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IR spectroscopy and antimicrobial activity of ethanolic flower extract of *Hibiscus rosa sinensis* Linn. (HIBISCUS Red)

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Abstract
Hibiscus rosa sinensis Linn. is one of the important medicinal as well as ornamental plant, belongs to family Malvaceae. It is commonly called Gurhal. The flower of *Hibiscus rosa sinensis* Linn. possess various activity such as anti - convulsents, antidiabetic, antipyretic, antioxidant, antifertility and antidiarrheal. IR spectroscopy of ethanolic flower extract shows the presence of functional groups like diallyl sulfoxide, sulfonamide, carbonyl, amide, gem dimethyl and flavonoids such as NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid], quercetin, flavone, myricetin and fisetin. This proves its correlation with antimicrobial activity.

Keywords: *Hibiscus rosa sinensis* Linn., IR spectroscopy, antimicrobial

Introduction
Hibiscus rosa sinensis Linn. (Malvaceae) is one of the important medicinal as well as ornamental plant. *Hibiscus rosa sinensis* is the national flower of Malaysia, called Bunga Raya in Malay (Verma, 2016; Pekamwar *et al*., 2013) [16, 12]. Vernacular name of *Hibiscus rosa sinensis* Linn. is Japa, Gurhal, Jusum, Beng etc. 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 at the base. The flowers are axillary solitary companulate and variously coloured red, pink, yellow and white (Kaushik *et al*., 1999) [7].

Material and Methods
Plant material (flower) of *Hibiscus rosa sinensis* Linn. were collected from Devi Ahilya Vishwavidhyalaya campus Indore in winter season.
The collected plant materials were identified with the help of different Floras viz. Flora of British India (Hookar, 1875), Flora of the Presidency of Bombay (Cooke, 1958) [1], Flora of Marathwada (Naik, 1998) [11] and Flora of Madhya Pradesh (Verma *et al.,* 1993) [15].
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) [9, 10]. To find out the flavonoids, chemical and functional groups of phytochemicals present in the extract, spectral studies were carried out by Infra Red Spectroscopy (Dyer, 1994; Silverstein *et. al.*, 1991; Silverstein and Webster, 2012; Dutta, 2000; Heneczkowski *et al.*, 2001) [4, 13, 3, 5]. Extract was tested for antimicrobial activity against human pathogenic bacteria by "Cup Borer Method" (Kavanagh, 1963; Cheesbrough, 1993) [8, 2]. The cultures of bacteria have been obtained from Microbial Type Culture Collection and Gene Bank Chandigarh. The name and culture number of bacteria are as follows - Gram positive Bacteria- *Staphylococcus aureus* ATCC 9144, *Bacillus subtilis* ATCC 6633. Gram negative Bacteria- *Escherichia coli* MTCC 739, *Salmonella typhi* ATCC 10749, *Pseudomonas aeruginosa* ATCC 25668, *Klebsiella pneumoniae* ATCC 33495.

Results and Discussion
The IR spectrum of *Hibiscus rosa sinensis* Linn. red flower shows weak peak at 609 cm^{-1} and 648 cm^{-1} is due to C-H bending vibrations, alkynes, sulfonic acid and NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid]. The presence of sharp peak at 879 cm^{-1}indicates the aromatic substitution, C-H bending vibrations, gem disubstituted olefinic group and the quercetin type of flavonoid. A very sharp peak at 1049 cm^{-1} shows the presence of diallyl sulfoxide, thiocarbonyl group and flavone. The strong peak at 1088 cm^{-1} indicates the presence of sulfur compound, thio carbonyl group and NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid] type of flavonoid. The sulfur containing groups are generally potent against gram positive and gram negative bacteria.
There is a peak at 1273 cm^{-1} which reveal the presence of amines, C-N vibrations and aromatic nature of compound. The weak peak at 1327 cm^{-1} indicates sulphonamides, aromatic nature of compound, gem dimethyl group and nitro compound. The peak shows the presence of flavonoid-Myricetin.
A variety of shoulder peak at 1381 cm^{-1}, 1419 cm^{-1} and 1450 cm^{-1} which indicates sulfur/ nitro compound, gem dimethyl group, ketones (carbonyl group), -OH bending vibrations and C-CH_3 bending. These peaks show the presence of myricetin and fisetin types of flavonoids.
The medium peak at 1651 cm^{-1} again confirms flavone. This peak indicates β – keto esters (enolic), amides and carbonyl stretching vibrations. The characteristic peak at 2893 cm^{-1} and

medium peak at 2978 cm^{-1} is due to C-H stretching vibrations and aldehydes. A clear hump at 3317 cm^{-1} and 3348 cm^{-1} are corresponding to amines and N-H stretching vibrations.

