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Hemisynthesis and evaluation of pharmacological activities of carvacrol-derivatives

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Hemi-synthesis, a process widely used in pharmacological research, consists of a modification in the chemical structure of a natural product in order to improve its activity and/or to reduce its side effects. Two carvacrol-derivatives (P1 and P2) have been synthetized using reactions of alkylation by binding alkan groups at the hydroxyl group of carvacrol. NMR analysis was performed for synthetized derivatives to confirm the success of the reactions. Then, cytotoxic activity, against two tumour cell lines (P-815 and MCF-7), and antibacterial activity of carvacrol, P1 and P2 were performed. Cytotoxicity was measured using the colourimetric methyl tetrazolium test (MTT) and antimicrobial activity was measured using the diffusion technique on solid media and the determination of CMI on liquid media. Our results show that chemical modifications made on carvacrol have no effect on its antitumor activity. However, an important decrease of its antibacterial activity was observed, especially for P1. Our results suggest that hydroxyl group at this position of the molecule may be responsible for carvacrol antibacterial activity, while the other parts of the molecule may be responsible for its antitumor activity. On the other hand, introduced modifications may affect mechanism of action of the molecules as well as its pharmacokinetics properties.

Keywords: Antibacterial, Carvacrol, Cytotoxicity, Hemi-synthesis, NMR analysis.

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Introduction

Hemisynthesis is an important process used to improve the efficiency and reduce the toxicity of chemical molécules[1-4]. In fact, providing modifications to the chemical structure of bioactive molecule could lead to change its pharmacokinetics and/or pharmacodynamics properties. This could affect its pharmacological effect by improving its activity and/or by reducing its toxicity[5,6]. The effect of chemical structure modification on pharmacological activity of molecules was reported in the literature. In fact, over the years, several structure-activity and side-effect relationships of fluoroquinolones, antibacterial class, have been developed. Subsequent developments produced quinolones with important improvements in either solubility (ofloxacin), antimicrobial activity (ciprofloxacin), prolonged serum half-life (pefloxacin), or reduced undesirable side-effects[7,8]. Also, Molecules with potential anticancer effect are among the products the most studied in this sense[3,4,9,10].

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Natural products are increasingly being explored because of their valuable therapeutic properties. However, like synthetic molecules, their systemic toxicity is a limit to their success[10-12]. In a previous study by the authors[13], it was reported that carvacrol, natural product of thyme essential oil, has an important *in vitro* and *in vivo* antitumoral effect. In order to increase its activity and/or decrease its toxicity on the normal cells, authors synthesized two carvacrol-derivatives by providing modifications to the chemical structure of this natural molecule. In this research, after synthesis of the carvacrol-derivatives, cytotoxic effect against tumour cell lines, and their antibacterial effect in comparison to that of carvacrol were tested.

Materials and Methods

Carvacrol-derivatives synthesis

Two carvacrol-derivatives (P1 and P2) have been synthetized using reactions of alkylation by grafting alkan at the hydroxyl group of carvacrol. The grafted alkans are the allyl-bromide (CH_2═CH─CH_2─Br) and 3-Br-propanoate d'ethyle (Br─CH_2─CH_2─CO─OEt).

The molecules obtained are P1 and P2, respectively. The reactions were realised as follow:

Exactly 0.5 g (3.3 moles) of carvacrol and 3.3 moles of natrium ethanoate were made in an erlenmeyer flask in the presence of 50 mL of THFA (Tetrahydrofolate) and agitated for 15 to 20 minutes. Afterwards, 3.3 moles of the alcan was added to the graft and continued to agitate until the end of the reaction. At time intervals, CCM layer chromatography is realized to monitor the progress of the reaction. This reaction is considered as finished when only one spot corresponding to the new synthesized product remains.

NMR analysis

^{1}**H** and ^{13}**C** NMR spectra were recorded in $CDCl_3$ and solution (unless otherwise specified) with TMS as an internal reference using a Bruker AC 300-MHz (^{1}**H**) or 75-MHz (^{13}**C**) instruments; chemical shifts are given in δ ppm downfield from TMS. Multiplicities of ^{13}**C** NMR resources were assigned by distortionless enhancement by polarization transfer (DEPT) experiments.

