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# Phytochemical Screening of *Hibiscus tiliaceus* by FTIR Spectroscopic Analysis

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## Abstract
Different parts of *Hibiscus tiliaceus* plant such as leaves, bark, flower and roots have medicinal properties and thus *H. tiliaceus* is potentially allelopathic and may produce compounds that are useful in Ayurveda. So to determine presence of various phytochemicals from the plant extracts, a simple, precise Preparative Thin layer chromatography was performed. Initially to confirm the presence of phytochemicals, qualitative tests for Tannins, Phlobatannins, Saponins, Flavonoids, alkaloids, Quinones, Coumarine, Triterpenoids, Cardi-glycosides, Anthraquinoacne glycosides, Steroids, Phytosterols were performed. The methanolic extract of leaves, bark, flower and root of *H.tiliaceus* upon separation using preparative TLC, showed separated bands. In this study, the most suitable solvent system for analysis was found to be chloroform: glacial acetic acid: methanol: water in the ratio 6:2:1:1 with the highest resolution power. Separated TLC bands, after purification were further analysed for their antimicrobial activity against pathogens by using agar cup method. Air dried samples were subjected to FTIR analysis. FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different extract.

**Keywords**
*H. tiliaceus*, allelopathic, TLC, FTIR, Infrared radiation.

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**INTRODUCTION:**
Nowadays 'Alternative medicine' is very common concept focused on the idea of using the plants and plant derived components for medicinal purposes [1]. The valuable medicinal properties such as antimicrobial, antioxidant of different plants are due to presence of several plant chemical constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol alkaloids, flavonoids, terpenoids. Among these plants *Hibiscus* (genus), Malvaceae contains several species many of which have been used medicinally and is comprises of about 250 species in tropics and subtropic [2]. *Hibiscus tiliaceus* (Belpata/Sea hibiscus) is evergreen, sprawling tree belonging to family **Malvaceae**. This plant is found along the eastern and western coast of India [3] [4]. One term 'Allelochemicals' means chemical that inhibit soil microorganisms and also have ability to inhibit

bacteria and fungi that are pathogenic to humans. This property is found in *H. tiliaceus* as this plant is used in traditional medicine in various parts of the world to treat infectious diseases or conditions that are caused by infectious organism [5]. This property may be due to the presence of certain phytochemical constitutes of plant.

## MATERIALS AND METHODS:

**Phytochemical investigation of aqueous extract of *H. tiliaceus* plants by qualitative tests:**

Plant part used: washed shade dried powdered form of leaves, bark, flowers and roots

Location of sampling: Mandovi beach, Ratnagiri, Maharashtra.

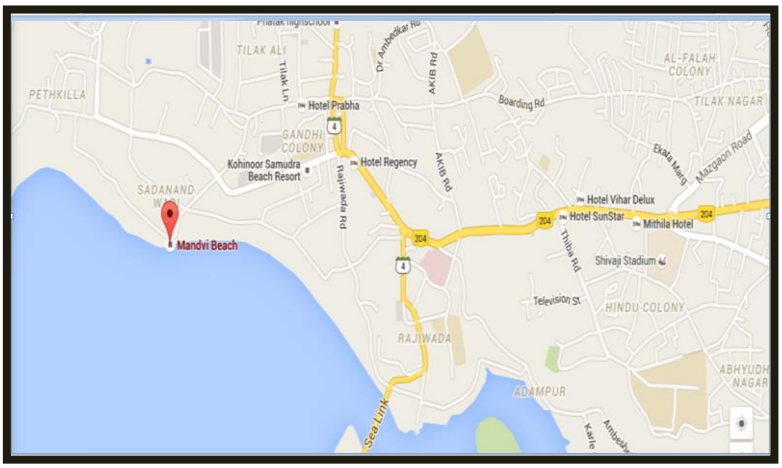



**Fig 1: Satellite map of Mandovi beach, Ratnagiri (Google image)**

**Preparation of plant extract**:

About 1 gm of leaf, bark, flower and root powder of *H.tiliaceus* was used to prepare 1% of leaf, bark, flower and root aqueous extract respectively. For this purpose, 1 gm of each powder was placed in conical flask and 100 ml Distilled water was added and plugged with cotton. The powder material was extracted with Distilled water for 24 hours at room temperature with continuous stirring. After 24 hours, the supernatant was collected by filtration and the extract was used for further experiments such as qualitative phytochemical testing.

**Qualitative tests for phytochemicals [6]:**

The aqueous extract of leaves, bark, flower and root was subjected to different chemical tests for detection of different phyto-constituents using standard procedure.

**Test for tannins (Ferric chloride) test**: 1 ml of sample was taken in a test tube and then 1-2 drops of 5% ferric chloride was added and observe for blue black colour, formation of blue colour indicated the presence of hydrolysable tannins

**Test for phlobatannins**: Crude extract of leaves was boiled with 2% aqueous HCl. The deposition of a red precipitation was taken as evidence for presence of phlobatannins

**Test for saponins**: Crude extract was mixed with 5 ml of D/W in a test tube and it was shaken vigorously. Formation of stable foam was taken as indication for the presence of saponins.

**Test for flavonoids**: 5ml of diluted ammonia solution were added to a portion of crude extract followed by addition of conc. $H_2SO_4$. A yellow colouration observed in each extract indicated presence of flavonoids. The yellow colouration disappeared on standing.

