2 |
| *E. foecalis* | 19±0.1 | 19±0.1 | 22±0.2 | 23±0.4 | 28±0.1 |
| *P. aeruginosa* | 15±0.2 | 17±0.2 | 18±0.1 | 19±0.1 | 35±0.4 |
| *S. aureus* | 17±0.2 | 17±0.1 | 18±0.3 | 19±0.2 | 35±0.1 |
| *S. epidermidis* | 18±0.4 | 18±0.2 | 19±0.1 | 19±0.1 | 35±0.4 |
| *K. pneumonia* | 13±0.1 | 15±0.3 | 17±0.2 | 18±0.3 | 32±0.3 |
| *G. vaginalis* | 10±0.2 | 11±0.1 | 12±0.3 | 14±0.1 | 18±0.3 |
| *C. albicans* | 11±0.1 | 14±0.2 | 15±0.2 | 17±0.1 | 30±0.1 |

**Note:** Standard drug used: Ciproflaxcin for G (+Ve) and G(-Ve) bacteria of concentration (5 µg/mL); Flucanozole for *Candida albicans* (5 µg/mL), Metronidazole (20µg/mL) for *Gardenerella vaginalis*. Data are means (n=3) ± standard deviation of three replicates

Table 5: Antimicrobial Activity of Aqueous Extract of Root

| Microorganisms | Diameter of zone of inhibition (mm) at different concentration levels | | | | Standard drug (µg/ml) |
|---|---|---|---|---|---|
| | n-hexane extract | | | | |
| | 10 mg/mL | 20 mg/mL | 30 mg/mL | 40 mg/mL | |
| *E. coli* | 12±0.2 | 14±0.2 | 15±0.1 | 15±0.3 | 32±0.2 |
| *E. foecalis* | 11±0.3 | 14±0.1 | 14±0.2 | 16±0.1 | 28±0.1 |
| *P. aeruginosa* | 11±0.1 | 14±0.2 | 15±0.1 | 16±0.4 | 35±0.4 |
| *S. aureus* | 13±0.2 | 13±0.1 | 14±0.2 | 14±0.3 | 35±0.1 |
| *S. epidermidis* | 14±0.1 | 14±0.3 | 18±0.2 | 21±0.1 | 35±0.4 |
| *K. pneumonia* | 13±0.2 | 14±0.4 | 14±0.1 | 17±0.1 | 32±0.3 |
| *G. vaginalis* | 6±0.2 | 10±0.1 | 12±0.3 | 12±0.1 | 18±0.3 |
| *C. albicans* | 6±0.1 | 9±0.2 | 11±0.2 | 12±0.1 | 30±0.1 |

**Note:** Standard drug used: Ciproflaxcin for G (+Ve) and G(-Ve) bacteria of concentration (5 µg/mL); Flucanozole for *Candida. albicans* ((5 µg/mL), Metronidazole 20 µg/ml for *Gardenerella vaginalis*. Data are means (n=3) ± standard deviation of three replicates

