CHAPTER IV

RESULTS

4.1 Physicochemical Properties of SBH

The physicochemical properties of SBH determined were pH, moisture content, total soluble solids (TSS, °Brix)), and color intensity.

4.1.1 pH

The pH of 100% pure honey, 50% and 10 % (w/v) is shown in Table 5. The pH of honey varies between 2.95 ± 0.2 to 3.57 ± 0 for all honey samples. Honey from sample C (*H. thoracica*) showed a lower pH of 2.95 than other samples. Diluting the honey samples to 10 and 50% slightly oncreased the pH values (3.32 ± 0.1 to 3.97 ± 0).

Table 5: pH values of SBH*

stingless bee honey samples

%SBH(w/v)	A	B	C	D	E
10	3.40 ± 0.1	3.97 ± 0	3.32 ± 0.1	3.40 ± 0	3.40 ± 0.1
50	3.01 ± 0.16	3.56 ± 0.2	2.89 ± 0.1	3.00 ± 0.2	3.14 ± 0.1
100	3.17 ± 0.1	3.57 ± 0	2.95 ± 0.2	3.02 ± 0	3.13 ± 0

* Honey samples: A= *Trigona* spp., B= *H. laevicea*, C= *H. thoracica*, D= *H. itama* and E= *H. terminata*.

4.1.2 Moisture Content, water activity and total soluble solid of SBH

The moisture content, water activity and total soluble solids of SBH samples are shown in Table 6. The percentage moisture content of SBH varies between 27.40 ± 1.14 of sample B (*H. laevicea*) to 37.07 ± 7.89 of sample C (*H. thoracica*). The

water activity (a_w) of honey samples ranged between 0.821 ± 0 for sample C and 0.792 ± 0 for sample E.

Table 6: Moisture content, a_w and total soluble solids (°Brix) of SBH*

Honey samples	Water content	a_w	°Brix
A	35.69 ± 0.09	0.820 ± 0	88.6 ± 6
B	27.40 ± 1.14	0.793 ± 0	73.4 ± 2
C	37.07 ± 7.89	0.821 ± 0	74.8 ± 2
D	33.32 ± 5.01	0.799 ± 0	74.8 ± 2
E	29.04 ± 3.21	0.792 ± 0	75.6 ± 7

* Honey samples: A= *Trigona* spp., B= *H. laevicea*, C= *H. thoracica*,

D= *H. itama* and E= *H. terminata*.

4.1.5 Color

The color of honey was varied from light yellow to dark amber or black. The mean values of five SBH samples color lightness L* and C* were determined upon the data that was given by computer connected to the colorimeter device as it reported in Table 7. As shown, there were significant differences between honey sample D and the other samples. Data was indicated for h^o shows that all SBH samples are around 62-85°, so the color is within yellow range. Sample E has the most saturated color. So that is why, it is present in dark yellow. Sample D related to the redness color (2.75), was the only one that was given in mince opposite the other samples. The results showed that most of the honeys samples are within the range of (yellow shades), with the wavelength of 400nm as a maximum absorption.

Table 7: The h^o value and C* value of SBH samples

Type of SBH sample	The value					
	l^*	a^*	b^*	h^o	C^*	Color
A	55.4	7.76	51.78	81.47681	52.35825	Yellow
B	55.48	8.77	55.48	81.0173	56.16888	Yellow
C	42.69	11.7	22.68	62.71201	25.52004	Black
D	61.75	2.75	33.86	85.35681	33.97149	Light Yellow
E	53.37	11.86	55.29	77.89321	56.54771	Dark yellow

