# USING *SENECIO LYRATIPARTITUS* AS A HAND DISINFECTANT AFTER ANAL ABLUTION

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## ABSTRACT

*Water kept in pans, pots and buckets placed in pit-latrine facilities of mostly Muslim communities and some Christian homes practicing anal ablution after defecation is contaminated with enteropathogenic diarrhoea causing pathogens. Ablution water samples from latrines of households practicing anal ablution in two cities, Kisumu in Kenya and Musoma in Tanzania, both located on the eastern shores of Lake Victoria in East Africa were found carrying Escherichia coli, Salmonella sp. and Klebsiella sp. In homes visited, no facilities for hand washing with clean water and soap after defecation and anal ablution were in place. This practice is definitely responsible for contaminating drinking water, food and utensils. Hands used for anal ablution can be disinfected with extracts of Senecio lyratipartitus plant also known as Senecio lyratus. An initial Kirby Bauer disk method performed with the extract to show activity using the methanol extract showed zones of inhibition of 15mm for E. coli, 14mm for Salmonella sp., 14mm for Enterobacter and 13 mm for Klebsiella. The agar well diffusion method was used to test the activity of n-hexane, ethyl acetate and chloroform extracts of dry leaves of S. lyratus were able to inhibit growth of E. coli, Salmonella sp., Klebsiella sp and Enterobacter aerogenes. The minimum inhibitory concentrations (MIC) obtained for crude methanol and crude ethyl acetate extracts were 31.5mg/mL for E. coli, 3.9mg/mL for Salmonella sp., 31.25mg/mL for Klebsiella sp. and 31.25mg/mL for E. aerogenes. For the n-hexane extract, the MIC were 7.81mg/mL for E.coli, 62.50mg/mL for Salmonella sp., 31.25mg/mL for Klebsiella sp. and 7.81mg/mL for Enterobacter sp. The MIC for crude chloroform extracts against E. coli, Salmonella sp., Klebsiella sp. and Enterobacter sp. (1.95mg/mL) is significant when compared with pure chloramphenical (7µg/mL) and compares favourably with pure that of synthetic biocides benzyldimethylhexylammonium chloride (0.98mg/mL) and benzyldimethyl hexadecylammonium chloride (0.98mg/mL) found in some hand disinfectants. S.lyratus grows widely in most parts of East Africa, and is currently not exploited for any commercial use. Since E. coli is the indicator organism for water quality, and S.lyratus extracts are showing activity against it the plant has potential for development as a hand sanitizer. Suitable formulation incorporating S.lyratus extract as a hand sanitizer in suitable packages are being worked out.*

Key words: ANAL ABLUTION, CONTAMINATED FOOD AND WATER, DIARRHOEA, ENTERIC PATHOGENS, HAND DISINFECTANT *SENECIO LYRATUS* ACTIVITY

## INTRODUCTION

It has been estimated that diarrhoea is responsible for over 1.8 million deaths of children in developing countries each year worldwide. The figure is higher than the combined mortality due to HIV/AIDS and malaria (Proctor and Gamble, 2011). Diarrhoea is the leading cause of morbidity and mortality in children in developing countries (WHO, 1964). Globally, there are about two billion cases of diarrhoea every year (WHO, 2011).

The Bill & Melinda Gates foundation and other organization such as World Health Organization (WHO) are supporting global efforts to find solutions to curb this scourge. It is generally recognized that major contributing factor to diarrhoea is the presence of Rotaviruses, *Shigellae,* enterogenic *Escherichia coli, Vibrio cholera, Campylobacter jejuni,* enteropathogenic *E. coli Salmonella* species and other viral agents (Vesikari *et. al.,* 1994; Chin, 2000) in drinking water and food (WHO, 2011). Many government health departments worldwide have advocated boiling drinking water and this has saved lives. In recent years Proctor & Gamble in collaboration with Centres for Disease Control and Prevention of the USA have developed the PUR technology to purify contaminated water (Rovner, 2011). The contents of PUR packets are: iron sulfate, a coagulant and flocculent which clarifies water and a chlorine releasing agent which removes enteric pathogens and worms. About 300 million packets in 63 countries have been released in the execution of an initiative called Children and Safe Drinking Water (CSDW) (Rovner, 2011).

While this is going on our research has found that one of the factors responsible for contaminating food and drinking water is the practice of anal ablution in the management of wiping or removing residual stool at anal endings after defecation. Three factors were observed. The first was that bare hands come in direct contact with stool in the process of washing it away. Secondly the water used for ablution was replete with enteric pathogens. Thirdly very few individuals washed their hands with clean water and soap after anal ablution. These settings were ideal for perpetuating contamination of hands and subsequently contamination of drinking water and food.

