

### 3. Results

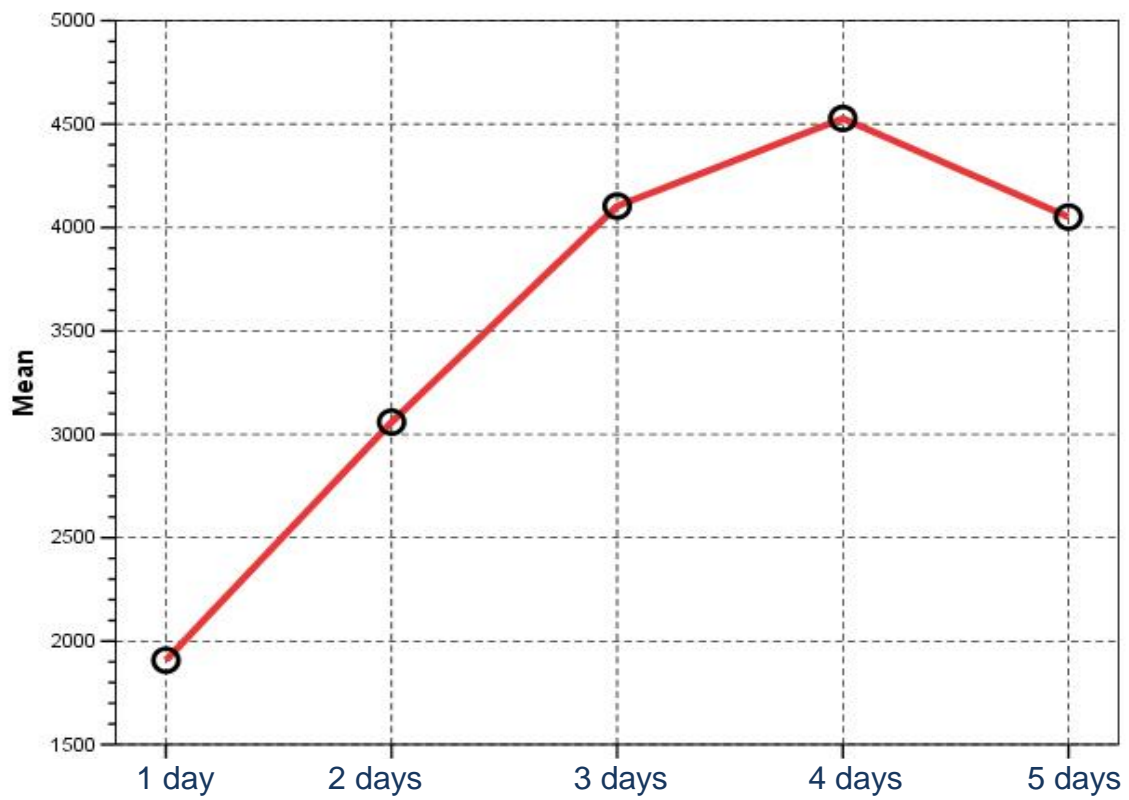
#### 3.1 Determination of logarithmic growth phase:

The parasite culture in RPMI 1640 medium with (10%) FCS and incubated at 26°C then counting the number of parasite daily by using haemocytometer during the 4 days after culture there is a statistically significant increase in mean parasite count from  $1908 \times 10^4$  promastigote/ml to a maximum mean count of  $4527 \times 10^4$  promastigote/ml. During the coming 5<sup>th</sup> day the mean count decreased to  $4051 \times 10^4$  promastigote/ml. The parasitic count significantly increase by a mean of  $2619 \times 10^4$  promastigote/ml after the 4<sup>th</sup> day compared to first day, the 4<sup>th</sup> day represent the log phase for *Leishmania tropica* promastigotes, table (3.1), figure (3.1),(3.2) and(3.3).

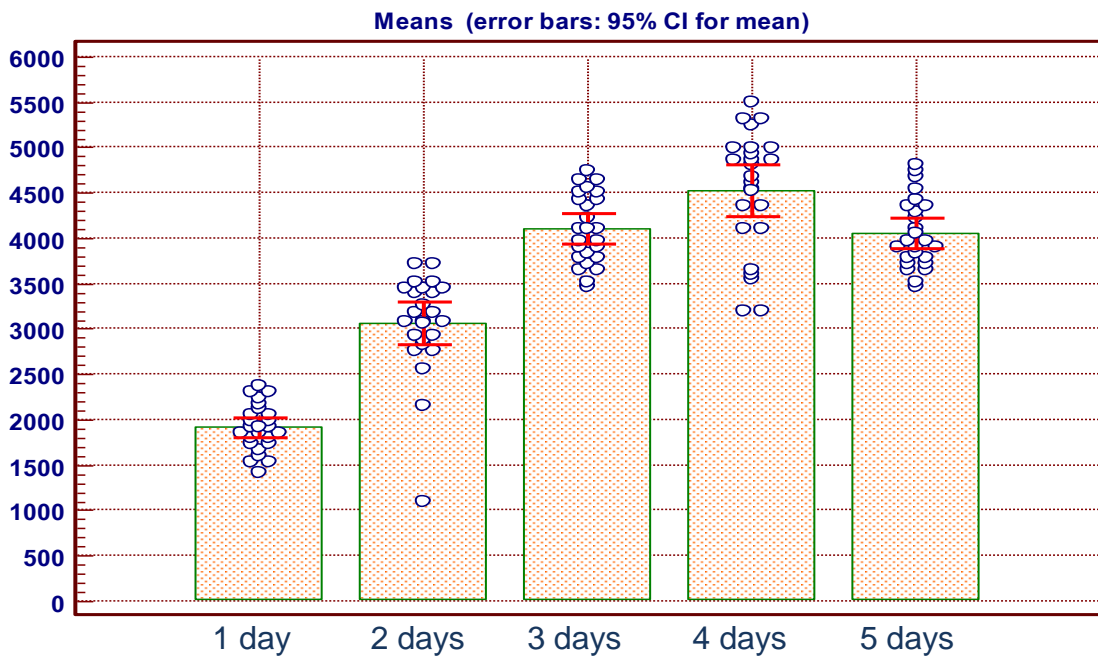
During the 2 days of culture (2<sup>nd</sup> and 3<sup>rd</sup> day) there is an increase in parasitic count of a mean of around  $1100 \times 10^4$  promastigote/ml compared to the day before, which is almost comparable in magnitude. During the 4<sup>th</sup> day there is still a statistically increase in mean count of  $424 \times 10^4$  promastigote/ml compared to the day before, the magnitude of change is however smaller than that observed in the previous 2 days. Finally during the 5<sup>th</sup> day there is a statistically significant reduction in mean count by  $470 \times 10^4$  promastigote/ml compared to the day before, table (3.2) and figure (3.4).

**Table 3.1: Changes in mean parasitic count ( $\times 10^4$  promastigote/ml) after culturing for 5 days.**

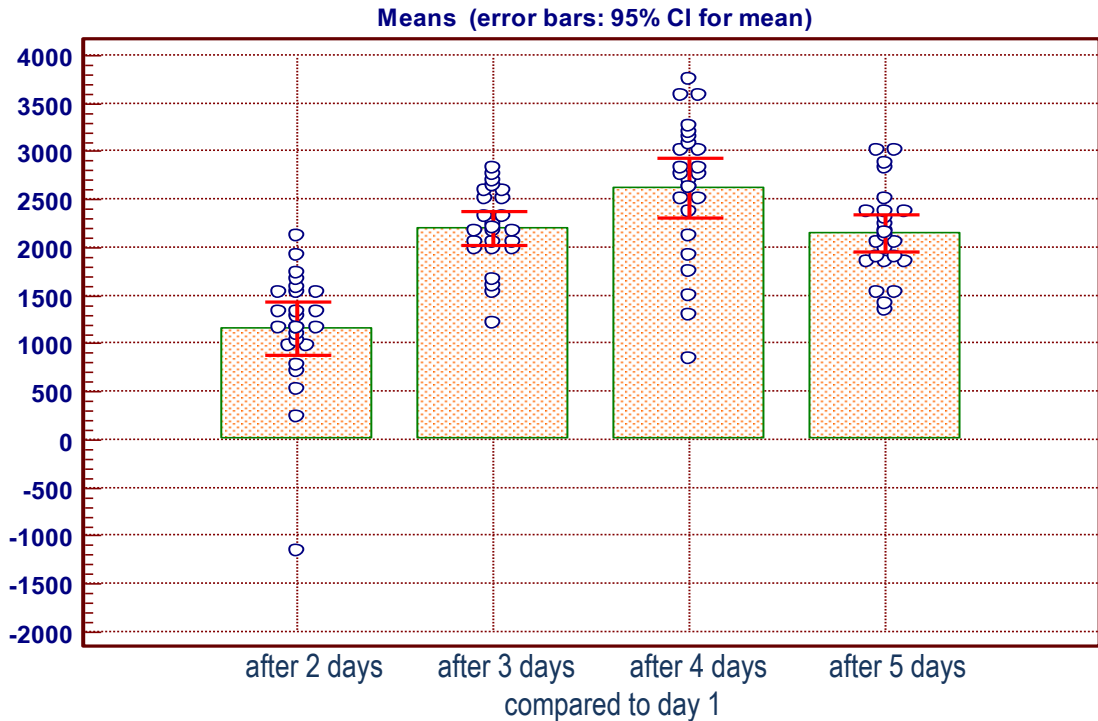
	Parasitic count after 1 day	Parasitic count after 2 days	Changes in parasitic count after 2 days compared to first day	Parasitic count after 3 days	Changes in parasitic count after 3 days compared to first day	Parasitic count after 4 days	Changes in parasitic count after 4 days compared to first day	Parasitic count after 5 days	Changes in parasitic count after 5 days compared to first day
Mean	1908	3059	1151	4104	2196	4527	2619	4051	2143
SE	53.2	114.9	133.5	80.6	84.0	137.4	150.9	79.9	96.4
N	24	24	24	24	24	24	24	24	24
<b>P (paired t-test)</b>			<b>&lt;0.001</b>		<b>&lt;0.001</b>		<b>&lt;0.001</b>		<b>&lt;0.001</b>



