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# Phytochemical screening, ultra violet and ir spectroscopy of ethanolic leaf extract of *Hibiscus rosa sinensis* Linn. (Hibiscus red)

**Shilpi Patel and Madhvi Adhav**

**Abstract**
*Hibiscus Rosa sinensis* Linn. (Malvaceae) is widely grown as an ornamental and medicinal plant. The present work deals with phytochemical screening, UV and IR spectroscopy of ethanolic leaf extract of *Hibiscus Rosa sinensis* Linn (Hibiscus Red). In phytochemical screening the extract shows the presence of flavonoids, glycosides, phytosterols, terpenoids, phenolic compound, carbohydrates, proteins, tannins, gum and mucilage. Alkaloids, saponins, anthoquinone, fixed oil and fats were totally absent. The UV and IR spectroscopy of ethanolic leaf extract of Hibiscus red shows the presence of Carbonyl group (ketone), α-β unsaturated amide and lactam, aromatic nature of compound, sulfurcompound, nitro compound, flavones, fistin, quercetin, NaQSA (Sodium Salts of Quercetin 5' Sulfonic Acid),, myricetin, chalcones and anthocyanin types of flavonoids. The above mention bioactive compound are mainly contributed in medicin-al utility of the plant.

**Keywords:** *Hibiscus Rosa sinensis* linn, phytochemical, ultra violet spectroscopy, flavonoids, chromophoric groups

## Introduction
*Hibiscus Rosa sinensis* Linn. (Malvaceae) is widely distributed throughout the world. It is an evergreen woody glabrous showy shrub with about 1.5-2.5 m height. Leaves are coarsely toothed above and entire below ovate bright green and 3 nerved base. The flowers are axillary solitary companulate. (Kaushik *et al.* 1999) [15] (Fig No, 01). Fresh leaves are taken orally in case of weakness and blood deficiency (Gupta *et al.* 2010). The leaves are prescribed by native of China in smallpox, paralysis (Durry, 2010; Caius, 1986) [4, 2] while Indian tribals have been using this plant as for same troubles and also used as diuretic, to cure menstrual disorders, to purity blood, to check high blood pressure, in stomach pain, in wound and for treatment of dandruff (Maheshwari, 2000; Trivedi, 2010) [18, 22].

## Material and Methods
Plant material (Leaves) of *Hibiscus Rosa sinensis Linn*. Were collected from Devi Ahilya Vishwavidhyalaya campus Indore in winter season.
The collected plant material were identified with the help of different Floras viz. Flora of British India (Hookar, 1875) [14], Flora of the Presidency of Bombay (Cooke, 1958) [3], Flora of Marathwada (Naik, 1998) [19] and Flora of Madhya Pradesh (Verma *et al.* 1993) [23].
To obtain ethanolic extract 100 gms of shade dried plant material was extracted with 500 ml of ethanol (95%) in "Soxhlet Extraction Apparatus". Finally the prepared plant material was macerated with water for 24 hrs. To obtain aqueous extract. Each extract was concentrated by distilling off the solvent (Kokate, 1994; Kokate *et al.* 2000) [16, 17].
The extract thus obtained was than subjected to preliminary phytochemical screening for identification of various plant constituents by methods suggested by (Finar, 1962; Farns worth 1966; Harbone 1973; Harborne *et al.* 1979) [7, 8, 10, 11]. To find out the flavonoids, chemical and functional groups of phytochemicals present in the extract, spectral studies were carried out by Ultra-Violet and Infra-Red Spectroscopy (Dyer, 1987; Silverstein *et al.* 1991; Silverstein and Webster, 2012; Dutta, 2000; Heneczkowski *et al.* 2001; Bohm, 1998; Harborne, 1975) [21, 20, 5, 13, 1, 12].

## Observations and discussion
### Phytochemical screening
The leaf extract of Hibiscus red shows the shows the presence of flavonoids, glycosides, phytosterols, terpenoids, phenolic compound, carbohydrates, proteins, tannins, gum and

mucilage. Alkaloids, saponins, anthoquinone, fixed oil and fats were totally absent (Table No. 01).

**UV Spectroscopy**

The UV specrum of *Hibiscus Rosa sinensis* Linn. (Red leaf) shows weak absorption bands at 340 nm and 325 nm is due to aromatic nature of compound, α-β unsaturated ketones and aldehydes. These weak bands indicate flavone and fisetin types of flavonoids.

A broad band at 270 nm indicates the presence of polyene-aldehydes, thiophene, carbonyl group, quercetin and anthocyanin.

