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Original Article

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF LANNA MEDICINAL PLANTS USED IN MAHOOG FORMULA

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ABSTRACT

Objectives: Antibacterial and antioxidant activities of Lanna medicinal plants used in Mahoog formula were investigated.

Methods: Dried powders of twenty five Lanna medicinal plants were extracted with ethanol using soxhlet's apparatus and with water by decoction method to obtain ethanolic and water extracts, respectively. Each extract was evaluated for antibacterial activity by agar diffusion technique and antioxidant activity by 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.

Results: Most ofLanna medicinal plant extracts were active against gram-positive bacteria. The extract of *Caesalpinia sappan* (heart wood) showed the highest inhibitory effect on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Interestingly,the extract of *Sindora siamensis* (stem) exhibited potent activity against *S. aureus* as same as *C. sappan* with MIC and MBC values of 0.049 and 0.098 mg/ml, respectively. The antioxidant activities revealed that the extract of *C. sappan* and *S. siamensis* possess significant free radical scavenging and reducing power.

Conclusion: Most of the medicinal plants consisted in Mahoog formula revealed antioxidant and antibacterial activities. Thedata obtained from the study will be used as a scientific evidence to support the pharmacological properties of medicinal plants used in Mahoog formula.

Keywords: Lanna medicinal plants, Mahoog, Antibacterial activity, Antioxidant activity.

INTRODUCTION

In Northern Thailand, there are plenty of Lanna Traditional Medicines used for treatments by traditional healers for a long time. It was found in our preliminary survey of folk medicines in Lanna communities that"Mahoog formula" is one of the most interesting ones. 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. Although the disease is not severe and occurs in frequently, it is a chronic disease if leaving untreated, and it may result in death [1]. Traditional medicine is one of the alternative ways to provide a supplement as well as treatment for this chronic disease.

To gather information from Lanna medical plants textbooks and from the in-depth interviewing traditional healers who specialize in the plants containing in Mahoog formula, from Chiang Mai, Chiang Rai and Lampang provinces. It was found that the characteristic symptoms of Mahoog are pain, inflammation and wound infection. Especially, when the wound is occurring, 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 the ROS produced in large amount at the site of the wound as a defense mechanism against invading bacteria, but also concurrent presence of free radicals may be hampered the process of wound healing, resulting in wound damage or microbial infection [2, 3]. In this study, the researcher is interested in investigating the antibacterial and antioxidant activities of Lanna medicinal plants in the Mahoog formula. The data obtained from the study will be used as a scientific evidence to support the pharmacological properties of Lanna medicinal plants in Mahoog formula.

MATERIALS AND METHODS

Plant materials

The medicinal plants containing in Mahoog formula were collected in Chiang Mai, Chiang Rai and Lampang provinces, Thailand. A list of the plants is presented in table 1. The identity of the Lanna medicinal plants was verified by a taxonomist and the voucher specimens were deposited in the Herbarium at the Faculty of Pharmacy, Chiang Mai University.

Materials

Ethanol 95 % was purchased from the Liquor Distillery Organization (Thailand). Ethanol AR grade and methanol were purchased from RCI Labscan (Thailand). Dimethyl sulfoxide (DMSO), Trolox, 2,2´-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich (Germany). Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) were purchased from Difco Laboratories (Detroit, MI, USA). Potassium persulfate was purchased from Unilab (Austria). Sterile Petri dish and 96-well microplates was purchased from Greiner bio-one (Austria).

Extraction of medicinal plants

The plant samples were cut into small pieces, dried at 50°C for 24 hours and then ground into powder. The medicinal plant powder was extracted with 95% ethanol by continuous extraction using soxhlet's apparatus and with water by decoction method. Each extract solution was filtered through Whatman filter paper No. 1 and then concentrated by rotary evaporator for ethanol extracts and freeze dryer for water extract.

Determination of antimicrobial activity

1) Microbial strains

Four species of bacteria i.e. 2 species of gram-positive and 2 species of 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.

2) Agar diffusion method

Agar-well diffusion assay was tested for microbial sensitivity to antibiotics. The method was described by Kirby-Bauer with some

modification [4]. Containing 1×10^8 CFU/ml was spread onto sterile Petri dish containing Tryptic Soy Agar (TSA) media. Each extract was dissolved in propylene glycol to the concentration of 50 mg/ml and autoclaved at 121°C, 15 psi for 15 min. Wells was 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. Propylene glycol was used as negative control while standard chloramphenicol 10 mg/ml and gentamicin 1 mg/ml were used as positive controls. Antibacterial activity was indicated by the presence of a clear inhibition zone around the wells. Tests were performed in triplicate.

