Bioprospecting the Phenolic and Flavonoid Content and Assessment of Antioxidant and Anti-Microbial Activity of leaf extracts of *Hibiscus callyphylus*

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Abstract

Nowadays, Plants are assessed for their traditional therapeutic properties, as a replacement or a superior option for chemical-based medications. In the similar context, the present work was designed for the Qualitative and quantitative evaluation of phytochemicals, anti-microbial, and antioxidant activities of the leaf extracts of *Hibiscus callyphylus* pertaining to its pharmacological utilisation. Qualitative preliminary phytochemical analysis depicted that alkaloids, phenols, carbohydrates, flavonoids, amino acids, tannins and terpenoids were present in these extracts. The maximum antioxidant activity was shown by methanolic extracts pertaining to the lowest IC50 values followed by aqueous, chloroform and petroleum ether extracts. Total phenolic and flavonoid content was also highest in methanolic solvent. HPLC analysis revealed that only two flavonoids viz. Rutin and Naringin and the representative phenols viz. Gallic acid, Caffeic acid, Ferulic acid, Cinnamic acid and Pyrogallol were present in *H. callyphylus*leaf extracts. The results showed that the tested plant extract possessed Naringin as a major flavonoid and pyrogallol as a major phenolic constituent.From the anti bacterial studies it can be concluded that inhibition activity of the extracts significantly depend upon the solvent used and microbial strain tested for susceptibility. Methanolic extract were found to be more effective against all the tested bacterial strains. The present work provides brilliant basic information regarding the phenols and flavonoids compounds present in the leaves of *H. Callyphylus* having excellent antioxidant and antimicrobial potential, which will play an important role for further research to isolate these phytochemicals for various purposes.

Keywords: Anti-bacterial activity; antioxidant activity; DPPH assay; flavonoids; phenols; phytoconstituent

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Introduction

In the recent years tremendous scientific exploration of plants has been done for various objectives. Plants have being assessed for their conventional therapeutic properties,as a substitute or an excellent option, intended for chemical based medications. In the past eras, plants and their products had been utilised by various systems such as natural preservatives, herbs, or spices in kitchen, flavouring agent as well as a remedy to cure deleterious human ailments. Consequently, the evaluation of phytoconstituents gains popularity and it has become the need of an hour for the disclosure of exceptionally effective and productive bio-remedial agents. (e.g. Roja et al, 2000). Moreover, It is no exaggeration to say that plants have an in-built system for the production of profound secondary metabolites under variable ecological conditions or illnesses. To adapt these biotic and abiotic stress conditions plants are coevolved with disease causingmicroorganism and developed various efficient chemical pathways against them. Henceforth, it is appropriate to expect that plants have bioactive components exhibiting antioxidant and antimicrobial activities. Additionally, World Health Organization (WHO) has witnessed that

conventional medication as secure therapeutic measure against the different diseases brought about by both microbial and non microbial agents(e.g. Geneva, 2011). Many research reports are likewise accessible supporting the antioxidant and antimicrobial properties of plant extracts. (e.g.Shyu et al, 2009; Jo et al, 2012; Voon et al, 2012).In light of the ancient practices, conventional utilizations and adequacy of this plant, present investigation was planned i.e.

i) Preliminary phytochemical screening

ii) Quantification of the total phenol and flavonoid content

iii) HPLC analysis of phenols and flavonoids

iv) Evaluation of the anti oxidant and anti microbial activities of *Hibiscus callyphylus* leaf extracts.

In view of this, it is expected that the discoveries of present investigation will give an appropriate base to the utilization of this plant in the pharmaceutical industries for the planning of plant based medicines.

Figure1. *Hibiscus callyphylus*

Materials and Methods

Collection of plant material

Fresh leaves of *H. callyphylus* with no apparent physical, insect, or microbial damage were collected from the Botanical garden of Punjabi University, Patiala. The leaves were properly washed to eliminate any dust particles from the surface and afterwards dried in shade. Leaves were then homogenized into fine powder and stored in air tight containers.

Extract preparation

For extraction of phytochemical compounds four solvents had been used i.e. Distilled water, Methanol, Chloroform and Petroleum ether. 5g of the powdered sample was mixed individually with 50 mL of solvent and placed overnight in an orbital shaker at 160 rpm at room temperature. Afterwards filtration was done through Whatman filter paper. Collected filtrates were measured and preserved in air tight glass bottles covered with aluminium foil and stored at 4^{o} C till further use.

