Agriculture and Natural Resources 50 (2016) 133–138

Contents lists available at ScienceDirect

Agriculture and Natural Resources

journal homepage: http://www.journals.elsevier.com/agriculture-and-natural-resources/

Original article

Spermicidal effects of lemon juice and juices from other natural products

Somsak Suthutvoravut,[a,*] Ourawan Kamyarat[b]

[a] Department of Obstetrics and Gynecology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand
[b] Chulalongkorn University, Pathumwan District, Bangkok 10300, Thailand

ARTICLE INFO

Article history:
Received 3 October 2014
Accepted 21 September 2015
Available online 9 June 2016

Keywords:
Lemon juice
Natural products
Spermicide

ABSTRACT

The study of spermicidal effects of lemon juice and juices from other natural products consisting of pineapple juice, apple juice and aloe vera juice, was carried out to develop methods of contraception using natural products. Semen was donated by 20 men from infertile couples who came to an infertility clinic at the Department of Obstetrics and Gynecology, Ramathibodi Hospital, Bangkok, Thailand from 1 November 2007 to 31 March 2010. Spermicidal effects were measured by observing changes in sperm viability, morphology and motility after the semen was mixed with lemon juice and the juices from the other natural products (pineapple juice, apple juice and aloe vera juice). Changes in sperm characteristics were compared with pure semen left at room temperature. After the semen was mixed with lemon juice, sperm were instantaneously immobilized and irreversibly deformed. A reduced spermicidal effect was observed when the semen was mixed with the other juices. A second, profound spermicidal effect was observed when semen was mixed with pineapple juice. The least effects were observed when the semen was mixed with aloe vera juice. This information can be used for the further development of natural barrier methods of contraception.

Introduction

The use of available household or commercial products as spermicidal agents for contraception has long been investigated. Umpierre et al. (1985) found that Coca-Cola had a spermicidal effect. However, the study of Hong et al. (1987) provided quantitative evidence that Coca-Cola and Pepsi-Cola have little, if any, spermicidal effect and thus their application as a postcoital douching is a practice lacking scientific foundation. Lemon juice solutions have been shown to immobilize sperm in the laboratory (Short et al., 2004), as has Krest Bitter Lemon drink (Nwoha, 1992). While the author of the Krest Bitter Lemon study suggested its use as a postcoital douche, this is unlikely to be effective, as sperm begin leaving the ejaculate (out of the reach of any douche) within 1.5 min of deposition. No published studies appear to have been done on the effectiveness of lemon juice preparations in preventing pregnancy, though they are advocated by some as 'natural' spermicide.

Short et al. (2004) from the University of Melbourne reported that lemon juice is not only an effective form of contraception, but also had effect on the HIV virus. The study of Sagay et al. (2009) on genital tract abnormalities among female sex workers who had douched with lemon/lime juice in Nigeria found that the practice of douching with citrus juice may be a risk factor for cervical dysplasia. They suggested that further studies to explore the association between douching with lime juice and cervical dysplasia are warranted in communities where this practice is common. Besides using lemon juice as a spermicidal agent, other natural products from plants have been studied to evaluate their effects on sperm (Farnsworth and Wall, 1982).

The current study investigated the spermicidal effects of lemon juice (*Citrus limon*) and juices of other natural products available—juice from pineapple (*Ananas comosus*), apple (*Malus estica*), and aloe vera (*Aloe vera*). Changes in sperm characteristics were compared with changes in control semen which was left at room temperature without mixing with any juices. The effects of the juices could lead to applicable information for the development of natural techniques of contraception.

Materials and methods

This research was approved by the Ethical Clearance Committee on Human Rights Related to Researches Involving Human Subjects,

* Corresponding author.
E-mail address: suthut22@yahoo.com (S. Suthutvoravut).

http://dx.doi.org/10.1016/j.anres.2015.09.004

Faculty of Medicine, Ramathibodi Hospital, Mahidol University (MURA 2007/194) issued on 14 June 2007.