**Test for alkaloid**: Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown coloured precipitate indicate the presence of alkaloids.

**Test for quinine**: Dil. NaOH was added to the 1 ml of crude extract. Blue green or red colouration indicates the presence of quinines.

**Test for coumarin**: 10% NaOH was added to the extract and chloroform was added for the observation of yellow colour which shows the presence of coumarin.

**Test for terpenoids (Salkowski test):** 5ml of extract was mixed with 2 ml of chloroform and 3 ml of conc. $H_2SO_4$ was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive result for the presence of terpenoids.

**Test for Cardiac glycosides**: 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 ml of conc. $H_2SO_4$. A brown ring of the interface indicates a deoxy sugar characteristics of cardenolides. A violet ring may appear below the

brown ring while in the acetic acid layer a greenish ring may form just gradually throughout thin layer.

**Test for Anthraquinones glycosides**: 3 ml of extract treated with dilute $H_2SO_4$, boiled and filtered and cold filtrate was treated with equal volume benzene. After separation of organic layer ammonia was added. Pink colour may appear which shows presence of anthraquinone glycosides.

**Test for steroids**: 2 ml of acetic anhydride was added to 0.5 ml of crude extract with 2 ml of $H_2SO_4$. The colour changed from violet to blue or green in sample indicates the presence of steroids.

**Test for phytosterols- Libermann- Burchard's test**: 2 mg of extract was dissolved in 2 ml of acetic anhydride heated to boiling cooling and then 1 ml of conc $H_2SO_4$.was added alongside of test tube. A brown ring formation at junction and turning upper layer to dark green colour confirmed presence of phytosterols.

**Preparative Thin Layer Chromatography:**

Optimized conditions:

The TLC was performed on precoated glass plate and thick plate

Sample: 10% Methanolic extract of leaves, bark, flower and root of *H.tiliaceus*

Solvent system used: chloroform: glacial acetic acid: methanol: water in the ratio 6:2:1:1

Silica gel G (Himedia) with calcium sulphate as binder was used to prepare thick preparative TLC plates. After making plates, were left overnight for air drying and then put at 100 $^oC$ for two hours to activate them. When plates were ready, methanolic extract was loaded on plate. The plates were dried and developed in a suitable solvent for rapid screening chloroform: glacial acetic acid: methanol: water in the ratio 6:2:1:1 [7]. The plates were then placed in iodine chamber which was pre saturated with iodine. Plates were kept in iodine chamber for few minutes to observe visible band.

**Extraction of separated TLC fractions:**

The compound from developed TLC bands were separated and recovered by scraping the adsorbent from the plate and eluting with a strong solvent chloroform.

**Antimicrobial activity of TLC separated phyto-compounds:**

The recovered separated phytochemical fractions were assessed for their antibacterial activity against the yeast *Candida albicans* and pathogenic bacteria such as *E. coli, S. aureus, Pseudomonas aeruginosa, S. pyogenes, S. typhi, Klebsiella pneumoniae, S. paratyphi B, P. vulgaris, P. mirabilis, M. furfur etc.* by using Agar Cup method. The cultures were obtained from Microbial Culture Collection, Department of Microbiology, Gogate Jogalekar College, Ratnagiri.

**UV-Vis spectroscopic analysis of TLC recovered fractions:**

The solutions were prepared separately by dissolving standard and phytochemical fractions in a methanol (After optimization of all conditions) for UV analysis and the absorbance is scanned from 250 nm to 500 nm using Perken Elmer UV-VIS lambda-25 spectrophotometer.

**FTIR Spectroscopic analysis of TLC recovered fractions:**

LABTRONICS, FT/IR-4100 type A, Serial number-C193161016, INDIA

FTIR analysis was performed using Perkin Elmet spectrophotometer system, which was used to detect the characteristics peaks and their functional groups. The peak values of the FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

**Table – 1: Measurable parameters for FTIR spectra.**

| Sr. No | Measurable parameter | Sr. No | Measurable parameter |
|---|---|---|---|
| 1 | Light source - Standard | 10 | Detector- TGS |
| 2 | Accumulation- Auto (512) | 11 | Resolution- 4 $cm^{-1}$ |
| 3 | Zero filling- On | 12 | Apodization- Cosine |
| 4 | Gain- Auto (64) | 13 | Aperture- Auto (7.1mm) |
| 5 | Scanning Speed - Auto(2mm/sec) | 14 | Filter- Auto(30000 Hz) |
| 6 | Data array type- Linear data type | 15 | Horizontle axis- Wavenumber( $cm^{-1}$) |
| 7 | Vertical axis- % T | 16 | Start- 349.053 $cm^{-1}$ |
| 8 | End- 7800.65 $cm^{-1}$ | 17 | Data interval- 0.964233 $cm^{-1}$ |
| 9 | Data points- 7729 | | |

**RESULTS AND DISCUSSION:**

According to previous research on phytochemical screening of *H. tiliaceus*, the results revealed that only tannins were present in the leaf extract, whereas alkaloids, reducing sugar and tannins were found in the bark extract. The phytochemical analysis of the leaf and bark extract of the *H. tiliaceus* showed the rich presence of different groups of secondary

metabolites [8]. All of these are known to possess medicinal activity as well as physiological activity.