Preliminary Phytochemical Analysis

Phytochemical screening was performed to identify various phytoconstituents such as alkaloids, flavonoids, steroids, tannins etc. in the aqueous, methanol, chloroform, and petroleum ether solvents as per standardized procedures as follows:

Test for Alkaloids (Wagner's reagent*)*

To 2mLof each extract, 1mL of Wagner's reagent (1 g of iodine and 2 g of potassium iodide in 100 mL of aqueous solvent)was added. The alkaloidspresence was indicated by the appearance of reddish brown precipitate. (e.g. Kokate et al, 2003)

Test for Flavonoids (Alkaline reagent test)

1 mL of 2N sodium hydroxide(NaOH) was added to 2mL of each extract. The appearance of yellow colourtion that disappeared after adding a few drops of dilute Hydrochloric acid depicted the presence of flavonoids in the solution. (e.g. Khandelwal, 2008)

Test for Tannins (Braymer's test)

10% ferric chloride solution was prepared and a few drops of it were added to 2mL of the plant extract. The presence of tannins was indicated by

the appearance of blue or greenish colour in the test tube. (e.g. Rani et al, 2017)

Test for Terpenoids (Salkowki's test)

2 mL of chloroform and few drops of conc. sulphuric acid were added to 1 mL of the leaf extract. Presence of red brown colour at the interface depicts the presence of terpenoids. (e.g. Mir et al, 2013)

Test for Carbohydrates (Fehling's test)

To 1 mL of leaf extract, 3-5 drops of Fehling A reagent and 5-10 drops of Fehling B reagent were added and then placed at 60 degree in water bath for 2-5 minutes. The appearance of brick red precipitates of cuprous oxide indicated the presence of reducing sugars. (e.g. Rani et al, 2017)

Test for Aminoacids (Ninhydrin test)

To 1 mL of extract solution add few drops of Ninhydrin and then placed in the boiling water bath for about 2 minutes. The purple coluration was observed which indicated the presence of amino acids. (e.g. Singh et al, 2013)

Test for Phenols (Ferric chloride test)

To 1 mL of the extract, 2 mL of distilled water was added followed by few drops of 10% ferric chloride. Appearance of deep blue or black colour confirmed the presence of phenols. (e.g. Hema et al, 2012)

Total Phenolic Content

The total phenolic content of all the leaf extracts were evaluated as per the Folin–Ciocalteu (FC) method with minor modifications (e.g. Singleton et al, 1965). The concentration taken for each test solution was 10mg/mL. Briefly, 2.0 mL of FC reagent was added in 400 µL of the sample extract. After that, the solution was incubated in the dark for 5 min at room temperature then 1 mL of sodium carbonate solution(7.5%, w/v) was added and afterwards incubatedat room temperature for 60 minutes. Afterwards, absorbance of the solution was estimated using a UV–visible spectrophotometerat 620 nm. A solution having 2mL of FC reagent, 1 mL Na2CO3 solution and 400 µL of extraction solvent was taken as blank. A suitable calibration curve of standard Tannic acid solution was developed. The TPC was calculated from calibration curve ($y = 0.001x+0.306$, $R^2 = 0.991$) and All the observations were given as mg Tannic acid equivalents (TAE) /gdry weight of plantsample.

Total Flavonoids Content

The total flavonoids content from the leaf extracts were determined utilising the aluminum chloride method with some minor modificationsas depicted by (e.g.Liu et al, 2008). The working test solution of all the extracts was having a concentration of 10mg/mL. In a nutshell, in 500 uL of the sample extract, 1.5 mL of methanol, 300 uL of aluminum chloride (10%, w/v) and 300 ul of 1 M potassium acetate were added and diluted with 5 mL of distilled water, the solution was mixed with vigorous shaking, andkept at room temperature for 30 minutes. The absorbance of the solution was recorded at a wavelength of 420 nm using a UV–visible spectrophotometer. A suitable calibration curve of standard Quercetin was prepared. All tests were performed in three replicates and TPC was calculated using the calibration curve ($y=0.001x+0.228$, $R^2 = 0.992$) and results of each extract was expressed as mg Quercetin equivalents (QE) / g dry weight of the plant sample.

