Indonesian Journal of Cancer Chemoprevention, February 2010
ISSN: 2088–0197
e-ISSN: 2355-8989

The Effect of Addition of Antioxidant to The Stability of Green Tea Water Extract As Anti Acne

Nadya Fadila Saib

Jalan Sangkuriang 11A/160 40135
Bandung - West Java, Indonesia

Abstract

There are so many potential effects that green tea, such as antibacterial activity against *Propionibacterium acnes*, that caused acne. In the previous study, green tea had been made in gel. In this study, formula of green tea water extract gel is developed with addition of antioxidant so that stability of the gel would increase. Gel of green tea water extract were made using Carbopol 940 with glycerin as the solvent. The chosen antioxidant was sodium sulfite. It is also combined with chelating agent, EDTA (Edetic Acid). The activity of green tea water extract gel was determined and tetracycline HCl gel was used as the reference. The addition of sodium sulfite 1% w/w into gel with Carbopol 940 1% w/w as the base prevent the change of color in more than eight weeks. Minimum Inhibitory Concentration (MIC) of green tea water extract without either sodium sulfite or EDTA was 1.20 ± 0.11 cm against *Propionibacterium acne*. MIC of green tea water extract with addition of sodium sulfite 1% w/w was 1.19 ± 0.28 cm. MIC of green tea water extract with addition of sodium sulfite 1% w/w and EDTA 0.1% w/w was 1.25 ± 0.22 cm. The addition of sodium sulfite 1% w/w caused viscosity of gel decrease 42.1%. The addition of sodium sulfite 1% w/w increased the color stability of green tea water extract. The addition of sodium sulfite 1% w/w didn't decrease its antibacterial activity against *Propionibacterium acnes*.

Keywords: green tea, anti acne, *Propionibacterium acnes*, gel, antioxidant.

INTRODUCTION

Today, many products are made from green tea, such as food products, beverages and cosmetics. It is associated with the benefits of green tea. Antimicrobial properties of green tea have been shown against some bacteria, including *Propionibacterium acnes*, which is the bacteria that cause acne.

In this research, gel formula of water extract of green tea was developed. Gel form was chosen because it has several advantages such as long contact time, more comfortable to be used and also reduce the risk of inflammation.

In a previous study, green tea gel changed its color in 14 days (Caroline, 2006). The color change of the stocks was caused by oxidation of green tea extract in the dosage. Oxidation reaction has already been prevented by using base without water. But that is not enough. Therefore, the antioxidant is needed. It is also important to determine the antimicrobial activity against *Propionibacterium acnes*.

*Corresponding author e-mail : nadyafadila@yahoo.com

MATERIALS AND METHODS

Tea leaves were collected from the Tea Research Centre and Kina plantation, Gambung, Ciwidey, West Java. Immediately after plucking, the tea leaves were dried in high temperature dryers. Crude green tea was then characterized through the determination of moisture content. Crude green tea powder was extracted using water solvent. Water extract was then freeze dried. After that, chromatogram pattern of the extract was determined using Thin Layer Chromatography (TLC).Next step is the determination of Minimum Inhibitory Concentration (MIC) of green tea water extracts against *Propionibacterium acnes* obtained from the Department of Microbiology, Faculty of Medicine, University of Indonesia in Jakarta. After the green tea water extract's MIC was obtained, a topical gel was then formulated. Its activity against *Propionibacterium acnes* was then tested and was compared with the activity gel tetracycline HCl. Finally, its performance was evaluated in 8 weeks.

RESULTS

MIC of green tea water extracts was 1% w / v. Its antibacterial activity was compared with the antibacterial activity of tetracycline HCl. Results showed that 0.5 grams of water extract of green tea had antibacterial activity against *Propionibacterium acnes* was almost equal to $0.6x10^{-3}$ grams terasiklin HCl.

In this study, Carbopol 1% w/w was used as the base with glycerin as the solvent. Formulas of green tea gel varied based on the concentrations of antioxidants that were added. Antioxidants are selected based on solubility, stability and compatibility with other excipients. Preferred antioxidants are sodium sulfite. Antioxidants are also combined with chelating agent, EDTA, as a form of synergism.

Each formula was tested against *Propionibacterium acnes*. Results of inhibition diameter of each formula obtained did not differ significantly. Each formula was evaluated for 8 weeks. Evaluations include acidity (pH), viscosity, occurrence of syneresis, growth of microorganisms, discoloration and odor. The results of each formula showed no syneresis occurred, no microorganisms, and no changes to the odor. Acidity and viscosity of the gel were relatively stable. During 8 weeks observation, the stocks with the addition of sodium sulfite 1% w/w were not having change in color.

