

INSECTICIDAL POTENTIALITY OF FLAVONOIDS FROM CELL SUSPENSION CULTURE OF
MARCHANTIA LINEARIS LEHM. & LINDENB AGAINST *SPODOPTERA LITURA* F.

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ABSTRACT: Bryophytes were diverse, primitive non vascular amphibious taxa distributed worldwide and form the second largest category of plants. Bryophytes synthesize an array of phytochemicals to combat against the inhospitable environmental conditions including predation, UV radiation, high temperature and pest and pathogens. The present investigation was undertaken to elucidate flavonoids from *in vitro* cell cultures of the liverwort *Marchantia linearis* Lehm. & Lindenb. its fractionation and analysis of insecticidal potentialities. Initially, callus culture was initiated from spores in MS/5 media containing growth regulators BAP and NAA at the concentration of 2 mg/L and 0.5 mg/L. Agitation of the friable callus at lower rpm bring about lower level of cell dispersion, on the contrary at higher rpm might have risk of cell collision that is why rpm was kept at moderate speed i.e., 110 rpm. Continuous sub culturing process substantially improves cell growth and biomass. In the second phase, the flavonoids were isolated from cell suspension cultures of *M. linearis* and were fractionated by TLC and HPLC PAD chromatogram, which revealed the presence of quercetin, luteolin, apigenin, rutin and kaempferol. *In vivo* insecticidal analysis revealed significant antifeedant, larvicidal and pupicidal activities at all the concentrations against 5th instar larvae of *Spodoptera litura*. The extract also exhibited feeding deterrent activity with *M. linearis*. Similarly, the nutritional parameters were also affected i.e., reduced ECI (Efficiency of conversion of ingested food) and ECD (Efficiency of conversion of digested food) and increased AD (Approximate digestibility) and metabolic cost for the larvae, when compared with the control. The consumption of the basal diet with the incorporation of flavonoids by *S. litura* larvae was not significantly different compared to the consumption of the control diet by the larvae. Faecal production reduced proportionally with concentrations of the extract.

Key words: Bryophytes, Cell suspension, Flavonoids, *Marchantia*, insecticidal

INTRODUCTION

Bryophyte, the simplest non-vascular plants and represents the second largest group in the plant kingdom. The Chinese, Indians, and Native Americans have used species of liverworts and mosses as herbal drugs for curing various skin disorders, fungal & bacterial infections, cardiovascular diseases, fever and cancer. Screening of biologically potential molecules, particularly the phytochemicals was an important aspect in the field of bryology. Many studies have reported the presence of high amounts of polyphenols, steroids, glycosides, tannins in bryophyte species (Klavina *et al.*, 2012). Antibiotics like compounds were also reported from these groups (Mishra *et al.*, 2014). Bryophytes synthesize an array of phytochemicals to combat against the inhospitable environmental conditions including predation, UV radiation, high temperature and pest and pathogens (Ferrazzano *et al.*, 2011). Polyphenols are polyhydroxylated secondary metabolites i.e., possess 12 to 16 phenolic hydroxyl groups and 5 to 7 aromatic rings per 1,000 kDa. They were categorized into subclasses like the flavonoids, phenolic acids and the stilbenoids. Seven pure flavonoids were isolated and identified from five moss species. The flavonoids were the flavones such as apigenin, apigenin- 7-O-triglycoside, lucenin-2, luteolin-7-O-neohesperidoside, saponarine, vitexin; and also the biflavonoid bartramia flavones (Basile *et al.*, (1999); (2003); Jiang *et al.*, (2006).

Currently, tissue culture has become an essential tool in supplying stable research materials for both chemists and botanists. Tissue culture studies on liverworts have been carried out on many species and the most intensely studied species was *Marchantia polymorpha*. Vujicic *et al.* (2011) developed a protocol to isolate secondary metabolites from axenic cultures of bryophytes. The flavonoids characterized in liverworts are mainly quercetin, luteolin, apigenin and their O- and C-glycosides. Calli of *Marchantia* species form fast-growing cell suspension cultures and are regarded as the most suitable *in vitro* culture to synthesize flavonoids under photo-mixotrophic growth conditions compared to other bryophytes.