DPPH Antioxidant assay

The DPPH radicals scavenging effect of leaf extracts was estimated according to the procedure described by (e.g.Sanchez-Moreno et al, 1998). Briefly, a stock solution (1mg/mL) of leaf samples was prepared in their respective solvents. Different dilutions ranging from 10-100 ug/mL were prepared. Then, to 1 mL of different concentrations of each sample, 1 mL of 0.3mM DPPH solution was added and the samples were incubated in dark for 30 minutes. Afterwards the absorbance was observed at 517 nm. Ascorbic acid was used as a standard and the absorbance of the standard was estimated in the similar way. All the observations were made in triplicates and the mean of the three reading was taken for final results. The outcomes acquired were

communicated as percentage inhibition of DPPH by utilizing the formula i.e. given below

Percent inhibition of DPPH = [(Abs.control-Abs.sample)/Abs.control]×100

where

$Abs_{control}$ is the absorbance of the DPPH solution without sample extract (control)

Abs_{sample} is the absorbance of the extract samples.

The final calculations were made in the form of IC 5o values.

HPLC Analysis

The phenol and flavonoids components of *H. callyphylus*leaf extracts in methanol were evaluated by HPLC analysis using a column Athena C18 250X 4 at 35 °C temperature.An initial concentration of 1000 ppm in methanol solvent was made for both standards and the samples. The working concentration used was 20 ppm. The leaf extract solutionswere given a pre treatment with 25% HCL for one hour in water bath at 60 degree temperature. Then the solution was filtered through a 0.22 µm filter disc and separately given an injection volume of 20 µLof all the standard and sample solutions. Eleven phenol standards i.e. Gallic acid, Pyrogallol, Caffeic acid, Resorcinol, Vanillic acid, Coumaric acid, Ferulic acid, Vanillin, Veratric acid, Coumarin , Cinnamic acid and nine flavonoids i.e.Rutin ,Naringin, Catechol , Myrecetin, Gossypin, Lutedin, Quercetin, Apegenin, Kampferol were evaluated. The flow rate was 1.5 mL per minute. The wavelength used for the detection of phenols and flavonoids was 280 nm and 254 nm respectively.The UV spectra in the form of chromatograms were observed and concentration of phenols and flavonoids in the samples was calculated using the following formula:

Concentration in sample = Area of sample/Area of standard × Conc. of standard.

Antibacterial susceptibility test (agar diffusion method)

Microorganisms and growth condition:

A total of five pathogenic bacteria (obtained from Botany department, Punjabi university, Patiala) were used for assessing the antibacterial activities of the leaf extracts in three solvents i.e. Methanol, Chloroform and Petroleum ether. Three Gram-positive (*Streptococcuss pneumoniae*, *Micrococcus luteus, Streptococcuss thermophilus*) and two Gram-negative (*Haemophilus influenza, Klebsiella pneumoniae*) bacteria were utilised to sdetermine antibacterial properties of leaf extracts. A loop full of all the pathogens acquired from respective stock cultures were inoculated (1% v/v) into 20mLnutrient broth medium followed by incubation at 37 °C (for 18 h) to obtain active cultures for further testing.

Extract preparation for microbial analysis

Extraction mainly using three solvents, Methanol, Chloroform and petroleum ether was performed. In brief, 5.0 g of each powdered sample was added in 50 mL of the solvent and placed on magnetic stirrer for 24 hrs at room temperature. After filtration, 50 mL of the solvent was added in the residues for re-extraction for around 24 hrs. The collected filtrates were mixed together and the solution was filtered by using Whatman filter paper. The alcoholic filtrates obtained were concentrated using a rotary vacuum evaporator at 50 °C. A stock solution of 100 mg/mL was prepared by dissolving the concentrated crude extracts in the DMSO solvent and stored at −20 °C until further utilization.

Culture preparation

Suspension of tested pathogens (10^8 CFU/mL) from active cultures was evenly distributed using sterile-glass spreader on nutrient agar medium plates. The agar plates were left undisturbed for around 1-2 hours to ensure full diffusion of the pathogens on agar plates. Wells were formed within the agar plates. The stock concentration of each extract was diluted further into three concentrations i.e. 50, 75 and 100 µg/mL in dimethyl sulfoxide (DMSO). Then, 5 µl of each dilution, Ampicillin (10 µg/mL) (positive control) and DMSO (negative control) was added in the wells to test the antimicrobial activity. The plates were incubated for 24 hours at 37 ºC temperature. Afterwards, the inhibition zone was measured and expressed in millimeters. Three replicates of each concentration were evaluated for final results.