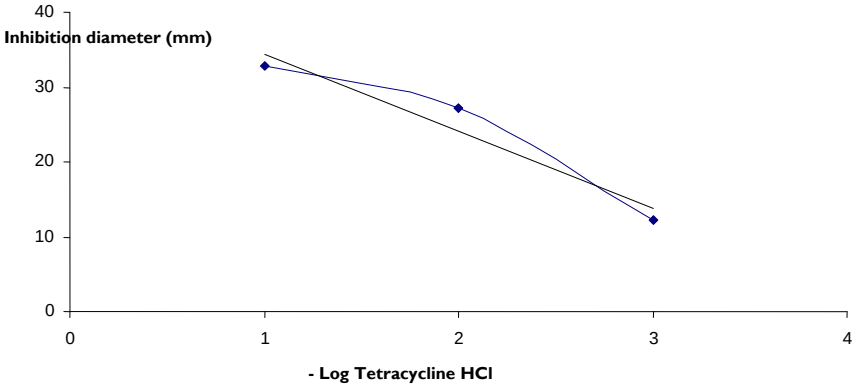

Figure 1. Activity of tetracycline HCl against *Propionibacterium acnes* with linear equation y = -10.25x + 44.623 and R^2 = 0.934

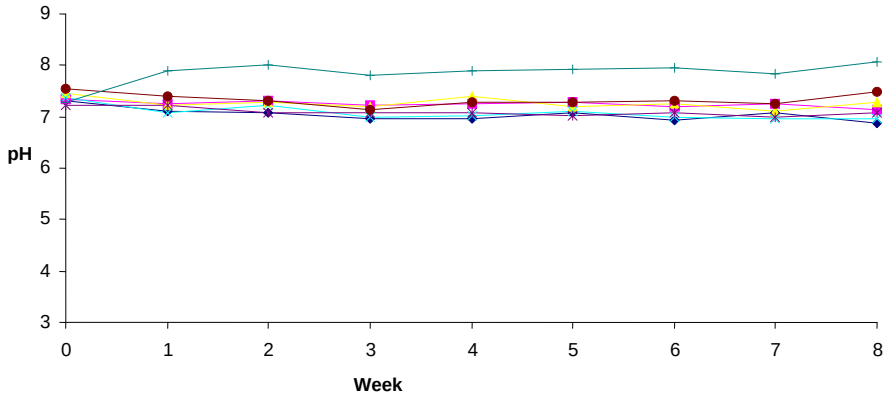

Figure 2 pH evaluation, F1 (♦), F2 (■), F3 (▲), F4 (×), F5 (✳), F6 (*), F7 (+)

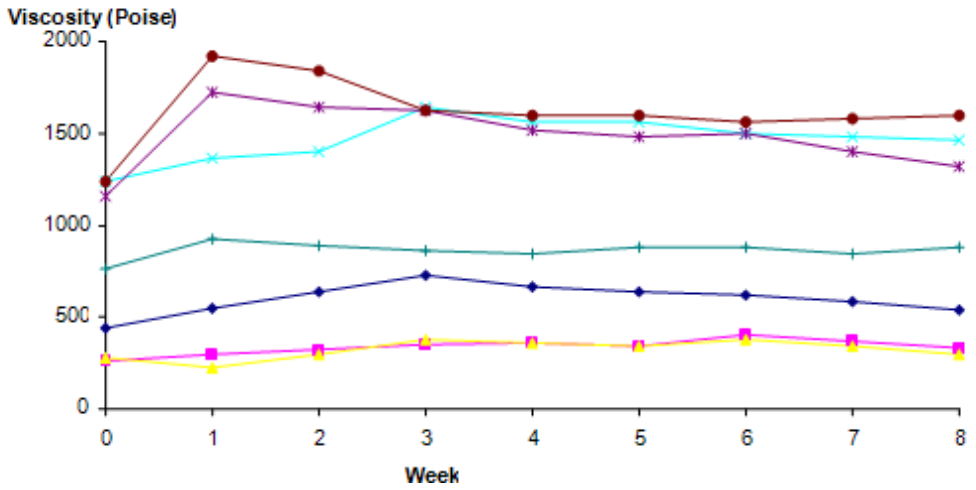

Figure 3 viscosity evaluation, F1 (♦), F2 (■), F3 (▲), F4 (×), F5 (*), F6 (*), F7 (+).

