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PHYTOCHEMICAL EVALUATION OF A MEDICINAL PLANT *COSTUS PICTUS* AND ITS ANTIBACTERIAL, ANTIOXIDANT ACTIVITY

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ABSTRACT

Nature has bestowed upon us plenty of herbs and plants, which are a source of medicines involved in maintaining human health. The word "herbs" comes from the Latin 'herba'. It means a medicinal plant. India has a rich cultural heritage of traditional medicines which chiefly comprised of a flourishing system of medicines like Ayurveda, Siddha, and Unani. It is considered not just as an ethnomedicine but also as a complete medical system that includes the physical, psychological, ethical, and spiritual well-being of man kind. Compounds extracted from different parts of the plants can be used in the treatment of diarrhoea, dysentery, cough, cold, fever, bronchitis, and cholera these can be derived from any part of the plant, like leaves, flowers, bark, roots, fruits, and seeds. According to the activity in plant metabolism, phytochemicals are grouped into primary and secondary metabolites. Primary metabolites such as sugars, amino acids, proteins, and chlorophyll are found in all plants while secondary metabolites comprise alkaloids, flavonoids, saponins, tannins, and phenolic compounds. The present research evaluates to screen for preliminary phytochemicals of secondary metabolites in *Costus pictus* leaf extracts.To evaluate the antimicrobial activity by using microorganisms such as *S. aureus, E. coli, S. typhi,* and *B. subtilis,*and the antioxidant activity using the DPPH method of radical scavenging activity of the leaves extract.

INTRODUCTION

Plants have been the principal tools of traditional medicine since time immemorial, however, only a small portion of botanical wealth is used in conventional medicines. In most developing countries, traditional medicine is practiced as an integral part of their culture. Rural areas still depend on herbal medicines for their primary health care and these conventional medicines have a place in day-to-day life. Plants with a long history in traditional medicinal systems are potential candidates for drug discovery. According to the activity in plant metabolism, phytochemicals are grouped into primary and secondary metabolites. These secondary constituents have therapeutic actions, which can be refined for the synthesis ofdrugs (1). Extraction and characterization of several bioactive compounds from these green factories gave birth to some high-active profile drugs.The antimicrobial activity will be evaluated using microorganisms such as *S. aureus, E. coli, S. typhi,* and *B. subtilis*. Antioxidant activity by using the DPPH method it is also known as "Free radical scavengers" protects the cells from damage caused by unstable free radicals, by inhibiting the oxidation reaction.

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Taxonomy of *C. pictus*:

Kingdom : Plantae
Division : Magnoliophyta
Class : Liliopsida
Subclass : Zingiberidae
Order : Zingiberales
Family : Costaceae
Genus : Costus
Species : pictus

MATERIALS AND METHOD

Collection of Plant Material: Fresh and healthy leaves of *C. pictus* were collected from in and around Thane city (Western Maharashtra, India).

Preparation of Plant Extract: Fresh leaves of *C. pictus* were well washed, shade-dried at room temperature, and ground into fine powder. The powdered sample is then stored in air-tight bottles for further analysis. The homogenized samples were extracted with ethanol, petroleum ether, and water. 20 gm of samples were dissolved separately in three different conical flasks with 150 ml of ethanol, petroleum ether, and water. The extraction was carried out for 48 hours in a rotary shaker at 150 - 160 rpm. The extracts were filtered using muslin cloth and the residue was removed. The filtrate was then evaporated

to dryness and the resulting paste-form extracts were stored in a refrigerator for future use.

Figure 1. *C. pictus* leaves

Figure 2. *C. pictus* leaves powder

Qualitative Analysis of Phytochemicals: The extracts of *C. pictus* were screened to analyze for the presence of following phytoconstituents like alkaloids, proteins, carbohydrates, phenols, flavonoids, saponins, glycosides, steroids, terpenoids and tannins (2).