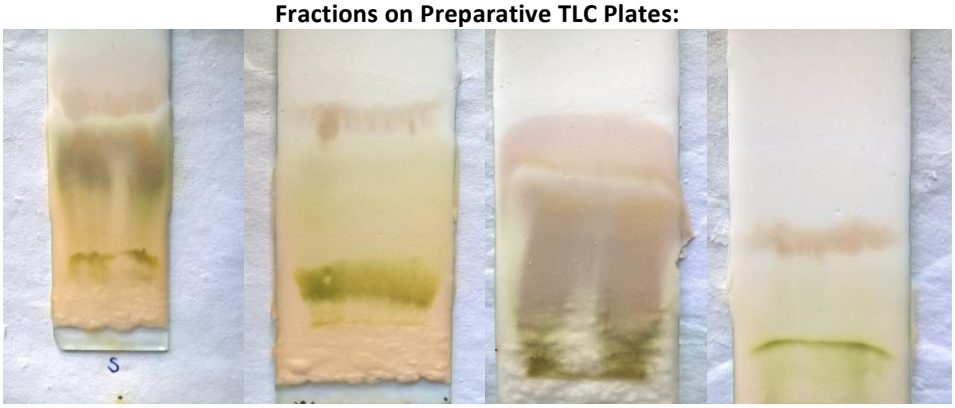
In present study, the phytochemical analysis indicates the presence of phytochemicals such as Tannins, Phlobatannins, Saponins, Flavonoids, alkaloids, Quinones, Coumarine, Triterpenoids, Cardiac glycosides, Anthraquinone glycosides, Steroids, Phytosterols in the aqueous extract of leaves, bark, flowers and roots of *H.tiliaceus* plant. The presence of tannins in leaf extracts may justify its traditional usage for the management of some infections and diseases such as diarrhea. Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents [8]. All four aqueous extract contained appreciable amount of 12 phytochemicals. These phytochemicals have been implicated in having diverse medicinal and pharmacological properties. Leaf and bark extract could be the potential candidates for determination of antitumor and anticancer properties [8].

**Table- 2: Results of qualitative phytochemical tests on *H.tiliaceus* plant**

| Sr. No. | Phytochemicals | Aqueous leaves extract | Aqueous bark extract | Aqueous flower extract | Aqueous root extract |
|---|---|---|---|---|---|
| 1 | Tannin | + | + | + | + |
| 2 | Phlobatannins | + | - | - | - |
| 3 | Saponin | + | + | + | + |
| 4 | Flavanoids | + | + | + | + |
| 5 | Alkaloids | + | + | + | + |
| 6 | Quinone | - | + | - | - |
| 7 | Coumarin | + | + | + | + |
| 8 | Terpenoids | + | - | + | - |
| 9 | Cardiac glycosides | + | - | - | - |
| 10 | Anthraquinone glycosides | + | - | + | - |
| 11 | Steroids | + | + | - | - |
| 12 | phytosterols | + | + | - | - |

Medicinal properties of plants are normally dependent on the presence of certain phytochemcial principles such as alkaloids, anthraquinones, glycosides, saponins, tannins and flavonoids which are the bioactive bases responsible for the pharmacological property [9].

**Fractions on Preparative TLC Plates:**



**(a) (b) (c) (d)**

**Fig 2: TLC Fractions of (a) leaf extract; (b) bark extract; (c) flower extract; (d) root extract**

TLC analysis suggests the presence of different kinds of phytochemicals in plant extracts. *Hibiscus tlilaceus* plant leaf extract showed four bands in TLC analysing system. Bark extract of revealed presence of two bands, flower and root extract showed three and one band on TLC respectively. TLC profiling of all extracts gives an impressive result that directing towards the presence of number of phytochemicals. Various

phytochemicals give different R*f* values in solvent system. This variation in R*f* values of the phytochemicals provides a very important clue in understanding of their polarity.

**Table-3: Rf values of TLC spots of plant extract**

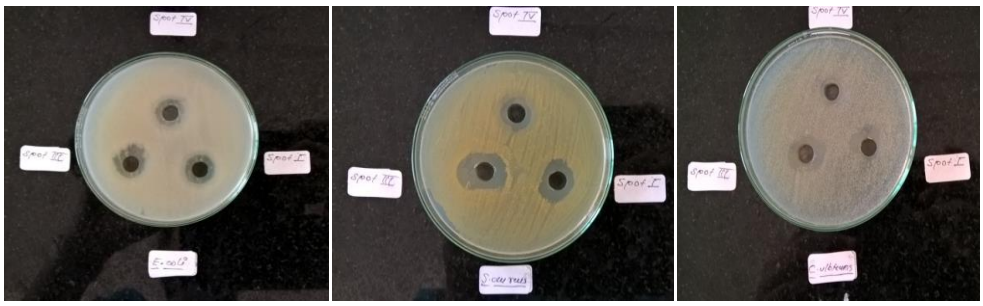
| Sr.no. | Plant extract | Fraction no. 1 Rf factor | Fraction no. 2 Rf factor | Fraction Rf factor | Fraction no.4 Rf factor |
|---|---|---|---|---|---|
| 1 | Leaf | 0.05 | 0.39 | 0.56 | 0.96 |
| 2 | Bark | 0.25 | 0.56 | - | - |
| 3 | Flower | 0.3 | 0.72 | 0.96 | - |
| 4 | Root | 0.88 | - | - | - |