In vitro cytotoxic effect of carvacrol and carvacrol-derivatives against tumour cell lines

The cytotoxic activity was studied against two tumour cell lines (P-815 (murine mastocytoma) and MCF-7 (Breast cancer). Cytotoxicity was measured using the colourimetric Methyl Tetrazolium Test (MTT) assay as described and modified by Tim Mosmann[14]. The target cells were washed twice and placed in 96-well microtiter plates (Bioster, Italy) at a density of $1.5.10^5$ cells/mL in 100 µL/well of culture medium (DMEM supplemented with 5% FCS and 1% of penicillin and streptomycin). Then, 100 µL of culture medium containing the specified concentration of the tested molecules was added in each well. After exposure of cells to serial concentrations of tested products for 48 h at 37°C and 5% CO_2, 100 µL of culture medium were carefully aspirated from each well and replaced by 20 µL of MTT solution (5 mg/mL of PBS). After incubation in the same conditions for 4 h, the plates were treated with a solution of HCl and isopropanol (24:1) to dissolve the blue intracellular formazan product. One hour later, the plates were read in a MicroELISA reader at 590 nm.

Antibacterial activity

In order to evaluate the impact of our chemical modifications on the antibacterial activity, the three molecules (carvacrol, P1 and P2) were tested against *Escherichia coli*, for antimicrobial activity using the diffusion technique on solid media and determination of CMI on liquid media.

Diffusion technique on solid media

In the experiments, bacterial suspensions of $1.5x10^8$ UFC/ mL or 0.5 McFarland density obtained from 15-18 h bacterial cultures developed on solid media was used. Solutions of the compounds in DMSO (dimethyl sulfoxide) was used. Exactly 10 µL of the compound solution was equally distributed on the paper filter disks (8 mm diameter) placed on Petri dishes previously seeded with the tested bacterial strain inoculum. The plates were then incubated for 24 h at 37°C. The results were recorded by measuring the zones of growth inhibition surrounding the cylinders. Control cylinders contained 100 mL of dimethyl sulfoxide (DMSO). In addition, gentamycin, ampicillin, and streptomycin were used as controls in the assay.

CMI determination by Broth microdilution method

This test was realised in order to establish the minimal inhibitory concentration (MIC) of the tested products (carvacrol, P1, and P2). The test was carried out in 96-well flat-bottom microplates with sterile covers. Preparation of the bacterial suspension began at the moment that growth was first observed on the solid medium. After agitation, the suspension was transferred into another tube containing sterile distilled water, until turbidity similar to the McFarland turbidity standard of 1 was achieved. Then, bacterial suspension was exposed to serial dilutions of the tested products. Microplate was sealed with plastic film and remained in the incubator at 37°C for 24 h. The MIC was then determined, defined as the lowest molecule concentration capable of 90% inhibiting bacterial growth.

Statistical analysis

The individual data values are presented as the arithmetic mean±SD (standard deviation). The statistical significance of the results obtained from *in vitro* studies was evaluated by the Student's t-test or by ANOVA at P <0.05, using STATISTICA software.

Results

Carvacrol-derivatives synthesis

Synthesis of the two carvacrol-derivatives was realised by reactions of alkylation. The molecules

Fig. 1 — a) The carvacrol-derivative P1 has been synthetized using reaction of alkylation by grafting the allyl-bromide (CH_2═CH─CH_2─Br) at the hydroxyl group of carvacrol; b) The carvacrol-derivative P2 has been synthetized using reactions of alkylation by grafting 3-Br-propanoate d'ethyle (Br─CH_2─CH_2─CO─OEt) at the hydroxyl group of carvacrol.

obtained (P1 and P2) (Fig 1, 2, 3) and NMR analysis results are represented below:

NMR^{1}H ($CDCl_3$) : δ(ppm) 1.33 (d, 6H, 2CH_3, J = 6.9Hz), 2.31 (s, 3H, CH_3), 2.95 (m, 1H, CH, J = 6.9Hz), 4.63 (dt, 2H, CH_2O, J = 1.6Hz et 5.1Hz), 5.36 (dq,1H,=CH, J = 1.5Hz, 10.5Hz), 5.52 (dq,1H,=CH, J = 1.7Hz, 17.2Hz), 6.16 (m, 1H, =CH), 6.78 (s, 1H, H-Ar), 6.83 (dd, 1H, H-Ar, J = 1.5Hz et 7.6Hz), 7.15 (d, 1H, H-Ar, J = 7.6Hz).

NMR^{13}C ($CDCl_3$): δ (ppm) 15.9 (CH_3), 24.2 (2CH_3), 32.0 (CH), 34.2 (CH_3), 68.8 (CH_20), 109.9 (=CH), 116.8(=CH_2), 117.9 (=CH), 124.3 (Cq), 130.5 (=CH), 133.9 (=CH), 147.8 (Cq), 156.7 (Cq).

