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IN VITRO EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF AN INDIAN MEDICINAL PLANT: *CELASTRUS PANICULATUS* WILLD

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ABSTRACT:
Aim: To evaluate the antibacterial efficacy, the presence of different phytoconstituents of the leaf extracts of Indian plant *Celastrus paniculatus* against 6 antibiotic-resistant bacteria along with the evaluation of their antioxidant potentiality.
Methods: The leaf extracts were prepared using 4 solvents, water, methanol, n-butanol, and acetone. The antibacterial property was evaluated using agar well diffusion and micro-broth dilution method. Qualitative phytochemical analysis was performed using standard protocols for each solvent extract to check for the presence of the secondary metabolites and phytochemicals which attributes to the antibacterial effectivity of *C. paniculatus*. Further, the antioxidant potentiality of all the 4 leaf-solvent-extracts was estimated using the DPPH method.
Results: The n-butanol leaf extract exhibited the highest antibacterial and antioxidant activity, followed by acetone, methanol extracts. The aqueous extracts exhibited negligible antibacterial activity.
Conclusion: This study validated the antibacterial properties of *C. paniculatus* leaf extracts against both gram-positive and negative bacteria and the phytochemical analysis revealed the presence of many secondary importance which can be attributed to its therapeutic properties. Hence, this plant can be further used as a complementary or alternative choice of drug for combatting multidrug-resistant bacterial pathogens.
Keywords: Antibiotic resistance; Medicinal plants; Celastrus Paniculatus; Antibacterial activity, Phytochemical Analysis; Antioxidant activity

Introduction

Antibiotic-resistant bacteria (ARB) are a major threat to the contemporary medical treatment regimen as they cripple the major surgical and infection control system. The spread of this ARB across all sectors of the society is a serious threat to global public health and it requires immediate attention from the government and all other major stakeholders.[1] Antibiotics are one of the major components of almost all types of surgery and chemotherapy, and without its usage, the treatment procedures are almost ineffective. ARB infections lead to prolonged hospitalization, increased treatment-cost, additional diagnostic tests with increased mortality and morbidity rates all over the globe. Commensals, *Staphylococcus aureus,* and *Escherichia coli* have epitomized the upgrade of genetic makeup deeply, in causing utmost clinical consternations, as *S. aureus/* MRSA is the superbug in the health domain. Multidrug-resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter baumanii* are causing untreatable urinary tract infections.[2] Today, the torrent of MDR pathogens has been recorded to get circulated in communities and escalate to hospitals, causing damnedest shenanigans of 'infection dynamics'.[3]

Since their control cannot be forsaken solely on antibiotics in antimicrobial stewardship, a new range of effective drugs are required to combat the drug/antibiotic resistance problem. Across the world, and especially in India, plants have been used as drugs in various forms and documented in several ancient literatures.[4] For example, the Ayurveda is an age-old medicinal system practiced in India from 200 BC, where various plant parts are used in drugs, solely or in a combination of other plants for treating almost all kinds of health ailments.[5] The modern medicine system also is dependent on plants for formulating drugs against various human diseases. Bioactive factors derived from the plants remain the basis for a large proportion of the commercial medication. One such plant which can be used for the control of ABR bacteria is *Celastrus paniculatus* Willd.[6]

C. paniculatus belongs to the family Celastraceae. It is a small to medium-sized woody species that is native to India and also widely distributed across countries like India, Malaysia, Thailand, China, Philippines, Northeastern parts of Australia. Its branches are cylindrical shaped or with slight tapering without substantial furrows or ridges. The young shoots and branches have foliage or branching that weeps creating softness to the plant. Leaves do not contain hairs, they are broadest below the middle and roughly twice as long as it is wide (ovate) and shows a pointed or tapering (acuminate) shape.[7,8] The flowers are yellowish-green in colour, borne in terminal and it possesses either stamens or carpels (unisex) but not both and is annually flowering. Fruits are capsule-globose shaped, 3-valved, 3-celled, 3-6 seeded. Seeds are ovoid or egg-shaped and brown in colour, which contains a reddish specialized outgrowth that completely covers it. Several parts of this plant such as roots, bark, leaves, and seeds are used for the treatment of many diseases and disorders. *C. paniculatus* is rich in therapeutic properties and it has been used for treating arthritis, asthma, beriberi, bronchitis, cancer, body pain, abdominal disorders, cardiac debility, and the plant also acts as an aphrodisiac and shows excellent brain tonic activities.[7-9]

