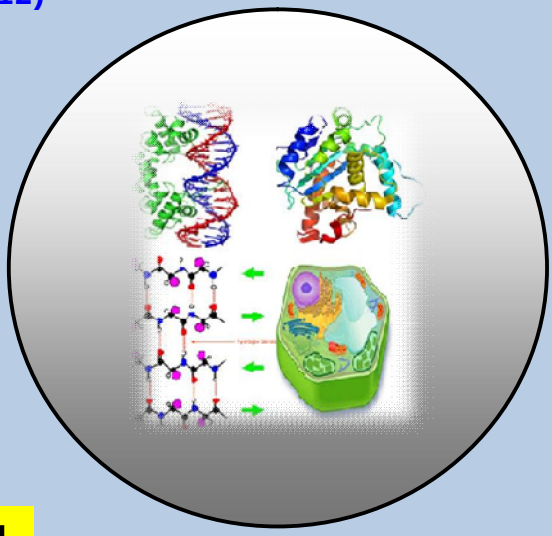
The *in Vitro* Antimicrobial Activity of Different Parts of *Corchorus olitorius* Extracts

By

Mahboob Ali Kalhoro, Tehmina Sohail, Sadia Farheen, Hina Imran, Beena Naqvi, Zahra Yaqeen and Yaqeenuddin

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Index Copernicus International Value
IC Value of Journal 4.21 (Poland, Europe) (2012)
Global Impact factor of Journal: 0.587 (2012)
Scientific Journals Impact Factor: 2.597



J. Biol. Chem. Research
Volume 31 (2) 2014 Pages No. 769-776

Journal of Biological and Chemical Research
(An International Journal of Life Sciences and Chemistry)

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 31, No. 2: 769-776 (2014)
(An International Journal of Life Sciences and Chemistry)

ISSN 0970-4973 (Print)
ISSN 2319-3077 (Online/Electronic)

Dr. Mahboob Ali
http:// www.jbcr.in
jbiolchemres@gmail.com
info@jbcr.in

RESEARCH PAPER
Received: 01/05/2014 Revised: 29/05/2014 Accepted: 02/06/2014

The *in Vitro* Antimicrobial Activity of Different Parts of *Corchorus olitorius* Extracts

Mahboob Ali Kalhoro, Tehmina Sohail, Sadia Farheen, Hina Imran, *Beena Naqvi, Zahra Yaqeen and Yaqeenuddin
Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi, Pakistan
***Food and Marine Resources Research Centre, PCSIR Labs Complex, Karachi, Pakistan**

ABSTRACT

The present study was conducted to investigate the antimicrobial activity of ethanol extracts of root, seed, leaf and stem of Corchorus olitorius (Linn). Theses extracts were tested for their antimicrobial activity by agar well diffusion assay against Staphylococcus aureus, Streptococcuc intermedius, Bacillus cereus, Bacillus pumilus Echrichia coli, Proteus mirabilis and Candida albican. Antimicrobial activity was evaluated at 100, 50, 25and 10mg/ml concentration of ethanol extract. Chloramphenicol was used as std. drug. The sensitivity of microorganisms to all the parts of plant was compared with each other and with standard antibiotic. Among all parts of plant ethanol extract of C. olitorius seed exhibited highest antibacterial effect against all of the microorganisms tested. Effectively was increased with concentrations and highest antibacterial activity was observed against C. albican with zone of inhibition of 29.16mm at 100mg/ml. Ethanol extract of C. olitorius roots presented good antibacterial activity with highest zone of inhibition of 20.5mm against S. aureus at 100mg/ml while leaf part of plant exhibited least antibacterial activity.
Keywords: Corchorus olitoriu, Antimicrobial activity, Chloramphenicol and Microrganisms.