Marchantia linearis Lehm & Lindenb. a thalloid liverwort with restricted distribution possess interesting ethnobotanical properties (Remesh and Manju, 2009). It is a traditional medicinal plant of Kani tribe of South Kerala. The plant possesses many therapeutic properties, especially against skin borne diseases and tumour (Remesh and Manju, 2009). In view of excessive utilization, sporadic distribution pattern and slow growth rate, the present study was undertaken to investigate the *in vitro* cell growth, the culture parameters and their effect on flavonoid synthesis in shake flask cultures of *Marchantia linearis* under photomixotrophic growth conditions. Subsequently, the total flavonoids were isolated, fractionated and quantified by TLC and HPLC PAD. Further, *in vivo* insecticidal potentiality of the flavonoids extracted from the cell suspension cultures of *M. linearis* against *Spodoptera litura* F. larvae were studied.

MATERIALS AND METHODS

Plant material

Fresh thalli of *Marchantia linearis* Lehm & Lindenb. collected from the Kallar river valley of Ponmudi hills, Kerala (Figure 1) and was authenticated by verifying with herbarium University Calicut and a specimen sheet was maintained in the department herbarium.

Spodoptera litura F.

Spodoptera litura was commonly known as Cluster caterpillar, Cotton leaf worm or Tobacco cutworm. The moth was distributed all over Asian countries and cause severe damages to wide range of crops (Figure 2a & b).

Axenic *in vitro* culture and regeneration of *M. linearis*

Mature spores from sporangia were selected as explants; they were disinfected with propamidine and pentamidine (5 µg/ml) and surface sterilized with 10% sodium hypochlorite + two drops of Tween- 20. The media employed were Knops, Gamborg, Murashiq skoog and 1/5 diluted MS medium supplemented with antibiotics. After germination, the effective explants were transferred onto different multiplication media, calli multiplying vigorously were transferred on to differentiation media of MS/5 with varying concentrations of NaH₂PO₄ (100 to 250 mg/L) containing growth regulators BAP (2 mg/L) and NAA (0.5 mg/L).

Establishment of cell suspension culture

To establish suspension cultures, 2-14 g green friable calli were aseptically transferred to MSK-2 liquid medium supplemented with varying growth regulators on a rotary shaker at 110 rpm (Kato *et al.*, 1980). Cells were continuously sub-cultured every 3 weeks at 23°C. Productivity (in mg/L/day) was calculated as the final yield of flavonoid (mg/L) divided by the total culture period (in days). Flavonoid productivity was optimized by standardizing various culture parameters like carbon source, growth regulators, light intensity, inoculum size, cations environmental stress and elicitors. Culturing was terminated as soon as the glucose in the medium was exhausted. Culture experiments were carried out in triplicates.

Fractination of flavonoids

The oven-dried cells were extracted with 10 ml of 60% aqueous methanol containing 20 mM sodium diethyldithiocarbamate (NaEDTC) as an antioxidant. They were homogenized with mortar and pestle before 2.5 ml 6 M HCl was added to each extract to give a 12.5 ml solution of 1.2 M HCl in 50% aqueous methanol. The extracts were refluxed at 90°C for two h known as flavonoid extract. The methanolic extracts of *M. linearis* were evaporated in vacuum and were subjected to purification by column chromatography. The highest flavonoid bearing fraction (1:3 ethyl acetate-methanol) was subjected to TLC. Samples and standards were applied on Aluminium plate pre-coated with Silica gel 60 of 0.2 mm thickness. 5% methanol in chloroform results the best solvent system for the separation of flavonoids compared to 30% ethyl acetate in hexane and 20% ethyl acetate in chloroform.

The TLC plates were dried with blow-dryer and were treated with Natural Product Reagent (1% methanol diphenylboryloxy ethylamine)/Polyethyleneglycol 4000 (5% ethanol polyethylene glycol 4000). Flavonoids produced orange-yellow bands or spots and were identified in terms of number of spots and the length of elution (Rf) using UV 254 and 366 nm by comparison with the standards such as apigenin, quercetin, rutin, kaempferol and luteolin (Calina *et al.*, 2013). Relative front (Rf) = Distance travelled by the solute from the origin / Distance travelled by the solvent from the origin.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) PAD analysis of flavonoids

