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PRELIMINARY PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF WHOLE PLANT EXTRACTS OF *CANTHIUM PARVIFLORUM* Lam.

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ABSTRACT

The present study is interpreting the existence of various phytoconstituents present in the different extracts of whole plant namely *Canthium parviflorum* Lam. These phytoconstituents were recognized by thin layer chromatography (TLC) using the suitable colour developing reagents by spraying on the silica gel 60 coated chromatogram plate, which showed the pertinent intensity of the colour determining the significant phytoconstituent. Antibacterial activity of various extracts had been carried out and the results were tabulated. From the consequences on comparing with standard drug ciprofloxacin, it was found that Ethyl acetate extract has shown potent effect against the gram+ive strains (*B.subtilis* and *S.aureus*) and gram-ive strains (*E.coli* and *K.pneumoniae*) employed in this study. At the same time, hydro alcohol extract also shows a significant effect on the same strains. A significant antifungal activity was shown by ethyl acetate extract, when comparing with ketoconazole against *A.niger*, *A.fumigatus* and *C.albicans*. Elevation in concentration of the above-mentioned extract has shown enhancement in zone of inhibition on bacterial and fungal strains.

Key words: *Canthium parviflorum* Lam, Phytoconstituents, Zone of inhibition.

INTRODUCTION

Canthium parviflorum Lam. (fam:- Rubiaceae) is commonly called as Carray cheddie in English, Kirma in Hindi and Mullukaarai in Tamil. It occurs in peninsular India, coramandel coast, dry plains and shrub forests of India and Srilanka. It is a thorny shrub with spreading branches. Its leaves are simple, small, obviate, opposite with interpetiolar stipules linear and axillary spines. The roots of this plant are traditionally used by the tribes of Orissa in treatment of swelling of neck and fruits in headache. This plant is reported for its pharmacological uses as an astringent, anthelmintic, antidysentric, antispasmodic and as a diuretic [1-6].

From the ethno medical survey we came to know that many people from vellore district are using the plant and its various parts traditionally practicing widely throughout those areas for various infections. Hence the whole plant was utilized for our present evaluation to study about the presence of various phytoconstituent and its concomitant activity.

MATERIALS AND METHODS

Collection of Plant Materials

The whole plant materials of *Canthium parviflorum* Lam was collected from vellore district near CMC Medical college in Tamilnadu state, which was identified and authenticated by Dr.S.M.Khasim, Assistant Professor, Department of Botany, Acharya Nagarjuna University in Guntur. One of the plant specimens had been planted in KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada and a voucher (No: KVSR/PCRL/No: 0052/BN) had been deposited after planted in the college herbal Garden.

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Chemical reagents

The chemicals and reagents used for the spraying in the TLC Chromatogram were procured from S.D.Fine Chemicals and B.D.H.Chemicals, Mumbai. The solvents were procured from chemical laboratory of KVSR Siddhartha college of Pharmaceutical sciences, Vijayawada.

Extraction and Preliminary Phytochemical Screening

The whole plant were collected and washed with running tap water. Then they were shade dried and pulverized to coarse particles by using the sieve no.40. About 200 grams of subjected to hot soxhlete extraction [7] apparatus with 700ml of pet ether (60-80ºC) for 48 hrs. Then the extraction was followed by one litre of benzene, 650 ml of ethanol (90%V/V) and 750ml of acetone for another 48 hrs. Finally the dried marc was subjected to cold maceration [8] by using 600mlof hydro alcohol (1:1) for 72 hrs. Finally the obtained extracts were filtered through muslin cloth. Then they were concentrated under reduced pressure and dried in vacuum condition to get a semisolid consistency whose yields are tabulated. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents [9-13] present in them. The results were observed and tabulated in table 1.

Experimental work

Antibacterial activity

Bacterial strains namely *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2019), *Escherchia coli* (NCIM 2065) and *Klebsiella pneumoniae* (NCIM2036) had been procured from the National Collection of Industrial Microorganism (NCIM), Pune, India for this evaluation. The stock culture was maintained on Mueller Hinton agar (MHA) medium at 37°C.

The anti-bacterial activity of the two extracts was performed by paper disc diffusion assay method [14]. The discs of uniform size (6mm) were prepared from Whatmann No.1 filter paper and were sterilized in hot air oven at 160°C for 1hr.Then the discs were impregnated with the MIC (minimum Inhibitory concentration) of different concentrations (50mg/ml, 100mg/ml and 200mg/ml) of various extracts and standard ciprofloxacin. The solvent DMF is used as a control. The plates were prepared by using MH agar media and the extracts of various dilutions were allowed to solidify and dried. Different wells [15] were prepared in the solidified agar plates and were labelled. Then a loopful of bacterial cultures was inoculated at the labeled spots and the plates were inoculated at 37°C for 24hrs and the zone of inhibition was observed. The results were observed and tabulated in table 2.

