International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 2 (2): 213-215, 2006
ISSN 1811-7775

The *in vitro* Antibacterial Activity of *Corchorus olitorius* Extracts

[1,2]Z.A. Zakaria, [2]M.N. Somchit, [3]H. Zaiton, [2]A.M. Mat Jais, [2]M.R. Sulaiman,
[1]W.O. Farah, [1]R. Nazaratulmawarina and [1]C.A. Fatimah
[1]School of Biotechnology and Life Sciences, Universiti Industri Selangor,
Jalan Zirkon A7/A, Seksyen 7, 40000 Shah Alam, Selangor, Malaysia
[2]Department of Biomedical Sciences, Faculty of Medicine and Health Science,
[3]Department of Food Technology, Faculty of Food Science,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract: The presence study was carried out to investigate on the possible antibacterial activity of methanol (MECO) and chloroform (CECO) extracts of *Corchorus olitorius* (Senaung betina) using the *in vitro* disc diffusion methods. The sterilized blank discs (6 mm diameter) was impregnated with 20 μL of the respective extract (in the concentration of 10,000; 20,000; 40,000 and 50,000 ppm) and tested against *Corneybacterium diphtheria*, *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Kosuria rhizophila*, *Shigella flexneri*, *Escherichia coli* (O 157), *Aeromonas hydrophila* and *Listeria monocytogenes*. The MECO and CECO, at all concentrations, were effective against *C. diptheria* and *K. rhozophila* with the latter also effective against *S. aureus* and *B. cereus*. At the concentration of 40,000 ppm and above, the MECO was effective only against *S. aureus*, *B. cereus* and *S. epidermidis* while the CECO was effective against *S. epidermidis, S. flexneri* and *A. hydrophila*. Based on this study, it was concluded that *C. olitorius* possesses antibacterial activity that is comparable to some of the standard antibiotics.

Key words: *Corchorus olitorius*, antibacterial activity, methanol extract, chloroform extrac, disc diffusion method

INTRODUCTION

Corchorus olitorius L., locally known to the Malaysia's Sabahan as Senaung betina, is an annual herb that belongs to the family Tiliaceae. Its leaves and roots eaten are as herbal medicine and as vegetable by local people in various part of middle and south east Asia[1]. Traditionally, its leaves are used in the treatment of gonorrhea, chronic cystitis, pain, fever and tumours[1, 2].

Recent study has demonstrated that the aqueous extract of *C. olitorius* possesses peripheral and central antinociceptive activities that might be mediated by the opioid receptor and enhanced at high temperature[3]. Further studies have also proven that *C. olitorius* possess anti-inflammatory and antipyretic activities[4] and that its antinociceptive activity is influenced by pH and α-amylase[5].

Based on the fact that there are no scientific research reporting on the antibacterial activity of this plant, we decided to take this opportunity to screen for its potential antibacterial activity. The aim of this study was to elucidate *C. olitorius* potential antibacterial activity using its methanol and chloroform extracts against a group of selected gram positive and gram negative bacteria.

MATERIALS AND METHODS

C. olitorius plants were collected from Shah Alam, Selangor, Malaysia and a voucher specimen (SK 963/04) was deposited at the Herbarium of Institute of Bioscience, Universiti Putra Malaysia, Malaysia.

Microorganisms tested in this study were *Corneybacterium diphtheria*, *Staphylococcus aureus*, *Bacillus cereus*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Kosuria rhizophila*, *Shigella flexneri*, *Escherichia coli*, *Aeromonas hydrophila* and *Listeria monocytogenes*.

Fresh leaves of *C. olitorius* were oven-dried for 24 h at 40°C according to the methods described by Somchit *et al.*[6] but with slight modifications. It was then ground into small pieces under sterilized condition and extracted with methanol or chloroform in the ratio of 1: 20 (w/v) for 24 h by using Soxhlet apparatus. The resultant extraction of both solvents was completely evaporated by using rotary evaporator machine[6]. The obtained crude extracts of methanol (MECO) and chloroform (CECO) were prepared into various concentrations (10,000, 20,000, 40,000 and 50,000 ppm) by dissolving the respective extract in

Corresponding Author: Zainul Amiruddin Zakaria, School of Biotechnology and Life Sciences, Universiti Industri Selangor, Jalan Zirkon A7/A, Seksyen 7, 40000 Shah Alam, Selangor, Malaysia
Tel: 603 55223590/603 55137959

dimethyl sulfoxide (DMSO). Twenty microliter of the respective MECO or CECO were then loaded into empty sterilized blank discs (6 mm diameter, Oxoid, UK). In addition, commercial antibiotic discs (Chloramphenicol; 30 μg μL^{-1}) were used for comparison.