Semen samples were obtained from 20 volunteers who came to the Infertility Clinic, Ramathibodi Hospital, Bangkok, Thailand from 1 November 2007 to 31 March 2010. The semen samples were collected in the Reproductive Biology Laboratory of Ramathibodi Hospital and then were allowed to liquefy for at least 20 min at 37 °C in an incubator and were analyzed within 60 min. One milliliter of semen was mixed with 1 mL of each natural product (1:1 by volume) consisting of pure and diluted lemon juice, pineapple juice, apple juice and aloe vera juice. Changes in the characteristics of sperm were observed and compared with control semen which was left at room temperature without mixing with any of the solutions. Sperm concentration, viability, morphology and motility were evaluated using standard techniques (Schrader et al., 1992; World Health Organization, 1999). Sperm morphology was scored using the Tygerberg Kruger strict criteria (Kruger et al., 1987). Volunteers were aged 25–55 yr with no history of medical diseases such as diabetes mellitus, hypertaion, hyper or hypothyroid or any autoimmune diseases and no history of chemotherapy or radiation. They had not been vasectomized and were not heavy smokers or heavy alcohol drinkers.

All 20 semen samples were processed and analyzed by researchers at the laboratory. A routine semen analysis was performed which included the following parameters: semen volume, sperm concentration, sperm viability, sperm morphology and sperm motility.

Sperm concentration

Measurement of sperm concentration was carried out under a microscope (40×) after the semen had been dropped into a Neubauer Hemocytometer. Scanning the slide and estimating the numbers of spermatozoa (both viable and non-viable) per field or part of a field equivalent to 1 mL gave an approximate sperm concentration in millions per milliliter.

Sperm viability

The viability of sperm was determined using eosin staining and examination under a microscope (100×). Two hundred spermatozoa were counted with a light or phase contrast microscope before differentiating the live (unstained) spermatozoa from the dead (stained) cells.

Sperm morphology

Morphological measurements of the sperm were undertaken by examining the sperm under a microscope (100×) after staining with methyl alcohol, eosin, and methylene blue. The percentage of normal morphological sperm was recorded. As each slide was examined systematically from one field of the microscope to the next, all normal spermatozoa were assessed and scored and the defects of the abnormal spermatozoa were noted. For normal morphology characteristics of sperm, the sperm must be vigorously motile and the sperm head must be a symmetrical, oval shape of the appropriate size (World Health Organization, 1999).

Sperm motility

Sperm motility was observed and classified. At least five fields of the microscope were assessed in a systematic way to classify 200 spermatozoa. The motility of each spermatozoon was graded *a*, *b*, *c* or *d*, according to whether it showed the following characteristics: *a* indicated rapid progressive motility (at least 25 μm/s at 37 °C and at least 20 μm/s at 20 °C); *b* indicated slow or sluggish progressive motility; *c* indicated non-progressive motility (less than 5 μm/s); and *d* indicated immotility.

Within a defined area of the fields indicated by lines A and B formed by a graticule in the focal plane of the microscope, all spermatozoa with grade *a* and *b* motility were counted first. Subsequently, the spermatozoa with non progressive motility (grade *c*) and immotile spermatozoa (grade *d*) were counted in the same areas. The numbers of spermatozoa in each category were tallied with the aid of a laboratory counter. The count of 200 spermatozoa was repeated on a separate 1 mL specimen from the same semen sample and the percentages in each motility grade from the two independent counts were compared and averaged. Sperm motility was also scored for statistical analysis. Levels *a*, *b*, *c* and *d* were allocated score of 3, 2, 1 and 0, respectively.

Preparation of juices of natural products

1) The lemons bought from a supermarket were cleaned and washed with soap and tap water. Then, each lemon was divided in half before squeezing to obtain the juice which was filtered through a clean cloth. About 2 mL of juice was obtained from one lemon. Diluted lemon juice was prepared by adding 1 mL of sterile water into 1 mL of fresh lemon juice (1:1 dilution). Only 1 mL of diluted lemon juice was mixed with semen.
2) The natural products consisting of apple, pineapple, and aloe vera were bought from a supermarket. All the fruits were washed and cleaned with soap and tap water. The skins of the fruits were peeled before dividing each fruit into small pieces of 2–3 mm^3, which were then ground using a grinding machine and squeezed to obtain the juice. The juices were then filtered through a clean cloth to eliminate the sediments.

Each type of the juice was prepared fresh just before mixing with semen. Data analysis was done using the SPSS Statistics 18.0 Mahidol package (SPSS Inc; Chicago, IL, USA). Comparison of qualities of sperm within and between groups was undertaken using the General Linear Model (GLM) for repeated measurement.

