

CHEMICAL COMPOSITIONS OF FINGERED CITRON PEEL (*CITRUS MEDICA* L. VAR. *SARCODACTYLIS*) AND ITS EFFECT ON THE AUTONOMIC NERVOUS SYSTEM

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ABSTRACT:

Background: In Thailand, Fingered Citron (*Citrus medica* L. var. *sarcodactylis*) is used in folk medicine as a tonic for antispasmodic conditions and as an inhaler. According to published research, there are no comprehensive scientific researches on the chemical composition and physiological effects of fingered citron; thus, the present study was designed. We investigated the chemical composition of fingered citron peel oil by GC-MS and its effect on the autonomic nervous systems (blood pressure, heart rate, skin temperature, and respiratory rate) and mood responses after inhaling.

Methods: The hydrodistillation technique was used to extract the essential oil from the fruit of fingered citron. The major chemical composition of the essential oil was limonene (65.11%) and gamma terpinene (34.89%). There were 30 volunteer participants for this experiment. The parameters of assessment were measured before and after using a paired *t*-test statistical procedure.

Results: The results revealed that fingered citron oil caused a significant increase in blood pressure, and the respiratory rate also showed a significant increase upon exposure to the inhaled oil. Furthermore, the oil increased positive emotions including the feelings of well-being, activeness, and freshness. The oil also significantly reduced negative emotions such as drowsiness.

Conclusion: This finding provides evidence of the stimulating effects of inhaling fingered citron peel oil.

Keywords: *Citrus medica* L. var. *sarcodactylis*; Autonomic nervous system; Stimulation Limonene

DOI:

Received: November 2016; Accepted: January 2017

INTRODUCTION

Currently, there are approximately 375 - 400 essential oils available for use by the therapeutic or clinical aromatherapies. They are widely used to promote relaxation or to target other symptoms. Essential oils are produced in glands, scales, canals and hairs of the aromatic florals [1]. The interest in medicinal plants has been increasing in recent years, especially for the care of symptomatic relevant to social impacts. Symptoms such as carminative antispasmodic and antidepressant are particularly relevant in Thailand, which has a hot climate almost all year round. This type of weather is a cause of

fatigue and dizziness. The Thai Traditional treatments use the herbal inhalant products to treat and decrease these symptoms. Fingered citron oil is a very popular material included in Thai Herbal inhalant products because its smell is not strong and is less irritating than other herbs [2].

Fingered citron (*Citrus medica* L. var. *sarcodactylis*) sometimes called Buddha's Hand, is a small tree, having a large fruit resembling a pineapple in shape [Figure 1]. The most important part of the citron is the peel. Most of the information researches indicate that limonene is the major component of the citrus plant group's peel, such as in oranges and pomelo [3, 4]. The analysis of fingered citron peel in each part of the world is different. For instance, in China, the analysis on fingered citron

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Cite this article as: Sayorwan W, Rumruay V. Chemical compositions of fingered citron peel (*Citrus medica* L. var. *sarcodactylis*) and its effect on the autonomic nervous system. *J Health Res.* 2017; 31(4): 307-13.
DOI:



Figure 1 Fingered citron (*Citrus medica* Linn. var. *sarcodactylis* Swing)

peel consists of *p*-coumaric acid, steroids, triterpenoids, limonin, and nomilin, [5, 6] and the analysis of fingered citron peel in Japan consists of limonene and gamma terpinene [7]. Furthermore, Bangladesh reported that fingered citron peel's main component is limonene and citral [8].

In folk medicine, a tonic using dried fruit are part of remedies for antispasmodic, antiemetic, expectorant and respiratory problems [9]. There are several studies reporting about antioxidant [10], anti-inflammatory [11, 12], and antimicrobial activities [13]. According to literature reviews, some essential oils extracted from citrus fruits were reported to have impacts on the autonomic nervous system and emotional states. Remarkably, the essential oil of fingered citron showed evidence of affecting the same areas. Although essential oils in citrus group studies supported the impact on the autonomic nervous system and emotional state, the findings were different for different types of oils. It was shown in the sweet orange oil (*Citrus sinensis*) research that after the sweet orange oil was being inhaled, it raised the blood pressure and increased the heart rate [14]. Neroli oil (*Citrus aurantium* L.), on the contrary, reduced systolic blood pressure [15].