Ethyl acetate-methanol (1:3) eluted flavonoid extract was subjected to RP-HPLC PAD analysis. The chromatographic system (Waters Company) consisted of Millennium 32 system software, Waters 717 plus Auto sampler, Model Waters Delta 600 pump, and Model Waters 2996 Photodiode Array Detector. Chromatographic separation was carried out by HIQ SIL C18 V reversed phase column (4.6 mm X 250 mm) packed with 5 µm diameter particles, the mobile phase is methanol- acetonitrile- acetic acid- phosphoric acid – water (200:100:10:10:200, V/V). The mobile phase was filtered through a 0.45 µm membrane filter (Millipore), and then de-aerated ultrasonically prior to use. Flavonoids such as apigenin (A), quercetin (Q), rutin (R), kaempferol (K) and luteolin (L) were quantified by a PAD following RP-HPLC separation at 254.5nm for Q, 345nm for L and A. The flow rate was 1ml/min, the injection volume was 25 µl and the column temperature was maintained at 30°C. The chromatographic peaks of the analytes were confirmed by comparing their retention times and UV spectra with those of the reference standards. Quantification was carried out by integration of the peak using external standard method (Nessa *et al.*, 2005).

Insecticidal assay

Effects of flavonoid on the development of *Spodoptera litura* larvae

The activities of flavonoids were assessed *in vivo* by feeding the 5th instars of *S. litura* on diet supplemented with flavonoid extract. Flavonoid extracts were incorporated into the larval base diet at concentrations of 1 to 3%. A set of controls was maintained, where the 5th instars were fed on base diet only. In all treatments the larvae were allowed to develop into pupae. Ten larvae were used for each treatment. For each replicate, survival of larval instar was recorded daily throughout the trail, and from day 3 onwards the larval weights were noted at an interval of three days. The efficacy of the flavonoid was determined by measuring the body weight and the number of larvae reaching pupal stage in comparison to the control.

Antifeedant activity

Antifeedant activity of the flavonoid extracts was studied using leaf disc method (Baskar *et al.*, 2011). Five replicates were maintained for each treatment with 10 larvae per replicate (total, n = 50). The antifeedant activity was calculated using the formula:

$$\frac{\text{Leaf area consumed in control} - \text{Leaf area consumed in treatment}}{\text{Leaf area consumed in control} + \text{Leaf area consumed in treatment}} \times 100$$

Larvicidal activity

Different concentrations of flavonoid extracts were applied using leaf dip method. The treated leaves were exposed to the larvae. After 24 h of treatment, the larvae were continuously maintained on the non-treated fresh castor leaves. Fresh castor leaves were provided at every 24 h. larval mortality was recorded after 96 h of treatment. Five replicates were maintained for each treatment with 10 larvae per replicate. Percent mortality was calculated. The experiment was conducted at laboratory temperature of 27 ± 2 °C with 14:10 light photoperiod and 75 ± 5% relative humidity.

$$\left. \begin{array}{l} \text{Abbott's corrected} \\ \text{mortality} \end{array} \right\} \frac{\% \text{ mortality in treatment} - \% \text{ mortality in control}}{100 - \% \text{ mortality in control}} \times 100$$

Pupicidal activity

Pupicidal activity was calculated by counting dead pupae from the total larvae.

Nutritional parameters

Nutritional parameters were compared among 5th instar larvae exposed to 1 to 3% flavonoid extract treated or control diet. The larvae, feces, and remaining uneaten diet were separated, dried and weighted. Nutritional indices of consumption, digestion and utilization of food were calculated, as described by Waldbauer (1968) and Farrar *et al.*, (1989). The nutritional indices, namely efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and approximate digestibility (AD) were calculated as follows: ECI $(\Delta B/I) \times 100$; ECD $[\Delta B/I - F] \times 100$; and AD $[(I - F)/I] \times 100$, where I = weight of food consumed, ΔB is change in body weight, and F = weight of feces produced during the feeding period. Metabolic cost (CM) was calculated as: $100 - ECD$.

Statistical analysis

Each data point was the mean of three replicates obtained from 3-5 independent experiments. All experimental data were analyzed by an analysis of variance (ANOVA). After confirming the significance of F values, the significance of the differences between the mean values was tested using ANOVA. Significant differences were considered at $P < 0.01$ probability levels.

