Chapter V

Discussion

It is wellknown that intensive use of an antibiotic is often followed by the appearance of resistant strains. In view of this propensity of bacteria to drug resistance, the search for new antibiotics continue unabated. In this connection, plants continue to remain a rich source of new therapeutic compounds (Ahmad et al, 1986).

In this study, eleven Thai medicinal plants were extracted with 95% ethanol and were tested for antibacterial activity against five test microganisms which caused respiratory tract infection. The activities of inhibition against each test organism were varied. More than 60% of the extracts showed activity against gram positive bacteria while less than 20% of them could inhibit *P. aeruginosa* ATCC 27853 and none of them showed activity against *K. pneumoniae* ATCC 10031. According to the weight yield and their antibacterial activities, seven plant extracts were selected and extracted with petroleum ether, chloroform and ethanol. The residues were tested for antibacterial activity. *Eleutherine palmifolia* (L.) Merr. extracted with either petroleum ether or chloroform and *Stephania glabra* (Roxb.) Miers. extracted with chloroform gave the high activity against test organisms. Among these three, *E. palmifolia* (L.) Merr. with petroleum ether extraction provided the highest antibacterial activity.

E. palmifolia (L.) Merr. with petroleum ether extraction was isolated by column chromatography technique for

antibacterial substance. The compound EP_2 was found and identified as naphthoquinone derivative. From IR spectrum of EP_2 (see Fig 10,p 112) in KBr disc showed two carbonyl absorption of quinone ring at 1650 cm^{-1} and 1660 cm^{-1}. The C-H stretching occurred at 2940 cm^{-1} and 3000 cm^{-1} and C=C stretching at 1585 cm^{-1} indicated that the aromatic was presented.

The mass spectra of EP_2 (see Fig 12, p 114) revealed the molecular ion at *m/z* 272 and the base peak at *m/z* 257. The main fragmentation of EP_2 were shown in mass spectra (Fig VI, p 84).

The molecular ion (M^+) were fragmented by loss methyl group mainly of the methoxyl and the other two methyl groups in the cyclic ether part. As EP_2 was naphthoquinone, the CO group would break from the quinone ring. There were rearrangement and inductive cleavage in cyclic ether part.

Its ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz, Fig 11, p 113) revealed a singlet due to an aromatic methoxyl group at δ 4.007 ppm. A pair of doublet (δ 7.288 and δ 7.745 ppm) revealed the proton of carbon near the methoxyl group, the proton para to the methoxyl substitution of the aromatic ring and a triplet at δ 7.651ppm showed the proton in the middle. A pair of doublets at δ 1.344 ppm (CH_3- 1') and δ 1.538 ppm (CH_3- 3') revealed the methyl groups of the pyran ring. The geminal protons expressed an axial proton (δ 2.233 ppm) and an equatorial proton (δ 2.694 ppm) at 2'-position. The multiplet at δ 3.93-3.99 ppm showed the proton of the H-1' and the quartet at δ 5.016 ppm revealed the proton of H-3' of the pyran ring. The position of methoxyl group on the naphthoquinone ring has to be confirmed by long-range selective

proton decoupling (LSPD) experiments in ^{13}C-NMR.

EP_2 is a new chemical compound which has never been found in plants. Although *E. palmifolia* (L.) Merr. was studied for the chemical constituents since 1950 (Schmid, Meijer, and Ebnöther, 1950; Schmid and Ebnöther, 1951; Komura et al., 1983; Chen, Huang, Wang, Li, Ding et al., 1984). Eleutherin and isoeleutherin have been isolated but the structure of eleutherin was different from EP_2. (Fig II, p 23 and Fig III, p 70) The physical properties of these two compounds were also different. For eleutherin, melting point was 175°C; $[\alpha]^{15}_D$ +346 in chloroform (Thomson, 1971) while EP_2 melting point was 166-167°C ; $[\alpha]^{25}_D$ -100 in chloroform. When eleutherin was run on TLC silica gel G plates using benzene : acetone (9:1) and petroleum ether : ethyl acetate : chloroform (67:33:10), the Rf values was showed at 0.57 and 0.40 (Bianchi and Ceriotti, 1975) while for EP_2, using the same solvent systems, the R_f values was at 0.39 and 0.24, respectively.

The antibacterial acitivity of EP_2 was tested against two gram positive bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes* which were the common cause of upper respiratory tract infection. The disc-susceptibility test to fifty isolated of each organism to five antibiotics were summerized in Table 20.

Table 20 The antimicrobial susceptibility against *staphylococcus aureus* and *Streptococcus pyogenes*

Antimicrobial agents	Percentage of susceptible strains	
	Staphylococcus aureus	*Streptococcus pyogenes*
Cephalothin	92	100
Clindamycin	96	100
Erythromycin	88	100
Penicillin G	6	100
Tetracycline	62	4

The susceptibility of S. *aureus* were 88-96% sensitive to clindamycin, cephalothin and erythromycin while they provided only 62% sensitive to tetracycline. Most of the strains were penicillin G resistant.

All isolated of S. *pyogenes* were 100% sensitive to cephalothin, clindamycin, erythromycin, and penicillin G but they were tetracycline resistant.

The MIC values of EP_2 were 40 µg/ml against S. *aureus* and 60 µg/ml against S. *pyogenes*. However, the MIC_{90} values against S. *aureus* and S. *pyogenes* were 29.2 µg/ml and 39.0 µg/ml, respectively.

The results from this study were in agreement with the results from the previous study (Ikekawa et al., 1967 ; Krishnaswamy and Purushothaman, 1980) which showed that other naturally occurring naphthoquinone from plants provided

the antibacterial activity. The growth of *S. aureus* was inhibited by juglone at the concentration of 50 µg/ml and by plumbagin at the concentration of 20 µg/ml.

Crude extract of *E. palmifolia* (L.) Merr. showed no activity against gram negative bacteria tested (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). This results supported the results from the study of Papageorgiou and others (1979) which showed that the two esters of alkannin, β,β-dimethylacrylic ester and β-acetoxy isovaleric ester, which exhibited no antibacterial properties against *Escherichia coli*. However, plumbagin showed the activity against the growth of gram negative bacteria by adding the substance to the nutrient agar (Krishnaswamy and Purushothaman, 1980). According to the structure of these compounds, it was possible that the highly substituted compound may cause no activity against gram negative bacteria. The further investigation of EP_2 against other gram negative and gram positive bacteria should be done both *in vitro* and *in vivo*.

Mostly, the naturally occurring naphthoquinone from plants showed antifungal activity such as plumbagin, juglone (Didry, Pinkas, and Dubreuil, 1986), alkannin (Papageogiou et al., 1979), tricrozarin A (Masuda et al., 1987), and 2-methoxy-1,4-naphthoquinone (Thatree Phadungcharoen et al., 1988). Some of them had anticancer properties such as plumbagin showed ED_{50} for fibrosarcoma in rats at 0.75 mg /kg body wt. and for P_{388} lymphocytic leukaemia, plumbagin was active at 4 mg/kg body wt. (Krishnaswamy and Purushothaman, 1980). Psychorubrin showed significant reproducible inhibitory activity against KB cells (Hayashi, Smith, and Lee, 1987). Antigermination, antiprotozoal and molluscicidal activity were also found in some naturally

occurring naphthoquinone from plants. According to the structure of EP_2 which related to the structure of other naturally occurring naphthoquinone, the further study on its other biological properties and pharmacological properties should be continued in order to explore its potentiality as probable antimicrobial agent.