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Comparative investigation on antimicrobial and phytochemical profiling of *Cyclea peltata* and *Tiliocora acuminate*

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ABSTRACT

Antimicrobial drug resistance is a major problem in worldwide, because the resistance microbial pathogens are emerged due to its genetic plasticity. Over usage of inappropriate antibiotics rose as a prominent factor for the development of multidrug resistant organisms. From the ancient time itself, plants are used as a principal source for medicinal agent/ drugs, which are lesser side effect compared to the commercially available synthetic drugs. The usage of traditional medicines and its related medicinal plants are helps to lead a healthy life. Here, we use two medicinal valuable plants, such as *Cyclea peltata* and *Tiliacora acuminate*, to determine the antimicrobial resistance capacity using various solvents. In this paper, water extracts were not revealed that much of inhibition against human pathogens when compared with other solvents, like, *C. peltata* give well determined zone of inhibition against *Bacillus subtilis* by chloroform and *Escherichia coli* while using ethanol, whereas for using *T. acuminata* showed better activity against *E. coli*, *Streptococcus pyogenes* (ethanol) and *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (chloroform).

1. INTRODUCTION

In the universe, plant domain encompasses huge variety of species that produce a diverse amount of active biomolecules with altered chemical structure and nature. From ancient times itself, the usage of medicinal and aromatic plants become a significant part in day today life and also in pharmaceutical world [1]. Now, they are gradually increased in food and cosmetic industry, as well as in the alternative medicines. Medicinal herbs or plants always sustain their property that creates a huge demand in market for the production of valuable drugs or compounds [2]. The relationship between human and their need of drugs is always demandable one in nowadays, because of the emergence and evolution of microbes. Specific usage of natural plant-based drugs for a specific disease or condition is a result of several years of struggles against the microbial pathogens. In this, drugs were prepared using several parts of plant or herb which are barks, seeds, fruits, roots, etc. [3]. In India, nearly 45,000 species of plants under 227 ethnic groups were occupied in various geographical and climatic zones with high traditional knowledge. The modern society people doesn't aware about the beneficiary values of the traditional medicinal system using naturally available herbs [4,5].

Cyclea peltata is belongs to Menispermaceae family which contain some alkaloids against hayatine, hayatinine, bisbenzyliso quinolone and berberines, saponins, etc., thus helps to treat various disease conditions [6,7]. This Menispermaceae family comprises maximum of climbing trees/plants those are available in tropical climatic condition used as a drug, because of its important pharmacological activity. *Cyclea peltata* is a slight twining plant with pilose, stem, and branches. The leaves are simple; they contain small flowers and greenish only on axillary panicles. Their root is irregular, cylindrical, and curved in shape, have grayish brown surface and the cortex are in white starchy [8]. Methanolic and ethanolic extract of *C. peltata* herb gives higher resistance action against several bacterial pathogens, such as *Staphylococcus aureus*, *S. haemolyticus*, *Klebsiella pneumonia*, and *Proteus vulgaris* [9]. *Cyclea peltata* have potential noble source for antibacterial agents and have an ability to treat microbial pathogens, especially isolates from diabetic wounds [10,11].

Around the tropical regions of Asia, nearly 28 species of *C. peltata* were estimated and all it was a climbing shrubs. Approximately,

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seven species of *C. peltata* are found in India, it has wide spectrum of medicinal properties, such as, antioxidant and antiulcer activity [12]. Activity and diuretic [13] that compound was extracted from the root. Type II diabetic activity was also identified using the aqueous extract of *C. peltata* root [14]. The nutritional and toxicological level of the *C. peltata* plant was confirmed the presence of bioactive molecules for various disease and condition [15].

Based on the traditional usage of *Tiliacora acuminata* is belongs to a Menispermaceae family, the whole plant was also used for various disease conditions and infections. In specific, it was used as an antipyretic, stimulator for cardiovascular and central nervous system alleviates spasms, and also used for casting the snake poisons. The extract of *T. acuminata* is used in some ayurvedic preparations used as an antidote and drug for snakebites [11]. Different solvent extracts of *T. acuminata* act as an antibacterial (*Proteus mirabilis*, *Enterococcus aerogenes*, *Pseudomonas aeruginosa*, *Escherichia coli* and *S. aureus*) and antifungal agent (Fusarium *oxysporum*, Aspergillus *niger*, *Aspergillus terreus*, *Rhizopus oxysporum*, and Candida *albicans*) [16].