IR spectroscopy reveals the presence of five types of flavonoids viz. NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid], quercetin, flavone, myricetin and fisetin (Fig No.01).

The observations of antimicrobial activity showed that ethanolic flower extract of Hibiscus Red having strong antibacterial activity against (*Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis* and *Staphylococcus aureus*) all the tested gram positive and gram negative bacteria. (Fig No. 02, Table No. 01)

Table 1: Antimicrobial activity of ethanolic extract of *Hibiscus rosa sinensis* Linn. (HIBISCUS Red)

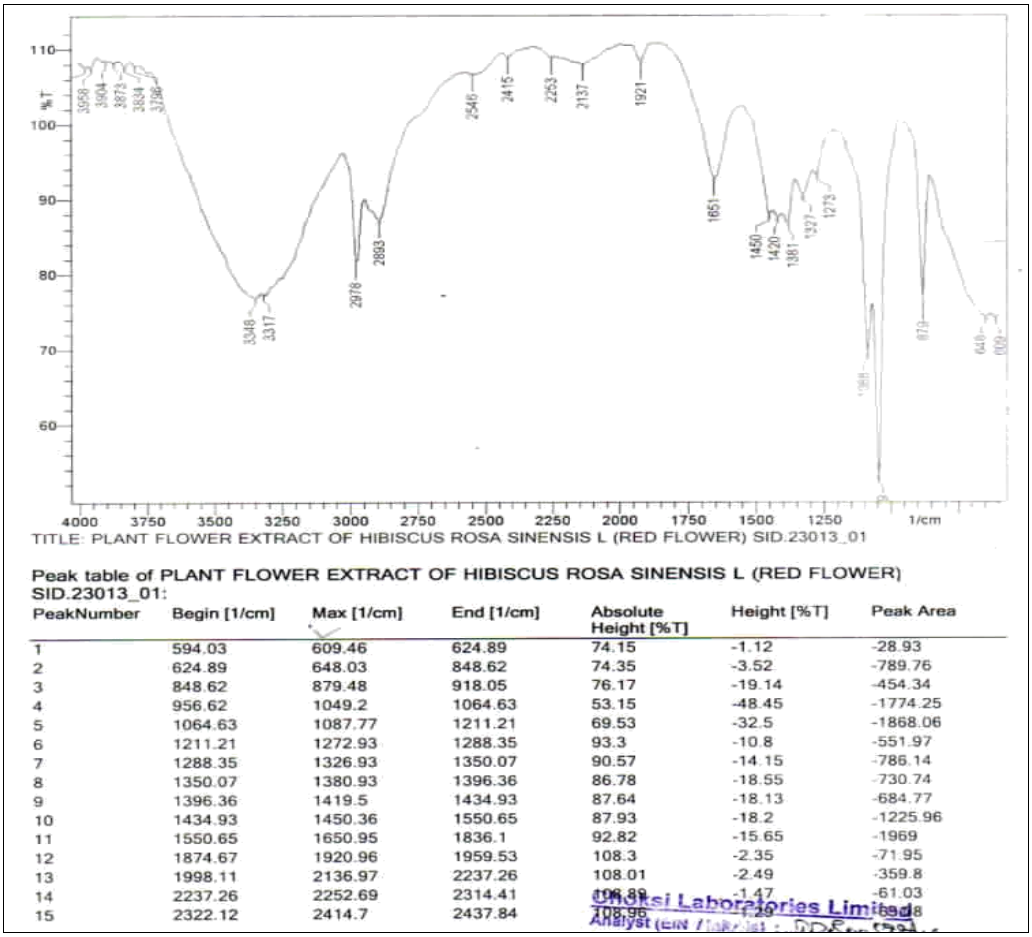
Quantity of extract in ml	Average diameter of zone of inhibition in mm					
	Escherichia coli.	***Salmonella typhi***	***Pseudomonas aeruginosa.***	***Klebsiella pneumoniae.***	***Bacillus subtilis.***	***Staphylococcus aureus.***
0.05	No zone	No zone	10	No zone	10	No zone
0.08	10	13	12	09	14	12
0.11	15	19	15	11	19	15
0.14	20	25	18	14	20	20
0.17	25	30	24	22	22	25
r	0.986394	0.978709	0.97824	0.970725	0.968246	0.970448