There is a strong band at 225 nm reveals the presence of furan, carbonyl group (ketones) and chalcone. The band at 210 nm shows the presence of acrolein and sulfoxide. This band again confirms carbonyl group and furan. The characteristic band at 204 nm is due to amides.

UV spectroscopy shows the presence of five types of flavonoids viz. flavone, fisetin, quercetin, anthocyanin and chalcones. (Fig No. 2, Table No. 2).

**IR Spectroscopy**

The IR spectrum shows the peak at 602 $cm^{-1}$, 633 $cm^{-1}$ and 802 $cm^{-1}$ indicates the presence of alkyne, C-H bending vibrations amides and quercetin.

The sharp peak at 879 $cm^{-1}$ is due to aromatic substitution, C-H bending vibrations, and gem disubsituted olefinic group. This peak again confirms the presence of quercertin.

The very sharp peak at 1041 $cm^{-1}$ shows the presence of sulfur compound, S=O strecting vibrations, thiocarbonyl group, sulfoxides and NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid]. The presence of sulfur compound, thiocarbonyl group and NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid] further supported by the strong peak at 1088 $cm^{-1}$. Sulfur compound prominently active against microbes. The peak at 1327 $cm^{-1}$ indicates aromatic nature of compound, sulphonamides, gem dimethyl group, nitro compound and myricetin type of flavonoids. The characteristic peak at 1381 $cm^{-1}$ again confirms the presence of C-$CH_3$ bending, nitro/sulfur compound, gem dimethyl group and myricetin. A shoulder peak at 1412 $cm^{-1}$ due to alkene. The medium peak at 1659 $cm^{-1}$ indicates the presence of carbonyl group and flavone. The presence of carbonyl group supports the antibacterial activity as generally these types of group are much potent against gram positive and gram negative bacteria.

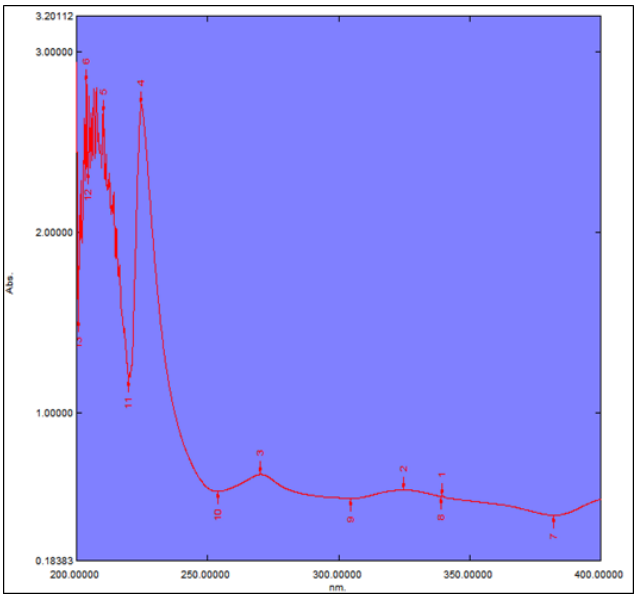
Appearance of peak at 2885 $cm^{-1}$ and 2978 $cm^{-1}$ reveals the presence of C-H stretching vibrations and aldehydes. There is a clear hump at 3340 $cm^{-1}$ is corresponding to amines and N-H stretching vibrations.

IR spectrum of *Hibiscus Rosa sinensis* Linn. (Red leaf) reveals the presence of four type of flavonoids viz. quercetin, NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid], myricetin and flavone (Fig No. 03, Table No. 03).

**Table 1:** Preliminary phytochemical screening of extract of *Hibiscus Rosa sinensis* Linn. (Hibiscus red)

| S No. | Plant constituents Test/Reagents | Result |
|---|---|---|
| 1 | **Alkaloids** | |
| | (i) Mayer's reagent | - |
| | (ii) Wagner's reagent | - |
| | (iii) Hager's reagent | - |
| 2 | **Carbohydrates** | |
| | (i) Molisch's test | + |
| | (ii) Benedict's reagent | + |
| | (iii) Fehling solution | + |
| 3 | **Types of Carbohydrates** | |
| | (i) Glucose | - |
| | (ii) Fructose | - |
| | (iii) Galactose | - |
| | (iv) Lactose | + |
| | (v) Starch | + |
| 4 | **Glycosides** | |
| | (i) Keller kiliani test | + |
| 5 | **Phytosterols** | |
| | (i) Liebermann's test | + |
| 6 | **Terpenoids** | |
| | (i) Salkowski test | + |
| 7 | **Fixed oils and Fats** | |
| | (i)Spot test | - |
| 8 | **Saponins** | |
| | (i) Foam test | - |
| 9 | **Phenolic Compounds** | |
| | (i) Ferric chloride solution | + |
| 10 | **Tannins** | |
| | (i) Lead acetate solution | + |
| 11 | **Proteins** | |
| | (i) Xanthoproteic test | + |
| | **(**ii) Biuret test | + |
| | **Amino acids** | |
| | (i) Ninhydrin reagent | + |
| 12 | **Flavonoids** | |
| | (i) Con. $H_2So_4$ + Magnesium ribbon | + |
| 13 | **Gums and Mucilages** | |
| | (i) Alcoholic precipitation | + |
| | (ii) Molisch's test | + |
| 14 | **Anthraquinones** | |
| | Borntrager's test | - |