2. MATERIALS AND METHODS

2.1. Collection of Test Sample (Plant material)

The healthy test plant materials of *C. peltata* and *T. acuminata* were collected from the Kolli hills in Tamil Nadu, South India. The test specimen was recognized with the help of local floras and the voucher specimen was deposited at Tamil university, Thanjaur, Tamil Nadu. The test sample material was allowed to shade dried, make a coarse powdered and stored at room temperature in air-tight container for further use.

2.2. Solvent Extraction of Plant Drug

The collected plant materials were allowed to wash with tap water and continue to wash with distilled water that helps to remove the contaminants from the surface of the sample. The finely powdered test material was allowed to the extraction process done with soxhlet. The powdered substance/compound of the test sample were sequentially extracted with two different solvents like chloroform and ethanol. The solvent extract was collected and concentrated to get dried plant extract.

2.3. Aqueous Extraction

Approximately, 100 g of dried powder of test sample was extracted using distilled water for 6 hours at low heat. Two hours between the extractions were filtered with the help of eight layers of muslin cloth and allowed to centrifuge at 5,000 rpm for 15 minutes. Finally, the supernatant was collected and the process was repeated twice. After 6 hours of process, the supernatant was concentrated to make the dried powder of plant extract. The percentage of extractive values was calculated by using the following formula.

$$\text{Percent Extract} = \frac{\text{Weight of dried extract}}{\text{Weight of dried material}}$$

2.4. Preliminary Phytochemicals Screening

For preliminary phytochemical screening, the extract of the whole plant was allowed to determine the various qualitative chemical tests that assist to analyze the presence of various phyto-constituents, such as glycosides, tannins, phytosterols, proteins, amino acids, flavonoids, and saponins [17,18].

2.5. Physicochemical Evaluation

The whole plant powder of *C. peltata* and *T. acuminata* was subjected to evaluate values of total ash content, acid insoluble ash, water soluble extract, alcohol soluble extract, and moisture content [19,20].

2.6. Microbiological Screening

Antimicrobial activities of different solvent extracts were analyzed by using disc diffusion method [21], later modified by Olurinola [22] and minimum inhibitory concentration (MIC) value was also calculated [23].

2.7. Disc Diffusion Method Assay

Disc diffusion method was used to test the antibacterial activity of the various solvent extracts of test plants against several bacteria. The leaf extracts were allowed to use to study the antibacterial activity. A loopful of bacterial pathogens was inoculated into 5 ml of nutrient broth medium. Incubate the culture plates for 24 hours at 37°C to get active strain.

Nutrient agar culture plates were prepared by pouring 15–20 ml of molten agar media into the sterile petriplates. After solidification of agar media, bacterial inoculums of MTCC strains, such as *E. coli* (MTCC 443), *S. aureus* (MTCC 3160), *P. aeruginosa* (MTCC 424), *Bacillus subtilis* (MTCC 441), *K. pneumonia* (MTCC 3384), and *Streptococcus pyogenes* (MTCC 442) were spread uniformly over the surface of the media and the inoculums was allowed to dry for 5–8 minutes. Then, the different concentrations of various solvent extracts (25 , 50 , 75, and 100 mg) were loaded on 10 mm sterile disc that was placed on the surface of media. The test extract/ compound were allowed to diffuse through the media for 5 minutes and the plates were allowed for incubation at 37°C for 24 hours.