In this perspective, the Indian ethnomedicinal plant *C. paniculatus is* evaluated for detailed phytochemical analyses involving chromatography of active principles. Active principles from plant extracts would help developing herbal preparations for uses complementary drugs for use along with regular antibiotics and antimicrobial stewardship program indented for empiric therapy as well as addressing instantaneous MDR bacterial infections. The current study evaluates the antibacterial efficacy of 4 different leaf solvent extracts of *C. paniculatus* against six ABR bacteria which were directly isolated from clinical samples. Apart from antibacterial efficacy, determination of the most lethal concertation of each extract was done in form minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Furthermore, qualitative phytochemical analysis was performed using standard protocols for each solvent extract to check for the presence of the secondary metabolites and phytochemicals which attributes to the antibacterial effectivity of *C. paniculatus*. Moreover, the antioxidant activity of all the leaf extracts was also evaluated. It is anticipated that this study should help in distinguishing the plant for further use in harnessing antibacterial for these antibiotic-resistant bacteria.

Materials & Methods

Collection and Processing plant material: Fresh young leaves of *C. paniculatus* plant (Figure 1) were collected from the Gandhamardhan hill range of Nurshinghanath, Odisha, in August 2019 and it was identified by its morphological features by consulting a local botanist and taxonomy expert. The fresh leaves were washed properly with pyrogen-free water and were shed dried and powdered in a mixer grinder. Further, the powdered leaves were stored in airtight polybags for future use.

Figure 1: *Celastrus paniculatus* Willd

Extraction: A mass of 15-gram powdered leaf samples was taken and extraction was carried out with the help of a Soxhlet apparatus by using different solvents. Four different solvents used for the extraction process, which were, n-butanol, acetone, methanol, and water respectively. After the extraction, solvent evaporation was done by using a rotary evaporator, where the solvent and extract got separated resulting in the yield of pure extract. Further, the final crude extracts were collected in a glass airtight vials at 4°C, till further use.

Test Micro-organisms: Antibacterial activity was tested against 6 strains of antibiotic-resistant bacteria of which 3 were Gram-positive (*Streptococcus pyogenes, Enterococcus faecalis, Staphylococcus aureus*) and rest 3 were Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa,* and *Acinetobacter baumanii*). The bacterial strains were obtained from the "Department of Microbiology, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Odisha, India". All the bacterial strains were antibiotic-resistant and were identified by using standard microbiological and biochemical tests.

Antibacterial efficacy test of plant extracts: Antibacterial potentiality of plant extracts against the 6 ABR bacterial pathogen was tested by the "agar-well diffusion method" where piperacillin-tazobactam 30 mg/mL as the standard positive control and 10% DMSO as a negative control, as previously detailed.[10] The zones of inhibition (ZI) formed by each extract against the 6 test bacteria were measured. The size of the ZI is always directly proportional to the antibacterial activity of the extract. The experiment was repeated thrice for confirmation and the results were recorded.

Determinations of MIC and MBC of plant extracts The MIC and MBC of the all the 4 solvent extracts were

determined by micro-broth dilution as described previously. [11]

Qualitative screening of the phytochemical constituents: The qualitative screening tests were performed for selected 4 solvent extracts used tests for anthraquinones, steroids/terpenes, flavonoids, carbohydrates, saponins, glycosides alkaloids, and resins, explained elsewhere.[11]

***In vitro* Antioxidant Assay**

The *in vitro* antioxidant properties of the 4 solvent leaf extracts from *C. paniculatus* were determined by using the DPPH method. One ml of DPPH solution in methanol (0.1 mMol/L^{-1}) was mixed with 3.0 ml of leaf extract in several concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml) and the mixture was incubated for 30 min at room temperature in dark and the absorbance was recorded at 517 nm using a UV -Visible spectrophotometer. Ascorbic acid was used as the standard to plot the standard curve. The antioxidant potentiality of these extracts were recorded as IC_{50}. The IC_{50} value is the concentration (in µg/ml) of extract that inhibits the formation of DPPH radicals by 50.[12]

Results

Determination of antibacterial activity (zone of inhibition), MIC and MBC values of different extracts of *C. paniculatus* leaves

