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Bioprospecting of Plant Molecules for Grasserie Disease Management in Silkworm, *Bombyx mori*

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Studies were conducted to identify and characterize the antiviral principles from plants against the grasserie disease of silkworm, *Bombyx mori* L. Eight partially purified fractions were separated from *Plectranthus amboinicus* using TLC. Among the partially purified fractions, Rf1 (0.98) and Rf5 (0.47) fractions with a larval mortality of 22.27 and 24.00 per cent were highly effective. Further purification of TLC fractions with RP-HPLC revealed two peaks with peak1 producing a larval mortality of 21.00 and 24.00 per cent respectively. The purified antiviral compounds obtained from RP-HPLC were characterized through LCMS. From the mass spectrum obtained, the compounds responsible for antiviral activity were characterized as 20-deoxocarnosol and 6 β-hydroxycarnosol belonging to diterpenoid. Studies for the estimation of biochemical constituents from *Psoralea corylifolia* through GCMS revealed the presence of eight compounds at different retention times (RT) *viz*., psoralen (RT 15.16 and 14.56 min), phenol (RT 18.33 min), eicosane (RT 8.19 and 11.05 min), undecane (RT13.58 min), eicosane - 2 methyl (RT15.87 min), decane 2,3,5,8 tetra methyl (RT11.62 min), bicylopentyl 2-one (RT 6.83 min) and 2-bromo dodecane (RT 14.07 min). Analysis of *P. amboinicus* through GCMS revealed the presence of carvacrol (RT 8.68 min), bicyclol (5.05 and10.32 min), eicosane (RT 11.05 min), 2- bromo dodecane (RT 13.55 min), dodecane (RT 8.20 min), phenol (RT 18.20 min), tridecanol 2-ethyl 2-methyl (RT 15.79 min), 2-methyl nonadecane (RT14.03 min) and methyl octadecane (RT11.6 min). The major compound eluted from the two botanicals might certainly contain antiviral principles as the hexane extract proved antiviral.

Keywords: *Bombyx mori,* grasserie, *P. amboinicus, P. corylifolia*, *in vivo* studies, mortality, economic parameters, active principles, LCMS, GCMS, metabolic profiling.

Disease occurrence in silkworm, *Bombyx mori* L. is a common feature in almost all silkworm growing countries of the world and the diseases are the major production constraints in Sericulture. Due to continuous rearing of silkworms, pathogens are persistent in the rearing environment as a result of which silkworms have become highly susceptible to diseases which accounts for 30-40 per cent loss in cocoon yield (Chandrasekharan *et al.,* 2006). Among the silkworm diseases, the viral disease-grasserie, causes great economic loss to sericulture farmers. In India, viral diseases alone cause 30-40 per cent crop loss (Nataraju *et al*., 1998). 20-40 per cent cocoon yield loss in Karnataka was mainly due to viral diseases caused by NPV and CPV (Chitra *et al*., 1975).

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Chemical disinfectants, bed disinfectants, antibiotics and botanicals are being used for disease management as an integrated approach. Among the various methods of disease management, use of plant molecules is appropriate for the current scenario because of their cost effectiveness and eco friendly nature. The efficacy of aqueous extract botanicals, viz., *Psoralea corylifolia* and *Plectranthus amboinicus* against grasserie disease of *B. mori* have been reported (Manimegalai and Chandramohan, 2006). Besides antimicrobial effects, growth promoting factors of botanicals were also demonstrated by several authors (Rajasekhar Gouda, 1991; Manimegalai and Chandramohan, 2006). Presences of viral inhibitors have been reported in various plant species (Verma *et al*., 1985). Hence, identification of antiviral compounds will be of great use to sericulture

industry especially for the management of diseases. Hence, in the presence study an attempt was made to explore the possibility of using plant molecules as an effective curative measure against grasserie disease of silkworm by way of identifying compounds using LCMS and GCMS analysis.

Materials and Methods

Laboratory bioassays were conducted to determine the efficacy of different compounds of *Plectranthus amboinicus* (Family: Lamiaceae) against the grasserie disease of silkworm cross breed, PM x CSR2 along with treated and untreated control. The polyhedral occlusion bodies were collected from grasserie infected fifth instar silkworm cadaver and purified by gradient centrifugation (Sugumori *et al*., 1990). The polyhedra in virus suspension were counted with the help of improved Neubauer haemocytometer using phase contrast microscope. Viral inoculum of 10^7 POB's/ ml was used for bioassays.