- **L:** sample's position on the lightness axis in colorimeter.
- **a:** sample's position on the green/ red axis in colorimeter.
- **b:** sample's position on the blue/yellow axis in colorimeter.
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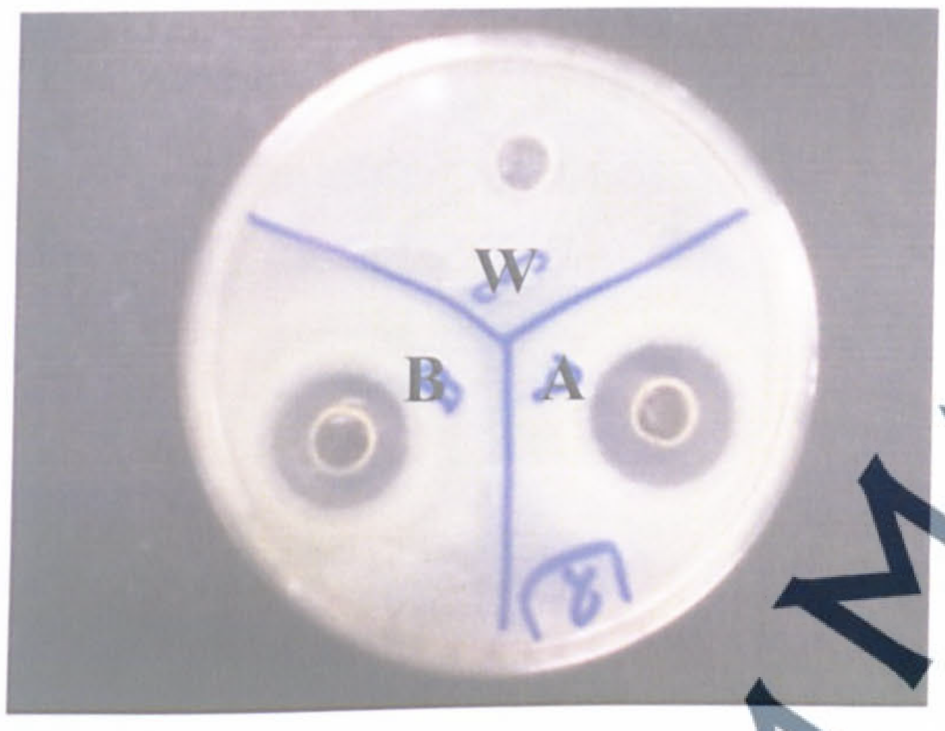
4.2 Agar Well Diffusion Assay

At 50% (w/v) honey samples inhibited the growth of the six pathogens (> 4 mm inhibitory zone) except samples C, D and E that failed to inhibit the growth of *S.* Typhimurium and *B. subtilis*. *P. vulgaris* was greatly inhibited by samples B. Honey sample B inhibited all the pathogens especially *P. vulgaris*, while honey A inhibited all except *S. aureus*, honey B inhibited all the pathogens but less than A except for *P. vulgaris*. Honey D inhibited *S. aureus* better than all other honey samples. Honey sample E has the lowest antimicrobial activity among all honey samples evaluated.

The 50% of filtered honey did not show the same capacity; the inhibitory zones were varied between 0 mm to 9 mm; *S. marcescens* and *S. aureus* were inhibited by all SBH samples. *P. vulgaris* and *B. subtilis* were not inhibited by all filtered SBH samples. Sample A has the heights antimicrobial activity against *S. aureus* with 9 mm inhibitory zone followed by sample C with 8 mm inhibitory zone. However, sample A allowed the growth of *E. coli* with 3 mm inhibitory zone. As a result, the filtered SBH has less activity against target bacteria than the unfiltered honey. This activity mostly referred to the large and less soluble molecular that has antimicrobial properties within SBH samples rather than the pH itself. However,

antimicrobial activity of SBH is not as the same as the common type of honey and further study needed to find the antimicrobial compound; and to understand the mechanism of SBH (Figure 9).



E. coli

Figure 9.1: Inhibitory zone of 50% (w/v) SBH A and B and water (W) against *E. coli* by agar-well diffusion test