The *in vitro* antibacterial potentiality of *C. paniculatus* leaves extracts was determined by the presence or absence of a zone of inhibition (Table 1, Figure 2a, 2b). Among the 4 extracts, n-butanol had significant antibacterial activity against 6 bacteria and formed clear ZI followed by acetone and methanol extract while the aqueous extract exhibited negligible antibacterial activity. N-butanol extract had the maximum ZI against *S. pyogenes* (19 mm) and the minimum against *A. baumanii* (13 mm). Similarly, the zone of inhibitions of the 4 solvent extracts against each of the 6 bacterial pathogens were recorded (Table 1). In general, the comparative analysis of the ZI suggests the gram-positive bacterial pathogens were more susceptible to leaf extracts than the gram-negative bacteria (Graph 1).

N-butanol extract had the minimum MIC value of 3.175 mg/ml against *S. pyogenes* whereas the maximum value of 25 mg/ml against *E. faecalis and A. baumanii.* Similarly, it had the minimum MBC value of 12.5 mg/ml against *S. pyogenes* whereas the maximum value of 50 mg/ml against *E. faecalis and A. baumanii.* Likewise, the MIC and MBC values of the acetone and methanol extracts were recorded (Table 1, Figure 3). The MIC and MBC values of aqueous extracts were not recorded as they had insignificant antibacterial activity. Further, the MIC and MBC values of positive control piperacillin-tazobactam (25 µg/ml) were also recorded for comparison (Table 1). The comparative graph of MIC and MBC values suggests, the n-butanol extract had maximum antibacterial activity and establishes the results exhibited in the agar-well diffusion method (Graph 2 & 3).

Table 1: Zone of inhibition (mm), MIC and MBC (mg/ml) values of different extracts of *C. paniculatus* leaves

Bacteria		n-Butanol	Acetone	Methanol	Aqueous	P&T
E. coli	**AWD**	15	13	11	0	**17**
	MIC	6.25	**25**	**25**	-	6.25
	MBC	25	**50**	**50**		25
S. aureus	**AWD**	**18**	16	17	4	**18**
	MIC	6.25	**12.5**	6.25		3.172
	MBC	**25**	**25**	**25**		12.5
E. faecalis	**AWD**	16	14	15	5	**17**
	MIC	**25**	**25**	6.25		3.175
	MBC	**50**	**50**	25		12.5
P. aeruginosa	**AWD**	**17**	15	12	0	**17**
	MIC	12.5	**25**	12.5		6.25
	MBC	25	**50**	25		25
A. baumanii	**AWD**	13	11	10	0	16
	MIC	**25**	**25**	**25**		6.25
	MBC	**50**	**50**	**50**		25
S. pyogenes	**AWD**	**19**	17	15	4	16
	MIC	3.175	**6.25**	**6.25**		3.175
	MBC	12.5	**25**	**25**		12.5

Note: AWD-Agar Well Diffusion (in mm); MIC- Minimum Inhibition Concentration (in mg/ml); MBC- Minimum Bactericidal Concentration (in mg/ml)

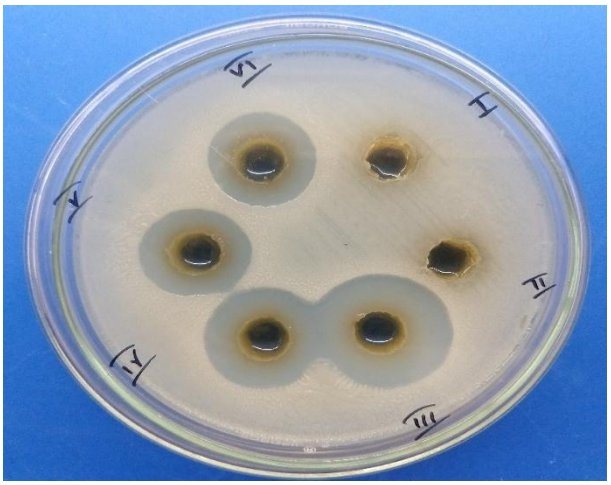


Figure 2a. Zone of inhibition formed by different leaf extracts against *S. pyogenes*