Fig. 2 — 2-(alloyloxy)-4-isopropy1-1-methylbenzene (P1).

NMR^{1}H ($CDCl_3$) : δ(ppm) 1.28 (d, 6H, 2CH_3, J = 6.9Hz), 1.36 (t, 3H, CH_3, J = 7.1Hz), 2.29 (s, 3H, CH_3), 2.91 (m, 1H, CH, J = 6.9Hz), 2.98 (t, 2H, CH_2, J = 6.8Hz), 3.64 (t, 2H, CH_2O, J = 6.8Hz), 4.28 (q, 2H, CH_2O, J = 7.1Hz), 6.73 (d, 1H, H-Ar, J = 1.5Hz), 6.78 (dd, 1H, H-Ar, J = 1.5Hz et 7.6Hz), 7.10 (d, 1H, H-Ar, J = 7.6Hz).

NMR^{13}C ($CDCl_3$): δ (ppm) 14.2 (CH_3), 15.5 (CH_3), 24.1 (2CH_3), 25.9 (CH_2), 33.7 (CH), 34.2 (CH_3), 61.4 (CH_2O), 68.8 (CH_2O), 113.2 (=CH), 118.6 (=CH), 121.3 (Cq), 130.9 (=CH), 148.3 (Cq), 153.9 (Cq), 171.2 (CO).

Fig. 3 — Ethyl 3-(5-isopropyl-2-methylphenoxy)propanoate (P2).

***In vitro* cytotoxic effect of carvacrol and carvacrol-derivatives against tumour cell lines**

The antitumor activity of carvacrol and its two derivatives was evaluated against two tumour cell lines (P-815 and MCF-7). The results obtained are summarized in Tables 1, 2, and 3. In order to compare the three molecules effects, their IC50 (i.e. the concentration at which 50% of the lytic activity was reached) was calculated. Against P-815, IC50 (in % v/v) values are 1.4 x 10^{-3}, 1.6 x 10^{-3} and 1.2 x 10^{-3} for carvacrol, P1 and P2, respectively. Against MCF-7, IC50 values are 1.65 x 10^{-3}, 1.8 x 10^{-3} and 2.05 x 10^{-3} for carvacrol, P1, and P2, respectively.

These results show that there is no significant difference in the effect of the three molecules against the tumour cell lines tested (P <0.05).

Table 1 — *In vitro* cytotoxic effect of carvacrol, P1 and P2 against P-815 tumour cell line

	Concentration (% v/v) 10^3			
	0.5	1.5	4.5	6.25
Carvacrol	28.23(±2.12)	51.12(±4.78)	58.05(±5.01)	61.76(±5.43)
P1	38.23(±3.09)	49.13(±3.22)	54.45(±2.17)	56.6(±6.09)
P2	40.43(±5.02)	54.5(±4.42)	56.71(±2.98)	56.89(±4.68)

Table 2 — *In vitro* cytotoxic effect of carvacrol, P1 and P2 against MCF7 tumour cell line

	Concentration (% v/v) 10^3			
	%Lysis			
	0.5	1.5	4.5	6.25
Carvacrol	31.77(±2.32)	88.16(±12.1)	89.71(±9.62)	94.24(±12.2)
P1	37.17(±3.12)	80.32(±7.12)	90.70(±9.94)	93.70(±13.1)
P2	24.49(±2.09)	83.10(±8.23)	88.65(±7.89)	90.34(±9.99)

Table 3 — IC50 (in % v/v x 10^3) values of the molecules against tumour cell lines tested (P <0.05)

	P-815	MCF-7
Carvacrol	1.4(± 0.12)	1.65(± 0.09)
P1	1.6(± 0.14)	1.8(± 0.11)
P2	1.2(± 0.12)	2.05(± 0.17)

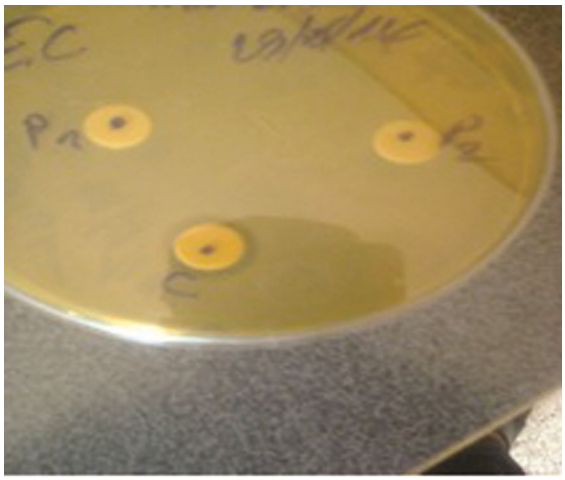

Fig. 4 — Antibacterial activity of carvacrol, P1 and P2 on solid media.