**Figure 3.1: line graph showing the mean count of parasites in culture medium within 5 days after Initial inoculation.**



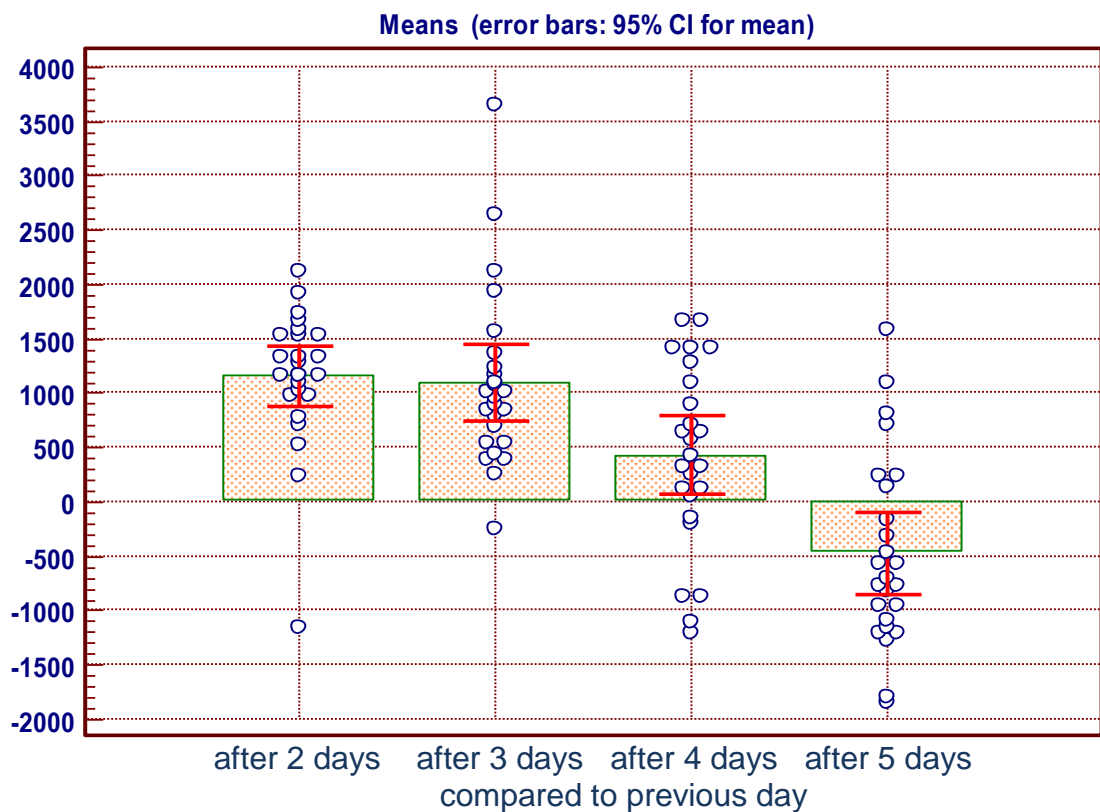
**Figure 3.2:** Dot diagram with error bars showing the mean (with its 95% confidence interval) parasitic count in culture during 5 days interval.



**Figure 3.3:** Dot diagram with error bars showing the mean (with its 95% confidence interval) change in parasitic count in culture during successive daily interval compared to first day.

**Table 3.2: The mean change in parasitic count in culture during successive daily interval compared to previous day.**

	Changes in parasitic count after 2 days compared to first day	Changes in parasitic count after 3 days compared to previous day	Changes in parasitic count after 4 days compared to previous day	Changes in parasitic count after 5 days compared to previous day
Mean	1151	1086	424	-476
SE	133.5	170.1	175.6	182.0
N	24	24	24	24
P (paired t-test)	<0.001	<0.001	0.024	0.015



**Figure 3.4: Dot diagram with error bars showing the mean (with its 95% confidence interval) change in parasitic count in culture during successive daily interval compared to previous day.**

## **3.2 The effect of increasing Concentration:**

The parasiticidal effect is the rate of parasitic killing after adding a certain dose of a specific experimental drug. It is calculated as the number of dead parasites/Total number of parasites (dead+alife) multiplied by 100 (Najim, 1998). This index ranges between a minimum of zero to a maximum of 100. The higher the index the stronger is the effect, appendix A.

### **3.2.1 Caffeic acid:**

The parasiticidal effect is significantly increased by a mean of  $24 \times 10^4$  promastigote/ml after adding 10 mg of Caffeic acid compared to baseline controls. After each successive increase in concentration the parasiticidal effect is significantly increased to reach a maximum increase by a mean of  $53 \times 10^4$  promastigote/ml compared to baseline control. The Cohen's d effect size is increased from 4.4 after 10 mg concentration to reach a maximum of 10.8 after the highest concentration of 30 mg. The concentration of added Caffeic acid showed a statistically significant positive strong linear correlation ( $r=0.93$ ) with parasiticidal effect. The regression model explaining the effect of Caffeic acid concentration was statistically significant and able to explain 87% of variation in the dependent variable (parasiticidal effect). For each one mg increase in Caffeic acid concentration the parasiticidal effect is expected to significantly increase by a mean of  $1.69 \times 10^4$  promastigote/ml as shown in table (3.3).

**Table 3.3: The changes in mean Parasiticidal effect (rate of Parasitic killing) after increasing dosage of Caffeic acid compared to baseline control (  $\times 10^4$  promastigote/ml).**

Caffeic acid	At baseline	Change after 10 mg dose		Change after 15 mg dose		Change after 20 mg dose		Change after 25 mg dose		Change after 30 mg dose	
		After 10 mg compared to baseline	After 10 mg dose	After 15 mg compared to baseline	After 15 mg dose	After 20 mg compared to baseline	After 20 mg dose	After 25 mg compared to baseline	After 25 mg dose	After 30 mg compared to baseline	After 30 mg dose
Mean	5	29	24	35	30	43	38	49	44	58	53
SE	0.51	1.46	1.6	1.2	1.23	1.6	1.75	1.27	1.3	1.33	1.59
N	24	24	24	24	24	24	24	24	24	24	24
Cohen's d (effect size) for changes after each dose compared to baseline =			4.4		6.7		6.6		9.4		10.8
P (paired t-test) =			<0.001		<0.001		<0.001		<0.001		<0.001

$r$  (dosage x rate of parasitic killing) = 0.93  $P < 0.001$

$R^2 = 0.87$

$P$  (Simple Linear Regression Model)  $< 0.001$

Regression coefficient for dosage = 1.69

### 3.2.2 Ferulic acid:

The parasitocidal effect is significantly increased by a mean of  $16 \times 10^4$  promastigote/ml after adding 10 mg of Ferulic acid compared to baseline controls. After each successive increase in concentration the parasitocidal effect is significantly increased to reach a maximum increase by a mean of  $50 \times 10^4$  promastigote/ml compared to baseline control. The Cohen's d effect size is increased from 5.7 after 10 mg concentration to reach a maximum of 9.6 after the highest concentration of 30 mg. The concentration of added Ferulic acid showed a statistically significant positive strong linear correlation ( $r=0.96$ ) with parasitocidal effect. The regression model explaining the effect of Ferulic acid concentration was statistically significant and able to explain 93% of variation in the dependent variable (parasitocidal effect). For each one mg increase in Ferulic acid concentration the parasitocidal effect is expected to significantly increase by a mean of  $1.65 \times 10^4$  promastigote/ml as shown in table (3.4).



**Table 3.4: The changes in mean Parasiticidal effect (rate of Parasitic killing) after increasing concentration of Feruleic acid compared to baseline control (  $\times 10^4$  promastigote/ml).**

Ferulic acid	At baseline	Change after 10 mg dose		Change after 15 mg dose		Change after 20 mg dose		Change after 25 mg dose		Change after 30 mg dose	
		After 10 mg dose	compared to baseline	After 15 mg dose	compared to baseline	After 20 mg dose	compared to baseline	After 25 mg dose	compared to baseline	After 30 mg dose	compared to baseline
Mean	5	21	16	28	23	36	31	46	42	54	50
SE	0.42	0.7	0.91	0.6	0.55	0.65	0.8	1.26	1.3	1.43	1.51
N	24	24	24	24	24	24	24	24	24	24	24
Cohen's d (effect size) for changes after each dose compared to baseline =			5.7		9.2		11.5		9.1		9.6
P (paired t-test) =			<0.001		<0.001		<0.001		<0.001		<0.001

$r$  (dosage x rate of parasitic killing) =0.96  $P < 0.001$

$R^2 = 0.93$

$P$  (Simple Linear Regression Model) < 0.001

Regression coefficient for dosage = 1.65

### 3.2.3 Syringic acid:

The parasitocidal effect is significantly increased by a mean of  $16 \times 10^4$  promastigote/ml after adding 10 mg of Syringic acid compared to baseline controls. After each successive increase in concentration the parasitocidal effect is significantly increased to reach a maximum increase by a mean of  $44 \times 10^4$  promastigote/ml compared to baseline control. The Cohen's d effect size is increased from 6.7 after 10 mg concentration to reach a maximum of 10.5 after the highest concentration of 30 mg. The concentration of added Syringic acid showed a statistically significant positive strong linear correlation ( $r=0.96$ ) with parasitocidal effect. The regression model explaining the effect of Syringic acid concentration was statistically significant and able to explain 93% of variation in the dependent variable (parasitocidal effect). For each one mg increase in Syringic acid concentration the parasitocidal effect is expected to significantly increase by a mean of  $1.47 \times 10^4$  promastigote/ml as shown in table (3.5).