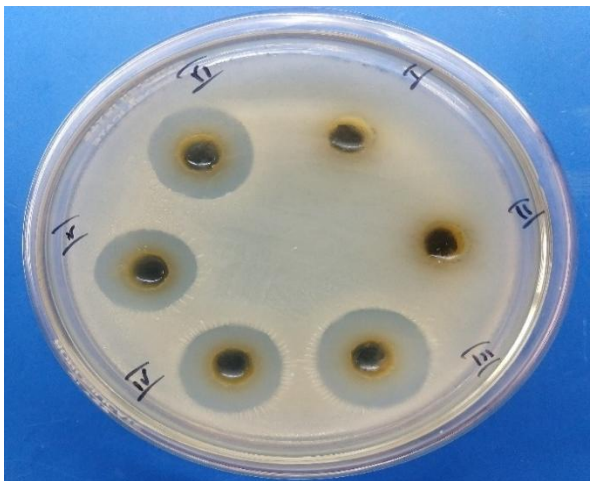
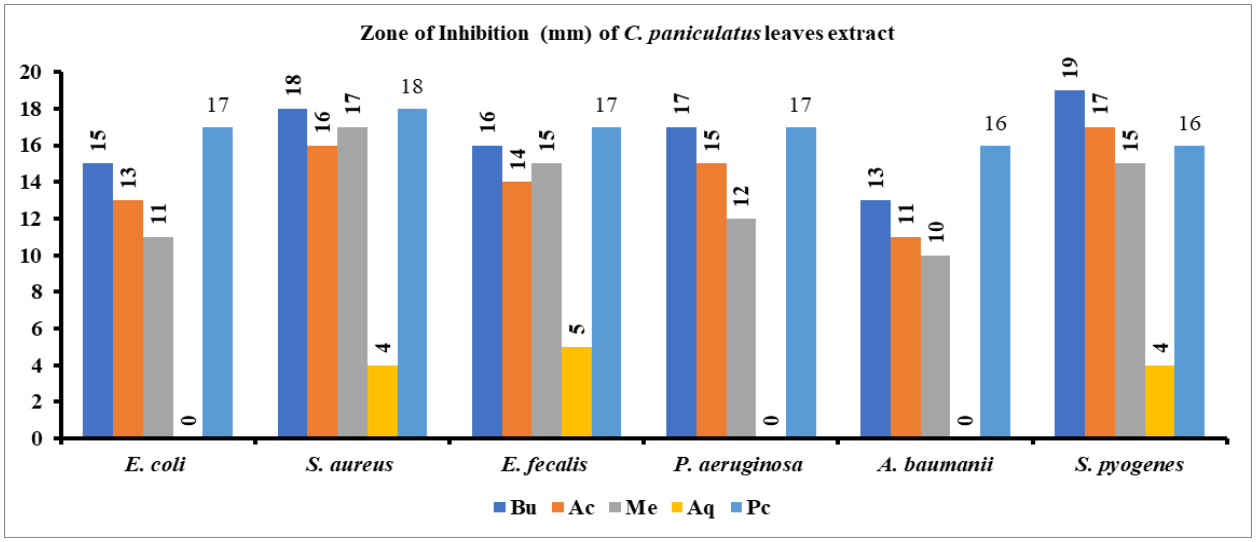


Figure 2a. Zone of inhibition formed by different leaf extracts against *E.faecalis*



Graph 1: Comparative analysis of the zone of inhibition (mm) values of *C. Paniculatus* leaves extract