Table I. Gel Formulas

Ingredients	Concentration % (w/w)						
	F1	F2	F3	F4	F5	F6	F7
Carbopol 940	1	1	1	1	1	1	1
Sodium Sulfite	1	1	1	0.01	0.01	0.01	-
EDTA	-	0.1	0.01	-	0.1	0.01	-
TEA	0.31	0.43	0.40	0.68	0.77	0.85	0.68
Green Tea Extract	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Glycerin (ad)	100	100	100	100	100	100	100

Table II. Activity of green tea water extract gels and Tetracycline HCl gel against Propionibacterium acnes

Formulas	Inhibition Diameter (cm)
Carbopol 940-gliceryn base	0
Carbopol 940-gliceryn + sodium sulfite 0.1% base	0
Carbopol 940-gliceryn + EDTA 0.01% base	0
Carbopol 940-gliceryn + sodium sulfite 0.1% + EDTA 0.01% base	0
F1	1.19 ± 0.28
F2	1.25 ± 0.22
F3	1.23 ± 0.19
F4	1.27 ± 0.21
F5	1.26 ± 0.13
F6	1.21 ± 0.26
F7	1.20 ± 0.11
Tetrasiklin HCl gel $0.6.10^{-3}$	1.34 ± 0.33

DISCUSSION

For the final formulation, the concentration of extract loaded in the gel is 0.5%. That concentration was five times the MIC. By using concentrations greater concentration than the MIC, it was expected to maintain antimicrobial activity of green tea water extract if the extract in a dosage form. Selections of antioxidants were added based on the solubility and compatibility of the gel base that will be created. Based on these considerations, the selected antioxidant is sodium sulfite.

EDTA was added to synergize antioxidants' work. EDTA as a chelating agent will bind the metal ions that allegedly contained in green tea extract. Metal ions catalyze the decomposition of green tea extract to form free radicals, which trigger oxidation reactions. EDTA is commonly used in Carbopol gels (Lund, 1994).

The different gel formula can be seen in Table I. Addition of sodium sulfite, which also is alkaline, made the addition of TEA be reduced, it is shown by the F1-F3 in the same table. The orientation of the base was done in previous studies, Carbopol 934 - glycerin (1%) (Caroline, 2006). In this study, Carbopol 940 was used. The different types of Carbopol used will not be a problem because there was no significant

difference regarding the compatibility. Some of monographs such as British Pharmacopoeia 2004 and Pharmacopoeia European 2005 state the same specifications of those two kinds of Carbopol. The difference between the types of Carbopol lies in gel viscosity that will be made. Because Carbopol 934 was rarely produced, Carbopol 940 was selected (Rowe, 2006).

Each green tea water extract gels were tested against *Propionibacterium acnes* and compared to tetracycline HCl gel $0.6x10^{-3}$ w/v, which was estimated to have antimicrobial activity equivalent to the water extract of green tea gel 0.5% w/v. The activity from gel base (with and without addition of antioxidants) to *Propionibacterium acnes* were also tested to show that antimicrobial activity is obtained only from the water extract of green tea.

Bases, either with or without the addition of antioxidants, showed no antimicrobial activity. While gels containing green tea water extract provides antimicrobial activity against *Propionibacterium acnes*. This indicates that the antimicrobial activity of green tea water extract gel to *Propionibacterium acnes* derived from green tea water extract. Value of inhibition diameter of F1-F7 and tetracycline hydrochloride gel did not differ significantly. It means the addition of sodium sulfite or EDTA did not increase or decrease the antibacterial activity of green tea water extracts against *Propionibacterium acnes*.

Further evaluation that includes observation of changes in color, pH and viscosity measurements and observations of the occurrence of syneresis, fungal growth and odors were tested. For the evaluation of syneresis, fungal growth and odors, all formulas gave the same result, while for the evaluation of the pH is shown in Figure 2.

Gel with a maximum concentration of sodium sulfite (F1-F3), 1%, gave the best color stability. However, those gels have lower viscosity. Compared with other formulas, gels with sodium sulfite 1% w/w (F1-F3), did not change their color up to eight weeks of observation. The addition of EDTA did not affect the color stability of gels.

