Chapter III

Result

3.1 Result of shoot induction and plantlet regeneration of *Nyctanthes arbor-tristis* L.

The excised internodes were cultured on MS medium containing with BA 0.1, 0.2, 0.3, 0.4, 0.5 mg/l, NAA 0.1, 0.2, 0.3, 0.4, 0.5 mg/l, sucrose 3%(w/v) and 0.8%(w/v) agar. After that subculture were carried out intervals of four weeks. The shoot induction was first observed in some treatment of internode within 8 weeks. Shoot formation were developed to small leaf and elongated from lateral buds for longer time. However, shoot tip were not regenerated for shoot and developed to pale-brown. Subsequently MS medium with BA 0.4 mg/l were induced 90% of shoot with highest yield. Furthermore BA 0.2, 0.3, 0.1, 0.5 mg/l were induced 30%, 30%, 40%, 40% of shoot with less significance, respectively. Nevertheless, only internodes cultured on GD medium with BA 0.2, 0.5 mg/l were induced 10% of shoot. On the other hand, BA 0.1, 0.3, 0.4 mg/l and almost of concentration of NAA were not induced.

Table 3.1 Effect of growth regulator by various BA and NAA on shoot induction from internodes in MS medium

Plant medium MS	Growth regulator	Concentration (mg/l)	No. of shoot	% of shoot
1	BA	0.1	3	30
2		0.2	4	40
3		0.3	4	40
4		0.4	9	90
5		0.5	3	30
6	NAA	0.1	2	20
7		0.2	1	10
8		0.3	0	0
9		0.4	0	0
10		0.5	0	0

Table 3.2 Effect of growth regulator by various BA and NAA on shoot induction from internodes in GD medium

Plant medium GD	Growth regulator	Concentration (mg/l)	No. of shoot	% of shoot
1	BA	0.1	0	0
2		0.2	1	10
3		0.3	0	0
4		0.4	0	0
5		0.5	1	10
6	NAA	0.1	0	0
7		0.2	0	0
8		0.3	0	0
9		0.4	0	0
10		0.5	0	0

3.2 Increasing of multiple shoots

Shoots were transferred to MS medium containing various concentration of BA and NAA for increasing multiple shoots after 4 weeks. The shoot were exhibited a cluster of green-bulb and were developed to leaflet. Then they were elongated to multiple shoots. The MS medium with BA 0.4 mg/l were established (average of shoot) at 5. Although MS medium with BA 0.4 mg/l and NAA 0.1 mg/l were higher (average of shoot) yield at 10. However, concentration of NAA to 0.2, 0.3, 0.4 mg/l were raised, multiple shoots were decreased. In addition to BA 0.3 mg/l and various concentration of NAA 0, 0.1, 0.2, 0.3, 0.4 mg/l were not observed shooting.

Table 3.3 Growth effect by regulator such as various BA and NAA, on shoot induction in MS medium for multiple shoots

Plant medium MS	Concentration BA (mg/l)	Concentration NAA (mg/l)	Average of shoot/tissues ± SD
1	0.4	0	5 ± 1
2	0.4	0.1	9 ± 1
3	0.4	0.2	3 ± 2
4	0.4	0.3	2 ± 1
5	0.4	0.4	1 ± 1
6	0.3	0	1 ± 1
7	0.3	0.1	1 ± 0
8	0.3	0.2	1 ± 0
9	0.3	0.3	1 ± 1
10	0.3	0.4	1 ± 0

3.3 Root induction and growth of seedling

Multiple shoots were used to obtain rooting in MS medium with NAA, BA and activated charcoal. The results were shown in Table 3.4 after 4 weeks. The shoots were wilted and leaf were fallen. After 6 weeks, shoots were germinated to leaflet in all conditions. MS medium with NAA 0.5 mg/l, BA 0.4 mg/l, MS without plant growth regulators and without activated charcoal produced callus at surface of medium and shoots. Subsequently condition with activated charcoal, shoot tip were changed to intense yellow. Therefore, all condition of medium were not induced roots and growth of plantlet.

Table 3.4 Influence of MS medium with growth regulator for roots induction

Plant medium	Concentration NAA (mg/l)	Concentration BA (mg/l)	Activated charcoal (g/l)	No. of root	% of root
MS	-	-	-	0	0
MS	-	-	1.0	0	0
MS	0.5	-	-	0	0
MS	0.5	0.4	-	0	0
MS	0.5	-	1.0	0	0
MS	0.5	0.4	1.0	0	0

Further rooting experiments were performed by transferring the regenerated shoot into MS culture with various concentration of 2,4-D and activated charcoal. Roots were observed initially after 4 weeks in MS medium with 2,4-D 0.1 mg/l as shown in Table 3.5 with 60% of rooting. Root induction were reduced when concentration of 2,4-D were increased (0.2 and 0.3 mg/l). The similar result of no root induction was found in MS medium containing activated charcoal.

Table 3.5 Influence of MS medium with 2,4-D for roots induction

Plant medium	Concentration 2,4-D (mg/l)	Activated charcoal (g/l)	No. of root	% of root
MS	0.1	-	6	60
MS	0.2	-	2	20
MS	0.3	-	0	0
MS	0.1	1.0	0	0
MS	0.2	1.0	0	0
MS	0.3	1.0	0	0

Figure. 3.1 Shoots induction from internode explants of *Nyctanthes arbor-tristis* L. supplemented with MS and BA 0.4 mg/l within 8 weeks.

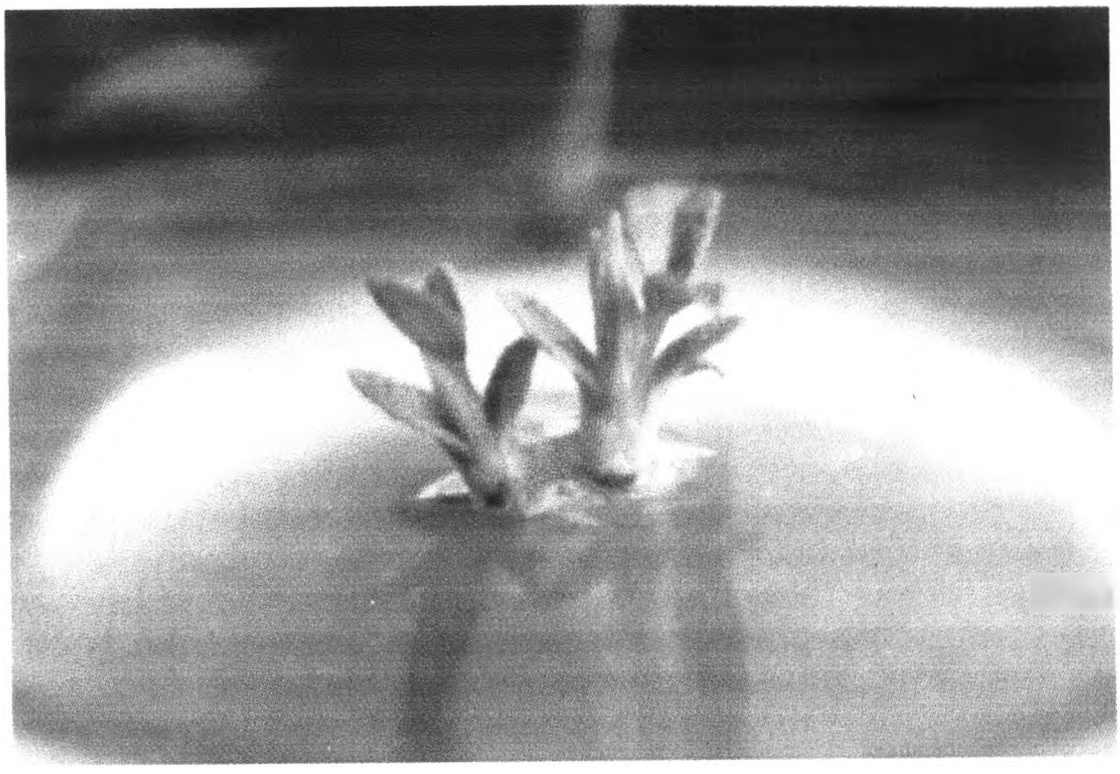


Figure. 3.2 Multiple shoots of *Nyctanthes arbor-tristis* L. supplement with MS, BA 0.4 mg/l and NAA 0.1 mg/l within 4 weeks from shoots induction