The management of anal ablution is certainly one of the elements of poor hygiene that has been overlooked in public health campaigns. Yet it can have disastrous effects if managed poorly. In an outbreak of cholera many people can be decimated without knowing the major pathways through which food and drinking water are contaminated.

An additional measure would be to introduce hand disinfectants which can be applied on hands after anal ablution or after defecation. There are already commercial hand sanitizers currently placed in hallways of hospitals. These are expensive and may not be affordable in poor countries and in rural areas. A natural hand sanitizer could come from extracts of *Senecio lyratipartitus. S. lyratipartitus* grows widely in East Africa. The plant is not poisonous and it can be easily processed into a syrup from which a hand lotion or gel can be formulated with a mineral oil. An antibacterial agent was reported to be one of the constituents of *S. lyratipartitus (*Kaberia, 1999*).*

## METHODS

Collection of *S.lyratipartitus* leaves and stems

Fresh leaves and stems of *S. lyratipartitus* were collected from the eastern periphery of Sotik town in Sotik Constituency of the Rift-Valley province in Kenya. The plant specimen was identical to that which was collected at Arusha in Tanzania by the main author in 1984 (Maradufu, 1984). The dried plant parts weighing 2.32 Kg were pulverized in a blender and soaked in a mixture of methanol: water (9:1) and left to stand for a day. Upon filtration and concentration in *vacuo,* the filtrate resulted in a green syrup weighing 152g (6.6%) of dry weight. The syrup was then successively extracted with n-hexane, ethyl acetate, chloroform, n-butanol and water. Upon removing residual solvents in a high vacuum pump, hexane extracts weighed 2.7g (0.12%) of dry weight; ethyl acetate extract weighed 1.4g (0.06%) of dry weight; chloroform extract weighed 18.7g (0.8%) of dry weight and the butanol extract weighed 21.3 g (0.9%) of dry weight. The remaining water fraction was saved. Small quantities of the crude extracts were then bioassayed for activity against *E.coli, Salmonella* sp. and *Klebsiella* sp.

Collection and Analysis of Water Samples Used for Anal Ablution

Anal ablution water kept in pots, pans and buckets found in latrine and toilet facilities from households in Muslim and non-Muslim communities of Kisumu city in Kenya and at Musoma Municipality in Tanzania were collected in plastic ampoules of 100 mL capacity and kept in a cool box and transported to the laboratory for refrigeration and analysis. Both towns are located on the eastern shores of Lake Victoria.

Bacteriological water analysis was carried out to confirm the presence of faecal coliform bacteria. The standard coliform count was carried out according to Atlas *et al* (1995). It involved three stage procedure; the first stage being the presumptive test to determine the Most Probable Number of coliform bacteria. The second stage is to confirm the presence of faecal coliform where by *E.coli* is the indicator for faecal contamination of water because it is found in the large intestine of virtually all people. The third stage completes the analysis with further biochemical tests: the IMViC (Indole, Methyl Red and Voges Proskauer and Citrate) tests.

The Presumptive Test to Determine the Most Probable Number of Coliform Bacteria

35.60 g of lauryl tryptose broth media was diluted in 1000mL distilled water. The solution was then dispensed into tubes containing inverted Durham tubes and autoclaved at 121$^{o}$C for 15 minutes. After sterilization the tubes were left at room temperature to cool before using.

Three double-strength lauryl tryptose broth and six single-strength lauryl tryptose broth tubes were set for each sample to be tested. The tubes were labelled according to the amount of water to be dispensed to it: 10mL, 1mL and 0.1mL. The bottle of water to be tested was mixed thoroughly. 10mL of water was transferred into each of the three double strength lauryl tryptose broth. 1mL of water was transferred into each of the three middle set of tubes, and 0.1mL to each of the last single-strength lauryl tryptose broth tubes. The procedure was repeated to all water samples. The tubes were then incubated at 35$^{o}$C-37$^{o}$C for 2 days. The tubes were examined and the number of tubes in each set that had more than 10% gas was recorded and the Most Probable Number (MPN) of coliform bacteria was determined (Atlas *et. al.,* 1995).