Results

Sperm concentration

In the control semen, the mean number of sperm concentration was stable over time at 55–64 $\times$ 10^6 per mm^3. After the semen was mixed with pure lemon juice, the mean concentration of sperm decreased instantaneously to zero and remained so. When semen was mixed with the other kinds of juice, the mean concentration of sperm decreased gradually and significantly ($p < 0.001$) when compared with the control. Among the other juices, the pineapple juice had the lowest mean concentration of spermatozoa (Table 1). When the mean concentrations of sperms were compared between each mixture of sperm and juice from the natural products, there were statistically significantly differences between each type of mixture except between semen mixed with diluted lemon juice and semen mixed with aloe vera juice (both not significant) as shown in Table 1.

Sperm viability

The viability of sperm in the control semen left at room temperature gradually decreased over time. Mean numbers of viable sperm were significantly different ($p < 0.001$) between the control semen and the semen mixed with each kind of juice. The viability of the sperm after mixing with lemon juice decreased immediately to

Table 1
Mean concentration of sperm ($\bar{x}$±SD) in control semen and semen mixed with lemon juice, diluted lemon juice, pineapple juice, apple juice and aloe vera juice over time.

Time (min)	Control(1) $\bar{x}$±SD ($\times 10^6$/mm^3)	Lemon(2) $\bar{x}$±SD ($\times 10^6$/mm^3)	Diluted lemon(3) $\bar{x}$±SD ($\times 10^6$/mm^3)	Pineapple(4) $\bar{x}$±SD ($\times 10^6$/mm^3)	Apple(5) $\bar{x}$±SD ($\times 10^6$/mm^3)	Aloe vera(6) $\bar{x}$±SD ($\times 10^6$/mm^3)
0	69.65 ± 16.40	0.00 ± 0.00	28.08 ± 8.93	5.85 ± 3.23	18.00 ± 10.48	30.05 ± 15.74
5	63.45 ± 18.14	0.00 ± 0.00	24.17 ± 6.78	4.05 ± 2.67	13.15 ± 8.36	25.65 ± 15.80
10	57.85 ± 16.21	0.00 ± 0.00	20.92 ± 5.69	2.95 ± 2.21	13.05 ± 9.78	21.85 ± 14.30
20	54.15 ± 16.40	0.00 ± 0.00	17.42 ± 3.80	1.75 ± 1.48	11.50 ± 10.09	18.55 ± 12.59
30	50.65 ± 18.45	0.00 ± 0.00	12.67 ± 3.82	1.10 ± 0.91	9.80 ± 6.58	13.65 ± 13.07
60	46.40 ± 17.56	0.00 ± 0.00	7.33 ± 2.74	0.60 ± 0.50	6.50 ± 5.15	9.40 ± 12.60

Note: Test of statistically significant difference within and between groups by GLM (General Linear Model) for repeated measurement. All differences were significant ($p < 0.001$) for control semen with semen mixed with each kind of juice and between semen–juice mixture (1)–(6), except between semen mixed with apple juice and with aloe vera juice (5) versus (6), ($p < 0.05$) and between semen mixed with diluted lemon juice and with aloe vera juice (3) versus (6), with the latter being not significant.

zero and remained the same over time. The pure lemon juice killed the sperm instantaneously but the diluted lemon juice (1:1 by volume) did not. The mean number of viable sperm was less when the semen was mixed with pineapple juice than when mixed with diluted lemon juice, apple juice or aloe vera juice. When the semen was mixed with diluted lemon juice, apple juice or the aloe vera juice, the same pattern of change was observed with higher mean numbers of viable sperm. There was a significant difference ($p < 0.001$) between the semen mixed with each type of juices, except for the semen mixed with diluted lemon juice and with apple juice and between the semen mixed with diluted lemon juice and with aloe vera juice (Table 2).

Sperm morphology

In the control semen, the mean percentage of sperm with normal morphology was 5.00 ± 0.80 percent and it decreased steadily over time. There were significant ($p < 0.001$) differences in the mean percentage of sperm with normal morphology between the control semen and semen mixed with the juices of natural products (Table 3). No sperm with normal morphology were observed immediately after semen was mixed with the pure lemon juice. There were significant ($p < 0.001$) differences in the mean percentage of normal morphology sperm for the semen mixed with lemon juice compared with semen mixed with other kinds of juice. The percentage of normal morphology sperm in the mixture of semen and diluted lemon juice was not significantly different from the semen mixed with pineapple juice and with apple juice. There was no significant difference between the semen mixed with apple juice and with aloe vera juice (Table 3).

