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Efficacy of Phytochemical and Antibacterial activity of *Corallocarpus epigaeus* Hook.f.

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Abstract: Qualitative and quantitative phytochemical analysis and antibacterial activity of hexane, petroleum ether, chloroform, acetone and methanol extracts of *Corallocarpus epigaeus* leaf, stem and tuber against various pathogens such as *Staphylococcus aureus, Escherichia coli*, *Klebsiella pneumoniae, Serratia marcescens* and *Pseudomonas aeruginosa* by disc diffusion method were analyzed in the present study. Phytochemical analysis recorded positive results for alkaloids, flavonoids, phenols, tannins, steroids, saponins, glycosides and terpenoids. Among the various extracts, methanol extracts of the investigated plant parts of *Corallocarpus epigaeus* were found to more effective against all the tested pathogens. The results of these studies revealed most valuable information and also support the continued sustainable use of these plants in traditional systems of medicine.

Key words: *Corallocarpus epigaeus,* antibacterial activity, phytochemical analysis.

Introduction

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in India. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency[1].

The use of plant extracts and phytochemicals both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency[2, 3, 4, 5, 6].

Considering this, an attempt has been made to investigate the qualitative and quantitative phytochemical analysis and antimicrobial activities of hexane, petroleum ether, chloroform, acetone and methanol extracts from leaf, stem and tuber of *Corallocarpus epigaeus. Corallocarpus epigaeus* (Cucurbitaceae) used in the treatment of chronic rheumatism, snake bite[7, 8]; asthma[9], dysentery and syphilitic disorders[10]. This study will also hopefully express new frontiers by improving the current

applications of this plant and provides a scientific basis for the traditional claims of their medicinal plant.

Materials and Methods

Plant materials

Different parts like leaf, stem and tuber of *Corallocarpus epigaeus* were collected during Nov 2009-Feb 2010 from Maruthamalai Hills, Coimbatore, Tamilnadu, India. The collected plant materials were identified and their authenticity was confirmed by Mathew[11] and Gambel[12] respectively. The voucher specimens were deposited in the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

Extraction of Plant Material

Various organic solvents were used for the extraction of bioactive compounds. The leaf, stem and tuber powders (10g) of *Corallocarpus epigaeus* were first extracted with petroleum ether for defatting in a Soxhlet apparatus. The defatted powdered sample of *Corallocarpus epigaeus* were dried and successfully extracted with hexane, petroleum ether, chloroform, acetone and methanol in a Soxhlet apparatus. The extracts obtained were completely evaporated by using vacuum rotary evaporator. The concentrated extracts were used for antibacterial activity.

Qualitatitve and Quantitative Analysis

The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures[13, 14, 15]. The flavonoid content was determined according to Jia *et al*,[16]. The steroid content was estimated by the methods of Harborne[13]. The total phenols and tannin contents were estimated by the methods of Sadasivam and Manickam[17].

Tested microorganisms

Antibacterial activity of crude extracts was tested against gram negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Serratia marcescens* and gram-positive *Staphylococcus aureus*. All the microbial cultures were procured from the Microbiology Laboratory, K.G Hospital, Coimbatore-641018. The stock cultures of bacteria were maintained on nutrient agar slants and fungi on potato dextrose agar slants at 4º C.

Antibacterial Assay

Antibacterial activity was demonstrated using a modification of the method originally described by Bauer *et al*., [18] which is widely used for the antibacterial susceptibility testing[19]. A loopful bacteria was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20µl) various crude solvent extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram positive and Gram negative bacteria. Respective solvents without plant extracts served as negative control. After the incubation period, the diameters of the inhibition zone around the plant extracts saturated discs were measured.

Statistical analysis

Statistical analysis was performed using statistical software package WINSAT 2007 in Microsoft Excel. The data were presented as Means ± S.E. Statistical analysis was performed using one way ANOVA, DMRT test was used for calculating for 5 % level of significance.