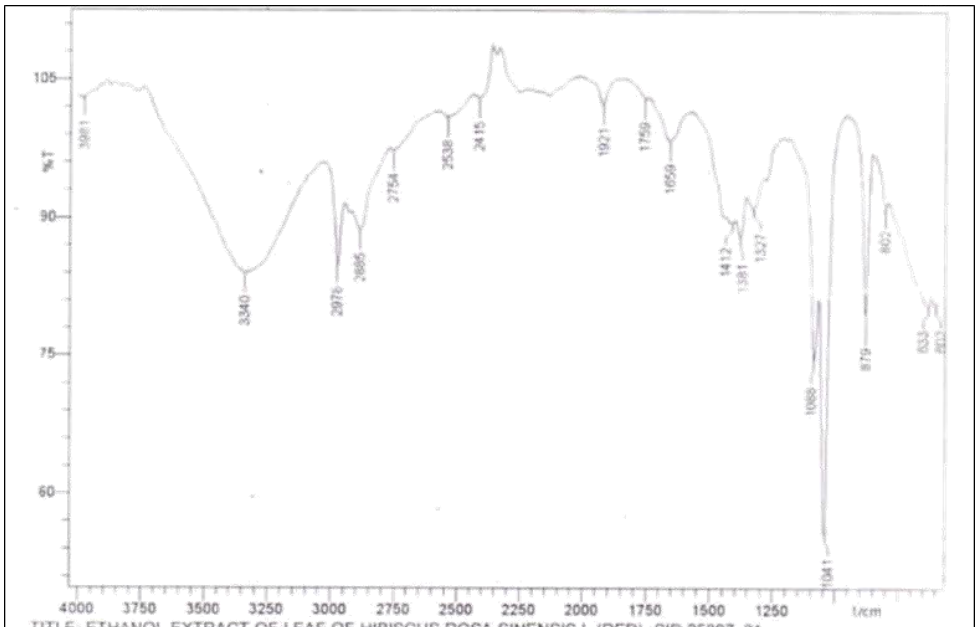
**Fig 1:** *Hibiscus Rosa sinensis* Linn. (Hibiscus red)




**Peak Pick**

| No. | P/V | Wavelength nm. | Abs. |
|---|---|---|---|
| 1 | ↑ | 339.60000 | 0.53915 |
| 2 | ↑ | 324.80000 | 0.57492 |
| 3 | ↑ | 270.20000 | 0.66067 |
| 4 | ↑ | 224.70000 | 2.71112 |
| 5 | ↑ | 210.10000 | 2.66027 |
| 6 | ↑ | 203.80000 | 2.83531 |
| 7 | ↓ | 382.20000 | 0.43527 |
| 8 | ↓ | 339.00000 | 0.53847 |
| 9 | ↓ | 304.50000 | 0.52894 |
| 10 | ↓ | 253.90000 | 0.56699 |
| 11 | ↓ | 219.90000 | 1.18941 |
| 12 | ↓ | 204.50000 | 2.33869 |
| 13 | ↓ | 200.80000 | 1.52383 |

**Fig 2:** UV spectrum of Hibiscus red




TITLE: ETHANOL EXTRACT OF LEAF OF HIBISCUS ROSA SINENSIS L (RED) SID.25997_01

Peak table of ETHANOL EXTRACT OF LEAF OF HIBISCUS ROSA SINENSIS L (RED) SID.25997_01:

