Antimicrobial Activity of Hydroxymatairesinol (HMR) Lignan[#]

Widad M.K. Al-Ani[*,1] and Fitua M. Aziz[*]

[*]Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, Al-Mustansiriyah University, Baghdad, Iraq.

Abstract

Lignans are natural products widely distributed in the plant kingdom. They are composed of two β-β-linked phenylpropane (shikimate-derived biogenetic subunits). Although the backbone of lignans is composed of phenylpropane units, there is enormous diversity in the structure of lignans leading to different classes of lignans, such as γ-butyrolactone derivatives, eg. Hymatairesinol, bicyclooctadiene derivatives, e.g. pinoresinol, tetrahydrofuran derivatives e.g.lariciresinol, di-arylbutandiol derivatives, e.g. secoisolariciresinol. Introduction of a further carbon –carbon linkage leads to a class of lignans collectively known as cyclolignans such as tetrahydro-naphthalene derivatives, for example podophyllotoxin. Lignans have a broad range of biological activities; many of them show significant antitumour, antimitotic, and antiviral effects. They also have cardiovascular effects, antimicrobial and insecticidal activities. The efflux mechanisms of bacteria to some antibiotics and resistance of bacteria to many antibiotics led to search for antibacterial compound of plant origin to overcome these problems. The antibacterial activity of HMR lignan was determined using the disc diffusion method and the MIC of the isolated compound was tested by the broth micro-dilution method. HMR lignan showed activity against *Staphylococcus epidermidis*(17 mm), *Candida albicans* (13mm), Proteussp(12mm) and *Klebsiella* sp (12mm).

Key words: Lignans, Hydroxymatiaresinol, Antimicrobial activity.

هيدروكسي ميتارزينول لكنان كمضاد للمايكروبات

وداد مصطفى كامل العاني[*،1] و فتوة منور عزيز

[*]فرع العقاقير والنباتات الطبية ، كلية الصيدلة ، الجامعة المستنصرية ،بغداد، العراق .

الخلاصة

اللكنان مركبات طبيعية ذات انتشار واسع في الطبيعة وهي تتكون في النباتات من ارتباط جزئيتين من phenylpropane. هناك تنوع كبير في تركيب اللكنان على الرغم من انها تتشابه في هيكلها الرئيسي مثل HMRوغيرها . اللكنان مركبات ذات فعالية بيولوجية متعددة فبعضها مضادة للسرطان وأخرى مضادة للبكتريا والفيروس وكذلك لها فعالية في علاج امراض القلب والأوعية الدموية. ازداد اهتمام الباحثين في السنوات الاخيرة في البحث عن مركبات مضادة للبكتريا ذات اصل نباتي طبيعي وذلك لازدياد ظاهرة المقاومة من قبل البكتريا للمضادات الحيوية وكذلك ظهور سلالات من البكتريا لها قابلية على سحب الدواء ورميه خارج الخلية مما يؤدي الى عدم فعاليته. يهتم البحث بتحديد نشاط ال HMRضد البكتريا بطريقة الانتشار. اظهر المركب نشاط ضد البكتريا متمثلا بوجود منع نمو البكتريا حول نقطة تواجد المركب النباتي.

مفتاح الكلمات : اللكنان، هيدروكسي ميتاريزينول، مضادات المايكروبات .

