WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 10, 288-298 Research Article ISSN 2278 – 4357

FORMULATION AND ANTIMICROBIAL ASSAY OF RED FRUIT (PANDANUS CONOIDEUS LAM.) EXTRACT AND OIL AS ANTIBACTERIAL HAND SOAP

Agnes Anania Triavika Sahamastuti*[1], Pietradewi Hartrianti[1] and Leonny Yulita Hartiadi[1]

Pharmacy Study Program, Indonesia International Institute for Life Sciences, Indonesia
Jalan Pulomas Barat Kavling 88, East Jakarta, Indonesia.

Article Received on
08 August 2018,

Revised on 28 August 2018,
Accepted on 18 Sept. 2018

DOI: 10.20959/wjpps201810-12473

***Corresponding Author**
Agnes Anania Triavika Sahamastuti
Pharmacy Study Program, Indonesia International Institute for Life Sciences, Jalan Pulomas Barat Kavling 88, East Jakarta, Indonesia.

ABSTRACT

Diarrhea is one of the major causes of death in children, especially in developing country. Insufficient sanitation, such as unwashed hand before eating, is considered as a big causative factor. Hence, the use of antibacterial hand soap is recommended. Antimicrobial activity of red fruit (*Pandanus conoideus* Lam), a native plant from Papua, has been recently demonstrated. The aim of this research is to optimize a hand soap formulation and evaluate its antimicrobial activity. Red fruits from Papua were macerated with 96% ethanol. The ethanol extract was evaporated using rotary evaporator, followed by liquid-liquid extraction with a mixture of n-hexane and water (1:1) to obtain n-hexane fraction. Meanwhile, crude red fruit oil was obtained separately. Hand soaps were prepared by incorporating either n-hexane fraction or crude red fruit oil in carbopol-based emulsion. Antimicrobial activity of crude red fruit oil and n-hexane fraction formulas were assessed on volunteers by counting the number of microbe colonies before and after hands washing. Our results showed that hand soap of crude red fruit oil and n-hexane fraction met the evaluation criteria. While crude red fruit oil hand soap exhibited similar antimicrobial activity compared to commercially available antibacterial hand soap, n-hexane fraction hand soap showed slightly lower antimicrobial activity. This research demonstrated that red fruit oil exhibited potential to be utilized as alternative natural antibacterial substance, specifically in hand soap formulation. Therefore, this study also showed the prospect of increasing economic value of red fruits.

KEYWORDS: Red fruit, hand soap, antimicrobial, sanitation.

INTRODUCTION

Indonesia is known as one of the country that has the highest number of biodiversity in the world. Despite the abundant natural resources, only about 5% of its natural resources have been explored, exploited and utilized.[1] This situation opens new challenges for researchers to explore Indonesia's biodiversity, especially for biomedical applications. On the other side, the discovery of new diseases and the evolution of the existing diseases also increase the demand for effective, safe and economical medicines.

According to UNICEF report[2], diarrhea is the fifth leading cause of death among children worldwide. Moreover, in Indonesia not less than 1,213 cases of diarrhea have been reported in 2015, with 2.47% case fatality rate (CFR).[3] This rate is still beyond the recommended target rate, which is less than 1% CFR. There are several factors which account for the occurrence of diarrhea such as viruses, bacteria, parasites, medications, lactose intolerance and other digestive disorders.[4] In fact, diarrhea is a water-borne disease, and its occurrence mostly due to insufficient sanitation, including unclean tap water, raw and unclean food and poor eating habits such as unwashed hand before eating.[5] This is somehow in line with Indonesian eating habits using bare hands and raw vegetables as part of the tradition. Unfortunately, research has found that Indonesia's river and lake water quality is poor, as its biological oxygen demand, fecal coli and total coliform are failed to meet the standard.[6]

This problem drives the needs to provide sufficient sanitation in order to prevent the occurrence of diarrhea. One alternative solution is by reducing the diarrheal-causing bacteria on hands and raw vegetables with antibacterial hand soap to kill the water-borne bacteria. There are several antibacterial soaps which have been marketed in Indonesia, such as Lifebuoy®, Nuvo®, Dettol®, Dee-dee®, Betadine®, etc. The active substances inside these soaps also vary; for example, triclosan, active silver, thymol, chloroxylenol, and povidone iodine. One of the most widely used antibacterial agent, triclosan has been utilized over the last 30 years.[7] However, recently the effectivity of triclosan has been questioned by FDA as studies have shown that triclosan was found to be not more effective than plain soap in decreasing the number of bacteria.[8] This has led to triclosan and other 18 antibacterial soap substances banned in US.[9]