As of this date, there are no experiments in Thailand about the chemical composition and autonomic nervous system for the essential oil from fingered citron peel have been published. Therefore, the primary objective of this study was to investigate essential oil from fingered citron peel by using a GC-MS and the autonomic nervous system effect i.e.; blood pressure, heart rate, breathing rate, skin temperature and an assessment of mood states using an inhalation technique.

MATERIALS AND METHODS

Plant material and essential oil isolation

The preparation of fingered citron has been set up at the Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University (Salaya Campus), Nakornpathom, Thailand, since June 2013. First, a botanist identified the sample. Then, the raw fresh fingered citron was transferred to Clevenger-type glass apparatus and the essential oil was isolated using the hydrodistillation technique.

Analysis of essential oil

The peel of *C. medica* L. var. *sarcodactylis* was ground into small pieces and was extracted using a Clevenger apparatus by the hydrodistillation technique. The oil was kept in a closed container at 4 °C and was analyzed the next day. A gas chromatography/mass spectrometry (GC/MS) analyzed the essential oil (1:100 in methanol). The analysis was performed using a Finnigan Trace GC Ultra gas chromatography equipped with ZB-5 capillary column (30 m x 0.25 mm x 0.25 μm) and interfaced to a Finnigan Trace DSQ MS detector. The oven temperature was ramped from 60 °C to 240 °C at a constant rate of 3 °C/min. The injection port stayed at 180 °C throughout the separation. The carrier gas was helium with a flow rate of 1 ml/min and a split ratio of 100:1. An electron ionization (IE) mode at 70 electron volts was used to carry out the MS. The chromatogram of the essential oil was obtained by using a full scan mass spectra with a scan range of 40-650 amu at 500.0 amu/second [16].

Participants

The Ethical Review Committee for the Center of Ethical Reinforcement for Human Research at Mahidol University endorsed the study and gave the ethical Permission No. MU IRB 2010/292.1310. The methodology strictly protected the participants' confidentiality and identity. The 30 participants

Table 1 Demographic data for the volunteers

Parameters	n	Minimum	Maximum	Mean	SD
Age	30	18	32	22.70	4.27
Height (cm)	30	152	177	167.43	6.82
Weight (kg)	30	46	71	58.57	6.38
Body Mass Index	30	18	25	21.33	2.10
Smell test	30	9	11	9.60	0.89

consisted of 15 males and 15 females with a body mass index ranging between 18-25 kg/m² (mean BMI 21.33±2.10). Their ages were between 18 and 32 years (mean age 22.70±4.27 years) as shown in Table 1. A screening with the n-butyl alcohol test (mean score 9.60±0.89) was carried out to detect the normal sense of smell [17]. Their personal health status including weight, height and blood pressure was also recorded. The participants who passed the screening procedure were non-smokers without any symptoms of upper respiratory infection, hypertension or cardiovascular disease [18].

According to the finding of previous studies, it had been found that the pleasantness of the oil odor could induce a variety of nervous system functions [19]. Therefore, before starting the experiment, subjects were requested to inhale the base oil with fingered citron and rate the level of pleasantness on a 5-point Likert scale. The participants who rated the pleasantness of the oil within 2-4 points were allowed to proceed with the experiment.

Acquisition of autonomic parameters and emotional state

The emotional state, and the autonomic nervous system (ANS) of the subjects such as blood pressure, heart rate, skin temperature and respiratory rate were recorded simultaneously. The ANS parameters were recorded by using the life scope 8 bedside monitor (Nihon Kohden, Japan). An emotion rating was used to create an emotion odor scale, which was developed by the researchers from the earlier research [20, 21]. This particular scale consisted of a 100 mm monopolar visual analog scale followed by five emotional states: pleasant (good), unpleasant (bad, uncomfortable, disgusted, frustrated, stressful), sensual (romantic), relaxed (relax, calm, drowsy), and refreshing (fresh, alert active).