r = correlation coefficient,

r = +1 perfect positive correlation, r = -1 perfect negative correlation

Conclusion

Flower extract shows the presence of functional groups like diallyl sulfoxide, sulfonamide, carbonyl, amide, gem dimethyl and flavonoids such as NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid], quercetin, flavone, myricetin and fisetin. Sulfonamides were the first antimicrobial agent (AMAs) effective against pyogenic bacterial infections (Tripathi, 2010). Various types of flavonoids viz. quercetin, NaQSA [Sodium Salts of Quercetin 5' Sulfonic Acid], flavone, myricetin and fisetin are reported in IR spectroscopy analysis of *Hibiscus rosa sinensis* Linn (Hibiscus red). They exhibit antimicrobial, anticancer, antioxidant, anti-inflammatory, cardiac stimulant activity (Dubey and Mishra, 2009; Kuntz *et al.*, 1999; Kokate *et al.*, 2014; Lemke *et al.*, 2013).



Peak table of PLANT FLOWER EXTRACT OF HIBISCUS ROSA SINENSIS L (RED FLOWER) SID.23013_01:

PeakNumber	Begin [1/cm]	Max [1/cm]	End [1/cm]	Absolute Height [%T]	Height [%T]	Peak Area
1	594.03	609.46	624.89	74.15	-1.12	-28.93
2	624.89	648.03	848.62	74.35	-3.52	-789.76
3	848.62	879.48	918.05	76.17	-19.14	-454.34
4	956.62	1049.2	1064.63	53.15	-48.45	-1774.25
5	1064.63	1087.77	1211.21	69.53	-32.5	-1868.06
6	1211.21	1272.93	1288.35	93.3	-10.8	-551.97
7	1288.35	1326.93	1350.07	90.57	-14.15	-786.14
8	1350.07	1380.93	1396.36	86.78	-18.55	-730.74
9	1396.36	1419.5	1434.93	87.64	-18.13	-684.77
10	1434.93	1450.36	1550.65	87.93	-18.2	-1225.96
11	1550.65	1650.95	1836.1	92.82	-15.65	-1969
12	1874.67	1920.96	1959.53	108.3	-2.35	-71.95
13	1998.11	2136.97	2237.26	108.01	-2.49	-359.8
14	2237.26	2252.69	2314.41	108.89	-1.47	-61.03
15	2322.12	2414.7	2437.84	108.96	-1.28	-88

Chokshi Laboratories Limited

Analyst (Sign / Initial) :

16	2437.84	2545.85	2568.99	106.67	-3.43	-328.73
17	2568.99	2893.01	2939.3	87.25	-22.45	-3942.29
18	2939.3	2977.87	3024.16	81.59	-28.01	-1704.44
19	3024.16	3317.32	3325.03	77.3	-31.91	-7031.62
20	3325.03	3348.18	3780.2	76.78	-32.39	-8284.62
21	3780.2	3795.63	3811.06	107.8	-0.86	-19.35
22	3811.06	3834.2	3857.35	107.88	-0.75	-20.25
23	3857.35	3872.78	3888.21	108.32	-0.26	-5.91
24	3888.21	3903.64	3926.78	108.37	-0.24	-5.09
25	3934.5	3957.64	3965.35	107.62	-1.16	-21.34
26	3965.35	3980.78	3996.21	107.61	-0.92	-21.85

Audit trail of PLANT FLOWER EXTRACT OF HIBISCUS ROSA SINENSIS L (RED FLOWER) SID.23013_01:

Fig 1: IR spectrum of Hibiscus red flower

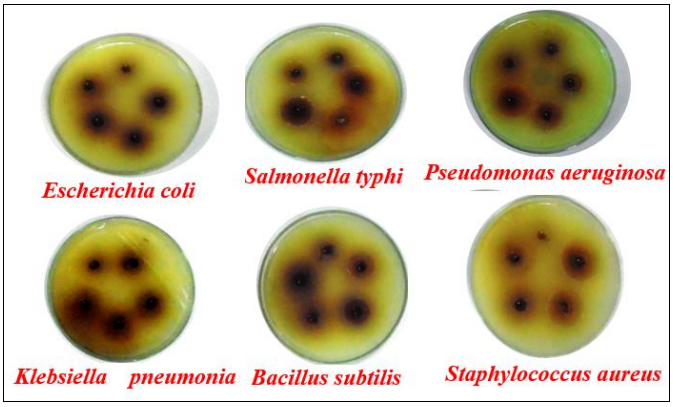


Fig 2

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