The Confirmed Test of Faecal coliform

37.46g of Eosin Methylene Blue (EMB) agar was diluted in 1000mL of distilled water. The solution was boiled to dissolve completely and autoclaved at 121$^{0}$ C for 15 minutes. After sterilization, it was cooled to about 50$^{0}$C and then poured onto petri dishes and left to solidify. One positive of lauryl tryptose broth tube from the presumptive test was selected from each of the strengths and streaked on the EMB plate. The quadrant streak method was used. The plates were incubated at 37$^{0}$C for 24 hours. The plates were examined for typical coliform colonies. The green metallic sheen colonies indicated the presence of *E.coli.*

The Completed Test of Faecal coliform

A single colony was selected and inoculated into Durham lauryl tryptose broth tubes and on nutrient agar slants. The tubes were incubated at 37 $^{0}$C for 24 hours. The Durham lauryl tryptose broth tubes were examined for gas formation and acid fermentation and the nutrient agar slant colonies were used for gram stain and biochemical tests; the IMViC tests.

The IMVIC Tests

*Indole test*

3g of tryptone broth media was diluted in 200mL distilled water and mixed thoroughly to dissolve. The solution was then dispensed into tubes and autoclaved at 121$^{0}$C for 15 minutes. After sterilization, the tubes were left at room temperature to cool before using. The tubes were inoculated with typical colonies from nutrient agar slants. The tubes were incubated at 35-37$^{0}$C for 245 hours. After incubation, 10 drops of Kovac´s reagent were added directly to the culture tubes.

*Methyl Red Test*

5.1g of MR-VP medium was diluted in 300mL distilled water and dispensed into tubes. The tubes were then autoclaved at 121$^0$C for 15 minutes. After sterilization the tubes were left at room temperature to cool before using. The MR-VP medium tubes were incubated with typical colonies from nutrient agar slants. The tubes were incubated at 35-37$^0$C for 24 hours. A few drops of methyl red solution were added to the culture tubes.

*Voges-proskauer test*

5.1g of MR-VP medium was diluted in 300 mL distilled water and dispensed into tubes. The tubes were then autoclaved at 121$^0$C for 15 minutes. After sterilization the tubes were left at room temperature to cool before using. The MR-VP medium tubes were inoculated with typical colonies from nutrient agar slants. The tubes were incubated at 35-37$^0$C for 24 hrs. After incubation, 1 mL of culture was aseptically transferred to a clean test tube. To 1mL of culture 0.5mL Barrits reagent A and 0.5 mL Barrits reagent were added and shaken vigorously for 30 seconds. The tubes were observed for gradual formation of a pink to red colour.

*Citrate utilization*

4.9g of Simmon's citrate medium was diluted in 200mL distilled water. The solution was boiled to dissolve completely and autoclaved at 121$^o$C for 15 minutes. After sterilization it was dispensed into tubes and placed into a slant position to solidify. The slants of Simmon's citrate were inoculated with colonies from the culture. The tubes were incubated at 37$^o$C for 24 hours. After incubation the colour of the tubes were observed.

Bioassay Procedures Against Microorganisms

Bioassay tests of crude *S.lyratipartitus* extracts (methanol, ethyl acetate, n-hexane, chloroform, n-butanol and aqueous) was determined by well-diffusion assay and Kirby Bauer disk diffusion assay. The bacteria used were obtained from the anal ablution water samples. The well diffusion was conducted in Mueller Hinton agar media overlaid with 2ml of 5% soft agar media which contained 100µl microbial suspension which was measured by spectrophotometer to 0.1 absorbance at 450nm of an overnight culture of the selected bacteria, 6 mm in diameter wells were cut into these agar plates and 100µl of the 500mg/mL extracts and biocides (benzyldimethylhexylammonium chloride and benzydimethylhexadecylammonium chloride) were placed into each well. Chloramphenicol and gentamicin disks were used as positive controls. The plates were incubated for 24 h at 37°C and subsequently examined for zones of inhibition.

Minimum Inhibitory Concetration (MIC)

The MIC values of *S.lyratipartitus* extracts and biocides (benzydimethylhexadecylammonium chloride and benzyldimethylhexylammonium chloride were determined using two-fold broth tube dilution to prepare extract concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.81, 3.91, 1.95 0.98 and 0.48 mg/mL. The extracts concentrations were prepared by dissolving in dimethylsulfoxide or methanol. 100µl of each extract was added to test tubes containing 10mL of sterile Nutrient broth. The tubes were then inoculated with 100µl of microbial suspension which was measured by spectrophotometer to 0.1 absorbance at 450 nm and incubated at 37 °C for 24 h. The MIC value was determined macroscopically after 24 hrs. of incubation.

