iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 4 **Issue:** VIII **Month of publication:** August 2016

DOI:

www.ijraset.com

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Volume 4 Issue VIII, August 2016
ISSN: 2321-9653
International Journal for Research in Applied Science & Engineering Technology (IJRASET)

In vitro Antimicrobial activity and Phytochemical analysis of *Cleome gynandra* Linn Leaf Extracts Against Human Pathogens.

J. Rajaselvam[1], Sr. M.R. Basil Rose[2]
Department of Zoology, Holy Cross College (Autonomous), Nagercoil, Tamilnadu, India

Abstract: ***The present study was carried out to investigate the antimicrobial and phytochemical analysis of different extracts by agar well diffusion method. Five bacterial pathogen such as Gram positive- Bacillus subtilis, Staphylococcus aureus and Gram negative - Escherichia coli, Pseudomonas aeruginosa, and Proteus mirabilis were used as test organisms. Among the extract prepared in four solvents ethanolic extracts were found to possess highest antimicrobial activity against E.coli, Proteus mirabilis, Pseudomonas aeruginosa. Acetone and chloroform extracts showed moderate inhibitory potency and no inhibitory activity was observed when tested in the aqueous extract. The phytochemical analysis revealed the presence of tannins terpenoids, saponin and protein.***

Key words: Cleome gynandra Linn, antimicrobial activity, phytochemical screening, agar well diffusion.

I. INTRODUCTION

Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et al*., 2008). A number of herbs with significant antimicrobial activity have been reported in different traditional literatures (Jones *et al*., 1996; Satish *et al*., 1999). Many medicinal plants are used daily in Ayurvedic practices. In India more than 7000 medicinal plants are known. According to a report of World Health Organization, more than 80% of World's populations depend on traditional medicine for the primary healthcare needs (Umamaheswari *et al*., 2008). The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity (Poovendran *et al*., 2011). Medicinal plants represent a rich source of antimicrobial agents. *Cleome gynandra* used as a medicinal plant is cosmopolitan in distribution. It grows as a weed in roadsides and in open grass lands. *Cleome gynandra* L.(Capparidaceae) is commonly known as 'Hurhur' and karaila. Different species of *Cleome* can be found in all. This study aims to evaluvate the antimicrobial activity and phytochemical constituents of different extracts of *Cleome gynandra* L. leaves against harmful human pathogens such as *Escherichia coli, Staphylococus aureus, Pseudomonas aeruginosa, Proteus mirabilis,* and *Bacillus subtilis*.

II. MATERIALS AND METHODS

Leaves of *C. gynandra* were collected from Kanyakumari District, Tamilnadu, India. Collected plant material was washed thoroughly in running tap water, shade dried in open air separately. The dried leaves were fine powdered and stored in polythene bags at room temperature until use. 1g/10 ml of dried powder of the plant were soaked separately in 100 ml of different solvents like acetone, ethanol, chloroform and water and allowed to stand for 48 h and filtered. The mixture was filtered using Whatmann No 1 filter paper. The extracts obtained were concentrated and stored in refrigerator.

III. SELECTION OF MICROORGANISMS

Escherichia coli, Proteus mirabilis, Pseudomonas aeroginosa (gram negative), *Staphylococcus aureus, Bacillus subtilis* (gram positive) were used for the study of antimicrobial activity. The bacterial cultures maintained on slants consisting of nutrient agar medium for 24 hours cultures were used in the antibacterial activity.

IV. PHYTOCHEMICAL SCREENING

Phytochemical screening was carried out to determine the presence of tannins, flavonoids, terpenoids, saponins, quinone, protein (Harbourne, 1973; Baker and Thormasberg, 1983; Sahm and Washington, 1990; Brindha *et al*., 1991).

V. ANTIBACTERIAL ASSAY

Antibacterial activity of the aqueous, acetone, ethanol and chloroform extracts of leaves of *C. gynandra* were tested using agar well

Volume 4 Issue VIII, August 2016
ISSN: 2321-9653
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diffusion method (Kartig *et al.*,1991). Bacterial culture having 108 CFU was spread on nutrient agar (NA) plate using swab. Wells of 4 mm diameter and about 2 cm apart were punched off with sterile cork borer and filled aseptically with 20 µl of leaf extracts. The inoculated plates were incubated at 37°C for 24 – 28 hrs. The antibiotic amikacin was used as standard for reference.