Separation of the active principles

To separate different active principles from the plant extracts, thin layer chromatographic (TLC) studies were conducted.

Preparation of plant extracts

Fresh leaves were thoroughly washed in running tap water followed by rinsing twice with distilled water. Leaves were then dried in shade and powdered. Powdered leaf sample was weighed and extracted with hexane in a soxhlet apparatus for six hours. Solvent free filtrate was collected and evaporated at room temperature. The residue was weighed and dissolved in the same solvent and stored in a refrigerator at 4^0C.

Preparation and activation of TLC plate

Silica gel–G (E-Merck) was used for preparing TLC plates of dimension, 20 x 20 cm. Finely powdered 20 g of silica gel -G was mixed thoroughly with 40 ml of distilled water. The slurry was then poured into TLC applicator and adjusted to 0.5 cm thickness. The applicator was then slowly moved onto the clean glass plate. Thus a fine uniform layer of 0.5 cm thick wet silica gel was formed on the entire glass plate. The glass plate was allowed to dry in the open air for one hour. Then, the glass plate with silica gel coating was activated by heating in the oven at 110°C for two hours. After activation of the TLC plate, 5 µl of the plant extract was spotted on the plate with the help of micropipette or capillary tube without disturbing the silica gel layer to assess the qualitative nature of the effective plant extracts (Sadasivam and Manickam, 2005).

Separation of compounds

Dichloromethane was used to separate the compounds. The solvent was poured in the TLC tank and the TLC plate was kept in it with approximately 0.5 mm immersed in the solvent at the bottom. The tank was closed with a glass lid so as to have the chamber completely filled with the solvent vapours. Within one hour, the solvent front reached the top of TLC plate. Then, the plate was removed from the tank and kept in open air at room temperature for the evaporation of solvent.

Observation of compounds

Visible observation

The TLC plate was dried and observed under bright light for the presence of phenols, flavanoids and alkaloids. All the spots were marked and Rf (Relative front) values were calculated using the formula.

$$R_f \text{ value} = \frac{\text{Distance moved by the solute from the origin}}{\text{Distance moved by the solvent from the origin}}$$

Observation under UV light

The dried TLC plate was exposed to UV light of 254 nm and the fluorescent spots were observed.

Purification of antiviral compounds through RP-HPLC

Compounds in TLC purified retention factors were separated using Shimadzu LC 8A RP – HPLC with C18 column. The pressure was set to 300 psi with the flow rate of 1.00 ml/min. UV wavelength of 245 nm was used for detection.

Two pumps, pump A consisting of 80 per cent methanol and pump B consisting of 20 per cent water were used to run the TLC purified compounds.

Characterization of compounds using LCMS

The LC- ESI -MS of the HPLC purified sample was recorded on a MICROMASS QUATTRO II triple quadrupole mass spectrometer having a JASCO PU-980 HPLC pump connected to it. The column was M/s. Nomura Chemical Co. Ltd. DEVELOSIL ODS, 150 × 4.6, 5μ m and solvent was methanol: water 85:15 eluted was given binary program at 1.0 mi/min. The PDA (JSCOMD-2010 plus multi wavelength detector) was monitored at 200-650 nm and reported at 205 nm. The 100 μl samples were injected into LC-flow through JASCO Autosampler-2051 and mass spectra were scanned in the range 110-1000 da in 2.5s. The ESI capillary was set at 3.5 kV and the cone voltage was 40 V. Dry nitrogen was used as the nebulizer (101 1/hr) and drying gas (250 l/hr.) The source temperature was set at 80ºC.

Administration of compounds from P. amboinicus to the larvae of B. mori.

Fresh mulberry leaves of V1 variety were dipped in viral suspension of 10^7 POBs/ml, shade dried and cut into pieces of one cm^2. The worms after second moult were fed with virus treated mulberry leaves. The treated leaves were provided during the first feeding on first day and thereafter the larvae were fed with normal leaves. On the next day, the leaves treated with compounds of *P. amboinicus* were fed to the worms at 800 ppm. Fresh leaves were dipped in required concentration of extracts and shade dried before feeding to silkworms. Botanicals were administered twice, once on the second day of third instar and the other on the first day of fourth instar. The worms fed with BmNPV alone served as treated control. Untreated control was also maintained. The larvae used for the experiment were reared as per the methods of (Krishnaswami *et al*., 1973). Observations were made on larval mortality, larval weight, cocoon weight and shell weight. From the data collected, shell ratio and survival percentage were computed.