Sperm motility or progression

Motility or progression of spermatozoa was categorized as previously described. The levels of sperm motility were unchanged at level *b* (score = 2) when the control semen was left at room temperature at 5 min, 10 min, 20 min and 30 min. After 60 min at room temperature, 40 percent of spermatozoa had progressively decreased to level *c* (score = 1) as shown in Table 4.

After mixing the semen with the pure lemon juice, all the sperm showed immediate immobility with scores of *d* (score = 0). After the semen had been mixed with the pineapple juice, 85 percent of spermatozoa had progressively decreased to level *c* at 0 min (mean score = 1.15 ± 3.66), 80 percent at 5 min (mean score = 1.05 ± 0.36), and 100 percent at 10 min (mean score = 1.00 ± 0.00). However, at 20 min, 30 min and 60 min, the percentages of sperm progression in level *c* were 95 percent (mean score = 0.90 ± 0.30), 60 percent (mean score = 0.60 ± 0.50), and 50 percent (mean score = 0.5 ± 0.51), respectively (Table 4). After the semen was mixed with apple juice, the percentage of sperm was 95 percent at level *b* (mean score = 1.90 ± 0.31) at 0 min, 5 min, 10 min and 20 min, respectively, and 5 percent at level *b* (mean score = 1.05 ± 0.22) at 30 min and 60 min, respectively (Table 4).

Sperm progression after mixing with the aloe vera juice was unchanged, that is, 100 percent at level *b* at 0 min and 5 min, respectively, then at 10 min, 15 percent of spermatozoa had decreased to level *c* (mean score = 1.85 ± 0.37), at 20 min, 35 percent were at level *b* (mean score = 1.65 ± 0.49), at 30 min, 75 percent were level *c* (mean score = 1.25 ± 0.44), and at 60 min, 90 percent were level *c* (mean score = 1.10 ± 0.31) as shown in Table 4. When the semen was mixed with the diluted lemon juice, the level of progression of spermatozoa was unchanged (100% at level *b*) at 0 min, 5 min and 10 min. After that, at 20 min, 50 percent of sperm decreased to level *c* (mean score = 1.50 ± 0.52) and 100 percent of sperm decreased to level *c* (mean score = 1.00 ± 0.00) at 30 min and 60 min, respectively (Table 4).

pH of juices from natural products

The spermicidal effects of juices of natural products varied by the type of natural product. Several different characteristics of these juices could account for their spermicidal effects. One of these characteristics was acidity (pH). Pure lemon juice had the lowest pH (pH = 1), which resulted in immediate and profound

Table 2
Mean number of viable sperm ($\bar{x}$±SD) in control semen and semen mixed with lemon juice, diluted lemon juice, pineapple juice, apple juice and aloe vera juice over time.

Time (min)	Control(1) $\bar{x}$±SD ($\times 10^6$/mm^3)	Lemon(2) $\bar{x}$±SD ($\times 10^6$/mm^3)	Diluted lemon(3) $\bar{x}$±SD ($\times 10^6$/mm^3)	Pineapple(4) $\bar{x}$±SD ($\times 10^6$/mm^3)	Apple(5) $\bar{x}$±SD ($\times 10^6$/mm^3)	Aloe vera(6) $\bar{x}$±SD ($\times 10^6$/mm^3)
0	71.25 ± 21.27	0.00 ± 0.00	29.50 ± 3.50	6.35 ± 3.96	20.10 ± 7.20	23.30 ± 15.69
5	65.45 ± 20.51	0.00 ± 0.00	22.25 ± 4.84	4.55 ± 2.70	17.95 ± 7.72	20.35 ± 12.51
10	61.95 ± 20.31	0.00 ± 0.00	18.85 ± 3.23	3.00 ± 2.32	14.95 ± 5.46	16.35 ± 9.82
20	58.70 ± 22.64	0.00 ± 0.00	16.75 ± 4.00	2.10 ± 1.62	12.40 ± 5.36	13.25 ± 6.69
30	54.75 ± 21.87	0.00 ± 0.00	12.67 ± 2.10	0.85 ± 0.67	9.15 ± 8.09	13.70 ± 9.50
60	51.00 ± 17.00	0.00 ± 0.00	7.83 ± 2.08	0.50 ± 0.51	6.25 ± 5.50	9.50 ± 4.93

Note: Test of statistically significant differences within and between groups by GLM (General Linear Model) for repeated measurement. All differences were significant ($p < 0.001$) for control semen with semen mixed with each kind of juice and between semen–juice mixture, except between semen mixed with diluted lemon juice and with apple juice, (3) versus (5) and between semen mixed with diluted lemon juice and with aloe vera juice, (3) versus (6), which were both not significant.