RESULTS AND DISCUSSION

In vitro culture of *Marchantia linearis*

Germination of the spores started within 8 days of their culture in the different media, such as Knop, GB, Murashige Skoog and diluted MS/5 basal medium supplemented with the antibiotics to suppress the growth of bacteria and fungi. The highest mean percentage of germination was observed with MS/5 diluted basal medium (66%). The proliferation of callus was continued until the fourth week of culture, and then the frequency of callus induction and size of the callus were calculated. Callus induced from the germ tube of *M. linearis* were dark green in colour (Figure 3a). Profound effect was observed on the multiplication of calli in the MS/5 media containing growth regulators BAP and NAA at the concentration of 2 mg/L and 0.5 mg/L respectively. The successfully multiplied calli were transferred to differentiation media [MS/5 medium with different concentrations of NaH_2PO_4 (100 to 250 mg/L)] containing growth regulators BAP (2 mg/L) and NAA (0.5 mg/L). After a period of 4 week of incubation, axillary proliferations were initiated from the green mass of cells (Figures 3b). For rhizoid induction, differentiated leafy thalli of *M. linearis* were transferred to the rooting medium containing different concentrations of IBA, *in vitro* regeneration of *M. linearis* were completed within an average of 12 weeks.

Cell suspension culture and optimization of flavonoid productivity

5 g of the friable callus was found optimal to form suspension in 12-14 days from inoculation time in the liquid medium. Agitation of the suspension at lower rpm bring about lower level of cell dispersion, on the contrary at high rpm might have risk of cell collision, that is why rpm was kept at moderate speed i.e., 110 rpm. *M. linearis* suspension cultures in the MSK-2 medium showed typical time course of flavonoid synthesis i.e., a positive correlation was observed between flavonoid content and cell growth (Figure 3 c & d). The culture was terminated as soon as glucose in the medium was exhausted. Culture temperature was a parameter which could regulate flavonoid synthesis. The trials revealed that 25°C was optimal than 15°C on flavonoid production. Carbon nutrient was known to affect culture parameters such as growth, primary metabolism and yield of phytochemicals. Highest cell mass and flavonoid production were obtained from medium supplemented with glucose followed by sucrose, fructose and galactose. Cells cultured in medium without supplement of glucose retained their viability (appeared green) but showed poor proliferation. Biomass and flavonoid synthesis peaked at the glucose concentration of 2-3%. Medium supplemented with phytohormones singly or with different combinations in flavonoid productivity were analyzed. Highest flavonoid productivity was obtained in cultures supplemented with 2,4-D. *Marchantia* cells were able to grow in the dark as well in light, with glucose as the carbon source (Su and Chiou, 1998). No significant changes were noted under different light intensities related to growth rate and biomass yield. However, intracellular flavonoid attained maximal level at the photon flux density around 20 $\mu\text{mol}/\text{m}^2/\text{s}$. Inoculum size (%) showed correlation with flavonoid yield and biomass production within the short culture period i.e., positive correlation was noticed with inoculum size with biomass and flavonoid productivity up to 12% followed by a decrease. Addition of Fe^{2+} enhanced flavonoid productivity. Environmental stresses such as osmotic stress, resulting from addition of NaCl or sucrose decreased the flavonoid productivity. Stoyanova-Bakalova *et al.*, (2009) revealed that environmental stress significantly regulate the synthesis of polyphenols. Variation in metabolites can be linked to ontogenetic transformation of plant metabolism. Methyl jasmonate and 2- (2-fluoro-6-nitrobenzylsulfanyl) pyridine-4-carbothioamide, which have been commonly used as elicitors in accelerating secondary metabolite production, were also examined. Both these elicitors induced intracellular flavonoid level. The present results were comparable with that of suspension culture of *Trifolium pretense* (Kasparov *et al.*, 2012). In the present study, flavonoid productivity was almost at par with biomass density during the culture period.

Identification of bioactive flavonoids from the cell suspension culture of *Marchantia linearis*

Many prominent orange yellow bands in the thin layer chromatograms were noticed with the extracts of *M. linearis*. The R_f values for the identified compounds in the extracts were 0.53 (apigenin), 0.91(quercetin), 0.32 (rutin), 0.72 (kaempferol) and 0.44 (luteolin) and also certain unknown compounds (Figure 4). Calina *et al.*, (2013) also noticed rutoside and hyperoside from the methanolic extracts of flowers from *Robinia pseudoacacia*. Shobha *et al.*, (2013) reported thin layer chromatogram of *Pimpinella anisum* with apigenin and luteolin, being more predominant among the many polyphenolic compounds.