Characterization of compounds using GCMS

Hexane was found to be an effective solvent for extraction and hence hexane extract was used for GCMS analysis. The extract was obtained using Soxhlet apparatus, filtered using 0.44 micron filter and fed to GCMS for separation of compounds.

GC-MS analysis was carried out by using Perkin Elmer - Clarus 500 GC-MS unit possessing a software libraries (NIST and WILEY) containing six lakh compounds

GC-MS requisites

Column type: PE-5 (equivalent to DB-5), Column length: 30 m, Carrier gas : Helium, Flow rate: 1 ml/min, Column temperature - Initial: 80 ºC, Final : 280 ºC Rate of temperature change : 10 ºC, Injector temperature: 230 ºC, Detector temperature : 280 ºC, Sample injection volume : 1 μl. The spectra were recorded and compared with the library.

Results and Discussion

Separation of antiviral principles through TLC

Extracts from leaves of *P. amboinicus* were subjected to TLC study. TLC using dichloromethane was employed for the separation of active compounds from the hexane extract of *P. amboinicus*. Six spots with Rf values of 0.98, 0.64, 0.47, 0.29, 0.22 and 0.16 were visualized under visible light and two spots with Rf values of 0.88 and 0.84 Rf values were visualized under UV light.

In vivo effect of P. amboinicus fractions from TLC against BmNPV

Mortality and survival

The treatments with Rf1 and Rf5 recorded significantly lower mortality (22.67 and 24.00 per cent). This was followed by Rf4 (28.00%), Rf3 (29.33%) and Rf8 (29.33%) which were on par with each other. The mortality was significantly higher in treated control (62.67%). Survival was found to

Table 1. *In vivo* effect of *Plectranthus amboinicus* fractions from TLC on mortality, survival and economic parameters of *Bombyx mori*

Sl. No	Treatment	Mortality (%)	Survival (%)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
1.	Rf_1	22.67 (28.41) **b**	77.33 (61.59) **b**	3.59 **a**	1.73 **ab**	0.30 **ab**	17.34 **ab**
2.	Rf_2	34.67 (36.07) **d**	65.33 (53.93) **d**	3.26 **bc**	1.67 **cd**	0.28 **c**	16.76 **c**
3.	Rf_3	29.33 (32.78) **c**	70.67(57.22) c	3.17 **c**	1.65 **de**	0.28 **c**	16.77 **c**
4.	Rf_4	28.00 (31.95) **c**	72.00 (58.05) **c**	3.29 **b**	1.68 **bc**	0.29 **bc**	17.26 **ab**
5.	Rf_5	24.00 (29.33) **b**	76.00 (60.67) **b**	3.62 **a**	1.71 **ab**	0.30 **ab**	17.54 **ab**
6.	Rf_6	37.33(37.66) **de**	62.67 (52.34) **de**	3.27 **bc**	1.63 **e**	0.28 **c**	17.17 **bc**
7.	Rf_7	40.00(39.23) **e**	60.00 (50.77) **e**	3.02 **d**	1.51 **f**	0.26 **d**	17.22 **bc**
8.	Rf_8	29.33 (32.78) **c**	70.67 (57.22) **c**	3.30 **b**	1.69 **ab**	0.29 **bc**	17.16 **bc**
9.	Treated control	62.67(52.34) **f**	37.33(37.66) **f**	2.98 **d**	1.64 **de**	0.26 **d**	15.85 **d**
10.	Untreated control	0.00 (0.91) **a**	100.00 (89.10) **a**	3.59 **a**	1.74 **a**	0.31 **a**	17.82 **a**
	SEd	0.8535	0.8535	0.0548	0.0245	0.0082	0.2802
	CD (P= 0.05)	1.7805	1.7805	0.1144	0.0511	0.0170	0.5844

Figures in parentheses are *arc sine* transformed values
In a column, means followed by same letters (s) are not significantly different (P=0.05).

be significantly higher in Rf1 (77.33%) and Rf5 (76.00%) which were on par, whereas it was very low in treated control (37.33%) (Table 1).

Economic parameters

The treatments with Rf5 and Rf1 of *P. amboinicus* recorded significantly higher larval weight of 3.62 g and 3.57 g. This was followed by Rf8 (3.30 g) and Rf4 (3.29 g) which were all on par. The treated control recorded significantly lower larval weight of 2.98g.

