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Anti-Cariogenicity of 2-Hydroxyethyl β-Undecenate from Cumin (*Cuminum cymium* L.) Seed

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Abstract This study was to assess the antimicrobial action of 2-hydroxyethyl β-undecenate purified from cumin (*Cuminum cymium* L.) seed against the oral anaerobe, *Streptococcus mutans*, which is associated with gingivitis, specifically focusing on the catabolic effect. 2-Hydroxyethyl β-undecenate inhibited the acid production and growth of *S. mutans* after 30 hr incubation at 50 mM. The glycolysis of *S. mutans* with glucose as substrate was similarly sensitive to 2-hydroxyethyl β-undecenate, with 70% inhibition of glucose utilization at 5 mM and 90% inhibition at 50 mM. In addition, this substance potently inhibited the glycolysis enzyme, glyceraldehyde-3-phosphate dehydrogenase (GADP); the phosphoenolpyruvate, glucose phosphotransferase (Glucose-PTS); and membrane ATPase, in a concentration dependent manner. The IC_{50} values for inhibition of GADP, Glucose-PTS, and ATPase were 1, 0.9, and 5 mM, respectively. Furthermore, 2-hydroxyethyl β-undecenate inhibited teeth calcium ion elution by 80% at 50 mM. These results suggest that 2-hydroxyethyl β-undecenate is a potent inhibitor of carbohydrate metabolism and the growth of *S. mutans* JC-2.

Keywords: 2-hydroxyethyl β-undecenate, cumin (*Cuminum cymium* L.) seed

Introduction

Oral disease is caused by dental caries that are induced by the demineralization of enamel and dentine mediated by the acid produced by plaque forming microbes that metabolize dietary sugar (1).

It is a well known fact that the direct cause of dental caries are the mutans streptococci such as *Streptococcus mutans, Streptococcus sobrinus* as well as anaerobic bacteria such as lactobacilli and actinomycetes in the vicinity of the disease lesions (2-4).

Among these microbes, streptococci can colonize the tooth surface and initiate plaque formation due to its ability to synthesize mucous extracellular polysaccharide from sucrose, mainly α-1,3 linked insoluble glucan and α-1,6 linked soluble glucan, using glucosyltransferase (GTase) (5).

In addition, these bacteria produce acids by fermenting vacious dietary sugars (6, 7). Efforts to remove *S. mutans* from the oral cavity by applying antibiotics such as chlorhexidine, penicillin, ampicillin, tetracyclin, erythromycine, and vancomycin are very effective. These substances, however, cause side effects such as speckled or spotted teeth, the appearance of tolerate microbes, and the dissemination of oral mucosa cells. Therefore, the search for safe natural antimicrobial agents specific to the oral cavity is important, and materials with antibiotic activity against *S. mutans* obtained from various plant species worldwide have been reported.

Limsong *et al.* (8) reported that the ethanol extract (0.2-0.5%) of the Thailand herbs *Andrographis paniculate, Camillia sinensis, Cassia alata, Psidium guajava, Harrisonia perfarata,* and *Streblu asper* decreased the adherence of *S. mutans* to the tooth surface. Hwang *et al.* (9) reported that isopanduratin isolated from *Kaempferia pandurata* Roxb has a minimum inhibitory concentration (MIC) of 4 mg/L to *S. mutans*. In addition, Yatsuda *et al.* (10) isolated various compounds from Mikania species plants that are used as folk-medicines in Brazil. Some of these compounds, namely 1-octadecene, diterpenic, and kaurenoic acid, were found to have MICs of 12.5-400 μg/mL against Streptococci.

In Turkey and India, the spice cumin has been used widely as a food additive for curries and other dishes. However, examples of studies on its antimicrobial effect in general or its effect on oral microbes, or caries-inducing bacteria, or its preventive effect on dental caries are not easy to find. Recently, we reported that 2-hydroxethyl β-undecenate from the Indian cumin (*Cuminum cymium* L.) seed (Fig. 1) showed superior antibacterial activity to the major causative bacteria of halitosis, *Fusobacterium nucleatum* and *Porphyromonas gingivali.* Also, it interfered effectively with the L-methionine-α-deamino-γ-mercaptomethane-lyase (METase) produced by these bacteria, and thus blocked the production of volatile sulfur compound (VSC) (11).