**Table 3.5: The changes in mean Parasiticidal effect (rate of Parasitic killing) after increasing concentration of Syringic acid compared to baseline control ( $\times 10^4$  promastigote/ml).**

Syringic acid	At baseline	Change after 10 mg dose		Change after 15 mg dose		Change after 20 mg dose		Change after 25 mg dose		Change after 30 mg dose	
		After 10 mg dose	compared to baseline	After 15 mg dose	compared to baseline	After 20 mg dose	compared to baseline	After 25 mg dose	compared to baseline	After 30 mg dose	compared to baseline
Mean	5	21	16	27	22	35	30	43	38	49	44
SE	0.38	0.57	0.72	0.7	0.83	0.7	0.74	1.25	1.28	1.16	1.3
N	24	24	24	24	24	24	24	24	24	24	24
Cohen's d (effect size) for changes after each dose compared to baseline =			6.7		7.9		10.7		8.4		10.5
P (paired t-test) =			<0.001		<0.001		<0.001		<0.001		<0.001

$r$  (dosage x rate of parasitic killing) = 0.96  $P < 0.001$

$R^2 = 0.93$

$P$  (Simple Linear Regression Model)  $< 0.001$

Regression coefficient for dosage = 1.47

### 3.2.4 4-Hydroxybenzoic acid:

The parasitocidal effect is significantly increased by a mean of  $13 \times 10^4$  promastigote/ml after adding 10 mg of 4-Hydroxybenzoic acid compared to baseline controls. After each successive increase in concentration the parasitocidal effect is significantly increased to reach a maximum increase by a mean of  $48 \times 10^4$  promastigote/ml compared to baseline control. The Cohen's d effect size is increased from 3.2 after 10 mg concentration to reach a maximum of 5.2 after the highest concentration of 30 mg. The concentration of added 4-Hydroxybenzoic acid showed a statistically significant positive strong linear correlation ( $r=0.89$ ) with parasitocidal effect. The regression model explaining the effect of 4-Hydroxybenzoic acid concentration was statistically significant and able to explain 78% of variation in the dependent variable (parasitocidal effect). For each one mg increase in 4-Hydroxybenzoic acid concentration the parasitocidal effect is expected to significantly increase by a mean of  $1.66 \times 10^4$  promastigote/ml as shown in table (3.6).

**Table3. 6: The changes in mean Parasiticidal effect (rate of Parasitic killing) after increasing concentration of 4-Hydroxybenzoic acid compared to baseline control (  $\times 10^4$  promastigote/ml).**

4-Hydroxybenzoic acid	At baseline	Change after 10 mg dose		Change after 15 mg dose		Change after 20 mg dose		Change after 25 mg dose		Change after 30 mg dose	
		After 10 mg dose	compared to baseline	After 15 mg dose	compared to baseline	After 20 mg dose	compared to baseline	After 25 mg dose	compared to baseline	After 30 mg dose	compared to baseline
Mean	6	19	13	27	22	36	31	46	41	54	48
SE	0.61	1.02	1.23	1.04	1.09	2.09	2.15	2.31	2.31	2.58	2.5
N	24	24	24	24	24	24	24	24	24	24	24
Cohen's d (effect size) for changes after each dose compared to baseline =			3.2		5.2		4.1		4.9		5.2
P (paired t-test) =			<0.001		<0.001		<0.001		<0.001		<0.001

$r$  (dosage x rate of parasitic killing) =0.89  $P < 0.001$

$R^2 = 0.78$

$P$  (Simple Linear Regression Model)  $< 0.001$

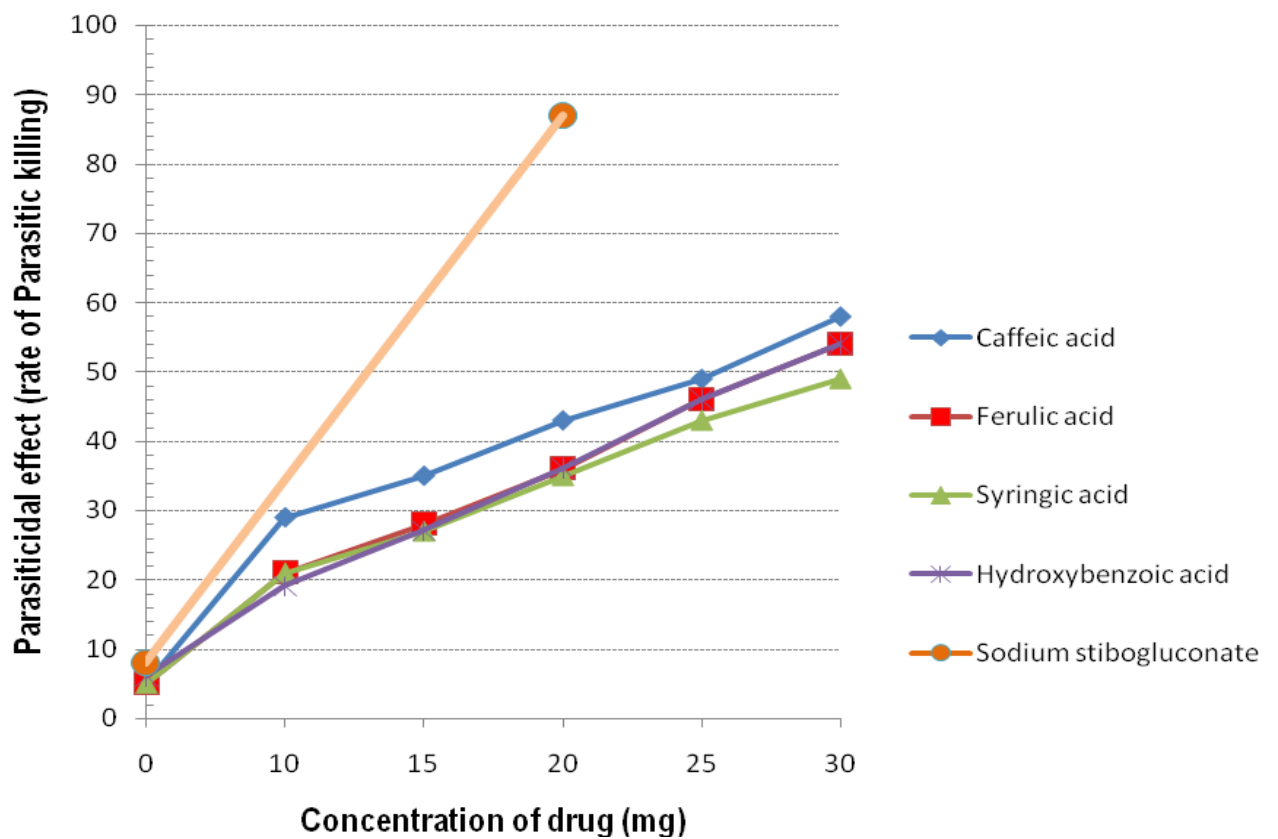
Regression coefficient for dosage = 1.66

### 3.2.5 Sodium stibogluconate:

The parasiticidal effect is significantly increased by a mean of  $79 \times 10^4$  promastigote/ml after adding 20  $\mu\text{g}$  of Sodium stibogluconate compared to baseline controls. The Cohen's d effect size reach 26.3 after 20 $\mu\text{g}$  dose as shown in table (3.7).

**Table 3.7: The changes in mean Parasiticidal effect (rate of Parasitic killing) after adding 20  $\mu\text{g}$  of Sodium stibogluconate compared to baseline control ( $\times 10^4$  promastigote/ml).**

Sodium stibogluconate	At baseline	After 20 $\mu\text{g}$ dose	Change after 20 $\mu\text{g}$ dose compared to baseline
Mean	8	87	79
SE	0.65	0.55	0.91
N	24	24	24
Cohen's d (effect size) for changes after each dose compared to baseline =			26.3
P (paired t-test) =			<0.001



**Figure 3.5: Line graph showing the linear trend of mean Parasitidal effect (rate of Parasitic killing) with dosage of 5 additives.**

### 3.3 Comparing the effect of 5 additives

There was no obvious or statistically significant difference in mean parasiticidal effect at baseline control for the first 4 additives: Caffeic acid, Ferulic acid, Syringic acid and 4-Hydroxybenzoic acid. The mean parasiticidal effect for 5<sup>th</sup> experiment baseline control (for sodium stibogluconate) was slightly higher ( $8 \times 10^4$  promastigote/ml) than the remaining 4 experiments ( $5$  to  $6 \times 10^4$  promastigote/ml). This difference would not affect the comparison of “*changes*” in parasiticidal effect after each specific concentration between different additives compared to baseline controls, table (3.8) and figure (3.6).