Rf1 and Rf5 of *P. amboinicus* recorded significantly higher cocoon weight (1.73 g, 1.71 g). This was followed by Rf8 (1.69 g). The treated control recorded significantly lesser cocoon weight of 1.64g

Shell weight was significantly higher when treated with Rf1 (0.30 g) and Rf5 (0.30 g) which were found to be on par with each other. This was followed by Rf4 (0.29 g) and Rf8 (0.29g).Treated control recorded significantly lesser shell weight of 0.26g.

Rf5 (17.54%), Rf1 (17.34%) and Rf4 (17.26%) of *P. amboinicus* were found to be on par with each other. The shell ratio was significantly low in treated control (15.85%)

In vivo effect of two effective P. amboinicus fractions from TLC on BmNPV

From the eight fractions of *P. amboinicus* screened against grasserie disease of *B. mori*, two fractions *viz.*, Rf 1 and Rf 5 were found effective. Confirmatory bioassays were carried out using these fractions individually and in combination and the results obtained are presented in Table 2.

Mortality and survival

Fractions, Rf1 and Rf5 recorded lower mortality of 21.33 and 22.67 per cent respectively and were on par with each other. Higher mortality of 65.33 per cent was recorded in treated control. Survival was significantly highest for Rf1 (78.67% and Rf5 (77.33%). Survival was found to be only 34.67 per cent in treated control (Table 2).

Economic parameters

Rf1 (3.37 g) and Rf5 (3.34 g) recorded significantly higher larval weight and were on par with each other. This was followed by (Rf1 and Rf5) combination fraction which recorded 3.25 g. The treated control recorded significantly lower larval weight of 3.14g.

Rf1 recorded significantly higher cocoon weight (1.49 g) followed by Rf5 (1.46 g) which were all on par with each other. The lowest cocoon weight of 1.37 g was recorded in treated control.

Shell weight was significantly higher in Rf1 (0.26 g) and Rf5 (0.25 g) which were on par with untreated control (0.26g) and significantly better than treated control (0.22 g)

The fractions Rf1 (17.45%), Rf5 (17.12%) and combination fraction Rf1&Rf5 (16.90%) recorded significantly higher shell ratio and were on par with each other. The lowest shell ratio was recorded in treated control (16.01%) (Table 2).

Table 2. *In vivo* effect of two effective *P. amboinicus* fractions from TLC on the mortality, survival and economic parameters of *B.mori* exposed to BmNPV

Sl. No	Treatment	Mortality (%)	Survival (%)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
1.	Rf_1	21.33 (27.49) **b**	78.67(62.51) **b**	3.37 **b**	1.49 **a**	0.26 **a**	17.45 **a**
2.	Rf_5	22.67 (28.41) **b**	77.33 (61.59) **b**	3.34 **b**	1.46 **ab**	0.25 **a**	17.12 **a**
3.	Rf_1 and Rf_5	28.00 (31.91) **c**	72.00 (58.09) **c**	3.25 **bc**	1.42 **bc**	0.24 **b**	16.90 **a**
4.	Treated control	65.33 (53.94) **d**	34.67(36.06) **d**	3.14 **c**	1.37 **c**	0.22 **c**	16.01 **b**
5.	Untreated control	0.00 (1.28) **a**	100.00 (88.72) **a**	3.53 **a**	1.51 **a**	0.26 **a**	17.21 **a**
	SEd	1.3470	1.3470	0.0548	0.0245	0.0056	0.2761
	CD (P = 0.05)	3.0013	3.0013	0.1221	0.0546	0.0124	0.6152

Figures in parentheses are arc sine transformed values
In a column, means followed by same letters (s) are not significantly different (P=0.05).

The results of preliminary separation and screening of phytochemical components in the present study showed two spots with Rf values, Rf1 (0.985) and Rf5 (0.474) from *P. amboinicus* compared to treated control (62.67%). Administration of these fractions also resulted in higher economic parameters.

