Hong Kong Student Science Project Competition 2023

Template of Extended Abstract (Investigation)
(Word Limit: 1,600 words, Pages: 3 pages only)

Team Number: [SBBC292]

Project Title: Can the antimicrobial properties of lichens be potentially utilized to create hand-sanitizers?

Project Type: Investigation (B)

To our best knowledge, there are no similar works in the field

I. Background

From applying lichen extracts for wound dressings by the Ancient Greeks, to relieving respiratory infections in East Asian countries, and even treating skin disorders such as eczema in Europe, lichens - a symbiotic cross between a fungi and cyanobacteria - contain many properties that are unique only to them. However, the specific reason behind this is still unknown, and although it is associated with the literary values of metabolite content, e.g. usnic acid which is in most lichen species, the number varies between 0.22~6.49% of their dry weight.

II. Objectives

Questioning the credibility of certain claims, we hope to investigate whether it is the lichen or the solvent through which its metabolites are extracted from which truly exhibits these properties, namely antibacterial. Moreover, we hope to see whether it could suit as an appropriate alternative to present commercially-sold hand sanitisers, as lichens are replenishable and therefore its production would be more environmentally friendly, as alcohol production requires the use of nonrenewable resources.

III. Hypothesis

The lichen extracts will demonstrate greater antimicrobial activity (measured through a greater zone of inhibition upon *E.coli* bacteria) than their pure-solvent counterparts. The extensiveness of the growth of bacteria can indicate the presence of an antimicrobial agent, and allow quantitative comparison between the different solution.

IV. Methodology

The following materials were used: Electronic balance ±0.001g (x1); Pestle and mortar ; Plastic weighing boats (x45); 10±0.5cm^3 Measuring cylinders (x3); Test tubes with bung (x45); Funnel (x3); Water bath at 40±0.1℃ (x1); Test tube rack (x3); Stock E. coli bacteria freshly prepared the night before; Inoculation loop (x5); Agar plates (x45); Sterile swabs (x45); Disposable test tubes (x5); Forceps (x3); Diffusion discs made from Whatman No.1 filter paper (x50); Micropipette 500.0±0.1µl (x1); Lighter (x3); Disinfectant solution (Dettol); Markers (x5); Vernier caliper (x1); Gloves (x10 pairs); 5ml syringe (x10); 100cm^3 and 200cm^3 beakers (x10 each); Heatproof mat; 2℃ Refrigeration; lichen (Lichen A = *Parmotrema perlatum*; Lichen B = *Cladonia cornuta*; Lichen C = *Cladia aggregata*) and the type of solvent used for extraction (for one extract respectively: Hexane 5.0±0.5cm^3; Propanol 5.0±0.5cm^3; Mix of 2.5±0.5cm^3 of Hexane and 2.5±0.5cm^3 of Propanol). Control variables were: Strain of E.coli bacteria used; Temperature and environment the agar plates were placed in; Procedure of the lichen extraction (controlling time and temperature); Volume of extract used; Type of diffusion disc used; Mass of lichen used; Volume of solvent to make the extracts; Surface area of the lichen. This was all to ensure a fair test to compare the independent values (varying solutions tested) were the only affecting factors upon the dependent variables. The methodology was split into three parts: lichen preparation, extraction of the secondary metabolites and the creation of solvent extracts, and antimicrobial testing on agar plates with subcultured *E.coli* bacteria.