Procedure

This research applied the A-B design experiment. It was found that sweet almond odor was given before fingered citron. According to the previous studies that sweet almond odor doesn't have an impact on autonomic nervous system [20, 21]. Experiments were conducted only in the

morning (8-12 am) [22] to reduce circadian variation. All activities were done in a silent room with an ambient temperature of 24±1°C and 40-50 % humidity. The height of the chair was adjusted to suit each subject. The ANS electrodes were attached to the appropriate positions after the subject sat comfortably in the adjustable armchair. Heart rate, skin temperature, and respiratory rate were recorded at every minute while systolic and diastolic blood pressures were recorded every 5-minute duration. The experiment consisted of three sessions. The first session served as a 10-minute baseline trial (resting period); the second and the third trials, the inhalation experiments, took 20 minutes each. Sweet almond oil was administered for the second trial, whereas 10% (v/v) fingered citron oil, diluted in sweet almond oil, was given in the third trial. Subjects had to rate their emotional states at the end of each test.

Statistical analysis

Data analysis on the effects of sweet almond oil and fingered citron assessments on physiological and mood states were calculated using the SPSS statistical package 17. A paired *t*-test was carried out on the data from autonomic parameters and emotional ratings.

RESULTS

Essential oil analysis

Essential oil hydrodistilled from the fresh fruit of *C. medica* L. var. *sarcodactylis* was found to be 1.25% w/w of the fresh peel weight. The GC-MS chromatogram of essential oil from the fresh fruit of *C. medica* L. var. *sarcodactylis* shown in Figure 2. To compute the percentage compositions, the GC peak area was used. The result revealed that fingered citron oil had two main ingredients: limonene at 65.11% and gamma terpinene at 34.89 % in Figure 3.

Autonomic nervous system response

Table 2 presented the mean and SD values of autonomic parameters in the experiment. The data on various ANS parameters were compared during inhalation of sweet almond oil and fingered citron

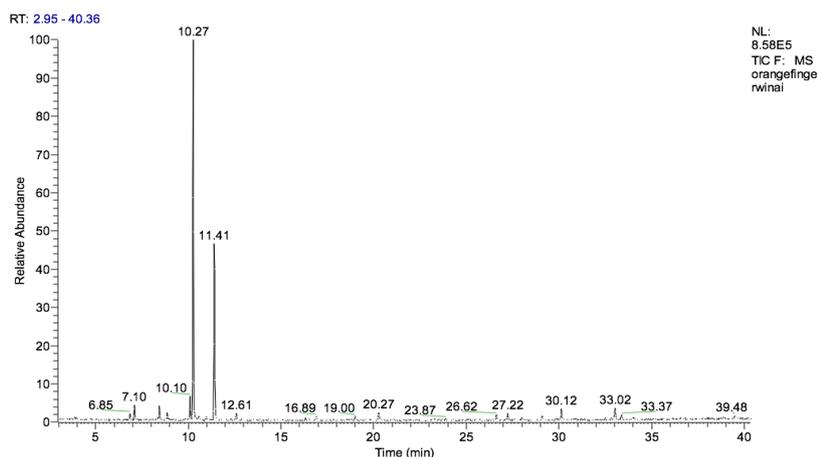


Figure 2 The GC chromatogram of fingered citron oil

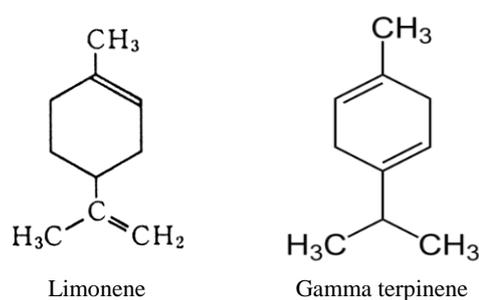


Figure 3 Chemical structures of two major components of essential oil from the peel *Citrus medica* L. var. *sarcodactylis*

Table 2 Mean and SD of ANS parameter change resting, sweet almond oil, and fingered citron

Parameters	Sweet almond oil		Fingered citron		P-value
	\bar{X}	S.D.	\bar{X}	S.D.	
Systolic blood pressure (mmHg)	106.48	8.04	107.91	8.86	0.039*
Diastolic blood pressure (mmHg)	64.01	6.25	68.97	6.96	0.010*
Heart rate (bpm)	65.39	8.63	65.45	8.67	0.814
Skin temperature (°C)	32.21	2.02	32.19	2.08	0.082
Respiratory rate (br/min)	16.12	2.17	16.68	2.33	0.019*