VI. RESULTS AND DISCUSSION

The presence of antibacterial substances in the higher plants is well established (Srinivasan *et al.*, 2001). In the present study reveals the antimicrobial activities of different solvent extracts of *Cleome gynandra* leaves against different bacterial strains(Table 1, plate-1). Their antimicrobial activity was assessed by the presence or absence of inhibition zone and zone diameters (mm). It was observed that the antimicrobial effect of plant extract varies from one plant to another in different regions of the world. This may be due to many factors such, as the effect of climate, soil composition, age, on the quality, quantity and composition of extracted product, different bacterial strains (Masotti *et al.*, 2003; Angioni *et al.*, 2006). From the results, the extract of C*leome gynandra* showed highest activity in ethanolic extract against *Escherichia coli* (17 mm), *Pseudomonas aeroginesa* (16 mm), and *Proteus mirabilis* (15 mm). The acetone extract of *C.gynandra* showed moderate inhibiting activity against *E.coli, Staphylococcus aureus, Pseudomonas aeruginosa* (13 mm) and least activity was observed against *Proteus mirabilis* (10 mm) and no activity was showed against *Bacillus subtilis*. The chloroform extract showed maximum inhibitory activity against *E.coli* (11 mm), *Proteus mirabilis* (11 mm), and less activity was observed against *Staphylococcus aureus* (8 mm), and *Pseudomonas aeruginosa* (8 mm). Chloroform extract was sensitive against *Bacillus subtilis*. Aqueous extract of *C.gynandra* showed no antibacterial activity. Different studies found that the type of solvent has an important role in the process of extracting. (Al - Zubaydi *et al*., 2009; Bedi *et al*., 2010; Bakht *et al*., 2011). Several authors have reported the antimicrobial activity of crude extracts of various plants (Oyeleke *et al*., 2000; Shilpa *et al*., 2009). Kumaraswamy *et al* ., (2012) reported the antimicrobial activity of *Bougainvillea spectabilis*. The similar finding was observed in *Aristolochia bracteata*, (Madhuri *et al*., 2012), *Mirabilis jalapa* Linn (Sharmila Shaik *et al* ., 2012). In the present study , the acetone, ethanol and chloroform extracts have shown antimicrobial activity . This may be due to the presence of terpenoids, tannins, saponins present in their extracts. Phytochemical constituents like tannins, flavonoids, terpenoids, saponins and proteins identified in *C.gynandra* are reported in Table 2 The antibacterial activity observed in *Cleome gynandra* L may be due to their substances present in them.

Plate 1. Zone of inhibition shown by *E. coli*

Plate 2. Zone of inhibition shown by *P. aeruginosa*

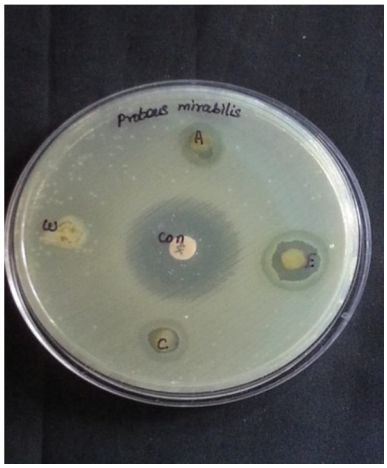

Plate 3. Zone of inhibition shown by *P.mirabilis*

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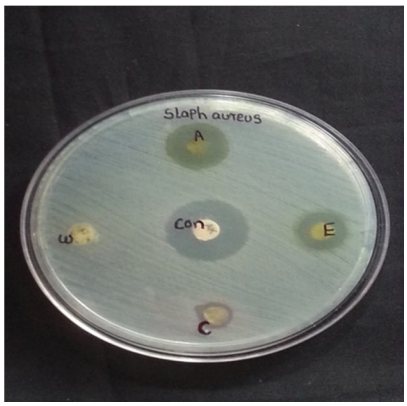

Plate 4. Zone if inhibition shown by *S.aureus*

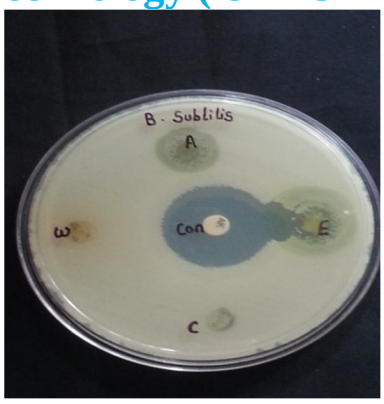

Plate 5. Zone if inhibition shown by *B.subtilis*

Table 1 : Antibacterial activity of *Cleome gynandra* leaf extract

S.No	Test organisms	Solvents				
		Acetone	Ethanol	Chloroform	Water	standard (Amikacin)
1.	*E. coli*	13 mm	17 mm	11 mm	-	16 mm
2.	*S. aureus*	13 mm	12 mm	8 mm	-	18 mm
3.	*P. aeroginosa*	13 mm	17 mm	8 mm	-	18 mm
4.	*P. mirabilis*	10 mm	15 mm	11 mm	-	19 mm
5.	*B. subtilis*	-	9 mm	-	-	24 mm