The present study was performed to examine the antibacterial and anti-caries effect of the compound isolated from cumin seed on the major causative bacteria of caries, *S. mutans*. Furthermore, in order to develop new therapeutic agents to prevent the overpopulation of *S. mutans,* the inhibition of *S. mutans* energy metabolism was correlated with bacterial viability.

Materials and Methods

Materials 2-Hydroxyethyl β-undecenate used in this study was isolated from cumin seeds as described, and after recharging with nitrogen, it was stored at 4°C until use (11).

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Culture conditions of *S. mutans* JC-2 *S. mutans* JC-2 used in our study were cultured in BHI broth (Brain Heart Infusion: Difco, Detroit, MI, USA) at 37°C in the presence of 5% CO_2 for 24 hr, and the stock organism was stored in BHI broth containing 5% CO_2 and 10% glycerol at -80°C.

The effect on the growth of *S. mutans* JC-2 and the production of acids The antibacterial activity of 2-hydroxyethyl β-undecenate on *S. mutans* JC-2 was determined after adding various concentrations of 2-hydroxyethyl β-undecenate (0.1-50 mM) to BHI broth. After the test strain was inoculated to a concentration of 4%(v/v) and cultured in a CO_2 incubator at 37°C for 30 hr, the bacterial growth was assessed spectrophotometrically by determing optical density at 620 nm. Also, a MIC assay was carried out by the cup cylinder diffusion method (12) using 250 μg of culture broth of *S. mutans* JC-2 (10^8 CFU/mL). The 6×10 mm cup cylinder was placed on the inoculated media and impregnated with 200 μg 2-hydroxyethyl β-undecenate at various concentrations (0.1-50 mM), at 37°C for 30 hr. MIC was determined by the presence or absence of the clear zone against the test organism. In order to measure pH changes due to various organic acids produced during the culture of *S. mutans* JC-2 in the presence of the 2-hydroxyethyl β-undecenate, the pH of the culture medium was measured by placing a pH meter (Orion SA 720; Thermo electron Co., Waltham, MA, USA) directly into the culture medium.

Measurement of the cell membrane permeability of *S. mutans* JC-2 The effect of 2-hydroxyethyl β-undecenate on the cell membrane permeability of *S. mutans* JC-2 was examined. Various amounts of 2-hydroxyethyl β-undecenate were added to BHI broth. *S. mutans* JC-2 was then inoculated and cultured at 37°C in a 5% CO_2 incubator for 24 hr, according to the method of Ryu *et al.* (13). The cultured suspension was centrifuged (10,000×g, 20 min) to recover the strain which was resuspended in 0.1 M potassium phosphate buffer (pH 7.0), and left at 37°C for 10 min. Cell permeability was measured by colorimetric quantification of the eluted cytoplasmic substance using a spectrophotometer at a wavelength of 260 nm.

The effect on monosaccharide metabolism ***Glucose utilization*****:** The glycolysis of *S. mutans* JC-2 was assayed by measuring the disappearance of glucose in the reaction mixture, using a modified procedure of Sheng *et al.* (14). The reaction mixture was 2 mM potassium phosphate buffer (pH 7.0) supplemented with 75 mM NaCl, 75 mM KCl, 2 mM $MgCl_2$, 10 mM glucose, and 2 mg/mL *S. mutans* JC-2 in a total volume of 5 mL. The pH of the reaction mixture was adjusted to 7.0 with 0.2 N NaOH. 2-Hydroxyethyl β-undecenate in the concentration range 0.5-50 mM was added, and the mixture was incubated at 37°C for 24 hr. The amount of glucose remaining in the mixture was measured by the colometric quantitation of reducing sugar.

The inhibitory effect on glyceraldehyde-3-phosphate dehydrogenase (GADP)**:** The inhibitory effect of 2-hydroxyethyl β-undecenate on the *S. mutans* JC-2 GADP was assayed according to the method of Crow and Wittenberger (15).