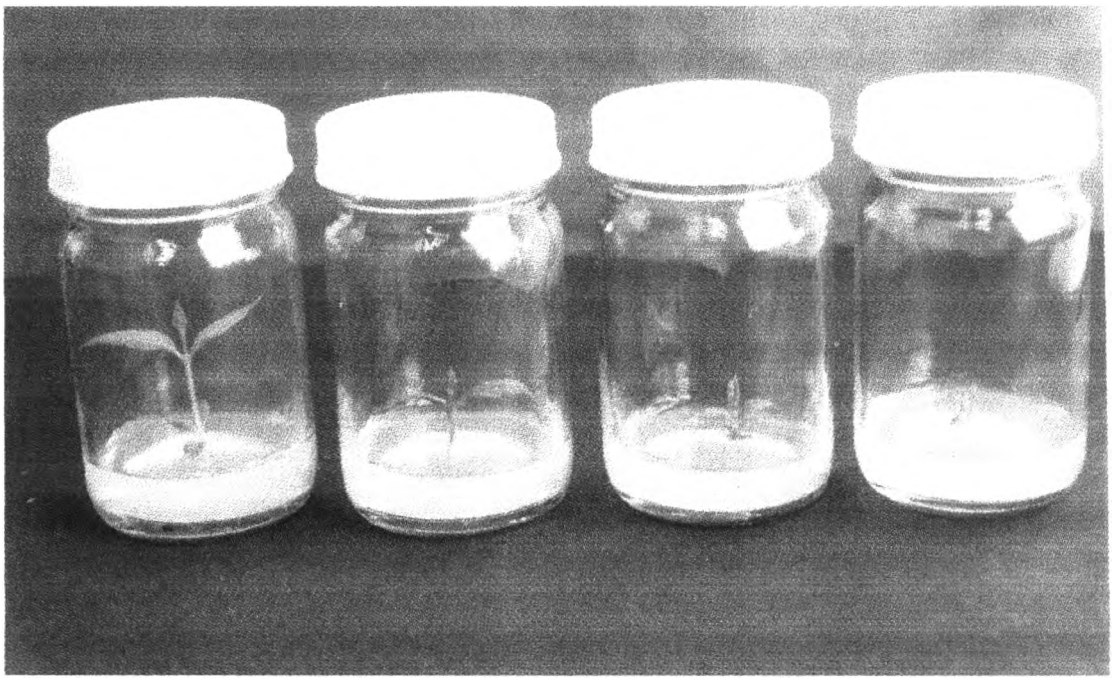


Figure. 3.3 Subculture of multiple shoots within 4, 6, 8, and 12 weeks from *Nyctanthes arbor-tristis* L. supplement with MS, BA 0.4 mg/l and NAA 0.1 mg/l

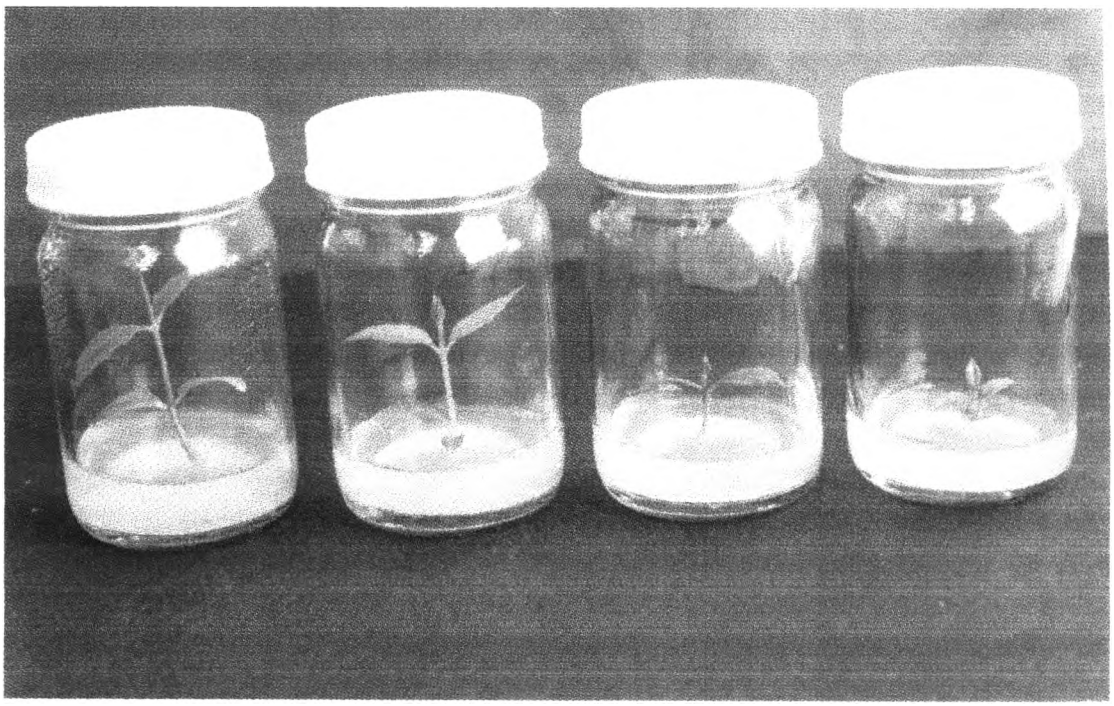


Figure. 3.4 Subculture of multiple shoots within 6, 8, 12, and 16 weeks from *Nyctanthes arbor-tristis* L. supplement with MS, BA 0.4 mg/l and NAA 0.1 mg/l

Figure. 3.5 Roots induction of *Nyctanthes arbor-tristis* L. supplement with MS and 0.1mg/l 2,4-D within 8 weeks from shoots culture

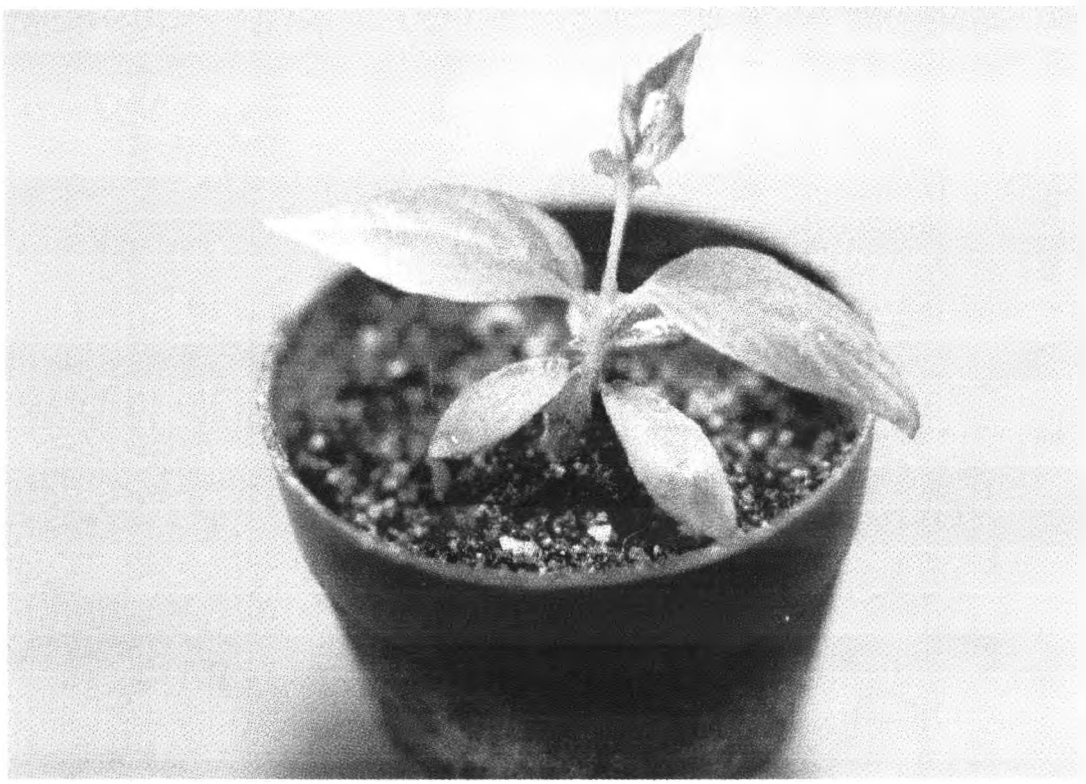


Figure. 3.6 Plantlet of *Nyctanthes arbor-tristis* L. from tissue cultures in nursery

3.4 Preparation of crude extracts from *Nyctanthes arbor-tristis* L.

The leaves and stems were dried completely in hot air oven with controlling at 50 °C for 48 hr. After that they were extracted with organic solvent according to the procedure described in Chapter II. The results of extraction were shown in Table 3.6

Table 3.6 Yield of crude extract in mother plants of *Nyctanthes arbor-tristis* L.

Plant part	Fresh weight (g)	Dry weight (g)	Total mass (g)	Yield (g)
Leaves	1,200	43	10.75	1.03
Stems	920	32	8.85	0.65

The leaves and stems were collected from *in vitro* and dried in hot air oven at 50 °C for 48 hr. The results of extraction were shown in Table 3.7

Table 3.7 Yield of crude extract from tissue culture of *Nyctanthes arbor-tristis* L.

Plant part	Fresh weight (g)	Dry weight (g)	Total mass (g)	Yield (g)
Leaves	10.32	0.86	0.227	0.018
Stems	9.68	0.64	0.194	0.015

Chemical constituents of crude extract by Thin Layer Chromatography

Preliminary study on the chemical constituents of crude extract from the leaves and stems was reported base on TLC system on silicagel plates in the solvent systems: (I) benzene – ethyl acetate (4:1) and solvent systems: (II) Toluene - ethyl acetate (93:7). The chromatograms were sprayed with H_2SO_4 50% (v/v). The crude extract from leaves and stems (mother plants) were showed two spots with Rf value of 0.22 and 0.27 respectively in solvent systems (II) as shown in Figure 3.7. The spots were scrapped from TLC plates for tested for β-sitosterol, trierpenoid and iridoids. The response to the test for β-sitosterol (changed colour green-blue) and trierpenoid (changed colour red-pink) from libermann-Burchards test method was positive respectively. After that crude extract from leaves and stems (tissue culture) were shown a spots with Rf value of 0.36 in solvent systems (II). The response to the test for β-sitosterol (changed colour green-blue) was positive respectively. However, all samples were shown no response to test for iridoids.

