

ANTIBACTERIAL AGENTS FROM THE SEEDS OF *PEUCEDANUM ZENKERI* L. (UMBELLIFERAE)

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ABSTRACT

Objective: In a search for natural substances with potential for the treatment of typhoid fevers and urogenital infections, the methylene chloride extract of the seeds of *Peucedanum zenkeri* was investigated.

Methodology: The extract was subjected to column chromatography leading to the isolation of seven compounds. Their structures were determined using modern 2D NMR techniques and by comparison with published NMR data. These compounds were tested against *Salmonella typhi*, *Salmonella paratyphi B*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, using both agar diffusion and broth dilution techniques.

Results: The compounds isolated were umbelliprenin (1), prangenin (2), imperatorin (3), isopimpinellin (4), bergapten (5), cnidilin (6) and stigmasterol (7). Among the above seven compounds, only two (1 and 2) exhibited antibacterial activity. For compound 1, the MIC value was 300 µg/ml against *P. aeruginosa*, *S. aureus*, *S. typhi* and *S. paratyphi B*. For compound 2, the MIC values varied between 200 and 300 µg/ml against all the bacteria strains tested.

Conclusion: These data suggest that *Peucedanum zenkeri* seed extract contains antibacterial agents which are active against *Salmonella* species causing typhoid and paratyphoid fevers, and some bacteria strains causing urogenital infections. The antibacterial activity of compound 2 appears to be due to the epoxide group present in its structure.

KEY WORDS: *Peucedanum zenkeri*, seeds, coumarins, antimicrobial activity, typhoid fever, urogenital infections.

Pak J Med Sci April - June 2010 Vol. 26 No. 2 314-318

How to cite this article:

MBAH JA, GATSING D, EFANGE SMN. Antibacterial agents from the seeds of *Peucedanum zenkeri* L. (Umbelliferae). Pak J Med Sci 2010;26(2):314-318.

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- * Received for Publication: August 20, 2009
- * Revision Received: January 29, 2010
- * Revision Accepted: January 31, 2010

INTRODUCTION

Typhoid, paratyphoid A and paratyphoid B fevers are caused by *Salmonella typhi*, *Salmonella paratyphi A* and *Salmonella paratyphi B*, respectively.¹ Typhoid fever continues to pose a marked public health problem in developing countries in general and in sub-Saharan Africa in particular, where it is endemic.² Non specific urogenital infections are caused by a variety of bacteria whose habitat is not limited to the urogenital tract. These bacteria are generally commensals which become pathogenic in debilitated persons or following a modification of their habitat.³ Among the bacteria causing non

specific urogenital infections are *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.⁴ Cases of resistance to the currently used antibiotics have been encountered with some strains of these bacteria.³ Therefore, there is the need to identify new and effective antimicrobial agents. Plants have provided useful leads for the development of drugs for the treatment of many illnesses. Many species of the genus *Peucedanum* such as *P. morisonii*,⁵ *P. japonicum*,⁶ *P. zenkeri*⁷ and *P. praeruptorum*⁸ have been reported for their antibacterial activity. Thus, we chose *P. zenkeri* for our study.

Peucedanum zenkeri (Umbelliferae) is an herb found in the West-Central African equatorial forest. The roots of *Peucedanum* species, known as Qian-Hu, are widely used in Chinese folk medicine as antitussive, expectorant, antipyretic and stomachic.⁹ Ngwendson *et al.*⁷ investigated the hexane extract of the seeds of *Peucedanum zenkeri* from which seven compounds were isolated, among which three (imperatorin, isopimpinellin and bergapten) showed antimicrobial activity against *Cryptococcus neoformans* and *Mycobacterium intracellulare*.

In a continuation of our search for therapeutic agents from natural sources with potential for the treatment of typhoid fevers and urogenital infections,^{1,2,10,11} we decided to investigate the methylene chloride extract of *Peucedanum zenkeri* which afforded seven compounds: umbelliprenin (1), prangenin (2), imperatorin (3), isopimpinellin (4), bergapten (5), cnidilin (6) and stigmaterol (7). These compounds were tested for their antimicrobial activity.