S. mutans JC-2 was cultured in BHI broth at 37°C for 18 hr, centrifuged, collected and washed with 500 mL of 0.05 M potassium phosphate buffer containing 1 mM dithiothreitol. The washed bacteria were frozen at -20°C for 24 hr, thawed, resuspended in a small amount of the identical buffer, and disrupted by ultrasound using a sonicator at the maximum output. The disrupted cells were removed by centrifuging at 15,000×g, and the supernatant was used as the crude enzyme solution. One mL crude enzyme was added to 1 mL of assay mixture {125 mM triethanolamine/HCl supplemented with One mM NAD^+, 5 mM sodium arsenate, and 5 mM cysteine/HCl buffer (pH 7.5)} and preincubated at 30°C for 10 min. The reaction was started by the addition of 2 mM DL-glyceraldehyde-3-phosphate and after 5 min the increase in absorbance at 340 nm associated with NADH production was recorded over 5 min. The amount of NADP in the supernatant was compared to control.

Phosphoenolpyruvate: glucose phosphotransferase (glucose-PTS) inhibitory effect**:** Glucose-PTS is an enzyme that plays a central role in the glucose metabolism by controlling the transport system of carbohydrates such as glucose, fructose, mannose, and 2-deoxyglucose to the inside of cells (16-18).

The inhibitory effect of 2-hydroxyethyl β-undecenate on *S. mutans* JC-2 glucose-PTS activity was assayed using the modified procedures described by Cochu *et al.* (19), Libermen *et al.* (20), and Bender *et al.* (21). The extraction of cell membrane for the measurement of glucose-PTS activity was carried out as follows. *S. mutans* was cultured in BHI broth supplemented with 20 mM glucose for 18 hr. The cell was harvested from the late-exponential phase of growth by centrifuging (10,000×g, 20 min), washed 3 times with 0.05 M potassium phosphate buffer (pH 7.0), and resuspended with 10^{-1} volume of the identical buffer supplemented with 5 mM $MgCl_2$. Five tenth g lysozyme and 2,000 U mutanolysin (Sigma Co., St. Louis, MO, USA), and perturbed by vigorous shaking at 37°C for 2 hr. The cell suspension was decryptified by centrifugation (10,000×g, 20 min). This bacteria pellet was resuspended with the identical buffer supplemented with 10 mM $MgCl_2$, and 1.0 M NaCl. Bacteria were lyzed and incubated at 30°C for 1 hr with DNase and RNase (10 μg/mL; Sigma Co.) to remove nucleotides. The cell membrane pellet was harvested by centrifugation (15,000×g, 20 min), and resuspended with the identical buffer.

Glucose-PTS levels were determined using an assay that utilizes the glucose analogue *O*-nitrophenyl β-glucopyranoside (ONPG; Sigma Chemical Co.). The standard reaction mixture contained 10 mM $MgCl_2$, 1 mM NaF, 5 mM phosphoenol-pyruvate (PEP; Sigma Chemical Co.), 10 mM ONPG, and decryptified cell membrane in a final volume of 1 mL. The reaction was stopped after 30 min at 37°C by the addition of 1 mL of 5% Na_2CO_3. The membrane was pelleted by centrifugation (10,000×g, 20 min), and *O*-nitrophenol (ONP) in the supernatant was measured by spectrometry at 420 nm. The amount of ONP was determined from a standard curve. The inhibiton of enzyme activity was assessed by adding 0.5-50 mM of 2-hydroxyethyl β-undecenate, and comparing the ONP level to that of control (without inhibitor).

ATPase inhibitory effect**:** Generally, Gram positive

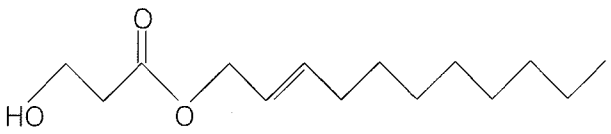

Fig. 1. The structure of 2-hydroxyethyl β-undecenate.

Streptococci lack the respiratory chain. Hence, ATP generated by substrate level phosphorylation is hydrolyzed by F_0F_1-ATPase. The cation potential generated by this enzymatic reaction is utilized in the various cation-coupled transports. This allows Streptococci to survive at conditions of low pH (22).