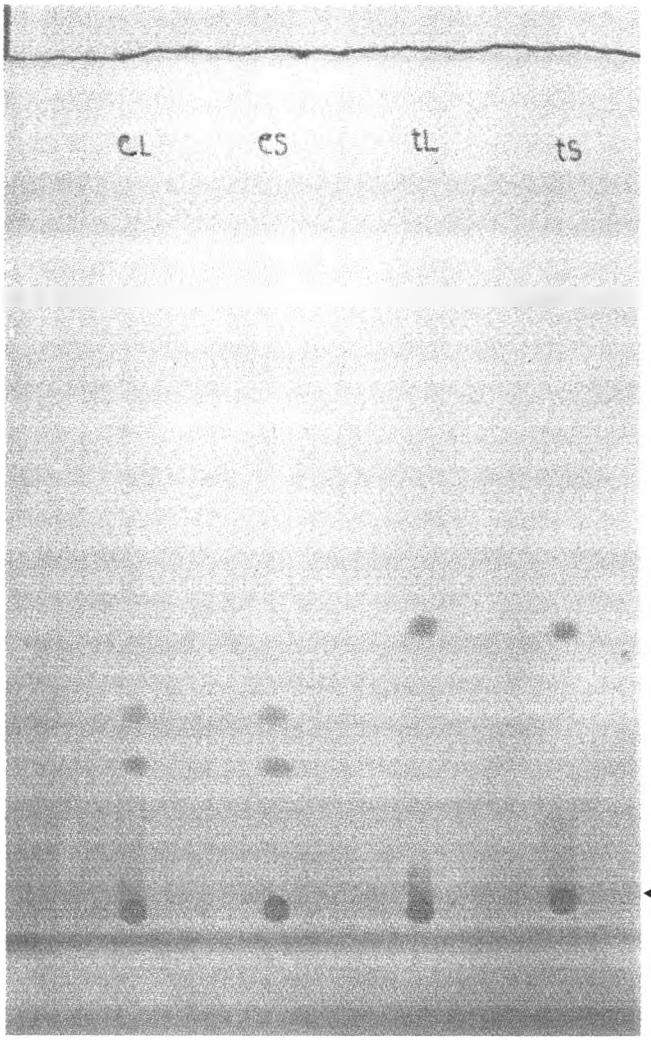


Figure. 3.7 TLC for crude extracts of *Nyctanthes arbor-tristis* L.

el: Crude extract from mother plants (leaves)

es: Crude extract from mother plants (stems)

tl: Crude extract from tissue culture (leaves)

ts: Crude extract from tissue culture (stems)

Qualitative by TLC of carotenoids

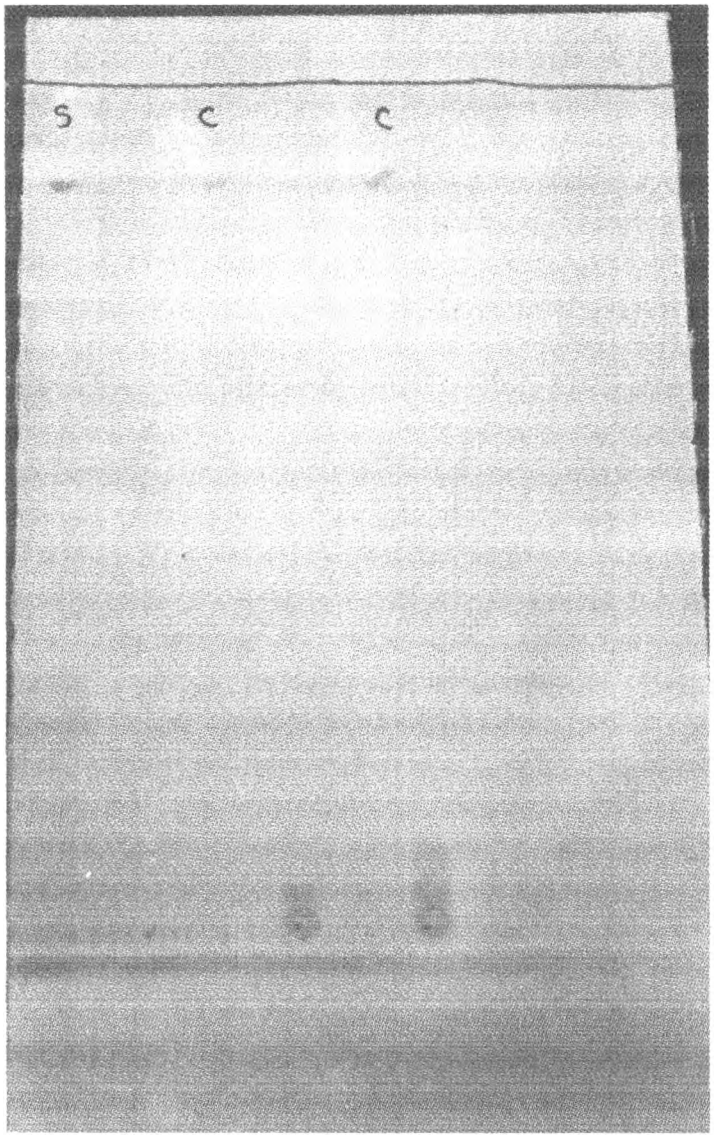


Figure. 3.8 Chromatogram of carotenoids extraction from *Nyctanthes arbor-tristis* L.

S: β-carotene standard

C: Extraction of carotenoids from *Nyctanthes arbor-tristis* L.

Quantitative method of carotenoids extraction

$$C = A/\varepsilon l$$

$$C = 2.608/2575x1$$

$$C = 1.013x10^{-3} \quad g/100\ ml$$

C = Concentration of total carotenoids in grams per 100 ml.

A = Absorbance at 450 nm

ε = Absorption coefficience of β-carotene at 450 nm.

in g/100 ml is 2575

l = Thickness of solution layer in centimeter

3.5 Antimicrobial activity of crude extract from *Nyctanthes arbor-tristis* L.

3.5.1 Antimicrobial activity by Inhibition zone method

Crude extract were dropped in a hole of penicillin carput and control using distilled water instead and incubated at 37 °C for 24 hr. The extract were diluted 0.15 mg/ml, 0.075 mg/ml, 0.0375 mg/ml (two-fold dilution) for 3 times. Clear zone were measured with diameter around a hole of penicillin carput. Antimicrobial test for *E. coli*, *Bacillus subsitlis*, *Aspergillus sp* and *Saccharomyces cerivisae* were shown in Table 3.8, 3.9, 4.0, 4.1 and Figure 3.9, 3.10, 3.11, 3.12, 3.13, 3.14, 3.15, 3.16, 3.17, 3.18, 3.19, 3.20, 3.21, 3.22, 3.23, 3.24 respectively.

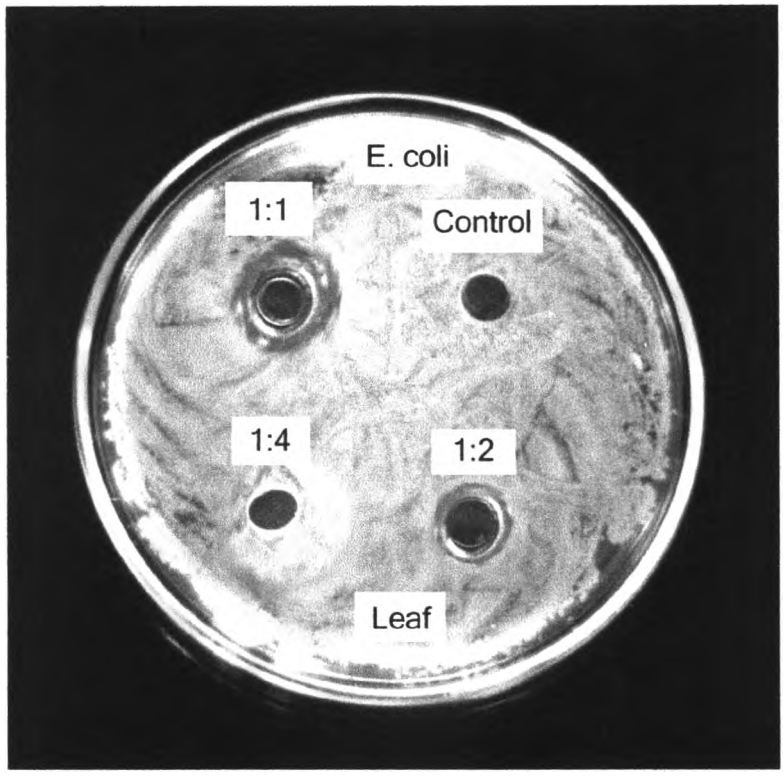


Figure. 3.9 Inhibition zone of crude extract (leaves) from mother plants on *E. coli*