At the end of incubation period, zones were formed around the disc and that was measured with transparent ruler scale in millimeter. Based on the zone diameter the inhibitory action and antibacterial susceptibility was ranked [24]. Inhibitory zones were measured and compared with the standards. Activity index of each solvent extracts was calculated and referred with European Committee on Antimicrobial Susceptibility test [25].

$$\text{Activity Index} = \frac{\text{Inhibition zone of sample}}{\text{Inhibition zone of the standard}}$$

2.8. Minimum Inhibitory Concentration (MIC)

The MIC value was determined by using micro dilution method done through serially diluted various solvent extracts of plant

sample. The extracts were diluted into altered concentrations of 0.125–8 mg/ml correspondingly with DMSO solution. Eventually, each tubes were filled with 1 ml of sterile nutrient broth medium and allowed inoculated with 0.1 ml of test organism (inoculums contains $1–2\times10^{-7}$ CFU/ml).

All the inoculated tubes were incubated aerobically at 37ºC for 24 hours, whereas the control tubes were maintained for each test. Inhibition of bacterial growth was observed in test tubes (No turbidity), which have the lowermost or minimum concentration of the test sample extract. This least concentration was measured as MIC [26].

2.9. Total Activity (TA) Determination

TA is the volume at which the test plant extract can be diluted with the potency to eradicate the growth of microorganisms. This was calculated by dividing the quantity of plant extract from 1 g plant material by the value of MIC of the same extract or compound isolated and that is expressed in ml/g [27].

Total Activity = Extract per gram dried plant part/MIC of extract

2.10. Statistical Analysis

Mean value and the standard deviation of the test were calculated for each test pathogens [28].

3. RESULTS AND DISCUSSION

The percentage yield and colour of the certain sequential extracts were mentioned in Table 1. About 42.98 ± 0.03% and 11.14 ± 0.1% yield was obtained from water extract of *T. acuminata* and *C. peltata*, respectively, and shows yellowish green colour, followed by ethanol extract and chloroform extract yield was 7.63 ± 0.29%, 2.78 ± 0.1% and 3.45 ± 0.13% and 2.42 ± 0.2%, respectively, and which was dark green. The extraction procedure should have efficiency to dissolve endogenous compounds that present in the plant sample for the further studies, thus showed in the physiochemical analysis depicted in Table 2. A recent study also supported the physiochemical properties of *C. peltata*, which also described the total ash, soluble and insoluble value of ash [29,30].

3.1. Physico-chemical Analysis

The results of Physico-chemical analysis is given in table 2.

3.2. Phytochemical Studies

3.2.1. Qualitative analysis

Examining the phytochemical compounds in medicinal test plants provides a bunch of traditional knowledge with insight to know how plants are medicinally effective and to understanding the chemical composition of leads to the development of new medicines. The phytochemical substances are identified by preliminary phytochemical analysis in aqueous extract and extracts obtained using organic solvent extract like ethanol and chloroform (Tables 3 and 4). According to the solubility of phyto-compounds present in the plant sample, the presence and absence of phyto-constituents were indicated.

Table 1: Colour and percentage yield of extract from *C. peltata* and *T. acuminata* using different solvent.

Solvent	Colour	Values % (w/w)	
		C. peltata	*T. acuminata*
Water extract	Yellowish green	2.78 ± 0.1	7.63 ± 0.29
Ethanol extract	Dark green	2.42 ± 0.2	3.45 ± 0.13
Chloroform extract	Dark green	11.14 ± 0.1	42.98 ± 0.03

Table 2. Physicochemical parameters of *C. peltata & T. acuminate.*

Ash values	Values % (w/w)	
	C. peltata	*T. acuminata*
Total ash	3.84 ± 0.1	5.87 ± 0.33
Acid insoluble ash	3.04 ± 0.2	4.89 ± 0.2
Water soluble ash	2.55 ±0.2	3.18 ± 0.13
Loss on drying	8.88 ± 0.3	9.87 ± 0.15
Alcohol soluble extractive value	5.47 ± 0.2	7.63 ± 0.29
Aqueous extractive value	11.14 ± 0.1	42.98 ± 0.03

Table 3. Preliminary phytochemical screening of *C. peltata* and *T. acuminata* (Lam.).