The effect of 2-hydroxyethyl β-undecenate on *S. mutans* JC-2 ATPase was assayed according to the method of Heinonen and Lathi (23). The cell pellet was added to 0.05 M potassium phosphate buffer (pH 7.0) containing 5 mM ATP and 10 mM $MgCl_2$, incubated at 37°C for 30 min, and then the reaction was terminated by adding cold 30% trichloroacetic acid. The amount of inorganic phosphate generated was quantitated colorimetrically at 640 nm. In addition, the enzyme inhibition was assessed by adding 2-hydroxyethyl β-undecenate in the concentration range 0.5-50 mM, preincubated with the enzyme. ATP was then added and after 30 min the amount of inorganic phosphate generated was compared with that generated without the inhibitor and the residual activity was measured.

The inhibitory effect on plaque formation on the human tooth surface 2-Hydroxyethyl β-undecenate was added to a captube containing 2 M sucrose and *S. mutans* JC-2 in order to measure its effect on the ability of glucan produced by *S. mutans* JC-2 to adhere to teeth. Teeth extracted from healthy individuals aged 20-30 were added, one to each tube, and incubated at 37°C for 3 days. The extracted tooth was then washed with cold water, unattached components were removed, and plaque formation was assessed.

Quantification of the calcium ion eluted from teeth In order to measure the amount of calcium released from the teeth, extracted teeth from healthy individuals aged 20-30 were fixed in epoxy resin, and the samples were prepared with the cutted tooth surface at the length and width 2×2 mm, surbmerged in the 2-hydroxyethyl β-undecenate solution for 30 min. And then washed with 1 mL distilled water 5 times. The collected distilled water was centrifuged (5,000×g, 20 min). Then, the absorbance of supernatant was read at 423 nm and the calcium ion concentration was calculated

Results and Discussion

The effect on the growth of *S. mutans* JC-2 and the production of acids MIC for 2-hydroxyethyl β-undecenate on the growth of *S. mutans* JC-2 was 0.5 mM (Fig. 2). At 5 mM the lag phase was increased and the growth in the log phase was decreased. Growth and acid production were clearly inhibited at 50 mM (data not shown). This result was similar to the findings of Koo *et al.* (24). that the MIC of *tt*-Farnesol and Baicalein against *S. mutans* JC-2 was 500 μM.

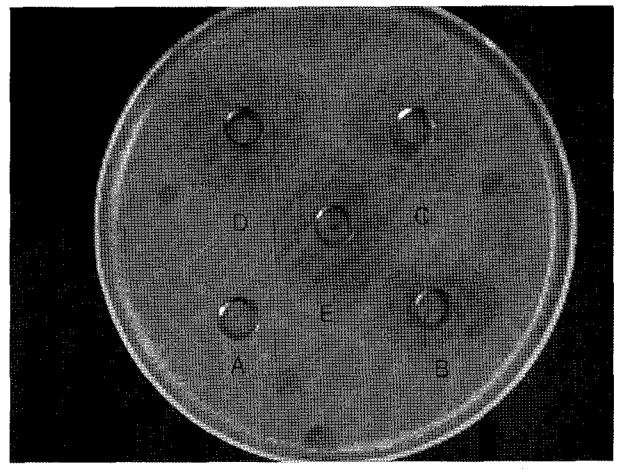

Fig. 2. MIC of 2-hydroxyethyl β-undecenate in *S. mutans* JC-2. A, Control ; B, 0.5 mM; C, 1 mM; D, 5 mM; E, 10 mM.

It has been reported that diverse substances which demonstrate antibiotic activity against *S. mutans*, such as anti-caries sugars, e.g., trehalose (25), matitol (26), xylitol (27), and Fuc-α(1 → 4)galNAc-α(2 → 6)NeuAc (13), and mineral components e.g., ascorbic acid and sodium nitrite (28) suppress acid production. This implies that low pH provides the optimal growth conditions for *S. mutans*. Anti-caries sugars have low acid fermentability so do not contribute to the acidity of the oral cavity and thus induce the death of oral microbes (13, 25-27). Also, NO and NO_2 form NO• radicals, and react with diverse substances such as O_2^-, Fe, -SH, etc., inducing the deamination of DNA and thus the death of bacteria (28, 29). Amino glycoside antimicrobial agents inhibit protein synthesis of the liposome system and thus inhibit m-RNA function (30).