Results and Discussion

Preliminary phytochemical analysis

The preliminary phytochemical screening of extracts of leaves revealed the presence of flavonoid, tannins, alkaloids, terpinoids, phenolic compound, amino acids and carbohydrates. Almost all the compounds were present in methanol solvents. Phenols and flavonoids were present in all the extracts. Amino acids were observed only in methanol solvent extracts.In aqueous extracts all other compounds were present except aminoacids and terpenoids. These findings conclude that methanol is the best solvent followed by aqueous, chloroform and petroleum ether for the extraction of phytochemicals. Results of this preliminary phytochemical investigation in the extract of *Hibiscus callyphylus* leaves were shown in the **(Table 1)**.

Table 1. Phytochemical investigations in the leaf extract of*Hibiscus callyphylus*

S.No	Phytochemical compounds	Leaf extracts			
		Methanol	Aqueous	Chloroform	Petroleum ether
1.	Alkaloids	+	+	-	-
2.	Flavonoids	+	+	+	+
3.	Amino acids	+	-	-	-
4.	Tannins	+	+	-	-
5.	Terpenoids	+	-	+	-
6.	Carbohydrates	+	+	-	-
7.	Phenols	+	+	+	+

The Present results depict similarity with various previous findings. Methanol is the best solvent for the extraction of phytochemical constituents (e.g. Kapoor et al, 2019). Flavonoids belong to the group of polyphenolic compounds and are typically known for their health promoting potentials such as anti-oxidant, anti-microbial properties (e.g. Boots et al, 2008). The plant extracts revealed the presence of numerous useful phyto-constituents exhibiting various medicinal properties (e.g. Njoku, 2009). Phenolic compounds in plant extracts were higher in concentration than the other phytochemicals such as alkaloids and flavonoids. (e.g. Hussain, 2011).

Total phenolic content

In the present study, the amount of total phenolic content was significantly variable among all the used extract solvents . Total phenols ranged from 5.6-35.1 mg GAE/g. The TPC of different extracts is given in (**Table 2**and **Figure 2 B**). The methanolic leaf extracts has maximum phenolic content (35.1 mg GAE/g) followed by Aqueous (10.6 mg GAE/g) and Chloroform (9.3 mg GAE/g). The petroleum ether extracts exhibited minimum phenolic content (5.6 mg GAE/g). Therefore, petroleum ether is the least recommended solvent for the extraction of phenolic compounds.

Total flavonoids content

The TFC in all the similar plant extracts were evaluated and a significant variation among all the solvents had been observed. Quantitative determination revealed that it ranges from 7.8-18.7 mg QE/g as shown in (**Table 2** and **Figure 2 C**). The maximum flavonoids conc. (18.7 mg QE/g) was observed in methanolic extracts followed by water (15.2 mg QE/gm) and chloroform (12.7 mg QE/g) and the minimum amount of flavonoids was present in petroleum ether (7.8 mg QE/g).

High level of total phenols, flavonoids, and anthocyanins has been observed in extracts of different plant parts in various solvents (e.g. Cai et al, 2004; Wijekoon et al, 2011; Yang et al. 2012; Gouveia et al, 2012), thus supporting the outcomes and elucidations done in the present

work. In addition to this, many studies reported a positive correlation between the presence of different phytoconstituents and antioxidant activities in different plant parts (e.g. flowers, fruits, leaves, seeds) and various extract solvents(e.g. Shyu et al, 2009; Voon et al, 2012;Wijekoon et al, 2011).

DPPH radical scavenging activity

Determination of antioxidant activity on the basis of scavenging effects of DPPH radical is a commonly used antioxidant assay. This is considered to be a convenient, rapid, and simple method as it is independent of the polarity of sample extracts and is effective for the antioxidant screening of large samples. (e.g. Magalhaes et al, 2008; Alam et al, 2012).

Antioxidant compounds and antioxidant activities of *Hibiscus callyphylus* leaf extracts were calculated in the form of IC 50 value. The IC 50 value of all the extracts is given in the **(Table 2)**. It was observed that the DPPH radical scavenging activity was maximum in methanol extracts as the IC50 value for the same was minimum i.e. 81.02±0.21 µg/mL. Petroleum ether extracts showed the minimum radical scavenging activity pertaining to the maximum IC 50 value i.e. 250.65±3.043 µg/mL. Ascorbic acid was used as a standard and the IC 50 value for Ascorbic acid was 9.54 ± 0.12 µg/mL.Similar results are graphically represented in (**Figure 2A)**. All the results given are mean of three individual replicates (n = 3 ± S.D.) The mean values followed by different letters in a row are significantly variable (p < 0.05) from one another.