The banned of the aforementioned substances highlights the needs to develop hand soap based from medicinal plants extract. Extract-based hand soap formulations have been developed and are commercially available. Studies have shown that extract-based hand soap formulations exhibited advantages. Soap containing n-hexane leaf extract of *Morinda morindoides* had stronger antifungal activity than the basic soap.[10] Hand soap formulation of liquorice root (*Glycyrrhiza glabra*) extract was found to be more effective in reducing the number of organisms from hands compared to commercial hand soap.[11] In addition to having a better antimicrobial activity, the addition of plant extract, such as chamomile extract, can also decreases the drying effect of hand wash liquids.[12]

One of Indonesia's medicinal plants that has the potential as active ingredient for hand soap is red fruit (*Pandanus conoideus* Lam). Red fruit is a native plant from Papua which has been traditionally used as a natural alternative to treat several diseases, such as cancer, rheumatoid arthritis, arteriosclerosis, stroke, and HIV/AIDS.[13] Red fruit contains saturated and unsaturated fatty acids. It is also rich in antioxidant, such as tocopherol and β-carotene.[14] Recent study reported antimicrobial activity of red fruit extract and its fractions against pathogenic bacteria, such as *Salmonella typhi*, *Bacillus cereus*, *Streptococcus pyogenes*, *Escherichia coli* and *Klebsiella pneumoniae* in *in vitro* setting.[13] Compounds that are responsible for antibacterial activity of red fruit have not yet been determined.

This research aimed to formulate red fruit oil and red fruit n-hexane fraction into a hand soap product that exhibit antimicrobial activity. Red fruit was extracted and fractioned using organic solvent and used as an active antimicrobial ingredient in hand soap formulation. Organoleptic observation, pH and viscosity measurement were performed as an evaluation of the products. Products that met the required specification were further tested for their antimicrobial activity in volunteers in order to determine red fruit extract and red fruit crude oil's potential as an alternative natural ingredient that can act as an antimicrobial agent. Finally, both antimicrobial activity results were compared with the commercial antibacterial hand soap as the positive control standard.

MATERIALS AND METHODS

Materials

Red fruit was obtained from Manokwari, West Papua, Indonesia. N-hexane was purchased from Merck (Germany) and 96% ethanol was obtained from PT. Acidatama Karanganyar Indonesia (Indonesia). Carbopol 940 was purchased from Sinolion (USA), while glycerin and

sodium laureth sulfate were purchased from PT. Sumi Asih Oleochemical (Indonesia). Methyl and propyl paraben were purchased from Sharon Laboratories (Israel). Nutrient agar, bacteriological agar, and potato dextrose broth was purchased from Liofilchem (Italia), Oxoid, and Becon (UK), Dickinson and company (USA), respectively. Crude red fruit oil was generously provided by CV. Made Mulya Asih (Indonesia).

Methods

Red fruit extraction. Red fruit was cleaned, freeze dried, and ground into powder. Red fruit powder was macerated for 24 hours with ethanol three times. The ethanol extract was evaporated and the concentrated extract was partitioned with a mixture of n-hexane and water in 1:1 ratio. N-hexane layer was taken and evaporated to produce n-hexane fraction.

Hand soap formulation of red fruit. Hand soap formulation was prepared according to Table 1. Carbopol was dispersed in distilled water and triethanolamine was added under continuous stirring of 600 rpm at room temperature until all carbopol formed gel. Glycerin, sodium laureth sulfate, metyl paraben, and propyl paraben were added subsequently. The mixture was mixed well and crude red fruit oil or n-hexane fraction was added under stirring until homogenous. Hand soap base was prepared accordingly without the addition of the red fruit oil or fraction.

Table 1: Hand soap formulation of crude red fruit oil, hexane fraction, and hand soap base.