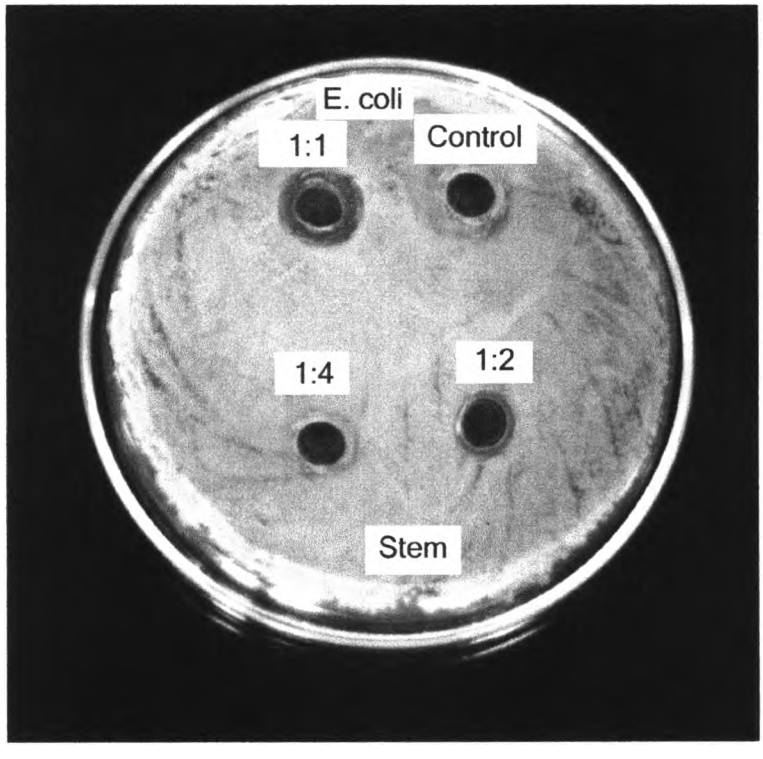
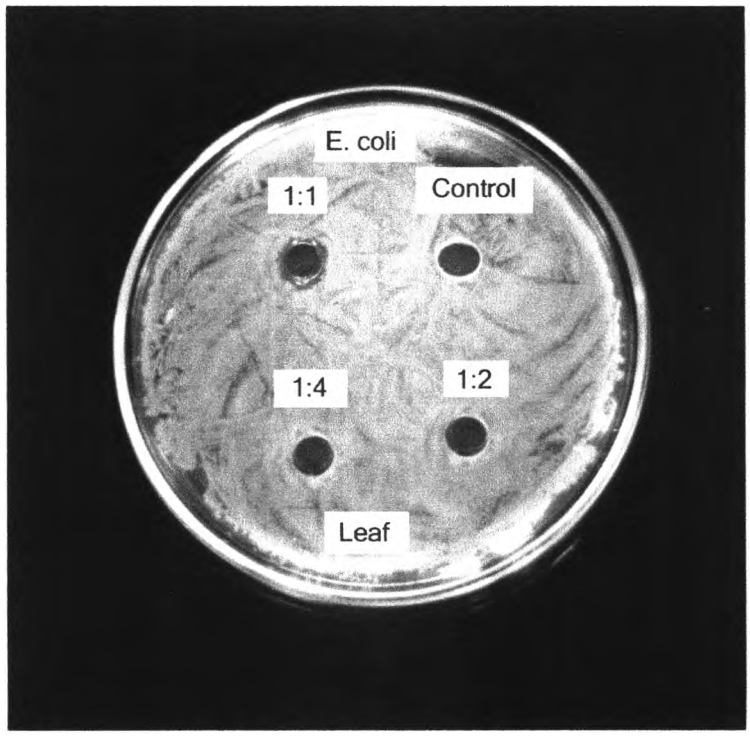


Figure. 3.10 Inhibition zone of crude extract (stems) from mother plants on *E. coli*

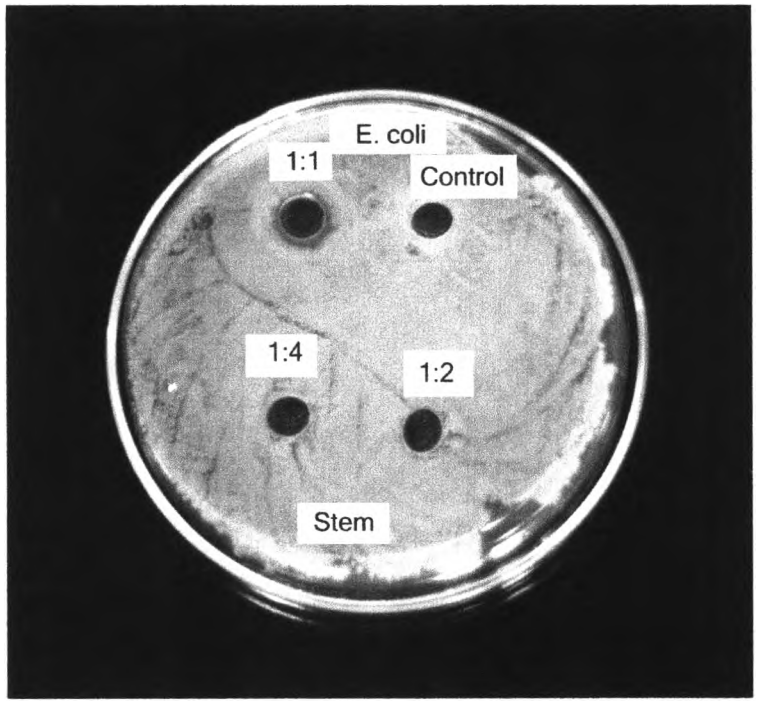


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.11 Inhibition zone of crude extract (leaves) from tissue culture on *E. coli*

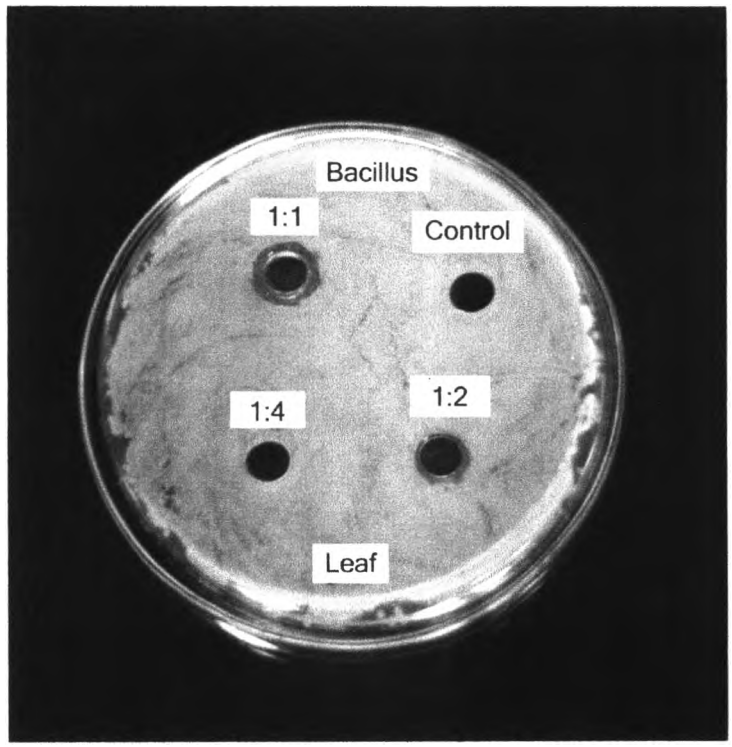


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.12 Inhibition zone of crude extract (stems) from tissue culture on *E. coli*

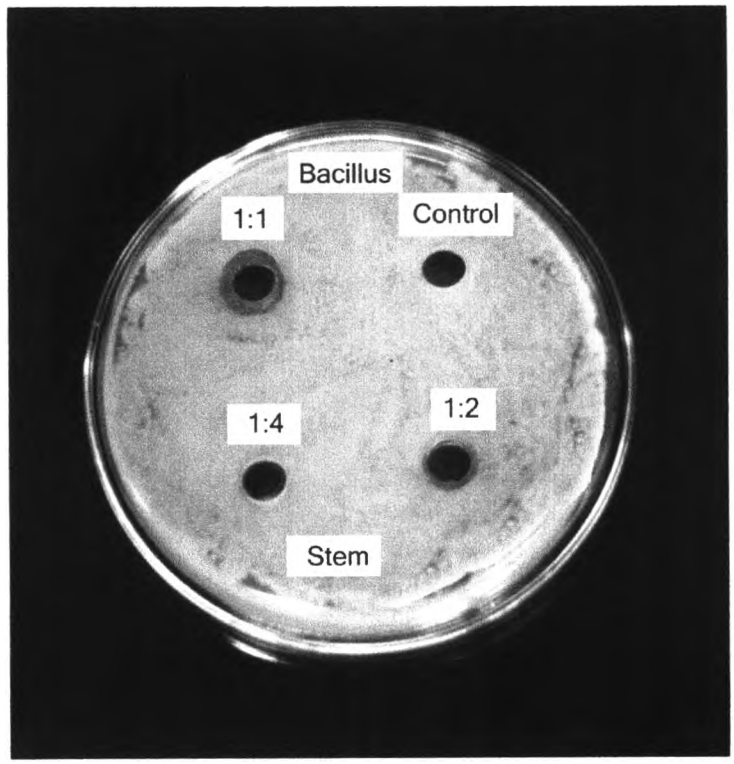


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.13 Inhibition zone of crude extract (leaves) from mother plants on *Bacillus substilis*