Table 2. DPPH antioxidant activity, Total phenolic and flavonoids content of leaf extracts of *H. callyphylus*

Parameters	Leaf extracts			
	Methanol	Distilled water	Chloroform	Petroleum ether
DPPH antioxidant activity (IC 50) µg/ml	81.02±0.21	86.62±0.45	132.75±3.93	250.65±3.043
TPC mg GAE/g	35.1	10.6	9.3	5.6
TFC mg QE/g	18.7	15.2	12.7	7.8

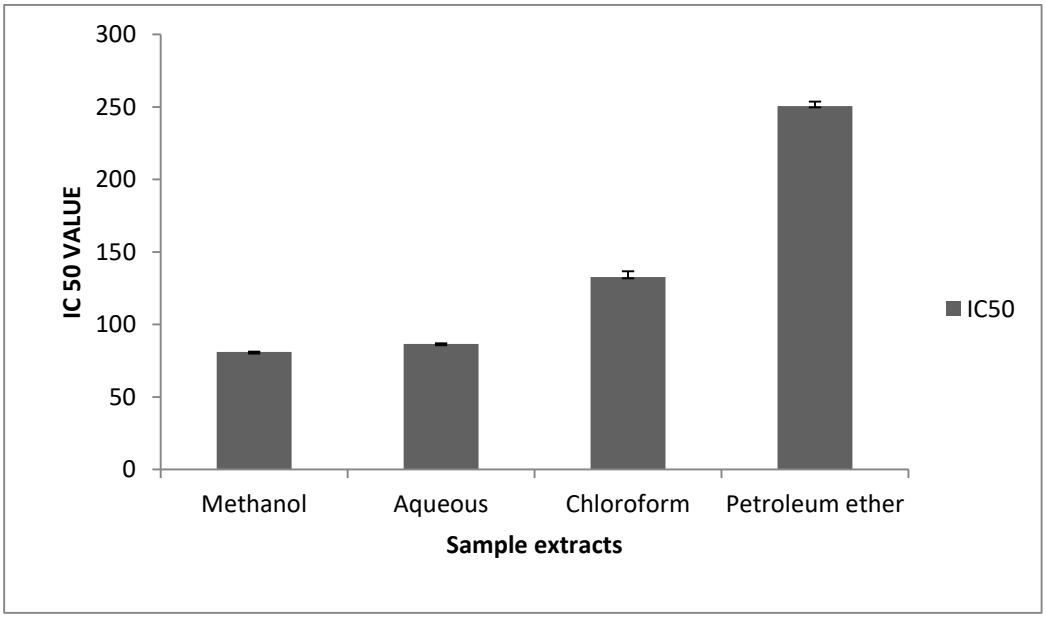

Figure 2 A. IC 50 values of leaf extracts in different

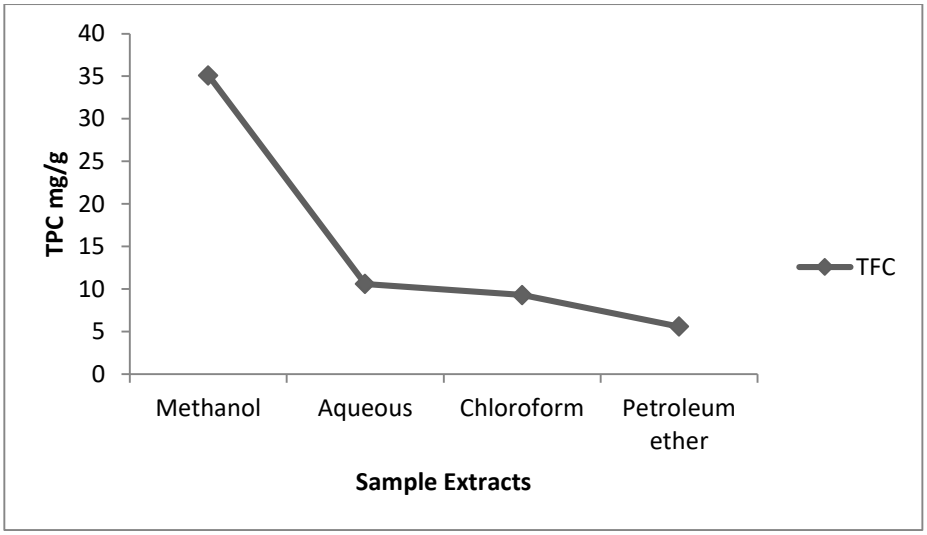

Figure 2B. TPC of leaf extracts in various

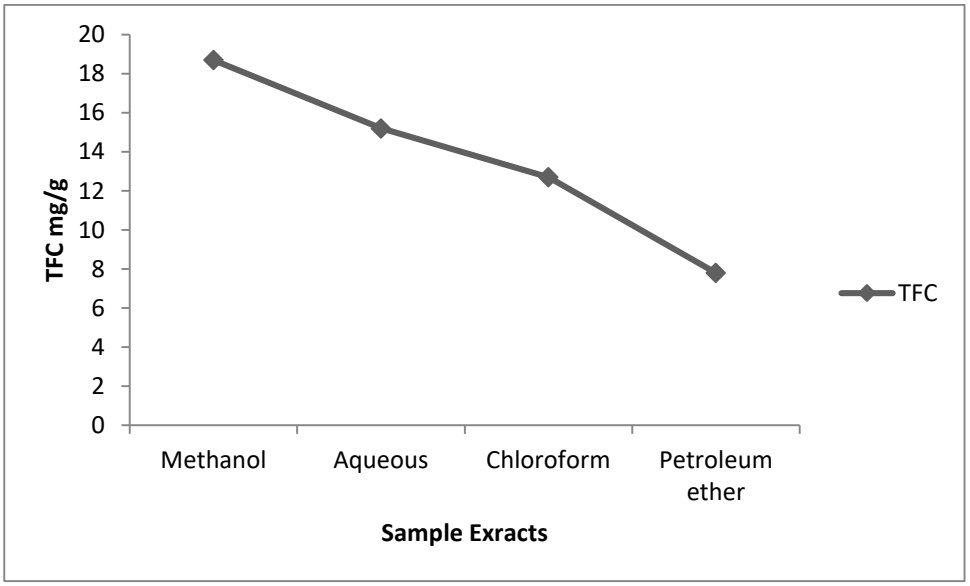

Figure 2 C: TFC of leaf extracts in various

Higher radical scavenging activity would be attributed due to the presence of phenols and flavonoids in the leaf extracts. It hasalso been observed that the different solvents used for extraction can contribute significantly to the antioxidant activities of the extracts (e.g. Tian et al, 2009; Wijekoon et al, 2011), which are in accordance with the present study as well. The phenols including flavonoids, tannins, flavonoland anthocyanins are most probably the major contributor to the observed antioxidant properties in various plant extracts. (e.g. Saeed et al, 2012)

