International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-2, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Antibacterial and Antioxidant Activities of Crude Ethanolic Extract and Solvent Fractions of *Leea rubra* Stems

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Manuscript No: IJPRS/V3/I2/00290, Received On: 03/06/2014, Accepted On: 07/06/2014

ABSTRACT

Leea rubra has been used as a Lanna Traditional Medicines for Mahoog. In this study, the antibacterial and antioxidant activities of crude ethanol extract and its different solvent fractions of the *L. rubra* were evaluated (namely Hexane fraction (HF), ethylacetate fraction (EF) and water fraction (WF). The antibacterial activities on 4 strains of microorganisms i.e. *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027 were carried out by using agar diffusion technique and the antioxidant activities were determined by using 2,2´-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical scavenging assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. Among the different fractions, the EF fraction exhibited higher antibacterial activity against gram-positive and antioxidant activity than the other fractions. Thus, the results suggest that *L. rubra* is a potential source of antibacterial and antioxidants and could serve as the base for drug development.

KEYWORDS

Leea rubra, Antioxidant Activity, Antibacterial Activity

INTRODUCTION

Leea rubra Blume ex Spreng is and erect or suberect soft-wooded shrub of family Leeaceae. Root and stem of this plant has been used as a Lanna Traditional Medicines for Mahoog. Mahoog is a group of intestinal diseases[1]. The exact cause of Mahoog disease is not well established, however there are many factors that provoke this disease including half-cooked and spicy food consumptions, drinking less water and hard work.

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The characteristic symptoms of Mahoog are pain, inflammation and wound infection. Especially, when wound is occurred, it is accompanied with pain, reddening and edema within a short time, which are the classical symptoms of inflammation. These symptoms are caused by releasing of eicosanoids, prostaglandins, leukotrienes, and reactive oxygen species (ROS). Not only is ROS produced in large amount at the site of wound as a defense mechanism against invading bacteria, but also at the same time, the presence of free radicals may be hampered the process of wound healing, resulting in wound damage or microbial infection[2-3]. Therefore, the objectives of the

present study were to investigate the antibacterial and antioxidant activities of crude ethanolic extract and solvent fractions of *L. rubra* stems. The data obtained from the study will be used as a scientific evidence to support the pharmacological properties of Lanna medicinal plants.

MATERIALS AND METHOD

Plant Materials

The plants used in the work were collected from Chiang Mai provinces, Thailand. The identity of the Lanna medicinal plants were verified by a taxonomist at Faculty of Pharmacy and the voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, Chiang Mai University.

Plant Preparation and Extraction

The stems of *L. rubra* were cut into small pieces, dried at 50°C for 24 hours and then ground into powder. The powder was successively extracted with 95% ethanol by soxhlet apparatus. The extract solutions were filtered through Whatman filter paper No. 1 and then the solvents were removed by using rotary evaporator (Eyela, USA). The crude ethanolic extract was sequentially partitioned with hexane (HF), ethylacetate (EF) and water (WF) to yield the following fractions: CF, EF, and WF fractions. These fractions were evaporated to dryness by using rotary evaporator.

Antibacterial Activity

Microorganisms

Four bacteria; 2 Gram-positive and 2 Gram-negative were used for the antibacterial assays. The following strains of microorganisms were used: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 9027.

Agar Diffusion Method

Diameter of zone of inhibition was determined using agar well diffusion technique modified from Kirby-Bauer method[4]. A swab of the bacteria suspension containing 1×10^8 cfu/ml was spread on to sterile Petri dish (*Greiner* bio-one, Austria) containing Tryptic Soy Agar (TSA; Difco, MI) media. Each extract was dissolved in propylene glycol to concentration 50 mg/ml and autoclaved at 121°C, 15 psi for 15 min. Wells were cut with sterile borer (6 mm) and 50 µl of the extracts were added into the wells. The plates were incubated at 37°C for 24 hours. The propylene glycol used as negative control while the standard chloramphenicol 10 mg/ml and gentamicin 1 mg/ml were used as positive controls. Antibacterial activity was indicated by the presence of clear inhibition zone around the wells. Tests were performed in triplicate.

Minimum Inhibitory Concentration (MIC)

The MIC was determined using microbroth dilution method. The extracts were dissolved in 50% DMSO (*Sigma-Aldrich*, Germany). The extracts were diluted by two-fold to obtain a concentration range 100–0.05 mg/ml with Tryptic Soy Broth (TSB; Difco, MI) in the 96-well microplates (*Greiner* bio-one, Austria). The microorganism suspension ($1x10^5$ cfu/ml) of 50 µl was added to the broth dilutions. These were incubated for 24 hours at 37ºC. MIC of each extract was taken as the lowest concentration that did not permit any turbidity of the tested microorganism.

Antioxidant Activity

ABTS Free Radical Scavenging Assay

The ABTS assay was performed using a modified method[5]. ABTS•+ stock solution was generated by oxidation of 7.0 mM ABTS (*Sigma-Aldrich*, Germany) with 2.45 mM potassium persulfate (UNILAB, Austria). The solution was protected from light and stored at room temperature for 12-16 hrs. The 2.0 ml of ABTS•+ working solution was mixed with 100 µl dilute extract, comparing it to Trolox (*Sigma-Aldrich*, Germany). After 3 min incubation at room temperature, the color reaction was measured at 734 nm using a UV/VIS spectrophotometer (*Varian Cary 1E* UV/Visible Spectrophotometer, USA). The results of ABTS assay were expressed as Trolox equivalent

antioxidant capacity (TEAC). This index is defined as gram of standard is equivalent to 1.0 gram of the extract.

DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging assay was done according to the method of Brand-Williams *et al*.[6] with some modifications. The 2.1 milliliters of reaction mixture containing 2.0 ml ethanolic DPPH (*Sigma-Aldrich*, Germany) and 100 µl diluted extract. The mixture was incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV/VIS spectrophotometer. The results were calculated in terms of TEAC. This index is defined as gram of standard is equivalent to 1.0 gram of the extract.

FRAP Assay

The FRAP assay was done according to Benzie and Strain[7] with some modifications. The 3.1 milliliters of reaction mixture containing 3.0 ml FRAP reagent and 100 µl diluted extract. The mixture was incubated in the dark for 4 min at 37°C. The absorbance was measured at 593 nm. The results were calculated in terms of TEAC. This index is defined as gram of standard is equivalent to 1.0 gram of the extract.

RESULTS AND DISCUSSION

Antibacterial Activity

In this study, the antibacterial activities of crude ethanolic extract and solvent fractions of *L. rubra* stems were evaluated against 2 gram-positive and 2 gram-negative bacterial. Chloramphenicol and gentamicin were used as Standards for bacteria at concentration 0.5 and 0.05 mg/well, respectively. The results showed that the crude ethanolic extract and its fractions were active against the gram-positive bacteria: *B. subtilis* and *S. aureus* (IZD=9.0±0.0 to 13.5±0.5 mm).None of extracts showed activity against the gram-negative bacteria: *E. coli* and *P. aeruginosa*. The zone of inhibition values are summarized in Table 1.

Diameter of well 6 mm, (-) no inhibition, Chloramphenicol 10 mg/ml and Gentamicin 1 mg/ml are the standards for bacteria (values are mean ± S.D. of three replicates, in difference in letter on column represented different statistic at 95%). Zone of Inhibition <10 mm: inactive; 10-13 mm: partially active; 14-19 mm: active; >19 mm: very active antibacterial activities[8]. The MIC values of different microbes, tested in a concentration range 100–0.05 mg/ml, are given in Table 2. All extracts showed activity against gram-positive bacteria, *B. subtilis* and *S. aureus*, with MIC ranging from 1.562-50.0 mg/ml.

Antioxidant Activity

The antioxidant activity of crude ethanolic extract and solvent fractions of *L. rubra* stems were evaluated by using ABTS, DPPH and FRAP method. The results of antioxidant activity were expressed as Trolox equivalent antioxidant capacity (TEAC) (Table 3). It was found that the antioxidant activity of EF fraction (TEAC=0.286±0.010 to 0.376±0.013) was higher than crude ethanol extract, HF and WF. While the WF showed low antioxidant activity in all method.

Table 1: Antibacterial activity of *L. rubra* in agar well diffusion method

Fraction	Inhibition zone diameter (mm)			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
Crude ethanolic	10.0±0.5[a]	9.0±0.0[a]	-	-
HF	11.5±0.5[b]	11.5±0.5[b]	-	-
EF	13.5±0.5[c]	13.5±0.5[c]	-	-
WF	9.5±0.0[a]	9.5±0.0[a]	-	-
Chloramphenicol	27.5±0.5	25.0±0.5	19.0±0.5	-
Gentamicin	38.0±0.5	33.5±0.5	31.5±0.5	28.5±0.5

Table 2: The minimum inhibitory concentration (MIC)

Fraction	MIC (mg/ml)			
	B. subtilis	***S. aureus***	***E. coli***	***P. aeruginosa***
Crude ethanolic	12.5	6.25	ND	ND
HF	1.562	1.562	ND	ND
EF	3.125	1.562	ND	ND
WF	50	25	ND	ND
Chloramphenicol	25.0	12.5	3.125	3.125
Gentamicin	0.50	0.25	1.00	1.00

ND= Not detected

Table 3: The antioxidant activity of hexane, ethylacetate and ethanol extracts of *L. rubra* root and stem

Fraction	Antioxidant activity (TEAC)		
	ABTS	**DPPH**	**FRAP**
Crude ethanolic	0.220 ± 0.024^{b}	0.136 ± 0.008^{c}	0.162 ± 0.009^{c}
HF	0.064 ± 0.005^{a}	0.117 ± 0.020^{b}	0.101 ± 0.016^{b}
EF	0.286 ± 0.010^{c}	0.295 ± 0.014^{d}	0.376 ± 0.013^{d}
WF	0.056 ± 0.016^{a}	0.030 ± 0.002^{a}	0.040 ± 0.001^{a}

Each value is mean ± S.D. of three replicates, in difference in letter on column represented different statistic at 95%)

CONCLUSION

In conclusion, the EF fraction of *L. rubra* stem showed the highest antibacterial activity against gram-positive (IZD=13.5.0±0.5 mm, MIC=1.562-3.125 mg/ml) and showed good antioxidant activities in ABTS, DPPH and FRAP method (TEAC=0.286±0.010, 0.295±0.014 and 0.376±0.013, respectively). The results of this study indicated *L. rubra* to have potential antibacterial and antioxidant activities that could be used as a scientific evidence to support the pharmacological properties of Lanna medicinal plants. This trial for these activities guided isolation led us knows that, the active principle(s) belongs to EF fraction.

ACKNOWLEDGMENTS

This work was supported by grants from the Department of Pharmaceutical Science, the Faculty of Pharmacy and the Graduate School, Chiang Mai University, Chiang Mai, Thailand.

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