1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.14 Inhibition zone of crude extract (stems) from mother plants on *Bacillus substilis*

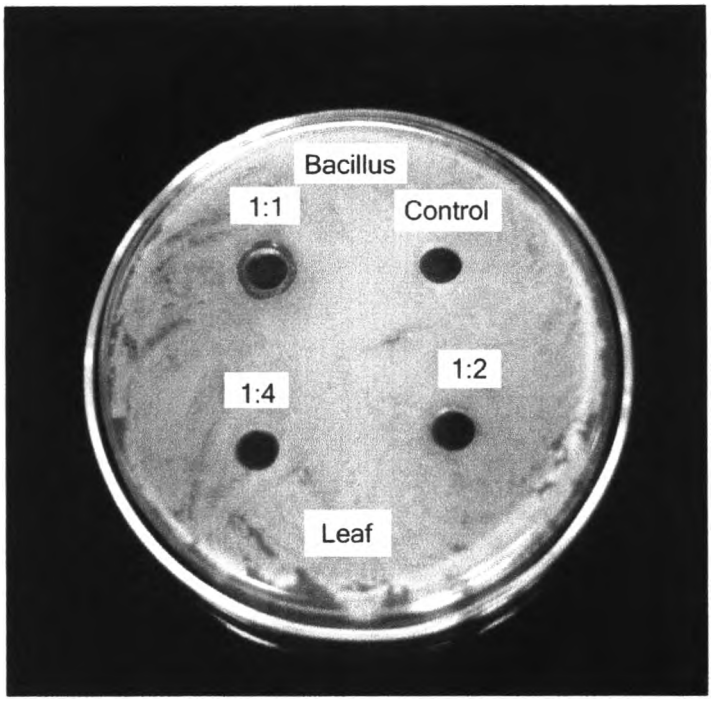


Figure. 3.15 Inhibition zone of crude extract (leaves) from tissue culture on *Bacillus substilis*

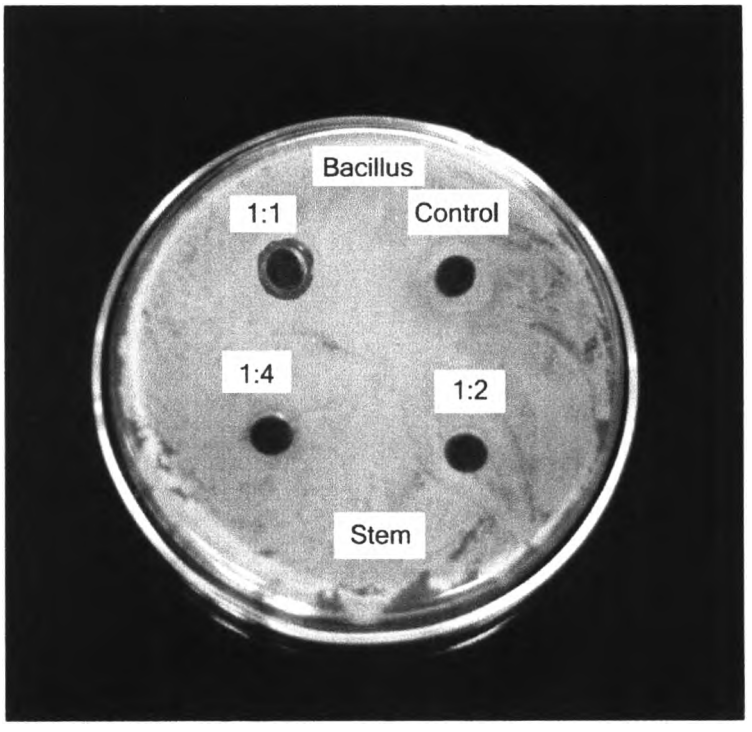
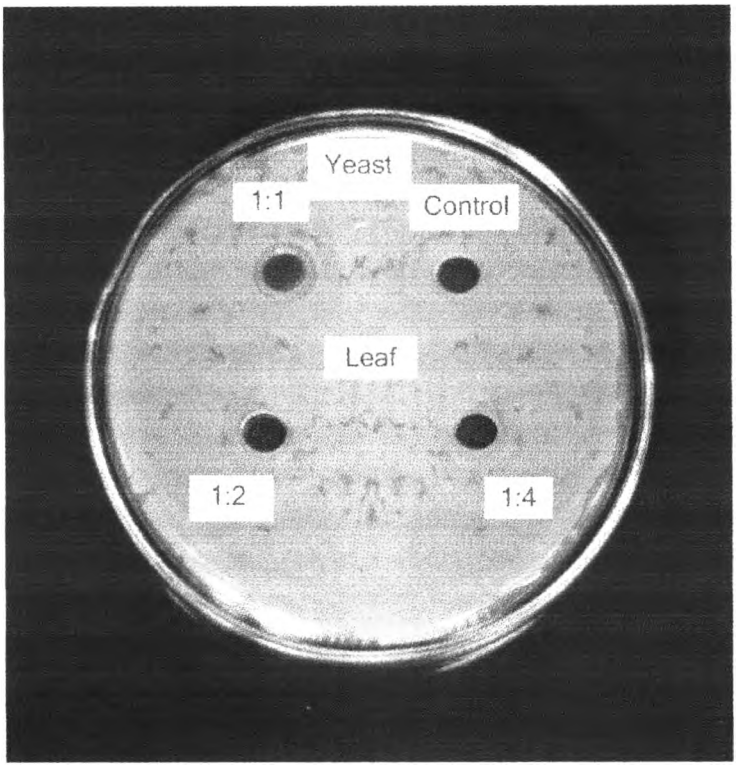


Figure. 3.16 Inhibition zone of crude extract (stems) from tissue culture on *Bacillus substilis*

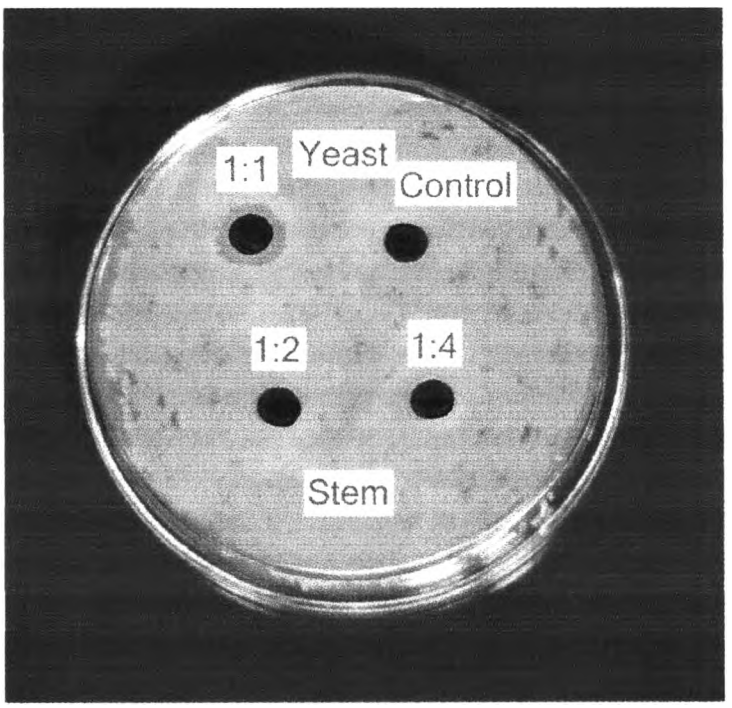


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.17 Inhibition zone of crude extract (leaves) from mother plants on *Saccharomyces cerevisiae*

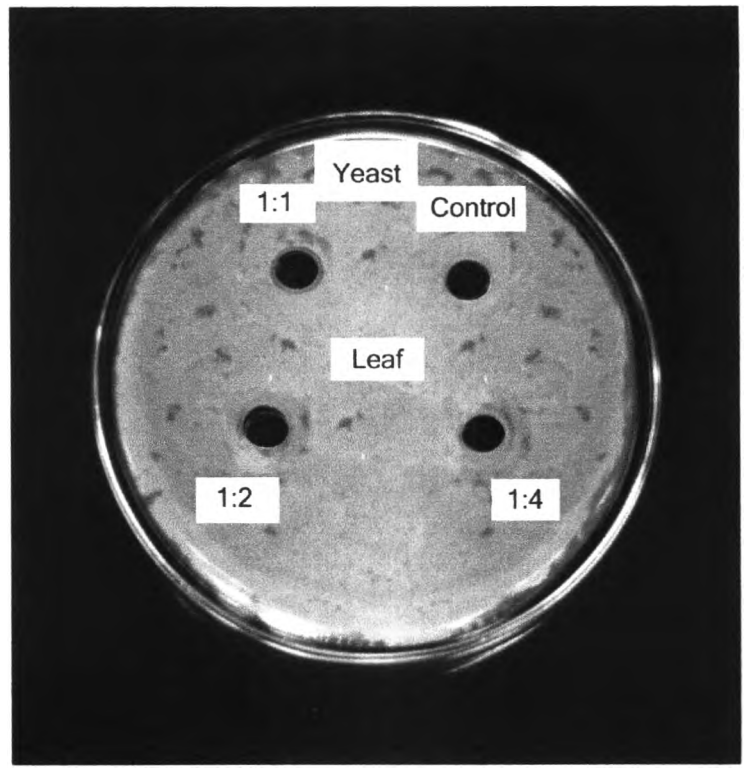


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.18 Inhibition zone of crude extract (stems) from mother plants on *Saccharomyces cerevisiae*