| PeakNumber | Begin [1/cm] | Max [1/cm] | End [1/cm] | Absolute Height [%T] | Width [1/cm] |
|---|---|---|---|---|---|
| 1 | 594.03 | 601.75 | 617.18 | 80.45 | 23.14 |
| 2 | 617.18 | 632.61 | 786.9 | 80.45 | 169.72 |
| 3 | 786.9 | 802.33 | 825.47 | 91.31 | 38.57 |
| 4 | 848.62 | 879.48 | 933.48 | 78.49 | 84.86 |
| 5 | 956.62 | 1041.48 | 1064.63 | 55.71 | 108.01 |
| 6 | 1064.63 | 1087.77 | 1203.49 | 74.4 | 138.86 |
| 7 | 1203.49 | 1326.93 | 1350.07 | 91.09 | 146.58 |
| 8 | 1350.07 | 1380.93 | 1404.07 | 87.75 | 54 |
| 9 | 1404.07 | 1411.79 | 1573.8 | 89.53 | 169.72 |
| 10 | 1573.8 | 1658.66 | 1743.52 | 98.41 | 169.72 |
| 11 | 1743.52 | 1758.95 | 1843.81 | 103.07 | 100.29 |
| 12 | 1843.81 | 1920.96 | 2013.54 | 102.39 | 169.72 |
| 13 | 2360.7 | 2414.7 | 2437.84 | 103.06 | 77.15 |
| 14 | 2437.84 | 2538.14 | 2576.71 | 101.03 | 138.86 |
| 15 | 2576.71 | 2754.15 | 2761.86 | 97.43 | 185.15 |
| 16 | 2761.86 | 2885.3 | 2947.01 | 88.73 | 185.15 |
| 17 | 2947.01 | 2977.87 | 3024.16 | 85.1 | 77.15 |
| 18 | 3024.16 | 3340.46 | 3741.63 | 84.06 | 717.47 |
| 19 | 3895.92 | 3980.78 | 3996.21 | 103.05 | 100.29 |

Audit trail of ETHANOL EXTRACT OF LEAF OF HIBISCUS ROSA SINENSIS L (RED) SID.25997

**Fig 3:** IR spectrum of Hibiscus Red

**Table 2:** UV spectroscopy of ethanolic leaf extract of *Hibiscus Rosa sinensis* Linn. (Hibiscus red)

| S. No. | Wavelength nm | Abs. | Chromophoric group | Types of flavonoids |
|---|---|---|---|---|
| 1 | 339.60 | 0.53915 | Aromatic | Flavone |
| 2 | 324.80 | 0.57492 | A-β unsaturated Ketones and aldehydes. | Flavone &Fisetin |
| 3 | 270.20 | 0.66067 | Polyene -aldehydes, Thiophene, Carbonyl group. | Quercetin, Anthocyanin |
| 4 | 224.70 | 2.71112 | Furan, Ketones, Carbonyl group. | Chalcones |
| 5 | 210.10 | 2.66027 | Furan, Carbonyl Group, Acrolein, Sulfoxide. | |
| 6 | 203.80 | 2.83531 | Amides | |

**Table 3:** IR spectroscopy of ethanolic leaf extract of *Hibiscus Rosa sinensis* Linn. (Hibiscus red)

| S. No. | Peak $cm^{-1}$ | Functional group | Types of Flavonoids |
|---|---|---|---|
| 1 | 602 | - | Quercetin |
| 2 | 633 | Alkyne, C-H bending | Quercetin |
| 3 | 802 | Amides | Quercetin |
| 4 | 879 | Aromatic substitution, C-H bending vibrations,gem-disubsituted,Olefinic group. | Quercetin |
| 5 | 1041 | Sulfur Compound, S=O Stretching vibrations, Sulfoxides. Thiocorbonyl group | NaQSA |
| 6 | 1088 | Sulfur Compound, Thiocorbonyl group | NaQSA |
| 7 | 1327 | Aromatic, Sulphonamides, gem- dimethyl group, Nitro Compound. | Myricetin |
| 8 | 1381 | C-$CH_3$ bending,Nitro/ Sulfur Compound, gem- dimethyl Group. | Myricetin |
| 9 | 1412 | Alkene | |
| 10 | 1659 | Carbonyl group | Flavone |
| 11 | 1759 | Amides, Carbonyl stretching vibratons. | - |
| 12 | 1921 | - | - |
| 13 | 2414 | - | - |
| 14 | 2538 | Carboxylic acid group, Inter molecular hydrogen bonding. | - |
| 15 | 2754 | C-H stretching vibration, Aldehydes. | - |
| 16 | 2885 | C-H stretching vibration, Aldehydes. | - |
| 17 | 2978 | Aldehydes. | - |
| 18 | 3340 | Amines, N-H stretching vibration. | - |
| 19 | 3981 | - | - |

## Conclusion

This investigation has gives preliminary information to determine the chemical composition of Hibiscus red leaf. The presence of chromophoric group, functional group, flavonoids, glycosides, phytosterols, terpenoids, phenolic compound, tannins is mainly contributed in medicinal utility of plant. The presence of these bioactive compounds in plant extract confirms the correct use of this plant in traditional medicinal system. It also holds for the production of novel drugs with isolation of specific compound.

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