- **Test for Alkaloids:** The plant extracts were treated with Dragendroff's reagent. The formation of a red precipitate indicates the presence of alkaloids.
- **Test for Proteins (Ninhydrin test):** The plant extracts were treated with 2 ml of 0.2% ninhydrin solution. The presence of violet coloration indicates amino acids and proteins.
- **Test for Carbohydrates:** 2 ml of Benedict's solution was added to each extract and boiled. The formation
- **Test for Phenols:** 2 ml of 2% ferric chloride solution was added to each extract. Blue-green or purple color formation indicates the presence of phenols.
- **Test for Flavonoids (Alkaline reagent test):** Each plant extract was mixed with 2 ml of 2% NaOH solution. The intense yellow color formed turns colorless-with the addition of a few drops of dilute hydrochloric acid indicating the presence of flavonoids.
- **Test for Saponins(Foam test):** The plant extracts were mixed with 5 ml of distilled water and shaken vigorously. The formation of stable foam indicates the presence of saponins.
- **Test for Glycosides (Salkowski's test):** 2 ml of chloroform was mixed with each plant extract. 2 ml of concentrated sulphuric acid was then added and shaken gently. A reddish-brown color indicates the presence of glycosides.
- **Test for Steroids:**Plant extracts were mixed with 2 ml of chloroform and concentrated sulphuric acid. A red color formed at the chloroform layer indicates the presence of steroids.
- **Test for Terpenoids:** 2 ml of chloroform was added to each extract and mixed well. 3 ml of concentrated sulphuric acid was added to each tube. The formation of a reddish-brown color indicates the presence of terpenoids.
- **Test for Tannins (Gelatine test):** 1% gelatine containing sodium chloride was treated with each plant extract. The formation of a white precipitate indicates the presence of tannins.

Preparation of Discs: The antimicrobial activity of the extracts was determined by the disc diffusion method. Discs of 4 mm diameter were cut out from Whatman No.1 filter paper. They were sterilized by autoclaving and stored in aseptic conditions. During the time of treatment, the disc was taken out with the help of sterile forceps. The concentrated extracts from the plant were pipetted out (40, 60, 80, and 100 µg/ml) and poured into a clean autoclaved Petri dish. The filter paper disc was placed in the Petri dish for 20 minutes to make the filter paper disc fully saturated with the extract.

Antibacterial Activity: Nutrient agar medium was prepared and transferred into sterile Petri plates. 25 µl of the standardized bacterial inoculum was spread on an agar medium using a sterile cotton swab. The discs impregnated with extracts at different concentrations (40, 60, 80, and 100 µg/ml) were placed on the inoculated agar medium. Amphicillin (10 µg/disc) was used as the standard to determine the sensitivity of each microbial species. All the Petri plates were incubated at 37°C for 24 hours. After the incubation period, growth inhibition was determined as the diameter of the inhibition zones around the discs. The growth inhibition diameter was an average of 4 measurements, taken in four different directions. All the tests were performed as triplicates.

Antimicrobial Activity: The Potato dextrose medium was prepared and transferred into sterile Petri plates. 200 µl of the standardized fungal inoculum was spread on an agar medium using a sterile cotton swab. The discs were impregnated in extracts at different concentrations (40, 60, 80 and100 µg/ml) and were placed on the inoculated agar medium. Tetracycline (10 µg/disc) was used as a reference standard to determine the sensitivity of each microbial species tested. All the Petri plates were incubated at 27°C for 72 hours. After the incubation period, growth inhibition was determined as the diameter of the inhibition zones around the discs. The growth inhibition diameter was an average of four measurements taken in four different directions. All the tests were performed in triplicate.

Antioxidant Activity: The antioxidant activities of medicinal plants are studied by using the DPPH method as they may be responsible for various bioactivities (3).

DPPH Radical Scavenging Assay: The hydrogen donating ability was examined in the presence of DPPH stable radical. One milliliter of 0.3 mM DPPH methanol solution was added to 1 ml of the plant extract (1000 µg/ml) at different

concentrations and allowed to react at room temperature. After 30 minutes the absorbance values were measured at 517 nm. The methanol solution was used as a blank and the DPPH solution (1.0 ml, 0.3 mM) with 1 ml methanol served as a negative control.

Ascorbic acid (1000 µg/ml) was taken as the positive control. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ of inhibition} = \frac{\text{A control} - \text{Atest}}{\text{A control}} \times 100$$

Where 'A control' was the absorbance of the control reaction and 'A test' was the absorbance in the presence of the extract/ standard. The mean values were obtained from triplicate analysis.The antioxidant activity of the extract was expressed as IC_{50}.

RESULT AND DISCUSSION

Preliminary Phytochemical Screening: The preliminary phytochemical screening of ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves is presented in Table 4.1. Among the three extracts, ethanol extract was found to be rich in biologically active compounds such as alkaloids, steroids, terpenoids, glycosides, carbohydrates, tannins, phenol, flavonoids, and proteins, followed by aqueous extract, which indicated the presence of compounds like alkaloids, terpenoids, glycosides, carbohydrates, phenols, flavonoids, saponins, and proteins.