Phytochemical test	*C. peltata*			*T. acuminata*		
	Solvents used					
	Water	Ethanol	Chloroform	Water	Ethanol	Chloroform
Alkaloid	+	+	+	–	+	–
Flavonoid	–	-	+	+	–	+
Steroid	+	-	-	–	+	+
Cardiac Glycoside	–	+	-	–	–	–
Terpenoid	–	+	-	–	–	–
Triterpenoid and Steroid	–	-	-	–	–	–
Phenol	–	+	+	+	+	–
Tannin	+	+	–	+	+	–
Saponin	–	+	–	+	-	–
Phlobatannin	–	–	–	–	–	–
Reducing sugar	–	–	–	+	+	–
Anthroquinone	–	–	–	–	–	–
Gum and Mucilage	–	–	–	+	+	+

(+) – Present and (−) – Absent.

Table 4. Quantitative phytochemical analysis of *C. peltata* and *T. acuminata* (Lam.).

Extracts	*C. peltata* (mg/ g)			*T. acuminata* (mg/ g)		
	Flavonoid	Phenol	Tannin	Flavonoid	Phenols	Tannin
Water	125 ± 0.637	40 ± 1.037	18 ± 1.354	75 ± 0.13	40 ± 0.431	27 ± 0.19
Ethanol	540 ± 1.354	46.4 ± 1.108	17 ± 0.637	245 ± 0.26	56 ± 1.09	39 ± 0.15
Chloroform	455 ± 0.25	23 ± 1.678	19 ± 0.245	160 ± 0.09	72.8 ± 0.45	8 ± 0.637

Values are expressed as mean ± SD, $n = 3$.

3.2.2. Quantitative analysis

Quantitative phytochemical investigation was done for total phenol, flavonoid, and tannin content availability in the test plant sample, which were responsible for the major pharmacological activity to treat several disease conditions. For the quantification analysis test, the plant extract consuming strong positive for

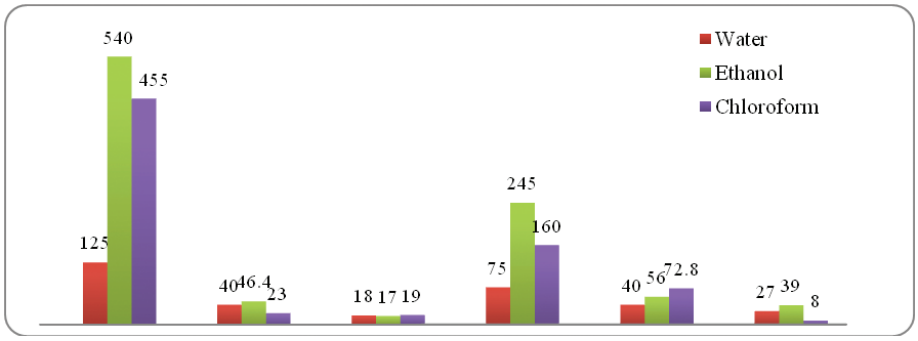

Figure 1: Quantitative phytochemical analysis of *C. peltata* and *T. acuminata* (Lam.).

Table 5. Screening of MIC (mg/ml) performance of different extracts of *C. peltata and T. acuminata* (Lam.) against pathogenic organisms.

Test microbes	*C. peltata*			*T. acuminata*		
	Solvents used					
	Water	Ethanol	Chloroform	Water	Ethanol	Chloroform
B. subtilis	–	0.200 ± 0.027	0.250 ± 0.007	–	0.500 ± 0.013	0.500 ± 0.009
S. aureus	–	–	–	–	0.125 ± 0.011	0.250 ± 0.005
S. pyogenes	–	0.500 ± 0.018	0.500 ± 0.014	–	0.500 ± 0.025	0.125 ± 0.007
E. coli	–	1.250 ± 0.024	1.125 ± 0.005	–	0.250 ± 0.012	0.250 ± 0.017
P. aeruginosa	–	0.125 ± 0.017	1.000 ± 0.024	–	0.500 ± 0.027	0.250 ± 0.032
K. pneumonia	5 ± 0.013	1.500 ± 0.043	0.250 ± 0.031	3.000 ± 0.024	0.500 ± 0.018	0.250 ± 0.019

Values are stated as mean ± SD, $n = 3$.

