12 A student carried out an investigation to compare the antibacterial effect of a garlic extract with that of three antibiotics, all at the same concentration.

(a) (i) To obtain the extract, a clove of garlic was cut into lots of small pieces and soaked in 0.1% ethanol for a long time.

Explain why this is an effective method of extraction.

(2)

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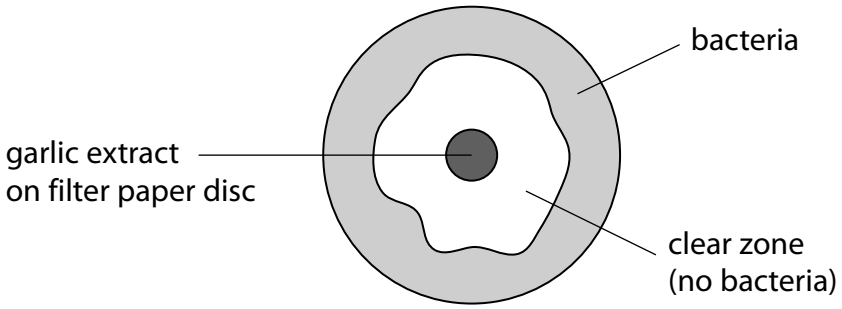
(ii) The diagram shows the effect of the garlic extract on bacteria growing on an agar plate.



The area of the clear zone was calculated by assuming it is a circle and estimating the diameter. The estimate made was 4.3 cm.

Calculate the estimated area of the clear zone.

(2)

Answer .....

(b) The results of the investigation are shown in the table.

Sample number	Estimated area of clear zone / mm^2			
	Antibiotics			Plant extract
	Chloramphenicol	Tetracycline	Streptomycin	Garlic
1	28	16	15	20
2	26	19	13	28
3	29	11	14	18
4	28	21	12	25
5	26	7	14	27
6	29	11	15	26
7	22	8	9	25
8	25	21	14	25
9	29	10	12	29
Mean	27	14	13	25
Standard deviation	2.37	5.54	1.90	3.60

These data were analysed using *t*-tests.

(i) Several statistical tests were available to the student to analyse these data, including the *t*-test, Chi squared and the correlation coefficient.

Explain why the *t*-test was chosen to analyse these data, rather than the other two tests.

(3)

(ii) Calculate the *t* value for the data to compare garlic with chloramphenicol, using the formula:

$$t = \frac{\left|\overline{x}^1 - \overline{x}^2\right|}{\sqrt{\left(\frac{\sigma_1^2}{n_1} + \frac{\sigma_2^2}{n_2}\right)}}$$

(3)

Answer .....

(iii) The table shows the critical values of *t* with 16 degrees of freedom.

Significance level (p)	0.20	0.10	0.05	0.01	0.001
Critical value of *t*	1.34	1.75	2.12	2.92	4.02

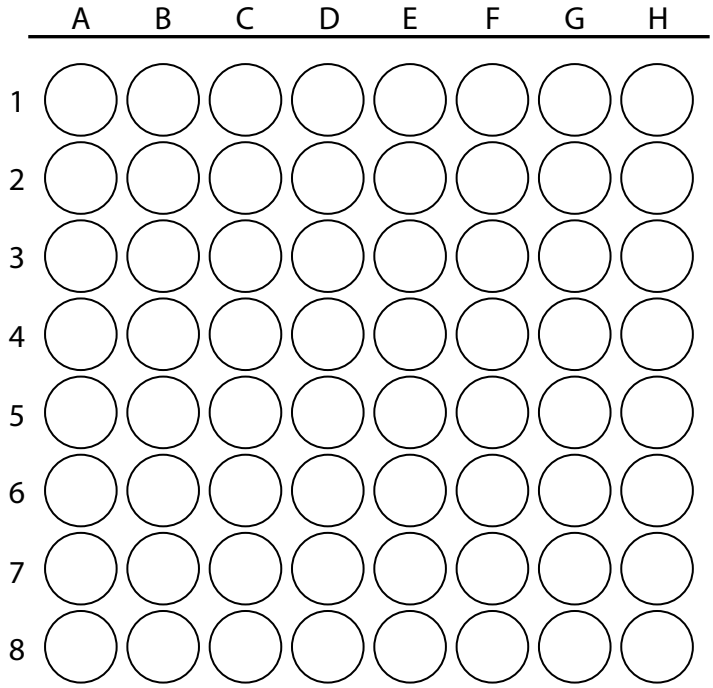
Use your value of *t* to test the validity of a stated null hypothesis.

(4)

(c) The size of the clear zone depends on variables other than the antibacterial properties of the substances used, such as size and solubility of the antimicrobial molecules in the extract.