HPLC analysis

HPLC analysis of flavonoids

In the present study, only methanol leaf extracts were analysed for the quantification of different phenols in leaf extracts of H. *callyphylus*using High Performance Liquid Chromatography (HPLC). Out of the nine standard flavonoids only two viz. Rutin (5.419µg/g) and Naringin (39.059 µg/g) were present in *Hibiscus* leaf extract. The results showed that the tested plant extract possessed Naringin as a major flavonoid constituent. All other flavonoids compounds were absent in leaf extract. The retention times, peak

area (including standards) and concentrations of various flavonoids identified in this study are depicted in the (**Figures 3A, 3B,** and **Table 3**)

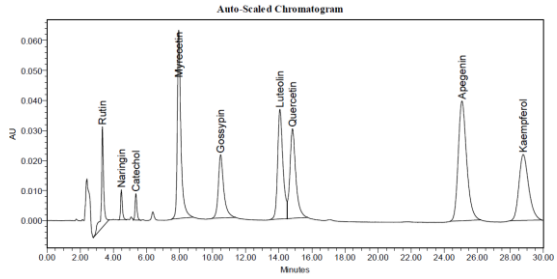

Figure 3A. High-performance liquid chromatogram of standard flavonoids

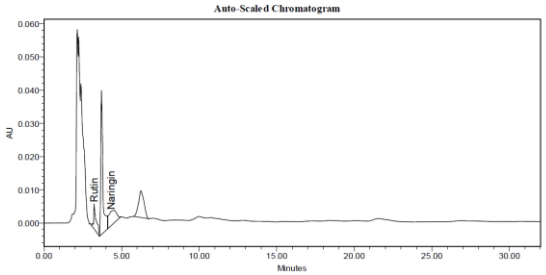

Figure 3B. High-performance liquid chromatogram of leaf extract.

Table 3. The retention times, peak area(including standards) and concentrations of various flavonoids identified in*Hibiscus callyphylus* methanol leaf extract (MLE).