Published by Society for Advancement of Science®

INTRODUCTION

During last decade, the rapid use of traditional medicines for primary health care of people in developing countries is increasing rapidly. The herbal medicines serve the health needs of about 80% of world's population especially for the people in the rural areas of developing countries [W. H. O, (2001)]. In view of this, need of new antimicrobial agents from medicinal plants are even more urgent in developing countries where infectious diseases and causative agents are developing an increase in resistance against commonly used antibiotics. Antimicrobial drug resistance in bacterial pathogens is a worldwide problem and as consequences control of these pathogenic organisms and its effective treatment remains as an important challenge [C.Kamaraj, A.A. Rahuman, C.Siva, M.Iyappan, and A.V. Kirthi (2012), and T.Sohail, Z.Yaqeen, H.Imran, Z.Rehman and N.Fatima (2013)].

Corchorus olitorius belongs to family *Tiliaceae* is an annual herb with selender stems known as a fiber crop. *C. olitorius* (jute) is a native plant of Tropical Africa, Asia, and has since spread to Australia, South America and some parts of Europe. It has different local names in different languages such as Tossa jute, Jute mallow, jews mallow, Bush okra and West African sorrel [G.Oboh, H.Raddatzand T.Henle, (2009) and A.Z.Tulio Jr ,K. Ose ,K. Chachin ,Y. Ueda, (2002)]. This crop is cultivated for jute production and its mucilaginous leaves are used in food as a vegetable [R.D.Meikle, (1977)].

In Africa and Asia *C. olitorius* leaves, seeds and roots are used as folk medicine. Traditionally its leaves are used in the treatment of pain, fever, tumor, chronic cystitis, gonorrhea, dysuria and toothache. Its cold infusion is used as a tonic to increase appetite and strength [D.K.Pal, M.Mandal, G.P.Senthilkumar, A.Padhiari (2006), J.Ndlovu, and A.J. Afolayan, (2008) and A.sharaf and S.A.R.Negm (2005)]. *C. olitorius* leaves contain antioxidative phenolic compounds, of which 5-caffeoylquinic acid is the most important. The mucilaginous polysaccharide in the leaves is rich in uronic acid (65%) and consists of rhamnose, galactose, glucose, galacturonic acid and glucuronic acid in addition to 3.7% acetyl groups. Jute fibre contains 45–84% α-cellulose, 12–26% hemicelluloses, 5–26% lignin, 0.2% pectin and 1–8% ash. The composition of *C. olitorius* leaves per 100g fresh edible portion is: water 80.4g (74.2–91.1), energy 243 kJ (58 kcal), protein 4.5g, fat 0.3g, carbohydrate 12.4 g, fiber 2.0g, Ca 360mg, P 122mg, Fe 7.2 mg, β-carotene 6410 µg, thiamin 0.15mg, riboflavin 0.53mg, niacin 1.2mg, ascorbic acid 80mg [W.-T.W .Leung, F.Busson, and C. Jardin (1968)]. Seeds are used as purgative and have been reported to possess estrogenic activity [M.Gupta ,U.K. Mazumder ,D.K. Pal and S. Bhattacharya (2003)]. On preliminary analysis seeds have been found to contain high content of hydrogen cyanide and cardenolide glycosides [T.Nakamura ,Y. Goda ,S. Sakai ,K. Kondo ,H. Akiyama and M. Toyoda (1998)]. Aqueous extract of seeds is reported to possess peripheral and central antinociceptive, anti-inflammatory and antipyretic activities [Z.A.Zakaria, C.A.Corazon, H.Asma, M.R.Sulaiman, A.K.Arifah, M.N.Somchit, A.M.MatJais, R.Johari, K.Kirisnaveni, D.Punnitharanni, M.Safarul and R.Valsala.(2005)].

Plant is found use full to man not only as food or as source of raw material for industry but also as a source of medicine [C.Azoro (2004), O.Erturk, H. Kati ,N. Yayli and Z. Demilrbag (2006)].
The purpose of this study was to determine and compare the antimicrobial activity present in different parts of *C. olitorius* against some microorganisms which are important pathogen and resistant to many antibiotics.

MATERIAL AND METHODS

Plant collection and authentication

The dried leave seed and root parts of *Corchorus olitorius* was purchased from local market of Karachi, Pakistan and was properly identified by Dr. Beena Naqvi, Plant taxonomist, Food and Marine Research Center, PCSIR Labs Complex, Karachi.