Composition	Crude red fruit oil formula (% w/w)	Red fruit hexane fraction formula (% w/w)	Hand soap base (% w/w)
Red fruit hexane fraction	-	1	-
Crude red fruit oil	1	-	-
Carbopol	1	1	1
Sodium laureth sulfate	4	4	4
Triethanolamine	1	1	1
Metyl paraben	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02
Glyserin	2	2	2
Distilled water	ad 100 g	ad 100 g	ad 100 g

Organoleptic observation. Physical evaluation of color and smell of hand soap was performed by visual and sensory inspection.

pH measurement. pH of hand soap was evaluated using pH meter (Ohaus, USA) that has been calibrated.

Viscosity measurement. Viscosity of hand soap was measured in triplicate using viscometer (Brookfield, USA). Spindel no.2 was used and measurement was carried out at 20 rpm.

Antimicrobial assay. Antimicrobial activity of hand soap of red fruit hexane fraction, crude red fruit oil, and hand soap base were assayed according to previous research with minor modification.[15] A commercial hand soap formulation containing chloroxylenol 0.175% w/w and salicylic acid 0.3% w/w was used as positive control. Nutrient agar (NA) and potato dextrose broth (PDB) were used as a media for bacterial and fungi growth, respectively. Bacteriological agar (15 g/L) was added into PDB (24 g/L) to allow PDB to solidify. NA (28 g/L) and PDB were sterilized prior to aliquoting each media into petri dishes. The assay was conducted on volunteers in a double-blind manner. Sterile cotton bud was soaked in 0.9% of sterile saline and was swabbed onto the palm of volunteer before and after handwashing. The cotton bud was then swabbed thoroughly onto the surface of NA or PDB petri dishes. Petri dishes containing NA was incubated for 24 hours at 37°C while petri dishes containing PDB was incubated for 48 hours at room temperature. Following incubation, colony of bacteria and fungi was counted using colony counter and the percentage of microbial decrease was calculated according to the following formula:

$$\%\ decrease\ of\ microbial\ count = \frac{Microbial\ count\ before\ handwashing - microbial\ count\ after\ handwashing}{Microbial\ count\ before\ handwashing}\ x\ 100\%$$

Statistical analysis. Percentage decrease of microbial count was analyzed using student's *t*-test and compared to commercial hand soap.

RESULTS

Extraction of red fruit. Crude oil of red fruit was obtained from CV. Made Mulya Asih with final yield percentage was about 50%, according to the distributor. Meanwhile the n-hexane fraction yield was known to be 13.19% of the dried ground red fruit.

Formulation of crude red fruit oil and n-hexane fraction hand soap. Crude red fruit oil and hexane fraction hand soap that were made was evaluated to ensure that the products meet the specification of hand soap. Crude red fruit oil and hexane fraction formula resulted in hand

soap with viscosity of 7,116 and 7,418 cps, respectively. This value is acceptable within the range of commercially available hand soap with higher viscosity.[16] pH of crude red fruit oil and hexane fraction formula were 6.29 and 6.02 which is suitable for topical application.[17] Both of the formula had a typical soap smell-like that might arise from sodium laureth sulfate. The color of crude red fruit oil was deep red and this affects the color of crude red fruit oil hand soap that visually had a reddish color (Fig. 1, Table 2). Meanwhile n-hexane fraction formula resulted a light orange colored hand soap.

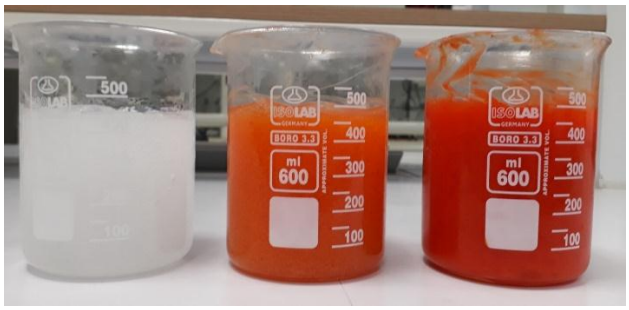

Figure 1: Results of hand soap formulas. Base of hand soap (left), n-hexane fraction formula (middle), and red oil formula (right) shown distinctive color.

Table 2: Hand soap physical evaluation of crude red fruit oil and red fruit hexane fraction.