**Table 3.8: The difference in mean Parasiticidal effect (rate of Parasitic killing) at baseline (control) between 5 study groups ( $\times 10^4$  promastigote/ml).**

Parasiticidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	Sodium stibogluconate	P (ANOVA)
Mean	5	5	5	6	8	<0.001
SE	0.51	0.42	0.38	0.61	0.65	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid = 1[NS]

Caffeic acid X Syringic acid = 1[NS]

Caffeic acid X 4-Hydroxybenzoic acid = 1[NS]

Caffeic acid X Sodium stibogluconate <0.001

Ferulic acid X Syringic acid = 1[NS]

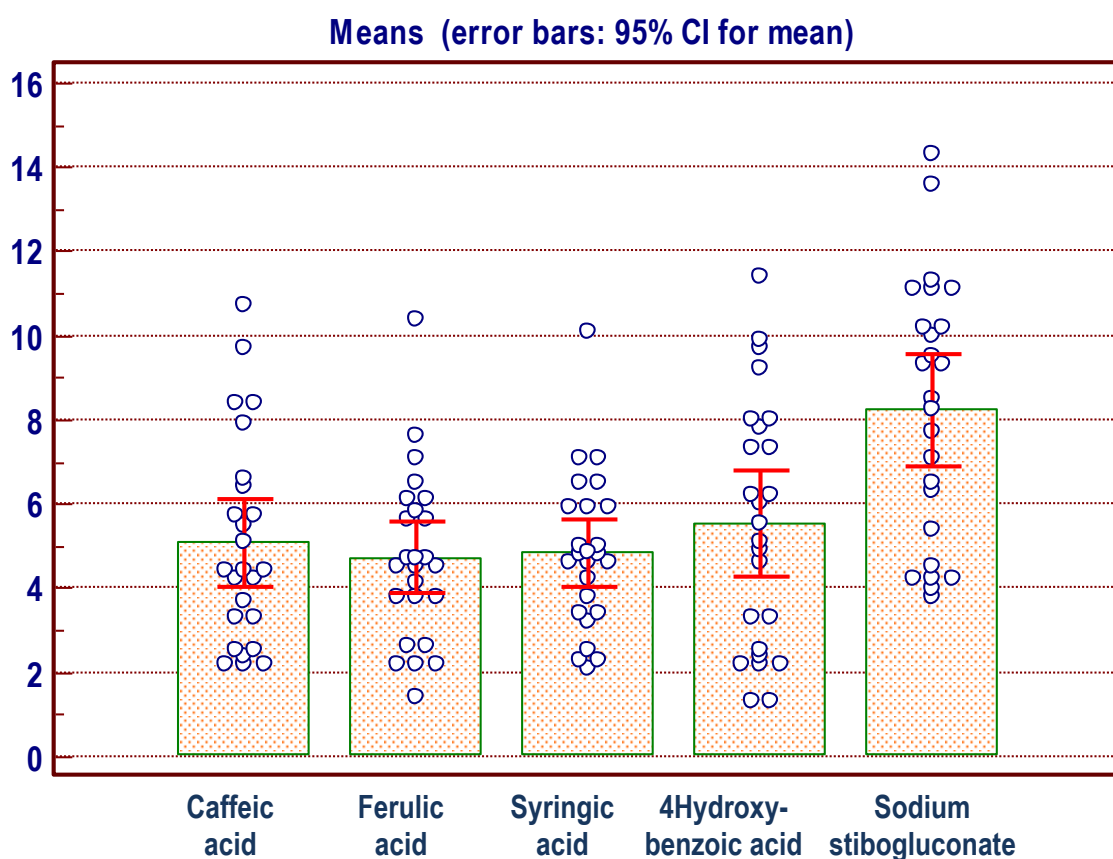
Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Ferulic acid X Sodium stibogluconate <0.001

Syringic acid X 4-Hydroxybenzoic acid = 1[NS]

Syringic acid X Sodium stibogluconate <0.001

4-Hydroxybenzoic acid X Sodium stibogluconate = 0.004



**Figure 3.6: Dot diagram with error bars showing the mean (with its 95% confidence interval) Parasiticidal effect (rate of Parasitic killing) at baseline (controls) between 5 study groups.**

### 3.3.1 After 10 mg concentration

There was a statistically significant difference in mean parasitocidal effect after 10 mg concentration between the 4 types of tested additives. The lowest parasitocidal effect was observed with 4-Hydroxybenzoic acid ( $19 \times 10^4$  promastigote/ml). It will therefore be used as the reference (comparison) group. The strongest effect was observed with Caffeic acid ( $29 \times 10^4$  promastigote/ml), resulting in a Cohen's d effect size of 1.6, which is significantly higher compared to the reference group. Both Ferulic and Syringic acid showed a slightly higher parasitocidal effect ( $21 \times 10^4$  promastigote/ml), which was not significantly higher than that of the reference group (4-Hydroxybenzoic acid). The Cohen's d effect size for both groups was of small magnitude (0.5), table (3.9) and figure (3.7).

**Table 3.9: The difference in mean Parasitocidal effect (rate of Parasitic killing) after a dosage of 10 mg of additive between 4 study groups( $\times 10^4$  promastigote/ml).**

Parasitocidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
After 10 mg dose					<0.001
Mean	29	21	21	19	
SE	1.46	0.7	0.57	1.02	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid <0.001

Caffeic acid X Syringic acid <0.001

Caffeic acid X 4-Hydroxybenzoic acid <0.001

Effect size (Cohen's d) of Caffeic acid compared to 4-Hydroxybenzoic acid=1.6

Ferulic acid X Syringic acid = 1[NS]

Ferulic acid X 4-Hydroxybenzoic acid = 0.56[NS]

Effect size (Cohen's d) of Ferulic acid compared to 4-Hydroxybenzoic acid=0.5

Syringic acid X 4-Hydroxybenzoic acid = 1[NS]

Effect size (Cohen's d) of Syringic acid compared to 4-Hydroxybenzoic acid=0.5

**Table 3.10: The difference in mean change in Parasitocidal effect (rate of Parasitic killing) after a dosage of 10 mg of additive compared to baseline (control) between 4 study groups (  $\times 10^4$  promastigote/ml).**

Parasitocidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
Change after 10 mg dose compared to baseline					<0.001
Mean	24	16	16	13	
SE	1.6	0.91	0.72	1.23	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid <0.001

Caffeic acid X Syringic acid <0.001

Caffeic acid X 4-Hydroxybenzoic acid <0.001

Effect size (Cohen's d) of Caffeic acid compared to 4-Hydroxybenzoic acid=1.6

Ferulic acid X Syringic acid = 1[NS]

Ferulic acid X 4-Hydroxybenzoic acid = 0.32[NS]

Effect size (Cohen's d) of Ferulic acid compared to 4-Hydroxybenzoic acid=0.6

Syringic acid X 4-Hydroxybenzoic acid = 0.67[NS]

Effect size (Cohen's d) of Syringic acid compared to 4-Hydroxybenzoic acid=0.6

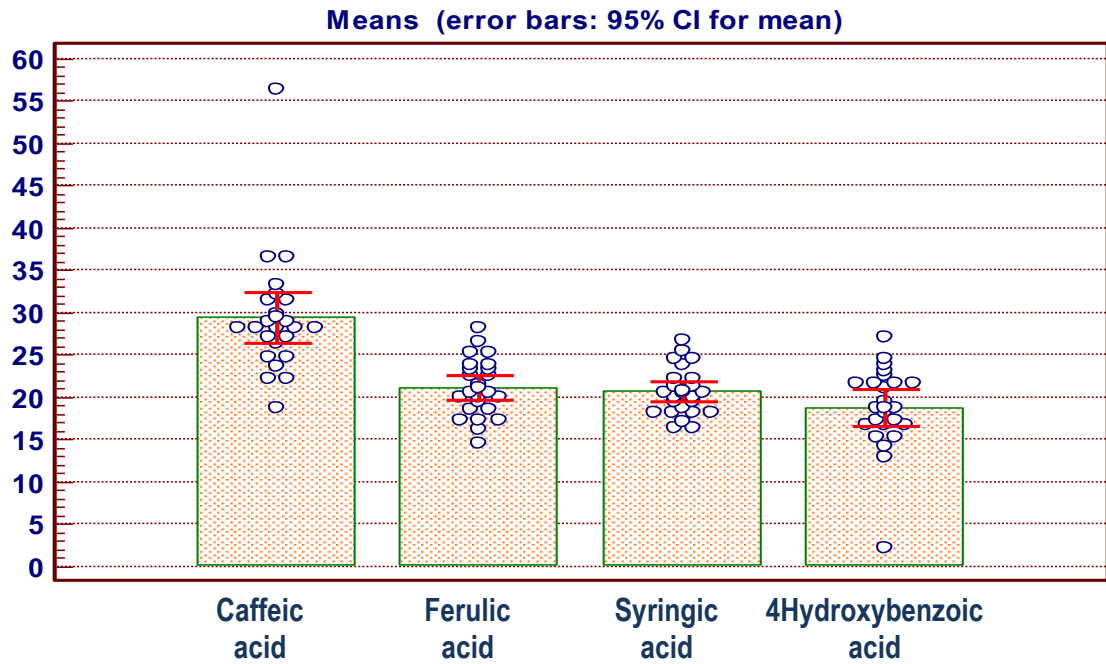


Figure 3.7: Dot diagram with error bars showing the mean (with its 95% confidence interval) Parasiticidal effect (rate of Parasitic killing) after a dosage of 10 mg of additive between 4 study groups.