Name	RT		Area		Concentration in leaf extracts (µg/g)
	Standard	MLE	Standard	MLE	
Rutin	3.343	3.223	317938	86140	5.419
Naringin	4.487	4.310	74008	144535	39.059
Catechol	5.363	-	68285	-	-
Myrecetin	7.955	-	934766	-	-
Gossypin	10.484	-	476544	-	-
Luteolin	14.070	-	817658	-	-
Quercetin	14.832	-	743174	-	-
Apegenin	25.077	-	1409175	-	-
Kaempferol	28.773	-	894549	-	-

HPLC analysis of phenols

Quantification of various flavonoids had been done by using High Performance Liquid Chromatography (HPLC). The representative phenols viz. Gallic acid, Caffesic acid, Ferulic acid, Cinnamic acid and Pyrogallol were present in *Hibiscus* leaf extract out of the total eleven phenols. The results showed that the tested plant extract possessed Pyrogallol (230.089 µg/g), Ferulic acid (10.390 µg/g), Gallic acid (2.047 µg/g), Caffeic acid(1.950 µg/g)), and cinnamic acid (0.231). Rest all phenol compounds were shown to be absent in these extracts. The major phenolic constituent present was Pyrogallol. The retention times, peak area (including standards) and concentrations of various phenols identified in this study are depicted in the (**Figures 4A, 4B** and **Table 4**).

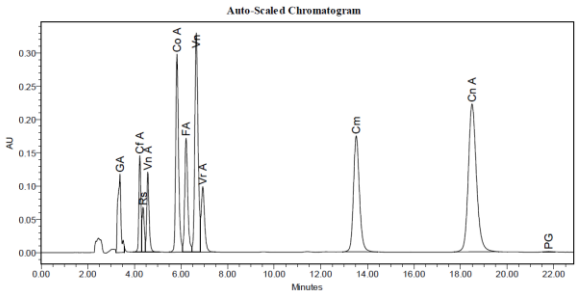

Figure 4A.High-performance liquid chromatogram of standard phenols

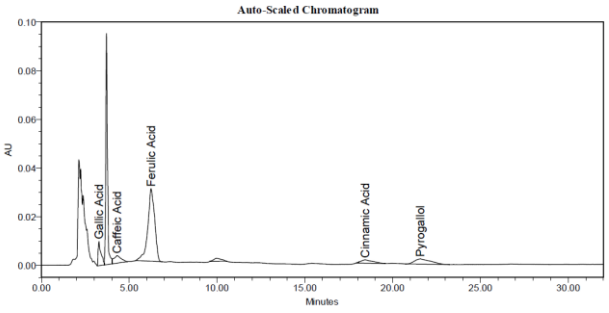

Figure 4B.High-performance liquid chromatogram of *Hibiscus* leaf extracts

Table 4.The retention times, peak area (including standards) and concentrations of various phenols identified in *Hibiscus callyphylus* methanol leaf extract.

Name	RT		Area		Concentration in leaf extracts (µg/g)
	Standard	MLE	Standard	MLE	
Gallic Acid	3.384	3.273	1091843	111753	2.047
Caffeic Acid	4.235	4.305	895855	87352	1.950
Resorcinol	4.376	-	432542	-	-
Vanillic Acid	4.577	-	833494	-	-
Coumaric Acid	5.829	-	2520886	-	-
Ferulic Acid	6.220	6.237	1559114	809935	10.390
Vanillin	6.657	-	3190068	-	-
Veratric Acid	6.936	-	988466	-	-
Coumarin	13.522	-	3100259	-	-
Cinnamic Acid	18.493	18.437	5391632	62272	0.231
Pyrogallol	21.778	21.571	11882	136696	230.089

Antibacterial activity

All the leaf extract showed significant zone of inhibition against all the tested bacterial strains. Among the different concentrations (50, 75 and 100 mg/mL), 100 mg/mL produced maximum inhibitory activity against all the tested microorganisms. The inhibition zone ranges from 1mm to 3.6 mm. Minimum zone of inhibition was observed against *M. leuteus*(2.3 mm) and maximum was observed against the *H. influenza* (3.6 mm) the activity shown by different concentrations against tested microbes is given in (**Table 5** and **Figure 5**). The results indicate that antibacterial activity of plants depends on the solvent used for extraction and tested bacterial strain. The inhibitory activity founds directly proportional to the concentrations of plant extracts. Ampicillin (Positive control) showed different inhibition zones against different bacterial species as given in the table 5 and the negative control (DMSO) depicted no inhibition zone against any microbe.