Preparation of plant extract

Dried leave, root and seed parts of *Corchorus olitorius* were washed and allow to air dry at room temperature.100 g of each part of plant was soaked separately in 70% ethanol (1 Liter) and kept at room temperature for 3 days. After every 24hs mixture was stirred by using a sterilized glass rod. The socked material was then filtered and evaporated on rotary evaporator at 45ºC under reduced pressure. Recovered solvent was again used for percolation for another three days. The process was repeated three times to obtain crude extract [P. I. Alade, and O.N. Irobi, (1993)].

Test Microorganisms

The antimicrobial activity was assessed against six bacteria *Staphylococcus aureus, Streptococcuc intermedius, Bacillus cereus, Bacillus pumilus*, *Echrichia coli, Proteus mirabilis* and one fungi *Candida albican.* All microorganisms used in the present study were clinically isolated and maintained on tryptic soya agar slants at 4ºC prior to testing.

Culture media and Inocula preparation

Tryptic soya broth and Tryptic soya agar (Merck) were used for microbial growth. 24hours old microbial cultures were appropriately diluted in sterile normal saline to obtain the cell suspension at 10^6 CFU/ ml.

Preparation of Solution

The crude ethanol extracts of seed, root and leaf parts of *C. olitorius* were dissolved in dimethylsulfoxide (DMSO) to give strength of 100mg/ml from which further dilutions of 50mg/ml, 25mg/ml and 10mg/ml were made in the same solvent. Chloramphenicol was used as reference standard (positive control) while DMSO used as negative control.

Antibacterial activity assay

The antimicrobial activity was carried out by agar well diffusion method [I.Ahmed, Z. Mehmood and F.Mohammad (1998)]. According to this method, 0.1 ml of diluted inoculums (10^6 CFU/ ml) of test organism was thoroughly mixed with 20 ml of molten sterile tryptic soya agar and poured in to pre-sterilized petri dishes under sterile condition. All plates were left to set at room temperature for 30-40 minutes.

A well of 6 mm diameter was made in the center of each seeded plates by using sterile cork borer. Holes were then filled aseptically with 0.1 ml of each concentration of test solutions (crude ethanol extract). Chloramphenicol is used as positive control. Negative control was done by DMSO. Antibacterial plates were incubated at 37ºC ±1ºC for 24 hours, antifungal assay plates were incubated at 28^{o}C ±2^{o}C for 48 hours. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the well. The diameter of inhibition zone was measured in millimeters by Vernier caliper. All tests were repeated three times to minimize test error.

Statistics

The data are analyzed as mean±S.E.M and compared by applying one way Anova Sigma Plot software version 11.0. The *p* value < 0.05 is considered as significant.

Result and Discussion:

Crude ethanol extracts of seeds leaves and roots of *C. olitorius* exhibited a varied degree of antimicrobial activity against wide range of microorganisms including *Staphylococcus aureus, Streptococcuc intermittence, Bacillus cereus, Bacillus pumilus, Echrichia coli, Proteus mirabilis* and one fungi *Candida albicans.* The results of antimicrobial activity are presented in Table-1, 2 and 3. DMSO is taken as negative control, which exhibited antimicrobial activity against gram-positive and gram negative bacteria up to 10mm zone of inhibition. Hence a zone of inhibition of 10mm and less is considered as no activity.

Table 1. Antibacterial activity of ethanol extracts of *C. olitorius* seed.

Sr. No.	Name of organism	100mg/ml	50mg/ml	25mg/ml	10mg/ml	Chloramphenicol Std.(10mg)	DMSO Negative control.
1	Staphylococcus aureus	28.83±1.04*	21.83±0.28*	20.16±0.76*	12.33±0.76*	32.16±2.75*	7.5±0.5
2	*Bacillus pumilus*	26.5±0.86*	18.16±1.25*	14.5±0.5*	9.3±1.25	28.16±0.28*	8±0.5
3	*Bacillus cerus*	28.83±0.5*	25.2±1.05*	18.16±1.04*	11.6±1.04*	29±0.5*	7.3±0.28
4	*Eschrichia coli*	26.25±0.5*	20.16±1.25*	15.5±.86*	6.6±.76	32.16±2.25*	7.76±0.25
5	*Streptococcus intermitance*	26.5±0.5*	21.53±.50*	15.56±.50*	6.7±.51	27.16±0.76*	7.83±0.76
6	*Clostridium sporangens*	25.1±0.36*	20.66±0.5*	17.83±.0.76*	11.33±0.5*	28.5±0.5*	7.36±0.32
7	*Candida albican*	29.16±0.76*	25.33±1.04*	19.33±0.76*	13.5±0.86*	32.33±2.51*	8.1±0.36