Results and Discussion

Qualitative Phytochemical Analysis

Phytochemical constituents of *C. epigaeus* leaf extract are presented in Table 1. Tests for tannins, steroids, triterpenoids, flavonoids, alkaloids and phenols were positive in both methanolic and chloroform extracts. Cardiac glycosides, glycosides, tannin, triterpenoids and steroids were detected only in the methanol and acetone extract while triterpenoids and steroids were detected in petroleum ether and hexane extract. Phlobatannins, anthraquinones, alkaloids, flavonoids and phenols were not detected in acetone extracts. However degree of precipitation of phytochemicals varies in all the extracts.

Methanol and chloroform extracts of *C. epigaeus* stem showed moderate precipitation for alkaloids, flavonoids, tannins, triterpenoids and steroids whereas cardiac glycosides, glycosides and saponins were detected only in methanol and acetone extracts (Table 1). Tests for alkaloids, flavonoids, phenols, tannins, phlobatannins, anthraquinones, glycosides, cardiac glycosides and saponins were negative in petroleum ether and hexane extracts while low precipitation (+) of alkaloids, flavonoids, phenols, glycosides, cardiac glycosides and saponins were observed in acetone extracts.

Positive tests for alkaloids, flavonoids, phenols, tannins, triterpenoids and steroids were present in chloroform, methanol and acetone extracts of *C. epigaeus* tuber while triterpenoids and steroidal components only were observed in petroleum ether and hexane extract. When compare to other extract, methanol shows high degree of precipitation (+++) of phytochemicals were noticed. Anthraquinones and phlobatannins were absent in all the extracts (Table 1).

Table 1: Qualitative phytochemical analysis of *Corallocarpus epigaeus*

Tests	Leaf					Stem					Tuber				
	Pet	Hex	Chlf	Acet	Met	Pet	Hex	Chlf	Acet	Met	Pet	Hex	Chlf	Acet	Met
Alkaloids:															
(i)Dragendroff's	-	-	++	-	++	-	-	++	+	++	-	-	++	++	+++
(ii)Wagner's	-	-	++	-	++	-	-	++	+	++	-	-	++	++	+++
(iii)Meyer's	-	-	++	-	++	-	-	++	+	++	-	-	++	++	+++
Flavonoids:															
(i)FeCl3	-	-	++	-	++	-	-	++	+	++	-	-	++	++	+++
(ii)Lead acetate	-	-	++	-	++	-	-	++	+	++	-	-	++	++	+++
(iii)NaOH	-	-	++	-	++	-	-	++	+	++	-	-	++	++	+++
(iv)Shinoda	-	-	++	-	++	-	-	++	+	++	-	-	++	++	+++
Phenols:															
(i) FeCl3	-	-	++	-	++	-	-	-	+	++	-	-	++	++	+++
(ii)Lead acetate	-	-	++	-	++	-	-	-	+	++	-	-	++	++	+++
Tannins	-	-	++	++	++	-	-	++	-	++	-	-	++	++	+++
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenoids:															
(i)Liberman's	+	+	++	++	++	+	+	++	-	++	+	+	++	++	+++
(ii)Salkowski	+	+	++	++	++	+	+	++	-	++	+	+	++	++	+++
Glycosides	-	-	-	+	++	-	-	-	+	++	-	-	-	-	+++
Cardiac glycosides															
(i)Liberman's	-	-	-	+	++	-	-	-	+	++	-	-	-	-	+++
(ii)Salkowski	-	-	-	+	++	-	-	-	+	++	-	-	-	-	+++
(iii)Keller-	-	-	-	+	++	-	-	-	+	++	-	-	-	-	+++
Steroids	++	++	++	++	++	++	++	++	+++	+++	++	++	++	++	+++
Saponins	-	-	-	-	-	-	-	-	+	++	-	-	-	-	+++

Note: + === Less precipitation, ++ == Moderate precipitation, +++ == Higher precipitation, - == Negative test