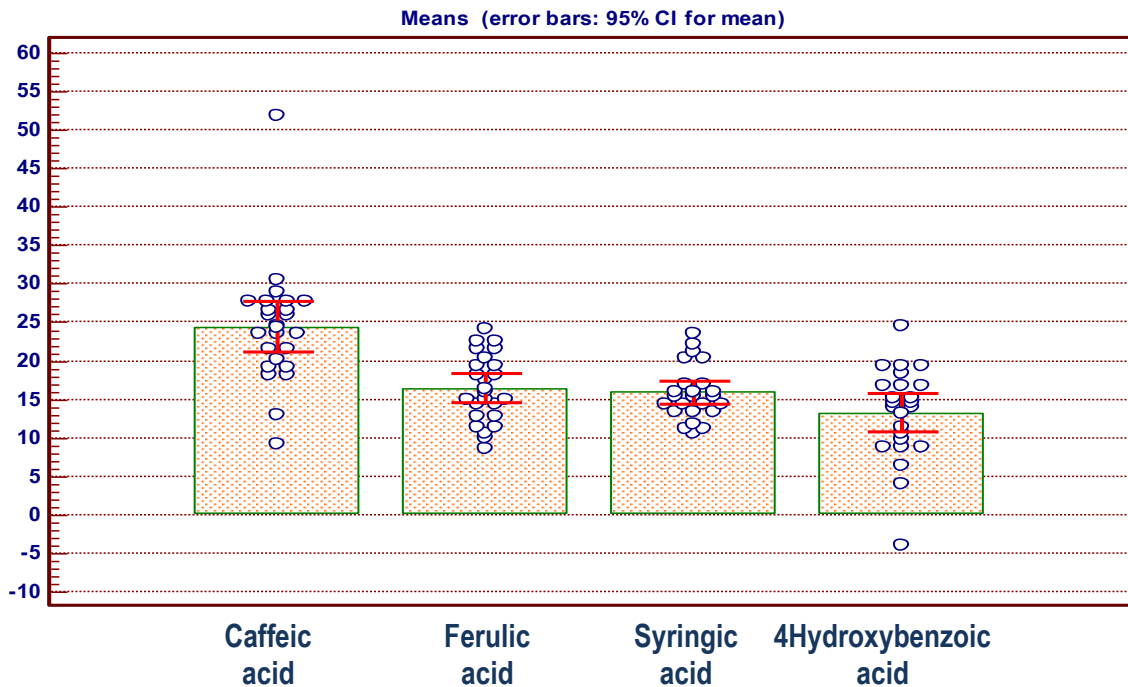


Figure 3.8: Dot diagram with error bars showing the mean change (with its 95% confidence interval) in Parasiticidal effect (rate of Parasitic killing) after a dosage of 10 mg of additive compared to baseline (control) between 4 study groups.

### 3.3.2 After 15 mg concentration

There was a statistically significant difference in mean parasitidal effect after 15 mg concentration between the 4 types of tested additives. The lowest parasitidal effect was observed with 4-Hydroxybenzoic acid ( $27 \times 10^4$  promastigote/ml). It will therefore be used as the reference (comparison) group. The strongest effect was observed with Caffeic acid ( $35 \times 10^4$  promastigote/ml), resulting in a Cohen's d effect size of (1.5), which is significantly higher compared to the reference group. Ferulic acid showed a slightly higher parasitidal effect ( $28 \times 10^4$  promastigote/ml), which was not significantly higher than that of the reference group (4-Hydroxybenzoic acid), syringic acid have the same paraticidal effect of 4-hydroxybenzoic acid ( $27 \times 10^4$  promastigote/ml). The Cohen's d effect size for both groups was of small magnitude (0.2, 0) respectively, table (3.11) and figure (3.9).

**Table 3.11: The difference in mean Parasiticidal effect (rate of Parasitic killing) after a dosage of 15 mg of additive between 4 study groups (  $\times 10^4$  promastigote/ml).**

Parasiticidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
After 15 mg dose					<0.001
Mean	35	28	27	27	
SE	1.2	0.6	0.7	1.04	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid <0.001

Caffeic acid X Syringic acid <0.001

Caffeic acid X 4-Hydroxybenzoic acid <0.001

Effect size (Cohen's d) of Caffeic acid compared to 4-Hydroxybenzoic acid=1.5

Ferulic acid X Syringic acid = 1[NS]

Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Effect size (Cohen's d) of Ferulic acid compared to 4-Hydroxybenzoic acid=0.2

Syringic acid X 4-Hydroxybenzoic acid = 1[NS]

Effect size (Cohen's d) of Syringic acid compared to 4-Hydroxybenzoic acid=0

**Table 3.12: The difference in mean change in Parasiticidal effect (rate of Parasitic killing) after a dosage of 15 mg of additive compared to baseline (control) between 4 study groups (  $\times 10^4$  promastigote/ml).**

Parasiticidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
Change after 15 mg dose compared to baseline					<0.001
Mean	30	23	22	22	
SE	1.23	0.55	0.83	1.09	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid <0.001

Caffeic acid X Syringic acid <0.001

Caffeic acid X 4-Hydroxybenzoic acid <0.001

Effect size (Cohen's d) of Caffeic acid compared to 4-Hydroxybenzoic acid=1.4

Ferulic acid X Syringic acid = 1[NS]

Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Effect size (Cohen's d) of Ferulic acid compared to 4-Hydroxybenzoic acid=0.2

Syringic acid X 4-Hydroxybenzoic acid = 1[NS]

Effect size (Cohen's d) of Syringic acid compared to 4-Hydroxybenzoic acid=0

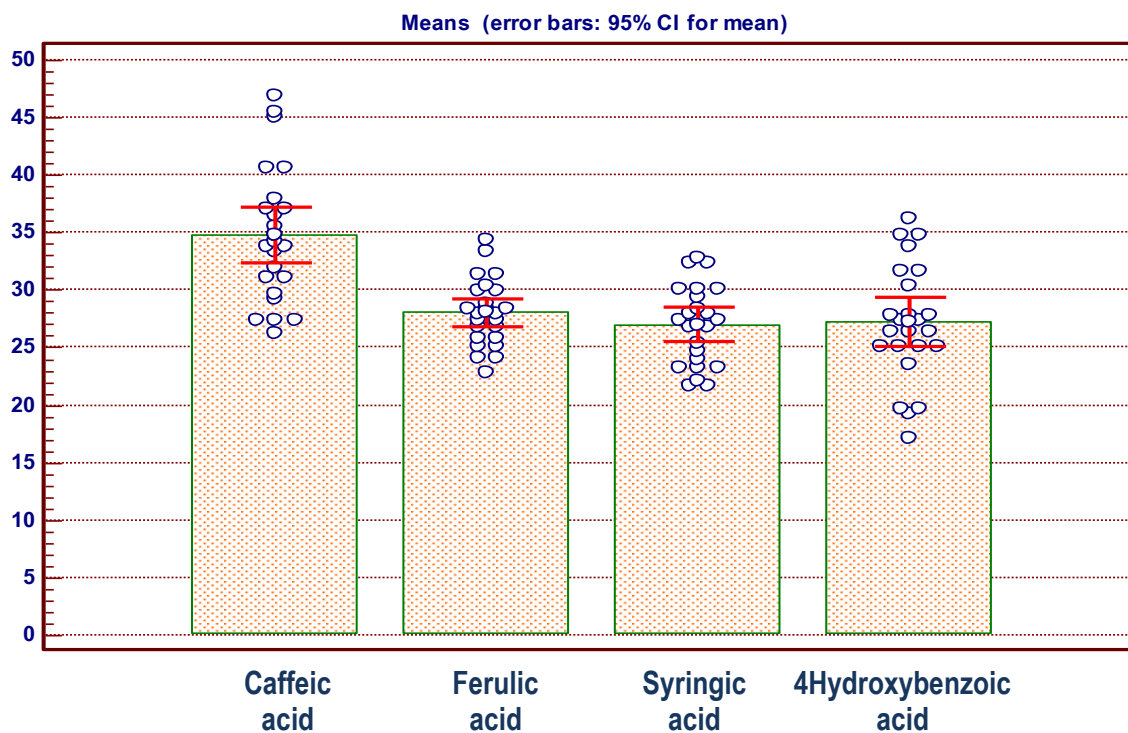


Figure 3.9: Dot diagram with error bars showing the mean (with its 95% confidence interval) Parasiticidal effect (rate of Parasitic killing) after a dosage of 15 mg of additive between 4 study groups.

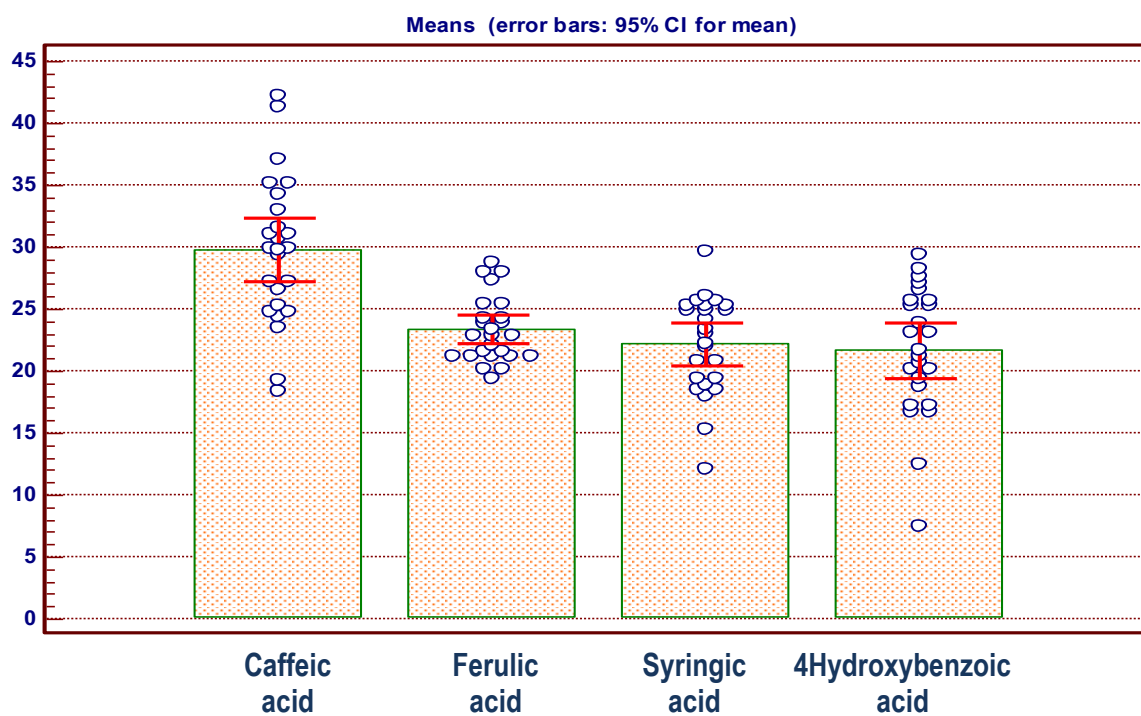


Figure 3.10: Dot diagram with error bars showing the mean change (with its 95% confidence interval) in Parasiticidal effect (rate of Parasitic killing) after a dosage of 15 mg of additive compared to baseline (control) between 4 study groups.