If concentration of sodium sulfite, which is alkaline, is increased, the amount of TEA that needs to be added is reduced. Besides acting as a base, TEA also acts as a viscosity-enhancing agent. So reducing the amount of TEA makes gel viscosity decreased. Addition of sodium sulfite 1% w/w caused the amount of TEA that was added as much as 36.80 to 54.40%, it caused the gel with the addition of sodium sulfite 1% decreased the viscosity of 42.11 to 65.79.

CONCLUSION

Addition of sodium sulfite 1% w/v in green tea water extract gel affected color changes in the gel. Addition of sodium sulfite or combinations with EDTA did not increase nor decrease the antibacterial activity of green tea water extract. In storage for eight weeks, no change in the color gel green tea water extract with the addition of sodium sulfite 1% w/w. There was no difference between each formula in color stability and results of other evaluations, which include the growth of microbes, syneresis, odor, pH and viscosity. Addition of sodium sulfite 1% w/v led to decrease of gel viscosity 42.11%.

REFERENCES

Ansel, H. C., 1989, *Pengantar Bentuk Sediaan Farmasi,* ed. 4, terjemahan Farida Ibrahim, UI-Press, Jakarta, 390-391.

Amila, 2003, Formulasi Teh Cepat Saji dari Ekstrak Air Teh Hijau dan Penentuan Kadar Polifenol Sediaan Secara Spektrofotometri Sinar Tampak dengan Pereaksi Folin Ciocalteu, *Skripsi sarjana*, Departemen Farmasi FMIPA ITB, Bandung, 34.

Bruneton, J., 1999, *Pharmacognosy, Phytochemistry Medicinal Plants*, Lavoiser, 381-387, 1076-1079.

Caroline, S., 2006, Formulasi Gel Ekstrak Air Teh Hijau dan Penentuan Aktivitas Antibakterinya terhadap Propionibacterium Acnes, *Skripsi sarjana*, Sekolah Farmasi-ITB, Bandung, 13, 18-21, 23-25.

Connors, K.A., 1986, *Chemical Stability of Pharmaceuticals, a Handbook for Pharmacists*, John Wiley & Sons, Inc, New York, 82-114.

Ditjen POM, Depkes RI, 1995, *Farmakope Indonesia*, edisi IV, Depkes RI, Jakarta 7-8.

Erawati, F., 1997, Telaah Fitokimia Ekstrak Air Teh Hijau dan Teh hitam (Camellia sinensis var. Assamica) dan Uji efek Antihiperkolesterolemia Pada Tikus Jantan, *Skripsi Sarjana*, Departemen Farmasi FMIPA ITB. Bandung, 23, 29.

Fessenden & Fessenden, 1991, *Kimia Organik*, jilid 1, ed.3, terjemahan Aloysius Hadyana Pudjaatmaka, Erlangga, Jakarta, 485.

Harry, R. G., 1973, *Harry's Cosmeticology, 6th ed., vol. 1*, Leonard Hill Books, London, 14–16, 557–561.

Katzung, B. G., 2004, *Farmakologi Dasar dan Klinik,* buku 3, terjemahan bagian Farmakologi Fakultas Kedokteran Universitas Airlangga, Salemba Medika, Jakarta, 166, 170.

Lund, W., 1994, *The Pharmaceutical Codex, Principles and Practice of Pharmaceutics*, 12th ed., The Pharmaceutical Press, London, 79.

Martin, A., J. Swarbrick, A. Cammarata, 1993, *Farmasi Fisik, Dasar-Dasar Kimia Fisik Dalam Ilmu Farmasetik*, ed. 3, terjemahan Yoshita, UI-Press, Jakarta, 1170-1173.

Martini, F. H., 2001, *Fundamentals of Anatomy & Physiology*, 5th ed., Prentice Hall, New Jersey, 155-156.

Pietta, P. G., 2000, Flavonoids as Antioxidants, J. Nat. Products, **63**(7), 1035-1041.

Rowe, R. C., P. J. Sheskey, and S. C. Owen (Eds.), 2006, *Handbook of Pharmaceutical Excipients*, 5th ed., The Pharmaceutical Press, London, 111-115, 260-263, 301-303, 691, 708-709, 794-795.

Swarbrick, J. and J.C. Boyland (Eds.), 1992, *Encyclopedia of Pharmaceutical Technology*, vol. 6, Marcel Dekker, New York, 415-436.

Wertz., P. W. And B. B. Michniak, 2000, *Sebum, in: Cosmeceutical–Drugs vs. Cosmetics*, P. Elsner and H. I. Maibach (Eds.), Marcel Dekker, Inc., New York, 48 – 53.