Quantification of total flavonoids by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) PAD

Extraction of flavonoids with 80% ethanol under the frequency of 100 kHz, at 25°C, the liquid-solid ratio of 10 ml/g with 15 min duration repeated thrice give the highest flavonoid yield (Figure 5). Good results were obtained with respect to repeatability, relative standard deviation (RSD) and recovery (97.27 - 99.68%). The major flavonoids identified and quantified in *M. linearis* were quercetin (487.65 µg/g), luteolin (587.8 µg/g), apigenin (256 µg/g), rutin (187 µg/g) and Kaempferol (146 µg/g).

Insecticidal properties of flavonoids of *Marchantia linearis* against *Spodoptera litura*

In vivo insecticidal analysis revealed optimal antifeedant activities of 51% with flavonoids of *M. linearis* at 3% concentration (Table 1). Meanwhile, at 5% concentration, the extracts revealed maximum antifeedant activities i.e., 68%. The flavonoids exhibited feeding deterrent activities significantly with *M. linearis*.

In addition, flavonoids of *M. linearis* at 3% showed 45% larvicidal and 40.8% pupicidal activities. (Table 1). The mortality rate was also higher at 3% concentration (65.6%) (Table 1). In addition, dose dependant decrease of larval body mass was noticed. i.e., 1% *M. linearis* flavonoid fed larval mass was 840 mg and at 3% it was further reduced to 503 mg ($p < 0.001$) (Table 2). Reduction in larval body mass was accompanied by growth retardation was also visualized (Figures 6 a & b).

Nutritional Parameters

Nutritional analyses revealed that flavonoids of bryophytes presented toxic effect, when ingested by larvae. Leaf coating assay of flavonoids reduced efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD). Parallely, approximate digestibility (AD) and metabolic cost (CM) were increased in the larvae of *S. litura* (Table 3). The consumption of the basal diet with the incorporation of flavonoids of *M. linearis* by *S. litura* larvae was different compared to the consumption of the control diet by larvae. Fecal production reduced proportionally with concentrations of the flavonoids for *S. litura* larvae than that of the control group. These results suggest that flavonoid in the extract act on the insect's intestinal tract or interfere with the digestion. Dietary utilization experiments show that flavonoid extracts, incorporated into basal diet, did not alter the consumption rate, but decreased the fecal production of *S. litura* larvae. An index of dietary utilization showed that ECI and ECD decreased when 3% flavonoid diet was employed. In the present study, the AD value for larvae of *S. litura* increased throughout the feeding period of the experiment; this finding suggests that, during this treatment, the food remained for a greater time in the insect's gut to allow the detoxification of the protein. This increased the exposure of the food to digestive enzymes, probably allowing an increased digestibility of the food in comparison to that seen in the control.

Table 1: Bioefficacy indices in terms of antifeedant, larvicidal, pupicidal and mortality of *S. litura* 5th instar larvae on 1, 2, 3 and 5% of flavonoids of *M. linearis* treated compared to control diet

Treatment	Percent antifeedant activity	Larvicidal activity (%)	Pupicidal activity (%)	Total mortality (%)
Control	2.5	1	0.89	0.99
1%	24.9	20.9	22	34
2%	38.6	32.4	31.5	53.6
3%	51	45	40.8	65.6
5%	68	59	40.8	71
F ratio I	785.39**	616.53**	389.38**	466.25**
ID	12.543**	11.332**	14.636**	14.654**
CD _(0.05) I	0.672	0.298	0.055	0.038
ID	2.565	2.281	2.09	2.09

Table 2: Body mass (mg) of *S. litura* 5th instar larvae on 1, 2 and 3% of flavonoids of *M. linearis* treated compared to control diet

Treatment	Body mass (mg) of <i>S. litura</i> 5 th instar stage (<i>M. linearis</i> treatment)
Control	925
1%	840
2%	628
3%	503
5%	396
F ratio I	8108.69**
ID	19.688**
CD _(0.05) I	0.256
ID	2.989