Formula	Viscosity (cps)	pH	N	Color	Smell
Crude red fruit oil formula	7116 ± 35	6.29 ± 0.01	3	Reddish	Soap smell
Red fruit hexane fraction formula	7418 ± 115	6.02 ± 0.02	3	Light orange	Soap smell

Antimicrobial activity of crude red fruit oil and red fruit hexane fraction hand soap. To assess whether crude red fruit oil and n-hexane fraction hand soap formula have antimicrobial activity, the percentage decrease of microbial count was evaluated. Antibacterial hand soap that is available at the market was used as a positive control with a percentage decrease of microbial count of 78.56 ± 19.53% (Fig. 2). This antimicrobial activity was similar to hand soap base that did not contain red fruit fraction or crude oil with a value of 73.02 ± 4.09% (Fig. 2). The significant antimicrobial effects of the hand soap base might be contributed by sodium laureth sulfate. Sodium laureth sulfate was used in the formula as much as 4% to produce foam. Previous reports revealed that sodium laureth sulfate had antimicrobial activity.[18] Interestingly, n-hexane fraction of red fruit formula had lower antimicrobial activity with 32.06 ± 18.93% of decrease in microbial count although the decrease was not statistically significant compared to commercially available antibacterial hand soap (Fig. 2). The decline in the antimicrobial activity could be caused by incompatibility of sodium laureth

sulfate towards compounds contained in red fruit fraction. On the other hand, crude red fruit oil formula had higher antimicrobial activity compared to n-hexane fraction group with 65.07 ± 7.65% decrease of microbial count (Fig. 2). This activity was not significantly different compared to commercially available antibacterial hand soap.

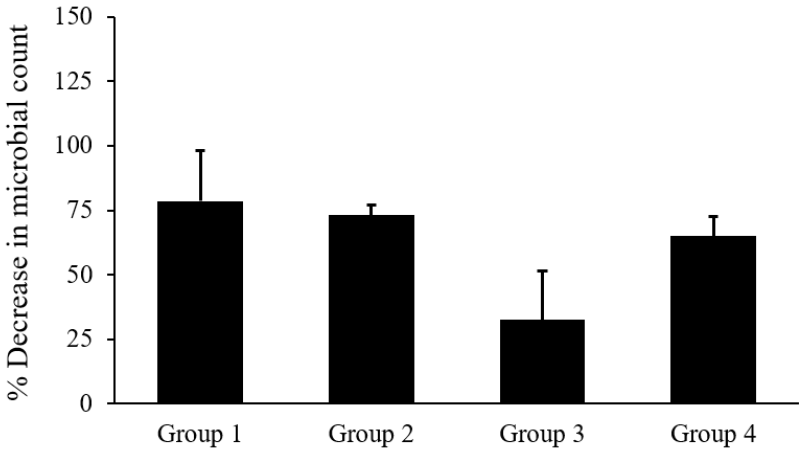

Figure 2: Antimicrobial activity of different hand soap formulations. Antimicrobial activity of commercially available antibacterial hand soap (group 1), hand soap base (group 2), n-hexane fraction of red fruit (group 3) and crude red fruit oil (group 4) formulas was evaluated. Columns represent mean ± S.E.M from n= 6 volunteers.

DISCUSSION

Thymol, chloroxylenol, triclosan, active silver, and povidone iodine are among the most commonly used active ingredients used in commercially available antibacterial hand soap. However, the effectivity of these active ingredients was shown to be equal to regular hand soap that lead to the withdrawal of these ingredients by FDA. Aside from its questionable effectivity, recent studies showed that triclosan is linked to allergic reaction and affects endocrine system.[19,20] These drawbacks of antibacterial active ingredients signify the need to find alternative active ingredients that are effective and safe. One of the sources that has potential antimicrobial activity are from natural products. Various medicinal plants, such as lemongrass, chamomile, and green tea have been shown to exhibit antimicrobial activity against bacteria.[21]

Previous research showed antimicrobial activity of red fruit against *Salmonella typhi*, *Bacillus cereus*, *Streptococcus pyogenes*, *Escherichia coli* and *Klebsiella pneumonia*[13], making red fruit as a potential candidate as active ingredient for antibacterial hand soap. In this research, red fruit was extracted, fractioned and used as the active ingredients in a

carbopol-based hand soap formulation. Physical evaluation of crude red fruit oil and n-hexane fraction formulas showed that both formulas met the specification of hand soap viscosity while the pH of the formulas is also suitable for skin. The ideal recommendation of soap pH is between 4.5-6.5 to avoid skin irritation.[17] Organoleptic observation that rely on sensory evaluation, showed that the color and smell of both formulas were acceptable. The organoleptic and pH evaluation of the hand soap formula are important as these parameters will affect whether the consumers will accept the product once the product is available to the market.