A new method was developed in which the minimum concentration of extract that causes inhibition of bacterial growth (Minimum Inhibitory Concentration, MIC), was found.

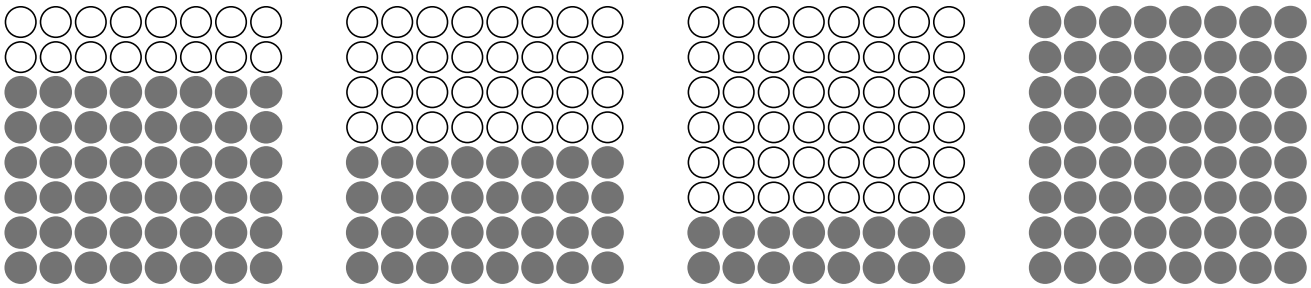
Samples of extract, bacteria (*E. coli*) and a respiration indicator were placed in a micro-titre tray.



1 = 4%
2 = 2%
3 = 1%
4 = 0.5%
5 = 0.25%
6 = 0.125%
7 = 0.0625%
8 = 0.0%

concentration of extract

The diagrams show the results obtained. The tubes are black when respiration occurs and clear when no respiration occurs.



rosemary geranium garlic cedar

(i) Analyse the data to explain the results of this experiment.

(2)

(ii) Give **two** changes that can be made to the procedure to get a more accurate measure of MIC.

(2)

(iii) It was concluded that plant extracts inhibit respiration of bacteria. This conclusion may not be valid because the investigation has limitations.

Describe how the investigation could be modified to reduce the effect of two named limitations.

(2)

(Total for Question 12 = 20 marks)

TOTAL FOR PAPER = 100 MARKS

Question Number	Acceptable Answer	Additional guidance	Mark
12(a)(i)	An explanation that makes reference to the following: • (small pieces) provides large surface area to volume ratio (1) • (use of ethanol for a long time means) the antibacterial substance is soluble in ethanol and more will be extracted (1)		**(2)**

Question Number	Acceptable Answer	Additional guidance	Mark
12(a)(ii)	$\pi 2.15^2$ (1) 14.5 cm^2 (1)		**(2)**

Question Number	Acceptable Answer	Additional guidance	Mark
12(b)(i)	An explanation that makes reference to the following: • the *t*-test assess the significance of the difference between the means of the two treatments (1) • Chi squared not appropriate because there are no expected values (1) • correlation coefficient not appropriate because the independent variable is discontinuous / not continuous (1)		**(3)**

Question Number	Acceptable Answer	Additional guidance	Mark
12(b)(ii)	$2.37^2 \div 9 = 0.62$ and $3.60^2 \div 9 = 1.44$ (1) $\sqrt{(0.62 + 1.44)} = 1.44$ (1) $(27 - 25) \div 1.44 = t = 1.39$ (1)	Correct answer gains full marks	**(3)**

Question Number	Acceptable Answer	Additional guidance	Mark
12(b)(iii)	An answer that makes reference to the following: • there is no significant difference between the clear area caused by garlic compared with that caused by chloramphenicol (1) • $p > 0.05$ (1) • difference due to chance (1) • therefore accept null hypothesis (1)	Allow marking points for the calculated value of *t* from the candidate	**(4)**

Question Number	Acceptable Answer	Additional guidance	Mark
12(c)(i)	An explanation that makes reference to the following: • suggests cedar wood oil has no anti-microbial effect on *E. coli* and all other oils do (1) • quoting at least two values from: rosemary 2%, geranium 0.5%, garlic 0.125% / manipulation of data to show relative effects (1)		**(2)**

Question Number	Acceptable Answer	Additional guidance	Mark
12(c)(ii)	An answer that makes reference to the following: • for cedar wood oil try concentrations above 4% (1) • for all the others, try concentrations below 0.0625% (1)		**(2)**

Question Number	Acceptable Answer	Additional guidance	Mark
12(c)(iii)	A description that makes reference to the following: • only one tray per species – need repeated measurements (1) • species – only used one species of bacteria / only used extracts from four plant species (1)		**(2)**

(Total for Question 12 = 20 marks)