**Antimicrobial activity of TLC separated phyto-compounds:**

**Table-4: Antimicrobial activity of isolated TLC bands of each part of *H.tiliaceus* with zone of inhibition**

| Sr. no | Pathogens | Zone of inhibition(mm) of TLC bands of *Hibiscus tiliaceus* plant parts | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Leaf TLC bands | | | | Bark TLC bands | | Flower TLC bands | | | Root TLC bands |
| | | 1 | 2 | 3 | 4 | 1 | 2 | 1 | 2 | 3 | 1 |
| 1 | *E.coli* | 16 | - | 14 | 12 | - | - | - | - | - | - |
| 2 | *S.aureus* | 16 | - | 18 | 15 | - | - | - | - | - | - |
| 3 | *S.pyogenes* | 13 | 13 | - | - | - | 13 | - | - | - | - |
| 4 | *S.faecalis* | 13 | 11 | - | - | - | - | - | - | - | - |
| 5 | *K.pneumoniea* | 14 | - | - | - | - | - | - | 10 | - | - |
| 6 | *S.typhi* | - | - | - | - | - | - | - | - | - | 13 |
| 7 | *P.aeruginosa* | - | 14 | 10 | - | - | - | - | 12 | 12 | - |
| 8 | *C.albicans* | 12 | - | 15 | 12 | - | - | - | - | - | - |

**Key: -, No zone**



**(a) (b) (c)**

**Fig 3: Antimicrobial activity of leaf TLC bands of *H.tiliaceus* against a) *E. coli* b) *S.aureus* c) *C.albicans***

In previous research on *H. tiliaceus*, the antibacterial activity of the methanol leaf extract of *H. tiliaceus* has been reported against Gram-positive bacteria of *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*. No inhibition was observed for Gram-negative bacteria of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella choleraesuis* [10]. In present study the TLC recovered fraction of methanolic leaf extract revealed antimicrobial activity against *E.coli*, *S.aureus* and *C. albicans* also. According to previous research, the ethanol extract of dried *H. tiliaceus* leaves showed activity against *S. aureus*, *E. coli* and *Salmonella paratyphi* [10]. The size of zone of inhibition for all microorganisms tested ranged from 10 to 18 mm. The TLC fraction of leaves, bark, flower and root were found to be active against only a few bacterial pathogen such as *E.coli, S.pyogenes, S.aureus, S.fecalis, K.pneumoniae, P.aeruginosa, C.albicans.* Isolated TLC bands of *H.tiliaceus* are found to be Antibacterial agents. *H.tiliaceus* exhibited remarkable antibacterial activity against *E.coli, S.pyogen, S.fecalis, Klebsiella and P.aeruginosa, S.aureus and yeast C.albicans.* Especially TLC bands

of leaves are found to be more effective against pathogens. Each bacterial strain has a different intrinsic growth rate and susceptibility to test bands and therefore diameter of inhibition zone may vary according to the strains, species.

**UV-Vis spectroscopic analysis of TLC recovered fractions:**

The qualitative UV-Vis spectrum profile of recovered TLC fractions were selected at wavelength from 200 to 700 nm. Most of the bands showed lamda max at 327nm and 329 nm. Different bands showed peaks at following wavelength with absorbance.

**Table-5: Absorbance and Lamda max of isolated TLC bands of each part of *H.tiliaceus***

| Sr. no | Bands | TLC bands of *Hibiscus tiliaceus* | |
|---|---|---|---|
| | | nm | Abs |
| 1 | Leaves | 329 | 0.513 |
| 2 | | 329 | 0.532 |
| 3 | | 329 | 0.542 |
| 4 | | 329 | 0.683 |
| 5 | Flower | 327 | 1.278 |
| 6 | | 329 | 0.848 |
| 7 | | 327.6 | 1.003 |
| 8 | Bark | 329 | 0.844 |
| 9 | | 329 | 0.900 |
| 10 | Root | 329 | 0.972 |

Peak analysis showed that wavelength of maximum absorbance (λmax) of isolated compounds from each of the band was found to be 327 nm in methanol extract which is very close to 316 nm which is of standard betulin in methanol extract. Spectral data shows that the isolated TLC bands has λmax which is mostly similar to standard Rutin (λmax – 329nm) [11]. Caffeic acid, Ferulic acid all of three are flavanoids. Rutin is a glycoside of the flavonoid quercetin. In humans, it attaches to the iron ion Fe2+, preventing it from binding to hydrogen peroxide, which would otherwise create a highly reactive free radical that may damage cells. Ferulic acid, like many natural phenols, is an antioxidant *in vitro* in the sense that it is reactive toward free radicals such as reactive oxygen species (ROS). ROS and free radicals are implicated in DNA damage, cancer and accelerated cell aging. If added to a topical preparation of ascorbic acid and vitamin E, ferulic acid may reduce oxidative stress and formation of thymine dimers in skin. The herbal products are usually a mixture of two or more herbs and responsible for a variety of action. The effect of herbal formulation depends on the amount of active constituent present in it. The raw material collected from the natural source may vary in the composition of active constituent responsible for the action. So it becomes necessary to quantify the active constituent in plant extracts. The modern method of analysis, such as chromatographic methods are costly and time consuming, where as a UV spectroscopic method is simple, rapid, sensitive, rugged, robust and gives results in a short duration of time.

