iJRASET
International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 12 **Issue:** I **Month of publication:** January 2024

DOI: https://doi.org/10.22214/ijraset.2024.57998

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 12 Issue I Jan 2024- Available at www.ijraset.com

Synthesis and Antibacterial Activity of PCMX Derivative

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Abstract: ***Para Chloro Meta Xylenol (PCMX) is an exceptionally broad spectrum bactericide and preservative with a long established and proven use in controlling bacteria, mildew and fungal growth in a wide range of applications (medical, domestic and industrial). Chloroxylenol, also known as para chloro meta xylenol, is an antiseptic and disinfectant which is used for skin disinfection and cleaning surgical instruments. It is also used within a number of household disinfectants and wound cleaners. Their analogues viz. hybrid / ester molecules also possess various biological activities which prompted us to synthesize a novel analogue for their future application as bioactive molecule. The synthesized compound was characterized by IR, 1HNMR, mass spectral data, elemental analysis and screened for its potential antibacterial activity against Gram positive and Gram negative bacteria. It shows a promising antibacterial activity.***

Keywords: Para chloro meta xylenol, antibacterial, antiseptic, disinfectant,1HNMR, ester molecule, Gram positive, Gram negative, bacterial cultures etc.

I. INTRODUCTION

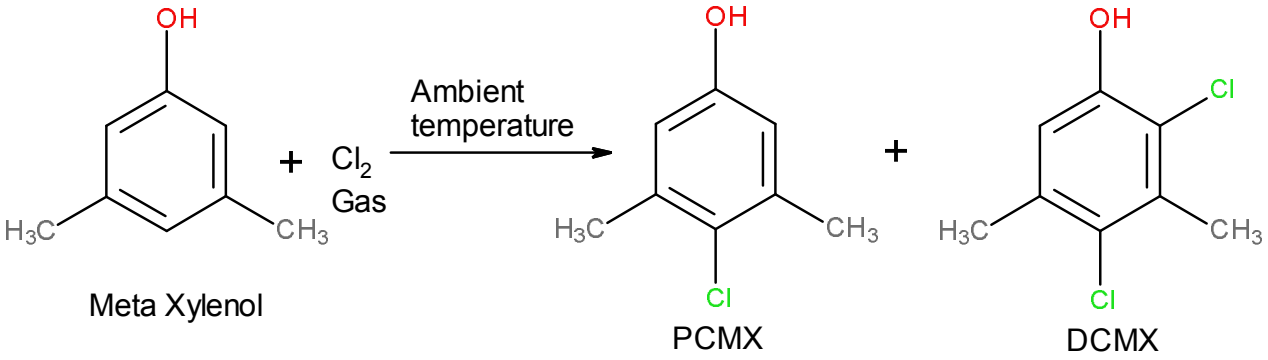
Phenolic phytochemicals are known to exhibit anti-inflammatory, antioxidant, anti-carcinogenic, anti-diabetic, anti-atherosclerosis and immunomodulatory activities in animals1,2. These are mostly polyphenols known as secondary plant metabolites3 present in plant and trees. One of such compound PCMX which is used as antiseptic, disinfectant and antimicrobial agent4,5. Chloroxylenol is an antimicrobial used to control bacteria, algae and fungi in adhesives, emulsions, paints and wash tanks. It also is used to sanitize bathroom premises, diaper pails, laundry equipment, human bedding and pet living quarters in households, hospitals and other institutions. Since it possesses various biological activity and in continuation to our earlier work6, we decided to make a library of compounds using various permutations and combinations to come up with a novel ester derivative of PCMX using conventional method. The objective of this study is to condense two molecules of the same disease domain to produce a more potent candidate in the same disease domain or to condense two molecules of different disease domain to produce mixed variety of those disease domains or to have drug candidate with entirely different disease domains. In the present work, in continuation to our earlier work7, we are condensing para chloro meta xylenol with 4-bromo benzoic acid under DCC / DMAP/ Pyridine reaction condition in dichloromethane as solvent at room temperature to yield desired ester derivative in 80 % yield. The resultant ester derivative evaluated for its potential antimicrobial activity.

II. RESULTS AND DISCUSSION

1) *Preparation of Para* chloro *meta* xylenol *PCMX:* It was prepared by passing chlorine gas through meta xylenol in xylene at ambient temperature to yield a mixture of para chloro meta xylenol (80 %, major) and dichloro meta xylenol (20 %, minor) since –OH group was activating and ortho, para directing. The PCMX was purified by crystallization and the mother liquor which was enriched in DCMX was used to prepare DCMX commercially.