Table 6. Screening TA performance of different extracts of *C. peltata* and *T. acuminata* (Lam.) against pathogenic organisms.

Test microbes	*C. peltata* (mm)			*T. acuminata* (mm)		
	Solvents used					
	Water	Ethanol	Chloroform	Water	Ethanol	Chloroform
B. subtilis	–	25.28	43.14	–	17.98	3.568
S. aureus	–	–	–	–	21.792	16.45
S. pyogenes	–	16.54	17.2	–	22.792	11.396
E. coli	–	32.67	17.54	–	45.84	17.78
P. aeruginosa	–	21.14	11.55	–	17.98	16.45
K. pneumonia	19.23	15.4	22.77	38.2	22.792	16.45

phenols, flavonoids, and tannin content were identified. The knowledge of the chemical elements of the given plants sample is desirable because such information will be valuable thing for the synthesis of multifaceted chemical substances. In poly herbal formulation, it was difficult to identify the biomarker and quantify it. Hence, this will helps to characterize the formulation total phenol and flavonoids, and tannins content (Fig. 1). Generally, steroids are extracted from natural sources by the extraction with organic solvents, in which they frequently dissolve in the aqueous solution, which is equally similar to the alcoholic solvents, whereas saponins were curiously stable to heat processing. Their biological activity is not reduced by normal boiling method, whereas the isolation of saponins from test plant material involves extraction of polar solvent after the removal of lipids, with petroleum ether and chloroform [30].

Phytochemical components using ethanolic extracts of *T. acuminata* shows the presence of alkaloid, steroid, phenol, tannins, saponins, reducing sugars, respectively, this results were also supported the same level of phytochemical appearances [29,31]. The presence of alkaloids, saponins, and terpenoids were detected using crude and chloroform extract of *C. peltata* [8] and another study also revealed that the presence of alkaloid, flavonoid, tannin, diterpene, and saponin using petroleum ether and ethanolic extracts of *C. peltata* [32]. Alkaloids, steroids, and flavonoids were extracted from aqueous extract of *C. peltata*; this result was highly supported by David *et al*. [30].

3.2.3. Antibacterial activity

The antimicrobial activity was screened because of the great medicinal of the test plant was highly relevant with the recent

Table 7. Antibacterial action of various extract of *Cyclea peltata* (Lam) against human pathogens.

Test microbes	Values	Solvents													
		Water					Ethanol					Chloroform			
		25 mg	50 mg	75 mg	100 mg	Mean value	25 mg	50 mg	75 mg	100 mg	Mean value	25 mg	50 mg	75 mg	100 mg	Mean value
B. subtilis	IZ ± S.D	–	–	–	–	–	11 ± 0.56	16 ± 0.23	21 ± 0.33	28 ± 0.34	9.95	–	10 ± 0.39	14 ± 0.23	20 ± 0.18	7.09
	AI	–	–	–	–		0.909	1.11	1.909	2.545	E-12	–	0.909	1.066	1.363	E-06
S. aureus	IZ±S.D	–	–	–	–	–	–	–	11 ± 0.24	13 ± 0.48	5.49	–	–	–	12 ± 0.27	0
	AI	–	–	–	–		–	–	0.131	0.8	E-04	–	–	–	1.003	
S. pyogenes	IZ ± S.D	–	10 ± 0.43	10 ± 0.23	11 ± 0.29	1.65	–	12 ± 0.42	14 ± 0.25	15 ± 0.42	7.38	11 ± 0.32	14 ± 0.07	16 ± 0.36	17 ± 0.18	3.66
	AI	–	0.476	0.476	0.6875	E-09	–	0.571	0.875	0.937	E-05	0.491	0.666	0.809	0.894	E-07
E. coli	IZ ± S.D	–	–	11 ± 0.26	12 ± 0.33	0.00137	–	12 ± 0.39	13 ± 0.27	15 ± 0.24	6.17	11 ± 0.09	13 ± 0.19	15 ± 0.25	18 ± 0.23	1.66
	AI	–	–	0.687	0.571		–	0.625	0.901	0.9375	E-07	0.491	0.786	0.943	1.123	E-05
P. aeruginosa	IZ ± S.D	–	–	–	10 ± 0.26	1.08	11 ± 0.19	13 ± 0.08	19 ± 0.23	25 ± 0.65	7.10	–	10 ± 0.25	11 ± 0.34	13 ± 0.14	3.04
	AI	–	–	–	0.416	E-06	0.458	0.556	0.791	1.375	E-10	–	0.416	0.458	0.556	E-08
K. pneumoniae	IZ ± S.D	–	–	10 ± 0.17	12 ± 0.13	6.40	–	11 ± 0.01	14 ± 0.13	16 ± 0.34	1.31	11 ± 0.09	16 ± 0.65	19 ± 0.34	22 ± 0.23	1.45
	AI	–	–	0.714	0.857	E-05	–	0.798	0.913	1.165	E-04	1.025	1.214	1.357	1.571	E-06