Table 3: Nutritional indices of *S. litura* 5th instar larvae on 1, 2 and 3% of flavonoids of *M. linearis* treated compared to control diet

Treatment	ECI (%)	ECD (%)	AD (%)	CM (%)
Control	26	29	78	92
1%	20.8	18.6	80.2	93.4
2%	14.4	15.2	86	95.6
3%	11	13	90	97
F ratio I	298.58**	325.33**	1129.38**	1200.25**
ID	15.69**	16.7**	8.55**	10.91**
CD _(0.05) I	0.35	0.02	0.69	0.02
ID	1.565	1.928	2.25	2.36



Figure 1. Thallus of *Marchantia linearis* Lehm & Lindenb.



Figure 2 a) Adult moth *Spodoptera litura* F. b) Caterpillar

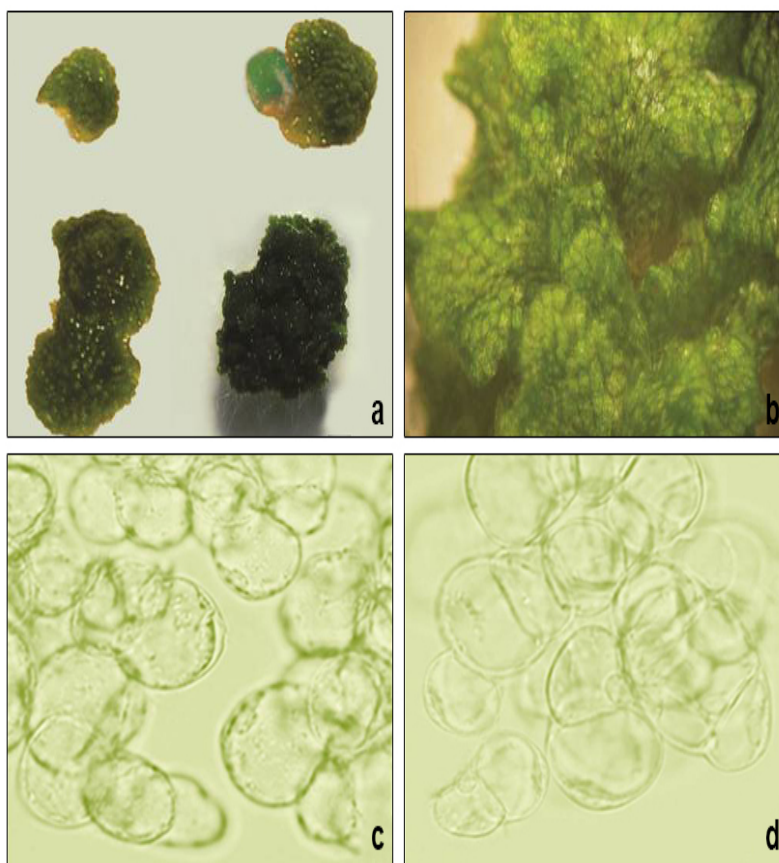


Figure Figure 3 a) Callus initiation and multiplication b) Thallus differentiation c & d) Stereo microscopic photograph of cell aggregates in suspension culture of *M. linearis*

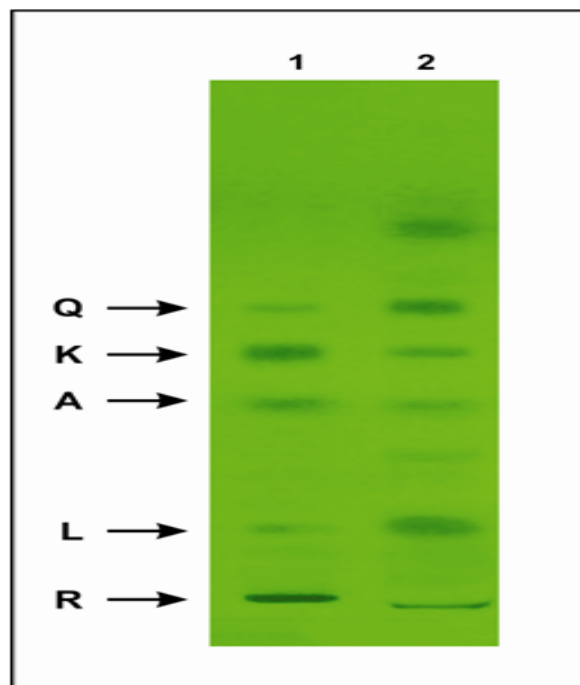


Figure 4. TLC chromatogram of flavanoids extracted from cell culture of *M. linearis*
 1) Marker, 2) *M. linearis*