* Significant difference, p -value < 0.05

Table 3 Mean and SD of emotional state change sweet almond oil and finger citron

Emotion	Sweet almond oil		Fingered citron		P-value
	\bar{X}	S.D.	\bar{X}	S.D.	
Good	66.85	18.94	76.38	16.90	0.003*
Bad	14.85	13.31	13.00	14.58	0.551
Active	41.10	20.83	64.57	23.29	0.000*
Drowsiness	51.85	10.78	39.10	24.94	0.032*
Fresh	48.35	20.65	69.31	22.74	0.000*
Relax	63.72	17.06	65.78	18.60	0.074
Stress	13.80	8.54	11.63	3.17	0.294
Uncomfortable	14.65	13.31	13.21	10.98	0.098
Romantic	47.80	21.56	48.42	13.66	0.785
Frustrated	13.84	1.95	12.42	3.57	0.298
Clam	61.73	16.80	62.21	13.73	0.598
Disgust	8.31	6.36	6.59	3.51	0.076

* Significant difference, p -value < 0.05

oil. Blood pressure and respiratory rate showed a significant increase upon exposure to fingered citron. The heart rate and skin temperature, on the contrary, showed no statistically significant change.

Emotional state response

Table 3 showed the mean and SD of mood state responses. In the third trial, exposure to fingered citron oil increased positive emotions including the feeling of well-being, activeness and freshness (p -value < 0.05). Furthermore, negative emotions such as drowsiness were significantly reduced (p -value < 0.05).

DISCUSSION

The GC-MS analysis showed that limonene (65.11%) and gamma terpinene (34.89%) were the two largest components of essential oil from the peel of *Citrus medica* L. var. *sarcodactylis*. The analysis of the components confirmed the results from Shiota's research, which was about finding the components of fingered citron peel conducted in Japan. Our results revealed that the two major components of fingered citron peel oil in this analysis were limonene and gamma terpinene as being studied in Japan [7]. Incidentally, when comparing the active amount of limonene found in this study with other research studies, our study yielded more limonene than many other studies in Asia: Korean (Peel = 52.54%) [12], Japan (Peel = 52.54%) [7] and China (Peel = 36.37%) [10]. In addition, the amounts of limonene in fresh leaves from Thailand have the same results as the peel oil: Thailand (Fresh leaf = 49.19%, Theraphong, et.al.) [13], Iran (Fresh leaf = 43.37%) [23], and Bangladesh (Fresh leaf = 18.36%) [8].

According to the literature reviews, each original article revealed unique biochemical composition results despite extracting from the same type of plant due to the differences in the stage of maturity and cultivated areas. The comparison of limonene in orange peel (*Citrus sinensis*) from Italy, North Iran, and Vietnam (Cai Be) showed that Vietnamese's orange peel yielded the maximum amount of limonene [24]. Therefore, the similar climate of Thailand and Vietnam played the major part in producing higher amounts of limonene in Thai fingered citron oranges. Additionally, this study selected the unripe green finger citron due to the results of Wu, et al. which revealed that unripe green fingered citron yielded more limonene than ripe fingered citron [10].

In Autonomic activities, fingered citron peel oil

inhalation significantly increased ANS arousal levels, blood pressure, and respiratory rate. These changes of the ANS parameters represent the functions of the sympathetic nervous system. Furthermore, after inhalation, the results demonstrated that subjects felt better, were more active and fresher. Consequently, negative emotions such as drowsiness were decreased. Studies related to genus citrus that affected stimulation also supported these results [3]. The studies include the research on sweet orange oil inhalation that increased stimulation levels [14], and it is also found that massaging forty healthy volunteers with kaffir lime oil (*Citrus hystrix*) had significantly increased in blood pressure and decreased in skin temperature than the control group [25].

In addition to previous studies, plants containing a high limonene level, such as lemon oil which is 65.11% of fingered citron oil, were reported to induce stimulating effects [26]. According to Santiago and his college experiment, it was found that Wistar rats showed increased systolic blood pressure and heart rate after being fed with d-limonene for four weeks [27]. This finding is similar to Heuberger and her college which reported that after inhalation of (+)-limonene in ten female healthy volunteers, systolic blood pressure, subjective alertness, and restlessness increased [28]. The Haze and colleague study used oil containing a high limonene level, such as peppermint oil, estragon oil, fennel oil and grapefruit oil. That study tested forty-three volunteers and found that after inhaling, relative sympathetic activity increased 1.5-2.5 times representing the low-frequency amplitude of systolic blood pressure when compared to the odorless inhaling [29].