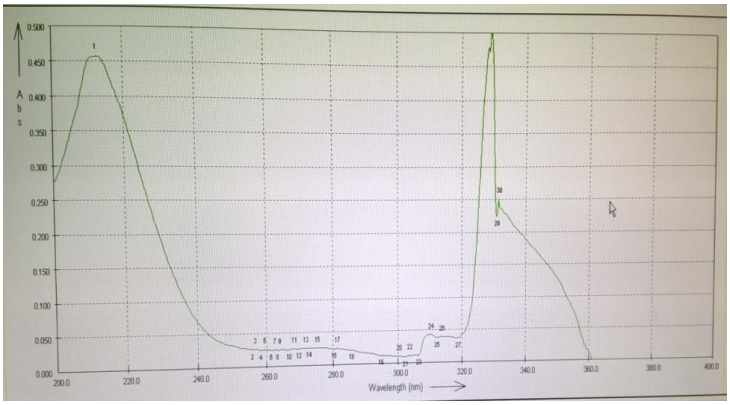



**Fig 4: UV spectrum of TLC band no 1 of *H.tiliaceus* leaves**

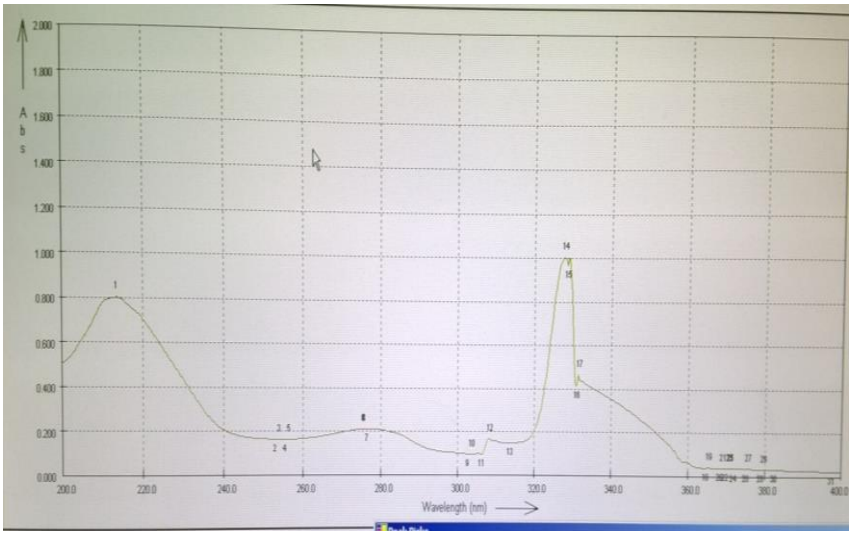



**Fig 5: UV spectrum of TLC band no 3 of *H.tiliaceus* flower**

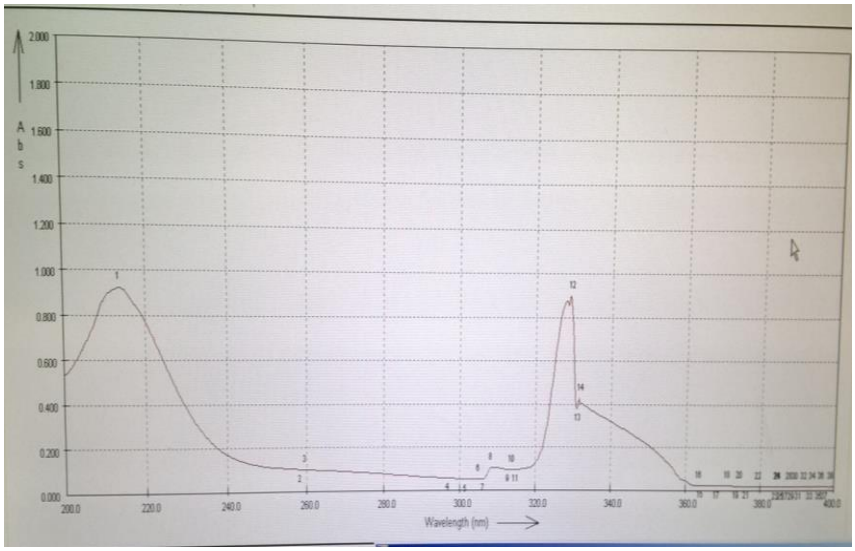



**Fig 6: UV spectrum of TLC band no .2of *H.tiliaceus* bark**

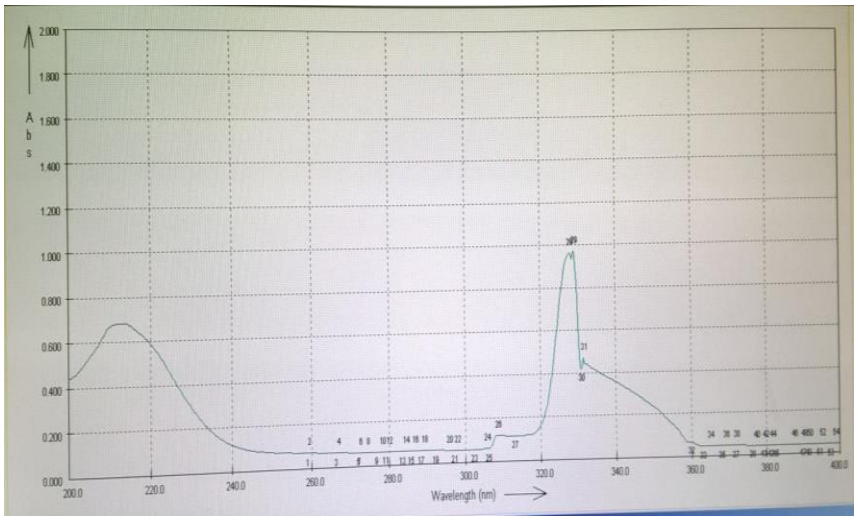



**Fig 7: UV Spectrum of band no 1 of root of *H.tiliaceus* plant**

**FTIR Spectroscopic analysis of TLC recovered fractions:**

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation [12]. The results of FTIR peak values and functional groups were represented in following table. FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different extract. Hence the extracts of *H*. tiliaceus may contains phytochemicals such as alkaloids, steroids, terpenoids and phenolics. These compound mostly shows antimicrobial and antioxidants activities.

**Table-6: Wavelength and respective functional groups of leaves of *H.tiliaceus***

| leaves | Band no. | Frequency ($cm^{-1}$) | Bonds | Functional groups |
|---|---|---|---|---|
| *H.tiliaceus* | Band no.1 | 3405.67 | O–H stretch, H–bonded | alcohols, phenols |
| | | 2916.81 | C–H stretch | alkanes |
| | | 2851.24 | C–H stretch | alkanes |
| | | 2515.69 | - | - |
| | | 2358.52 | N- H stretch | Acid unhydride |
| | | 1619.91 | N–H bend | 1° amines |
| | | 1488.78 | N–O asymmetric stretch | nitro compounds |
| | | 1113.69 | C–N stretch | aliphatic amines |
| | | 3393.14 | N–H stretch | 1°, 2° amines, amides, |
| | | 2471.33 | - | - |