### 3.3.3 After 20 mg concentration

There was a statistically significant difference in mean parasitocidal effect after 20 mg concentration between the 4 types of tested additives. The lowest parasitocidal effect was observed with Syringic acid ( $35 \times 10^4$  promastigote/ml). It will therefore be used as the reference (comparison) group. The strongest effect was observed with Caffeic acid ( $43 \times 10^4$  promastigote/ml), resulting in a Cohen's d effect size of (1.3), which is significantly higher compared to the reference group. Both Ferulic and 4-hydroxybenzoic acid showed a slightly higher parasitocidal effect ( $36 \times 10^4$  promastigote/ml), which was not significantly higher than that of the reference group (Syringic acid). The Cohen's d effect size for both Ferulic acid (0.3) and for 4-hydroxybenzoic acid (0.1), table (3.13) and figure (3.11).



**Table 3.13: Table 3.14: The difference in mean Parasiticidal effect (rate of Parasitic killing) after a concentration of 20 mg of additive between 4 study groups ( $\times 10^4$  promastigote/ml).**

Parasiticidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
After 20 mg dose					<0.001
Mean	43	36	35	36	
SE	1.6	0.65	0.7	2.09	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid = 0.001

Caffeic acid X Syringic acid <0.001

Effect size (Cohen's d) of Caffeic acid compared to Syringic acid=1.3

Caffeic acid X 4-Hydroxybenzoic acid = 0.002

Ferulic acid X Syringic acid = 1[NS]

Effect size (Cohen's d) of Ferulic acid compared to Syringic acid=0.3

Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Syringic acid X 4-Hydroxybenzoic acid = 1[NS]

Effect size (Cohen's d) of 4-Hydroxybenzoic acid compared to Syringic acid=0.1

**Table 3.15: The difference in mean change in Parasiticidal effect (rate of Parasitic killing) after a concentration of 20 mg of additive compared to baseline (control) between 4 study groups ( $\times 10^4$  promastigote/ml).**

Parasiticidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
Change after 20 mg dose compared to baseline					<0.001
Mean	38	31	30	31	
SE	1.75	0.8	0.74	2.15	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid = 0.004

Caffeic acid X Syringic acid <0.001

Effect size (Cohen's d) of Caffeic acid compared to Syringic acid=1.2

Caffeic acid X 4-Hydroxybenzoic acid = 0.003

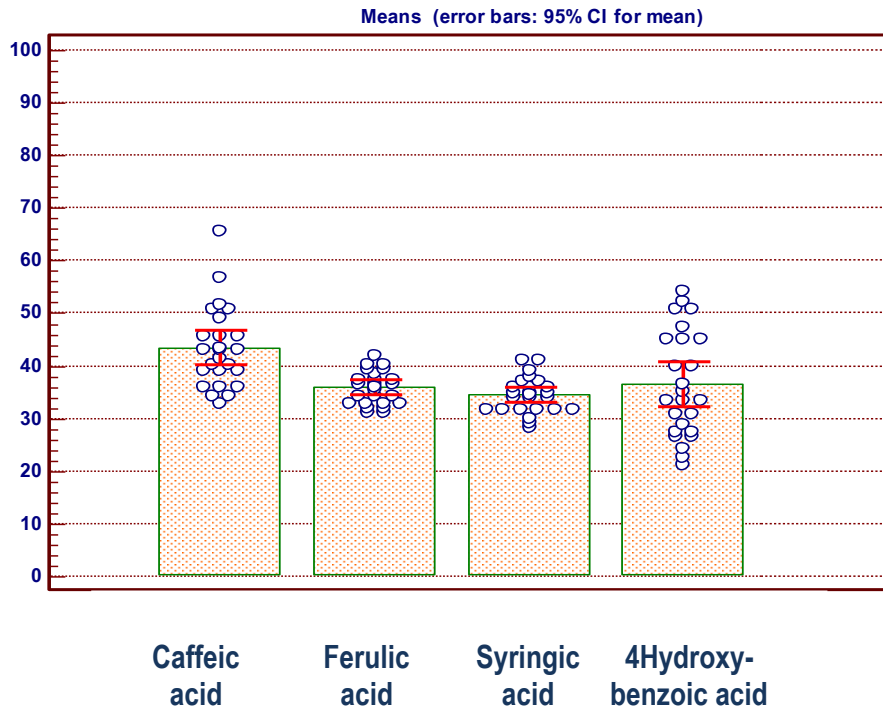
Ferulic acid X Syringic acid = 1[NS]

Effect size (Cohen's d) of Ferulic acid compared to Syringic acid=0.3

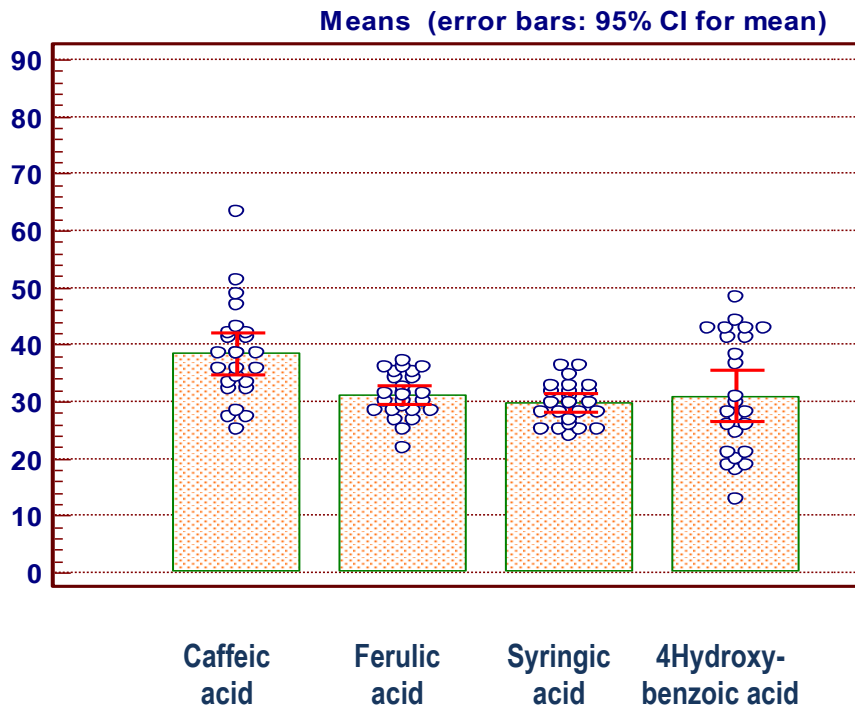
Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Syringic acid X 4-Hydroxybenzoic acid = 1[NS]

Effect size (Cohen's d) of 4-Hydroxybenzoic acid compared to Syringic acid=0.1



**Figure 3.11:** Dot diagram with error bars showing the mean (with its 95% confidence interval) Parasiticidal effect (rate of Parasitic killing) after a dosage of 20 mg of additive between 4 study groups.



**Figure 3.12:** Dot diagram with error bars showing the mean change (with its 95% confidence interval) in Parasiticidal effect (rate of Parasitic killing) after a dosage of 20 mg of additive compared to baseline (control) between 4 study groups.

### 3.3.4 After 25 mg concentration

There was a statistically significant difference in mean parasitocidal effect after 25 mg concentration between the 4 types of tested additives. The lowest parasitocidal effect was observed with Syringic acid ( $43 \times 10^4$  promastigote/ml). It will therefore be used as the reference (comparison) group. The strongest effect was observed with Caffeic acid ( $49 \times 10^4$  promastigote/ml), resulting in a Cohen's d effect size of (1), which is significantly higher compared to the reference group. Both Ferulic and 4-hydroxybenzoic acid showed a slightly higher parasitocidal effect ( $46 \times 10^4$  promastigote/ml), which was not significantly higher than that of the reference group (Syringic acid). The Cohen's d effect size for both groups was of small magnitude (0.5, 0.3) respectively, table (3.16) and figure (3.13).

**Table 3.16: The difference in mean Parasitocidal effect (rate of Parasitic killing) after a concentration of 25 mg of additive between 4 study groups(  $\times 10^4$  promastigote/ml) .**

Parasitocidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
After 25 mg dose					0.08[NS]
Mean	49	46	43	46	
SE	1.27	1.26	1.25	2.31	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid = 1[NS]

Caffeic acid X Syringic acid = 0.06[NS]

Effect size (Cohen's d) of Caffeic acid compared to Syringic acid=1

Caffeic acid X 4-Hydroxybenzoic acid = 1[NS]

Ferulic acid X Syringic acid = 0.92[NS]

Effect size (Cohen's d) of Ferulic acid compared to Syringic acid=0.5

Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Syringic acid X 4-Hydroxybenzoic acid = 0.87[NS]

Effect size (Cohen's d) of 4-Hydroxybenzoic acid compared to Syringic acid=0.3

**Table 3.17: The difference in mean change in Parasitocidal effect (rate of Parasitic killing) after a concentration of 25 mg of additive compared to baseline (control) between 4 study groups (  $\times 10^4$  promastigote/ml) .**

Parasitocidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
Change after 25 mg dose compared to baseline					0.1[NS]
Mean	44	42	38	41	
SE	1.3	1.3	1.28	2.31	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid = 1[NS]

Caffeic acid X Syringic acid = 0.09[NS]

Effect size (Cohen's d) of Caffeic acid compared to Syringic acid=0.9

Caffeic acid X 4-Hydroxybenzoic acid = 1[NS]

Ferulic acid X Syringic acid = 0.86[NS]