Table 3
Mean percentage of normal morphology of sperm ($\bar{x}$±SD) in control semen and semen mixed with lemon juice, diluted lemon juice pineapple juice, apple juice and aloe vera juice over time.

Time (min)	Control(1) $\bar{x}$±SD (%)	Lemon(2) $\bar{x}$±SD (%)	Dil lemon(3) $\bar{x}$±SD (%)	Pineapple(4) $\bar{x}$±SD (%)	Apple(5) $\bar{x}$±SD (%)	Aloe vera(6) $\bar{x}$±SD (%)
0	5.00 ± 0.80	0.00 ± 0.00	3.75 ± 0.97	3.80 ± 1.61	3.50 ± 1.28	4.15 ± 1.31
5	4.15 ± 0.67	0.00 ± 0.00	2.75 ± 0.97	3.45 ± 1.57	3.10 ± 1.02	3.60 ± 1.19
10	3.35 ± 0.67	0.00 ± 0.00	1.67 ± 0.65	2.70 ± 1.22	2.45 ± 0.89	2.90 ± 0.91
20	2.70 ± 0.57	0.00 ± 0.00	1.33 ± 0.65	2.15 ± 0.93	2.05 ± 0.69	2.40 ± 0.82
30	2.05 ± 0.39	0.00 ± 0.00	0.17 ± 0.39	1.55 ± 1.00	2.00 ± 0.80	1.75 ± 0.72
60	1.40 ± 0.50	0.00 ± 0.00	1.17 ± 0.39	0.95 ± 0.69	0.95 ± 0.40	1.15 ± 0.49

Note: Test of statistically significant differences within and between groups by GLM (General Linear Model) for repeated measurement. All differences were significant ($p < 0.001$) for control semen with semen mixed with juices and between each kind of semen–juice mixture, except for semen mixed with diluted lemon juice and with pineapple juice (3) versus (4), between semen mixed with diluted lemon juice and with apple juice (3) versus (5) and between semen mixed with apple juice and with aloe vera juice (5) versus (6), which were all not significant.

Table 4
Mean score of sperm progression in control semen and semen mixed with lemon juice, diluted lemon juice pineapple juice, apple juice and aloe vera juice over time.

Time (min)	Control(1) $\bar{x}$±SD (score)	Lemon(2) $\bar{x}$±SD (score)	Dil. lemon(3) $\bar{x}$±SD (score)	Pineapple(4) $\bar{x}$±SD (score)	Apple(5) $\bar{x}$±SD (score)	Aloe vera(6) $\bar{x}$±SD (score)
0	2.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	1.15 ± 3.66	1.90 ± 0.31	2.00 ± 0.00
5	2.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	1.05 ± 0.36	1.90 ± 0.31	2.00 ± 0.00
10	2.00 ± 0.00	0.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.00	1.90 ± 0.31	1.85 ± 0.37
20	2.00 ± 0.00	0.00 ± 0.00	1.50 ± 0.52	0.90 ± 0.30	1.90 ± 0.31	1.65 ± 0.49
30	2.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.00	0.60 ± 0.50	1.05 ± 0.22	1.25 ± 0.44
60	1.60 ± 0.50	0.00 ± 0.00	1.00 ± 0.00	0.50 ± 0.51	1.05 ± 0.22	1.10 ± 0.31

Note: Score of sperm progression: level $a = 3$, level $b = 2$, level $c = 1$, level $d = 0$. Test of statistically significant difference within and between groups by GLM (General Linear Model) for repeated measurement. All differences were significant ($p < 0.001$) for the control semen with semen mixed with different juices and between each semen–juice mixture (1)–(6), except between semen mixed with diluted lemon juice and with pineapple juice (2) versus (3) and between semen mixed with apple juice and with aloe vera juice, (5) versus (6), which were both not significant.

spermicidal action. Diluted lemon juice had pH = 2 at the beginning and increased in pH after 10 min and later. Pineapple juice which had the second best spermicidal effects had pH = 3 at the beginning and increased to pH = 4 at 5 min and 10 min and then to pH = 5 later. Apple juice had pH = 4 at the beginning and pH = 5 for most of the time after that. Alove vera juice had pH = 6 during most of the experiment (Fig. 1).