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Reaction Scheme :



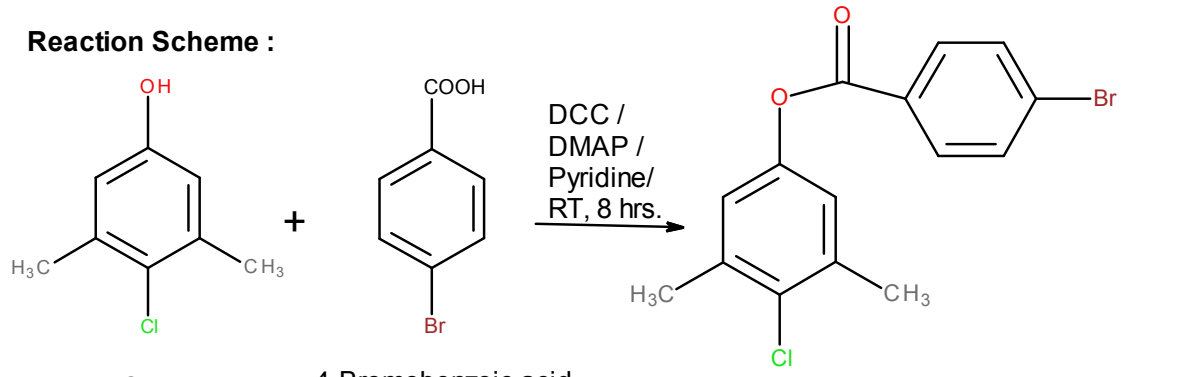
Meta Xylenol PCMX DCMX

PCMX and DCMX were purified by crystallization. The above procedure can be scaled up to get more quantities of PCMX and DCMX.

2) *Characterization of Para Chloro Meta Xylenol (PCMX) :* Pale yellow solid; Molecular Formula C8H9ClO; Melting Range 114 – 1160C; 1H NMR (500 MHz, CDCl3) δppm:- 2.34 (s, 6H, 2 x –CH3), 5.32 (brs, 1H, -OH, D2O exchangeable), 6.52 (d, J = 2.6 Hz, 2H, meta coupling); IR (KBr) cm-1: 3350 (Tertiary phenolic –OH group), 2900 – 2800 (-CH stretching), 1600 (aromatic), 850 – 550 (C-Cl stretching);
3) *Characterization of Dichloro Meta Xylenol (DCMX):* Pale yellowish brown solid; Molecular Formula C8H8Cl2O; Melting Range 92 – 960C; 1H NMR (500 MHz, CDCl3) δppm :- 2.36 (s, 6H, 2 x –CH3), 5.62 (brs, 1H, -OH, D2O exchangeable), 6.74(s, 1H, ArH); IR (KBr) cm-1: 3450 (Tertiary phenolic –OH group), 2900 – 2800 (-CH stretching), 1600 (aromatic), 850 – 550 (C-Cl stretching);

Diversification of PCMX to its ester derivative:- It was prepared by following general method as depicted below.

Reaction Scheme :



PCMX (1) 4-Bromobenzoic acid (2) (4-chloro-3,5-dimethylphenyl)-4'-bromobenzoate (3)

To a stirred solution of PCMX (1 g, 0.006385 moles, 1 eq.) in dichloromethane (30 mL) was added pyridine (0.3 ml, 0.003192 moles, 0.5 eq.), DMAP (0.040 g, 0.0003192 moles, 0.05 eq.), DCC (1.71 g, 0.008300 moles, 1.3 eq.) and 4-bromo benzoic acid (1.67 g, 0.008300 moles, 1.3 eq.) respectively at room temperature and stirred for next 8 hrs. As the reaction proceeds, the by-product urea derivative precipitates out of the reaction mixture and floats on the reaction surface. The reaction was monitored by TLC for the completion of reaction.

Work up : The organic layer filtered through Buchner funnel, to get rid of by product urea derivative. The residue washed with 5 ml of dichloromethane. The mother liquor (organic layer) was concentrated under reduced pressure to minimum, preadsorbed on silica gel and purified by column chromatography (SiO2, 100 – 200 mesh) with increase in concentration of ethyl acetate in petroleum ether to yield pure compound in 80 % yield. The purified compound was unambiguously characterized by IR, 1H NMR, elemental analysis and mass spectroscopy.