Table 1: Lists of medicinal plants used

Family name	Scientific name	Part used	Voucher No.
Acanthaceae	*Thunbergia laurifolia*	stem	004895
Annonaceae	*Anomianthus dulcis*	stem	007575
Apocynaceae	*Aganosma marginata*	stem	003385
Bignoniaceae	*Millingtonia hortensis*	stem	004528
Caesalpiniaceae	*Caesalpinia sappan*	heart wood	002276
	Sindora siamensis	stem	003605
Celastraceae	*Celastrus paniculatus*	stem	007361
Combretaceae	*Combretum deciduum*	stem	005698
	Combretum quadrangulare	stem	007346
	Terminalia bellerica	stem	007198
Dipterocarpaceae	*Shorea obtusa*	stem	007101
Erythroxylaceae	*Erythroxylum cuneatum*	root	001391
Euphorbiaceae	*Croton crassifolius*	root	009378
	Trigonostemon reidioides	root	023171
Leeaceae	*Leea indica*	stem	003792
	Leea rubra	stem	005978
Papilionaceae	*Derris scandens*	stem	001965
	Pterocarpus macrocarpus	stem	007385
Rhamnaceae	*Ventilago denticulata*	stem	023175
	Ziziphus cambodiana	stem	023177
	Ziziphus mauritiana	stem	023176
	Ziziphus oenoplia	stem	006162
Sapindaceae	*Schleichera oleosa*	stem	007275
Ulmaceae	*Holoptelea integrifolia*	stem	006963

Minimum inhibitory concentration (MIC)

Determination of MIC using the microbroth dilution method was applied on extracts that already proved for their high efficacy against tested microorganisms. The extracts were dissolved in 50% DMSO and diluted by twofold to obtain a concentration range of 100–0.05 mg/ml with Tryptic Soy Broth (TSB) in the 96-well microplates. 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. Tests were performed in triplicate.

3) Minimum bactericidal concentration (MBC)

Those wells used in the MIC studies and did not show any turbidity in the bacteria were determined for MBC. An aliquot of the suspension (0.02 ml) was spread onto TSA and incubated at 37°C for 24 hours. The MBC was the lowest concentration which the initial inoculums were killed as 99.9% or more. Tests were performed in triplicate.

Determination of antioxidant activity

1) The 2,2´-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS)free radical scavenging assay

The ABTS radical scavenging assay was performed by the modified method from Pellegrini N *et al.*, 1999 [5]. ABTS•+stock solution was generated by oxidation of 7.0 mM ABTS with 2.45 mM potassium persulfate. The mixture was stored in the dark at room temperature for 12-16 hrs. The solution was diluted with ethanol and the absorbance was measured at the wavelength of 734 nm (absorbance = 0.70-0.90±0.05) before use. Then, 20 µl of the different sample concentrations were added into test tubes and mixed with 80 µl of ethanol including 2 ml of ABTS radical solution. The mixture was left at room temperature for 3 minutes and the absorbance was measured at 734 nm. The results of the ABTS assay were expressed as Trolox equivalent antioxidant capacity (TEAC). This index is defined as milligram of standard equivalent to 1.0 gram of the extract.

2) The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH free radical scavenging assay was tested following the method described by Brand-Williams *et al.* [6] with some modifications. Two thousand and one hundred microliters of reaction mixture containing 2.0 ml ethanolic DPPH 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 milligram of standard equivalent to 1.0 gram of the extract.

3) Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was investigated according to Benzie and Strain [7] with some modifications. Three thousand and one hundred microliters 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 milligram of standard equivalent to 1.0 gram of the extract.

RESULTS AND DISCUSSION

The antibacterial activity of ethanolic extracts(EE) and water extracts (WE) of 25 Lanna medicinal plants was evaluated against 2 gram-positive and 2 gram-negativebacteria species. Chloramphenicol and gentamicin were used as standards for bacteria at concentration 0.5 and 0.05 mg/well, respectively. The results showed that all of Lanna medicinal plant extracts were active against the gram-positive bacteria especially *B. subtilis*, the size of inhibition range from 7.0-33.3 mm. The ethanolic and water extracts of *M. hortensis*, water extract of *C. paniculatus*, *L. rubra*, *L. indica*, *A. marginata*, *T. laurifolia* and *Z. cambodiana* and ethanolic extract of *E. cuneatum* and *O. horridus* did not show inhibition zone against *S. aureus*. Most of the extracts revealed no inhibitory effect on gram-negative bacteria except the inhibition of *C. sappan* against *Pseudomonas aeruginosa* and *Escherichia coli* and the inhibition of *E. coli* from *D. scandens*. It has been reported that gram-negative

bacteria had low susceptibility to plant extracts compared to gram-positive bacteria [8,9]. The low inhibitory effect of gram-negative bacteria might be due to lipopolysaccharides in the outer membrane.