Table 2: Quantitative phytochemical analysis of *Corallocarpus epigaeus*

Samples tested	Flavonoids (mg/g)	Steroids (mg/g)	Tannins (mg/g)	Phenols (mg/g)
Leaf				
Petroleum ether	0.24^{c}±0.47	0.46^{b}±0.52	0.15^{b}±0.31	0.10^{c}±0.20
Hexane	0.27^{c}±0.37	0.45^{b}±0.67	0.17^{b}±0.41	0.14^{c}±0.55
Chloroform	0.86^{b}±0.61	0.63^{a}±0.29	0.31^{b}±0.12	0.76^{b}±0.58
Acetone	0.74^{b}±0.51	0.45^{b}±0.44	0.38^{b}±0.32	0.47^{c}±0.64
Methanol	0.74^{b}±0.57	0.62^{a}±0.56	0.60^{a}±0.34	0.83^{b}±0.58
Stem				
Petroleum ether	0.27^{c}±0.32	0.36^{b}±0.66	0.16^{b}±0.21	0.17^{c}±0.29
Hexane	0.43^{c}±0.41	0.31^{b}±0.35	0.21^{b}±0.38	0.35^{c}±0.69
Chloroform	0.84^{b}±0.64	0.68^{a}±0.32	0.33^{b}±0.24	0.64^{b}±0.53
Acetone	0.92^{b}±0.45	0.48^{b}±0.41	0.22^{b}±0.20	0.54^{b}±0.57
Methanol	1.04^{a}±0.49	0.55^{a}±0.59	0.67^{a}±0.66	0.97^{b}±1.03
Tuber				
Petroleum ether	0.33^{c}±0.43	0.27^{b}±0.49	0.29^{b}±0.12	0.40^{c}±0.36
Hexane	0.39^{c}±0.91	0.45^{b}±0.41	0.30^{b}±0.15	0.34^{c}±0.55
Chloroform	0.74^{b}±0.75	0.55^{a}±0.44	0.66^{a}±0.59	0.80^{b}±0.35
Acetone	0.72^{b}±0.55	0.55^{a}±0.52	0.43^{b}±0.22	0.62^{b}±0.52
Methanol	1.19^{a}±0.82	0.70^{a}±0.41	0.95^{a}±0.46	1.00^{a}±0.71

Values are expressed as Mean±Standard Error of 3 replicates. Means followed by a common letter aren't significantly different at the 5% level by DMRT

Quantitative phytochemical analysis

Table 2 depicts that, chloroform leaf extract of *C. epigaeus* had greatest content of flavonoids (0.86±0.61mg/g) and steroids (0.63±0.29 mg/g). Least content of tannin (0.60±0.34 mg/g) and phenols (0.83±0.58 mg/g) found in methanol extract. In stem and tuber, high content of flavonoids (1.04±0.49 and 1.19±0.82 mg/g), tannins (0.67±0.66 and 0.95±0.46 mg/g) and phenols (0.97±1.03 and 1.00±0.71 mg/g) was recorded in methanol extract while steroids were observed in chloroform (0.68±0.32 and 0.70±0.41 mg/g).

The results of phytochemical analysis of *C. epigaeus* were investigated and are summarized in Tables1 and 2. The results show that the plants are rich in alkaloids, flavonoids, triterpenoids and steroids. The presence of these bases in the investigated plants account for their usefulness as medicinal plants. The degree of precipitation of secondary metabolites varies in solvents. This may be due to various degrees of solubility of different solvents for different phytoconstituents.

Alkaloids are heterogeneous group compounds which contain one or more nitrogen atom in acyclic system. These are widely used in medicinal purposes which have positive and negative effects even to human beings. Most of the plants have alkaloids in different organs with different chemical configurations[20]. Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, developed resistance against diseases and endurance against stress[21]. They also have a protective role in animals[22]. High degree precipitation of alkaloids found in the methanol and chloroform extracts of *C. epigaeus* tubers. The present result coincides with the view of Jain *et al*.[23] who found high degree of alkaloid precipitation in methanol and chloroform extracts of *Cocculus hirsutus*. Many researchers reported that the presence of alkaloids in the plants cure asthma[24], snake bite[25] and skin diseases [26]. The presence of alkaloid may be the reason why the infusion of tuber paste *C. epigaeus* are given orally in village areas to cure asthma, skin diseases and snake bite.

Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom[27]. Flavonoids have been refereed to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergies, virus and carcinogens. They show antiallergic, anti-inflammatory, antimicrobial and anticancer activity[28]. Flavonoids are found in methanol, chloroform and acetone extracts of different parts of *C. epigaeus* (except acetone extracts of leaf) and shown different degree of precipitation. This results can be correlated with the result of Siciliano *et al*.[29] who detected and quantified eight flavonoids, three C-glycosyl and five O-glycosyl flavones in roots, leaves, stems and fruits of *Sechium edule* and reported highest amount of total flavonoids in the leaves, followed by roots and finally by stems.

Phenols are reported antitumour agents and to exhibit antiviral and antimicrobial activities[30], hypotensive effects[31] and antioxidant properties[32]. Methanol extracts of tubers of *C. epigaeus* showed highest phenolic content. The presence of the phenolic compounds in these studied samples proved that they had antimicrobial and antifungal effect. High degree precipitation of phenols was observed in methanol leaf extracts of *C. epigaeus*. Similar reports were reported in methanol leaf extracts of *oxalis corniculata*[33].

Moderate degree of precipitation of tannin was observed in methanol extract, while it was found to be absent in petroleum ether extract of *C. epigaeus*. Similar works were reported by Sule *et al*.[34] in the leaf extract of *Olax manni*. Presence of tannins in acetone and methanol tuber extract of *C. epigaeus* was observed, while it was found to be absent in petroleum ether extract. The present findings were similar to the report of Nataraj *et al*.[35] in *Amorphophallus paeoniifolius*. It was reported that tannins possess physiological astringent properties which hasten wound healing and ameliorate inflamed mucous membrane [36] and also it could prevent the growth of microorganism by precipitating microbial protein. So that nutritional protein available for them is ceased[37]. High content of tannin was observed in the tuber of *C. epigaeus*. This may be the reason why most of the people are used these plants for the treatment of insect bites.

Glycosides get hydrolysed and released phenolics that are toxic to microbial pathogens[38]. Presence of moderate degree of precipitation of cardiac glycosides was identified in methanol leaf extract of *C. epigaeus*. The present investigations were contradictory to Ramkumar *et al*.[39] and Adegoke and Adebayo-tayo[40] who reported low degree of precipitation of cardiac glycosides in methanol leaf extract of *Gymnema montanum* and *Lasienthera africanum* respectively. Cardiac glycosides were completely absent in hexane leaf extract of *C. epigaeus* while it was reported in methanol extract. The present report was contrary to the findings of Aiyelaagbe and Osamudiamen [28] who observed cardiac glycosides in leaves extract of hexane and methanol of *Mangifera indica*.

The adequate report of steroids in various studies indicated their importance and interest in

pharmacy due to their relationship with the compounds such as sex hormones, especially in the development of female contraceptive pill. High degree precipitation of steroids was found to be present in methanol leaf extract of *C. epigaeus*. The present works are in agreement with Raghavendra *et al*.[33] who reported the presence of steroids in methanol leaf extract of *Oxalis corniculata*. Presence of steroids was noted in the hexane leaf extract of *C. epigaeus*. The present investigations are corroborated with Ajaiyeoba[41] and Aiyelaagbe and Osamudiamen[28] who observed the presence of steroids in the leaves of *Parkia bicolor*, *P. biglobosa* and *Mangifera indica* respectively.

Saponins are terpene glycosides. Saponin is useful in medicine and pharmaceutical industry due to its foaming ability that produces frothy effect. Saponin is also used in the manufacture of shampoos, insecticides and various drug preparations and in synthesis of steroid hormones[42]. Generally saponins are toxic, but Price *et al*.[43] showed that consumption of saponins by human beings may be beneficial in reducing heart disease (by binding of saponins with plasma membrane and cholesterol). According to Shahidul Alam *et al*.[44] the presence of steroidal saponins could develop resistance to viral diseases. Finar [45] reported that, saponins had expectorant action which is very useful in the management of upper respiratory tract inflammation. So these plants may be used to treat various ailments.