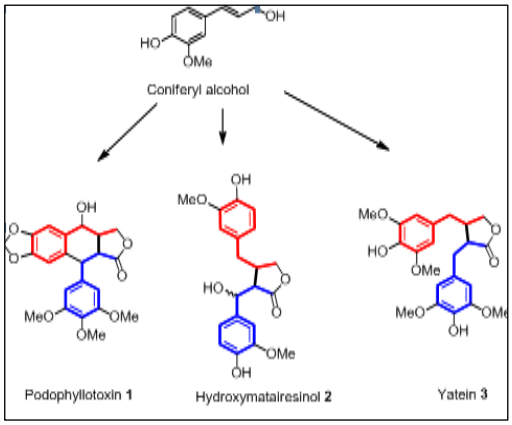
Introduction

Lignans are natural products widely distributed in the plant kingdom. They are composed of two β-β-linked phenylpropane (shikimate-derived biogenetic subunits). Lignans are biosynthesized by the coupling of two conifer [(1)] alcohol units through a one electron oxidative coupling process (Figure 1).Lignans have a broad range of biological activities; many of them show significant antitumour, antimitotic, and antiviral effects. They also have cardiovascular effects, antimicrobial and insecticidal activities[(2)].In the early 1980s, it was suggested that a diet rich in HMR lignan may help prevent breast cancer through conversion to enterolactone (ENL) [(3)].An epidemiological study indicated that ENL found in the serum of women with no previous history of breast cancer but not in the serum of women who have such a history. This study led to the suggestion that a diet high in lignans may have chemopreventive effects[(4)]. HMR lignans usually occur in the form of glycosides in the fiber of cereals and some fruit like apricot[(5)].The knots of the Norway spruce, from Finland, are considered to be a rich source of HMR. Concentrations of HMR up to 20% have been found in some knots, making the Norway sprucea source of HMR for experimental studies[(6)].The precipitation of (-)-HMR by potassium acetate as a potassium a adduct from a concentrated ethanolic extract, by Freudenberg, is a superior method for large-scale production[(7)].

Based on oral presentation in the fourteenth professional and scientific conference of the syndicate of Iraqi pharmacists 19-21 March 2013

[1]Corresponding author E-mail:wmkalani@yahoo.com

Received: 4/5/2013
Accepted: 9/6/2013



Figure(1):Biosynthesis and example of lignans

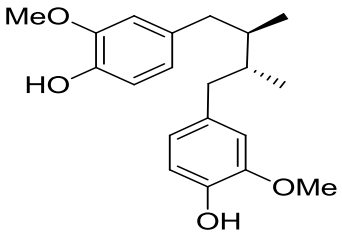
HMR had stronger lipid peroxidation inhibition capacity than any other lignan or flavonoid tested[8].HMR was compared to the well-known antioxidants TROLOX (a water-soluble vitamin E derivative), butylated hydroxyanisole, and butylated hydroxytoluene in terms of their ability to inhibit lipid peroxidation, inhibit LDL oxidation, and scavenge superoxide and peroxyl radicals. HMR was found to be the strongest antioxidant, more effective than butylated hydroxyanisole or butylated hydroxytoluenein all assays and stronger than TROLOX in all assays except in the lipid peroxidation inhibition assay in which the compounds were almost equally active[9].

The oestrogenic activity of HMR was investigated by measuring their effects on growth and apoptotic markers in the human oestrogen-sensitive cell line MCF-7 in comparison to oestradiol (E2). HMR and ENL targeted the same intracellular mechanisms acted upon by E2 but had a milder effect. This weak oestrogenic activity was blocked by the anti-oestrogenic drug tamoxifen[10].It was found that HMR decreases flushing in women during menopause, which indicates that this compound mimics oestrogen activity[11].

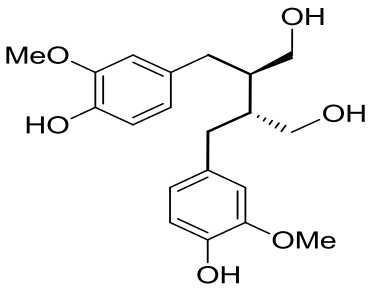
Searching for antimicrobial natural products have been acquired a considerable importance nowadays due to the development of multiple drug resistance and efflux mechanisms of bacteria to many antibiotics [12].Side effects associated with use of synthetic antibiotics are frequently more reported than that of natural products[13].

Three lignans were isolated and characterized from *Larreatridentata* and compounds were tested against 16 bacterial species/strains. Results showed that: dihydroguaiaretic (**4**) acid had activity towards methicillin resistant (MR) *Staphylococcusaur-eus*and multidrug-resistant (MDR) strains of *Mycobacteriumtuberculosis* [14].

The relationship between the stereochemistry and antimicrobial activity of butane-type lignans was clarified by Satoshi *et al*. All stereoisomers of dihydroguaiaretic acid (**4**) showed both antibacterial and antifungal activity. No activity of any stereoisomer of secoisolariciresinol (**5**) was apparent[15].

