PLATE - 5.1

Effect of ethanolic rhizome extract of *Kaempferia galanga* on various bacteria by Disc diffusion method

a. Control

b. *Staphylococcus aureus*

c. Control

d. *Streptococcus faecalis*

e. Control

f. *Escherichia coli*

g. Control

h. *Enterobacter aerogenes*
PLATE - 5.2

Effect of ethanolic rhizome extract of *Kaempferia galanga* on various bacteria by Disc diffusion method

a. Control
b. *Bacillus subtilis*
c. Control
d. *Bacillus cereus*
e. Control
f. *Salmonella typhi*
g. Control
h. *Klebsiella pneumoniae*
Effect of methanolic rhizome extract of *Kaempferia galanga* on various bacteria by Disc diffusion method

a. *Staphylococcus aureus*
b. *Streptococcus faecalis*
c. *Enterobacter aerogenes*
d. *Escherichia coli*
e. *Bacillus subtilis*
f. *Bacillus cereus*
g. *Klebsiella pneumoniae*
h. *Pseudomonas aeruginosa*
Effect of ethanolic leaf extract of *Kaempferia galanga* on various bacteria by Disc diffusion method

a. *Staphylococcus aureus*
b. *Streptococcus faecalis*
c. *Escherichia coli*
d. *Enterobacter aerogenes*
e. *Bacillus cereus*
f. *Bacillus subtilis*
g. *Klebsiella pneumoniae*
h. *Salmonella typhi*
PLATE - 5.5

Effect of methanolic leaf extract of *Kaempferia galanga* on various bacteria
by Disc diffusion method

a. *Staphylococcus aureus*

b. *Streptococcus faecalis*

c. *Bacillus cereus*

d. *Bacillus subtilis*

e. *Escherichia coli*

f. *Enterobacter aerogenes*

g. *Klebsiella pneumoniae*

h. *Pseudomonas aeruginosa*

i. *Salmonella typhi*

j. *Vibrio cholerae*
Effect of ethanolic root extract of *Kaempferia galanga* on various bacteria by Disc diffusion method

a. *Staphylococcus aureus*
b. *Bacillus subtilis*
c. *Klebsiella pneumoniae*
d. *Escherichia coli*
e. *Bacillus cereus*
f. *Streptococcus faecalis*
g. *Salmonella typhi*
h. *Enterobacter aerogenes*
i. *Pseudomonas aeruginosa*
j. *Vibrio cholerae*
PLATE - 5.7

Effect of ethanolic rhizome extract of *Kaempferia galanga* on various bacteria by Agar Well method

a. *Staphylococcus aureus*
b. *Streptococcus faecalis*
c. *Enterobacter aerogenes*
d. *Escherichia coli*
e. *Bacillus subtilis*
f. *Bacillus cereus*
g. *Klebsiella pneumoniae*
h. *Salmonella typhi*
PLATE - 5.8

Effect of ethanolic leaf extract of *Kaempferia galanga* on various bacteria by Agar Well method

a. *Staphylococcus aureus*
b. *Streptococcus faecalis*
c. *Bacillus cereus*
d. *Escherichia coli*
e. *Enterobacter aerogenes*
f. *Bacillus subtilis*
g. *Klebsiella pneumoniae*
h. *Salmonella typhi*
PLATE - 5.9

Effect of ethanolic root extract of *Kaempferia galanga* on various bacteria by Agar Well method

a. *Staphylococcus aureus*
b. *Streptococcus faecalis*
c. *Salmonella typhi*
d. *Pseudomonas aeruginosa*
e. *Enterobacter aerogenes*
f. *Bacillus cereus*
g. *Enterobacter aerogenes*
h. *Bacillus subtilis*
Effect of ethanolic rhizome extract of *Kaempferia galanga* on various bacteria by Streak method

A. *Staphylococcus aureus*

B. *Streptococcus faecalis*
Effect of ethanolic rhizome extract of *Kaempferia galanga* on various bacteria by Streak method

**A. Bacillus subtilis**

**B. Bacillus cereus**
PLATE - 5.12

Effect of ethanolic leaf extract of *Kaempferia galanga* on various bacteria by
Streak method

A. *Staphylococcus aureus*

B. *Streptococcus faecalis*
PLATE - 5.13

Effect of ethanolic root extract of *Kaempferia galanga* on various bacteria by Streak method