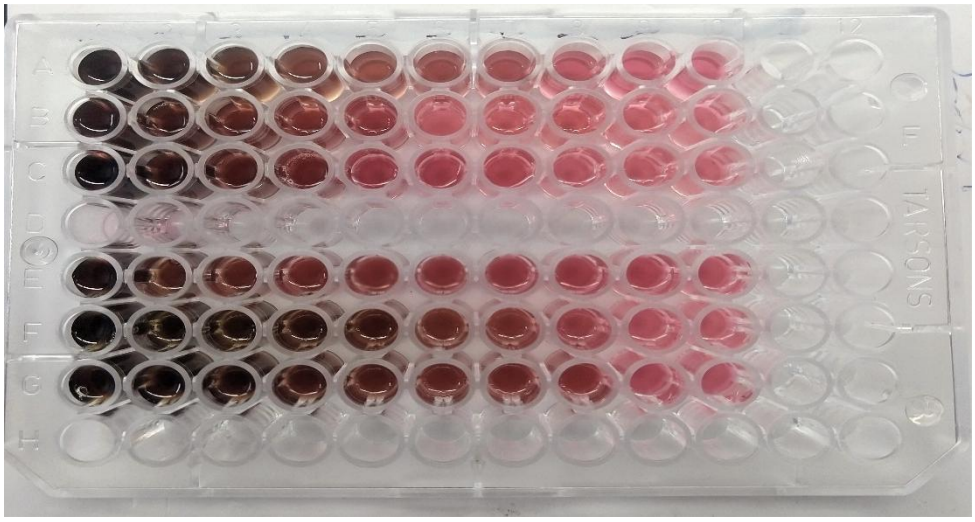
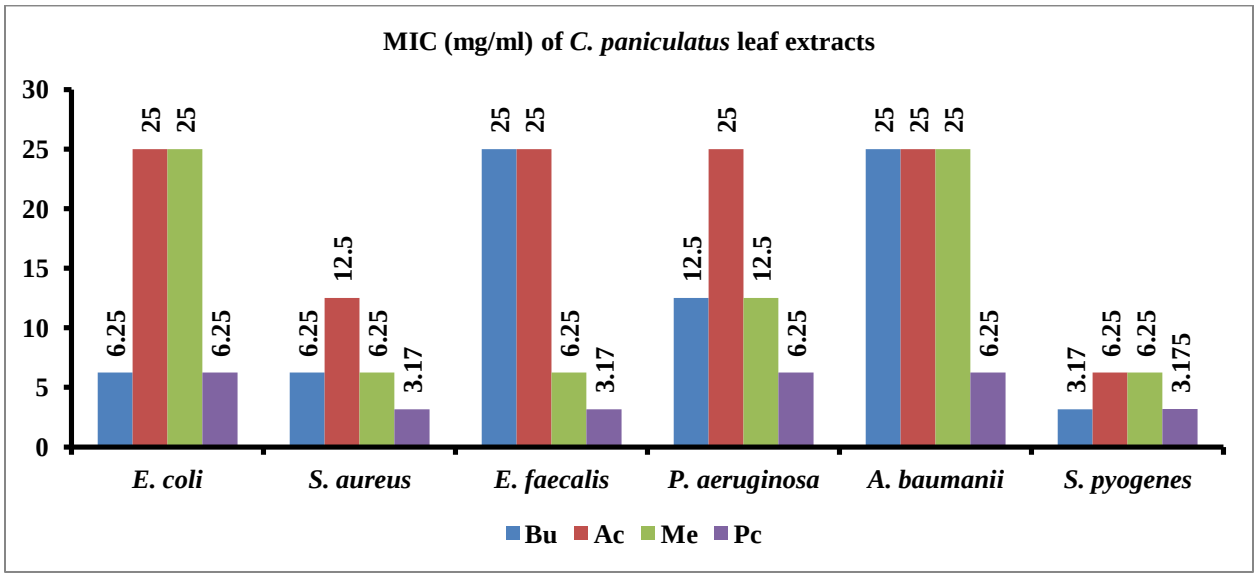
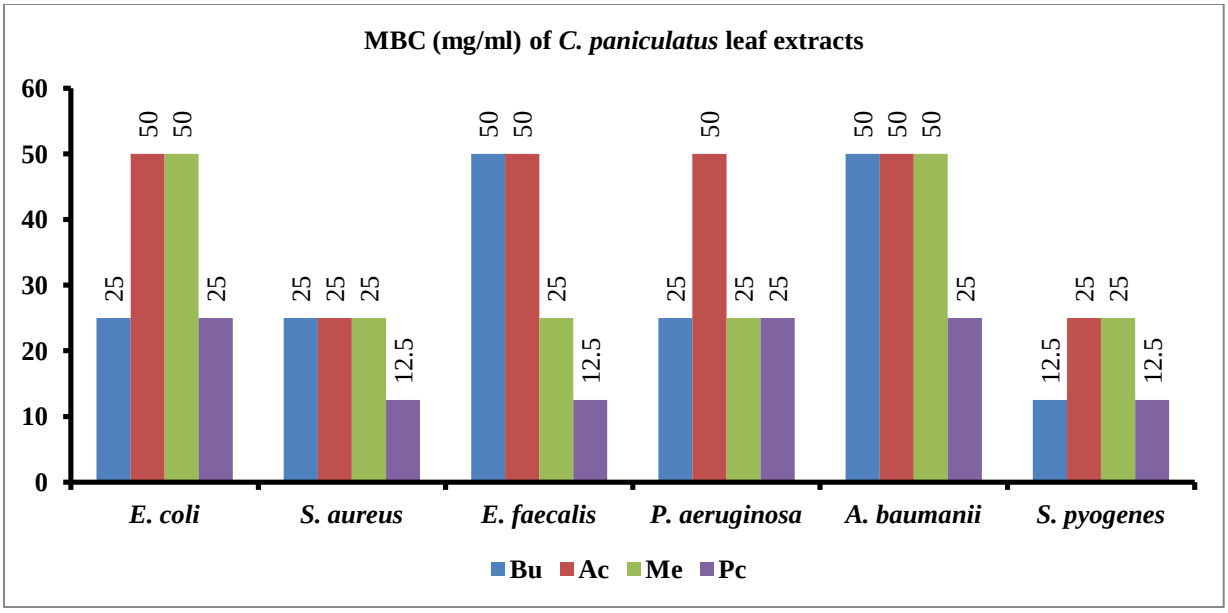