## RESULTS AND DISCUSSION

In developed countries, toilet tissues are used to wipe anal endings after defecation. Usually, this exercise is followed by hand washing with soap and clean water. In many developing countries, the majority of the people cannot afford buying toilet paper and therefore resort to using waste paper, or plant leaves, grass and other cellulosic materials to wipe their anal endings. In many of these poor countries, provision of water and soap for hand washing in toilets does not exist. In fact, toilets or latrines are the most neglected places. These facilities are poorly constructed and lack adequate ventilation and lighting. In Muslim communities, anal endings are washed with water with bare hands. Many people of other faiths in Africa and Asia have adopted this Muslim tradition.

Considering that 20% of the world population is made up of Muslims (CIA, 2012) and assuming that 10% of these are privileged to practice hand washing with soap and clean water after anal ablution there remains the 90% or about 1.2 billion Muslims who do not have access to clean water and soap after anal ablution. If to this figure are added those who are not Muslims in India, Philippines, Indonesia, Africa, and other places, then close to 3 billion people practice anal ablution without recourse to clean water and soap.

Of the 100 households visited in Kisumu in Kenya and at Musoma in Tanzania, both towns located on the eastern shores of Lake Victoria, none was found to wash hands with soap and clean water after anal ablution. 92% of ablution water collected and assayed carried *E.coli, Salmonella sp. and Klebsiella sp.* (Figure 1).

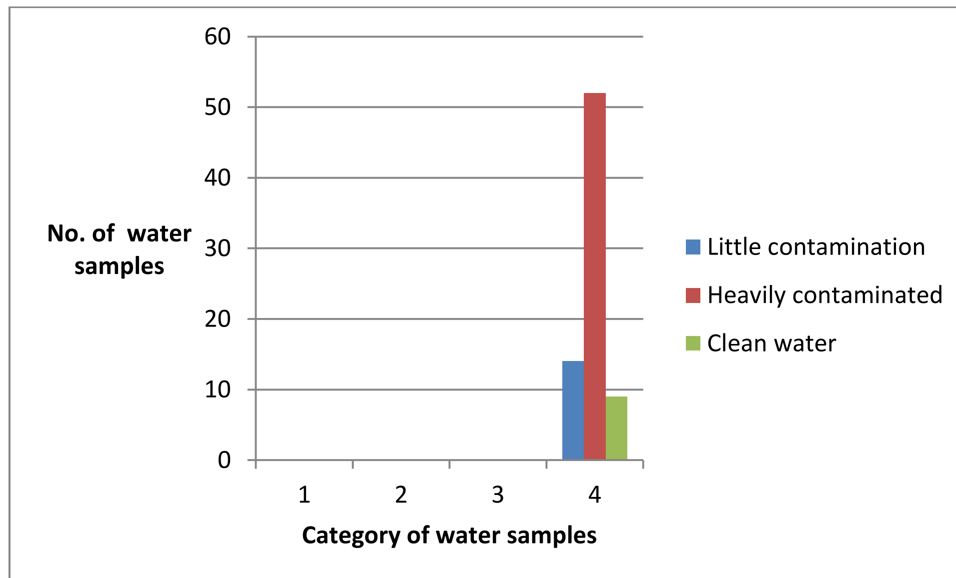


Fig.1: Categorization of the level of contamination for water samples by Presumptive test collected from two towns.

For lack of facilities other pathogens responsible for diarrhoea such as viruses could not be assayed but were assumed to be present in varying proportions. Pathogenic enterobacteria were certainly on the hands of those practicing anal ablution. This was certainly the major route through which food and drinking water were contaminated causing recurrence of diarrhoea in populations. This report is the first of its kind to reveal the connection between the practice of poor management of anal ablution and the prevalence of diarrhoea in poor populations.

Table 1. shows the distribution of deaths annually due to diarrhoeal diseases worldwide (WHO 2012). There is certainly a correlation between deaths due to diarrhoea and populations believed to practice anal ablution. According to this report the whole of Africa, with a population of 1,070 million, experiences 760,037 deaths annually predominantly Muslim, while the Eastern Mediterranean, with a population of 273 million, registers 567,111 deaths annually.