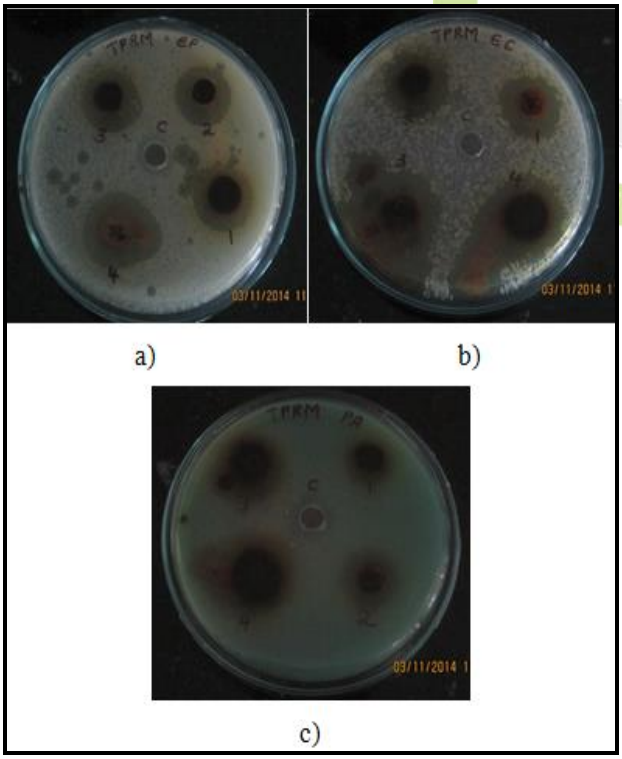



Figure 2: Zone Of Inhibitions Obtained For methanolic extract A) *E. foecalis*; B) *E.coli*; C) *P. aerugenosa*

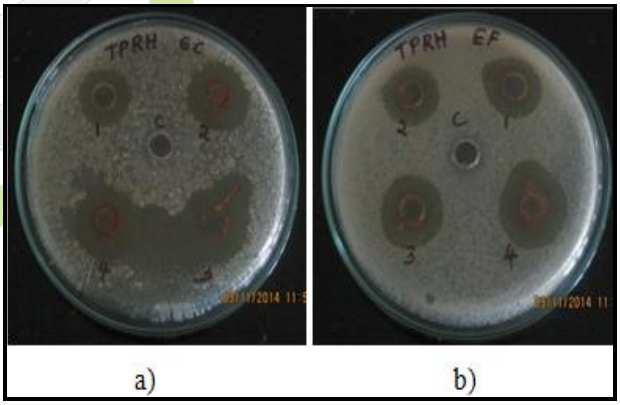



Figure 3: Zone Of Inhibition for n-Hexane Extract of A) *E.coli*; B) *E.foecalis*

The test concentrations of all the extracts were in the range of 10 to 40 mg/mL for antimicrobial activity. The inhibition pattern was observed to be varied with the sort of microorganism employed, solvent type and their concentration of extract. The zone of inhibition increased with the concentration of extract used. The tested samples were used in the range of 1 to 4 mg/well. The zone of inhibitions for all the concentrations of the extracts was shown in tables 2-4.

The inhibition zones were formed to be in the ranges from 10±0.2 to 23±0.4 mm for hexane extract, 10±0.2 to 23±0.4 mm for ethyl acetate extract, 6±0.3 to 28±0.1mm for methanol and 6±0.1 to 21±0.1mm aqueous extracts. Among the tested extracts the maximum zone of inhibition (28±0.1mm.) was found for methanol.

Maximum zone of inhibition, 28±0.1mm, was observed for methanolic extract at 40 mg/mL concentration (Table 3). The antifungal activity of this extract against *Candida albicans* (concentration 40 mg/mL) produced 19±0.1mm zone of inhibition.

The same extract showed in16±0.2mm zone of inhibition on *Gardenerella vaginalis.* The antimicrobial activity of n-Hexane, ethyl acetate, water extracts were lesser when compared to methanol extract of *Thespesia populnea* root (Fig. 3).The Methanolic extract contributes the highest zone of inhibition (28±0.1mm and 25±0.2mm) for *E.coli and P.aeruginosa* however the same extract inhibited *S.epidermidis* poorly(15±0.1mm).

**Minimum Inhibitory and Bactericidal Concentrations**

The MICs of the root extracts observed were analysed and they were found to be in range from 0.06 mg/mL to 2mg/mL for bacterial strains and 0.25 mg/mL to 2mg/mL for *Candida albicans*. In case of bacterial strains, methanol extract showed potent activity against the *E.coli*, *P.aeruginosa* having MICs of 0.062 mg/mL and 0.125 mg/mL, respectively (Table 6).

Similarly the methanol extracts showed the lowest MICs against C*andida albicans* when compared to other extracts. The MBCs were observed to be in the range of 0.125 mg/mL to 0.5 mg/mL for bacterial strains and 0.25 to 2 mg/mL for *Candida albicans*. Similarly the methanol extract showed the lowest MBCs against *Candida albicans* when compared to the other extracts. *P-values* calculated by excel software found to be $p<0.05$ evaluates significant values.