Alkaloids, steroids, terpenoids, glycosides, phenols, and flavonoids are the compounds found in petroleum ether extract.

Table 1. The qualitative phytochemical analysis of *C. pictus* leaf extracts

Phytochemicals	Solvents		
	Ethanol	Aqueous	Petroleum ether
Alkaloids	+	+	+
Steroids	+	-	+
Terpenoids	+	+	+
Glycosides	+	+	+
Carbohydrates	+	+	-
Tannins	+	-	-
Saponins	-	+	-
Phenols	+	+	+
Flavonoids	+	+	+
Proteins	+	+	-

Antimicrobial Activity of *C. pictus* Leaf Extracts: Ethanol, aqueous, and petroleum ether extracts of *C. pictus* were screened for their antimicrobial potency against 10 medically important pathogenic microorganisms.The antimicrobial assay was performed by agar disc diffusion method. Antibacterial andantifungal activity of *C. pictus* extracts were compared with standard antibiotics, Ampicillin, and Tetracycline respectively. The activity was determined by measuring the diameter of the zone of inhibition. All the bacterial and fungal strains were collected from KMCH and freshly sub-cultured on Nutrient Agar and Potato Dextrose Agar for 24- 48 hours at 37°C and 25°C respectively.

Antibacterial Activity of *C. pictus* Leaf Extracts: The ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves weretested for their antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* with Ampicillin(10 µg) as positive control. The results obtained in evaluating the antibacterial activity of *C. pictus* leaf extracts against selected bacteria are listed in Table 4.2. and the activity of the extracts at 100 µg/ml concentration is represented in Graph 3.The Zones of inhibition are recorded in millimeters.

Ethanol extract recorded the highest inhibition zone (18.017±0.076 mm) against *S. typhi* while less inhibition zone (10.033±0.058 mm) was observed against *B. subtilis*. Ethanol extract of *C. pictus* leaves showed activity (11.617±0.104 mm) against *E. coli*, whereas it failed to inhibit the growth of *S. aureus* and *P. aeruginosa*. Aqueous and petroleum ether extract of *C. pictus* leaf exhibited antibacterial activity against all the tested pathogenic bacteria. Aqueous extract showed maximum activity (14.100±0.100 mm) against *B. subtilis* and the minimum being (8.867±0.058 mm) recorded against *P. aeruginosa*. The extract showed moderate antibacterial activity (11.033±0.058 mm), (11.000±0.100 mm), (10.033±0.058 mm) against *E. coli, S. aureus,*and *S. typhi* respectively. Petroleum ether recorded the highest activity (10.167±0.058 mm) against *B. subtilis* and the least activity (9.083±0.076 mm) against *S. aureus*. The extract exhibited moderate activity (10.083±0.076 mm), (10.117±0.104 mm), (10.033±0.058 mm) against *E. coli, S. typhi,*and *P. aeruginosa* respectively.

Table 2. The antibacterial activity of ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves against selected microbes

Bacteria	**Zone of inhibition in mm**			
	Concentration (µg/ml)			
	40	**60**	**80**	**100**
	Ethanol			
E. coli	8.033±0.058	10.033±0.058	11.150±0.050	11.617±0.104
B. subtilis	8.450±0.050	9.100±0.100	10.033±0.058	
S. aureus	-	-	-	-
S. typhi	10.083±0.076	12.083±0.076	15.100±0.100	18.017±0.076
P. aeruginosa	-	-	-	-

Aqueous				
E. coli	9.083±0.076	9.483±0.029	10.067±0.058	11.033±0.058
B. subtilis	10.067±0.058	11.083±0.076	13.100±0.100	14.100±0.100
S. aureus	8.100±0.100	9.150±0.304	9.500±0.100	11.000±0.100
S. typhi	7.117±0.104	8.033±0.058	8.483±0.029	10.033±0.058
P. aeruginosa	7.067±0.058	7.783±0.029	8.083±0.076	8.867±0.058
Petroleum ether				
E. coli	8.083±0.076	8.583±0.029	9.117±0.029	10.083±0.076
B. subtilis	7.100±0.100	8.033±0.058	9.000±0.100	10.167±0.058
S. aureus	7.200±0.050	7.750±0.050	8.033±0.058	9.083±0.076
S. typhi	8.033±0.058	8.400±0.100	9.150±0.050	10.117±0.104
P. aeruginosa	8.117±0.104	8.550±0.050	9.067±0.058	10.033±0.058
Standard				
E. coli	11.133±0.153			
B. subtilis	20.100±0.100			
S. aureus	18.183±0.161			
S. typhi	9.033±0.058			
P. aeruginosa	25.950±0.050			
Each value represents the mean ±SD of three replicated experiments; - = no inhibition				