Antibacterial activity

Diffusion on solid media

Reading of results was performed by measuring the microbial growth inhibition zones around the filter disks impregnated with the testing compounds. The results show an important antibacterial effect of carvacrol with an inhibition zone of 12 mm. However, P1 and P2 have no effect against the tested bacteria since no inhibition zone was observed (Fig. 4).

Broth microdilution method

Broth microdilution method in 96-well microplates was conducted in order to establish the minimal inhibitory concentration (MIC). Comparing antibacterial effect of the tree tested molecules, our results show that carvacrol has the most important effect. In fact, the minimal inhibitory concentrations (MICs) of carvacrol, P1, and P2 were 3.45, 6.54 and >12.5 v/V respectively.

Discussion

Hemi-synthesis is a process widely used in research in the field of pharmacology[15]. It consists of a modification in the chemical structure of a natural product in order to improve its pharmacokinetics and/or pharmacodynamics properties and, subsequently, to hence its activity and/or to reduce its side effects[16-21]. In this sense, two carvacrol derivatives (P1 and P2) were synthesized by alkylation (Fig. 1, 2, 3) and evaluated for their antitumor and antibacterial activities.

Our results show that antitumor activity of our derivatives, obtained by the binding of two alkyls at the carvacrol hydroxyl group, has not been changed (Tables 1, 2 3). However, an important decrease of antibacterial activity was observed, especially for P1 (Fig. 4). Thus, elimination of hydroxyl group has no effect on antitumor activity but strongly decrease antibacterial activity, which means that OH at this position of the molecule may be responsible of carvacrol antibacterial activity. On the other hand, the absence of inhibition zone for P1 and P2 in the test of diffusion on solid media could be explained by the decrease in the capacity of the synthetized derivates to diffuse on solid media because of their chemical modification. Thus, we can say that introduced chemical modifications may affect mechanism of action of the molecule as well as its pharmacokinetics properties.

The involvement of hydroxyl group in the carvacrol activity is not approved by all the researchers. In fact, some of them believe that the presence of a free hydroxyl group is essential for its antimicrobial activity[22]. The structure-activity relationship study demonstrated that antibacterial activity of the curcumin analogues was critically dependent upon the aromatic hydroxyl group[23]. Others, unlike the previous, reported that the hydroxyl group of carvacrol itself is not essential for antimicrobial activity[24]. The same study reported that hydroxyl group of carvacrol is not essential for activity but has special features that add to the antimicrobial mode of action of carvacrol which was related to membrane disruption. In fact, the

hydrophilic part of the molecule interacts with the polar part of the membrane, while the hydrophobic benzene ring and the aliphatic side chains are buried in the hydrophobic inner part of the bacterial membrane[24]. Ultee *et al.*[25], proposed a mechanism of action for carvacrol based on the phenolic compounds acidity. In this mechanism, the loss of the proton gradient motive force, over the bacterial membrane[25]. This result suggests, may be, that carvacrol hydroxyl group, at least at this position and in this environment, is not necessary for its mechanism of action as antitumor agent, but play an important role in antibacterial activity and pharmacokinetics properties. In fact, previous study reported that cytotoxic activity of carvacrol is related to the apoptotic mode of action[13]. On the other hand, concerning the role of OH in antitumor activity, Wilbour reported that ring hydroxylation or removal of the terminal hydroxyl group have only modest effects on activity against L1210 leukaemia. These data also suggest that primary and ring hydroxyl groups do not markedly influence either anticancer activity or potency[26].