The antimicrobial activity of n-hexane fraction and crude red fruit oil formulas was assessed on volunteers by comparing the number of microbial colony before and after washing hands with hand soap. Hand soap that is available on the market has high antimicrobial effect as it causes a decline in microbial count close to 80%. Interestingly, the hand soap base that did not contain n-hexane fraction or crude red fruit oil exhibited a comparable antimicrobial effect to commercially available hand soap. This is likely due to the presence of sodium laureth sulfate, a surfactant that has been reported to have antimicrobial activity.[18] Interestingly, the addition of n-hexane fraction of red fruit to the hand soap base caused a reduction in antibacterial activity. Compounds contained in the n-hexane fraction possibly interact with sodium laureth sulfate that leads to a reduction in the antimicrobial effectivity. When the antimicrobial activity of n-hexane fraction formula was compared to crude red fruit oil formula, the crude red fruit oil formula had higher microbial activity. This result might be explained by different hydrophobicity of n-hexane and crude oil extract. Extraction using n-hexane will draw out more hydrophobic compounds compare to crude oil. Therefore, more sodium laureth sulfate needed to stabilize n-hexane extract in hand soap formulation. Due to less sodium laureth sulfate existed to solubilize bacterial cell wall, the antibacterial activity of n-hexane fraction was decreased.

Previous research by Indrawati S[13] showed that red fruit inhibits the growth of various of pathogenic bacteria, demonstrating that red fruit contains compounds that have antimicrobial activity. The research also showed that water fraction of red fruit gave the largest inhibition zone. On the contrary, our result showed that crude red fruit oil that has lipophilic properties had a better antimicrobial activity. This differences may also be caused by the variability of the compounds contained in the red fruit that was obtained by Indrawati S[13] and the author. It has been shown that the compounds present in natural products are influenced by area in

which the products are grown. Different growing areas have distinct nutrients composition in the soil. Since these nutrients are utilized for plant metabolism to produce secondary metabolite compounds in plants, the same natural products that are grown in different areas may have distinct composition or type of compounds in the products.[22]

Differences in antimicrobial activity between n-hexane and crude red fruit oil may indicate that compounds that are responsible for the antibacterial activity of red fruit belong to class of compounds that have high solubility in oil. Although the antibacterial activity of red fruit has been demonstrated, the compounds that are responsible for the antibacterial activity needs to be elucidated. Our result narrows down the possibility of the compounds that mediate the antibacterial action of red fruit, which likely to be present in the crude oil.

CONCLUSION

In the present study, hand soap formulation of n-hexane fraction and crude red fruit oil were made. Both of the formulas fulfilled the physical requirements of viscosity and pH for topical use. However, both crude red fruit oil and n-hexane fraction formulas exhibited similar reduction in microbial count compared to SLS base only. This might come from the incompatibility between SLS and the extract, since a lot of SLS molecules stabilized the oil base of red fruit instead of disrupting the bacterial cell walls. Therefore, further studies needed to determine the best hand soap base which can fully exhibit the antibacterial activity of red fruit. Nevertheless, crude red fruit oil hand soap showed comparable antibacterial activity to the commercial antibacterial hand soap. Hence, this result suggests that the crude red fruit oil formula is suitable for commercialization. Despite the potential for commercialization, further studies are needed to determine and isolate compounds that are responsible for antimicrobial activity of red fruit. The isolation of the antimicrobial compound from red fruit would serve as an alternative active ingredient for hand soap and fill the gap following the banned of synthetic active ingredients by FDA.

ACKNOWLEDGEMENT

Authors gratefully acknowledge Indonesia International Institute for Life Sciences, Indonesia, for the internal research funding support. We also thank B. Santoso for providing red fruit from Papua, CV. Made Mulya Asih for providing crude red fruit oil, and all the volunteers who were involved in the antibacterial assay of hand soap.