Therefore, we judgment be in need of to be concrete elucidate for antimicrobial activity to the acid production and growth of *S. mutans* JC-2.

The measurement of cell membrane permeability of *S. mutans* JC-2 The effect of various concentrations of 2-hydroxyethyl β-undecenate on the cell membrane permeability of *S. mutans* JC-2 was examined, and the eluted cytoplasmic component was colometrically quantitated (Fig. 3). As the concentration of 2-hydroxyethyl β-undecenate increased, the cell membrane permeability increased sharply. This effect could be due to the hydroxyl group present in 2-hydroxyethyl β-undecenate inhibiting or inactivating the permease that is involved in the permeability of substances present in the cell membrane of *S. mutans* JC-2. This disturbs or destructs the selective permeability of the cell membrane. Moreover, it was judgmented that a long chain fatty acid mediates an effect on the formation of cell membrane. This result is similar to the findings of Bard *et al.* (31) that terpenes with a long carbon chain, such as *tt*-Fornesol, destroy the cell wall and thus induce cell death, and the findings of Imokswa *et al.* (32) that sphingolipid and cerebroside have anti-bacterial activity. In other words, 2-hydroxyethyl β-undecenate, formesol, and spingolipide all have a long chain unsaturated fatty acid group with an -OH group within the molecule.

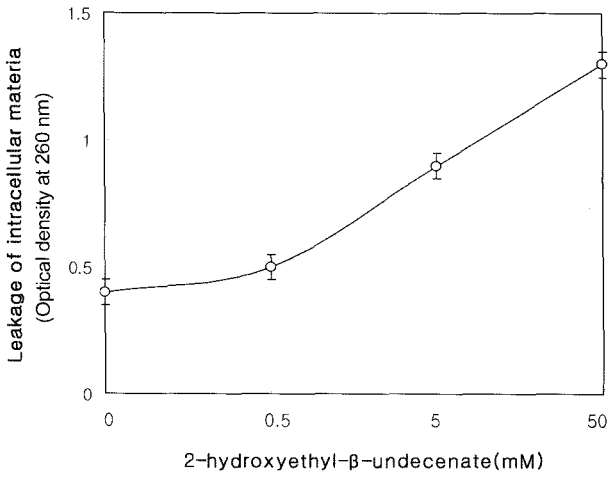

Fig. 3. Effect of 2-hydroxyethyl β-undecenate on the cellular permeability of *S. mutans* JC-2.

The effect on monosaccharide metabolism ***The utilization of glucose*****:** The amount of residual glucose was increased in a dose-dependent manner by 2-hydroxyethyl β-undecenate (Fig. 4). 70% glucose was not utilized and remained in the culture medium when 5 mM, 2-hydroxyethyl β-undecenate was added to *S. mutans* JC-2, and at 50 mM, 2-hydroxyethyl β-undecenate, over 90% glucose remained. This result was in agreement with the antibacterial effect. Hence, it was determined that 2-hydroxyethyl β-undecenate affects carbohydrate metabolism. According to the reports of Reizer and Peterkofsky (33) and Yamada (34), Gram positive bacteria such as oral streptococci mediate the translocation of glucose influxed to cells by PEP-glucose-PTS, and produce lactic acid through the Enbden-Meyerhof-Parnas pathway. Additionally, glucose is actively transferred by ATP-dependent glucokinase, and is metabolized to glucose-6-phosphate.

In addition, the result is similar to the results reported by Ryan and Kleinberg (35) that the rate of the glucose utilization of non-arginolytic bacteria such as *S. mutans* is faster than that of arginolytic bacteria such as *S. mitior,* due to the rate of glycolytic activity, and consequently, the rate of the decrease of pH is also faster.

In contrast to the finding that the ID_{50} of Zn^+ ion of Streptococci glycolysis was 0.01 mM (14), we found a relatively high concentration of 2-hydroxyethyl β-undecenate was required for inhibition of Streptococci glycolysis. Nevertheless, this compound was found that 2-hydroxyethyl β-undecenate reacts very sensitively on the glycolysis of *S. mutans* JC-2 using glucose as a substrate

The inhibitory effect on GADP**:** GADP is an enzyme that specifically acts on 2-hydroxyethyl β-undecenate and mediates oxidation to 1,3-diphosphoglycerate, and it is a rate limiting enzyme in the Embden-Meyerhof-Parnas carbohydrate pathway (15).

