ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

ANTIMICROBIAL ACTIVITY IN ROOTS EXTRACT OF *CYPERUS ROTUNDUS L.* ON PATHOGENIC ORGANISMS

Wanjare P. D. and Patil U.S.

G.S. GawandeMahavidyalaya ,Umarkhed DistYavatmal (M.S.) India.
Department of Botany, Bhartiya Mahavidyalaya, Amravati (M.S.) India,
Corresponding Author-wanjare.gsg@gmail.com

ABSTRACT

The increasing prevalence of drug-resistant pathogens has gained the attention of pharmaceutical and scientific communities towards potential antimicrobial agents from plant derived sources. The present research work has been undertaken to study the antimicrobial activity of the chloroform extract of CyperusrotundusL. against some human pathogens like *Escherichia coli,Pseudomonas aeruginosa, Salmonella typhimurium Staphylococcus aureus,Shigellaflexneri, Streptococcus pneamoniae,Klebsiella pneumonia* and fungi *Aspergillus niger* by using agar well diffusion method. Inhibition zones ranged between 4.l2 ± 0.22 - 16.24 ± 0.11 mm.Roots extract inhibited the growth of all tested microorganisms with large zones of inhibition .The standard antibiotics chloramphenicol and miconuzole nitrate were found to have zone of inhibitions 10.05 ±0.12-24.12 ±0.20 mm at the concentration of 30 ug/ml. In contrast, the inhibition zone of chloroform (negative control) was almost zero for all the tested microorganisms. The spectrum activity of chloroform extract of this plant could be a possible source to obtain new and effective herbal medicines to treat various infectious diseases.

KEYWORDS: Antimicrobial activity, *Cyperusrotundus*L.,*chloroform* extract, human pathogens, zones of inhibition

INTRODUCTION

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine (Dubey et al. 2011).Since antiquity, many plant species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides. saponins. flavonoids, steroids, tannins, alkaloids and terpeonoids which are utilized to combat the disease causing pathogens (Kamali 2010: Lalitha et al- 2010; Hussain et al, 2011).With the advancement in Science and Technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs (Preethi et al. 2010). Antibiotics are indisputably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections (Shanna, 2011). Despite the huge number of antimicrobial agents for various purposes that already exist the search for

ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

new drugs is a continuous task since the target microorganisms often develop new genetic variants which subsequently become resistant to available antimicrobial agents (Enneet al.2001: Westh et al. 2004). The world's attention is now increasing directed toward plant sources for developing antimicrobial drugs, since natural products are considered safer than synthetic ones (Kim et al. 1999; Alagesaboopathi, 2011), According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (Ahmad, 2001). Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimenio et al 2000). There are several published reports describing the antimicrobial activity of various crude plant extracts (Igoli et al. 2005; Alzoreky, 2003). It is estimated that there are about 2.5 million species of higher plants and the majority of these have not yet been examined for their pharmacological activities (Ram el al 2003).

The different herbal plant extracts are traditionally has been used as anticancer antioxidant, antiulcer, analgesic and antidiabetic (Pankaj, 2011),and they also having the antiparasitic, antifungal, antibacterial, antimalarial activity, analgesic and anti-inflammatory aclivity (Acharyya et al. 2011),Different species of *Cyperrotundus*are used as a folk medicine for the treatment of various ailments such as skin diseases, intestinal parasites and warts. It has been reported that *Cyperrotundus*possesses antidiarrhoeal and antidysenteric activity (Shailili et al, 8008).*Cyperrotundus*L.belongs to the family Cyperaceae. Perennial herbs,30 -50 cm tall;stolons wiry ending in ellipsoid tuber; stem trigonous . Leaves:sheaths glabrous; blade linear,cariaceous , long-acuminate.Umbles compound; involucral bract 4-5 spreading; spikes ovoid,with 8-10 spikelets. (Freeman, 2005). The present research was set up to determine the antimicrobial activity in roots extract of Cyperusrotundus against some pathogenic bacteria and fungi.

MATERIAL AND METHODS

Chemicals and Plant collection

The following ingredients were used for the preparation of nutrient agar media and Potato dextrose media: Agar, Peptone, Sodium chloride. Beef extract, Potato, dextrose water. All other chemicals and analytical reagents were purchased from Hi-media, India, unless stated otherwise. Mature plants of *Cyperusrotundus,* used for this study was collected from Field area of yavatmal district (M.S.) India.

ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

Preparation of the plant extract

The fresh plants were collected from Field area of yavatmal district (M.S.) India and identified with the help of flora and well known taxonomist. The roots were washed for 2-3 times with tap water and finally with distilled water. Further air dried in shade for ten days and then dried in an oven at 60°C for one to two days, and finally milled to obtained a coarse powder (Sieve no.80). About 100 grams of powdered material was extracted by maceration in chloroform (400 mL) for 14 days with frequent agitation (Freitas et al. 1991; Qaisar et al. 2012; Venkatanagaraju. 2014). The mixture was filtered through clean muslin cloth followed by double filtration with WhatmanNo.l filter paper and the filtrate was concentrated by rotary evaporation under vacuum (vacuum pressure: 500 N/m^2) al 40^0C until a volume of about 15 mL waste reached. Next the concentrate was poured into glass Petri-dishes and brought to dryness in an oven at 60°C The obtained paste like mass was then stored in paraffin. Sealed petri-dishes in a dark cabinet. The extracts were reconstituted by dissolving in chloroform 10 the required concentrations. The reconstituted extracts were maintained at 2-8°C.

Test microorganisms and growth media

Pure cultures of all experimental bacteria; *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Shigeillaflexneri*, *Streptococcus pneumonia,Klebsiella pneumonia* and fungi *Aspergillus niger* were obtained from the microbial type Culture Collection and Gene Bank Institute of Microbial Technology (IMTECH), Chandigarh. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by sub culturing regularly on the same medium and stored at 4^0C before use in experiments.

Determination of the antimicrobial activity

Agar well-diffusion method was followed to determine the antimicrobial activity (Castello et al. 2002; Didry el al, 1998; Esimone el al, 1998). Nutrient agar (gm/1: beef extract, 3g; peptone. 5g; sodium chloride, 5g; agar, 20g) and Potato Dextrose Agar (39 gm/l) plates were swabbed (sterile

ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

cotton swabs) with 24h old-broth culture (10^6-10^8 bacteria CFU ml^{-1}) of respective bacteria and fungi. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of plant extract was prepared at a concentration of 100 mg/mL About 100 ml of plant extracts was added with sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without plant extract.30ug/ml chloramphenicol, and 30ug/ml miconazole nitrate were also used at positive controls for bacteria and fungi, respectively. The plates were incubated at 37^0C for 24h for bacteria pathogens and 37°C for 48h fungal pathogens. The diameter of the inhibition zone (mm) around each well was measured and express as antimicrobial activity. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Statistical analysis

The results of the experiment are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple pair wise comparison tests to assess the statistical significance.

RESULTS AND DISCUSSION

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growlh of microorganism (Kelmanson et al. 2000; Ahmad et al, 2001), These compounds have significant therapeutic application against human pathogens including bacteria. fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Guleria, 2006; Zakaria et aL 2007). Therefore, medicinal plants are finding their way into pharmaceuticals, neutralceuticals and food Supplements.

In the present investigation, the inhibitory effect of *Cyperusrotuntus*roots m

chloroform extract was evaluated against both fungal and bacterial strains. The antimicrobial activity was determined by using agar well diffusion method and the results as summarized in Table 1.Methanolic extract (100.00 mg/ml) of the fruits displayed good antibacterial activity against

ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

Table No. 1 :Cyperusrotundus Root the show Zone of Inhibition (mm)

Microorganisms	**Zone of Inhibition (mm)**		
	Chloroform	**Chloramphenicol**	**Miconazole nitrate**
Staphylococcus aureus	10.46 ± 0.14	12.12 ± 0.14	ND
Escherichia coli	13.12.± 0.18	20.10.± 0.22	ND
Aspergillus niger	16.24 ± 0.11	ND	24.12 ± 0.20
Salmonella typhimurium	8.06 ± 0.28	10.05 ± 0.12	ND
Shigellaflexneri	4.12.± 0.22	12.10 ± 0.16	ND
Streptococcus pneumoniae	8.20 ± 0.16	14.16 ± 0.10	ND
Klebsillapneumoniae	6.02 ± 0.22	10.18 ± 0.32	ND
Pseudomonas aeruginosa	10.22 ± 0.12	14.10 ± 0.26	ND

ND: Not determined. The inhibition zone diameter was taken as an average value of triplicate plates for each microorganism at 100 uL of 100 mg/ml crude extract, 30 ug/ml of chloramphemcol and 30 ug/ml of miconazole nitrate.

Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus, Shigellaflexneri, Streptococcus pneumonia, Klebsiellapneumoniae and fungi *Aspergillus niger*.chloroform extract inhibited the growth of all tested microorganisms with large zones of inhibition ranged from 4.12 ± 0.22 - 16.24 ± 0.11 mm.The standard antibiotics chloramphenicol and miconazole nitrate were found to have zone of inhibitions 10.05 ± 0.12-24-12 ±0.20 mm at the concentration of 30 ug/ml In contrast, the inhibition zone of chloroform l (negative control) was almost zero for all The tested microorganisms. The large inhibition zones exhibited by the extract against *Aspergillus niger*justificd the plant use in the treatment of fungal infections.

CONCLUSION

Bacterial and fungal infections can be treated with the *Cyperusrotundus,*since it exhibited favourable antibacterial and antifungal activities. On the basis of the present study, further phytochemical and pharmacological studies will be needed to isolate the bioactive

ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

compound(s) and investigate the antimicrobial activities against a wider range of pathogenic microorgnisms.

ACKNOWLEDGEMENTS

The authors are grateful to the Principal BharatiyaMahavidyalaya, Amravati for providing laboratory facilities and support for this research work.

REFERENCES

Acharyya S, Rathore DS, Sundeep Kumar HK, Panda N (2011) Screening of *Anhocephaluscadamba*(Roxb.)Miq. Root for Antimicrobial and Anmdmintic activities. Int J of Res Pharma Biomed Sci2(l):2229-2234

Ahmad I, Beg AZ (2001) Antimicrobial and phytochemical studies on 45 Indian medicinal plants inst multiple drug resistant human pathogens. J EthanoPharma74:l 13-123

Alagesaboopathi C (2011) Antimicrobial Potential and Phytochemical Screening of *Andrographisaffinis*Nees an Endemic Medicinal Plant from India.Int J of Pharma and Pharmaceutical Sci3 (2):I57-159

Alzoreky NS, Nakahara K (2003) Antibacterial activity of extracts from some edible plants commonly consumed in Asia, Int J Food Microbio180:223-230

Ambhore J. S. (2014) Some Ethinomedicinal Plants Used by Triabls in Shriwardhan Taluka of Raigad District (M.S) Multilogic in Science,2014; 2 (7): 17-21

Caslello MC, Phatak A, Chandra N. Sharon M (2002) Antimicrobial activity of crude extracts from plant parts and corresponding of *Bixaorellana L.* Indian J ExpBiol40(12):l378-1381

Didry N, Durcuil L, Trotin F, Pinkas M (1998) Antimicrobial activity of the aerial parts of *Droserapellata*on oral bacteria. J Ethno Pharmacol 60:91-96

Dubey R, Dubey K, Sridhar C, Jayaveera KN (2011) Human Vaginal Pathogen Inhibition Studies on Aqueous, Methanolic and Saponins Extracts of Stem Barks of *ZiziphusMauritiana.* Int J Pharm SciRes2(3): 659-663

Enne VI, Livermore DM, Stephens P (2001) Hal LMC Persistence of sulphonamide resistance in *Escherichia coli* in the UK despite national prescribing restriction. The Lancet 28:1325-1328

Esimone CO, Adiutwu MV, Okonta JM (1998) Preliminary antimicrobial screening of the ethanolic extract from the lichen *Usneasubfloridans*(L). J Pharmaceutic Res Dev 3(2):99-102

ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

Freeman CC (2005) Coccoloba (Polygonaceae) in Flora of North America Norih of Mexico, New York and Oxford 5(2):483-484

Freitas JC. Presgrave FF, Fingola MA (1991) Toxicological study of the molluscicidal latex of *Euphorbia splendens*irritant action on skin and eye. Paumganten, Memorias do InsitutoOswaldo Cruz87:88

Guleria S, Kumar A (2006) Antifungal activity of some Himalayan medicinal plants using direct bio autography. J Cell Mol Bio 5:95-98

Hussain H, Badawy A, Elshazly A, ELsayed A, Krohn K. Riaz M. Schulz B (2011) Chemical Constituents and Antimicrobial Activity of *Salix subserrata.* Rec Nat Prod 5(2):33-37

Igoli JO, OgajiTA,Tor-Anyiin INP (2005) Traditional Medicine Practice amongst the Igede People of Nigeria. Part II. Afr J TradComplAltern Med 2:134-152

Kamali HH, Amir EL (2010) Antibacterial Activity and Phytochemical Screening of Ethanolic Extracts Obtained from Selected Sudanese Medicinal Plants. Curr Res J of Bio Sci2(2): 1 43-146

Ladda R. G, Aradwad R. P, Ambhore J. S., (2011) Studies on herbal medicinal plants in Marathwada region (MS) India. Bioscience Discovery, 2013;4(2):211-213.