Results express as mean± stdev. * *p*< 0.005, **

This study not only indicates the presence of antibacterial substances in the different parts of *C. olitorius* but also shows comparison of antimicrobial activity among these parts. Among all three extracts, ethanol extract of seeds exhibited highest antibacterial activity with a zone of inhibition ranging from 25.10mm to 29.16mm at concentration 100mg/ml against *Staphylococcus aureus, Streptococcuc intermittence, Bacillus cereus, Bacillus pumilus, Echrichia coli, Proteus mirabilis* and *Candida albicans.* Significant activity was observed at 50mg/ml and 25mg/ ml with zone range of 18.16mm to 25.2mm and 14.5mm to 20.16mm respectively. At 10 mg /ml concentration mild activity was observed against all microorganisms tested ranging from 6.6mm to 12.33mm zone of inhibition.

Table 2. Antibacterial activity of ethanol extracts of *C. olitorius* roots.

Sr. No.	Name of organism	100mg/ml	50mg/ml	25mg/ml	10mg/ml	Chloramphenicol Std.(10mg)	DMSO Negative control.
1	*Staphylococcus aureus*	20.5±0.5*	15.83±0.76*	11±0.5*	6.83±0.28	32.16±2.75*	7.5±0.5
2	*Bacillus pumilus*	19.43±0.60*	14.5±0.5*	9.33±1.25	6.7±0.25	28.16±0.28*	8±0.5
3	*Bacillus cerus*	20±0.5*	13.33±1.04*	8.66±0.76	6.9±0.36	29±0.5*	7.3±0.28
4	*Eschrichia coli*	18.03±0.45*	13.23±0.75*	9.6±0.76	6.13±0.63	32.16±2.25*	7.76±0.25
5	*Streptococcus intermitance*	17.83±.0.28*	11.76±0.64*	8.3±0.32	6.76±0.25	27.16±0.76*	7.83±0.76
6	*Clostridium sporangens*	15.1±.36*	10.5±0.5	7.1±0.36	-	28.5±0.5*	7.36±0.32
7	*Candida albican*	18.83±0.76*	14.06±0.40*	9.6±0.76	6.9±0.36	32.33±2.51*	8.1±0.36

Results express as mean± stdev. * $p< 0.005$, **

Ethanol seed extract showed highest antimicrobial activity at 100mg/ml against *S. aureus* i.e. 29.16mm. These findings are at variance to another study which reported that methanol seed fraction of *C. olitorius* exhibited a broad spectrum of antibacterial activity at concentration ranges 50-150ug/ml while other fractions (petroleum ether, chloroform) had no such activity [A.H.S.Abou Zeid (2002)]. These variations in results are may be due to the solvent used in extraction. The polarity of solvents used for extraction is reported to play a significant role in the exhibition of antimicrobial activity of plant extracts [J.Parekch, N. Karathia and S.Chanda, (2006)].

Table 3. Antibacterial activity of ethanol extract of *C. olitorius leaves*

Sr. No.	Name of organism	100mg/ml	50mg/ml	25mg/ml	10mg/ml	Chloramphenicol Std.(10mg)	DMSO Negative control.
1	*Staphylococcus aureus*	19.5±0.5*	15.66±0.76*	11.16±1.04*	6.83±0.28	32.16±2.75*	7.5±0.5
2	*Bacillus pumilus*	16.16±1.25*	13.16±0.76*	10.18±0.76*	----------	28.16±0.28*	8±0.5
3	*Bacillus cerus*	22.16±0.76*	18.33±1.04*	10.83±1.04*	7.06±.11	29±0.5*	7.3±0.28
4	*Eschrichia coli*	18.76±0.75*	12.83±0.76*	10.33±0.76*	6.66±0.28	32.16±2.25*	7.76±0.25
5	*Streptococcus intermitance*	12.16±0.28*	9.5±0.60	8.1±0.28	---------	27.16±0.76*	7.83±0.76
6	*Clostridium sporangens*	10.83±1.04	----------	---------	----------	28.5±0.5*	7.36±0.32
7	*Candida albican*	18.66±0.76*	13.16±0.28*	11±0.5*	6.7±0.25	32.33±2.51*	8.1±0.36