Table 2: Antibacterial activity of 25 Lanna medicinal plants by agar well diffusion method

Families/ Scientific names	Zone of inhibition (mm)							
	B. subtilis **ATCC 6633**		***S. aureus*** **ATCC 25923**		***E. coli*** **ATCC 25922**		***P. aeruginosa*** **ATCC 9027**	
	EE	**WE**	**EE**	**WE**	**EE**	**WE**	**EE**	**WE**
Acanthaceae *T. laurifolia*	8.8±0.8	7.0±0.0	8.0±0.5	-	-	-	-	-
Annonaceae *A. dulcis*	13.0±0.9*	9.2±0.8	10.8±0.8*	11.0±0.5	-	-	-	-
Apocynaceae *A. marginata*	7.5±0.5	7.0±0.0	8.5±0.5	-	-	-	-	-
Bignoniaceae *M. hortensis*	9.0±1.0	7.3±0.6	-	-	-	-	-	-
Caesalpiniaceae *C. sappan* *S. siamensis*	32.3±0.6*	33.3±1.5*	36.0±1.0*	36.3±0.6*	13.3±0.3*	14.0±0.5	12.8±0.3	11.2±0.3
	16.2±0.3*	13.5±0.5*	17.7±0.3*	16.5±0.5*	-	-	-	-
Celastraceae *C. paniculatus*	10.8±0.8*	8.2±0.3	9.2±0.6	-	-	-	-	-
Combretaceae *C. deciduum* *C. quadrangulare* *T. bellerica*	13.0±0.9*	9.3±0.8	18.0±0.5*	15.8±0.3*	-	-	-	-
	10.7±0.3*	10.0±1.0	8.2±0.3	14.6±0.3*	-	-	-	-
	14.2±0.8*	8.2±0.3	20.1±0.8*	16.3±0.6*	-	-	-	-
Dipterocarpaceae *S. obtusa*	16.2±0.6*	13.5±0.5*	15.6±0.3*	13.8±0.6*	-	-	-	-
Erythroxylaceae *E. cuneatum*	10.0±0.5	7.0±0.5	-	7.7±0.6	-	-	-	-
Euphorbiaceae *C. crassifolius* *T. reidioides*	9.3±0.6	8.0±1.0	7.5±0.5	7.5±0.5	-	-	-	-
	9.2±0.3	7.0±0.5	13.5±0.9*	10.5±1.0	-	-	-	-
Leeaceae *L. indica* *L. rubra*	9.5±0.5	7.0±0.0	7.5±0.5	-	-	-	-	-
	12.5±0.9*	7.7±0.6	8.5±0.9	-	-	-	-	-
Papilionaceae *D. scandens* *P. macrocarpus*	12.5±0.5*	8.0±0.0	10.5±0.5*	7.5±0.5	9.0±0.0[a]	-	-	-
	9.7±0.3	7.0±0.0	12.5±0.5*	12.2±0.8*	-	-	-	-
Rhamnaceae *V. denticulata* *Z. cambodiana* *Z. mauritiana* *Z. oenoplia*	15.0±1.0*	10.3±0.6	15.0±0.5*	8.0±0.5	-	-	-	-
	9.0±0.5	7.0±0.0	7.0±0.0	-	-	-	-	-
	13.3±0.6*	10.0±0.5	13.5±0.9*	10.0±0.5	-	-	-	-
	10.5±0.0	9.0±0.0	10.5±0.0*	9.0±0.0	-	-	-	-
Rubiaceae *O. horridus*	10.0±1.0	8.0±1.3	-	8.0±1.0	-	-	-	-
Sapindaceae *S. oleosa*	13.0±0.5*	12.0±0.5*	17.0±0.9*	17.5±0.5*	-	-	-	-
Ulmaceae *H. integrifolia*	8.0±0.0	7.0±0.0	8.5±0.5*	7.0±0.0	-	-	-	-
Chloramphenicol[a]	27.5±0.5		25.0±0.5		-		-	
Gentamicin[a]	-		-		31.5±0.5		28.5±0.5	