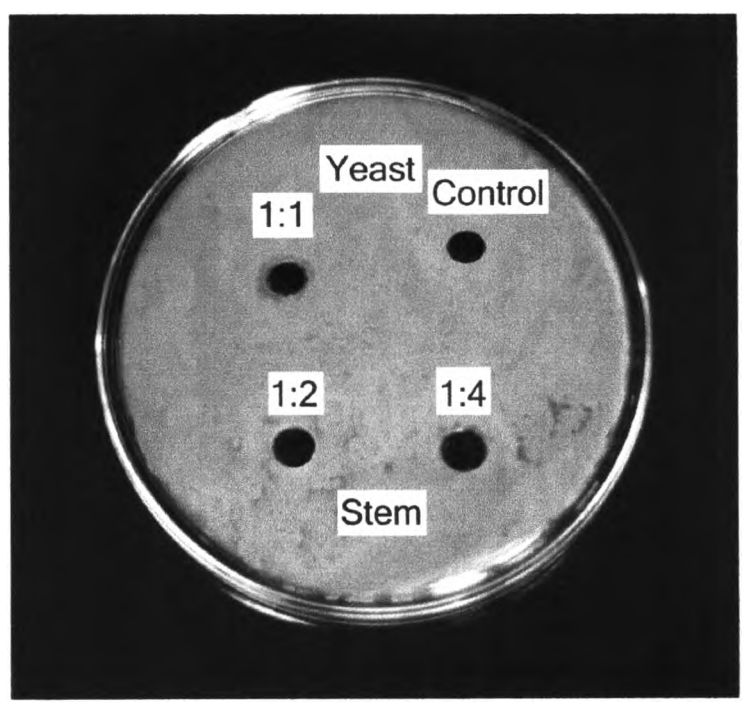


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.19 Inhibition zone of crude extract (leaves) from tissue culture on *Saccharomyces cerevisiae*

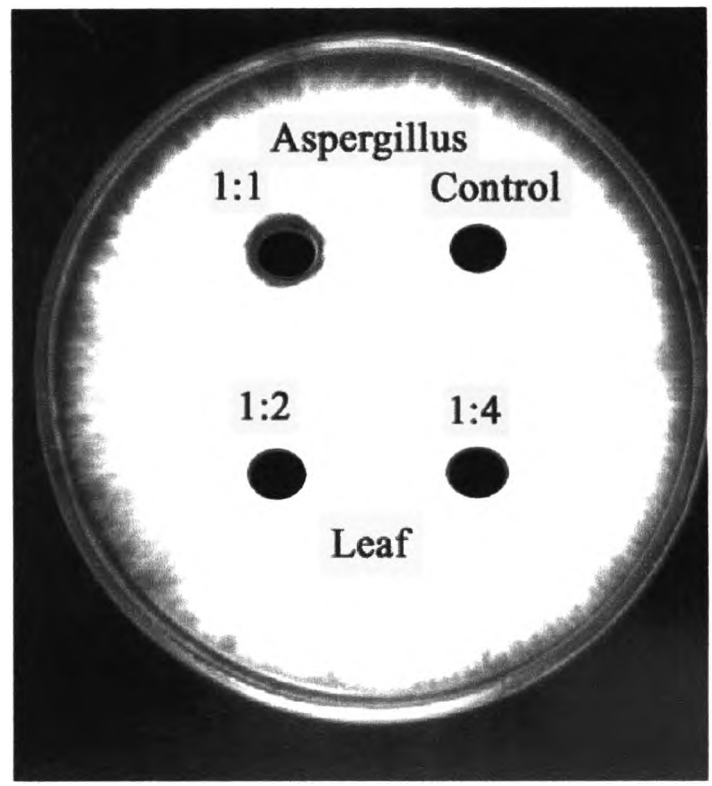


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.20 Inhibition zone of crude extract (stems) from tissue culture on *Saccharomyces cerevisiae*

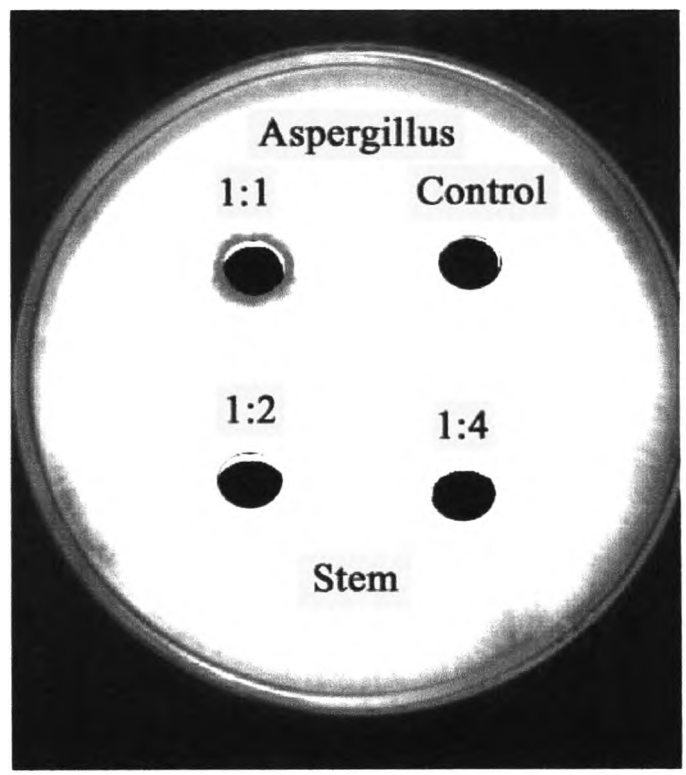


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.21 Inhibition zone of crude extract (leaves) from mother plants on *Aspergillus sp.*

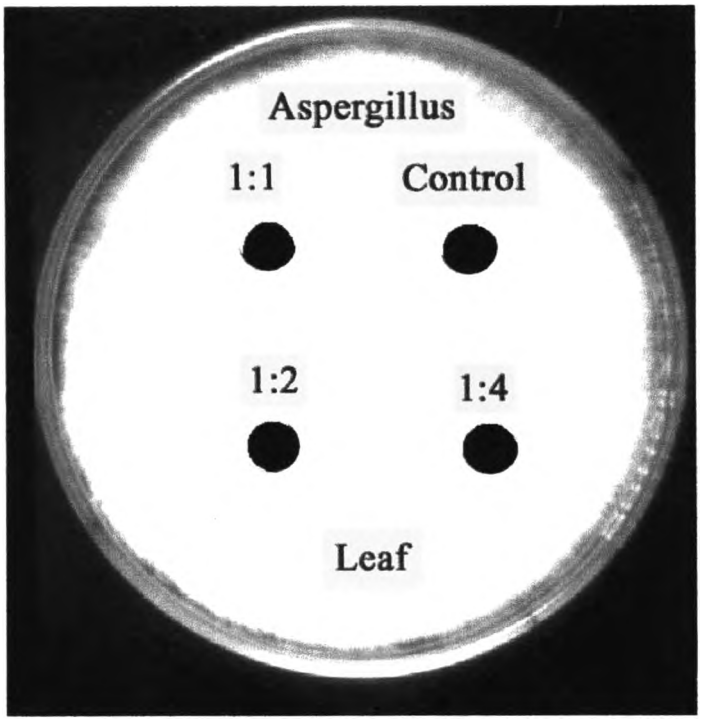


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.22 Inhibition zone of crude extract (stems) from mother plants on *Aspergillus sp.*