Methanol stem extract of *C. epigaeus* showed the presence of saponin, while it was absent in chloroform extract. The above result was coincidence with the report of Moorthy *et al.*[46] who observed saponins in methanol stem extract of *Mallotus philippinensis* and absent in chloroform extract. *C. epigaeus* tuber extract of methanol showed high degree of precipitation of saponins, while it was completely absent in acetone extract. The present investigation was contradictory to Nataraj *et al*.[35] who noted the absence of saponins in both methanol and acetone extracts of *Amorphophallus paeoniifolius*.

Methanol leaf and stem extract of *C. epigaeus* showed the presence of triterpenoids. The present works are in agreement with Akinmoladun *et al*. [47, 48] who identified the presence of triterpenoids in the leaves and stems of *Chromolaena odorata* and *Alstonia boonei* respectively. Triterpenoids were observed in petroleum ether, acetone and methanol tuber extracts of *C. epigaeus*. Similar inference was obtained by Nataraj *et al*.[35] in *Amorphophallus paeoniifolius*, when they used all the three solvents to extract the triterpenoids from the tuber.

Mahato and Sen[49] reported that terpenoids had antitumour, anticancer, antiviral, antimicrobial and anti-inflammatory activities. Dymock[50], Kottaimuthu[8] and Swarnkar and Katewa[10] who reported that *K. foetidissima* and *C. epigaeus* were very effective in the treatment of asthma, snake bite and diarrhoea respectively. To site this, researchers reported that the presence of terpenoids cure bronchial asthma[51], diarrhoea and bacillary dysentery[52]. Researchers also reported the presence of terpenoids in Cucurbitaceae members[53, 54]. The above results confirm the medicinal properties of triterpenoids present in the study materials. The results of the present study supported the therapeutical potency of *C. epigaeus*.

Antimicrobial activity

The results of antibacterial activities of *C. epigaeus* leaf of various extracts tested against organisms are depicted in Tables 3 and 4. In this investigation petroleum ether and hexane extract remains inactive against test isolates. The chloroform and acetone extract showed significantly equal activity against *S. aureus* (9.33 and 9.67mm at 100% concentration) similar activity was also observed in *P. aeruginosa* by chloroform and methanol extract (7.03 and 7.06mm at 100% concentration). When compared to methanol and acetone extracts, the chloroform extract showed maximum zone of inhibition against *S. marcesense* (7.67mm at 100% concentration) whereas with *E. coli* methanol extract (11.33mm at 100% concentration) shows maximum zone of inhibition. The organism *K. pneumoniae* was resistant to all extracts. The 25-75% concentrations of acetone stem extract of *C. epigaeus* showed significant increase in the zone of inhibition while 100% concentration showed lesser inhibition against *P. aeruginosa* and *S. marcesense* (Tables 3 and 4) similarly the hexane extract against *S. marcesense* showed increase in the zone of inhibition upto 50% concentration and then there was a sudden decline of inhibition was noted. *K. pneumoniae* was extremely resistant to all extracts. The chloroform extract showed maximum zone of inhibition against *S. aureus* (11.33mm at 100% concentration) while in *P. aeruginosa* the methanol extract (9.00mm at 100% concentration) showed higher activity. The chloroform and acetone extract showed equal zone of inhibition against *P. aeruginosa* (5.90; 5.68mm at 100% concentration) whereas in *E. coli* methanol, chloroform and acetone extract (6.00; 6.73 and 6.13 mm at 100% concentration) showed significantly equal activity. Inhibitory effect of petroleum ether and hexane was observed only against the bacteria *S. marcesense*. The results from Tables 3 and 4 depicted that, *C. epigaeus* tuber extract showed that the antibacterial activity was linearly increased with

increasing concentration levels of all extracts except petroleum ether that showed decline in the zone of inhibition at 100% concentration against *S. marcesense*. Methanol, chloroform and acetone extracts of 100% concentration showed maximum zone of inhibition against *S. marcesense* (21.76; 20.31 and 18.46mm) whereas, moderate zone of inhibition was observed in the order of *S. aureus* (12.17 and 11.50 and 11.50mm), *P. aeruginosa* (10.07 and 8.00 and 6.93mm) *E. coli* (7.67 and 7.33 and 6.37mm). *K. pneumoniae* was extremely resistant to all extracts similarly as seen in *C. epigaeus* leaf and stem.