Taken together, our results suggest that hydroxyl group is responsible of carvacrol antibacterial activity and the other parts of the molecule are responsible of its antitumor activity. Thus, in order to improve cytotoxic activity of carvacrol, the chemical modifications must be executed at the other parts of the molecule (methyl groups and benzen ring) by alkylation or introduction of halogen groups. Some natural monoterpens, like carvacrol, were explored in this sense. In fact, derivatives of carvone, carveol, and limonene were designed and synthesized via chlorination, nucleophilic substitution, and reduction. It was shown that the introduction of 4-(2-methoxyphenyl) piperazine to carvone, carveol or limonene significantly increased their antiproliferative effect that was correlated with ERK activation and p21 (waf1) induction[27]. Also, the number and environment of hydroxyl groups in a molecule may also have an impact on its antitumor activity as it has been shown by the study of Iwasaki *et al.*[28]. Regarding carvacrol, it has been reported that four derivatives of carvacrol: 4-(hydroxymethyl) -5-isopropyl-2 methylphenol,4,4'methylenebis (5isopropyl2methyl) phenol, 4-allyl-6-(hydroxymethyl)-2-methoxyphenol, and 4-(hydroxymethyl)-2-isopropyl-5-methylphenol were synthesized and tested for their pharmacological activities. The obtained derivatives showed remarkably better antioxidative properties and the majority of synthesized compounds had dose-dependent antiproliferative effects on human uterine carcinoma cells (HeLa)[29,30]. It must be noted here, that antiproliferative activity of carvacrol derivatives is improved when the chemical modifications are made on the other parts of the molecule and not on the hydroxyl group, which agrees with the present hypothesis.

Conclusion

Two new carvacrol derivatives have been hemi-synthesized by binding two different alcans on the hydroxyl group of this natural product extracted from thyme essential oil. The hemi-synthesized molecules were then tested for their antibacterial and antitumor effects in a comparison to the original product (carvacrol). Our results revealed that hydroxyl group of carvacrol in this position and this environment may have a role to play in antibacterial activity of carvacrol but not in its antitumor activity. These finding suggests that, in order to improve its antitumor effect, the chemical modifications must be provided on the other parts of the natural molecule and/or by increasing the number or changing the position of the hydroxyl group. The nature of alcans to bind could also improve this effect.

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References

1 Hidmi A, Salah Z, Ariqat S and Nasereddin A, *Chemical modification of curcumin enhances its anti-cancer, anti-inflammatory and anti-leishmania activities*, 14th International Conference on Generic Drugs and Biosimilars & 9th Global Experts Meeting on Neuropharmacology, 15-16 Nov, 2018, (Berlin, Germany), 2018.

2 Hou J, Fu J, Zhang Z and Zhu H, Biological activities and chemical modification of cafeic acid, *J Med Sci*, 2011, **38**(6), 546-552.

3 Routier S, Mérour J Y, Dias N, Lansiaux A, Bailly C, *et al.*, Syhthesis and evaluation of novel phenylcarbazoles as potential anticancer agents, *J Med Chem*, 2006, **49**(2), 789-799.

4 Jacquemard U, Dias N, Lansiaux A, Bailly C, Logé C, *et al.*, Synthesis of 3, 5-bis (2-indolyl) pyridine and 3-[(2-indolyl)-5-phenyl]-pyridine derivatives as CDK inhibitors and cytotoxic agents, *Bioorg Med Chem*, 2008, **16**(9), 4932-4953.

5 Huang G, Chen X and Huang H, Chemical modifications and biological activities of Polysaccharides, *Curr Drug Targets*, 2016, **17**(15), 1799-1803.

6 Magyar J, Szentandrássy N, Bányász T, Fülöp L, Varró A, *et al.*, Effects of terpenoid phenol derivatives on calcium

current in canine and human ventricular cardiomyocytes, *Eur J Pharmacol*, 2004, **487**(1-3), 29-36.

7 Percival A, Impact of chemical structure on quinolone potency, spectrum and side effects, *J Antimicrob Chemother*, 1991, **28(**suppl_C), 1-8.

8 Domagala J M, Structure-activity and structure-side-effect relationships for the quinolone antibacterials, *J Antimicrob Chemother*, 1994, **33**(4), 685–706.

9 Zhiyan X, Morris-Natschke S L and Lee K H, Strategies for the optimization of natural leads to anticancer drugs or drug candidates, *Med Res Rev*, 2016, **36**(1), 32-91.

10 Liu D and Chen Z, The effects of cantharidin and cantharidin derivates on tumour cells anticancer agents, *Med Chem*, 2009, **9**(4), 392-396.

11 Kok S H, Chui C H, Lam W S, Chen J, Lau F Y, *et al.*, Synthesis and anti-cancer activity of benzothiazole containing phthalimide on human carcinoma cell lines, *Bioorg Med Chem*, 2008, **16**(7), 3626-3631.