Table 1: Distribution of Deaths annually due to diarrhoeal diseases worldwide (WHO 2012).

| WORLD REGION | ESTIMATES OF DEATHS | % OF WORLD TOTAL | TOTAL POPULATION (IN MILLION) |
|---|---|---|---|
| Africa | 760,037 | 40 | 1,070 |
| Americas (Including Western Europe) | 63,403 | 3 | 962 |

| | | | |
|---|---|---|---|
| Eastern Mediterranean | 567,111 | 25 | 273 |
| South East Asia | 681,457 | 26 | 1,585 |
| Western Pacific | 125,644 | 6 | 644 |

Bioassay tests of crude solvent extracts of *Senecio lyratus* previously known as *Senecio lyratipartitus* (Agnew 1994) against colonies of *E.coli, Salmonella sp. Enterobacter sp.* and *klebsiella* sp. shows clearly that *S. lyratus* is active against these organisms. As shown in Table 2. extremely sensitive zones of inhibition were observed for gentamicin against *E.coli* (34 ± 1.35), *Salmonella* sp. (32 ±0.47), *Klebsiella* sp.(31 ± 1.08), *Enterobacter* (33± 1.39); chloramphenicol against E.coli (34 ± 0.40), *Salmonella* sp. (33 ±1.19), *Klebsiella* sp.(32 ± 1.15), *Enterobacte*r (34± 0.40); benzydimethylhexadecylammonium chloride against E.coli (20 ± 0.41); benzyldimethylhexylammonium chloride against E.coli (22 ± 0.44) and Enterobacter ( 20 ± 0.57). Very Significant zones of inhibition were observed for methanol extract against all organisms and ethyl acetate extract against *E.coli* and *Salmonella* sp. while chloroform extract was very sensitive against *E.coli, Enterobacter* and *Klebsiella.* The aqueous extract showed sensitivity against *Salmonella* and *Klebsiella* sp. The aqueous extract showed no sensitivity against *E.coli* and *Enterobacter* sp. From the results above the most sensitive extract was chloroform extract followed by methanol then ethyl acetate and hexane extracts. The chloroform and methanol extracts are suitable candidates for preparation of a hand sanitizer to be used after anal ablution. Other investigators working on the same plant reported strong activity of the plant extracts against *E.coli* (Kaberia 1999).

Table 2. Zone of inhibition in mm (± SE) of 500 mg/ml of various extracts of *Senecio lyratus(lyratipartitus)* and control drugs, and selected biocides against selected bacteria.

| Organisms / Antimicrobials | *Escherichia coli* | *Salmonella* sp. | *Enterobacter* Sp. | *Klebsiella sp.* |
|---|---|---|---|---|
| *Senecio lyratus* Methanol Extract | 17 ± 0.40 | 19 ± 0.58 | 16 ± 1.15 | 15 ± 0.57 |
| *Senecio lyratus* Ethyl Acetate Extract | 16 ± 0.37 | 17 ± 0.29 | 14 ± 0.59 | 14 ± 0.52 |
| *Senecio lyratus* Hexane Extract | 15 ± 0.20 | 17 ± 0.36 | 14 ± 0.65 | 14 ± 0.39 |
| *Senecio lyratus* Chloroform Extract | 17 ± 0.49 | 20 ± 0.52 | 19 ± 0.55 | 18 ± 0.90 |
| *Senecio lyratus* Butanol Extract | 11 ± 0.51 | 12 ± 0.41 | 13 ± 0.35 | 11 ± 0.36 |
| *Senecio lyratus* Aqueous Extract | 7 ± 0.36 | 12 ± 0.98 | 8 ± 0.89 | 12 ± 0.62 |
| Benzyldimethylhexadecylammonium chloride | 20 ± 0.41 | 19 ± 0.49 | 18 ± 0.36 | 17 ± 0.24 |
| Benzyldimethylhexylammonium chloride | 22 ± 0.44 | 18 ± 0.69 | 20 ± 0.57 | 19 ± 0.32 |
| Gentamycin | 34 ± 1.35 | 32 ± 0.47 | 33 ± 1.39 | 31 ± 1.08 |
| Chloramphenicol | 34 ± 0.40 | 33 ± 1.19 | 34 ± 0.40 | 32 ± 1.15 |

Key: ± SE - Standard Error

According to Babu *et al* (2011) diameter of ≤ 8 mm indicates that organisms are not sensitive to the material tested. Diameter of 9-14 mm indicates that the organisms are sensitive to the material tested. Diameter of 15-19 mm indicates that the organisms are very sensitive to the material test. Diameter of ≥ 20 mm indicates that the organisms are extremely sensitive to the material tested.