A. *Staphylococcus aureus*

B. *Bacillus subtilis*
Effect of ethanolic rhizome extract of *Kaempferia galanga* on various fungi by Disc diffusion method

a. Control

b. *Aspergillus niger*

c. Control
d. *Aspergillus flavus*

e. Control

f. *Aspergillus fumigatus*

g. Control

h. *Candida albicans*
Effect of methanolic rhizome extract of *Kaempferia galanga* on various fungi by Disc diffusion method

a. Control

b. *Aspergillus niger*

c. Control

d. *Aspergillus flavus*

e. Control

f. *Candida albicans*

g. Control

h. *Aspergillus fumigatus*
Effect of ethanolic rhizome extract of *Kaempferia galanga* on various fungi by Agar Well method

a. Control  
b. *Aspergillus flavus*  
c. Control  
d. *Aspergillus niger*  
e. Control  
f. *Aspergillus fumigatus*  
g. Control  
h. *Candida albicans*
Effect of methanolic rhizome extract of *Kaempferia galanga* on various fungi by Agar Well method

a. Control

b. *Aspergillus fumigatus*

c. Control
d. *Candida albicans*

e. Control

f. *Aspergillus flavus*

g. Control

h. *Aspergillus niger*
Effect of ethanolic rhizome extract of *Kaempferia galanga* on various fungi by Streak method

A. *Aspergillus niger*

B. *Aspergillus flavus*
PLATE - 5.19

Minimum Inhibitory Concentration of ethanolic extracts of

*Kaempferia galanga*

a. Rhizome extract

b. Leaf extract
PLATE - 3.1

Collection of different accessions of *Kaempferia galanga* for RAPD analysis

a. KGL 01 - Nileswaram
b. KGL 02 - Kannur
c. KGL 03 - Iritti
d. KGL 04 - Manachira
e. KGL 05 - Bathery
f. KGL 06 - Perinthalmanna
g. KGL 07 - Palakkad
h. KGL 08 - Vellanikkara
Collection of different accessions of *Kaempferia galanga* for RAPD analysis

a. KGL 09 - Mala  
b. KGL 10 - Aroormuzhy  
c. KGL 11 - Kothamangalam  
d. KGL 12 - Kuttikkanam  
e. KGL 13 - Mannanam  
f. KGL 14 - Kanjirapilly  
g. KGL 15 - Varkala  
h. KGL 16 - Palode
PLATE - 3.3

Kerala Political and Physical Map

a. Kerala Political Map showing *Kaempferia galanga* collection sites
b. Kerala physical Map showing Geographical Regions
PLATE - 3.4

RAPD product of sixteen accessions of *Kaempferia galanga* by different primers

a. Primer S - 61
b. Primer S - 62
c. Primer S - 63
PLATE - 3.5

RAPD product of sixteen accessions of *Kaempferia galanga* by different primers

a. Primer S - 64
b. Primer S - 65
c. Primer S - 66
RAPD product of sixteen accessions of *Kaempferia galanga* by different primers

a. Primer S - 68
b. Primer S - 69
c. Primer S - 70
RAPD product of sixteen accessions of *Kaempferia galanga* by different primers

a. Primer S - 71
b. Primer S - 73
c. Primer S - 74
PLATE - 3.8

RAPD product of sixteen accessions of *Kaempferia galanga* by different primers

a. Primer S - 75
b. Primer S - 76
c. Primer S - 80
PLATE - 1.1

Habit of *Kaempferia galanga*

a.  *Kaempferia galanga* with smaller leaves
b.  *Kaempferia galanga* with larger leaves
c.  Flowers
d.  *Kaempferia galanga* with medium sized leaves
e. & f.  *Kaempferia galanga* showing roots, rhizomes and leaves
g.  Leaves
h.  Rhizome
PLATE - 2.1