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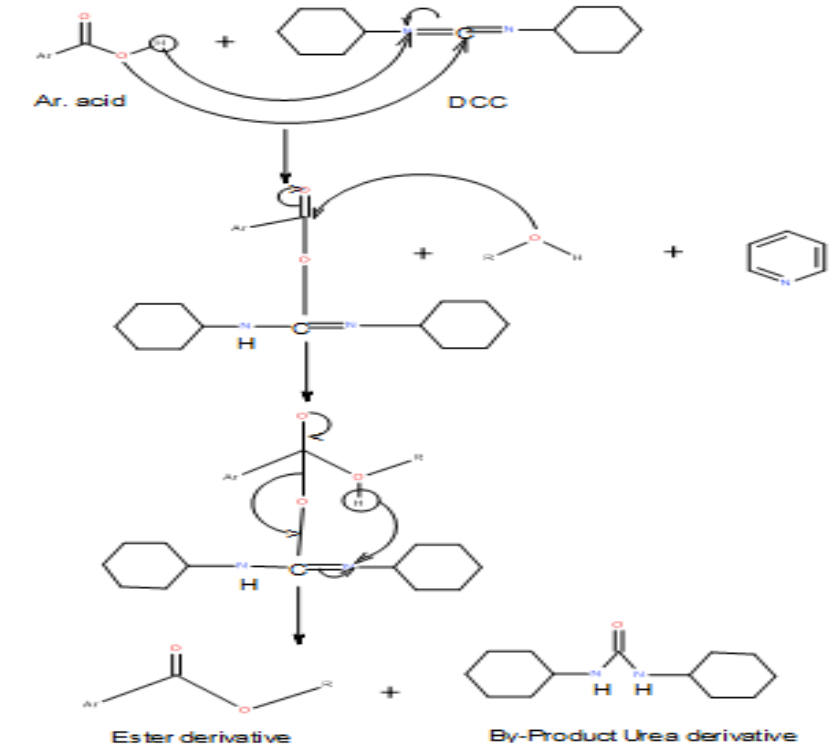
4) *Characterization of (4-chloro-3,5-dimethylphenyl)-4'-Bromobenzoate:-* Off white solid; Molecular Formula C15H12BrClO2; Melting Range 133 – 1350C; 1H NMR (500 MHz, CDCl3)∂ppm :- 2.32 (s, 6H, 2 x –CH3 from p-chloro meta xylenol moiety), 6.87 (d, J = 1.5 Hz, 2H, meta coupling), 7.57 (dd, J = 7.0 Hz, 2.0 Hz, 2 H, ortho coupling and meta coupling respectively for *p*-bromo benzoic acid moiety), 7.96 (dd, J = 6.5 Hz, 2.0 Hz, 2 H, ortho coupling and meta coupling respectively for *p*-bromo benzoic acid moiety); IR (KBr) cm-1: 2950 – 2800 (-CH stretching), 1734 (ester >C=O), 1588 (aromatic), 700 – 750 (C-Cl stretching), 500 – 600 (C-Br stretching); TOFMS : Mass calculated for C15H12BrCl35O2 (M+) = 338 and (M + 2)+ = 340 in the ratio 1 : 1; Elemental Analysis, Requires C 53.13 % H 3.62 % Br 23.39 % Cl 10.45 % O 9.41 % Found C 53.10 % H 3.65 % O 9.38 %.

a) *1H NMR Analysis:* The peak resonating at 2.32 ppm appeared as a singlet integrating for six protons corresponding to two methyl groups from para chloro meta xylenol moiety. The region 6 – 8 ppm corresponds to aromatic protons. The peak at 6.87 appeared as a doublet integrating for two aromatic protons with coupling constant 1.5 Hz corresponding to meta coupling due to symmetry of PCMX moiety. The signal resonating at 7.57 ppm appeared as a doublet of a doublet with coupling constants 7.0 Hz & 2.0 Hz integrating for two aromatic protons corresponds to ortho and meta coupling respectively from *para*-bromo benzoic acid moiety. The most downfield signal resonating at 7.96 ppm appeared as a doublet of doublet with coupling constants 6.5 Hz & 2.0 Hz integrating for two aromatic protons corresponds to ortho and meta coupling respectively *para*-bromo benzoic acid moiety.

b) *IR Analysis:* In original PCMX, tertiary phenolic –OH group appears at 3350 cm-1. Similarly, in original p-bromo benzoic acid the acid carbonyl group appears at 1710 cm-1 as sharp peak. However, when condensation reaction is carried out between PCMX and *p*-bromo benzoic acid using DCC as a dehydrating agent, the peak at 3350 cm-1 corresponding to tertiary phenolic –OH and acid carbonyl peak at 1710 cm-1 disappears and peak due to ester carbonyl at 1735 cm-1 appears with the elimination of water molecule indicating that reaction takes place at this position resulting in the formation of desired ester molecule. In addition, peaks at 2924 – 2849 cm-1 corresponds to methines and at 1588 cm-1 corresponds to aromatic functionality.