Effect size (Cohen's d) of Ferulic acid compared to Syringic acid=0.6

Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Syringic acid X 4-Hydroxybenzoic acid = 1[NS]

Effect size (Cohen's d) of 4-Hydroxybenzoic acid compared to Syringic acid=0.3

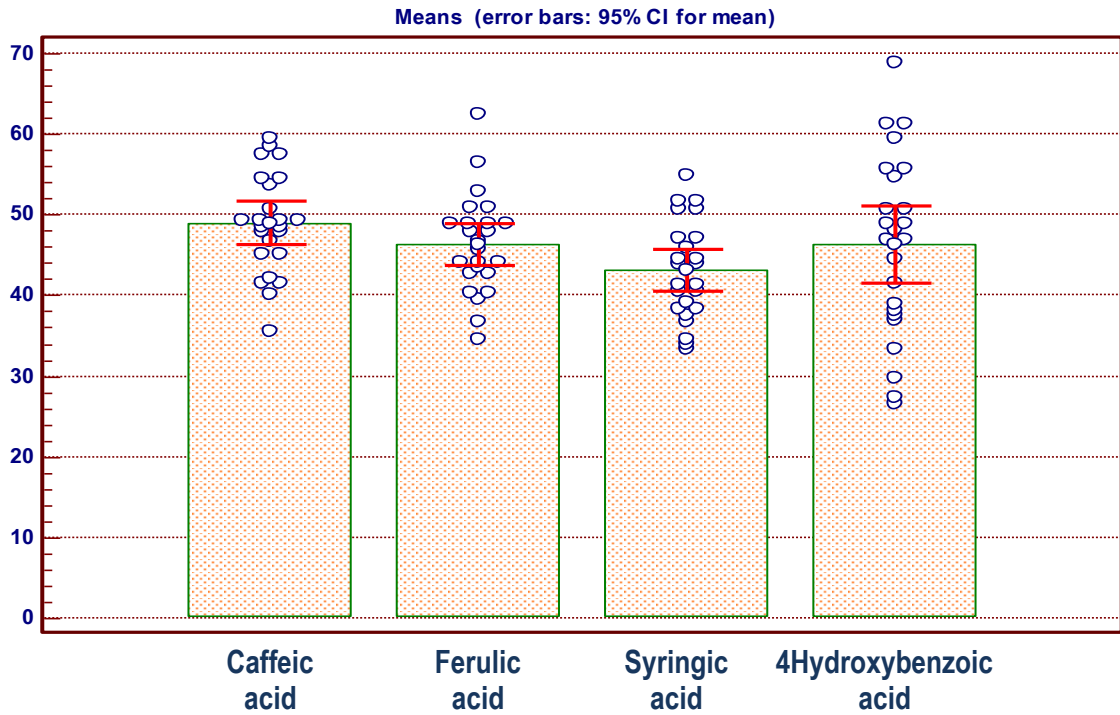


Figure 3.13: Dot diagram with error bars showing the mean (with its 95% confidence interval) Parasiticidal effect (rate of Parasitic killing) after a dosage of 25 mg of additive between 4 study groups.

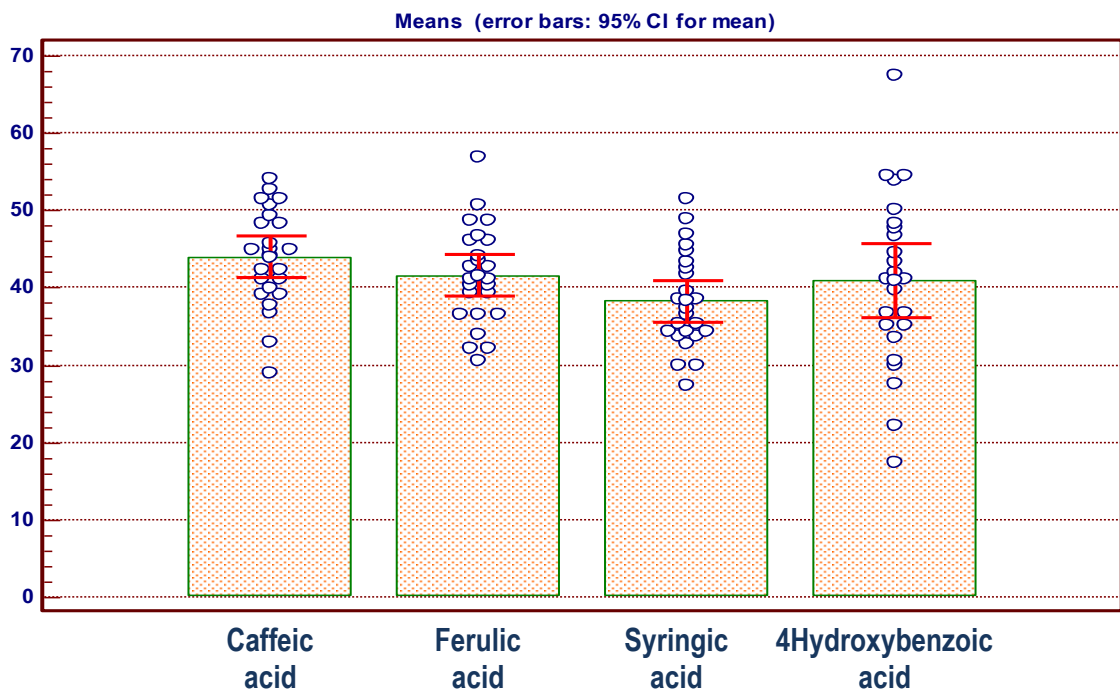


Figure 3.14: Dot diagram with error bars showing the mean change (with its 95% confidence interval) in Parasiticidal effect (rate of Parasitic killing) after a dosage of 25 mg of additive compared to baseline (control) between 4 study groups.

### 3.3.5 After 30 mg concentration

There was a statistically significant difference in mean parasitocidal effect after 30 mg concentration between the 4 types of tested additives. The lowest parasitocidal effect was observed with Syringic acid ( $49 \times 10^4$  promastigote/ml). It will therefore be used as the reference (comparison) group. The strongest effect was observed with Caffeic acid ( $58 \times 10^4$  promastigote/ml), resulting in a Cohen's d effect size of (1.5), which is significantly higher compared to the reference group. Both Ferulic and 4-hydroxybenzoic acid showed a slightly higher parasitocidal effect ( $54 \times 10^4$  promastigote/ml), which was not significantly higher than that of the reference group (Syringic acid). The Cohen's d effect size for both groups was of small magnitude (0.8, 0.5) respectively, table (3.18) and figure (3.15).

**Table 3.18: Table 3.19: The difference in mean Parasitocidal effect (rate of Parasitic killing) after a dosage of 30 mg of additive between 4 study groups(  $\times 10^4$  promastigote/ml) .**

Parasitocidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
After 30 mg dose					0.003
Mean	58	54	49	54	
SE	1.33	1.43	1.16	2.58	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid = 1[NS]

Caffeic acid X Syringic acid = 0.002

Effect size (Cohen's d) of Caffeic acid compared to Syringic acid=1.5

Caffeic acid X 4-Hydroxybenzoic acid = 0.77[NS]

Ferulic acid X Syringic acid = 0.11[NS]

Effect size (Cohen's d) of Ferulic acid compared to Syringic acid=0.8

Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Syringic acid X 4-Hydroxybenzoic acid = 0.17[NS]

Effect size (Cohen's d) of 4-Hydroxybenzoic acid compared to Syringic acid=0.5

**Table 3.20: The difference in mean change in Parasitocidal effect (rate of Parasitic killing) after a dosage of 30 mg of additive compared to baseline (control) between 4 study groups (  $\times 10^4$  promastigote/ml) .**

Parasitocidal effect (rate of Parasitic killing)	Caffeic acid	Ferulic acid	Syringic acid	4Hydroxybenzoic acid	P (ANOVA)
Change after 30 mg dose compared to baseline					0.007
Mean	53	50	44	48	
SE	1.59	1.51	1.3	2.5	

P (Bonferroni t-test) for difference in mean between:

Caffeic acid X Ferulic acid = 1[NS]

Caffeic acid X Syringic acid = 0.004

Effect size (Cohen's d) of Caffeic acid compared to Syringic acid=1.3

Caffeic acid X 4-Hydroxybenzoic acid = 0.6[NS]

Ferulic acid X Syringic acid = 0.12[NS]

Effect size (Cohen's d) of Ferulic acid compared to Syringic acid=0.9

Ferulic acid X 4-Hydroxybenzoic acid = 1[NS]

Syringic acid X 4-Hydroxybenzoic acid = 0.38[NS]