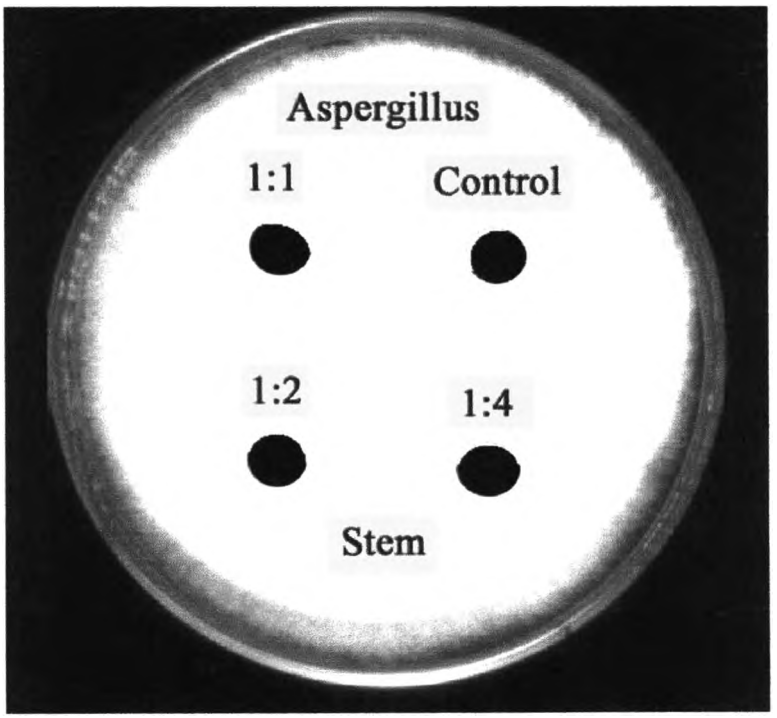


1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.23 Inhibition zone of crude extract (leaves) from tissue culture on *Aspergillus sp.*



1:1- 0.15 mg/ml

1:2- 0.075 mg/ml

1:4- 0.0375 mg/ml

Figure. 3.24 Inhibition zone of crude extract (stems) from tissue culture on *Aspergillus sp.*

Table 3.8 Result of antibacterial activity from crude extract on *E. coli*

Samples	Average diameter (mm.)		
	0.15 mg/ml	0.075 mg/ml	0.0375 mg/ml
Leaves (mother plants)	15.2	8.6	-
Stems (mother plants)	10.3	7.3	-
Leaves (tissue culture)	8.5	-	-
Stems (tissue culture)	7.6	-	-

Table 3.9 Result of antibacterial activity from crude extract on *Bacillus substilis*

Samples	Average diameter (mm.)		
	0.15 mg/ml	0.075 mg/ml	0.0375 mg/ml
Leaves (mother plants)	12.3	10.3	-
Stems (mother plants)	11.4	7.5	-
Leaves (tissue culture)	6.2	-	-
Stems (tissue culture)	5.8	-	-

Table 3.10 Result of antiyeast from crude extract on *Saccharomyces cerevisiae*

Samples	Average diameter (mm.)		
	0.15 mg/ml	0.075 mg/ml	0.0375 mg/ml
Leaves (mother plants)	6.3	-	-
Stems (mother plants)	5.8	-	-
Leaves (tissue culture)	5.2	-	-
Stems (tissue culture)	5.2	-	-

Table 3.11 Result of antifungus from crude extract on *Aspergillus sp*

Samples	Average diameter (mm.)		
	0.15 mg/ml	0.075 mg/ml	0.0375 mg/ml
Leaves (mother plants)	6.2	-	-
Stems (mother plants)	5.3	-	-
Leaves (tissue culture)	-	-	-
Stems (tissue culture)	-	-	-

3.5.2 Antimicrobial activity by Growth inhibition method

Effect of extract from leaves and stems of mother plants and tissue culture on growth of *E. coli*, *Bacillus subsitlis*, *Aspergillus sp.* and *Saccharomyces cerivisae* by following growth at various time as shown from Figure 3.25, 3.26, 3.27 and 3.28.

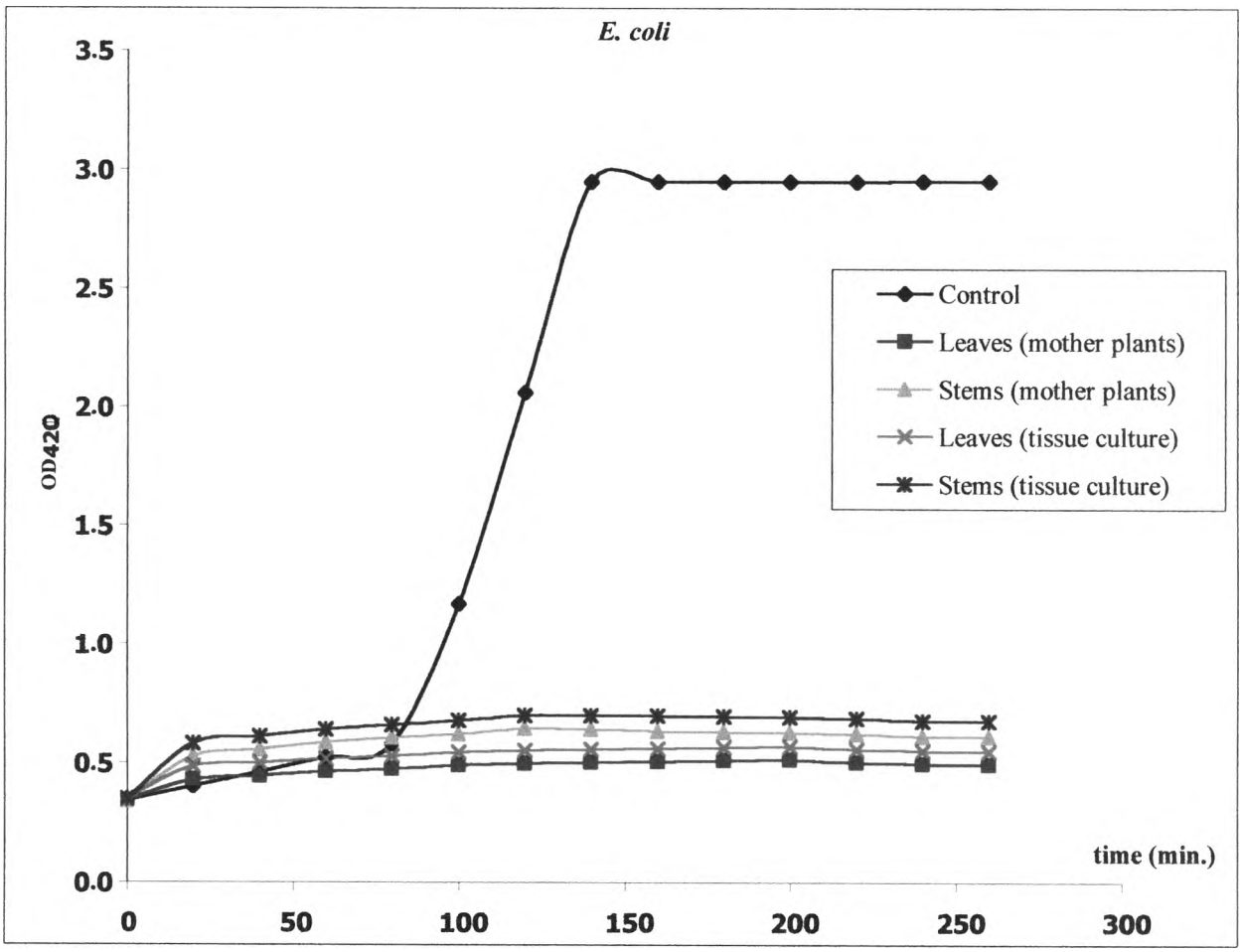


Figure. 3.25 Growth inhibition from crude extract of *Nyctanthes arbor-tristis* L. on *E. coli*