Though Neroli oil (*Citrus aurantium* L.) is one of the citrus plant groups, it has linalool as its main component. Linalool is in the essential oils that have a relaxation effect, for example, lavender [20]. In this study of 27 participants; 13 subjects were in the controlled group that inhaled sunflower oil, and 14 subjects inhaled Neroli. The physiological parameters showed a significant lower pre- and post-procedural systolic blood pressure in the Neroli inhalation group than in the controlled group [15]. The relationship between body and emotion demonstrates clearly that active and fresh feelings were associated tightly with the sympathetic system, leading to a secretion of norepinephrine from the peripheral nerve. The secretion stimulated the SA

node and increased the heart rate, influencing the vascular constriction causing high blood pressure [30].

CONCLUSION

In conclusion, our study showed a stimulating effect with fingered citron peel oil inhalation that correlated directly to the autonomic nervous system's activities and emotions. This research proves that fingered citron peel oil needs to be included in the stimulating essential oil group. Further research should be conducted using brain wave measurements for a complete analysis.

ACKNOWLEDGEMENTS

Financial support for this study came from grants from Praboromarajchanok Institute and Kanchanabhishek Institute of Medical and Public Health Science Technology. The authors are grateful to Miss Thidarat Duangyod for GCMS protocol recommendations and Mr. Ralph M. Green for his editorial corrections.

CONFLICTS OF INTERESTS

All authors have none to declare.