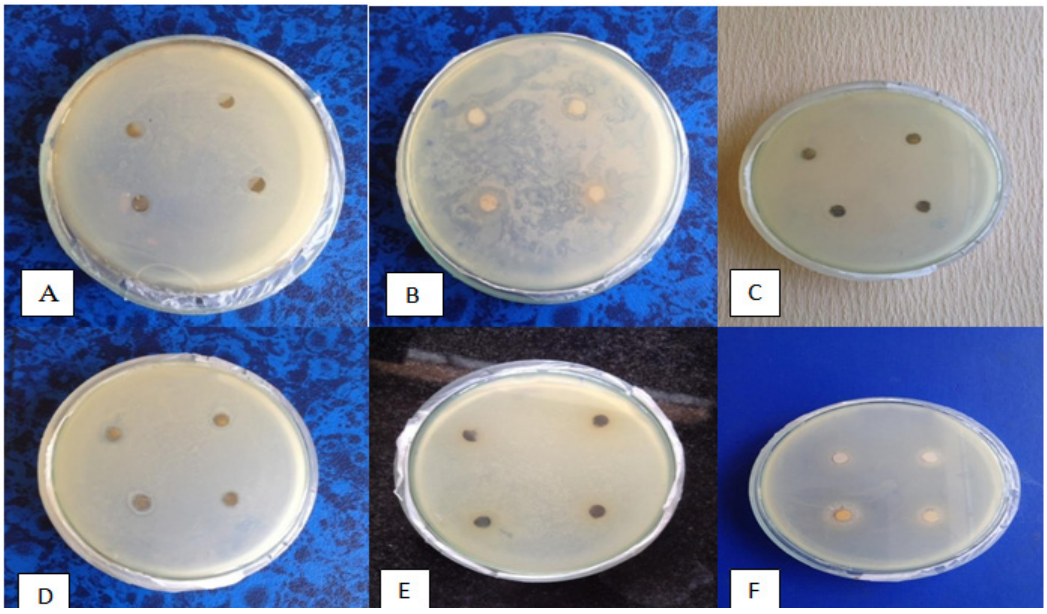


Figure 3. The Antibacterial activity of *C. pictus* leaf extracts

Antibacterial activity shows against different crude extracts, (A)*Bacillus subtilis*- petroleum ether extract, (B)Escherichia coli - aqueous extract, (C) *Pseudomonas aeruginosa*- petroleum ether extract,(D)*Pseudomonas aeruginosa*-petroleum ether extract, (E)*Salmonella typhi*- ethanol extract, (F) *Bacillus subtilis*- aqueous extract.

Antioxidant Activity of *C. pictus* Leaf Extracts

Excess generation of free radicals can also lead to athero sclerosis,cancer,inflammatory joint disease, asthma,diabetes,and degenerativeeye disease. Antioxidants protect cells from damage caused by unstable free radicals. The antioxidant activities of medicinal plants are studied as they may be responsible for various bioactivities (3). In the present study, ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves were evaluated for their antioxidant activities. Three methods, DPPH,FRAP, and Total antioxidant activity,were used for the investigation.

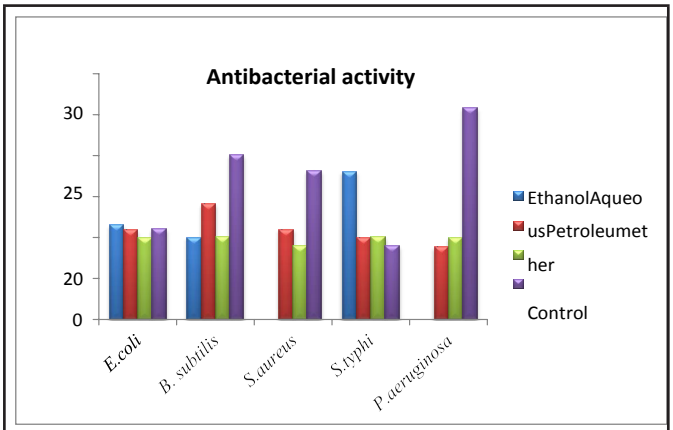
DPPH Radical Scavenging Assay

The DPPH free radical method has been widely used to determine the antioxidant activity of plant extracts based on the reduction of DPPH, a stable free radical. DPPH scavenging ability of ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves were determined as shown in Table 4.8 and Graph 5.The results are compared with the standard ascorbic acid. IC50 value was determined for each extract as well as forthe standard. The DPPH scavenging ability of the ethanol extract (26 µg/ml) and aqueous extract (22 µg/ml) showed