| | | 2358.52 | N- H stretch | Acid unhydride |
| | Band no 2. | 1868.68 | C=O stretch | Acid unhydride |
| | | 1554.34 | N–O asymmetric stretch | nitro compounds |
| | | 1450.21 | C–C stretch (in–ring), C–H bend | Aromatics, alkanes |
| | | 1056.8 | C–N stretch | aliphatic amines |
| | Band no 3 | 3545.49 | - | - |
| | | 3401.82 | O–H stretch, H–bonded | alcohols, phenols |
| | | 3246.57 | O–H stretch, O–H stretch, H–bonded | carboxylic acids, alcohols, phenols |
| | | 2926.45 | C–H stretch | alkanes |
| | | 2515.69 | | |
| | | 2359.48 | N- H stretch | Acid unhydride |
| | | 1866.76 | C=O stretch | Acid unhydride |
| | | 1786.72 | C=O stretch | Acid unhydride |
| | | 1617.02 | N–H bend | 1° amines |
| | | 1506.13 | N–O asymmetric stretch | nitro compounds |
| | | 1118.51 | C–N stretch, C–O stretch | aliphatic amines, alcohols, carboxylic acids, esters, ethers |
| | Band no.4 | 3394.1 | O–H stretch, H–bonded, N–H stretch | alcohols, phenols, 1°, 2° amines, amides |
| | | 2513.76 | - | - |
| | | 2361.41 | - | - |
| | | 1514.81 | N–O asymmetric stretch | nitro compounds |
| | | 1121.4 | C–O stretch, C–N stretch | alcohols, carboxylic acids, esters, ethers, aliphatic amines |
| | | 799.35 | =C–H bend, N–H wag, C–H “oop”, C–Cl stretch | Alkenes, 1°, 2° amines, aromatics, alkyl halides |

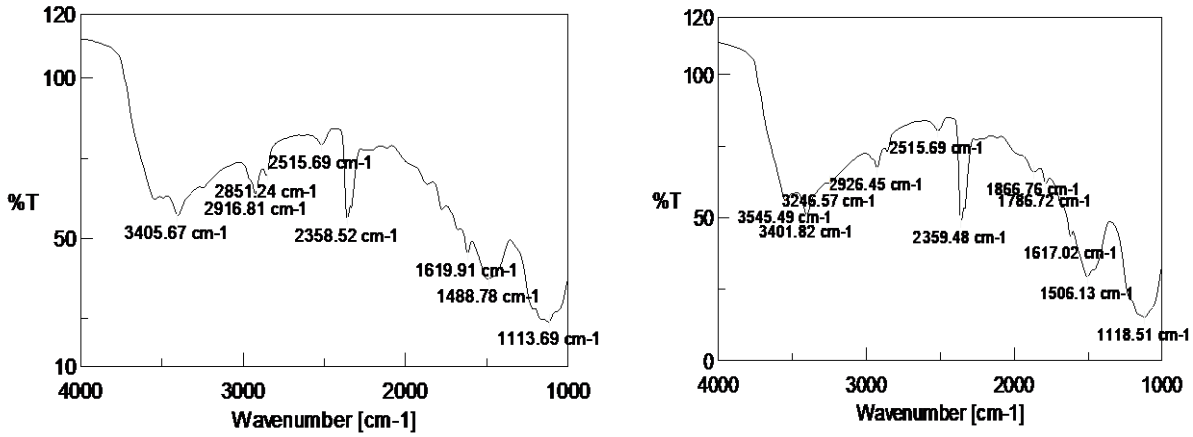



**Fig 8: FTIR spectrums of chromatographic band no.1 and 3 of methanolic extract of *H.tiliaceus* leaf**

**Table –7: FTIR peak values and functional groups of chromatographic bands of methanolic extract of bark of *H.tiliaceus* and *Thespesia populnea***