REFERENCES

- Battagli S. The complete guide to aromatherapy. 2nd ed. Zillmere: Perfect Potion; 2004.
- Hongratanaworakit T. Essential oil and aromatherapy. 2nd ed. Nonthaburi: PS Print; 2012.
- Hongratanaworakit T, Buchbauer G. Autonomic and emotional responses after transdermal of sweet orange oil in humans: placebo-controlled trial. *Int J Essential Oil Therapeutics*. 2007; 1(1): 29-37.
- Babazadeh-Darjazi B. Comparison of peel volatile components of citron and pummel (*Citrus sp.*). *Intl Res J Appl Basic Sci*. 2013; 5(6): 682-9.
- Feng Y, Cheng L, Liang C. Studies on the constituents of *Citrus medica* L. var. *sarcodactylis* (Noot.) Swingle. *Chin J Nat Med*. 2004; 2(1):149-51.
- Yin F, Lou F. Studies on the constituent of *Citrus medica* L. var. *sarcodactylis*. *Zhongguo Yaoxue Zazhi*. 2004; 3: 20-21.
- Shiota H. Volatile components in the peel oil from fingered citron (*Citrus medica* L. var. *sarcodactylis* Swingle). *Flavour Frag J*. 2006; 5(1): 33-7.
- Bhuiyan MNI, Begum J, PK, Rahman MS. Constituents of peel and leaf essential oils of citrus medica L. *J Sci Res*. 2009; 1(2): 387-92.
- Meena AK, Kandala A, Rao MM, Panda P, Reddy G. A Review on citron - pharmacognosy and medicinal uses. *Int Res J Pharmacy*. 2011; 2(1): 14-9.
- Zhen W, Hong L, Yong Y, Yong Z, Dawei T. Variation in the components and antioxidant activity of *Citrus medica* L. var. *sarcodactylis* essential oils at different stages of maturity. *Ind Crop Prod*. 2013; 46: 311-6.
- Chan YY, Li CH, Shen YC, Wu TS. Anti-inflammatory principles from the stem and root barks of *Citrus medica*. *Chem Pharm Bull (Tokyo)*. 2010 Jan; 58(1): 61-5.
- Kim KN, Ko YJ, Yang HM, Ham YM, Roh SW, Jeon YJ, et al. Anti-inflammatory effect of essential oil and its constituents from fingered citron (*Citrus medica* L. var. *sarcodactylis*) through blocking JNK, ERK and NF-kappaB signaling pathways in LPS-activated RAW 264.7 cells. *Food Chem Toxicol*. 2013 Jul; 57: 126-31. doi: 10.1016/j.fct.2013.03.017
- Theanphong O, Songsak T, Mingvanish W. Chemical composition and antimicrobial activity of the essential oil from *Citrus medica* L. var. *sarcodactylis* (Sieber) Swingle Leaf. *Mu J Pharm*. 2008; 35 (1-4): 57-61.
- Hongratanaworakit T, Buchbauer G. Human behavioral and physiological reactions to inhalation of sweet orange oil. *Acta Hort*. 2005; 679: 75-81.
- Hu PH, Peng YC, Lin YT, Chang CS, Ou MC. Aromatherapy for reducing colonoscopy related procedural anxiety and physiological parameters: a randomized controlled study. *Hepatogastroenterology*. 2010 Sep-Oct; 57(102-103): 1082-6.
- Hübschmann HJ. Handbook of GC/MS: fundamentals and applications. 2nd ed. Weinheim: Wiley-VCH; 2009. p.133-7.
- Cain WS. Testing olfaction in a clinical setting. *Ear Nose Throat J*. 1989 Apr; 68(4): 316, 22-8.
- Saeki Y, Shiohara M. Physiological effects of inhaling fragrances. *International Journal of Aromatherapy*. 2001; 11(3): 118-25. doi: 10.1016/S0962-4562(01) 80047-3
- Brauchli P, Rüegg PB, Etzweiler F, Zeier H. Electrocortical and autonomic alteration by administration of a pleasant and an unpleasant odor. *Chem Senses*. 1995 Oct; 20(5): 505-15.
- Sayorwan W, Siripornpanich V, Piriyaunporn T, Hongratanaworakit T, Kotchabhakdi N, Ruangrunsi N. The effects of lavender oil inhalation on emotional states, autonomic nervous system, and brain electrical activity. *J Med Assoc Thai*. 2012 Apr; 95(4): 598-606.
- Sayorwan W, Ruangrunsi N, Piriyaunporn T, Hongratanaworakit T, Kotchabhakdi N, Siripornpanich V. Effects of inhaled rosemary oil on subjective feelings and activities of the nervous system. *Sci Pharm*. 2013 Apr-Jun; 81(2): 531-42. doi: 10.3797/scipharm.1209-05
- Sztajzel J. Heart rate variability: a noninvasive electrocardiographic method to measure the autonomic nervous system. *Swiss Med Wkly*. 2004 Sep; 134(35-36): 514-22. doi: 2004/35/smw-10321
- Behzad Babazadeh D. The effect of seasonal variation on citron (*Citrus medica*) Leaf components. *Journal of Current Research in Science*. 2014; 2(2): 285-90.
- Quoc Le PT, MyVo A, Nguyet Trinh TM. Volatile constituents of essential oil from *Citrus sinensis* grown in Tien Giang Province Vietnam. *Asian Journal of Food and Agro-Industry*. 2011; 4(3): 183-6.

25. Hongratanaworakit T, Buchbauer G. Chemical composition and stimulating effect of *Citrus hystrix* oil on humans. *Flavour Fragr J*. 2007; 22(5): 443-9. doi: 10.1002/ffj.1820
26. Nijjima A, Nagai K. Effect of olfactory stimulation with flavor of grapefruit oil and lemon oil on the activity of sympathetic branch in the white adipose tissue of the epididymis. *Exp Biol Med (Maywood)*. 2003 Nov; 228(10): 1190-2.
27. Santiago JVA, Jayachitra J, Shenbagam M, Nalini N. D-limonene attenuates blood pressure and improves the lipid and antioxidant status in high fat diet and l-name treated rats. *Journal of Pharmaceutical Sciences and Research*. 2010; 2(11): 752-8.
28. Heuberger E, Hongratanaworakit T, Bohm C, Weber R, Buchbauer G. Effects of chiral fragrances on human autonomic nervous system parameters and self-evaluation. *Chem Senses*. 2001 Mar; 26(3): 281-92.
29. Haze S, Sakai K, Gozu Y. Effects of fragrance inhalation on sympathetic activity in normal adults. *Jpn J Pharmacol*. 2002 Nov; 90(3): 247-53.
30. Hongratanaworakit T. Physiological effects in aromatherapy. *Songklanakarinn Journal of Science and Technology*. 2004; 26(1): 118-25.