12 Hansch C and Verma R P, Understanding tubulin/ microtubule-taxane interactions: a quantitative structure-activity relationship study, *Mol Pharm*, 2008, **5**(1), 151-61.

13 Jaafari A, Mouse H A, M'bark L A, Tilaoui M, Elhansali M, *et al.*, Differential antitumor effect of essential oils and their major components of *Thymus broussonettii*: relationship to cell cycle and apoptosis induction, *Herba Polinica*, 2009, **55**(2), 36-50.

14 Mosmann T, rapid colourimetric assay for cellular growth and survival: Application to proliferation and cytotoxic assay, *J Immunol Methods*, 1983, **65**, 55-63.

15 McCluskey A, Ackland S P, Bowyer M C, Baldwin M L, Garner J, *et al.*, Cantharidin analogues: synthesis and evaluation of growth inhibition in a panel of selected tumour cell lines, *Bioorg Chem*, 2003, **31**, 66-77.

16 Ferrera-Suanzes M, Prieto V, Medina-Olivera A J, Botubol-Ares J M, Galán-Sánchez F, *et al.*, Synthesis of degraded limonoid analogues as new antibacterial scaffolds against Staphylococcus aureus, *Antibiotics*, 2020, **9**, 488.

17 Casertano M, Menna M and Imperatore C, The ascidian-derived metabolites with antimicrobial properties, *Antibiotics*, 2020, **9**, 510.

18 Vuong T V, Natural products and their derivatives with antibacterial, antioxidant and anticancer activities, *Antibiotics*, 2021, **10**(1), 70.

19 Ridha H, Imad A H, Bouchama A, Boukenna L, Aissaoui M, *et al.*, Green hemi-synthesis of novel thiazole derivatives from *Ammodaucus leucotrichus* Coss. & Dur. and Cuminum cyminum, *J Mol Struct*, 2022, **1265**, 133376.

20 Sabour A, El asbahani A, Bentahar S, Taleb M A, Lacherai A, *et al.*, Synthesis of some Thymol derivatives for enhanced antibacterial activity, *Mor J Chem*, 2019, **7**, 748-757.

21 Sameut A, Zanndouche S, Boumaza C, Dikes C and Ziani B C, Chemical synthesis and hemi-synthesis of novel benzimidazoles derivatives using microwave-assisted process: Chemical characterization, bioactivities and molecular docking, *Chem Proc*, 2021, **3**(1), 71.

22 Ben Arfa A, Combes S, Preziosi-Belloy L, Gontard N and Chalier P, Antimicrobial activity of carvacrol related to its chemical structure, *Lett Appl Microbiol*, 2006, **43**, 149–154.

23 Kim M K, Park J C and Chong Y, Aromatic hydroxyl group plays a critical role in antibacterial activity of the curcumin analogues, *Nat Prod Commun*, 2012, **7**(1), 57.

24 Veldhuizen E J, Tjeerdsma-van Bokhoven J L, Zweijtzer C, Burt S A and Haagsman H P, Structural requirements for the antimicrobial activity of carvacrol, *J Agric Food Chem*, 2006, **54**(5), 1874-1879.

25 Ultee A, Bennik M H and Moezelaar R, The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen bacillus cereus, *Appl Environ Microbiol*, 2002, **68**(4), 1561-1568.

26 Leopold W R, Shillis J L, Mertus A E, Nelson J M, Roberts B J, *et al.*, Anticancer activity of the structurally novel antibiotic CI-920 and its analogues, *Cancer Res*, 1984, **44**(5), 1928-1932.

27 Chen J, Lu M, Jing Y and Dong J, The synthesis of L-carvone and limonene derivatives with increased antiproliferative effect and activation of ERK pathway in prostate cancer cells, *Bioorg Med Chem*, 2006, **14**(19), 6539-6547.

28 Iwasaki K, Zheng Y W, Murata S, Ito H, Nakayama K, *et al.*, Anticancer effect of linalool via cancer-specific hydroxyl radical generation in human colon cancer, *World J Gastroenterol*, 2016, **22**(44), 9765-9774.

29 Ignas G and Petrikaite V, Relationship between antioxidant and anticancer activity of trihydroxyflavones, *Molecules*, 2017, **22**(12), 2189

30 Mastelić J, Jerković I, Blazević I, Poljak-Blazi M, Borović S, *et al.*, Comparative study on the antioxidant and biological activities of carvacrol, thymol, and eugenol derivatives, *J Agric Food Chem*, 2008, **56**, 3989–3996