Diameter of well 6 mm, (-) no inhibition, Chloramphenicol 10 mg/ml and Gentamicin 1 mg/ml are the standardsfor bacteria (values are mean±S. D. of three replicates). *Significant difference at p<0.01 (in column). EE, ethanolic extract; WE, water extract.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using microbroth dilution method were investigated in 5 Lanna medicinal plants; *C. sappan*, *S. siamensis*, *T. bellerica*, *L. rubra* and *S. oleosa*, the results are shown in table 3. The ethanolic extract of the *C. sappan* gave very low MIC and MBC values against *S. aureus* and *B. subtilis* (MIC, MBC values were 0.05, 0.10and 0.40, 0.40 mg/ml, respectively). The extracts of*S. siamensis* and *S. oleosa* also revealed low MIC and MBC values against *S. aureus* and *B. subtilis* (MIC, MBC values were 0.05-6.25, 0.10-12.50 mg/ml). MIC, MBC values against *S. aureus* and *B. subtilis* of *T. bellerica* and *L. rubra* were 3.12-12.50, 6.25-12.50 mg/ml.

The ethanolic and water extracts of *C. sappan* heart wood showed the highest inhibitory effect on *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. The results were similar to that reported by Kim *et al.* and Srinivasan *et al.* [10, 11]. The heart wood of *C. sappan* showed inhibitory effect against *S. aureus*, *E. coli*, *Streptococcus faealis*, *Salmonella typhi*, *Enterobacter aerogenes*, *P. aeruginosa*, *Aspergillus niger* and *Candida albican*. Interestingly, the MIC and MBC of the extract of *S. simensis* suppressed *S. aureus* was equal to the extract of *C. sappan* (MIC 0.05 mg/ml and MBC 0.10 mg/ml).

T. bellerica fruit extract has been reported its antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *Streptococcus pneumoniae*, *S. typhi*, *S. typhimurium*, *Yersinia enterocolitica*and *C. albican* [12]. The MIC value of crude and methanol *T. bellerica* fruit extracts against *S. aureus* were 300 and 250 µg/ml, respectively. In our study, the stem extract of *T. bellerica* showed an inhibitory effect against only gram-positive bacteria (*S. aureus* and *B. subtilis*). It may be due to different active components were contained in each part of the plant [13].

Table 3: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Ethanolic extract from	MIC (mg/ml)		MBC (mg/ml)	
	S. aureus ATCC 25923	*B. subtilis* ATCC 6633	*S. aureus* ATCC 25923	*B. subtilis* ATCC 6633
C. sappan	0.05±0.00	0.40±0.00	0.10±0.00	0.40±0.00
S. siamensis	0.05±0.00	12.50±0.00	0.10±0.00	12.50±0.00
T. bellerica	3.12±0.00	12.50±0.00	6.25±0.00	12.50±0.00
L. rubra	6.25±0.00	12.50±0.00	12.50±0.00	12.50±0.00
S. oleosa	0.10±0.00	6.25±0.00	0.20±0.00	12.50±0.00

Values demonstrated in this table are mean±SD of MIC and MBC (mg/ml). Experiments were triplicately done.

The antioxidant activity of ethanolic extracts and water extracts of 25 Lanna medicinal plants was evaluated by using ABTS, DPPH and FRAP method. The results of antioxidant activity were expressed as Trolox equivalent antioxidant capacity (TEAC) (table 4). It was found that the ethanolic extracts and water extracts of all medicinal plants showed antioxidant activity.

The ethanolic extract of *C. sappan* exhibited the highest antioxidant activity in all methods (TEAC = 1003.86-1358.53 mg Trolox/g extract), followed by *S. obtosa* (TEAC = 666.07-1116.04 mg Trolox/g extract) and *S. siamensis* (TEAC = 651.59-975.58 mg Trolox/g extract), respectively.

The water extracts of *C. sappan* also showed the highest antioxidant activity in all method (TEAC = 695.45-1147.55 mg Trolox/g extract), followed by *S. obtosa* (TEAC = 597.40-860.92 mg Trolox/g extract) and *S. siamensis* (TEAC = 538.19-650.03 mg Trolox/g extract), respectively. The results of the extract of *C. sappan* in this study corresponded to Badami*et al.* [14], Batubara *et al.* [15], Wetwitayaklung *et al.* [16] and Hu *et al.* [17].