| Bark | Band no. | Frequency ($cm^{-1}$) | Bands | Functional groups |
|---|---|---|---|---|
| *H.tiliaceus* | Band no.1 | 3394.1 | O–H stretch, H–bonded, N–H stretch | alcohols, phenols, 1°, 2° amines, amides |
| | | 2262.09 | | |
| | | 1874.47 | | |
| | | 1566.88 | | |
| | | 1045.23 | C–N stretch | aliphatic amines |
| | Band no.2 | 3398.92 | O–H stretch, H–bonded, N–H stretch | alcohols, phenols, 1°, 2° amines, amides |
| | | 2513.76 | | |
| | | 1860.01 | | |
| | | 1552.42 | N–O asymmetric stretch | nitro compounds |
| | | 1121.4 | C–O stretch, C–N stretch | alcohols, carboxylic acids, esters, ethers, aliphatic amines |
| | | 789.707 | N–H wag, C–H “oop”, C–Cl stretch | 1°, 2° amines, aromatics, alkyl halides |

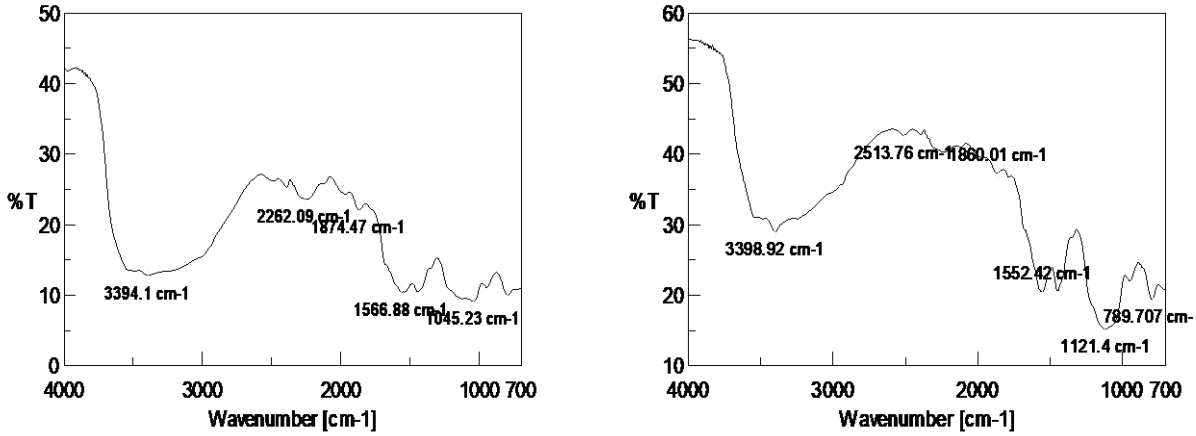



**Fig 9: FTIR spectrums of chromatographic band no.1, 2 of methanolic extract of *H.tiliaceus* bark**

**Table -8: FTIR peak values and functional groups of chromatographic bands of methanolic extract of flowers of *H.tiliaceus* and *T.populnea*.**

| Flower | Band no. | Frequency ($cm^{-1}$) | Bonds | Functional groups |
|---|---|---|---|---|
| | | alcohols, phenols, 1°, 2° amines, amides | O–H stretch, H–bonded, N–H stretch | alcohols, phenols, 1°, 2° amines, amides |
| | O–H stretch, H–bonded, N–H stretch | - | - | - |
| | - | - | - | - |
| | - | nitro compounds | - | - |
| | N–O asymmetric stretch | alcohols, carboxylic acids, esters, ethers, aliphatic amines | C–C stretch (in–ring) | aromatics |
| | C–O stretch, C–N stretch | 1045.23 | C–O stretch, C–N stretch | alcohols, carboxylic acids, esters, ethers, aliphatic amines |
| 3394.1 | | | | |
| 2513.76 | | 2508.94 | - | - |
| 2361.41 | | 2349.84 | - | - |
| 1514.81 | | 1570.74 | - | - |
| 1121.4 | | | | |
| | Band no.2 | 1131.05 | C–O stretch, C–N stretch | alcohols, carboxylic acids, esters, ethers, aliphatic amines |
| | | 941.092 | =C–H bend, O–H bend, | Alkenes, carboxylic acids |
| | | 861.06 | =C–H bend, N–H wag, C–H “oop” | Alkenes, 1°, 2° amines, aromatics |
| | Band no.3 | | | |
| | | 799.35 | =C–H bend, N–H wag, C–H “oop”, C–Cl stretch | Alkenes, 1°, 2° amines, aromatics, alkyl halides |