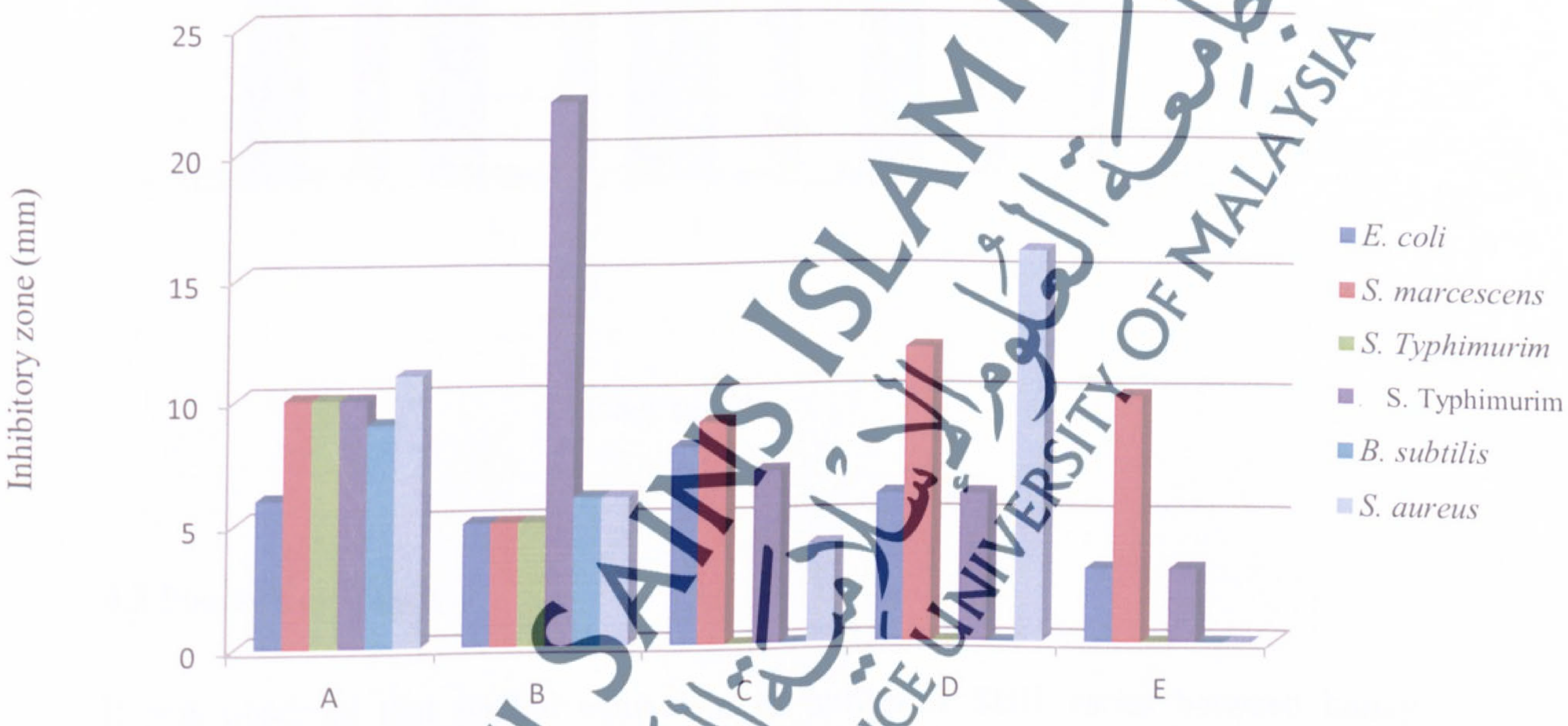
S. marcescens

S. Typhimurium

Figure 9.2: Inhibitory zone of 50% (w/v) filtered SBH A, B, C and D against *S. marcescens* and *S.* Typhimurium by agar well diffusion test

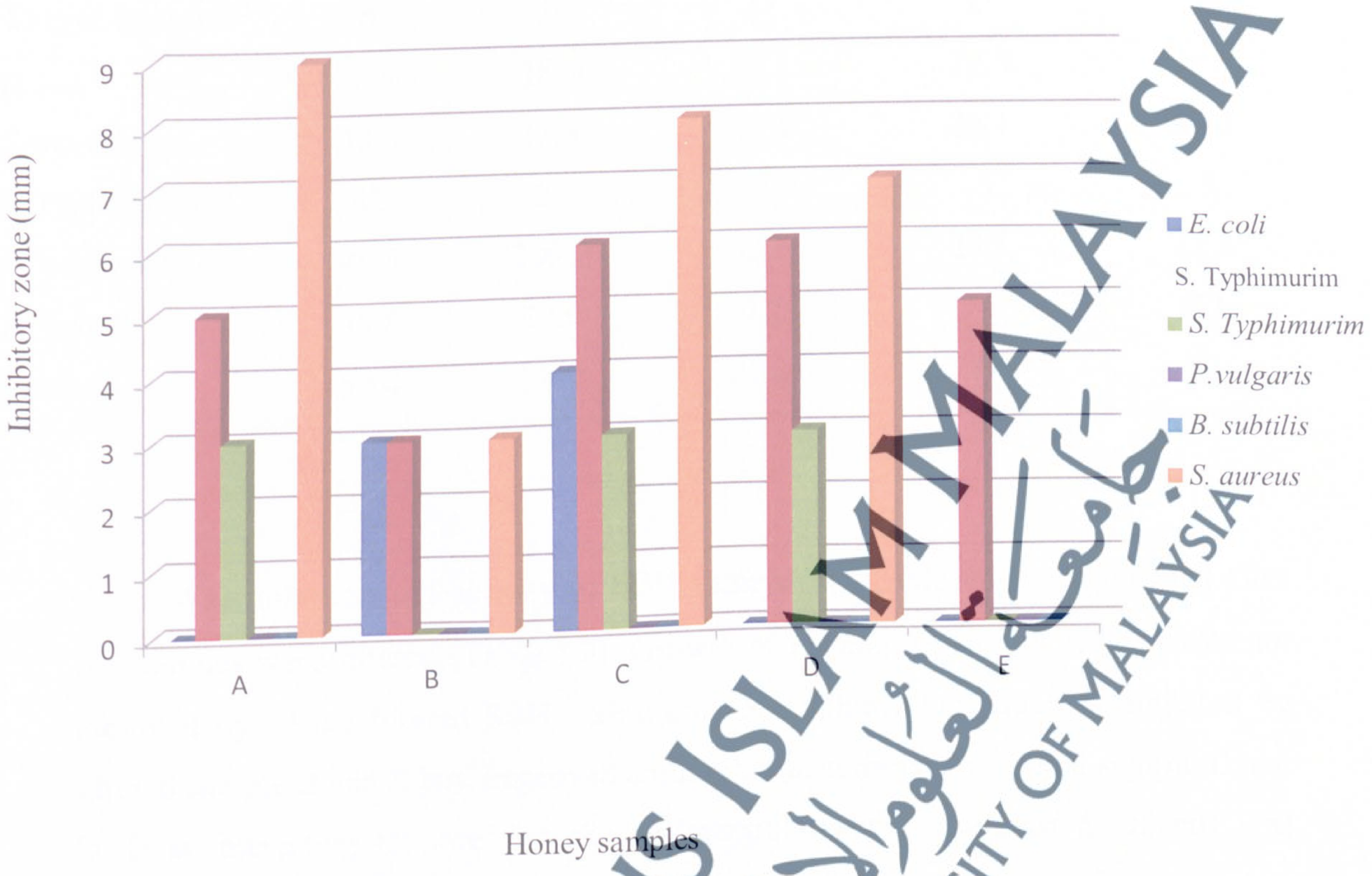
It was observed that the growth inhibitory activity of all honey samples was moderate to the target bacteria with growth inhibitory zone below 10 mm in diameter (Figure 10). Honey sample B inhibited *P. vulgaris* (22 mm), sample A and D inhibited *S. aureus* (11 mm and 16 mm, respectively) and sample D inhibited *S. marcescens* (12 mm). Honey sample C showed poor antimicrobial activity against the target bacteria especially against *S.* Thypimurium and *B. subtilis*. Similarly, Honey sample D was not effective to inhibit growth of *S.* Thypimurium, *B. subtilis* and *S. aureus*.