METHODOLOGY

Plant material: *Peucedanum zenkeri* was collected in November 2002 from the cultivation farm of a botanist, Dr. Wirmum Clare, Director of the Medicinal Foods and Plants in Bamenda.

Test bacterial and culture media: The test microorganisms, *Salmonella typhi*, *Salmonella paratyphi B*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus*, were obtained from the Medical Bacteriology Laboratory of the Pasteur

Centre, Yaounde, Cameroon. The culture media used, namely Salmonella-Shigella agar (SS agar), Mueller Hinton and Selenite Broth, were supplied by International Diagnostics Groups PLC, Topley House, 52 Wash Lane, Bury, Lancashire BL96 AU, UK. SS agar was used for the isolation of the *Salmonella* species and for the screening of contaminants when preparing the inoculum. Mueller Hinton agar and Selenite broth were used for antibacterial tests.

Extraction and Isolation: The seeds (2.4 kg) were ground and macerated in dichloromethane (10 l) for six days. Removal of solvent yielded 100 g of dark brown extract. Column chromatography of this extract on silica gel eluting with gradients of ethyl acetate (EtOAc) in hexane (hex) afforded ten main fractions based on their TLC profiles. Fraction 2 (obtained with 10% EtOAc/hex) was further chromatographed on silica gel and finally gel permeated via Sephadex LH-20 (hex/CH₂Cl₂ [6:4]) to afford umbelliprenin (1, white crystals, 210 mg) and stigmaterol (7, white crystals, 120 mg). Fraction 3 (obtained with 20% EtOAc in hex) was chromatographed with SiO₂ and eluted with gradients of EtOAc in hexane. Subfractions 10-21 were further chromatographed on silica gel while eluting isocratically with hex/EtOAc (98:2) leading to imperatorin (3, pale yellow powder, 50 mg) and cnidilin (6, yellowish crystals, 30 mg). Fraction 4 (obtained with 40% EtOAc) were filtered and crystals chromatographed on silica gel eluting isocratically with hex/CH₂Cl₂/acetone (96:2:2), subfractions 2-3 afforded pale yellow powder of bergapten (5, 65 mg), while subfractions 9-10 gave isopimpinellin (4, white powder, 50 mg). Fraction 5 (obtained with 60 % EtOAc) was repeatedly chromatographed on silica gel and gel permeation via Sephadex LH-20 (CH₂Cl₂/Methanol (MeOH) [95:5]) to yield prangenin (2, yellowish crystals, 200 mg).

Antimicrobial assay: The antibacterial activity was determined using both agar diffusion and broth dilution techniques as previously described by Cheesbrough.³ Agar diffusion susceptibility testing was done using the disc method. All the compounds were dissolved in DMSO. The discs were charged with 200 µg of

the various compounds to be tested. Ciprofloxacin (Sigma) was used as the standard drug (1 µg as disc potency).

The compounds that induced zones of inhibition (i.e. compounds 1 and 2) were further studied using broth dilution technique, and the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values were determined. The concentrations of the compounds in the tube ranged from 300 to 37.5 µg/ml and those of ciprofloxacin ranged from 16 to 0.5 µg/ml.

RESULTS

The structures of the isolated compounds (Fig-1) were determined by comparison of their spectral data with those reported for umbelliprenin,¹² prangenin,¹³ imperatorin,¹³ isopimpinellin,¹⁴ bergapten,¹⁴ cnidilin¹⁵ and stigmasterol.¹⁶ The ¹³C NMR data of prangenin (2) were in close agreement with those of imperatorin (3) except for the absence of signals at δ 119.7 and 139.2 due to epoxidation of this double bond in 2.