IZ: Inhibition zones in mm; S.D: Standard Deviation; AI: Activity Index; -: No zone formation.

Table 8. Antibacterial action of various extracts of *Tiliacora acuminata* (Lam) against human pathogens.

Test microbes	Values	Solvents													
		Water					Ethanol					Chloroform			
		25 mg	50 mg	75 mg	100 mg	Mean value	25 mg	50 mg	75 mg	100 mg	Mean value	25 mg	50 mg	75 mg	100 mg	Mean value
B. subtilis	IZ ± S.D	–	–	10 ± 0.13	12 ± 0.26	0.00276	–	12 ± 0.23	14 ± 0.34	18 ± 0.56	3.68	–	10 ± 0.06	12 ± 0.48	14 ± 0.24	2.07
	AI	–	–	0.909	1.090		–	1.090	1.272	1.727	E-06	–	0.909	1.090	1.272	E-04
S. aureus	IZ ± S.D	–	–	–	11 ± 0.21	0.00228	–	14 ± 0.24	16 ± 0.06	22 ± 0.24	1.12	13 ± 0.23	15 ± 0.32	16 ± 0.31	17 ± 0.03	5.30
	AI	–	–	–	0.987		–	0.933	1.066	1.466	E-04	0.786	0.989	1.066	1.234	E-06
S. pyogenes	IZ ± S.D	–	–	–	13 ± 0.24	8.44	10 ± 0.29	12 ± 0.23	14 ± 0.42	21 ± 0.29	6.90	–	12 ± 0.07	14 ± 0.29	16 ± 0.18	1.01
	AI	–	–	–	0.812	E-04	0.476	0.571	0.666	1.005	E-10	-	0.571	0.666	0.793	E-04
E coli	IZ ± S.D	–	–	10± 0.42	12± 0.1	0.00357	10± 0.27	12± 0.24	16± 0.09	24± 0.23	3.24	13± 0.27	14± 0.25	16± 0.65	18± 0.09	2.29
	AI	–	–	0.712	0.940		0.656	0.75	1.09	1.562	E-08	0.812	0.583	1.09	1.125	E-05
P. aeruginosa	IZ ± S.D	–	–	10± 0.23	11± 0.12	8.52	–	10± 0.13	14± 0.25	17± 0.08	8.55	12± 0.12	14± 0.25	15± 0.19	17± 0.08	4.19
	AI	–	–	0.416	0.458	E-07	–	0.416	0.583	0.708	E-09	0.5	0.583	0.625	0.708	E-08
K. pneumoniae	IZ ± S.D	–	–	11 ± 0.27	13 ± 0.38	0.027	10 ± 0.23	13 ± 0.24	16 ± 0.25	21 ± 0.24	3.14	13 ± 0.25	15 ± 0.14	16 ± 0.27	17± 0.09	1.86
	AI	–	–	0.5	0.928		0.717	0.928	1.142	1.428	E-09	0.928	1.015	1.142	1.214	E-05