Ethanol root extract showed significant antimicrobial activity at 100 mg/ml concentration against all microorganisms used ranging from 15.1mm to 22.16mm and moderate activity was found at 50mg/ml concentration with zone range of 10.5mm to 15.83mm. At 25mg/ml and 10mg/ml all microorganisms used showed zone of inhibition less than 10mm that considered as no activity except *S. aureus* which exhibited zone of inhibition of 11.0mm.

The ethanol extract of leaves showed significant activity against all bacteria tested at 100mg/ml except *S. intermittence* and *C. sp*orangens which showed mild activity i.e. 12.16mm and 10.83mm respectively. It is also reported in another study that ethanol extract of leaves possessed antibacterial activity at very high concentrations (150 and 100mg/ml). This mean that the active compound of *C. olitorius* is present in very low amount thus requiring the use of large amount of the crude extract [A.J.Senu, E.M. Olusola, O.O Afolashade and B.S. Ajoke, (2012)]. The presence of antibacterial activity in *C. olitorius* was supported by previous workers who reported that *C. olitorius* is used as potential antibacterial agent against the infection of *C. diphtheria, S. aureus, B. cereus, S. epidermidis* and *K. rhizophila* [Z.A.Zakaria, M.N. Somchit, H. Zaiton, A.M. Mat-Jais, M.R. Suleiman, W. Farah, R. Nazaratul Marawana and C.A.Fatimah, (2006)]. Leaf extract also exhibited the strong activity against yeast *C. albican* with zone size 18.66mm. These results are in alignment with work of another scientist who reported that petroleum ether extract of *C. olitorius* leaves exhibited strongest inhibition activity against *C. albican* [I.Semra, S. Piliz and C. Ferdag, (2007)]. The presence of coumarin compounds in *C. olitorius* leaves may be responsible for this inhibition effect. The coumarine compounds and volatile compounds present in leaves are effective against microorganisms [S.Burt (2004)].

At 50mg/ml leaf extract exhibited moderate activity with zone size ranging from 9.5 to 18.33 except *clostridium sporangens* which showed resistance. The extract showed very mall zones of inhibition at 25mg/ml ranging from 8.1mm to 11.16mm which are negligible and at 10mg/ml all microorganisms showed resistance.

CONCLUSION

The present study indicated that crude ethanol extract of leaf, root and seed part of *C.olitorus* showed different degree of antimicrobial activity against the microorganisms used. Seed part was found most effective among all parts. Antibacterial activity of seed extract was equivalent to traditional antibiotic used to treat infections caused by the pathogenic microorganisms tested. It is also concluded that selection of proper solvent can enhance the specific activity. This plant could be a source of new antibiotic compounds. Further work is required to isolate the secondary metabolites from the extracts studied in order to find specific antibacterial activity.

ACKNOWLEDGEMENTS

The authors are thankful to Mr. Muhammad Haris for the collection/Purchase of samples.

REFERENCES

A.H.S. Abou Zeid, 2002. "Stress metabolites from Corchorus olitorius L. leaves in response to certain stress agents," Food Chemistry, 76,187

A.J. Senu, E.M. Olusola, O.O Afolashade and B.S. Ajoke,2012. "*Globabal Research Journal of Microbiology*," 2,113.

Asharaf and S.A.R. Negm, 2005. "Pharmacological study of Corchorus olitoriusL. Seed with special reference to its cardiovascular activity," Plant Foods for Human Nutr, 17 (4),305.

A.Z.Tulio Jr, K. Ose, K. Chachin and Y. Ueda, 2002. "Effects of storage temperatures on the postharvest quality of jute leaves (Corchorus olitorius L.)," Postharvest Biology and Technology, 26,329.