In vitro multiplication of shoots from rhizome bud explants of *Kaempferia galanga*

a. Multiple shoots proliferation from rhizome bud explants on MS medium supplemented with BAP 1.0 mg/l and KN 3.0 mg/l
b. Multiple shoots proliferation from rhizome bud explants on MS medium supplemented with BAP 1.0 mg/l and KN 3.0 mg/l
c. Multiple shoots proliferation from rhizome bud explants on MS medium supplemented with BAP 1.0 mg/l and KN 3.0 mg/l
d. Elongation of shoots after 20 days of inoculation with two subcultures on same medium with the hormone.
e. Elongation of shoots after 20 days of inoculation with two subcultures on same medium with the hormone.
f. Rooting of *in vitro* elongated shoots in same medium with the same growth regulators.
g. Rooting of *in vitro* elongated shoots in same medium with the same growth regulators.
h. Well developed *in vitro* derived root system and complete plantlet.
i. Regenerated plantlet acclimatized to green house conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1.
j. Acclimatized cups shifted gradually to normal climate, after 3 weeks.
In vitro multiplication of shoots from rhizome disc explants of *Kaempferia galanga*

a. Multiple shoots proliferation from rhizome disc explants on MS medium supplemented with BAP 2.0 mg/l and KN 1.0 mg/l

b. Multiple shoots proliferation from rhizome disc explants on MS medium supplemented with BAP 2.0 mg/l and KN 1.0 mg/l

c. Multiple shoots proliferation from rhizome disc explants on MS medium supplemented with BAP 2.0 mg/l and KN 1.0 mg/l

d. Elongation of shoots on MS + BAP 2 mg/l + NAA 1.0 mg/l.

e. *In vitro* raised single shoot rooted in MS medium containing BAP 2.0 mg/l and KN 1.0 mg/l

f. *In vitro* raised single shoot rooted in MS medium containing BAP 2.0 mg/l and KN 1.0 mg/l

g. *In vitro* raised single shoot rooted in MS medium containing BAP 2.0 mg/l and KN 1.0 mg/l

h. *In vitro* raised multiple shoot rooted in MS medium containing BAP 2.0 mg/l and NAA 1.0 mg/l

i. Regenerated plantlet acclimatized to greenhouse conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1.

j. Well developed plantlets transferred to normal climate, after 5 weeks.
In vitro multiplication of shoots from shoot tip explants of *Kaempferia galanga*

a. Induction of shoots from shoot tip explants on MS medium supplemented with BAP 2.0 mg/l + NAA 1.0 mg/l after 15 days of inoculation.

b. Initiation of multiple shoot buds on same medium, after 20 days of inoculation.

c. Multiplication of shoots on same medium (25 days of culture).

d. Multiplication of shoots on same medium (25 days of culture).

e. Multiplication of shoots on same medium (25 days of culture).

f. Elongation of *in vitro* raised shoots on MS medium augmented with GA$_3$ 1.0 mg/l + BAP 2.0 mg/l.

g. Elongation of *in vitro* raised shoots on MS medium augmented with GA$_3$ 1.0 mg/l + BAP 2.0 mg/l.

h. Elongation of *in vitro* raised shoots on MS medium augmented with GA$_3$ 1.0 mg/l + BAP 2.0 mg/l.

i. *In vitro* raised shoots rooted in half strength MS medium containing NAA 2.0 mg/l.

j. Well developed *in vitro* derived root system and complete plantlet

K. Regenerated plantlet acclimatized to green house conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1.
PLATE - 2.4

Direct shoot regeneration from leaf sheath explants of *Kaempferia galanga*

a. Leaf sheath explants cultured on MS medium supplemented with BAP 2.0 mg/l + NAA 1.0 mg/l.

b. Initiation of shoot buds from leaf sheath explants, after 15 days of culture on same medium with plant growth regulator.

c. Multiplication of shoots (25 days of culture).

d. Elongation of *in vitro* raised shoots on MS medium augmented with BAP 2.0 mg/l + NAA 1.0 mg/l (20 days old elongation medium).

e. Elongation of *in vitro* raised shoots on MS medium augmented with BAP 2.0 mg/l + NAA 1.0 mg/l (20 days old elongation medium).

f. *In vitro* raised single shoot rooted in half strength MS medium containing NAA 2.0 mg/l.

g. Well developed *in vitro* derived root system and complete plantlet

k. Regenerated plantlet acclimatized in paper cup under green house conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1.

l. Well developed plantlets transferred to normal climate, after 6 weeks.
Effect of different concentrations and combinations of plant growth regulators on callus induction, colour and nature of the callus from various explants of *Kaempferia galanga* cultured on MS supplemented medium

a. Induction of callus from rhizome bud explant at 2, 4-D 2.0 mg/l + BAP 1.0 mg/l (within 15 days).