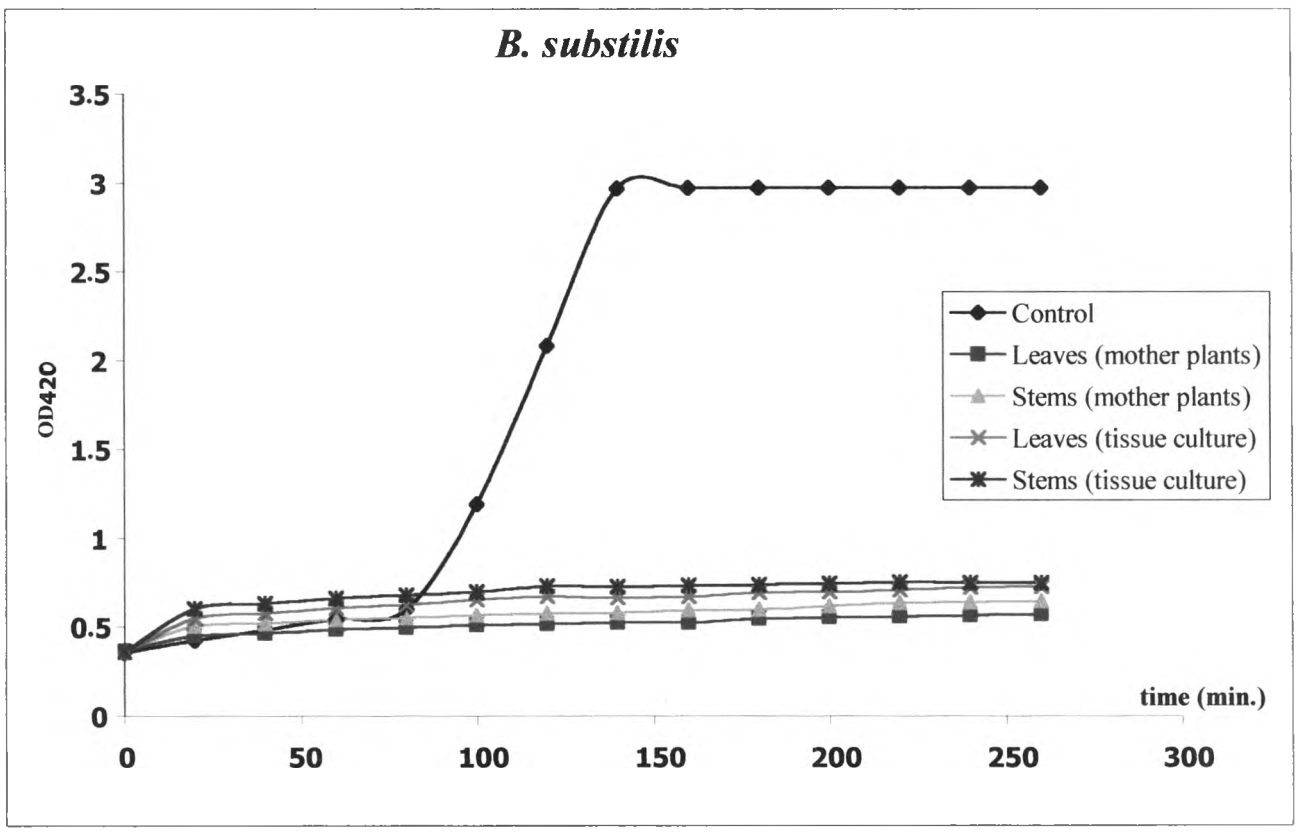


Figure. 3.26 Growth inhibition from crude extract of *Nyctanthes arbor-tristis* L. on *Bacillus substilis*

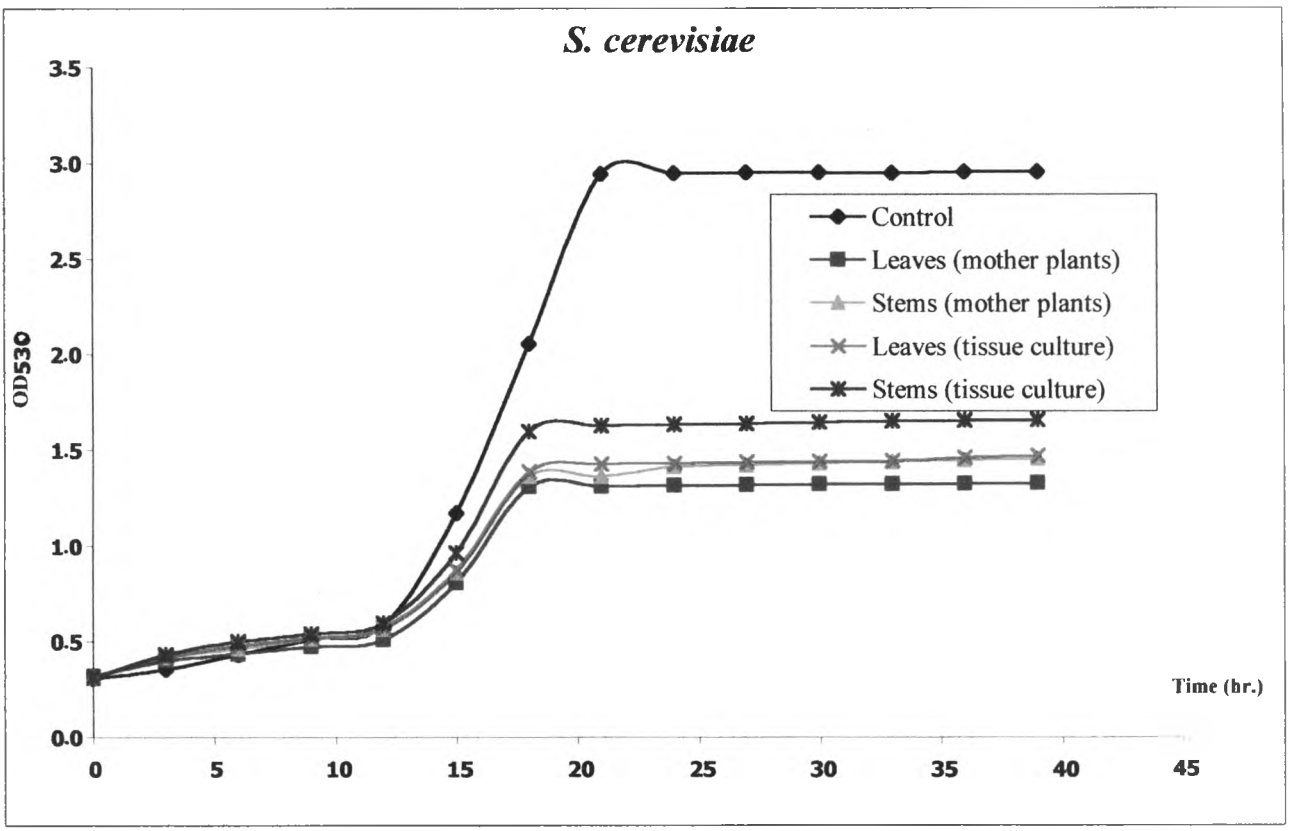


Figure. 3.27 Growth inhibition from crude extract of *Nyctanthes arbor-tristis* L. on *Saccharomyces cerevisiae*

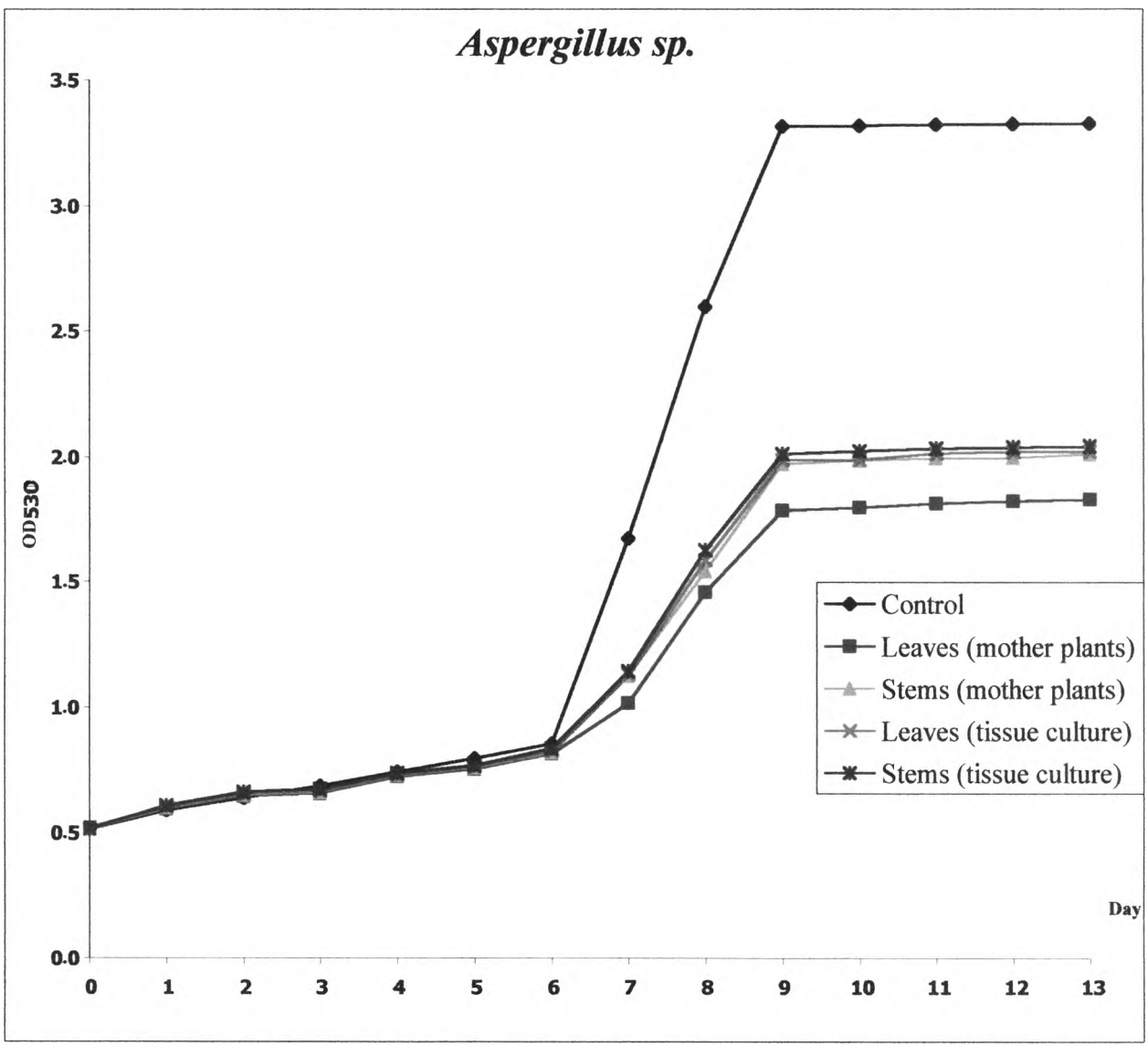


Figure. 3.28 Growth inhibition from crude extract of *Nyctanthes arbor-tristis* L. on *Aspergillus* sp.