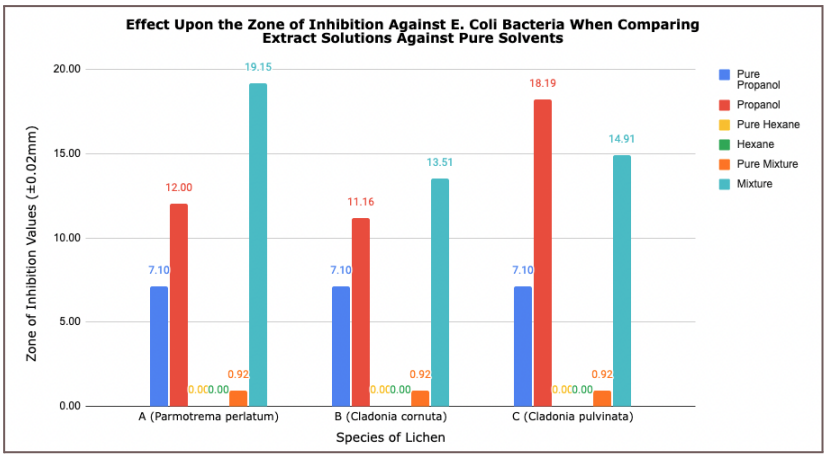
V. Results

Lichen A	[D.V.] Zone of Inhibition (±0.02mm)							
[I.V.] Solvent Added (500 ±1.25µl)	Trial 1		Trial 2		Trial 3		Trial 4	
Hexane (H)	0.00	0.00	0.00	0.00	0.00	N/A	N/A	N/A
Propanol (P)	10.99	11.04	11.40	11.46	12.88	14.25	N/A	N/A
Mixture (H+P)	10.87	11.22	27.90	20.59	69.40	25.16	N/A	N/A
Pure Solvent H	0.00	0.00	0.00	0.00	0.00	N/A	N/A	N/A
Pure Solvent P	0.38	0.00	0.79	N/A	0.00	0.50	N/A	N/A
Pure Solvent M	0.00	0.00	0.00	0.00	0.00	3.94	N/A	N/A

Lichen B	[D.V.] Zone of Inhibition (±0.02mm)							
[I.V.] Solvent Added (500 ±1.25µl)	Trial 1		Trial 2		Trial 3		Trial 4	
Hexane (H)	0.00	0.00	0.00	0.00	0.00	N/A	N/A	N/A
Propanol (P)	11.64	12.38	15.34	55.95	4.91	11.52	N/A	N/A
Mixture (H+P)	11.46	16.33	41.40	20.27	7.55	11.95	N/A	N/A
Pure Solvent H	0.00	0.00	0.00	0.00	0.00	N/A	N/A	N/A
Pure Solvent P	5.31	6.16	2.54	5.73	0.00	0.50	N/A	N/A
Pure Solvent M	0.00	0.00	0.00	0.00	0.00	1.58	N/A	N/A

Lichen C	[D.V.] Zone of Inhibition (±0.02mm)							
[I.V.] Solvent Added (500 ±1.25µl)	Trial 1		Trial 2		Trial 3		Trial 4	
Hexane (H)	0.00	0.00	0.00	0.00	0.00	N/A	N/A	N/A
Propanol (P)	16.62	26.42	17.35	9.62	26.97	12.25	109.36	18.10
Mixture (H+P)	13.20	16.62	12.57	15.90	16.62	14.52	N/A	N/A
Pure Solvent H	0.00	0.00	0.00	0.00	0.00	N/A	N/A	N/A
Pure Solvent P	3.27	3.46	3.80	3.20	60.82	3.20	N/A	N/A
Pure Solvent M	0.00	0.00	0.00	0.00	0.00	0.00	N/A	N/A

Above is the raw data collected, which was further condensed by, using the diameters of each spot made from the respective solutions added to the diffusion discs cut with equal diameters of 6.3mm (which was subtracted from the raw data values), the zone of inhibition was calculated. Necessary outliers highlighted in red were removed from the data processing, and a graph was created outlining the final values referenced.



The raw data mean values for each respective solution and lichen was subtracted by the value of the zones of inhibition for the pure solvents alone. Although percentage differences were calculated, they were unrepresentative of the values expressed, and hence the absolute values were used as a point of comparison.