6. Ladda R.G., Ambhore J.S. &Aradwad R.P. (2013)Studies On Identification of Traditional Medicinal Plants Used as Remedies On Piles by Traditional Practitioners. International Journal of Science and Nature.2013;4 (1):212-213

7. Ladda R.G. and Ambhore J.S. Use of Medicinal Plants in Remedies On Jaundice by Traditional Ayurvedic Practitioners in Maharashtra. Multilogic in Science.2014;7 (2) ;51-52

Kelmanson JR, Jager AK, VaanStaden J (2000) Zulu medicinal plants with antibacterial activity, J EthanoPharmacol 69:241-246

Kirn H, Park SW. Park JM, Moon K.H. Lee CK (1999) Screening and isolation of antibiotic resistance inhibitors from herb material Resistant Inhibition of 21 Korean plants. Nai Prod Sci 1:50-54

Laliiha P, **Araihi KA,** Shubashini K, Hemalatha, S, Jayamhi P (2010) Antimicrobial Activity and Phytochemical Screening of an Ornamental Foliage Plant *Pothosaurea*(Linden ex Andre). An InUofChemll2):63-7l

Nascimento GGF, Lacatelli J, Freitas PC, SilvaGL (2000) Antibacterial activity of plant extracts and phyto chemicals on antibiotic-resistant bacteria. BrazJ Microbiol31:886-891

ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

PankajG.Kaushik P (2011) In *vitro* Evaluation of Antibacterial Activity of Various Crude Leaf Extracts of Indian Sacred Plant, *Ocimum sanctum L.* British Microbiology) Research Journal 1(3):70-78

Preethi R. Devanathan VV, Loganalhan M (2010) Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants against Food Borne Pathogens.Adv in Bio Res 4(2): 122-125

Qaisar M, Gilani SH Farooq S. Rauf A, Pen'eez S (2012) Preliminary Comparative Phylochemical Screening of *Euphorbia Species.*American-Eurasian Journal of Agricultural & Environmental Sciences 12(8):1056-I060

Ram AJ, Bhakshu LM, Raju RRV (2003) In vitro antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases, J Ethno Pharmacol90:333-357

Shailili Moreno M, Oscar Crescente V. William Henriquez G, Gustavo Liendo P. HemandoHerrera M (2008) Three constituents with biological activity from *Coccolobauvifera*seeds. Scientific Journal of the Experimental Faculty of Sciences16(1);84-89

Sharma A (2011) Antibacterial activity of ethanolic extracts of some arid zone plants. Int J of PharmTechRes3(1):2S3-286

Venkatanagaraju E, Divakar G (2014) Antimicrobial activity of *Euphoribia*milli leaves extract. International Journal of Pharmacy Research 2(2); 135-140

Westh R Zinn CS, Rosdahl VT (2004)An international multicenler study of antimicrobial consumption and resistance in *staphylococcus aures*isolates from 15 hospitals in 14 countries, Microb Drug Resist10:169-176
Zakaria Z, Sreenivasan S, Mohamad M (2007) Antimicrobial Activity of *Piper ribesaides*Root Extract against *Staphylococcus aureus.* J App BiolSci 1(3):87-90
hitp://plantyflor-blogspot.in/2010/11/coccoloba-uvifera.html (25/04/2014)

Table No. 1 :Cyperusrotundus Root the show Zone of Inhibition (mm)

Microorganisms	**Zone of Inhibition (mm)**		
	Chloroform	**Chloramphenicol**	**Miconazole nitrate**
Staphylococcus aureus	10.46 ± 0.14	12.12 ± 0.14	ND
Escherichia coli	13.12.± 0.18	20.10.± 0.22	ND
Aspergillus niger	16.24 ± 0.11	ND	24.12 ± 0.20
Salmonella typhimurium	8.06 ± 0.28	10.05 ± 0.12	ND
Shigellaflexneri	4.12.± 0.22	12.10 ± 0.16	ND

ISSN: 2278-4632
Vol-10 Issue-6 No. 15 June 2020

Streptococcus pneumoniae	8.20 ± 0.16	14.16 ± 0.10	ND
Klebsillapneumoniae	6.02 ± 0.22	10.18 ± 0.32	ND
Pseudomonas aeruginosa	10.22 ± 0.12	14.10 ± 0.26	ND

ND: Not determined. The inhibition zone diameter was taken as an average value of triplicate plates for each microorganism at 100 uL of 100 mg/ml crude extract, 30 ug/ml of chloramphemcol and 30 ug/ml of miconazole nitrate.