Table 3: Antibacterial activity of *Corallocarpus epigaeus*

Sample tested	Diameter zone of inhibition in mm														
	Staphylococcus aureus					*Escherichia coli*					*Klebsiella pneumoniae*				
Leaf	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**
Petroleum ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	6.00^{c}	6.83^{f}	8.17^{f}	9.33^{g}	0.00	0.00	3.67^{e}	5.13^{d}	7.43^{c}	0.00	0.00	0.00	0.00	0.00
Acetone	0.00	0.00	5.50^{g}	7.83^{g}	9.67^{f}	0.00	3.00^{e}	4.93^{c}	5.27^{d}	6.47^{e}	0.00	0.00	0.00	0.00	0.00
Methanol	0.00	4.50^{e}	8.17^{e}	9.50^{d}	10.17^{e}	0.00	.33^{a}	7.23^{a}	10.93^{a}	11.33^{a}	0.00	0.00	0.00	0.00	0.00
Stem															
Petroleum ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	6.00^{c}	9.33^{c}	10.00^{c}	11.33^{c}	0.00	0.00	4.03^{d}	5.20^{d}	6.73^{d}	0.00	0.00	0.00	0.00	0.00
Acetone	0.00	5.00^{d}	6.50^{f}	8.83^{e}	9.00^{g}	0.00	0.00	4.20^{d}	5.57^{c}	6.13^{e}	0.00	0.00	0.00	0.00	0.00
Methanol	0.00	3.30^{f}	8.50^{d}	9.50^{d}	10.83^{d}	0.00	3.60^{d}	4.73^{c}	5.73^{c}	6.00^{e}	0.00	0.00	0.00	0.00	0.00
Tuber															
Petroleum ether	0.00	1.07^{g}	1.00^{i}	1.02^{h}	2.10^{h}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	3.00^{f}	2.16^{h}	1.40^{h}	1.00^{i}	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	0.00	6.82^{b}	9.17^{c}	10.17^{c}	11.50^{b}	0.00	4.07^{c}	5.67^{b}	6.17^{b}	7.33^{c}	0.00	0.00	0.00	0.00	0.00
Acetone	0.00	4.90^{e}	9.50^{b}	10.50^{b}	11.50^{b}	0.00	4.53^{b}	5.57^{b}	6.00^{b}	6.37^{e}	0.00	0.00	0.00	0.00	0.00
Methanol	0.00	8.18^{a}	10.50^{a}	11.50^{a}	12.17^{a}	0.00	3.83^{d}	5.83^{b}	6.07^{b}	7.67^{b}	0.00	0.00	0.00	0.00	0.00

Values are expressed as Mean of 3 replicates. Means followed by a common letter aren't significantly different at the 5% level by DMRT.