Preparation of microorganism culture: The above-mentioned bacteria were incubated at 37°C±0.5 for 24 h after injection into nutrient broth. Mueller Hinton Agar (MHA) (Oxoid, UK), sterilized in a flask and cooled to 40-50°C was poured, in the volume of 15 mL, into sterilized Petri dishes (diameter of 9 cm) and allowed to harden under room temperature. This is followed by homogenous distribution of 0.1 mL bacteria cultures (10^6 bacteria per mL) onto medium in Petri dishes. Discs loaded with extracts were then positioned on the solid agar medium by pressing slightly[7]. Petri dishes were placed in incubator according to their respective growth temperature and condition for 18 to 24 h. At the end of the period, inhibition zones formed was measured in mm. The study was performed in triplicate and the formation of the inhibition zones were compared with those of antibiotic discs.

RESULTS AND DISCUSSION

As can be seen from the Table 1, the MECO at the concentration between 10,000 and 50,000 ppm, was found to be effective against *C. diphtheriae* and *K. rhizophila* with the inhibition zone ranging from 14.0 to 18.0 mm, respectively. Other than the two bacteria, the CECO was also very effective against *S. aureus*. The inhibition zone of the CECO, at the concentration between 10,000 and 50,000 ppm, against *C. diphtheriae*, *K. rhizophila*, *B.cereus* and *S. aureus* ranged between 12.3 to 16.0, 12.0 to 17.0, 9.0 to 13.0 and 15.0 to 19.0 mm, respectively. The extracts high effectiveness is indicated by the presence of activity at the lowest concentration (10,000 ppm) used (Table 1).

The MECO was also found to show positive activity at the concentration of 20,000 or 40,000 ppm and above, against *B. cereus* and *S. aureus* and *S. epidermidis*, respectively. On the other hand the CECO was found to show positive inhibitory effect, at the concentration of 40,000 ppm, against *S. epidermidis*. However, the MECO and CECO extracts were found to give negative results when treated against *P. vulgaris*, *S. flexneri*, *E. coli* (O 157), *A. hydrophila* and *L. monocytogenes* (Table 1).

This study has demonstrated the potential used of *C. olitorius* as an antibacterial agent against the infection of *C. diphtheria*, *S. aureus*, *B. cereus*, *S. epidermidis* and *K. rhizophila*. The CECO was also found to be more effective against the said bacteria when compared to the MECO indicated by the presence of activity in the lowest concentration of CECO used (10,000 ppm) (Table 1).

The traditional used of *C. olitorius* for treatment of various ailments by local people in India, Egypt and Philippines[1] but lack of scientific papers to prove on those claims has lead to this study. Present preliminary study on the aqueous extract of its leaves has demonstrated a potential antinociceptive[3] and anti-inflammatory and anti-pyretic[4] activities. The reason for not using the aqueous extract in this study is because the leaves were found to release sticky mucus once soaked in DH_2O. Furthermore, the disc impregnated with the aqueous extract was found not to block but improved bacteria growth, which is observed even on the disc itself. This observation may be attributed to the extract sticky condition, which is suggested to take sometimes to dry and thus, provide enough moisture for bacteria growth. Even though we have dried the disc for one week under sterilized condition, the sticky appearance of the extract was still observed indicating the presence of moisture that might influence bacteria growth.