C. Kamaraj, A.A. Rahuman, C. Siva, M. Iyappan, and A.V. Kirthi, 2012. "Evaluation of antibacterial activity of selected medicinal plant extracts from south India against human pathogens," Asian *Pac J Trop Dis.,* S,296.

C. Azoro, 2004. "Towards effective integration of herbal medicine into Nigerian medical practice," Nigerian J Res. Production, 3,132.

D.K. Pal, M. Mandal, G.P. Senthil Kumar and A. Padhiari, 2006. "Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed," *Fitoterapia*, 77,589.

G. Oboh, H. Raddatz and T. Henle, 2009. "Chracterization of antioxidant properties of hydrophilic and lipophilic axtracts of jute(Corchorus olitorius)leaf," Int J Food Sci *Nutr* ,2,124.

I. Ahmed, Z. Mehmood and F. Mohammad, 1998. "Screening of some Indian medicinal plants for their antimicrobial properties," J Ethnopharmacol, 62,183.

I. Semra, S. Piliz and C. Ferdag, 2007. "Antibacterial and Antifungal Activity of Corchorus olitorius L. (Molokhia) Extracts," IJNES, 3, 39.

J. Ndlovu and A.J. Afolayan, 2008. "Nutritional analysis of the south African wild vegetable Corchorus olitorius," Asian J Plant Sci, 7 (6): 615.

J. Parekch, N. Karathia and S.Chanda, 2006. "Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegate* L.bark," Afr.J.Biomed Res, 9, 53.

M. Gupta, U.K. Mazumder, D.K. Pal and S. Bhattacharya, 2003. "Onset of puberty and ovarian steroidogenesis following adminstration of methanolic extract of Cuscuta reflexa Roxb. stem and Corchorus olitorius Linn. seed in mice," J. Ethnopharmacol , 89, 55.

O. Erturk, H. Kati, N. Yayli and Z. Demilrbag, 2006. "Antimicrobial properties of silence multifida (Adams) rohrb.Plant extract," Turkish J.Biol, 17.

P. I. Alade, and O.N. Irobi ,1993. "Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana," J. Ethnopharmacol, 39, 171.

R.D. Meikle, 1977. "In *Flora of Cyprus*," Vol.1,Bentham-Moxon Trust, p. 832.

S. Burt, 2004. "Essential oils: their antibacterial properties and potential applications in foods-a review," INT J FOOD MICROBIOL, 94, 223.

T. Nakamura, Y. Goda, S. Sakai, K. Kondo, H. Akiyama and M. Toyoda,1998. "Cardenolide Glycosides from Seeds of Corchorus olitorius," Phytochemistry, 49, 2097.

T. Sohail, Z. Yaqeen, H. Imran, Z. Rehman and N. Fatima, 2013. "Effect of metal salts on antibacterial activity of *Zingiber officinale* extract," J. Chem. Soc. Pak, 35, 804.

World Health Organization, 2001. "General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine," WHO, Geneva, Switzerland, p.1.

W.T.W. Leung, F. Busson and C. Jardin, 1968. "Food composition table for use in Africa," FAO, Rome, Italy, 306 pp.

Z.A. Zakaria, C.A. Corazon, H. Asma, M.R. Sulaiman, A.K. Arifah, M.N. Somchit, A.M. MatJais, R. Johari, K.Kirisnaveni, D. Punnitharanni, M. Safarul and R. Valsala, 2005. "The analgesic, anti-inflammatory and antipyretic activities of *Corchorus olitorius* in mice," Poster oresented at 20th Scientific Meeting of the Malaysian Society of Pharmacology and Physiology, 25-27th April 2005,

Z.A. Zakaria, M.N. Somchit, H. Zaiton, A.M. Mat-Jais, M.R. Suleiman, W. Farah, R. Nazaratul Marawana and C.A. Fatimah, 2006. "The invitro antibacterial activity of Corchorus olitorius Extracts," Int. J. of Pharmacology, 2 (2), 2013.

Corresponding author: Dr. Mahboob Ali Kalhoro, Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi, Pakistan
Email: kalhoro786pk@yahoo.com