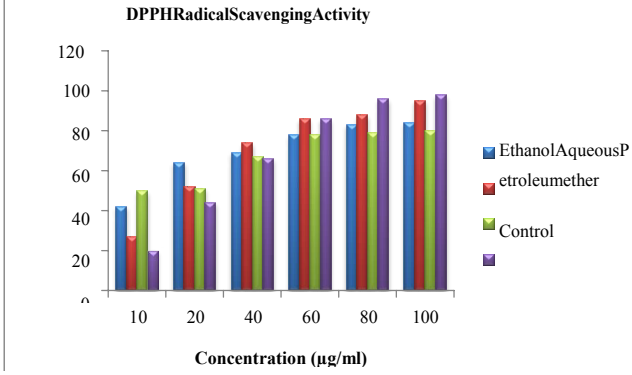
Table 3. The antimicrobial activity of ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves against selected microbes

Bacteria	Zone of inhibition in mm			
	Concentration (µg/ml)			
	40	60	80	100
	Ethanol			
E. coli	8.033±0.058	10.033±0.058	11.150±0.050	11.617±0.104
B. subtilis	8.450±0.050	9.100±0.100	10.033±0.058	10.033±0.058
S.aureus	-	-	-	-
S. typhi	10.083±0.076	12.083±0.076	15.100±0.100	18.017±0.076
P. aeruginosa	-	-	-	-
	Aqueous			
E. coli	9.083±0.076	9.483±0.029	10.067±0.058	11.033±0.058
B. subtilis	10.067±0.058	11.083±0.076	13.100±0.100	14.100±0.100
S.aureus	8.100±0.100	9.150±0.304	9.500±0.100	11.000±0.100
S. typhi	7.117±0.104	8.033±0.058	8.483±0.029	10.033±0.058
P. aeruginosa	7.067±0.058	7.783±0.029	8.083±0.076	8.867±0.058
	Petroleum ether			
E. coli	8.083±0.076	8.583±0.029	9.117±0.029	10.083±0.076
B. subtilis	7.100±0.100	8.033±0.058	9.000±0.100	10.167±0.058
S.aureus	7.200±0.050	7.750±0.050	8.033±0.058	9.083±0.076
S. typhi	8.033±0.058	8.400±0.100	9.150±0.050	10.117±0.104
P. aeruginosa	8.117±0.104	8.550±0.050	9.067±0.058	10.033±0.058
	Standard			
E. coli	11.133±0.153			
B. subtilis	20.100±0.100			
S.aureus	18.183±0.161			
S. typhi	9.033±0.058			
P. aeruginosa	25.950±0.050			
Each value represents the mean ±SD of three replicated experiments; - = no inhibition				

significantly lower IC50 values when compared with ascorbicacid. However, the standard reference compound, ascorbic acid gave a better DPPH scavenging ability than the petroleum etherextract (50µg/ml) with IC50of 39µg/ml.The scavenging activity of extracts increased with increasing concentration.



Graph.1 The antibacterial activity of C. pictus leaf extracts at higher concentrations