TLC analysis of phenolic acids in *Ginkgo biloba* leaves showed that cold water extract in n-butanol: acetic acid: water (4:1:5) solvent system exhibited three fluorescent spots (Rf 0.62, 0.76 and 0.88) under UV light and three spots (Rf 0.50, 0.63 and 0.83) under visible light (Ellnanin and Zgorka,1999)

Girijashankar and Thayumanavan (2005) identified three spots with Rf values 0.43, 0.22 and 0.1 in cold water extract of *Lawsonia inermis* which was effective against *Rhizoctonia solani*. They also reported the efficacy of three spots in methanol extract (Rf 0.94, 0.86 and 0.48) against *Pythium aphanidermatum* and *Macrophomina phaseolina*. Akila (2006) reported that the TLC fraction of (Rf 0.20 and Rf 0.16) aqueous extract of *Datura metel* showed complete inhibition of mycelial growth of fusarium wilt in banana.

High-performance liquid chromatography (HPLC) studies

Upon injection of 20 µl of the effective sample solution from TLC fraction of *P. amboinicus* into HPLC column, two peaks were obtained from each of the two effective fractions (Rf1 and Rf5) (Table 3).

Table 3. HPLC peaks obtained from different TLC fractions of *P. amboinicus*

TLC Fraction	Peak number	Retention time (minutes)
Rf1	1	3.8
	2	4.6
Rf5	1	3.9
	2	4.6

In vivo effect of HPLC purified compounds from P. amboinicus

Purified compounds of *P. amboinicus* obtained from HPLC were subjected to bio assays and the results obtained are presented in Table 4.

Mortality and survival

Peak 1 of *P. amboinicus* recorded significantly lower mortality of 21.33% followed by peak 2 (24.00%). Compared to higher mortality recorded in treated control (64.00%). Survival was more in peak 1 (78.67%) which was followed by peak 2 (76.00%). Treated control recorded significantly lower survival of 36.00 per cent.

Economic parameters

Peak 1 of *P. amboinicus* recorded significantly higher larval weight of 3.26g followed by peak 2 (3.23 g) which were on par. The lowest larval weight was recorded in treated control (3.08 g)

Peak 1 (1.52 g) and peak 2 (1.48 g) of *P. amboinicus* recorded significantly higher cocoon weight and were on par. Treated control recorded significantly lesser cocoon weight of (1.40 g)

Peak 1 (0.2 6g) and peak 2 (0.25 g) of *P. amboinicus* recorded significantly higher shell weight and were on par next to untreated control (0.28 g). The treated control recorded lower shell weight of 0.22 g

Table 4. *In vivo* effect of HPLC purified compounds from *P. amboinicus* on the mortality, survival and economic parameters of *B. mori* exposed to BmNPV

Treatment	Mortality (%)	Survival (%)	Larval weight (g)	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
P1	21.33 (27.49) **b**	78.67(62.51) **b**	3.26 **ab**	1.52 **b**	0.26 **b**	17.10 **a**
P2	24.00 (29.33) **c**	76.00 (60.67) **c**	3.23 **b**	1.48 **b**	0.25 **b**	16.89 **a**
Treated control	64.00 (53.13) **d**	36.00 (36.87) **d**	3.08 **c**	1.40 **c**	0.22 **c**	15.71 **b**
Untreated control	0.00 (1.43) **a**	100.00 (88.57) **a**	3.38 **a**	1.61 **a**	0.28 **a**	17.39 **a**
SEd	0.6526	0.0532	0.0245	0.0062	0.2738	
CD (P= 0.05)	1.5050	1.5050	0.1227	0.0565	0.0144	0.6314

Figures in parentheses are arc sine transformed values
In a column, means followed by same letters (s) are not significantly different (P=0.05).

Peak 1(17.10%) and peak 2 (16.89%) of *P. amboinicus* recorded significantly higher shell ratio when compared to treated control (15.71%) (Table 4).

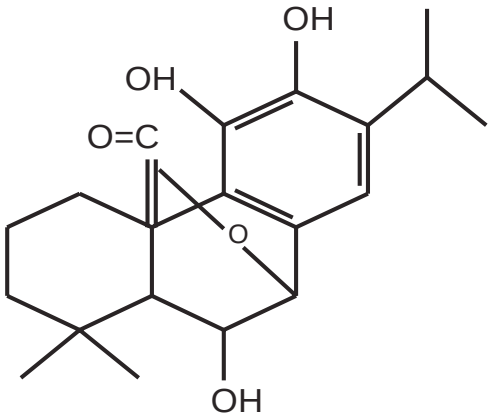
Hsu *et al*. (2001) identified the compounds, daidzein, genistein and biochanin from *P. corylifolia* with RP-HPLC using flavone as internal standard. RP-HPLC analysis of aqueous extract of *L. inermis* exhibited four peaks with retention times 4.51, 5.99, 7.19 and 17.78 min (Girijashankar and Thayumanavan, 2005). They also reported that RT values 4.51 and 5.99 were super imposable with the RT of standards for tannic acid (4.67min) and catechol (6.05min) respectively in the presence of tannic acid and catechol in the detectable amounts. Presence of phenolic compounds in the *L. inermis* leaf extract along with phytochemical components resulted in *in vitro* fungal inhibition.