Antifungal Activity

The fungal species had also been procured from the National Collection of Industrial Microorganism (NCIM), Pune, India for evaluating antifungal activity. The prepared semisolid extracts were subjected for antifungal screening. The paper disc method is the popular method, which is used to perform this screening. The Sabouraud Dextrose Maltose Hiveg Agar (SDMHA) [16] was sterilized by moist heat sterilization using autoclave at 120°C for 30 minutes and the medium was inoculated (1ml/100ml of medium) after it exhibits room temperature by using the suspension of the fungal species namely *Aspergillus niger* (NCIM 596), *Aspergillus fumigatus* (NCIM 519) and *Candida albicans* (NCIM 3100). The impregnated disc papers were Whatmann filter paper no.40 was utilized. The extracts having a concentration level of 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml in dimethyl sulfoxide (DMSO) were prepared and then placed on solidified medium. Then the plates were preincubated for 1hr at room temperature and further incubated for 48 hrs at 37°C using Incubator (Kemi (Pvt) India Ltd, Ernakulam, Kerala). Then the zone of inhibition was observed. The lowest concentration of various extracts required for inhibiting the growth was considered as minimum inhibitory concentration (MIC) of those extracts against the fungal strains. The results were tabulated in table-2. In this evaluation ketoconazole is used as standard drug for comparative evaluation of those extracts.

RESULTS AND DISCUSSIONS

From the obtained results, the phytochemical evaluation showed that alkaloids are mostly present in acetone extract and hydroalcohol extract. Saponins are intensly present in ethanol and hydroalcohol extract. Fixed oils and fats are highly present in petroleum ether and benzene extract. Flavones and Flavonoids are most intense in pet ether, benzene and acetone extract. Gums and Mucilages are highly present in ethanol, ethyl acetate and hydro alcohol extracts. Phenolics and Poly Phenolics are mostly present ethanol, acetone and hydro alcohol extracts. Tannins highly present in pet ether, benzene, ethyl acetate and hydro alcohol extracts but moderately present in ethanol and acetone extracts.

From the zone of inhibition, it is evident that the ethyl acetate and hydro alcohol extracts of *Canthium parviflorum* Lam showed significant anti-bacterial activity against gram+ve and gram-ve bacteria on comparison with standard ciprofloxacin. Also by increase in concentrations of both the extracts showed increase in the anti-bacterial activity. Also the antifungal activity of ethyl acetate and hydroalcohol extracts showed a significant effect against *A.niger* and *A. fumigatus*. But ethyl acetate extract has excellent effect against C.albicans than hydro alcohol extract. Destruction of the microbial colonies was already reported by production of oxygen free radical (Ö) and superoxide dismutase (SOD) [17] which are released and this stimulation is due to the unopsonised surface adherent in the outer layer of bacteria and fungal species as reported by Fischer and Adams. In conclusion it is noted that the activity of the extracts is due to the interaction of plant molecules such as alkaloids, phenolics and polyphenolics and their derivatives with calcium current by electron transport chain [18] reaction on those microbial cells which produce complication and results in respiratory burst [19] of those microbial cells as reported by Rebut.

Table 1: Preliminary phytochemical evaluation of roots and rhizomes extract of *Canthium parviflorum* Lam

S.No	Name of the Phytoconstituents	Pet-ether extract	Benzene extract	Ethanol extract	Acetone extract	Ethyl acetate extract	Hydro alcohol extract
1	Alkaloids	-	-	-	++	-	++
2	Carbohydrates and Glycosides	-	-	+	+	++	++
3	Fixed oils and fats	++	++	-	+	+	-
4	Flavones and Flavonoids	++	++	-	++	-	+
5	Gums and Mucilages	-	-	++	+	++	++
6	Phenolics and Poly Phenolics	-	-	++	++	+	++
7	Proteins and Aminoacids	-	-	-	-	-	+
8	Saponins	-	-	++	-	-	++
9	Steroids	++	++	+	+	-	-
10	Tannins	++	++	+	+	++	++
11	Triterpenoids	-	-	-	+	+	++
12	Amount (grams)	1.2210	0.6984	0.9218	1.46	1.3994	1.1312
13	%yield w/w	6.14	2.15	3.64	8.52	7.88	5.33

(++) denotes more intensity; (+) denotes moderate intensity; (-) denotes no intensity

Table 2: Antibacterial activity of various crude extracts of *Canthium parviflorum* Lam

Various crude extracts and various concentrations (mg/ml)	Zone of inhibition against bacterial strains (mm)			
	B. subtilis **(NCIM 2063)**	***S. aureus*** **(NCIM 2019)**	***E. coli*** **(NCIM 2065)**	***K. pneumonia*** **(NCIM 2036)**
Pet-ether extract (PE) 50 100 200	- - 1	- 1 1	3 - 3	- 2 2
Benzene extract (BE) 50 100 200	- - -	- - 1	3 4 7	- - 2
Ethanolic extract (EE) 50 100 200	- 2 2	- - -	1 3 4	2 2 2
Acetone extract (AE) 50 100 200	- 2 2	- - 1	- - -	- - 3
Ethylacetate extract (EAE) 50 100 200	12 15 22	13 20 25	8 14 23	7 12 18
Hydro alcohol extract (HAE) 50 100 200	11 14 18	12 16 20	8 13 18	9 13 17
Ciprofloxacin (CP) 50 100 200	15 19 26	15 19 27	11 17 25	10 15 21
DMF	5	5	5	5

(-) denotes no inhibition

Table 3: Antifungal activity of various crude extracts of *Canthium parviflorum* Lam.