Table 2 : Preliminary phytochemical analysis of *Cleome gynandra* L. leaf extracted with different solvents

Phytochemicals	Acetone	Chloroform	Ethanol	Water
Tannins	_	+	+	+
Flavonoids	_	_	_	_
Terpenoids	+	+	+	_
Saponin	+	+	_	+
Quinones	_	_	_	_
Protein	+	+	+	_

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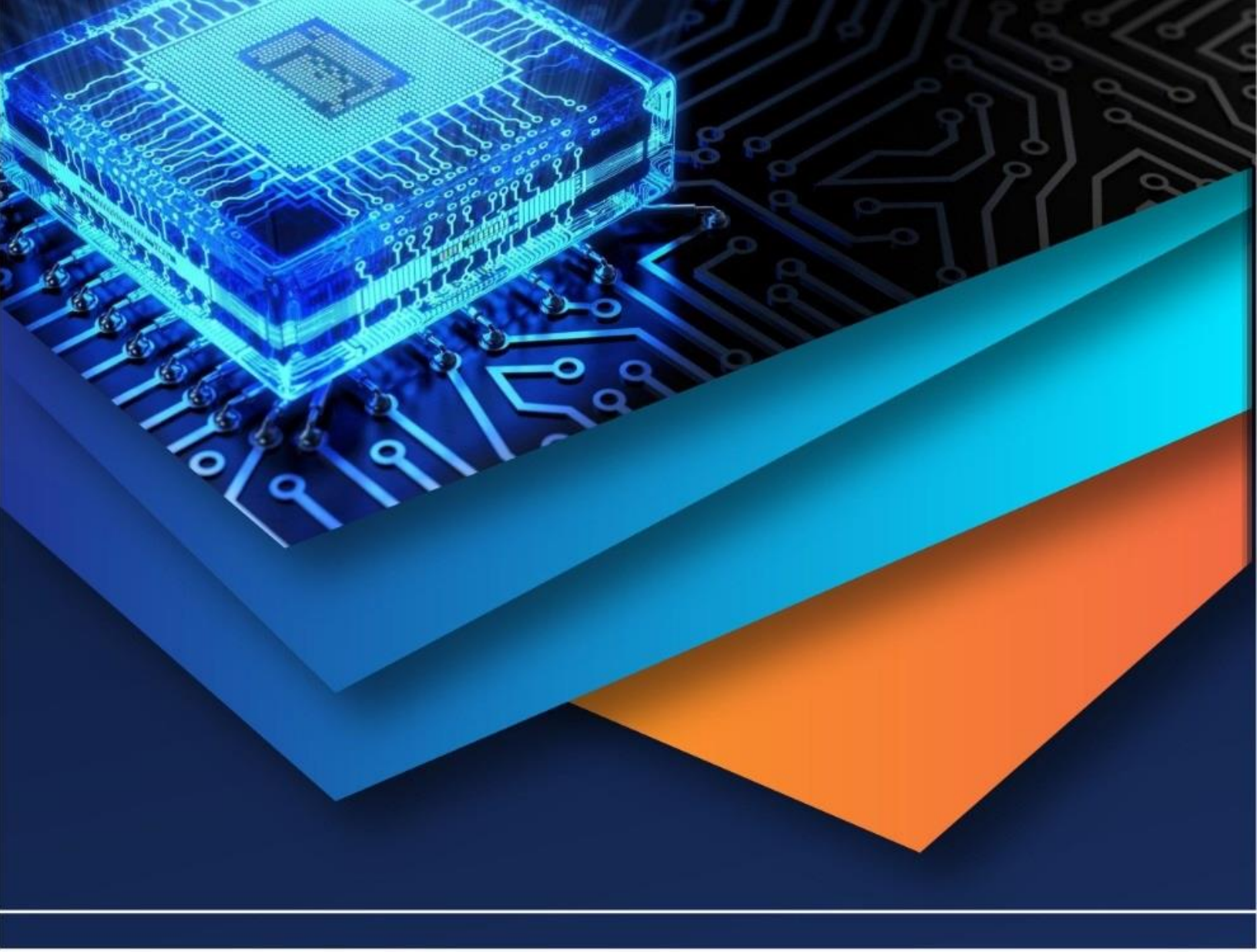

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