Table 4: Antibacterial activity of *Corallocarpus epigaeus*

Sample tested	Diameter zone of inhibition in mm									
	Serratia marcescens					*Pseudomonas aeruginosa*				
Leaf	**C**	**25%**	**50%**	**75%**	**100%**	**C**	**25%**	**50%**	**75%**	**100%**
Petroleum ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Hexane	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Chloroform	2.50^{d}	4.63^{f}	5.63^{g}	6.00^{g}	7.67^{f}	0.00	2.43^{e}	4.00^{e}	6.00^{d}	7.03^{d}
Acetone	1.16^{g}	2.56^{h}	3.50^{i}	0.00	0.00	0.00	0.00	0.00	3.20^{g}	5.90^{f}
Methanol	3.00^{c}	3.00^{g}	4.33^{h}	5.00^{h}	6.17^{h}	0.00	3.67^{c}	5.33^{c}	6.33^{d}	7.06^{d}
Stem										
Petroleum ether	2.00^{e}	1.50^{j}	2.15^{k}	2.35^{k}	3.00^{i}	0.00	0.00	0.00	0.00	0.00
Hexane	2.09^{e}	2.00^{i}	3.12^{j}	1.05^{l}	1.20^{k}	0.00	0.00	0.00	0.00	0.00
Chloroform	4.17^{a}	6.50^{e}	7.00^{f}	8.13^{f}	9.00^{e}	0.00	0.00	0.00	4.20^{f}	5.90^{f}
Acetone	2.00^{e}	7.50^{d}	8.17^{e}	9.17^{e}	7.00^{g}	0.00	4.23^{b}	6.77^{b}	7.13^{c}	5.68^{f}
Methanol	3.50^{b}	7.93^{d}	8.90^{d}	11.07^{d}	12.00^{d}	0.00	0.00	6.50^{b}	8.00^{b}	9.00^{c}
Tuber										
Petroleum ether	2.00^{e}	1.10^{k}	1.58^{l}	3.25^{i}	1.00^{k}	0.00	1.07^{f}	1.00^{g}	1.20^{h}	2.10^{g}
Hexane	2.05^{e}	1.35^{k}	2.15^{k}	2.50^{j}	2.88j	0.00	3.00^{d}	2.16^{f}	1.40^{h}	1.00^{h}
Chloroform	3.83^{b}	16.72^{b}	17.33^{b}	19.20^{b}	20.31^{b}	0.00	4.13^{b}	6.53^{b}	7.17^{c}	8.00^{b}
Acetone	1.93^{f}	14.47^{c}	15.43^{c}	17.49^{c}	18.46^{c}	0.00	3.00^{d}	4.63^{d}	5.00^{e}	6.93^{e}
Methanol	3.40^{c}	19.04^{a}	19.95^{a}	20.34^{a}	21.76^{a}	0.00	5.90^{a}	8.37^{a}	9.67^{a}	10.07^{a}

Values are expressed as Mean of 3 replicates. Means followed by a common letter aren't significantly different at the 5% level by DMRT.

Antimicrobial activity of *C. epigaeus* was summarized in Tables 3 and 4. In the present work, methanolic extract showed higher inhibitory effect while chloroform and acetone showed moderate inhibitory activity. These observations may be attributed to two reasons; first, the nature of biological active of components whose activity can be enhanced in the presence of methanol; second, the stronger extraction capacity of methanol could have produced greater number of active constituents responsible for antimicrobial activity. Generally, gram negative bacteria are more resistant than gram positive bacteria[55]. But findings of this present work are different. Here, the significant antibacterial activity was seen against both gram positive and gram negative bacteria. *K. pneumoniae* was completely resistant to the plant extracts. This could be due to the fact that the microorganisms possess a mechanism for detoxifying the active principles in the extract or bacteria are known to posse's mechanism by which they convert substances that inhibit their growth into non toxic compounds. The results of Kandhasamy and Arunachalam [56, 57] agreed with present results who reported inhibitory effect of methanolic tuber extracts of *Zehnaria scabra* and *Typhonium trilobatum* against *K. pneumoniae*. Studies of Nair *et al*.[58] also reported the resistance of *K. pneumoniae* in methanolic extracts of *Sapindus emarginatus*. The most susceptible bacteria observed in *S. marcescens* and *S. aureus*. This is different from previous studies of Kandhasamy and Arunachalam[57] and Bonjar [59] who reported that no inhibitory effect was observed in methanol and chloroform tuber extracts of *Typhonium trilobatum* and *Zingiber officinale* against *S. marcescens*. The different in plant part, botanical varieties and geographical origin may contribute different results.

The extracts of *C. epigaeus* clearly reveal the antibacterial nature and it is an evident that the findings of the present study can provide a basic concept for synthesizing a new drug. This activity may probably due to the presence of bioactive compounds in the plant parts. Several phytochemicals like flavonoids, phenolics, tannins, terpenoids and glycosides are effective antimicrobial substances against a wide range of microorganisms [60, 61, 62]. Thus, this study ascertains the value of the plant, which would be of considerable interest to the development of new drugs. It will also help to isolate new antibiotic substances that control the infectious disease causing microbial pathogens.

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