Characterization of antiviral compounds from effective botanicals through LCMS

The HPL chromatogram of *P. amboinicus* recorded one major peak with a retention time at 2.7. A total of seven mass spectra were recorded. HPLC peaks with retention time of 2.986 and 3.546 recorded mass spectra showing base peaks at m/z 316. The compound was identified as 20 – deoxocarnosol, a diterpenoid derivative. A loss of methyl group showed a peak at m/z 301. There was no other major peaks in the mass spectrum. Another compound was identified as 6-ß-hydroxycarnosol from the HPLC

Fig 1. Chemical structures of 6β hydroxycarnosol and 20-deoxocarnosol from *P. amboinicus*

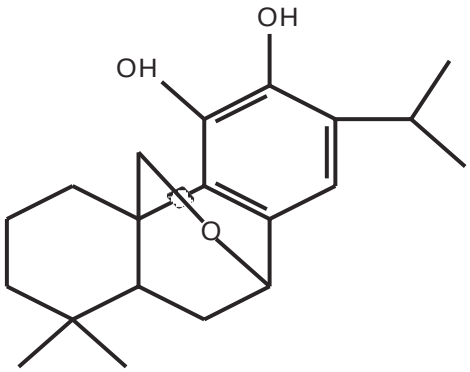
6β-hydroxycarnosol



Molecular formula: $C_{20}H_{26}O_5$

$(M-H)^+$: 346

20- deoxocarnosol



Molecular formula : $C_{20}H_{28}O_3$

M^+ : 316

$(M-CH_3)^+$: 301

In the present research, purified antiviral compounds obtained from RP-HPLC were characterized through LCMS. From the mass spectrum obtained, antiviral compounds, *viz*., 20-deoxocarnosol and 6β-hydroxycarnosol belonging to diterpenoid group were characterized.

The presence of colenol D, colenol E,colenone, barbatusol, 6 β-hydroxycarnosol and plectrin from *P. amboinicus* were reported by Rastogi and Mehrotra (1993). Similarly, Rastogi and Mehrotra (1997) reported the presence of bavacoumestan A, bavacoumestan B, bakuchicin, corylifonol and isocorylifonol from *P. corylifolia*.

Abdel *et al*. (2002) reported that diterpenoids were the most common secondary metabolites in *Plectranthus*. Khatune *et al*. (2004) characterized psoralidin, bakuchicin, psoralen and angelicin from seeds of *P. corylifolia*.

The antiviral effect of these compounds may be due to their ability to form complexes with viral DNA, altering the pH of the gut, binding with proteinaceous virions or blocking the pores in the peritropic membrane of the gut. Phillipson and Neill (1987) reported that the mechanism of action of terpenoid in berberin and harmane is due to their ability to incalate with DNA.

According to Ya *et al*. (1988) the mode of action of phenol are related to the ability of plant compounds to inactivate microbial adhesion, enzyme, cell envelope, transport protein and forming complexes with polysaccharides. Keating *et al*. (1989) reported that phenols may bind directly the proteinaceous virions and subsequently interfere with host cell interactions. Felton and Duffey (1990) found that chlorogenoquinone, a powerful oxidizing agent covalently bonded to the occlusion bodies of NPV and significantly reduced the solubility ultimately impairing the release of infective virions in the midgut.

Samuel Manohar Raj (1994) reported that the leaf extracts of *P. corylifolia* possessed some phenolics, which may have acted as viral inhibitors in avoiding infection by BmNPV. Similarly, Manoharan (1996) reported that leaves of *Acacia suma*, *Caesalpinia* coriaria and *Terminalia tomentosa* had higher amount of tannins and phenols and the polyhedra exposed to these extracts showed higher rate of aggregation with lesser mortality due to BmNPV. The aggregation of polyhedra in the midgut lumen of *B. mori* due to the administration of the aqueous extract of *P. amboinicus* was documented by Ranganatha *et al.* (2004) by way

of histopathological studies.They also observed partial disruption of epithelial layer of peritrophic membrane.