Figure 10: Antimicrobial activity of 50% (w/v) of SBH against target bacteria



A different antimicrobial activity was observed when the 50% (w/v) SBH was filtered. The antimicrobial activity of all honey was reduced to below 9 mm inhibitory zone (Figure 11). Growth of *P. vulgaris* and B. subtilis was not inhibited by filtered SBH A, B, C, D and E. Similarly, growth of *E. coli* was not inhibited by filtered SHB A, D, and E; while S. Typhimurium was not inhibited by filtered SBH B and E. Although the antimicrobial activity against *S. aureus* was low, all the SBH samples showed activity against these bacteria except sample E.

Figure 11: Antimicrobial activity of 50% (w/v) of filtered SBH against target bacteria



4.3 Phenol Equivalent

It was observed that phenol equivalent of unfiltered SBH varies between honey samples and target bacteria. SBH A and B showed phenol equivalent ranging from 21 to >100 for all target bacteria indicating broad spectrum antimicrobial activity (Table 8.1). In contrast, SBH C, D and E inhibited four of the target bacteria except *S.* Typhimurium and *B. subtilis*.

Table 8.1: Phenol Equivalent of 50% (w/v) SBH

	Unfiltered SBH samples (50% w/v)				
Target bacteria	A	B	C	D	E
E. coli	22.5	18.6	30.3	22.5	10.8
S. marcescens	38.3	18.8	34.4	46.1	38.3
S. Typhimurim	47	21	-5	-5	-5
P. vulgaris	48.5	109.7	33.2	28.1	12.8
B. subtilis	48.8	32.6	0.2	0.2	0.2
S. aureus	50.4	28.9	20.3	71.9	3.1

The antimicrobial activity of SBH samples was reduced or diminished after the samples were filtered (Table 8.2). Growth of *P. vulgaris* and *B. subtilis* was not inhibited by all the filtered SBH. Similarly, *S.* Typhimurium was not inhibited by filtered sample B and E but, improved antimicrobial activity for filtered sample C and D. It is interesting to note that the antimicrobial activity against *S. aureus* was increased for filtered SBH C and D.

Table 8.2: Phenol Equivalent of 50% (w/v) Filtered SBH

	Filtered SBH samples (50% w/v)				
Target bacteria	A	B	C	D	E
E. coli	0.9	10.8	14.7	0.9	14.7
S. marcescens	18.8	11	22.7	22.7	18.8
S. Typhimurim	10.6	-5	10.6	10.6	-5
P. vulgaris	-2.5	-2.5	-2.5	-2.5	-2.5
B. subtilis	0.2	0.2	0.2	0.2	0.2
S. aureus	41.8	16	37.5	33.2	33.2