The most significant features of this methodology are (a) good accessibility of the reagent and its stability (b) a stoichiometric amount of reagent can be used by direct weighing, avoiding excess (c) no evolution of hazardous vapours during the reaction (d) the total elimination of the use of toxic organic solvents (e) a simple experimental procedure (g) good control over the outcome of the reaction by varying the amount of reagent (h) less expensive and (i) very simple reaction work up with avoidance of by-product. The aforesaid protocol thus provides an improved procedure for the synthesis of useful hybrid derivatives having important pharmaceutical, agricultural and other physicochemical properties.

Probable mechanism for fused /hybrid molecules :



III. EXPERIMENTAL

Melting points were uncorrected. 1H NMR spectra were recorded at 500 MHz on a Varian spectrometer and Mass spectra on TOF MS ES mode. Elemental analysis was carried out as a percentage on a Thermofinnigan, Flash EA 1112 series, Italy.

A. Chromatographic System

1) *Column Chromatography:* For column chromatography 100 – 200 mesh Acme grade silica gel is used. The crude reaction mixture is concentrated under reduced pressure to yield crude mass which is preadsorbed on silica gel and purified by column chromatography with increase in concentration of Ethyl acetate in Petroleum ether. The fractions having similar 'rf" values were pooled together, concentrated and subjected for characterization using various spectroscopic techniques.
2) *Thin Layer Chromatography:* TLC plates were prepared using silica gel G (ACME, BOMBAY). Pet. ether: EtOAc (95 : 5) was used as the solvent system.
3) *Radial Chromatography:* The circular glass plates of thickness 1 mm, were prepared by using silica gel (PF254, E. MERCK, 50 g) in cold distilled water (105 ml). For elution, gradually increasing concentrations of EtOAc in pet ether were employed.

B. Biological Activity

1) Antibacterial Activity using ditch plate method8:- Conc. 100 µg/ml
2) The synthesized molecules were screened for their antibacterial activity using ditch plate method at 100 µg/ml concentration against Gram positive (Staphylococcus aureus) and Gram negative (Escherichia coli) bacterial species qualitatively. The results of the antibacterial activities are summarized in Table 1.
3) Theory: One of the many ways to test the anti-bacterial activity of compounds / drugs is ditch plate method. Ditch plate method is a preliminary method to screen the test compounds / drugs for their potential as antimicrobials. In this method, the compound to be tested for antimicrobial activity is seeded in the agar plate and the test organisms are streaked across.
4) Procedure: A ditch 10mm wide was cut into sterile MH agar plate. The test drug / compound was added to 5 ml molten MH agar butt at 400C and this mixture was poured into the ditch and allowed to solidify. The ditch should be made in level with the rest of the agar by pouring the mixture. The different bacterial cultures are streaked perpendicular to the ditch using nichrome wire loop. The plate is then incubated at 370 C for 24 hours.
5) The results were observed as inhibition of bacterial growth on the ditch as well as adjacent to the ditch .
6) RESULTS: The following test samples showed anti-bacterial activity against the organisms mentioned in the following Table.

SAMPLE NO.	ACTIVE AGAINST
1 (PCMX)	Staphylococcus aureus [Gram positive] Salmonella typhi [Gram negative] Klebsiella pneumonia [Gram negative] Corynebacterium diphtheriae [Gram positive] Escherichia coli [Gram negative]
Ampicillin (Std. drug)	Staphylococcus aureus [Gram Positive] Corynebacterium diphtheriae [Gram positive] Proteus vulgaris [Gram negative] Salmonella typhi [Gram negative]
2 (4-Bromo Benzoic Acid)	Staphylococcus aureus [Gram positive] Corynebacterium diphtheriae [Gram positive] Escherichia coli [Gram negative]
3	Staphylococcus aureus [Gram positive] Salmonella typhi [Gram negative] Klebsiella pneumonia [Gram negative] Corynebacterium diphtheriae [Gram positive] Escherichia coli [Gram negative]