Table 5. Antimicrobial activity of methanolic extracts of *Hibiscus* leaf extracts.

Microorganism	Methanol			Ampicillin
	50mg/mL	75mg/mL	100mg/mL	0.1mg/mL
S. pneumonia	-	1.6	3.3	8.3
S. thermophilus	-	1.3	3.3	8.0
K. pneumonia	1.0	2.1	3.2	9.0
H. influenza	-	1.0	3.6	8.6
M. luteus	-	1.2	2.3	7.6

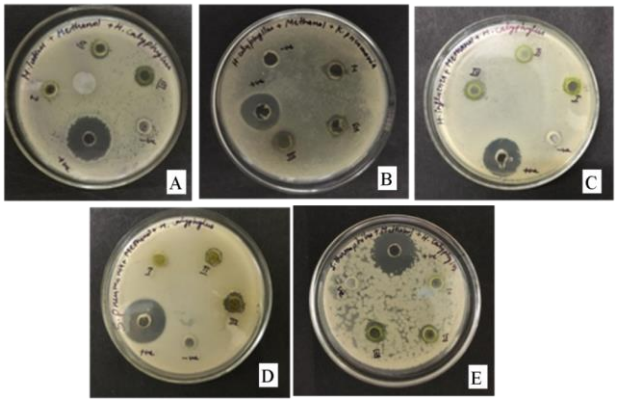

Figure 5. Plates showing antimicrobial activity of methanol leaf extract against different bacterial strains

A. Micrococcus luteus B. *Klebsella pneumonia* C. *Haemophilia influenza*

D. *Streptococcus pneumonia* E. *Streptococcus thermophilus*

Previous reports have shown that the plant extracts exhibit higher antibacterial activities against Gram-positive bacteria as compared to Gram-negative bacteria (e.g.Kabuki et al, 2000; Tian et al, 2008). However, in the present study, leaf extracts of *H. callyphylus* inhibited both Gram-positive and Gram-negative pathogens almost in the similar way. Polyphenols, flavonoids and tannins present in the leaf extracts would be responsible for the antibacterial activity as all these compounds are produced in various plant parts to show defensive mechanisms against various biotic stresses.On the basis of previous available reports, methanol extracts result in high extraction amounts having strong antibacterial properties (Quarenghi et al, 2000; Mann et al, 2011; Jo et al, 2012).

Conclusion

It can be concluded that among the five solvents used for extraction, methanol solvent was found to be more effective, followed by aqueous, chloroform and petroleum ether. The reason behind this might be the more solubility of photochemical compounds of *H. callyphylus*with methanol than other solvents. Both Total phenolic and flavonoids content were found to be highest in methanol leaf extracts. The antioxidant potential of the extract was also found to be highest in methanol solvent as it has minimum IC50 values.The extracts of leaves in different solvents were found to cause maximum antimicrobial activity toward *H. influenza*, followed by *S. pneumonia*, *S. thermophilus*, *K. pneumonia*and *M. luteus*. Hence, the plant can be considered as a potential source of novel herbal medicines that can be beneficial for the therapeutic industries. However, more detailed research is required to explore its potential by isolating components that have antioxidant and antimicrobial properties. The present study can also provide excellent basic information regarding the phenols and flavonoids compounds present in the leaves of *H. Callyphylus* which will play an important role for further research to isolate these phytochemicals for various purposes

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Abbreviations list

S.No.	Abbreviation	Full form
1.	g	Gram
2.	Mg	Milligram
3.	mL	Millilitre
4.	µg	Microgram
5.	µL	Microlitre
6.	QE/g	Quercetin Equivalent
7.	Ppm	Parts per million
8.	Rpm	Rotations per minute
9.	GAE/g	Gallic Acid Equivalent
10.	CFU/ml	Colony Forming Units
11.	i.e.	That is
12.	viz	For example
13.	e.g.	For example
14.	V/V	Volume per volume
15.	W/V	Weight per volume
16.	IC 50	Inhibitory concentration

17.	DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
18.	TPC	Total phenolic content
19.	TFC	Total flavonoids content
20.	TAE	Tannic avid equivalent
21.	HCL	Hydrochloric acid
22.	DMSO	Dimethyl sulfoxide
23.	MLE	Mallic acid equivalent
24.	RT	Retention time
25.	Abs.	Absorbance

Conflict of Interest

All the authors declare that there are no conflicts of interest regarding the publication of this research.

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