Seven compounds were isolated from the seeds of *P. zenkeri* and tested against *Salmonella typhi*, *Salmonella paratyphi* B, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* at the disc potency of 200 µg, using agar diffusion technique. Among the

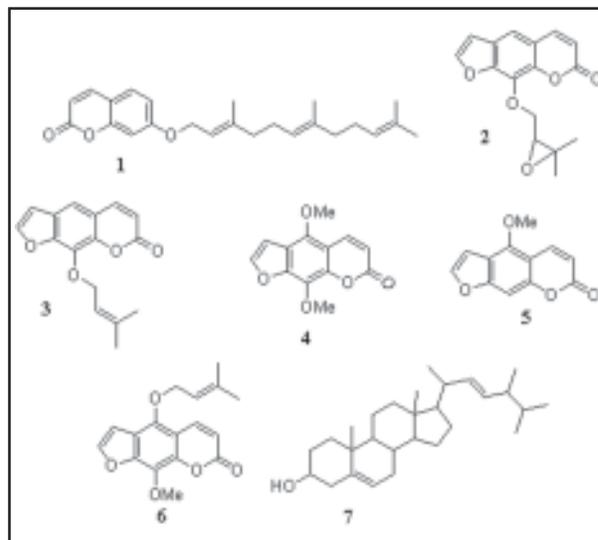


Fig-1: Structures of the compounds isolated.

seven compounds tested, only two [umbelliprenin (1), prangenin (2)] were active at the disc potency tested. The data obtained showed that compound 1 was not active against *P. aeruginosa* and *S. paratyphi* B, whereas it showed antibacterial activity against *E. coli*, *K. pneumoniae*, *S. aureus* and *S. typhi*. As far as compound 2 is concerned, it showed antibacterial activity against all the strains used (Table-I).

Compounds 1 and 2, which showed antibacterial activity against the bacteria strains used, were further studied using broth dilution technique and the following results were obtained:

Table-I: Diameters of inhibition of bactericidal strains by the compounds isolated from the seeds of *P. zenkeri*.

Compound	Disc potency(µg)	Bacteria and diameters of zones of inhibition (mm)					
		EC	PA	KP	SA	ST	SPB
1	200	10	NA	8	8	7	NA
2	200	10	9	8	7	7	8
3	200	NA	NA	NA	NA	NA	NA
4	200	NA	NA	NA	NA	NA	NA
5	200	NA	NA	NA	NA	NA	NA
6	200	NA	NA	NA	NA	NA	NA
7	200	NA	NA	NA	NA	NA	NA
Ciprofloxacin (Standard)	1	13	12	8	11	9	10

Tabulated values of diameters of zones of inhibition are means of two determinations. NA= Not Active; ST=*Salmonella typhi*, SPB = *Salmonella paratyphi* B, EC=*Escherichia coli*, PA=*Pseudomonas aeruginosa*, KP=*Klebsiella pneumoniae*, SA=*Staphylococcus aureus*, 1 = Umbelliprenin, 2 = prangenin, 3 = imperatorin, 4 = isopimpinellin, 5 = bergapten, 6 = cnidilin, 7 = stigmasterol.

for compound 1, the MIC values were greater than 300 µg/ml against *E. coli* and *K. pneumoniae*, and 300 µg/ml against the other bacteria strains. The MBC values were greater than 300 µg/ml against all the bacteria strains tested; for compound 2, MIC values were 300 µg/ml against *P. aeruginosa*, *S. typhi* and *S. paratyphi B*, 250 µg/ml against *E. coli* and 200 µg/ml against *K. pneumoniae* and *S. aureus*. MBC values were greater than 300 µg/ml against *P. aeruginosa*, *S. typhi* and *S. paratyphi B*, and 300 µg/ml against the remaining bacteria strains (Table-II).

DISCUSSION

Among the seven compounds isolated from the seeds of *P. zenkeri*, Umbelliprenin (1), prangenin (2) were the only compounds found to exhibit antimicrobial activities against the bacteria strains used. Compound 3 did not show any antibacterial activity against these bacteria strains. From the structure of compound 3, it can be seen that it differs from compound 2 in that the double bond present on compound 3 is epoxidised in compound 2. Thus, the antibacterial activity of compound 2 may be attributed to the epoxide group present in its structure.