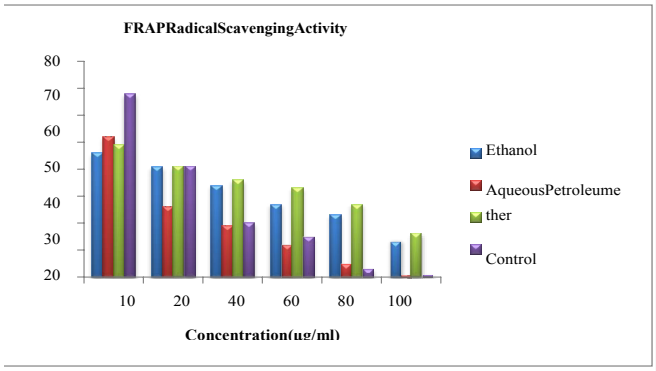


Graph 3. The DPPH radicals cavenging activity of *C.pictus* leaf extracts

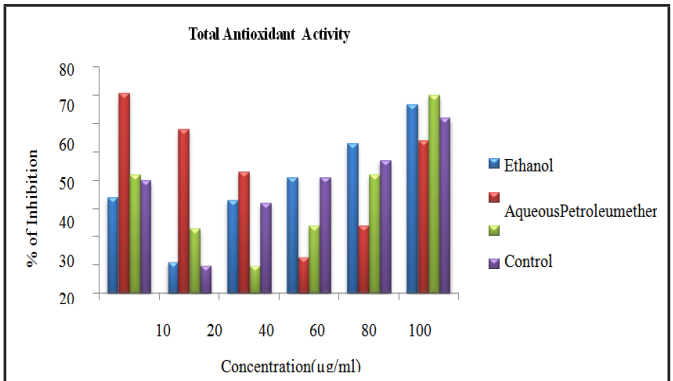
FerricReducing AntioxidantPotential (FRAP)of*C. pictus* Leaf Extracts:

The ferrous ion chelatinga bilities of ethanol, aqueous,and-petroleume ther extracts of *C. pictus* leaves are summarized in Table 4.9 and Graph 6. IC50 value of each extract was compared with that of standard, ascorbic acid. Among the extracts,the aqueous extract had a higher Fe^{2+} chelating effect

(12.54 µg/ml) whereas, ethanol and petroleum ether extracts exhibited IC50 41.90 µg/ml and 62.50 µg/ml respectively. The standard, ascorbic acid showed ferrous ion chelating ability of 00IC5014.13 µg/ml.



Graph 4. The FRAP radicals cavenging activity of *C. pictus* leaf extracts



Graph 5. The Total antioxidant activity of *C. pictus* leaf extracts

Total Antioxidant Activity of *C. pictus* Leaf Extracts

The total antioxidant activity of ethanol, aqueous, and petroleum ether extracts of *C. pictus* leaves is shown in Table 4.10

Table 4. The percentage inhibition of DPPH Radical Scavenging activity of *C. pictus* leaf extracts

S.No	Concentration(µg/ml)	%of Inhibition			
		Ethanol	Aqueous	Petroleum ether	Standard
1	10	42	27	50	20
2	20	64	52	51	44
3	40	69	74	67	66
4	60	78	86	78	86
5	80	83	88	79	96
6	100	84	95	80	98
IC50Value(µg/ml)		26.28	22	50	39.66

Table 5. The percent age inhibition of FRAP Radical Scavenging activity of *C. pictus* leaf extracts

S.No	Concentration (µg/ml)	%of Inhibition			
		Aqueous	Ethanol	Petroleumether	Standard (Ascorbicacid)
1	10	52	46	49	68
2	20	26	41	41	41
3	40	19	34	36	20
4	60	12	27	33	15
5	80	5	23	27	3
6	100	1	13	16	1
IC50Value(µg/ml)		12.54	41.90	62.50	14.13

Table 6. The Total antioxidant activity of *C. pictus* leaf extracts

S.No	Concentration(µg/ml)	Aqueous	Ethanol	Petroleumether	Standard (Ascorbicacid)
1	10	34	71	42	40
2	20	11	58	23	10
3	40	33	43	10	32
4	60	41	13	24	41
5	80	53	24	42	47
6	10034	67	54	70	62
IC50Value (µg/ml)		72.5	31.89	92.84	44.31

and Graph 7. The IC50 value was determined for each extract and the standard. The aqueous extract showed total antioxidant activity of IC50 31.89 µg/ml, whereas ethanol and petroleum ether extracts showed IC50 72.75 µg/ml and 92.84 µg/ml,respectively.The antioxidant capacity of Quercetin(44.31µg/ml) has been used as the reference standard.

CONCLUSION

Due to their biological and medicinal activities, herbal medicines and plant products play an important role in the healthcare systems of the remaining 20%, who reside in developedcountries (4). Bioactive compounds or phytochemicals are non-nutritive Chemicals naturally occurring in plants that have defense mechanisms and protect from various ailments (5) and are produced during the normal metabolic processes. The present research evaluates and screens for preliminary phytochemicals of secondary metabolites in *C. pictus* leaf extracts, such as alkaloids, flavonoids, saponins, tannins, and phenolic compounds (6). To evaluate the antimicrobial activity using microorganisms such as *S. aureus, E. coli, S. typhi,* and *B. subtilis,* and the antioxidant activity using the DPPH method of radical scavenging activity of the leaves extract.

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