Discussion

This study confirmed that the natural products from plants are likely to make practical fertility-regulating agents. In the past, lists of plants were investigated to discover some practical and safe contraception. Plants are definitely sources of many useful and widely-employed drugs, and so practical fertility-regulating agents are likely to be discovered eventually from these sources (Farnsworth and Wall, 1982).

Farnsworth and Wall (1982) noted that the mechanisms of the agents that inhibit fertility by changing the characteristics of human sperm include disruption of spermatozoon plasma membranes (nonoxynol 9). Numerous enzymatic systems in viable sperm are also susceptible to inhibition, such as those involving glycolysis and energy productive myosin contraction. A large number of plants have been randomly selected and screened for spermicidal activity *in vitro* and several appear promising. For example, oleanolic acid and saponins in roots and seeds of several plants were found to have spermicidal effects (Farnsworth and Wall, 1982).

The current study confirmed a previous study that lemon juice immobilized sperm in the laboratory (Short et al., 2004) as did Krest Bitter Lemon drink. The mechanisms for this action are postulated to be due to its high acidity (pH = 2) which destroys spermatozoon plasma membranes. The study of Nwoha (1992) also found that the alkalinity of all drinks decreases spermicidal action. Coca-Cola and Krest bitter lemon juices may achieve very high

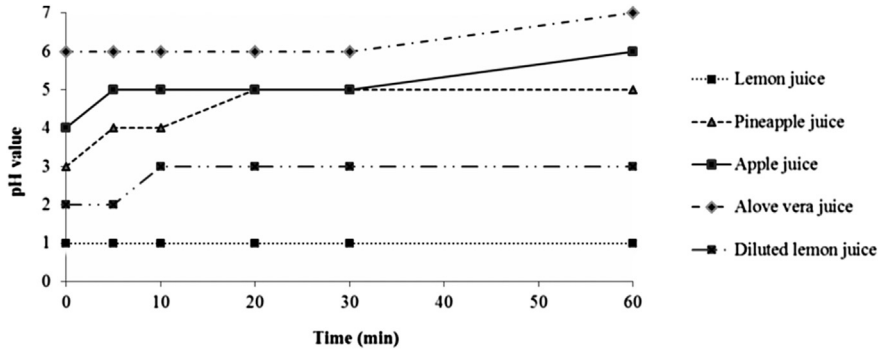

Fig. 1. pH from juices of natural products.

efficacy if used as a post-coital douche, especially in the impoverished and densely populated third worlds. While the authors of the Krest Bitter Lemon study suggested its use as a postcoital douche, it is unlikely to be effective as no published studies have been done on the effectiveness of lemon juice preparations in preventing pregnancy, though they are advocated by some as a 'natural' spermicide (Short et al., 2004). Proper applications of spermicidal agents in conjunction with other barrier methods (such as a cervical cap or diaphragm or a tampon soaked with lemon juice or any kind of juice which has spermicidal effects, inserted into upper vagina just before intercourse) might be likely to have contraceptive effects, especially in an emergency and where no other method is available.

The study of Sagay et al. (2009) from the University of Jos, Nigeria on genital tract abnormalities among female sex workers who douched with lemon or lime juice, showed that the practice may be a risk factor for cervical dysplasia and further studies to explore the association between vaginal douching with lime juice and cervical dysplasia are warranted in communities where this practice is common. However, daily intravaginal administration of lime juice to macaque monkeys for one month causes no vaginal pathology (Sagay et al., 2009).

Nowadays, intravaginal lemon and lime juice douches are used by women in Nigeria to protect themselves from pregnancy and supposedly from sexually transmitted infections (Short et al., 2004). They advocated that lemon juice is not only an effective form of contraception, but also had an effect on the HIV virus. His study was based on his finding that intravaginal lemon juice applied prior to intercourse had been used as a contraceptive by women around the Mediterranean for more than 300 years. His findings also confirmed the contraceptive properties of lemon juice by showing that a 20 percent final concentration of lemon juice in a fresh human ejaculate irreversibly immobilized 100 percent of sperms within 30 s. In the current study, the diluted lemon juice (1:1 by volume) was not as effective as the pure lemon juice.