The above results show that the base molecule PCMX has anti-bacterial activity against both the bacterial cultures. Its derivative viz.3 was active against both Staphylococcus aureus (Gram positive bacteria) and certain cultures of Gram negative bacteria. Thus, 3

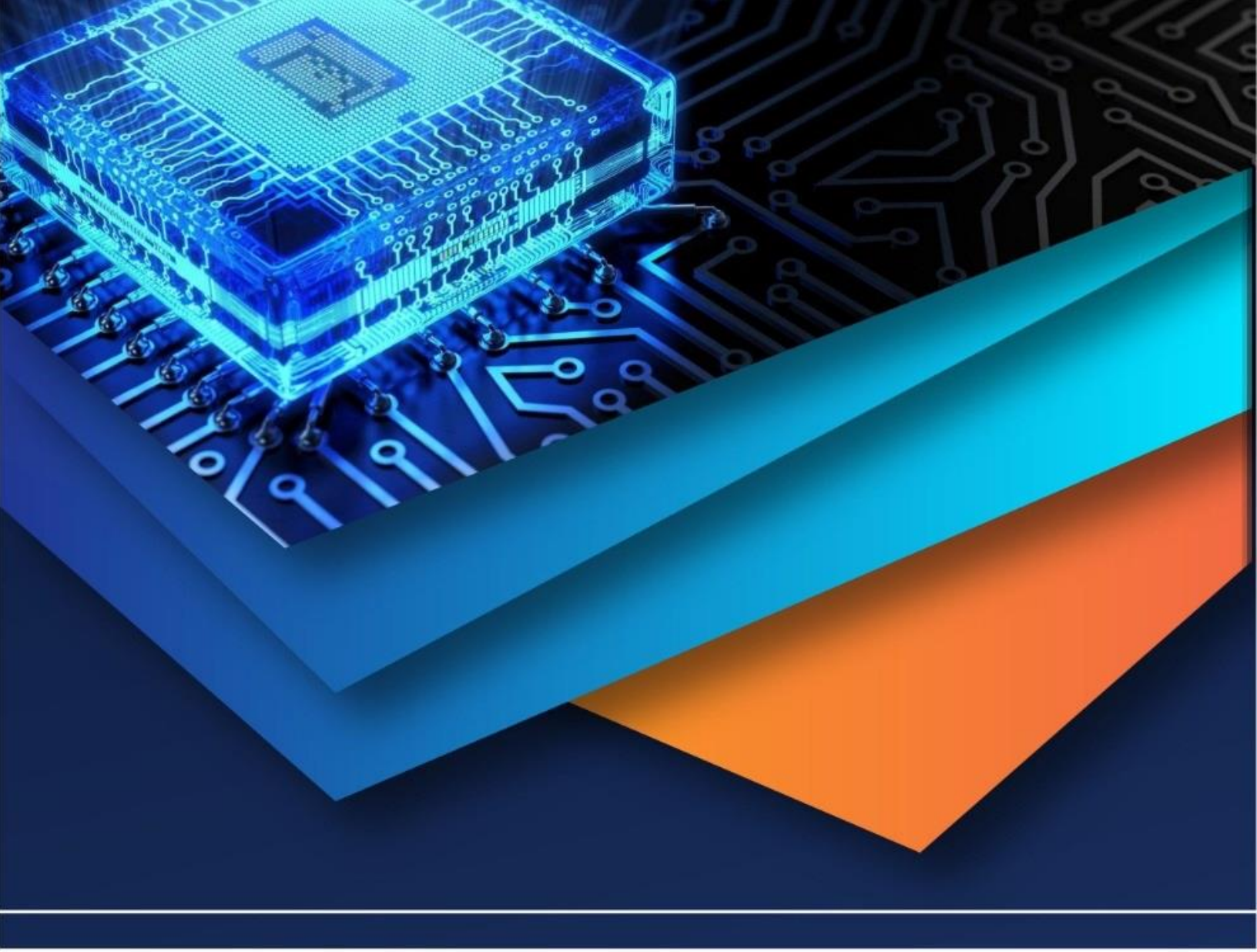
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is a potential antibacterial candidate. In depth analysis of this compound through structure activity relationship studies would provide further insight and can be an interesting topic of future studies.

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