The results revealed that *P. amboinicus* compounds were highly effective in reducing the morality of *B.mori* due to BmNPV. It may be due to the more number of hydroxyl groups attached to the phenolic group. This was also supported by Geissman (1963) who reported that the toxicity of phenols to microorganisms is related to the site and number of hydroxyl groups in phenols.

Characterization of compounds from effective botanicals through GCMS

GCMS analysis revealed the presence of psoralen (RT 15.16 and 14.56 min), phenol (RT 18.33 min), eicosane (RT 8.19 and 11.05 min), undecane (RT13.58 min), eicosane-2 methyl (RT15.87 min), decane 2,3,5,8 tetra methyl (RT11.62 min), bicylopentyl 2-one (RT 6.83 min), 2-bromo dodecane (RT 14.07 min) from *Psoralea corylifolia.*

The two major compounds that were eluted from *P. corylifolia* were psoralene and phenols. The psoralen (also called psoralene) is the parent compound in a family of natural products known as furanocoumarins. Psoralens can inactivate the pathogens (Hudson et al., 1993). The psoralen compounds, when activated, increased phenylalanine ammonia lyase (PAL) activity, a key enzyme in the phenylpropanoid pathway is involved in the biosynthesis of phenolic compounds (Bowles, 1990) which act as a secondary metabolite in defence against insects and pathogens .This enzyme participates in five metabolic pathways namely tyrosine metabolism, phenylalanine metabolism, nitrogen metabolism, phenyl propanoid biosynthesis, and alkaloid biosynthesis. Increased accumulation of oxidative enzymes such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), pathogenesis-related (PR) proteins, chitinase, β-1,3-glucanase and phenolics were observed to bring about systemic resistance in banana (*Musa* spp.) against *Banana bunchy top virus* (Mathiyazhagan Kavino *et al*., 2007). So, in the present study also these psoralen compounds might have increased the PAL activity, which might have led to the biosynthesis of phenolic compounds. These phenols are a group of substances that are poisonous to many organisms like plants, micro-organisms and some insects. Harborne (1994) has explained how natural plant phenols play a role in plant resistance to microbial infection. Rastogi and Mehrotra (1995) isolated psoralen and angellicin from. *P. corylifolia* .The presence of oleic acid and linoleic acid in P*. corylifolia* was reported by Huang *et al*. (2000). Singh and Himadri Panda (2005) reported the presence of coumesterol, psoralen, angellicin and daidzein from *P. corylifolia.*

The compounds obtained from *Plectranthus amboinicus* were carvacrol (RT 8.68 min), bicyclol (5.05 and10.32 min), eicosane (RT 11.05 min), 2- Bromo dodecane (RT 13.55 min), dodecane (RT 8.20 min), phenol (RT 18.20 min), tridecanol 2-ethyl 2-methyl (RT 15.79 min) 2-methyl nonadecane (RT14.03 min) and methyl octadecane (RT11.6 min). Rastogi and Mehrotra (1993) reported the presence of carvacrol, limonene, thymol, euginol, γ-terpinene, β-selinene, p-cymene, α-pinene, myrcene, β-pinene and an unidentified phenolic fraction from *P. amboinicus*. Prudent *et al*. (1995) reported carvacrol, 1, 3 hexadiene, 3-hexanol, alpha-farnesene and alpha-mucurolene as main constituents of *P. amboinicus*. Similarly the compounds, thymol, carvacrol, 1, 8-cineole, p-cymene and terinen-4-ol and an unidentified compound were reported from *P. amboinicus* (Gurdip singh *et al*., 2001).

Carvacrol, or isopropyl-o-cresol, $C_6H_3CH_3$ (OH) (C_3H7) [1.2.4], is the principal constituent of *P. amboinicus* which has been found to be a powerful antiseptic and has been used in products to combat bacteria. Carvacrol has antifungal and anthelmintic activities, although weaker than those of thymol, a chemically-similar phenol found in thyme. The hexane extract of the plant *Lippia multiflora* was found to be the most active, while the methanol extract exhibited no antimicrobial activity. The isolated carvacrol

from the hexane fraction showed tremendous antimicrobial activity and is used in the treatment of disease conditions due to microbes (Kunle *et al.,* 2003).

Having explored the antiviral principles from *P. amboinicus* and *P. corylifolia*, the next step is to go in for developing bio-formulations for the management of grasserie disease of *B. mori.* utilizing the antiviral principles.

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