Table 6: MIC and MBC of Different Solvent Extracts of *Thespesia Populnea* Root

| Name of the organism | Solvent extracts | | | | | | | | Standard drug (µg/mL) |
|---|---|---|---|---|---|---|---|---|---|
| | Hexane | | Ethyl acetate | | Methanol | | Aqueous | | |
| | MIC mg/mL | MBC mg/mL | MIC mg/mL | MBC mg/mL | MIC mg/mL | MBC mg/mL | MIC mg/mL | MBC mg/mL | |
| *E.coli* | 0.5 | 0.5 | 0.25 | 0.5 | 0.062 | 0.125 | 1 | 2 | 0.058 |
| *E.foecalis* | 0.5 | 1 | 0.5 | 1 | 0.25 | 0.5 | 2 | 3 | 0.026 |
| *P.aeruginosa* | 0.5 | 1 | 0.5 | 1 | 0.125 | 0.25 | 1 | 2 | 0.078 |
| *S.aureus* | 0.5 | 1 | 0.5 | 1 | 0.25 | 0.5 | 1 | 2 | 0.035 |
| *S.epidermidis* | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 | 0.5 | 1 | 2 | 0.043 |
| *K.pneumonia* | 0.5 | 1 | 0.5 | 1 | 0.25 | 0.5 | 1 | 3 | 0.124 |
| *Gardenerell avaginalis* | 0.7 | 1 | 0.25 | 1 | 0.125 | 0.25 | 1 | 2 | 0.062 |
| *Candida albicans* | 0.6 | 0.75 | 0.5 | 0.5 | 0.25 | 0.5 | 2 | 3 | 0.028 |

Standard drug used: Ciprofloxacin for G (+Ve) and G(-Ve) bacteria and Fluconazole for C*andida albicans*, metronidazole for *Gardenerella vaginalis*

MIC: Minimum Inhibition Concentration, MBC: Minimum Bacterial Concentration.

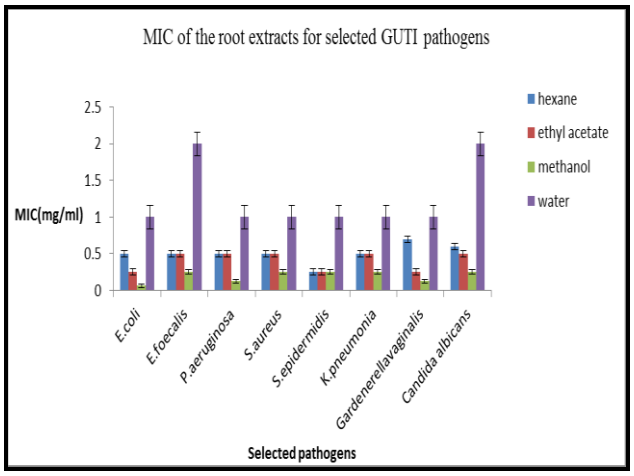



Figure 3: Minimum Inhibition Concentration of Root Solvent Extracts for Selected Pathogens

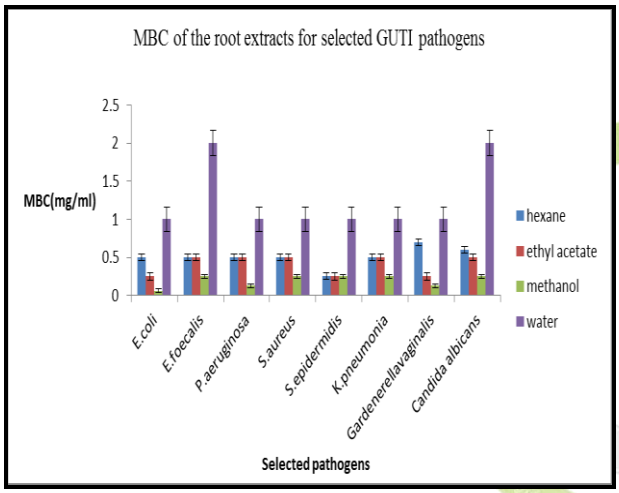



Figure 4: Minimum Bacterial Concentration of Root Solvent Extracts for Selected Pathogens

## Thin layer Chromatographic Analysis

Thin layer chromatographic analysis of the methanol extract showed presence of different compounds (Fig. 3) under long and short UV-wavelengths.