2-Hydroxyethyl β-undecenate decreased GAPD activity in a dose-dependent manner (Fig. 5) with an IC_{50} of 1 mM. Fifty mM 2-hydroxyethyl β-undecenate inhibited 97% of the enzyme activity. This result is similar to the report of Sheng *et al.* (14) that the IC_{50} of Zn^+ ion was 0.9 mM.

The importance of this enzyme in carbohydrate metabolism was described by Crow and Wittemberger (15), who found that *S. mutans* has both NAD^+ and $NAPD^+$ specific GADP, but lacks two types of oxidase in the hexose mono-phosphate pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Therefore, GADP plays an important role in the formation of 1,3-diphosphoglycerate for the production of ATP (15).

In addition, this enzyme is a central enzyme in the treatment of parasitic infections such as leishmaiasis caused by the protozoa related to Leishmania and Trypanosomia caused by the flagella of Trypanosoma species. 3-Piperonyl-coumarin inhibits the activity of GADP (36), as does the NAD^+ derivate N-naphtalenemethyl-2'-methoxy-benzeamido-β-NAD^+ (37). Therefore, it can be seen that the inhibition of this enzymes activity is an effective way to inhibit the growth of harmful microbes, and so 2-hydroxyethyl β-undecenate may be applicable to various infectious diseases.

Phosphoenolpyruvate: Glucose phosphotransferase (Glucose-PTS) inhibitory effect**:** Glucose and other

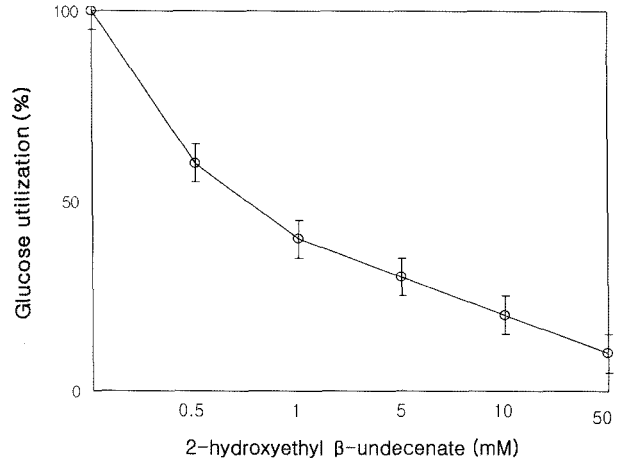

Fig. 4. Effect of 2-hydroxyethyl β-undecenate on glucose utilization of *S. mutans* JC-2.

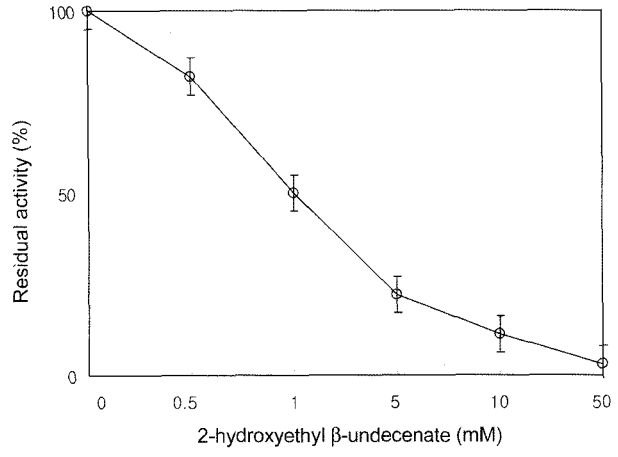

Fig. 5. Effect of 2-hydroxyethyl β-undecenate inhibition on glyeraldehyde-3-phosphate dehydrogenase in *S. mutans* JC-2 sugar metabolism.

mono- and disaccharides, manitol, sorbitol, and other hexitols, are transported to the inside of *S. mutans* by the phosphoenolpyruvate: glucose phosphotransferase-mediated mechanism and then phosphorylated (38, 39). In the absence of glucose-PTP, the influx of sugar to the inside of cells is blocked, as seen in glucose-PTS$^-$ mutans which lack this enzyme (40). Glucose-PTS has been shown to play an essential role in the carbohydrate metabolism of *S. mutans* (41).