Results for minimum inhibitory concentration (MIC) are shown in table 3. It can be seen that extracts of *S. lyratus* are active and compare favourably with synthetic biocides: benzyldimethylhexylammonium chloride

and benzyldimethylhexadecylammonium chloride. Purification of the crude plant extracts can improve the activity. Alternatively for reasons of costs the crude extracts can be formulated into gels and directly applied for hand sanitization. This will then be affordable for low income communities.

Table 3: Minimum Inhibitory Concentration (MIC) in mg/mL of various extracts of *Senecio lyratus (lyratipartitus)* and antimicrobial agents against selected bacteria

| Antimicrobials \ Organisms | *Escherichia coli* | *Salmonella* sp. | *Enterobacter* sp. | *Klebsiella sp.* |
|---|---|---|---|---|
| *Senecio lyratus* Methanol Extract | 15.63 | 1.95 | 1.95 | 31.25 |
| *Senecio lyratus* Ethyl Acetate Extract | 15.63 | 1.95 | 15.63 | 15.63 |
| *Senecio lyratus* Hexane Extract | 3.91 | 15.63 | 15.63 | 3.91 |
| *Senecio lyratus* Chloroform Extract | 1.95 | 1.95 | 1.95 | 1.95 |
| *Senecio lyratus* Butanol Extract | 15.63 | 7.81 | 15.63 | 15.63 |
| *Senecio lyratus* Aqueous Extract | 62.5 | 31.25 | 62.5 | 31.25 |
| Benzyldimethylhexadecylammonium chloride | 0.98 | 0.98 | 0.98 | 0.98 |
| Benzyldimethylhexylammonium chloride | 0.98 | 0.98 | 0.98 | 0.98 |
| Chloramphenicol | 7 µg/mL | 7 µg/mL | 7µg/mL | 7 µg/mL |
| Gentamicin | 20 µg/mL | 20µ/mL | 20 µg/mL | 20 µ/mL |

The lowest MIC values (7µg/mL) for chloramphenicol for all organisms, (20 ug/mL) for gentamicin for all organisms, (0.98 mg/mL) were the same for the biocides benzyldimethylhexadecylammonium chloride and benzyldimethylhexylammonium chloride. The *Senecio lyratipartitus* chloroform extract showed the lowest MIC value (1.95mg/mL) for all the organisms. The methanol extract had an MIC of 1.95 mg/mL against *Salmonella* sp. and *Enterobacter* sp. but 15.63mg/mL against *E.coli* and 31.25mg/mL against *Klebsiella*. The ethyl acetate extract showed MIC of 15.63mg/mL against *Klebsiella* and *Enterobacter*, and *E.coli* but 1.95mg/mL against *Salmonella*. The results were the same for the n-butanol extract. The aqueous extract showed the least activity. These results show that the chloroform extract was the most active at the lowest concentration and should therefore be considered in the formulation of a dermatological complication hand sanitizer.

*S.lyratus* leaves and stems are eaten by goats and other ruminants. It is therefore not toxic. Crude water extracts of *S.lyratus* have been applied on scalps of young children with perfect healing outcomes (Maradufu 1984). Developing *S.lyratus* as a hand disinfectant would be cheaper than other. The plant is not commercially exploited for any product. The plant grows profusely during the rainy season. Commercial hand sanitizers are available, some effective as a natural biocide and of these contain up to 62% ethanol, and are effective against bacteria and a large proportion of viruses (Rotter 1999). However, continued use of alcohol based hand sanitizers may lead to skin irritation, cracking or flaking and skin defatting (Baker 2012, Larson 2006). Isopropyl alcohol based hand sanitizers are even more effective (White et al 2003). Low concentrations of benzalkonium chlorides such as benzyldiemethylhexylammonium chloride and benzydimethylhexadecylammonium chloride used in this experiment are also ingredients of some commercial hand sanitizers but besides being effective against bacteria, there are indications that they are hazardous, and are rated as having level 7 in the Cosmetics Safety Database.

Considering the above *S.lyratus* stands a chance of being developed as a safe and renewable hand disinfectant. It can be used alone or in a mixture with low content of alcohol (ethanol) to maximize sanitization outcomes.

## CONCLUSION

This is the first report which shows that there is a link between the practice of anal ablution and contamination of water and food. Hands that are used for anal ablution carry enteric organisms which finally land in drinking water and food. Application of a hand sanitizer would drastically cut down levels of enteric pathogens reaching food and drinking water. Extracts of *Senecio lyratus* have the potential to be developed as non toxic, affordable, renewable and environmentally friendly biocides in the control of common organisms responsible for diarrhoea. This would help to save lives.

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