The best solvent - quantified through the greatest individual-factor caused zone of inhibition observable - for Lichen A was the Mixture (2.5cm^3 of hexane and 2.5cm^3 of propanol); for Lichen B was the Mixture; and for Lichen C was the pure 5cm^3 of propanol. This can be theorized to be due to the composition of the Lichen. Mentioned in the introduction, the presence of metabolites means there can vary in type, quantity, composition, and optimum conditions, and hence, each lichen can be extremely different. Keeping this in mind, we look at the solvents: Hexane (a nonpolar covalent solvent); propanol (a polar solvent of weaker polarity than water and lesser solubility to water than ethanol); and Mix (immiscible, keeping both polar and nonpolar traits as like-dissolves-like).

Strengths lied in the following: three different species of lichens were tested, allowing for further analysis into the possible variation of antibacterial properties for different types of lichen; positive controls of pure solvents (Hexane, Propanol and Mix) were included along with negative controls (pure water), allowing for extensive comparisons; the preliminary testing helped for experimental practice. Limitations were the following: only having three repeats for each extract tested; uneven pipetting regions; accuracy of the zone of inhibition test; species of lichen used.
However, the research gave crucial implications: the possible reasoning behind the results we obtained could be summarized due to the substance composition of each lichen species Lichen A (*Parmotrema perlatum*) has been noted to contain atranorin complexes, which are semi-polar and hence, would favour the Mixture (with both hexane and propanol); Lichen B (*Cladonia cornuta*) has been proven to be chemically similar to *Cladonia coniocraea*, as it contains fumarprotocetraric acid which shifts more towards favoring nonpolar substances, and also salazinic or quaesitic acid which has the tendency to either be extremely polar or nonpolar. This could have been the reason why the Mixture led to the greatest zone of inhibition recorded; Lichen C (*Cladonia pulvinata*) is known for having Psoromic acid, a bioactive molecule found specifically in lichen which was recently discovered to have antibacterial effects against Mycobacterium tuberculosis as it inhibited the bacteria-associated enzymes. As Psoromic acid was proven ethanol-soluble, it can be predicted that perhaps due to its covalent-nature, it can bind to the polar solvent of Propane.

VI. If your team will compete for the Sustainable Development Award, please indicate the specific sustainable development goal the project is related to, and provide justification for competing for this award. (Word Count: 296/300 words)

SDG: 3, 13
Based on the instruction manual posed by the World Health Organisation on how mass-produced hand sanitizers are often made, calculations were carried out in order to determine the approximate value of energy used in the process of manufacturing 10L of hand sanitizer. Assumptions were made that only the energy in Joules needed solely for conversion of materials are compared. We were able to estimate approximately 126,212,182 J of energy is consumed for the production of 10L of ethanol-based hand sanitizers, and approximately 282,689,782J of energy is consumed for the production of 10L of isopropyl alcohol-based hand sanitizers. Comparing this with the values of the energy used for our theoretical hand sanitizer - 269,025,000J and 145,140,570J respectively - the lichen and propanol was more energy-efficient than the ethanol-based, whereas lichen and mix was more energy-efficient than both. Moreover, moisturizing beads - small paint-packed pockets contained in commercially-sold hand sanitizers to prevent dry skin - can be replaced by the lichen filtrate themselves, as they are renowned for their high water capacity, sources showing that they can hold up to 3,360% of their dry weight with water. This indicates how utilizing the lichen itself can be used in the production of these theoretical hand sanitizers as a possible eco-friendly alternative to the harmful microplastics often used in these products.
Hence, as commercially-sold hand sanitizers are conventionally made with almost purely of isopropyl alcohol, we thought that lichen extracts could pose as an alternative to this with less volumes of propene and propanol used - since both solutions are extracted from crude oil which is an unreplenishable and unsustainable resource, using natural derivatives of antimicrobial compounds such as lichens which can may potentially innovate a new path for antiseptics in the future.

VII. Conclusion

The best-performing lichen and solvent combination - as seen through the bar chart values of highest zone of inhibition values - was Lichen A (*Parmotrema perlatum*) with the Mixture (2.5cm^3 of Hexane and 2.5cm^3 of propanol), the next being Lichen C (*Cladonia pulvinata*) with the propanol. Most notably, it could be seen that none of the pure solvents on their own performed better than when the lichen was added, hence justifying the value of our research.