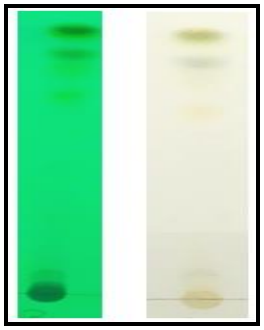


Figure 5: Thin Layer Chromatograms of methanol extract

The $R_f$ values for each component was measured and furnished in table 8.

Table 8: Chromatogram of TPR: Observed Color Bands and R*f* Values of Methanolic Extracts

| **Petroleum ether : Ethyl acetate (9:1)** | | **Ethyl acetate : toluene: formic acid (4.5:2.5:0.75)** | |
|---|---|---|---|
| **Anisaldehyde-$H_2SO_4$** | | **Ethanolic $FeCl_3$** | |
| **Colour spot/ band observed** | **R*f*** | **Colour spot/ band observed** | **R*f*** |
| Violet-blue | 0.65 | Blue | 0.70 |
| Light yellow/bluish violet | 0.78 | Brown | 0.85 |
| Dark blue-violet | 0.81 | - | - |

## GC-MS Analysis

The compounds were identified from the recorded mass spectrum by comparing the existance of mixture of compounds spectra with masses National institute of Standards and Technology (NIST) library having more than 62000 patterns. The resultant chromatogram showed different peaks (table-9), the methanol extract showed 15 assorted components in the GC-MS spectrum. In the gas chromatogram, the retention time eluted as a relative concentration of compounds present in root extract. The relative concentrations identified by the height of peaks (fig 6) in mass spectra compared chromatogram 4H-1-Benzopyran-4-one,3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy- at retention time of 14.96 gave the large chromatographic peak whereas Tetratetracontane at retention time of 6.31 showed the lowest peak as shown in fig.6. A spectrum of unknown compounds was compared with the NIST library. The name, molecular weight and structure of the possible compounds were ascertained[15].