Table 4: Antioxidant activity of ethanol and water extracts of 25 Lanna medicinal plants

Families/ Scientific name	Antioxidant activity (TEAC)					
	ABTS		DPPH		FRAP	
	EE	WE	EE	WE	EE	WE
Acanthaceae *T. laurifolia*	134.40±6.13	97.40±0.05	76.98±0.12	68.06±0.14	310.29±1.02	108.98±0.12
Annonaceae *A. dulcis*	444.08±4.40	194.11±0.68	319.08±0.17	141.14±0.04	103.71±0.35	100.56±0.16
Apocynaceae *A. marginata*	363.14±0.78	179.58±0.15	183.05±0.14	144.08±0.06	192.43±0.12	138.69±0.33
Bignoniaceae *M. hortensis*	192.90±3.07	92.68±0.62	132.57±0.08	39.60±0.21	80.81±0.09	49.89±0.06
Caesalpiniaceae	1358.53±2.55	902.90±0.20	1003.86±0.52	695.45±0.60	1279.66±0.46	1147.55±2.42
C. sappan	975.58±6.03	650.03±1.80	651.59±008	553.84±0.45	916.16±3.05	538.19±2.34
S. siamensis						
Celastraceae *C. paniculatus*	95.33±0.05	123.06±0.15	54.92±0.09	108.21±0.02	51.03±0.08	103.19±0.08
Combretaceae	444.68±0.17	726.14±0.68	510.23±0.11	442.41±1.00	418.37±0.84	558.38±1.24
C. deciduum	169.19±3.10	505.37±2.88	111.45±1.68	455.02±0.07	188.56±0.47	269.72±0.52
C. quadrangulane	506.40±4.97	562.07±0.87	369.35±0.08	424.93±1.14	633.33±0.96	400.93±0.56
T. bellerica						
Dipterocarpusceae *S. obtusa*	868.67±0.22	860.92±0.99	666.07±0.18	702.80±0.40	1116.04±3.38	597.40±0.89
Erythroxylaceae *E. cuneatum*	331.24±7.68	106.71±0.73	243.80±1.02	135.66±0.30	189.96±0.37	66.33±1.49
Euphorbiaceae	91.58±6.14	49.15±0.23	76.37±0.08	39.65±0.08	95.72±0.19	35.26±0.53
C. crassifolius	361.62±1.98	31.84±0.31	75.85±0.07	22.57±0.02	175.79±0.16	33.54±0.64
T. reidioides						
Leeaceae	162.46±3.98	104.79±0.10	114.40±0.14	120.90±0.05	50.61±0.15	77.54±0.06
L. indica	212.71±3.20	227.84±0.62	140.09±0.02	218.20±0.13	133.66±0.26	103.59±0.28
L. rubra						
Papilionaceae	398.25±1.44	155.94±0.24	187.63±0.04	156.04±0.14	312.34±0.36	119.51±0.23
D. scandens	420.84±0.52	321.30±1.86	203.49±0.04	243.64±0.06	171.64±0.67	155.53±0.29
P. macrocarpus						
Rhamnaceae	262.14±0.17	114.17±0.10	159.50±0.19	92.07±0.23	117.04±0.26	72.43±0.11
V. denticulata	171.96±3.83	122.17±0.08	108.39±0.03	114.10±0.14	69.63±0.06	82.01±0.14
Z. cambodiana	830.65±0.48	350.95±0.21	412.04±0.06	294±0.28	223.25±0.16	229.03±0.30
Z. mauritiana	251.50±2.10	203.59±0.76	143.59±0.11	170.94±0.12	69.96±0.27	197.85±0.14
Z. oenoplia						
Rubiaceae *O. horridus*	115.64±3.19	139.72±0.53	110.43±0.90	104.39±0.07	68.03±0.18	110.81±0.06
Sapindaceae *S. oleosa*	209.81±4.68	243.83±1.20	325.79±0.04	238.38±0.07	106.35±0.15	285.94±0.16
Ulmaceae *H. integrifolia*	179.13±3.27	55.18±0.41	31.36±0.04	31.98±0.02	101.12±0.03	52.70±0.18

Each valueis mean±SD. of three replicates. EE, ethanolic extract; WE, water extract.

CONCLUSION

Mahoog formula, a Lanna Traditional Medicines in Northern Thailand, has been claimed as a remedy for Mahoog disease in Lanna communities. This study was designed to evaluate the antibacterial and antioxidant activities of some medicinal plants used in Mahoog formula. Among 25 Lanna medicinal plants, *C. sappan* heart wood extracts showed the highest antibacterial activity against gram-positive and gram-negative and antioxidant activity. *S. siamensis* and *S. obtosa* showed the inhibitory effect on *S. aureus* and *B. subtilis* and revealed potent antioxidant activity. Most of Lanna medicinal plant extracts were active against gram-positive bacteria and showed antioxidant activity. The results of this study indicated that Lanna medicinal plant is a potential source of antioxidant relevant to wound infection in Mahoog disease.

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CINFLICT OF INTERESTS

Declared None

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