Figure 2: Determination of MIC and MBC values of *C. paniculatus* leaf extracts by micro-broth dilution method.



Graph 2: Comparative analysis of the MIC (mg/ml) values of *C. paniculatus* leaves extract



Graph 3: Comparative analysis of the MBC (mg/ml) values of *C. paniculatus* leaves extract

Qualitative phytochemical analysis

The preliminary phytochemical analysis suggests that n-butanol extract contained the maximum secondary metabolites such as alkaloids, glycosides, terpenoids, carbohydrates, saponins, tannins, flavonoids, steroids and anthraquinones except for resins. This attributes to its excellent antibacterial properties. Similarly, the qualitative phytoconstituents screening of the acetone, methanol and aqueous extracts were recorded (Table 2). The aqueous extracts had the minimum number of secondary metabolites which substantiates the results obtained in the agar well diffusion method,

TABLE 2: Phytochemical analysis of 4 solvent leaf-extracts of *C paniculatus* leaves.

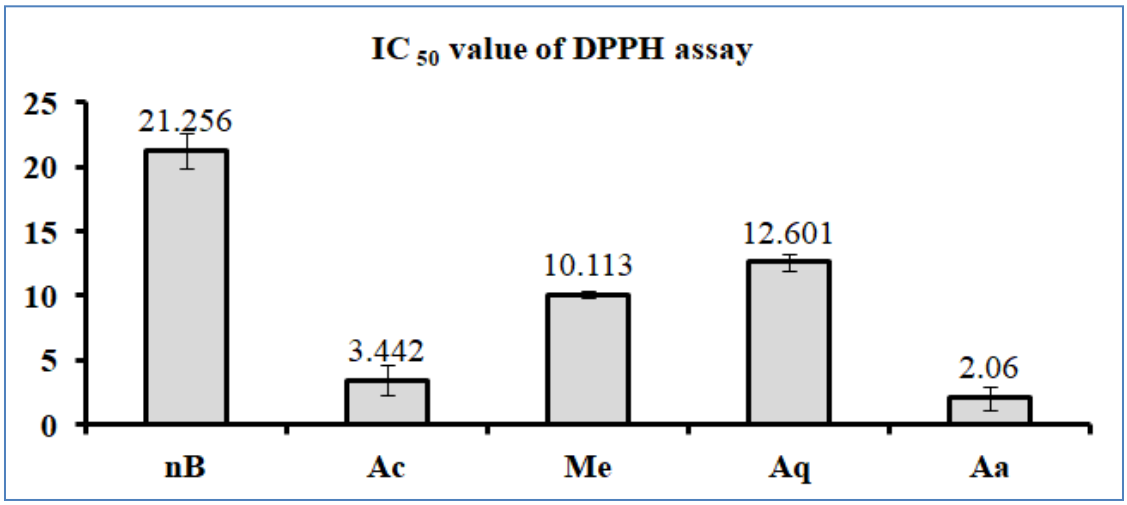
Extracts	Alkaloids	Resins	Glycosides	Terpenoids	Carbo-hydrates	Saponins	Tannins	Flavonoids	Steroids	Anthraquinones
N-butanol	+	—	+	+	+	+	+	+	+	+
Acetone	—	+	+	+	+	+	—	—	+	—
Methanol	+	+	+	—	+	+	+	—	+	+
Aqueous	+	—	—	—	—	—	+	+	+	+

Determination of antioxidant activity.