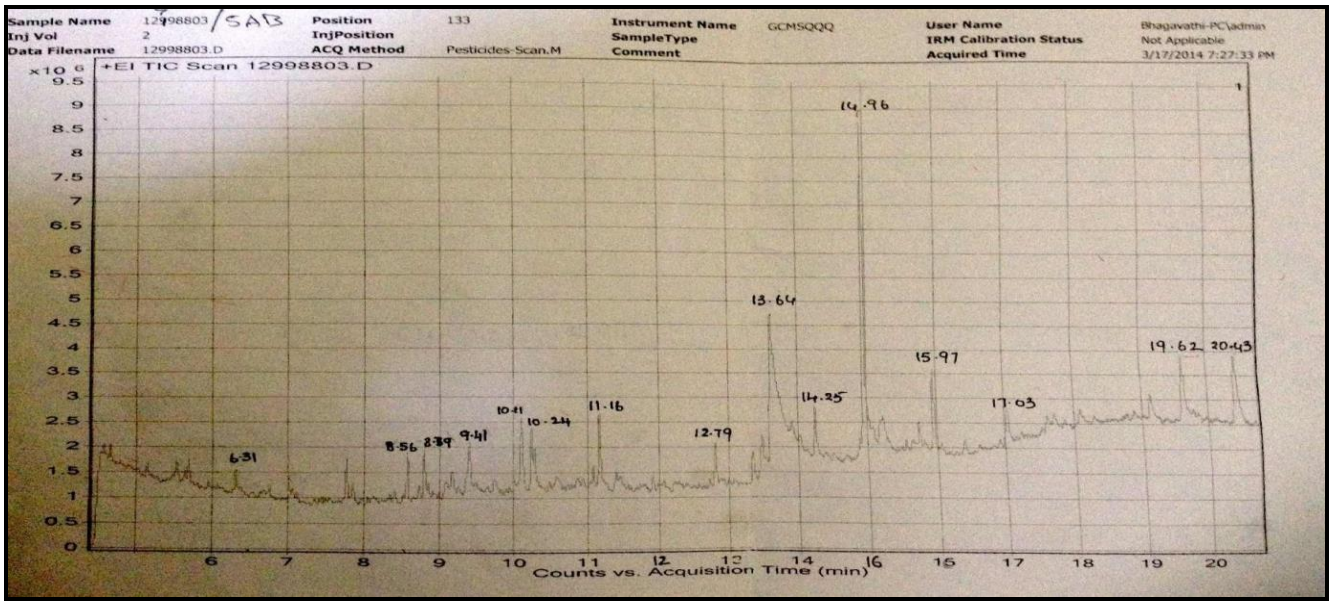


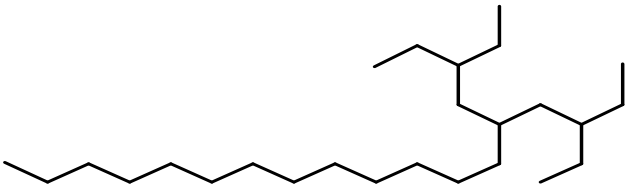
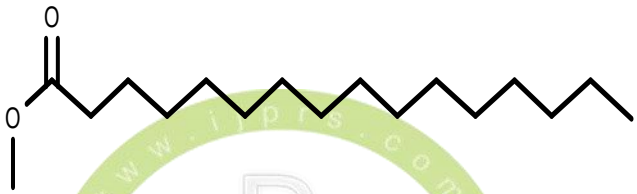
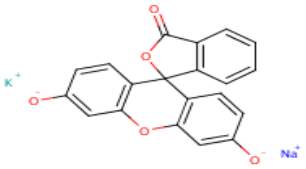
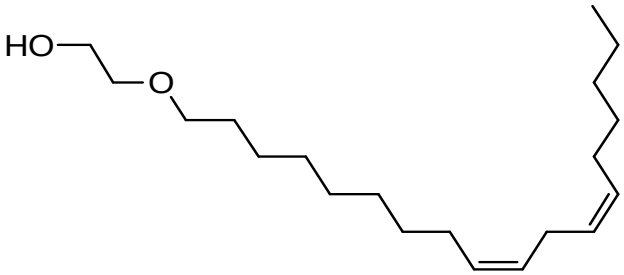

Figure 6: Chromatogram for GC-MS of Methanolic Root Extract of Thespesia Populnea