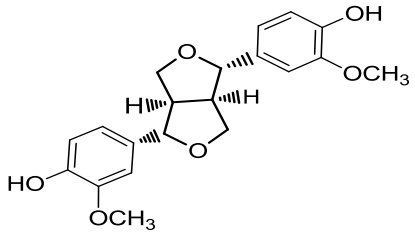


4

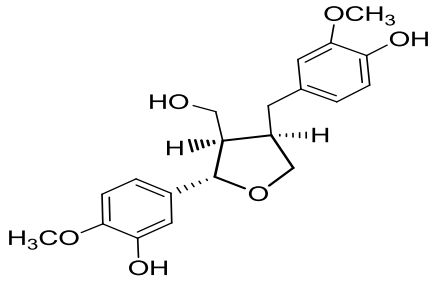


5

Lignans secoisolariciresinol **5**, pinoresinol **6** and Lariciresinol **7**, were isolated from MeOH extract from *Araucaria araucana* wood for the first time in this species and their structures determined with spectroscopic methods. The antimicrobial activities of these compounds were determined for the bacteria *Citrobacter* sp., *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Staphylococcus aureus*,and *Pseudomonas aeruginosa*, and for the white rooting and staining fungi *Mucor miehei*,*Paecilomyces variotii*, *Ceratocystis pilifera*, *Trametes versicolor*, and *Penicillium notatum*, in addition, the MeOH extract was evaluated against *Aspergillus niger*, *Candida albicans*,*Fusarium moniliforme*, *F. sporotrichum* and *Trichophyton mentagrophytes*. The most sensitive bacteria against pinoresinol were the Gram-positive. However, secoisolariciresinol exhibited antifungal activity against wood rooting[16].



6



7

Materials and Methods

General

HMR potassisum adduct from Linea[R] Company was converted to free HMR lignan by dissolving in phosphate buffer pH 4.5.

Free HMR from potassium adducts

The food supplement HMR potassium adducts (1 g) was dissolve in minimum amount ofaqueous ethanol. Phosphate buffer pH 5(100 mL) was added and the pH was adjusted to 4.5. The mixture was stirred for 15 minutes and the free HMR was extracted with ethyl acetate (3 x 100 mL). The combined ethyl acetate layers were dried with anhydrous magnesium sulphate, evaporated under vacuo to give free HMR (7.8 g, 78% yield). The purity of the lignan was tested by TLC (DCM / ethanol, 7%).

Screening of antibacterial activity

HMR was dissolved in methanol to obtain final concentrations of (100, 50, and 25) mg / mL and sterilized by filtration through a 0.45 µm membrane filter. The Muller-Hinton agar was inoculated with one of the tested bacteria. Methanol was used as a control to eliminate solvent effect. Ciprofloxacin (5 µg/ disc) was used as a positive control.

The agar diffusion method was used to determine antimicrobial activity of the tested lignan (HMR).Six mm diameter wells were punched in to the agar. Inocula were obtained from overnight cultures on nutrient agar slants at 37 ^{o}C and diluted in sterile saline solution to a final concentration of $10^{(6)}$ colony forming uints (cfu)/ mL (adjusted according to the turbidity of 0.5 Mc farland scale tube).

Results

In this study HMRlignan showed an efficient antimicrobial activity against most of the bacteria and fungi used except *Pseudomonasaeruginosa* .This bacteria showed resistance to all concentration used. The most sensitive microorganism was *Staphylococcus epidermidis* (inhibition zone, 17, 15, 8 mm) and *Candida albicans* showed (inhibition zone, 13, 12, 10 mm). *Klebsiella sp* and *Proteus* sp. also showed sensitivity to all concentrations. *Escherichia coli* was sensitive to a high concentration only (100 mg/ mL). The result of our study revealed that HMR lignan posses antimicrobial properties as antibiotic principles (Table 1)

Table (1): Inhibition zones of bacterial growth produced by HMR lignan

Microorganisms	Concentrations (mg/mL)/ Inhibition zone in mm				
	100 mg/mL	**50 mg/ mL**	**25 mg/ mL**	**Solvent (C)**	**ciprofloxacin**
Proteus sp.	12	9	7	-	35
Pseudomonasaeruginosa	-	-	-	-	27
Escherichia coli	8	-	-	-	32
Staphylococcus aureus	8	7	-	-	19
Candida albicans	13	12	10	-	-
Klebsiella sp	12	9	7	-	-
Staphylococcus epidermidis	17	15	8	-	-

Methanol as a solvent showed no antimicrobial activity against the bacteria, while HMR lignan showed a good inhibition zone for both (*Staphylococcus epidermidis)* and *Klebsiella sp* (Figure 2)**.**

Figure (2): Inhibition zone for s*taphylococcus epidermidis*

Discussion

Natural products have been found to be an efficient antimicrobial compounds especially those with phenolic group. It is necessary to investigate the activity of the diphenolic HMR lignan to improve the quality of health care. Many organisms develop resistance to antibiotics which lead to search for novel antimicrobial agents. References 14 and 15 focus on the antimicrobial activity of butane type lignan therefor HMR is also a good example for those butane type antimicrobial lignans.