Various crude extracts and various concentrations (mg/ml)	Zone of inhibition against fungal strains (mm)		
	A.niger (NCIM 0596)	*A.fumigatus* (NCIM 0519)	*C. albicans* (NCIM 3100)
Pet-ether extract (PE)			
62.5	-	-	02
125	03	-	04
250	03	-	05
500	04	-	07
1000	04	-	18
Benzene extract (BE)			
62.5	-	-	-
125	-	01	-
250	-	01	01
500	-	02	01
1000	-	02	01
Ethanolic extract (EE)			
62.5	-	-	-
125	-	01	-
250	01	01	-
500	01	02	01
1000	03	02	01
Acetone extract (AE)			
62.5	-	05	-
125	-	08	09
250	-	11	10
500	03	14	12
1000	04	16	14
Ethylacetate extract (EAE)			
62.5	12	10	12
125	16	14	15
250	18	19	17
500	21	23	20
1000	26	29	24
Hydro alcohol extract (HAE)			-
62.5	09	09	-
125	10	13	06
250	13	14	11
500	16	19	14
1000	19	24	-
Ketoconazole (KC)			
62.5	13	10	12
125	19	14	17
250	20	19	22
500	23	23	26
1000	26	29	29
Dimethyl sulfoxide (DMSO)	5	7	6

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References

1. Warrier PK, Nambiar VPK and Raman Kutty C.In: Indian Medicinal Plants, Vol-I. Orient Longman Publishers Ltd, Madras. 1994, 366.
2. The Drug Controller. In: The wealth of India-Raw Materials. Publication and Information of directorate.Council of Scientific and Industrial Research. Vol-III. New Delhi.1992, 210.
3. Nadkarni KM. In: Indian Materia medica. Vol-I. Popular Prakashan Publishers, Bombay.1976, 264.

4. Vaidya Bhagwan Dash and Kanchan gupta KV.In: Materia medica of Ayurveda. Jain Publishers, New Delhi.1991, 295.
5. Guha Bakshi DN, Sensarm P and Pal DC. In: A Lexicon of Medicinal Plants in India, Naya Prakash Publishers, Calcutta.1999, 359.
6. Kirtikar KR and Basu BD. In: Indian Medicinal Plants. Vol-II.1975, 1284.
7. Khandelwal KR. In:Practical Pharmacognosy technique and experiments. 2nd edn. Nirali Prakashan Publishers, Pune.2000, 149-156.
8. Harborne JB.In: Phytochemical methods. A guide to modern techniques of plant analysis, Chapman and Hall Publishers, London. 1973, 182-189.
9. Peache and Tracey MV. In: Modern methods of plants analysis. Springler and Veriag Publishers, Berlin. 3, 1955, 321-322.
10. Franswotrh NR. Phytochemical investigation on *Cassia tora. Journal of Pharmaceutical Sciences*, 55(3), 1966, 225-269.
11. Anes Ahmad Siddqui and Mohamad Ali. In: Practical pharmaceutical chemistry, CBS publishers, New Delhi.1997, 127-137.
12. Tyler VE, Brady LR and Robert JE. In: Pharmacognosy. Lea and Febiger Publications, Philadelphia. 9th edn, 1988, 77-79.
13. Kokatae CK.In: Practical Pharmacognosy. Vallabha Prakashan Publications, New Delhi. 3rd edn. 1991, 107-111.
14. Forbes BA, Sahn DF and Weissfeld AS,In: Diagnostic Microbiology. 10th edn, Bailley and Scott, Mosby Publication, USA.1998, 252-258.
15. Kierby WMM and Bauer AW, Antibiotics susceptibility testing by a standardized single disc method. *Journal of Clinical Pathology*. 45, 1996, 493.
16. Seher G, Turgut – Balik D and Nazmi G. Antimicrobial activites and some fatty acids of turmeric, ginger root and Linseed used in treatment of infectious diseases. *World Journal of Agricultural sciences*, 2(4), 2006, 439-442.
17. 17. Devalou ML, Elliot GR and Regelmann WE. Oxidative response of human neutrophils, monocytes and alveolar macrophages induced by unopsonised surface adherent staphylococcus aureus. *Infectious Immunology*, 55, 1987, 2398.
18. Chai HB and Doke N.Superoxide anion generation: A response of potato leaves to infection with phytophthora infestations. *Phytopathology*, 77, 1987, 645.
19. Rebut-Bonneton C, Bailly S and Pasquier C. Superoxide anion production in glass – adherent polymorphonuclear leukocytes and its calcium movement. *Journal of Leukocyte Biology*, 44, 1988, 402.