Even though the current study does not involved identification of the chemical constituents presence in the leaves extract, it is possible to relate the observed activity

Table 1: The antibacterial activity of methanol and chloroform extracts of *Corchorus olitorius* determined by disc diffusion method

	Concentration (ppm)							
	MECO				CECO			
Bacteria	10 K	20 K	40 K	50 K	10 K	20 K	40 K	50 K
C. diphtheriae	+++	+++	++++	++++	++	+++	+++	+++
S. aureus	-	-	++++	++++	+++	+++	++++	++++
B. cereus	-	+	++	++	+	++	+++	++
P. vulgaris	-	-	-	-	-	-	-	-
S. epidermidis	-	-	++++	+++	-	-	++	++
K. rhizophila	++	++	+++	+++	++	++	++++	++++
S. flexneri	-	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-
A. hydrophila	-	-	-	-	-	-	-	-
L. monocytogenes	-	-	-	-	-	-	-	-

IZ = Inhibition zone, - = No inhibition zone, + = IZ ≤ 9.0 mm, ++ = 9.0 mm < IZ ≤ 13.0 mm, +++ = 13.0 mm < IZ ≤ 16.0 mm
++++ = 16.0 mm < IZ ≤ 20.0 mm, Except for *P. vulgaris* (IZ = 9 mm), Chloramphenicol gave inhibition zone of ≤20 mm against all bacteria

to the presence of tannins, flavonoids and glycosides[8], which have been known to occur abundantly in leaves of all plants.

Furthermore, present results also demonstrated that the polar, expected to be found in MECO and non-polar, expected to be found in CECO, compounds to be more effective in the treatment of Gram-positive bacteria rather than Gram-negative ones.

Finally, it was concluded that present results provided a basis for isolation of antibacterial compounds of interest from *C. olitorius*. Further study is being carried out to isolate and identify the antibacterial compounds present in both extracts of *C. olitorius*.

ACKNOWLEDGEMENTS

The authors would like to thank Universiti Industri Selangor for the research grant (Project Code Number: 03018; Vote Number: 3090103018) and Universiti Putra Malaysia for the facilities.

REFERENCES

1. Zeghichi, S., S. Kallithkara and A.P. Simopoulus, 2003. Nutritional Composition of Molokhia (*Chorchorus olitorius*) and Stamnagathi (*Cichorium spinosum*). In: Plants in Human Health and Nutrition Policy (Eds. Simopoulus A.P. and C. Gopalan). Karger, Basel, pp: 1-21.
2. Abu-Hadid, A.F., M.Z. El-Shinawy, A.S. El-Bethagy, S.A. Gaafer and M. Medany, 1994. Studies on the Production of Off-season Jew's Mallow (Molokhia) in Egypt. Egypt. J. Hortic., 21: 187-193.
3. Zakaria, Z.A., M. Safarul, R. Valsala, M.R. Sulaiman, C.A. Fatimah and A.M. Mat Jais, 2005. Influence of temperature on the opioid-mediated antinociceptive activity of *Corchorus olitorius* L. in mice. Naunyn Schmiedebergs Arch. Pharmacol., (In Press).
4. Zakaria, A.Z., C.A. Corazon, H. Asma, M.R. Sulaiman, A.K. Arifah, M.N. Somchit, A.M. Mat Jais, R. Johari, K. Kirisnaveni, D. Punnitharanni, M. Safarul and R. Valsala, 2005. The analgesic, anti-inflammatory and antipyretic activities of *Corchorus olitorius* aqueous extract in mice. Poster presented at the 20th Scientific Meeting of the Malaysian Society of Pharmacology and Physiology, 25-27th April 2005, Penang, Malaysia.
5. Zakaria, Z.A., C.A. Corazon, M.N. Somchit, A.M. Mat Jais, M.R. Sulaiman, A.K. Arifah, R. Johari., S. Pubalan, M. Neelendran, M. Safarul and R. Valsala, 2005. Effects of various receptor antagonists, enzymes, temperature and pH on the antinociceptive activity of aqueous extract of *Corchorus olitorius* in mice. Poster presented at the 20th Scientific Meeting of the Malaysian Society of Pharmacology and Physiology, 25-27th April 2005, Penang, Malaysia.
6. Somchit, M.N., I. Reezal, E.I. Nur and A.R. Mutalib, 2003. *In vitro* antimicrobial activity of ethanol and water extract of *Cassia alata*. J. Ethnopharmacol., 84: 1-4.
7. Sundar, R.K., 1996. Antibacterial activity of some medicinal plants of Papua New Guinea. Intl. J. Pharmacognosy, 34: 223-225.
8. Ogunleye, D.S. and S.F Ibitoye, 2003. Studies of antimicrobial activity and chemical constituents of Ximenia americana. Trop. J. Pharm. Res., 2: 239-241.