Investigations into spermicidal effects of juices from natural products have two major benefits. First, if the spermicidal effects are significant, they could be used as a component of a barrier contraceptive method when they are soaked into a tampon that is then inserted into the upper vagina just before sexual intercourse. Because juices are available in households or nearby markets, this type of contraception can be suitable for use as an emergency method. Second, as these juices are all natural products, their toxicity would be nonexistent or mild when compared to synthetic products.

Penniston et al. (2008) discussed lemon juice and its components from which the following information is sourced. Lemon juice had the lowest pH at 1–2 because of citric acid which is the main component. Lemons are a rich source of vitamin C, providing 64 percent of the Daily Value in a 100 g serving whereas other essential nutrients are not present. As with other citrus fruits, they have a substantial concentration (about 47 g/L in juice) of citric acid. Lemons contain numerous phytochemicals, including polyphenols and terpenes (Rauf et al., 2014). Morton (1987) studied pineapple, one of the fruits from a warm climate and provided the following information. In addition to being used as food and drink, pineapple juice contains bromelain which is composed of a variety of substances, including peroxidase, acid phosphatase, calcium, and protease inhibitors. However, the main active ingredients are two enzymes known as fruit and stem bromelain, which cause break down dietary proteins, easing the body's digestive burden. Commonly applied as a meat tenderizer, when used appropriately, bromelain can tenderize overly inflamed and fibrin-congested muscles and connective tissues with its enzymes. Bromelain's fibrinolytic properties can contribute to thinning the blood; however, those on blood-thinning medications must be careful, especially when consuming any part of the bromelain-rich core. Furthermore, bromelain has the ability to enhance the absorption of other nutrients and drugs due to its ability to modulate intestinal permeability. This can be a good thing, for instance, if one is trying to absorb more of a therapeutic herb or nutrient, but a bad thing if one does not wish to disrupt the delicate pharmacokinetics of the bodily absorption and distribution of potent drugs. Bromelain has even been found to be superior to the highly toxic chemotherapy agent 5-fluorouracil as an anti-tumor agent in preclinical research (Báez et al., 2007). Pineapple juice, due to its low pH and other constituents in the juice, has been found to be effective at inactivating rotavirus, while honeydew and papaya juice failed (Yap et al., 2008). In the current study, pineapple juice was found to be the second most potent spermicide after the pure lemon juice. The role of pineapple juice as a spermicidal agent needs further investigation.

Eisele and Drake (2005) discussed apple juice and its ingredients and provided the following information. Apple juice usually has a pH in the range 3.37–4.24. Its main ingredient that contributes to its acidity is malic acid; although it also contains citric and quinic acid at the average concentration of 11.9 mg/100 mL and 41.8 mg/100 mL respectively, the concentration of malic acid is as high as 847.7 mg/100 mL. Apple juice also contains high concentrations of potassium and phosphate.

Atherton (1998) studied the *Aloe vera* plant and its components and provided the following information. *Aloe vera* contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids. The active acid components of *Aloe vera* are ascorbic acid in leaves, and glutaminic acid, aspartic acid, aloetic acid, fomic acid, palmitic acid and estearic acid in plants. Minerals in *Aloe vera* include calcium, magnesium, potassium, zinc, phosphorus, manganese and aluminum.

Shelton (1991) studied the therapeutic properties of *Aloe vera* and provided the following information. Aloin is a compound found in the exudate of some *Aloe* species and was a common ingredient in over-the-counter (OTC) laxative products in the United States until 2002 when the Food and Drug Administration banned it because the companies involved in its manufacture failed to provide the necessary safety data. *Aloe vera* has potential toxicity, with side effects occurring at some dose levels both when ingested or applied topically. Although toxicity may be less when the aloin is removed by processing, *Aloe vera* that contains aloin in excess amounts may induce side effects. Aloe vera juice has been marketed to support the health of the digestive system, but there is neither scientific evidence nor regulatory approval to support this claim and the extracts and quantities typically used for such purposes appear to be dose-dependent for toxic effects (Cosmetic Ingredient Review Expert, 2007).

Among the four tests of sperm characteristics, sperm concentration and sperm viability are two characteristics which showed apparent changes from control semen to semen mixed with the juices of natural products. On the contrary, sperm morphology and sperm progression showed less significant changes. Semen mixed with lemon juice produced immediate sperm death, morphological changes and immobility. Pineapple juice produced the second most potent spermicidal effects especially when sperm concentration and sperm viability were considered. Apple juice and diluted lemon juice showed comparable effects when mixed with semen. Aloe vera juice showed the least effects on sperm characteristics.