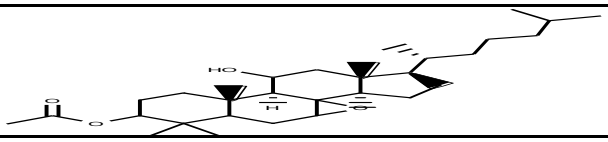
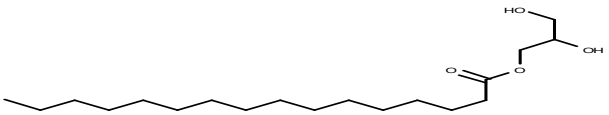
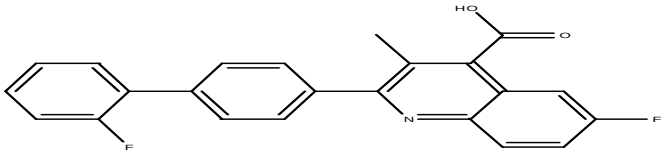
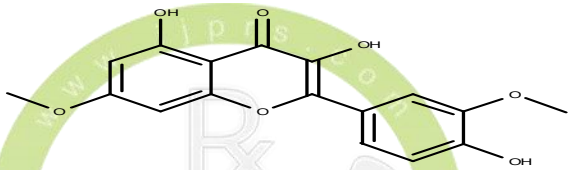
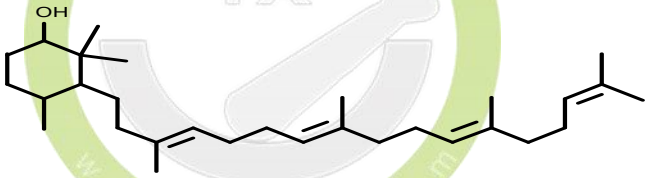
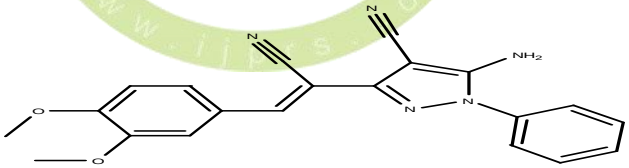
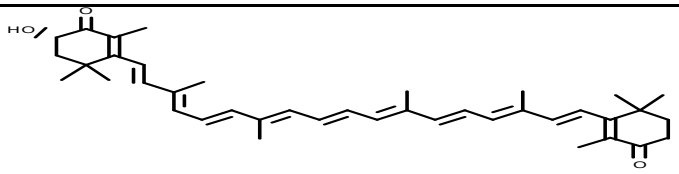
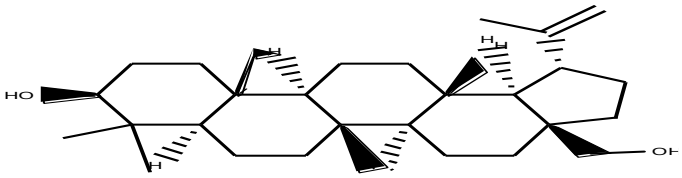
Table 9: Compounds Identified in the Mixture of Methanol Root Extract of Thespesia Populnea

| S.No | Component name | Chemical formula | Molecular weight | Retention time |
|---|---|---|---|---|
| 1 | Tetratetracontane | $C_{44}H_{90}$ | 618 | 6.31 |
| 2 | Octadecane, 3-ethyl-5-(2-ethylbutyl) | $C_{26}H_{54}$ | 366 | 8.56 |
| 3 | Hexadecanoic acid, methyl ester | $C_{17}H_{33}O_2$ | 269 | 8.79 |
| 4 | Heptadecane, 9-hexyl- | $C_{23}H_{48}$ | 324 | 9.41 |
| 5 | Spiro[isobenzofuron-1(3H),9′-(9H)xanthene]-3-one | $C_{20}H_2O_3$ | 290 | 10.11 |
| 6 | Ethanol, 2-(9,12-octadecadienyloxy)-,(Z,Z)- | $C_{20}H_{38}O_2$ | 310 | 10.24 |
| 7 | 17-pentatriacontene | $C_{35}H_{70}$ | 490 | 11.16 |
| 8 | 7,8-epoxylanostan-11-ol,3-acetoxy | $C_{32}H_{54}O_4$ | 502 | 12.79 |
| 9 | Glycerol-1-palmitate | $C_{19}H_{38}O_4$ | 330.5 | 13.64 |
| 10 | Brequinar | $C_{23}H_{15}F_2NO_2$ | 375 | 14.25 |
| 11 | 4H-1-Benzopyran-4-one,3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy- | $C_{17}H_{14}O_7$ | 330.29 | 14.96 |
| 12 | 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol | $C_{30}H_{52}O$ | 428 | 15.97 |
| 13 | 5-Amino-3-[1-cyano-2-[3,4-dimethoxyphenyl]vinyl]-1-phenyl-1H-pyrazole-4-carbonitrile | $C_{15}H_{13}N_5O_2$ | 295.29 | 17.03 |
| 14 | Astaxanthin | $C_{40}H_{52}O_4$ | 596 | 19.62 |
| 15 | Betulin | $C_{30}H_{50}O_2$ | 442 | 20.43 |