b. Greenish white, friable and nodular callus produced by 2, 4-D 2.0 mg/l + BAP 1.0 mg/l on rhizome bud explants (after 30 days.)

c. Inductions of callus from young leaf explant at NAA 2.0 mg/l + BAP 1.0 mg/l (within 25 days).

d. Yellow green semi friable callus produced by NAA 2.0 mg/l + BAP 1.0 mg/l (within 45 days).

e. Induction of callus from young rhizome disc explants at 2,4-D 2.0 mg/l + BAP 1.5 mg/l (within 20 days.)

f. Pale yellow, friable callus produced by 2, 4-D 2.0 mg/l BAP 1.0 mg/l on rhizome disc explant (within 30 days).

g. Induction of callus from leaf sheath explant at 2,4-D 2.0 mg/l + BAP 1.0 mg/l (within 15 days).

h. Greenish white, more friable callus produced by 2,4-D at 2.0 mg/l + BAP 1.0 mg/l on leaf sheath explants (within 45 days).
PLATE - 2.6

Induction of callus, shoot regeneration and complete plant establishment from rhizome bud explants of *Kaempferia galanga*

a. Callus induction from rhizome bud after two week after culture on MS medium supplemented 2, 4-D 2.0 mg/l + BAP 1.0 mg/l.

b. Callus induction on MS medium fortified with 2, 4-D 2.0 mg/l + BAP 1.0 mg/l after 15 - 20 days of culture.

c. Callus regeneration on MS medium containing BAP 2.0 mg/l + NAA 1.0 mg/l, after a month culture.

d. Callus with regenerated shoots, after 40 days of culture.

e. Elongation of *in vitro* raised shoots on half strength MS medium augmented with GA$_3$ 1.0 mg/l + BAP 1.5 mg/l, after 20 days of elongation medium.

f. *In vitro* raised shoots rooted in MS medium containing NAA 1.0 mg/l.

g. Regenerated plantlet acclimatized to green house conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1.
PLATE - 2.7

Induction of callus, shoot regeneration and complete plant establishment from rhizome disc explants of Kaempferia galanga

a. Induction of callus from rhizome disc explants on MS medium fortified with at 2, 4-D 2.0 mg/l + BAP 1.5 mg/l (within 20 days.)

b. Callus with regenerated shoots on MS medium with NAA 1.0 mg/l + BAP 2.0 mg/l, after 25 days of culture.

c. Callus with regenerated shoots on MS medium with NAA 1.0 mg/l + BAP 2.0 mg/l, after 25 days of culture.

d. Elongation of in vitro raised shoots on MS medium augmented with GA3 1.0 mg/l + BAP 1.5 mg/l, after 20 days of elongation medium.

e. Rooting of in vitro shoots on half strength MS medium containing NAA 1.0 mg/l.

f. Well developed in vitro derived root system and complete plantlet.

g. Regenerated plantlet acclimatized to green house conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1.
Induction of callus, shoot regeneration and complete plant establishment from leaf sheath explants of *Kaempferia galanga*

a. Induction of callus from cut ends of leaf sheath segments on MS medium supplemented with 2,4-D 2.0 mg/l + BAP 1.0 mg/l (within 15 days).

b. Multiple shoots developed from the subcultured callus in MS medium containing BAP 1.5 mg/l + NAA 1.0 mg/l.

c. Multiplication and elongation of shoots on MS medium fortified with GA\textsubscript{3} 1.0 mg/l + BAP 1.5 mg/l, after 20 days of elongation medium.

d. Multiplication and elongation of shoots on MS medium fortified with GA\textsubscript{3} 1.0 mg/l + BAP 1.5 mg/l, after 20 days of elongation medium.

e. Rooting of *in vitro* shoots on half strength MS medium containing NAA 1.0 mg/l.

f. Regenerated plantlet acclimatized to greenhouse conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1.

g. Well developed plantlets transferred to normal climate, after 6 weeks.
PLATE - 2.9

Induction of callus, shoot regeneration and complete plant establishment from leaf explants of *Kaempferia galanga*

a. Leaf cuttings one week after culture on MS medium supplemented with NAA 2.0 mg/l + BAP 1.0 mg/l

b. Induction of callus from cut ends of leaf segments on MS medium supplemented with NAA 2.0 mg/l + BAP 1.0 mg/l, after three week of culture.