2-Hydroxyethyl β-undecenate inhibited glucose-PTS activity with an IC_{50} of 0.9 mM (Fig. 6). Fifty mM 2-hydroxyethyl β-undecenate caused 91% inhibition of enzyme activity. This result concurs with our finding that glucose utilization decreased in a dose-dependent manner (Fig. 5), and demonstrates that 2-hydroxyethyl β-undecenate exerts antibacterial activity by mediating an effect on the influx of saccharides to cells during carbohydrate metabolism.

Glucose-PTS is the enzyme system that transfers phosphoryl group influx to cells as follows:

EI: HPr + PEP HPr-P + Pyruvate (1)

EII/EIII: HPr-P + Sugar Sugar-P + HPr (2)

In reaction (1), the enzyme I (EI) hydrolyzes phosphoenopyruvate (PEP) and dissociates to a low molecular weight phosphoryl group and heat-stable protein (HPr). In reaction (2), glucose is transferred via the permease enzyme II (EII) bound to the membrane, and it is phosphorylated by enzyme III (EIII). The presence of this glucose-PTS enzyme system in *S. mutans* has been reported by Ellwood *et al.* (42).

In addition, Liberman and Bleiwis (43) examined the role of PEP and ATP on the phosphorylation of glucose, fructose, and mannose, and they reported that glucose accepts phosphoryl groups from PEP at a ratio of 1:1, fructose accepts phosphoryl groups at a ratio of 7:3, and mannose at a ratio of 10:2. This implies that depending on the type of substrates used, the inhibition of glucose-PTS and the growth of bacteria are different. It was determined that the inhibition by 2-hydroxyethyl β-undecenate is due to the inhibition of the *S. mutans* JC-2 glycolysis.

ATPase inhibition effect: The activity of membrane ATPase was measured by varying the concentration of 2-hydroxyethyl β-undecenate and reacting with fresh membrane, and as shown in Fig. 7, it was found that the activity decreased dose-dependently, with IC_{50} value of 5 mM. ATPase present in plasma membrane is involved in the release of cytoplasmic protons and the control of internal pH. The major enzyme involved in the translocation activity is F_1F_0-ATPase, and when *S. mutans* grows in an acidic environment, the activity of this enzyme is increased (44, 45).

When *S. mutans* produces acid rapidly or grows in an acidic environment, there is a difference between the external and cytoplasmic pH. This difference plays an extremely important role in an acid-sensitive system such as the glycolytic system (46).

Magalhaes *et al.* (47) reported that the molecular weight of ATPase of *S. mutans* is 100 kDa, and that it is inhibited by vanadate that the P-type and G, K^+-ATPase inhibitor.

In addition, it result showed similar to the report of Suzuki *et al.* (48), who found that 100 μM dicyclohexylcarbodiimide (DCCD) decreased ATPase activity by 50%, and that the release of protons and the growth rate of a mutant that had defective F_1F_0-ATPase activity (50% of wild type) were slower than that of wild type bacteria.

Therefore, it was determined that 2-hydroxyethyl β-undecenate inhibits ATPase of *S. mutans* JC-2, disrupts the pH gradient between the cytoplasm and the external environment, mediates an effect on the glycolysis of oral streptococci that is activated in acidic conditions, and thus inhibits the growth of microbes.

The inhibitory effect on plaque formation on human tooth surface The effect on the adherence of glucan produced by *S. mutans* JC-2 to human extracted teeth was examined. As shown in Fig. 8, 2-hydroxyethyl β-undecenate demonstrated excellent inhibition of plaque formation, so it should be good at preventing tooth caries.