Antimicrobial substances are considered as bactericidal agents when the ratio MBC/MIC <4 and bacteriostatic agents when the ratio MBC/MIC > 4.^{11,17} For compound 1, it cannot

be specified, since its MBC values were not precisely determined. As far as compound 2 is concerned, the ratio MBC/MIC < 4, suggesting that it may be classified as bactericidal agent against *E. coli*, *K. pneumoniae* and *S. aureus*. However, its class cannot be specified as far as *P. aeruginosa*, *S. typhi* and *S. paratyphi B* are concerned, since MBC values against these bacteria were not precisely determined. Besides, the compounds 1 and 2 showed antimicrobial activity against both Gram-negative rods (e.g. *K. pneumoniae*, *P. aeruginosa*, *E. coli*, *S. typhi* and *S. paratyphi B*)³ and Gram-positive cocci (e.g. *S. aureus*),³ indicating that these substances may be broad spectrum antibacterials. These results are in line with those of Ngwendson *et al.*⁷ who reported the antimicrobial activities of three compounds (imperatorin, isopimpinellin and bergapten) isolated from the hexane extract of the seeds of *Peucedanum zenkeri* against *Cryptococcus neoformans* and *Mycobacterium intracellulare*.

The activity of ciprofloxacin (standard drug) against all the bacteria strains tested was much greater than those of compounds 1 and 2. The MIC and MBC values of ciprofloxacin were generally more than 100 times lower than those of these active compounds, suggesting that compounds 1 and 2 may be about 100 times less active than ciprofloxacin against the bacteria used.

Table-II: Inhibition parameters (MIC, MBC) of active compounds (1 and 2), isolated from the seeds of *P. zenkeri*, against bacteria strains tested.

Compound	Parameters	Bacteria strains					
		EC	PA	KP	SA	ST	SPB
1	MIC (µg/ml)	>300	300	>300	300	300	300
	MBC(µg/ml)	>300	>300	>300	>300	>300	>300
	MBC/MIC	ND	ND	ND	ND	ND	ND
2	MIC (µg/ml)	250	300	200	200	300	300
	MBC(µg/ml)	300	>300	300	300	>300	>300
	MBC/MIC	1.2	ND	1.5	1.5	ND	ND
Ciprofloxacin (Standard)	MIC (µg/ml)	2	2	2	2	2	2
	MBC(µg/ml)	5	5	8	5	8	5
	MBC/MIC	2.5	2.5	4	2.5	4	2.5

MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration.

ND=Not Determined; ST =*Salmonella typhi*, SPB=*Salmonella paratyphi B*, EC=*Escherichia coli*, PA=*Pseudomonas aeruginosa*, KP=*Klebsiella pneumoniae* and SA=*Staphylococcus aureus*

1 = Umbelliprenin, 2 = prangenin.

In order to ascertain the therapeutic efficacy of the active compounds, *in vivo* studies should be conducted; i.e., typhoid fever or urogenital infection should be induced in animal models to test the therapeutic effect of the compounds. Moreover, toxicological studies should be done to assess the safety of the users of these compounds.

CONCLUSIONS

In the light of the foregoing, it is clear that the methylene chloride extract of the seeds of *Peucedanum zenkeri* contains antimicrobial principles [i.e. Umbelliprenin (1) and prangenin (2)] which are active against both Gram-negative and Gram-positive bacteria, causative agents of typhoid fevers and urogenital infections. The antibacterial activity of compound 2 appears to be due to the epoxide group present in its structure. Besides, other compounds namely imperatorin (3), isopimpinellin (4), bergapten (5), cnidilin (6) and stigmaterol (7) were also isolated from this extract, though they did not show any antibacterial activity against the bacteria stains used.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from the Faculty of Science (University of Buea) Research Grant, TWAS grant N^o: 07-301 RG/CHE/AF/AC-UNESCO FR: 3240184293 and the ICBG grant from the Fogarty International Center. We acknowledge Viboh Ruth for running the NMR data for the compounds and Dr. Wirmum Clare for plant collection. We also wish to express our gratitude to Dr/Mrs Fonkoua M. Marie-Christine, Medical Bacteriology Laboratory, Pasteur Centre, Yaounde, Cameroon, for her cooperation.

Authors Contribution: The chemical aspect of the work was done by James A. MBAH and Simon M.N. EFANGE, whereas the microbiological (or antimicrobial) aspect was done by Donatien GATSING.

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