Effect size (Cohen's d) of 4-Hydroxybenzoic acid compared to Syringic acid=0.4

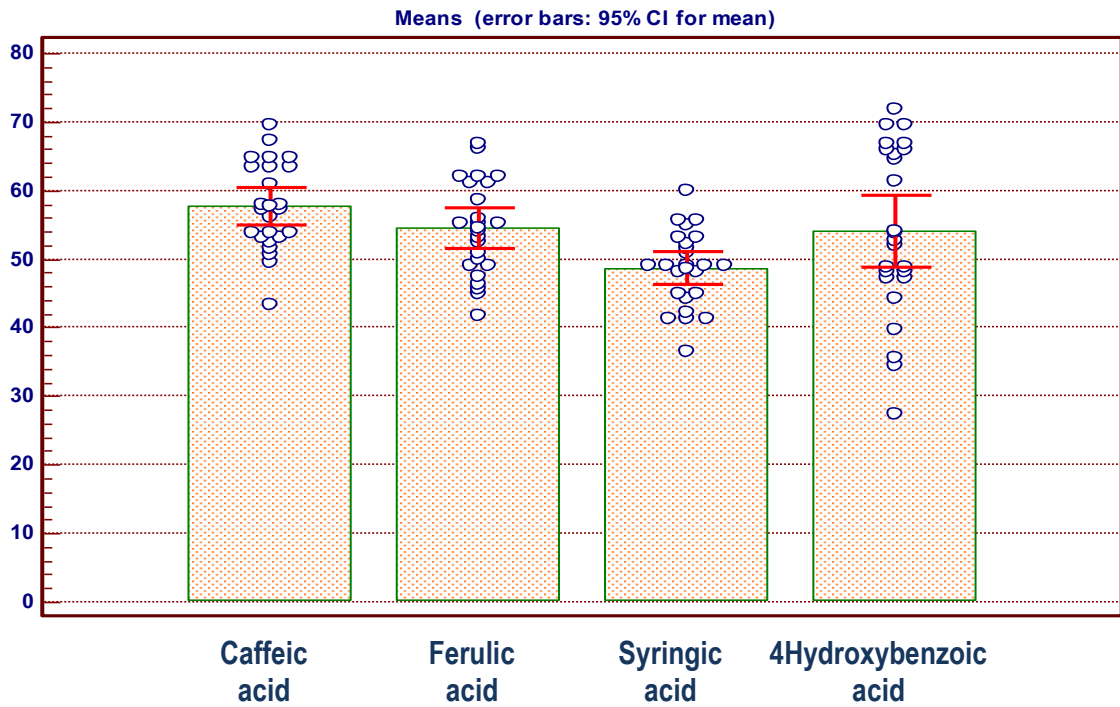


Figure 3.15: Dot diagram with error bars showing the mean (with its 95% confidence interval) Parasiticidal effect (rate of Parasitic killing) after a dosage of 30 mg of additive between 4 study groups.

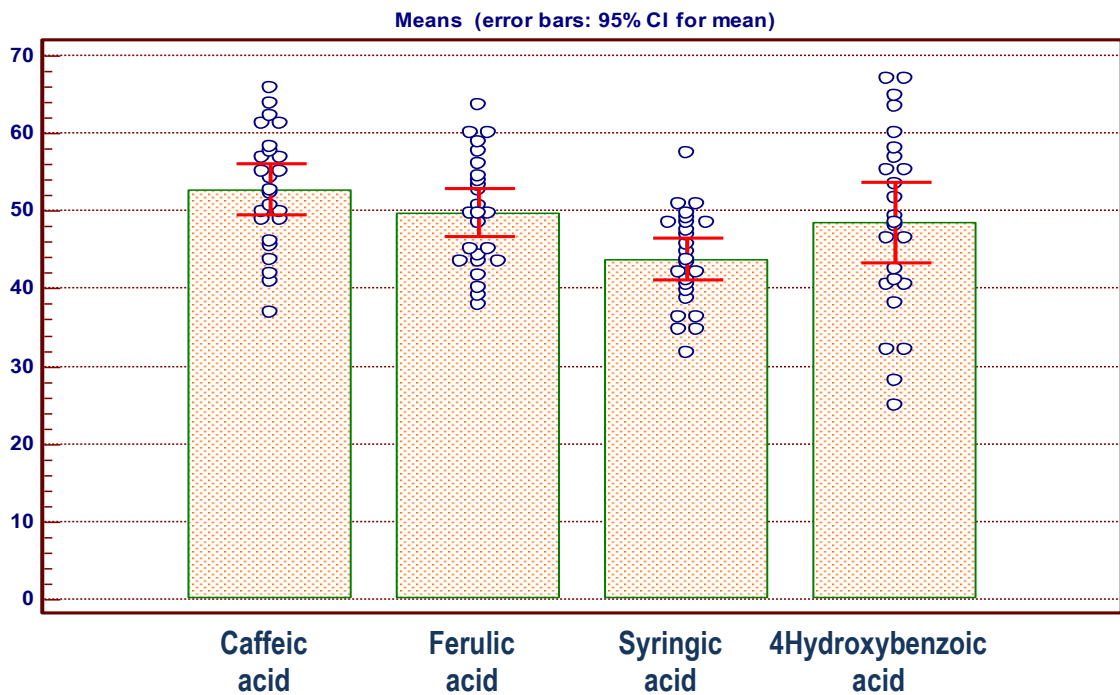


Figure 3.16: Dot diagram with error bars showing the mean change (with its 95% confidence interval) in Parasiticidal effect (rate of Parasitic killing) after a dosage of 30 mg of additive compared to baseline (control) between 4 study groups.



Descriptive tables that should be taken to appendix, because they only help to display the original data (table 3.12 to 3.17)

## Appendix A

**Table 3.21: (The number of parasite  $\times 10^4$  promastigote/ml)**

<b>Caffeic acid</b>	<b>Count of living Parasites</b>	<b>Count of dead Parasites</b>	<b>Total count of Parasites</b>	<b>Parasitocidal effect (rate of Parasitic killing)</b>
At baseline				
Mean	5030	264	5294	5
SE	116.74	24.75	111.95	0.51
N	24	24	24	24
After 10 mg dose				
Mean	3027	1227	4253	29
SE	136.55	52.35	157.66	1.46
N	24	24	24	24
After 15 mg dose				
Mean	2587	1352	3939	35
SE	104.86	44.65	121.58	1.2
N	24	24	24	24
After 20 mg dose				
Mean	2451	1872	4323	43
SE	84.09	72.4	79.32	1.6
N	24	24	24	24
After 25 mg dose				
Mean	2296	2203	4499	49
SE	62.75	62.46	61.57	1.27
N	24	24	24	24
After 30 mg dose				
Mean	1979	2707	4685	58
SE	69.76	80.85	76.46	1.33
N	24	24	24	24

**Table 3.22: (The number of parasite  $\times 10^4$  promastigote/ml)**

<b>Ferulic acid</b>	<b>Count of living Parasites</b>	<b>Count of dead Parasites</b>	<b>Total count of Parasites</b>	<b>Parasitocidal effect (rate of Parasitic killing)</b>
<b>At baseline</b>				
Mean	5142	259	5400	5
SE	91.03	23.43	99.85	0.42
N	24	24	24	24
<b>After 10 mg dose</b>				
Mean	3453	931	4384	21
SE	49.1	37.35	65.15	0.7
N	24	24	24	24
<b>After 15 mg dose</b>				
Mean	2947	1152	4099	28
SE	35.2	34.24	55.62	0.6
N	24	24	24	24
<b>After 20 mg dose</b>				
Mean	2680	1496	4176	36
SE	49.23	30.77	56.39	0.65
N	24	24	24	24
<b>After 25 mg dose</b>				
Mean	2011	1739	3749	46
SE	68.15	73.87	97.76	1.26
N	24	24	24	24
<b>After 30 mg dose</b>				
Mean	1763	2108	3871	54
SE	68.04	78.24	92.02	1.43
N	24	24	24	24

**Table 3.23: (The number of parasite  $\times 10^4$  promastigote/ml)**

<b>Syringic acid</b>	<b>Count of living Parasites</b>	<b>Count of dead Parasites</b>	<b>Total count of Parasites</b>	<b>Parasitocidal effect (rate of Parasitic killing)</b>
<b>At baseline</b>				
Mean	4941	251	5192	5
SE	93.97	20.37	95.27	0.38
N	24	24	24	24
<b>After 10 mg dose</b>				
Mean	3325	867	4192	21
SE	53.17	25.55	57.14	0.57
N	24	24	24	24
<b>After 15 mg dose</b>				
Mean	3088	1147	4235	27
SE	41.18	38.12	57.74	0.7
N	24	24	24	24
<b>After 20 mg dose</b>				
Mean	2576	1365	3941	35
SE	37.4	37.28	54.33	0.7
N	24	24	24	24
<b>After 25 mg dose</b>				
Mean	2221	1691	3912	43
SE	60.36	73.99	95.25	1.25
N	24	24	24	24
<b>After 30 mg dose</b>				
Mean	2069	1968	4037	49
SE	64.42	75.71	99.71	1.16
N	24	24	24	24

**Table 3.24: (The number of parasite  $\times 10^4$  promastigote/ml)**

<b>4-Hydroxybenzoic acid</b>	<b>Count of living Parasites</b>	<b>Count of dead Parasites</b>	<b>Total count of Parasites</b>	<b>Parasiticidal effect (rate of Parasitic killing)</b>
<b>At baseline</b>				
Mean	5085	299	5384	6
SE	64.8	33.29	62.15	0.61
N	24	24	24	24
<b>After 10 mg dose</b>				
Mean	4051	947	4998	19
SE	79.93	58.31	107.37	1.02
N	24	24	24	24
<b>After 15 mg dose</b>				
Range	(2816 to 3968)	(704 to 1792)	(3648 to 5312)	(17.1 to 36.2)
Mean	3475	1301	4776	27
SD	347.7	276.5	413.4	5.1
SE	70.98	56.44	84.38	1.04
N	24	24	24	24
<b>After 20 mg dose</b>				
Mean	3080	1780	4860	36
SE	114.95	118.96	118.32	2.09
N	24	24	24	24
<b>After 25 mg dose</b>				
Mean	2424	2144	4568	46
SE	93.68	134.72	111.77	2.31
N	24	24	24	24
<b>After 30 mg dose</b>				
Mean	2051	2429	4480	54
SE	110.88	127.85	89.35	2.58
N	24	24	24	24

**Table 3.25: (The number of parasite  $\times 10^4$  promastigote/ml)**

<b>Sodium stibogluconate</b>	<b>Count of living Parasites</b>	<b>Count of dead Parasites</b>	<b>Total count of Parasites</b>	<b>Parasiticidal effect (rate of Parasitic killing)</b>
At baseline				
Mean	2728	246	2974	8
SE	53.35	20.63	56.39	0.65
N	24	24	24	24
After 20 $\mu\text{g}$ dose				
Mean	431	2888	3319	87
SE	18.11	62.8	65.95	0.55
N	24	24	24	24