Pure lemon juice had profound spermicidal effects. Other juices from natural products showed less effective spermicidal effects to varying degrees. Pineapple juice had a greater effect than apple juice, aloe vera juice, and diluted lemon juice. However, the apple

juice and diluted lemon juice had slow but significant spermicidal effects over a longer period. The aloe vera juice produced the least spermicidal effects.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Acknowledgment

This study was partially supported by the Chulalongkorn University Graduate School's Scholarship to commemorate the 72nd anniversary of his Majesty the King Bhumibol Adulyadej.

References

Atherton, P., 1998. Aloe vera revisited. Br. J. Phytother. 4, 76–83.
Báez, R., Lopes, M.T., Salas, C.E., 2007. In vivo antitumoral activity of stem pineapple (*Ananas comosus*) bromelain. Planta Med. 73, 1377–1383.
Cosmetic Ingredient Review Expert, 2007. Final report on the safety assessment of *Aloe andongensis* extract, *Aloe andongensis* leaf juice etc. Int. J. Toxicol. 26, 1–50.
Eisele, T.A., Drake, R.S., 2005. The partial compositional characteristics of apple juice from 175 apple varieties. J. Food Compos. Anal. 18, 213–221.
Farnsworth, N.R., Wall, D.P., 1982. Current status of plant products reported to inhibit sperm. Res. Front. Fertil. Regul. 2, 1–16.
Hong, C.Y., Shieh, C.C., Wu, P., Chiang, B.N., 1987. The spermicidal potency of Coca-Cola and Pepsi-Cola. Hum. Exp. Toxicol. 6, 395–396.
Kruger, T.F., Acosta, A.A., Anibal, A., Simmons, K.F., Swanson, R.J., Kathryn, F., 1987. New method of evaluation sperm morphology with predictive value for human *in vitro* fertilization. Urology 30, 248–251.
Morton, J., 1987. Pineapple. In: Morton, Julia F. (Ed.), Fruits of Warm Climates, pp. 18–28. Miami, FL.
Nwoha, P., 1992. The immobilization of all spermatozoa in vitro by bitter lemon drink and the effect of alkaline pH. Contraception 46, 537–542.
Penniston, K.L., Nakada, S.Y., Holmes, R.P., Assimos, D.G., 2008. Quantitative assessment of citric acid in lemon juice, lime juice, and commercially-available fruit juice products. J. Endourol. 22, 567–570.
Rauf, A., Uddin, G., Ali, J., 2014. Phytochemical analysis and radical scavenging profile of juices of *Citrus sinensis*, *Citrus anrantifolia* and *Citrus limonum*. Org. Med. Chem. Lett. 7, 5.
Sagay, A.S., Imade, G.E., Onwuliri, V., Short, R.V., 2009. Genital tract abnormalities among female sex workers who douche with lemon or lime juice in Nigeria. Afr. J. Reprod. Health 13, 37–45.
Schrader, S., Chapin, R.E., Clegg, E.D., Davis, R.O., Fourcroy, S.L., Katz, D.F., 1992. Laboratory methods for assessing human semen in epidemiologic studies: a consensus report. Reprod. Toxicol. 6, 275–279.
Shelton, M., 1991. Aloe vera, its chemical and therapeutic properties. Int. J. Dermatol. 30, 679–683.
Short, R.V., Scott, G., McCoombe, C., Clare, M., Eman, N., Suzanne, C., 2004. Lemon and lime juice as potent natural microbicides. In: Abstract Presented at the 15th International AIDS Conference, Bangkok, Thailand.
Umpierre, S.A., Hill, J.A., Anderson, D.J., 1985. Effect of coke on sperm motility. New Engl. J. Med. 313, 1351–1352.
World Health Organization (WHO), 1999. WHO Laboratory Manual for the Examination of Human Semen and Sperm-cervical Mucus Interaction, fourth ed. Cambridge University Press, New York, NY, USA.
Yap, K.L., Ong, C.X., Ong, K.C., 2008. Survival of SA11 rotavirus in fresh fruit juices of pineapple, papaya, and honeydew melon. J. Food Prot. 71, 1035–1037.