c. Multiple shoots developed from the subcultured callus in MS medium containing BAP 2.0 mg/l + NAA 1.0 mg/l.

d. Multiplication and elongation of shoots on MS medium fortified with GA$_3$ 1.0 mg/l + BAP 1.5 mg/l, after 20 days of elongation medium.

e. Multiplication and elongation of shoots on MS medium fortified with GA$_3$ 1.0 mg/l + BAP 1.5 mg/l, after 20 days of elongation medium.

f. Rooting of *in vitro* shoots on half strength MS medium containing NAA 1.0 mg/l.

g. Well developed *in vitro* derived root system and complete plantlet.

h. Well developed plantlets transferred to normal climate with river sand, garden soil and farmyard manure in the ratio of 1:2:1, after 6 weeks.
Acclimatization, hardening and transplantation of in vitro regenerated plantlets of Kaempferia galanga

a. Regenerated plantlets hardened and acclimatized in mist chamber with river sand, garden soil and farmyard manure in the ratio of 1:2:1, after 3 weeks.
b. Acclimatized cups shifted gradually to normal climate, after 6 weeks.
c. Well developed plantlets transferred into nursery bags, after 8 weeks.
d. Regenerated plantlet in pots after three months.
e. Six month old regenerated plant with well developed rhizomes, roots and leaves.
f. Six month old regenerated plant with well developed rhizomes, roots and leaves.
g. Six month old regenerated plants
h. Six month old regenerated plants
PLATE - 2.11

Somatic embryos obtained from both direct and indirect (through callus phase and cell suspension culture) somatic embryogenesis from leaf sheath and rhizome bud explants of *Kaempferia galanga*.

- **a, b & c.** Embryogenic callus showing somatic globular embryos from rhizome bud explants after 3 weeks in culture medium supplemented with BAP 0.5 mg/l and NAA 1.0 mg/l.

- **d, e & f.** Small, round globular somatic embryos directly produced from leaf sheath surface, after 4 weeks in culture medium supplemented with BAP 0.5 mg/l and NAA 1.0 mg/l.

- **h, i, j, k & l.** Mature embryos, dividing stage established in cell suspension culture of MS liquid medium with 0.5 mg/l BAP + 1.0 mg/l NAA, after 4 weeks of culture.

- **m, n, o & p** Scutellar shaped somatic embryos established in cell suspension culture of MS liquid medium with 0.5 mg/l BAP + 1.0 mg/l NAA, after 4 weeks of culture.

- **q, r, s & t.** Banana and club shaped somatic embryos established in cell suspension culture of MS liquid medium with 0.5 mg/l BAP + 1.0 mg/l NAA, after 4 weeks of culture.
Direct somatic embryogenesis and plant regeneration from leaf sheath explants of *Kaempferia galanga*

a. Early globular embryos formed at cut ends of the leaf sheath explant in 20 days on induction medium (BAP 0.5 mg/l and NAA 1.0 mg/l)

b. Four weeks old culture of globular stage embryos on MS medium with BAP 0.5 mg/l and NAA 1.0 mg/l showing direct differentiation of somatic embryos.

c. Matured globular embryos on MS medium with BAP 0.5 mg/l and NAA 1.0 mg/l

d. Matured globular embryos-enlarged view

e. *In vitro* germination of somatic embryos on germination medium.

f. *In vitro* germination of somatic embryos on germination medium.

g. *In vitro* germination of somatic embryos on germination medium.

h. Regenerated plantlet acclimatized to greenhouse conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1, after 6 weeks.
Indirect somatic embryogenesis and plant regeneration from rhizome bud explants

a. Induction of embryogenic callus from rhizome bud explant on MS medium fortified with 1.0 mg/l 2, 4-D + 0.5 mg/l BAP, after 15 days of culture.

b. Embryogenic callus with earlier stages of somatic embryos on MS medium containing 0.5 mg/l 2, 4-D + 0.2 mg/l BAP within 30 days of culture.

c. *In vitro* germination of somatic embryos on germination medium.

d. *In vitro* germination of somatic embryos on germination medium.

e. Rooting of *in vitro* derived shoot on germination medium.

f. Regenerated plantlet acclimatized to green house conditions with river sand, garden soil and farmyard manure in the ratio of 1:2:1, after 6 weeks.

g. Acclimatized cups shifted gradually to normal climate, after 6 weeks.