REFERENCES

1. Beers, H.W., Indonesia: Resources and their technological development, Kentucky; University Press of Kentucky, 2015.
2. Committing to Child Survival: A Promise Renewed: Progress Report 2015, Compilation prepared by Unicef, https://www.unicef.org/publications/index_83078.html.
3. Kepmenkes, R., Profil kesehatan Indonesia, Jakarta; Kementrian Kesehatan Republik Indonesia, 2015.
4. Guandalini, S. and H. Vaziri. Diarrhea: diagnostic and therapeutic advances. Springer Science & Business Media, 2010.
5. Hays, J [Internet]. Hepatitis, Diarrhea and Water-Borne Diseases; c2018 [cited 2018 Sept 9]. Available from: http://factsanddetails.com/world/cat57/sub381/item2147.html.
6. Bank, A.D., Indonesia: Country Water Assessment, Philippines; Metro Manilla, 2016.
7. Bhargava, H. and P.A. Leonard. Triclosan: applications and safety. American journal of infection control, 1996; 24(3): 209-218.
8. Aiello, A.E., E.L. Larson, and S.B. Levy. Consumer antibacterial soaps: effective or just risky? Clinical Infectious Diseases, 2007; 45(Supplement_2): S137-S147.
9. Food and Drug Administration [Internet]. Safety and effectiveness of consumer antiseptics; topical antimicrobial drug products for over-the-counter human use. Health and Human Services. Final Rule. Federal Register; c2018 [cited 2018 August 1]. Available from: https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Reports/EconomicAnalyses/UCM510753.pdf
10. Touré, A., et al. In vitro antifungal activity of the soap formulation of the hexane leaf extract of morinda morindoides (morinda; rubiaceae). Tropical Journal of Pharmaceutical Research, 2010; 9(3).
11. Patel, A., et al. Formulation and evaluation of Herbal Hand wash containing Ethanolic extract of Glycyrrhiza glabra root extract. Research Journal of Pharmacy and Technology, 2017; 10(1): 55.
12. Wasilewski, T., A. Seweryn, and M. Krajewski. Improvement in the safety of use of hand dishwashing liquids through the addition of hydrophobic plant extracts. Journal of surfactants and detergents, 2016; 19(6): 1315-1326.
13. Indrawati, I. Sensitivity of pathogenic bacteria to buah merah (Pandanus conoideus Lam.). AIP Conference Proceedings, 2016: 1744(1).

14. Rohman, A., et al. Antioxidant activity, total phenolic, and total flavaonoid of extracts and fractions of red fruit (Pandanus conoideus Lam). International Food Research Journal, 2010; 17(1): 97-106.
15. Radji, M., H. Suryadi, and D. Ariyanti. Uji efektivitas antimikroba beberapa merek dagang pembersih tangan antiseptik. Pharmaceutical Sciences and Research (PSR), 2012: 4(1): 1-6.
16. Macgilp, N.A., et al. US Patent, US5296158A, 1994.
17. Ali, S.M. and G. Yosipovitch. Skin pH: from basic science to basic skin care. Acta dermato-venereologica, 2013; 93(3): 261-269.
18. Orafidiya, L.O., et al. The formulation of an effective topical antibacterial product containing Ocimum gratissimum leaf essential oil. International Journal of Pharmaceutics, 2001; 224(1-2): 177-183.
19. Wong, C. S. M., & Beck, M. H. Allergic contact dermatitis from triclosan in antibacterial handwashes. Contact Dermatitis: Short Communication, 2001; 45(5): 307-307.
20. Hinther, A., Bromba, C. M., Wulff, J. E., & Helbing, C. C. Effects of triclocarban, triclosan, and methyl triclosan on thyroid hormone action and stress in frog and mammalian culture systems. Environmental science & technology, 2011; 45(12): 5395-5402.
21. Hammer, K.A., C.F. Carson, and T.V. Riley. Antimicrobial activity of essential oils and other plant extracts. Journal of applied microbiology, 1999; 86(6): 985-990.
22. Ormeño, E. and C. Fernandez. Effect of soil nutrient on production and diversity of volatile terpenoids from plants. Current bioactive compounds, 2012; 8(1): 71-79.