The diameter of inhibition zone of the antibacterial agents the lignans and ciprofloxacin were different according to the kinds, concentrations and purity.

The mechanism of action of the antimicrobial (HMR) is due to the toxic effect or may be impair variety of enzyme systems including those involved in energy production and structural component synthesis[17].

Excess acid should be avoided during the libration of free HMR from the potassium adduct due to cyclization of HMR to the cyclolignan (Figure 3)[18].

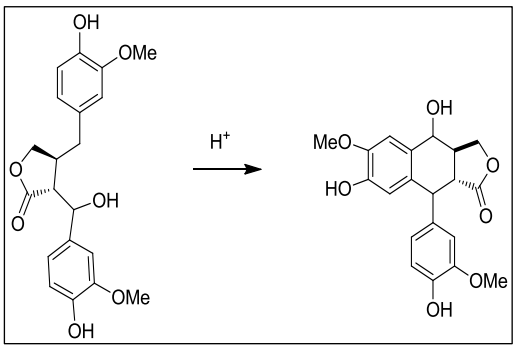


Figure (3): Cyclization of HMR to cyclolig-nansin acidic media

Conclusion

HMR lignan shoswed an efficient antimirobial activity it shows antimicrobial activity against most of the bacteria and fungi used. This result together with the results reported in the literatures support the idea of using natural compounds as sources for antimicrobial drug to overcome the resistance and efflux mechanism of microbe to antibiotics.

References

1. Haworth, R. D., Ann. Rep. Prog. Chem. 1936; 33; 26.
2. MacRae, W. and Towrs, G. Phytochemistry ,1984; 23: 1207
3. Axelson, M, Sjovall J, Gustafsson, B. and Setchell, K, Nature ,1982; 298:659.
4. Ingram D, Sanders, K. Kolybaba, M. and Lopez, D. Lancet 1997; 9083: 990.
5. Milder, I., Arts, C., Putti, B., Venema, D., and Hollman, P, B. J. Nut. 2005;93; 393).
6. Holmbom, B., Eckerman, C., Eklund, P., Hemming, J. Nisula, L., Reunanen, M., Sjoholm, R., Sundberg,A., Sundberg K. and Willfor, S., Phytochem. Rev. 2003;2:313.
7. Freudenberg, K. and Knof, L., Chem. Ber. 1957; 90: 2857.
8. US patent, 700547 B2; 2006.
9. Ahotupa, M., Ruutu, M., and Mantyl, A., Clin. Biochem. 1996; 29:139.
10. Cosentino, M., Marino, F., Ferrari, M., Rasini, E., Bombelli, R., Luini, A., Legnaro, M., Delle Canne, M., Luzzani, M., Crema, F., Paracchini, S., Lecchini, S., Pharmacol. Res. 2007; 56: 140.
11. Kangas, L., Saarinen, N., Mutanen, M., Ahotupa, M., Hirsinummi, R., Unkila, M., Perala, M., Soininen, P., Laatikainen, R., Korte, H., Santti, R., Eur. J. Cancer Prev. 2002 ;2: 48.
12. Kawahi, Y. Etal, Smith, E., Williamson, E.M., Zloh, M. and Gibbons, S.Phytotherapy Research, 2005; 19 (6). 538.
13. Bishnu, J., Sunil, L., Anuja, S., Kathmandu University Journal of Science, Engineering and Technology, 2009;5:143.
14. Favela, H JM, García AGarza-González E, Rivas-Galindo VM, , Camacho-Corona MR, PhytotherRes.2012;15:4660.
15. Satoshi, Y., Toshiya, M. , Takuya S, ,Yuya K, Maya O, Tatsushi S, A, Shiori T, Manami Y, Taro K, Koichi A, Masafumi M., Bioscience, biotechnology, andbiochemistry, 2009; 72: 2981.

16. Carlos L. Cespedesa, J. Guillermo Avilab, Ana M. Garcıab, Jose Becerrad, Cristian Floresd, Pedro Aquevequed, Magalis Bittnerd, Maritza Miguel Martinezc, and Mario Silvad, Z. Naturforsch., 2006; 61: 35.

17. Conner, D.E. and Beuchat , sensitivity of heat stressed yeasts to essential oils of plants, Applied Environ. Microbiol. 1984; 47: 229.

18. Willfore, S., Reunanen, M., Eklund , B., Sjoholm, R .Kronberg, Farminm , P.; Pietarenen, S. and Holmbom, B., Holzforschu, 2004;45: 345 .