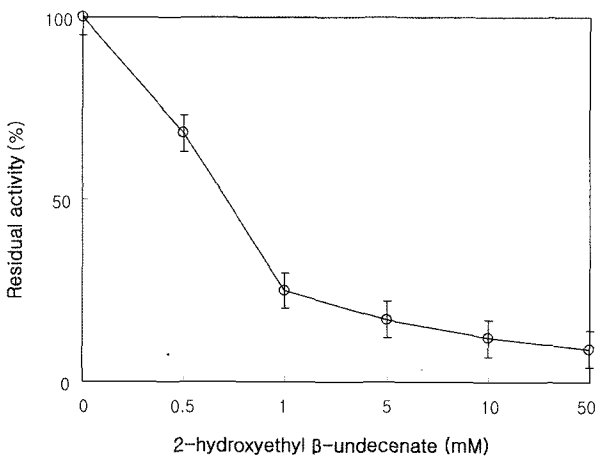

Fig. 6. Effect of 2-hydroxyethyl β-undecenate on glucose-PTs activity in *S. mutans* JC-2 sugar metabolism.

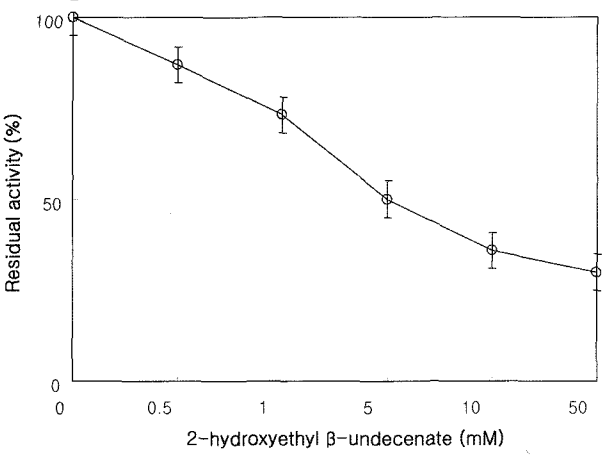

Fig. 7. Effect of 2-hydroxyethyl β-undecenate on the activity of *S. mutans* P-type ATPase.

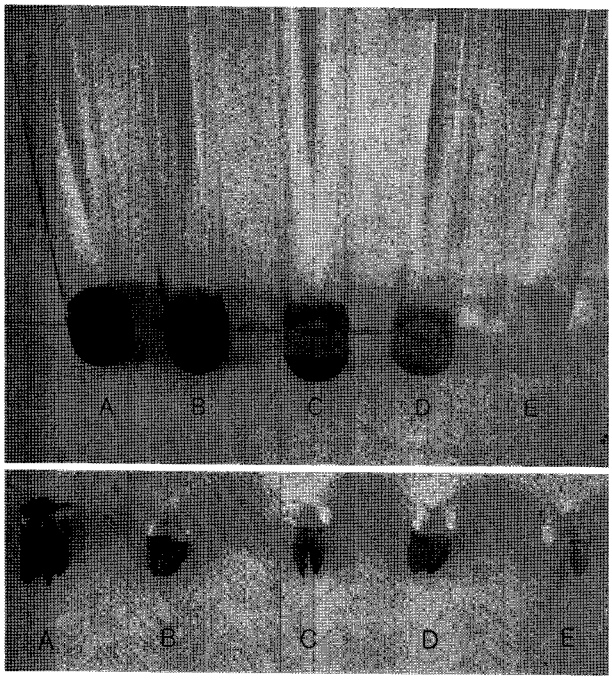

Fig. 8. Effect of 2-hydroxyethyl β-undecenate on plaque formation on extracted human teeth. A, Control; B, 1 mM; C, 5 mM; D, 10 mM; E, 50 mM.

Table 1. Effect of 2-hydroxyethyl-β-undecenate on calcium ion elution from extracted human teeth

2-hydroxyethyl-β-undecenate (mM)	Eluted calcium ion (%)
Control	0.45
1	0.45
5	0.40
10	0.27
50	0.20

The quantification of calcium ion eluted from the teeth Table 1 shows the results of amount of calcium ion eluted from extracted teeth in the presence of 2-hydroxyethyl β-undecenate at concentrations ranging from 0 to 50 mM. The amount of calcium ion eluted was inhibited by 56% by 50 mM 2-hydroxyethyl β-undecenate compared to control. Thus we conclude that 2-hydroxyethyl β-undecenate purified from cumin seeds has good anticaries activity and so potentially is of commercial value.

Acknowledgments

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