IZ: Inhibition zones in mm; S.D: Standard Deviation; AI: Activity Index; -: No zone formation.

years analysis. An infection was also increased to the great extent and resistant against antibiotics, becomes an ever aggregation of therapeutic problem. The results exposed that the variability in inhibitory concentrations of each extract against a given bacteria. The inhibition rate of bacterial growth was highly proportional to dose dependent since the inhibitory action of the extract was found to increase with an increase in concentration against all bacterial strains as evidenced by the higher zone of inhibitions at higher concentrations of different solvent extracts. Antimicrobial activity (assessed in terms of inhibition zone, TA and activity index) of the crude extracts, tested against selected microorganisms were recorded (Tables 5–8).

Streptomycin used as a standard antibiotic at the concentration of 30 µg/disc exhibited higher diameters of inhibition than other extracts. Totally, three extract of different concentrations of the selected plants were tested to analyze the bioactivity. Alcoholic extracts exhibited substantial antimicrobial potential against test microbes than chloroform and water extracts. Most pathogenic organism are used in the investigation was *S. aureus* against which the plant extracts showed better *C. peltata* exposed extreme zone of inhibition against *B. subtilis* (chloroform) and *E. coli* (ethanol), whereas for using *T. acuminata* showed better activity against *E. coli*, *S. pyogenes* (ethanol) and *Klebsiella pneumoniae*, *P. aeruginosa*, and *S. aureus* (chloroform) (Table 6).

3.3. Minimal Inhibitory Concentration

The minimum inhibitory concentration (mg/ml) values of different extracts of *C. peltata* and *T. acuminata* (Lam.) against pathogenic organisms are depicted in table 5.

3.4. Total Activity

In the present examination, *in vitro* antibacterial ability of the crude aqueous, ethanol, and chloroform extracts of *C. peltata* (Lam.) against pathogenic organisms showed better activity (zone of inhibition) using various concentration level. The *C. peltata* ethanolic showed maximum effect on bacterial pathogens about (32 mm—*E. coli*; 25 mm—*B. subtilis*) and chloroform (43—*B. subtilis*; 17—*S. pyogenes* and *E. coli*) (Table 6). This inhibitory action of *C. peltata* extract result was also supported by several studies [9,10,33]. Methanol and ethanol extract of *T. acuminata* showed significant inhibitory action against both gram positive and negative bacteria, when comparing with hexane and ethyl acetate extracts. Nearly, 14 and 16 mm of zone of inhibition was observed using 600 and 1,200 µg/100 µl of methanol extract of *T. acuminata* extracts against *K. pneumoniae* and *P. aeruginosa* [34,35]. This result was highly supported our study results, *E. coli*, *K. pneumonia*, and *S. pyogenes* was inhibited by the ethanolic extract of *T. acuminate*, respectively (Tables 7 and 8). The minimum inhibitory concentration is an inhibition using lower concentration; therefore, there is a slight inhibitory difference identified using disk diffusion method.

Steroids in *C. peltata* could be highly responsible for the antimicrobial activity against *S. aureus*, Bacillus *Cereus*, and *E. coli*. Equally, the presence of steroid and amino acid in *C. peltata* could accountable to its higher antimicrobial activity showed against *E. coli*. *Tiliacora acuminata* exhibited a lesser antimicrobial activity against *S. aureus*. However, some studies established that the methanol extract of *C. peltata* is extremely active against *S. aureus*, *Staphylococcus epidermidis*, and *B. subtilis*. Calendula *officinalis* was the species that showed the maximum variability of secondary metabolites and it also exposed antimicrobial activity against all the bacterial pathogens studied [36].

4. CONCLUSION

This study is helps to known about the phytochemical constituents in *C. peltata* and *T. acuminata* plants. Here, we quantify the phytochemistry of both plants and its inhibitory action against bacterial pathogens. All the extracts from the test plant sample indicated variable amounts of antimicrobial activity on the microorganisms tested and these plants were more effective than traditional antibiotics which we used to combat the pathogenic microorganisms studied.

CONFLICT OF INTEREST

The authors declare that they do not have any conflicts of interest.

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