Leaf extracts of *C. paniculatus* exhibited a concentration-response relationship in DPPH scavenging. The increase in concentration was synchronous with the increase in the scavenging capacity. Lower the IC_{50} value greater of its scavenging activity. As a positive control, Ascorbic acid had the highest scavenging activity with an IC_{50} value of 2.06 ± 0.92 µg/ml. Comparing IC_{50} values of all the extracts, it was recorded, that the acetone extract had more scavenging activity than rest three extracts. The IC_{50} value of the n-butanol extract was 21.256 ± 1.34089. Further, the acetone, methanol and aqueous extracts had the scavenging activity of IC_{50} value 3.442 ± 1.156734, 10.113 ± 0.242192 and 12.601 ± 0.636684 respectively. From the above readings and the graph, it was concluded that the n-butanol extract of *C. paniculatus* leaves had the highest scavenging activity than others (Table 3, Graph 4).

Table 3: Antioxidant activity of *C. paniculatus* leaf extracts in the DPPH method

Extracts/ Standard	IC_{50} value in µg/ml
n-Butanol	21.256 ± 1.34089
Acetone	3.442 ± 1.156734
Methanol	10.113 ± 0.242192
Aqueous	12.601 ± 0.636684
Ascorbic acid	2.06 ± 0.92



Graph 4: IC_{50} value of different extracts of *C. paniculatus* leaf extracts by DPPH method

Discussion

The literature published in the field of ethnobotany indicates that rustics and tribal people traditionally use plant parts and their extracts in their primary health care system.[13-15] Because of poverty, the modern medicinal system is inaccessible to tribal mass in most states in India. Research is needed to use that knowledge for the preparation and formulations of plant drugs, and many such crude formulations are in use by several pharmaceutical companies and their products are found increasingly popular.[17] Such products can cater to the need of rural mass and those can be economically viable too. Further, that knowledge must be exploited by Indian scientists for drug targeting against MDR strains of certain bacterial pathogens; otherwise, several infectious diseases may become increasingly notorious and invincible soon .[14-16]

Plants produce a huge number of phytochemicals or better known as secondary metabolites for their defense.[17] However, the pharma sector has exploited on 10% of the naturally occurring chemical for drug formulation.[17] Some such as terpenes (diterpenes and triterpenes) give plants their odors; others (quinines and tannins) are responsible for plant pigments. Other plant products like flavonoids, alkaloids, phenols and phenolic acids (caffeic and chlorogenic acid) and essential oils (camphor and cineole). These secondary metabolites attributes to the therapeutic properties and as found in our study *C. paniculatus* is rich in secondary metabolites and phytochemicals.[18, 19]

The use of medicinal plants not only restricted to the leaves but in many ancient literature different parts such as roots, seeds, barks, fruits have also been used. In some cases, a combination of different parts and or the whole plant is also used. [20] *C. paniculatus* is one such well-known plant used is a traditional or herbal medicinal system for wound healing, memory enhancement, antiseptic, antioxidant, irregular

menstrual cycle, and other pharmacological agents. The various extracts of Extensive research are being done throughout the world of *C. paniculatus* for its neuro-therapeutic properties. The oil extract from the seeds of *C. paniculatus* known as "Jyotismati oil" is well known for its neuro-regenerative capacity. Hence, this plant is a potential source of novel drugs that can be exploited in the pharmaceutical industries.[21, 22]

Conclusion

N-butanol extract was found to be positive for most of the qualitative phytochemical screening test. It contained almost all the phytochemicals including reducing sugar, proteins, flavonoids, saponins, anthraquinones, and steroids. Methanol and acetone leaf extract were moderately positive, whereas the aqueous extract was found mostly negative. The study concluded that the Indian plant, *C. paniculatus* is rich secondary metabolites and it can be used in the production alternative and complementary drugs, which can be used in treating various human health ailments and combating antibiotic-resistant bacteria.

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