Table 10: Therapeutic Use of Biologically Active Compounds Identified in Thespesia Populnea Root Extract

| S.No/Component Name | Structure | Properties |
|---|---|---|
| **1**.Tetratetracontane |  | fuel |
| **2.** Octadecane, 3-ethyl-5-(2-ethylbutyl) |  | olatile |
| **3.** Hexadecanoic acid, methyl ester |  | Antioxidant<br>Hypocholesterolenic<br>Pesticide<br>5-α reductase inhibitor |
| **4.** Heptadecane, 9-hexyl- |  | Hydrocarbon |
| **5.** Spiro[isobenzofuron-1(3H),9′-(9H)xanthene]-3-one |  | Wood stain<br>Solven dye |
| **6.** Ethanol, 2-(9,12-octadecadienyloxy)-,(2,2)- |  | Anti-inflammatory<br>Anticancer<br>Hypochlorestorelenic<br>Antihistamine<br>Antinematode<br>Antiandrogeic<br>Anticoronary<br>Hepatoprotectve<br>5-α reductase inhibitor |

| | | |
|---|---|---|
| **7.** 17-pentatriacontene |  | Anti-inflammatory<br>Anticancer<br>Antibacterial<br>Antiarthritic |
| **8.** 7,8-epoxylanostan-11-ol,3-acetoxy |  | Antimicrobial |
| **9.** Glycerol-1-palmitate |  | Triacyl glycerol |
| **10**. Brequinar |  | Immunosuppressive agent<br>Antineoplastic<br>Enzyme inhibitor |
| **11.** 4H-1-Benzopyran-4-one,3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy- |  | Antibacterial |
| **12.** 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol |  | Fuel |
| **13.** 5-Amino-3-[1-cyano-2-[3,4-dimethoxyphenyl]vinyl]-1-phenyl-1H-pyrazole-4-carbonitrile |  | Pesticide |
| **14.** Astaxanthin |  | Anti-oxidant<br>Anti-ageing<br>Anti-cancer<br>Cardiovascular disease |
| **15. Betulin** |  | Anticancer<br>Antiviral<br>Antiflu<br>Antiseptic<br>Psoriasis<br>Eczema<br>Prostaglandin synthesis inhibitor |

## DISCUSSION

Although previous findings reported the antimicrobial activity of the root, current research provides an extended study on antimicrobial activity of *Thespesia populnea* root against GUTI pathogens for the first time. Previous studies include pharmacognostic and phytochemical standards of root[16], antioxidant activity[17], anti-inflammatory activity and antimicrobial activity of root[18]. Senthil et.al reported that the ethanol extracts of root produced maximum zone of inhibition against *Staphylococcus aureus* and *Escherichia. coli* of 27±0.2mm and 24±0.5mm, respectively whereas the lowest inhibition zone for *Pseudomonas aerugenosa* of 22±0.2mm at 250 µg/mL concentration. For *Candida. albicans* at 250 µg/mL concentration zone of inhibition produced was 22±0.4mm[18]. In the present investigation, at this concentration the methanolic extract exhibited 28±0.1, 25±0.2 and 19±0.1 mm of inhibition zones against *Escherichia. coli*, *Pseudomonas aerugenosa* and *Candida albicans*