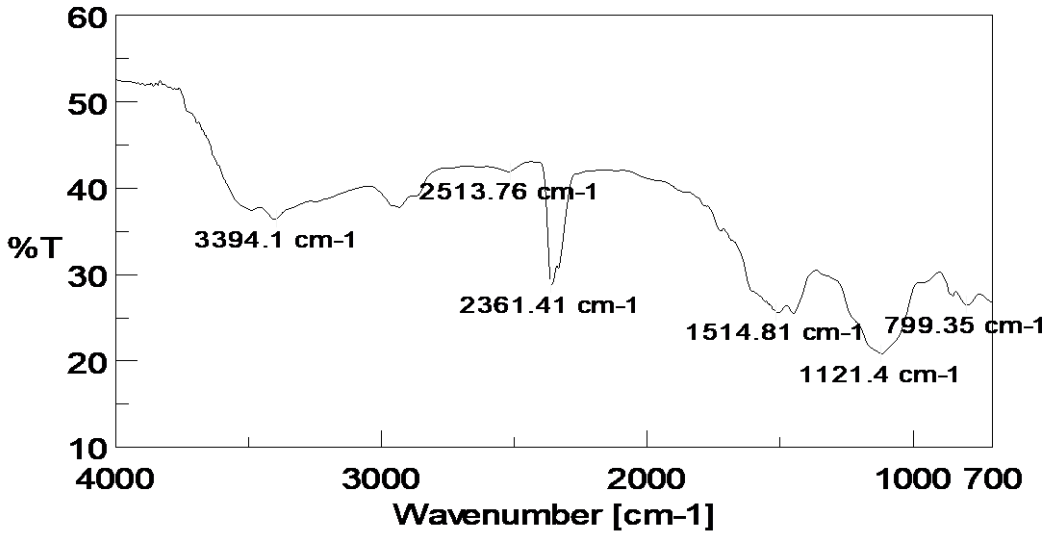



**Fig 10: FTIR spectrums of chromatographic band no. 3 of methanolic extract of *H.tiliaceus* flower**

**Table - 9: FTIR peak values and functional groups of chromatographic bands of methanolic extract of roots of *H.tiliaceus* and *T.populnea*.**

| Root | Band no. | Frequency ($cm^{-1}$) | Bonds | Functional groups |
|---|---|---|---|---|
| *H.tiliaceus* | Band no.1 | 3398.92 | O–H stretch, H–bonded, N–H stretch | alcohols, phenols, 1°, 2° amines, amides |
| | | 2920.66 | C–H stretch | alkanes |
| | | 2357.55 | | |
| | | 1724.05 | C=O stretch, C=O stretch, C=O stretch, C=O stretch | carbonyls (general), carboxylic acids, aldehydes, saturated aliphatic, α⍰β –unsaturated esters |
| | | 1444.42 | C–C stretch (in–ring) | aromatics |
| | | 1121.4 | C–O stretch, C–N stretch | alcohols, carboxylic acids, esters, ethers, aliphatic amines |

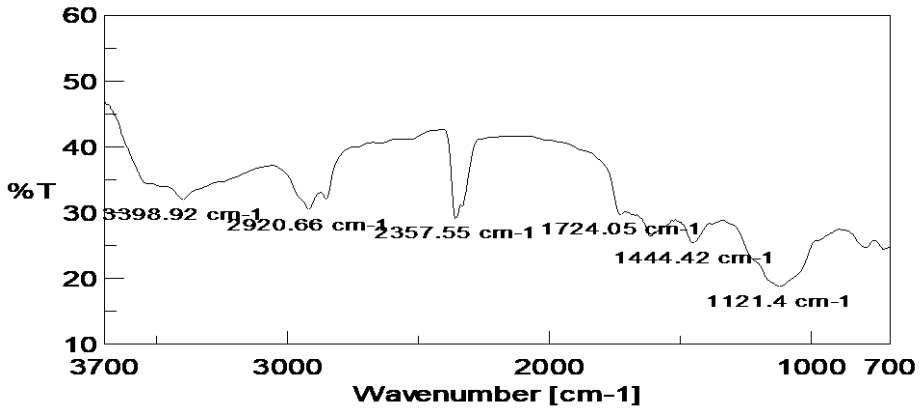



**Fig 11: FTIR spectrum of chromatographic band no.1 of methanolic extract of *H.tiliaceus* root**

Among the functional groups observed in the extracts, OH group was found to be present frequently in almost all TLC recovered fractions. As OH group has got the ability of forming hydrogen bonding capacity, presence of OH group particularly in extract probably indicates the higher potential of methanol extract towards inhibitory activity against microorganisms [13]. In the present study, FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different extracts. Hence the leaf, bark, flower and root extracts of *H.tiliaceus* may contain phytochemicals such as alkaloids, cyanogenic glycosides, nonprotein amino acids, glucosinolates, triterpenoids, saponin, steroids, coumarin, flavonoids, polyamines, aromatic amines, tannins as well as certain peptide and proteins i.e. protease inhibitors, amylase inhibitors etc. These phytochemicals have been shown to possess antimicrobial, antioxidant activities. Detailed information about phytochemical will be useful for the development of standardization parameters, isolation of bioactive compounds from plant extracts, screening of preclinical and clinical investigation, manufacturing of herbal formulations and also distinguishing it from its closely related species [14].

**CONCLUSION:**

Qualitative phytochemical screening of four parts (leaves, bark, flower and root) of *Hibiscus tiliaceus* plant reveals presence of various phytochemicals. Developed chromatogram shows presence of various phytochemicals. According to FTIR Spectrum analysis, plant contains rich presence of phytochemicals such as alkaloids, phenolic acids, flavonoids, terpenoids. So the plant could be potential sources of natural antimicrobial agents, antioxidants that could have medicinal and therapeutic values.

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