The phytochemical screening of *Thespesia populnea* root extracts revealed the existence of flavonoids, phenols, steroids, saponins and tannins as shown in Table 1. The strong antibacterial and antifungal activity was due to the presence of tannins. The flavonoids, phenols, steroids and saponins found to be used in synthetic drugs as starting materials. Alkaloids significantly used as anesthetics, stimulants, analgesics and antibacterial[19]. In the present study, among all the solvent extracts the methanol based extract exhibited the highest total phenolic and flavonoid content than the rest. Hence methanol is the best solvent system. However, much literature on scientific data on antimicrobial activity of *Thespesia populnea* root extract is not available. The antimicrobial assay showed that the methanol extract has significant inhibition against the selected GUTI pathogens whereas n-hexane, ethyl acetate and aqueous extracts have low zone of inhibition comparatively. The results of this study showed that the extracts having MIC values less than 0.25 mg/mL were considered as strong pathogenic inhibitor, 0.25 to 0.5 mg/mL as moderate inhibitors and more than 0.5 as weak inhibitors. Senthil et al, identified as MIC of root extract for *E.coli* was 25 mg/mL at 250 mg/mL concentration and for *C.albicans* was 750 mg/mL at 2000 mg/mL concentration[18]. In the present study minimum inhibitory concentration for *E.coli* and *C.albicans* reported 0.062 mg/mL and 0.25 mg/mL, respectively.

Hence, the screening of phytochemical constituents acts as an initial step for identifying the potential of active compounds. The methanol extract of *Thespesia populnea* root subjected to GC-MS analysis for further investigation. From GC fractions of the extract 15 components were identified through mass spectrometry. Due to strong extraction capacity of methanol the solvent is responsible for the leaching out of various bioactive compounds. Based on ethnobotanical databases, results revealed the presence of Tetratetracontane, Octadecane,3-ethyl-5-(2-ethylbutyl), Hexadecanoic acid, methyl ester, Heptadecane,9-hexyl-, Spiro[isobenzofuron-1(3H),9′-(9H)xanthene]-3-one, Ethanol,2-(9,12-octadecadienyloxy)-,(Z,Z)-, 17-pentatriacontene, 7,8-epoxylanostan-11-ol,3-acetoxy, Glycerol-1-palmitate, Brequinar, 4H-1-Benzopyran-4-one,3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-, 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15 tetraenyl)-cyclohexanol, 5-Amino-3-[1-cyano-2-[3,4- dimethoxyphenyl]vinyl]-1-phenyl-1H-pyrazole-4-carbonitrile, Astaxanthin and Betulin.

Several studies reported that the secondary metabolites of plants possess therapeutic benefits. Among the identified phytochemicals, 17-pentatriacontene, Hexadecanoic acid, methyl ester, 7,8-epoxylanostan-11-ol,3-acetoxy, Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-, 4H-1-Benzopyran-4-one,3,5-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-, 5-Amino-3-[1-cyano-2-[3,4- dimethoxyphenyl] vinyl]-1-phenyl-1H-pyrazole-4-carbonitrile and Betulin has the properties of antimicrobial, anticancer, enzyme inhibitor and decreases cholesterol. Brequinar used as

immunosuppressive agent to avoid graft rejection. Astaxanthin, Hexadecanoic acid, methyl ester, Betulin has antioxidant and anticancer properties. Spectrum profile of GC-MS confirmed the compounds possessing flavonoids, alkaloids, alcohols, biphenyls, terpenes, pyrazoles, triacylglycerols, fatty acid esters, linolenic acid and carotenoids etc. has antimicrobial activity. From a recent report the carotenoids were found to be important antioxidant molecules in humans[24,25,26,27].

Based on this study, methanolic extract paves the way for developing several treatment regimens and identification of adulterants. Hence the present investigation on the root supports prominently in developing novel antimicrobial drugs.

## CONCLUSION

The phytochemical analysis of the extracts revealed the existence of various constituents including flavonoids, phenols, steroids, saponins and tannins and these are capable of inhibiting bacteria and fungi growth. GC-MS analysis of methanolic root extract identified 15 bioactive compounds. Carotenoids, alkaloids and flavonoids found to have considerable antimicrobial potential which makes the plant as a competent drug resource. The screening results showed that the methanol extract of the root is highly effective towards inhibiting the pathogens involved in genitourinary tract infections (GUTI). This could be helpful for discovering new antibiotic resistant antimicrobial drugs to combact the antibiotic resistant strains.

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