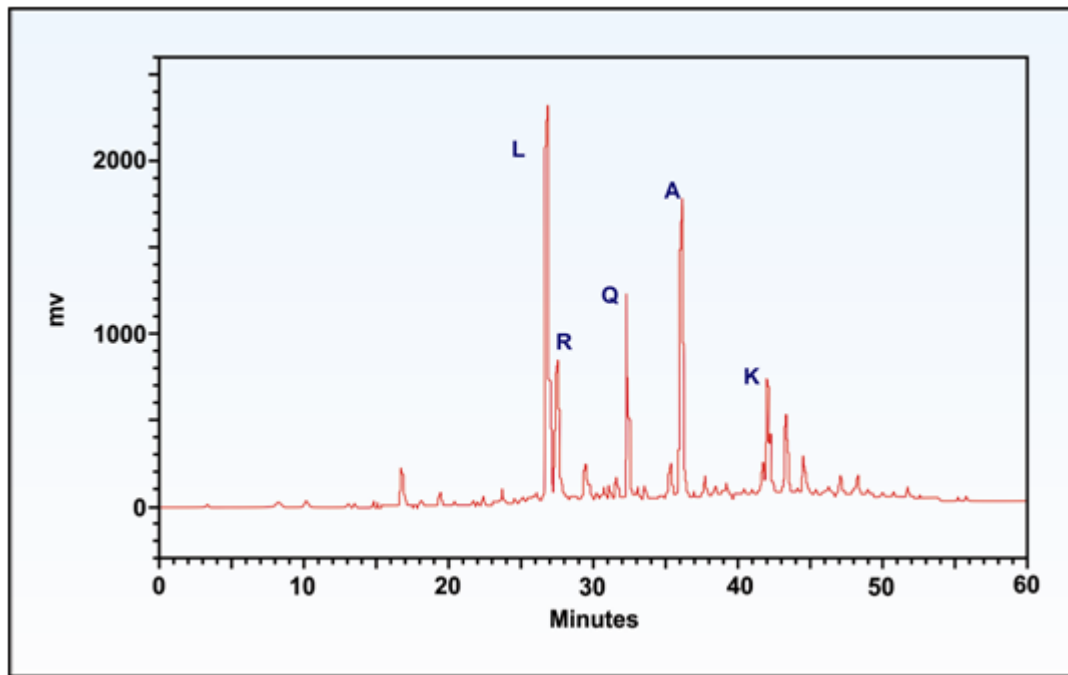


Figure 5. HPLC - PAD Chromatogram of flavonoids of *M. linearis* L - Luteolin, R - Rutin, Q - Quercetin, A – Apigenin & K – Kaempferol



Figure 6 a & b. Larvicidal activity in *Spodoptera litura* against flavonoid extracts of a & b) *M. linearis*

The present investigation revealed insecticidal activities of flavonoids of *M. linearis*. Baskar *et al.*, (2011) reported that ethyl acetate extract of *Aristolochia tagala* contain steroids, phenols, flavonoids and alkaloids, which showed potential insecticidal activity against *S. litura*. Also Arannilewa *et al.*, (2006) reported insecticidal potential of methanolic extract of *Aristolochia ringens* followed by food poisoning, contact poisoning and repellency against *Sitophilus zeamais*. Baskar *et al.*, (2010) also reported antifeedent potential at 5% concentration of ethyl acetate extract of *Couroupita guianensis* against *Helicoverpa armigeras. arborea* displayed potential insecticidal activity against eggs and nymphs of the pest (Cruz-Estrada *et al.*, 2013). de Oliveira *et al.*, (2012) reported dose-dependent insecticidal and repellent effects of *Vitex cymosa* and *Eschweilera pedicellata* extracts against *Sitophilus zeamais* adults. Feeding deterrents or antifeedant ability of leaf and seed extracts of *Azadirachta indica* was confirmed against fourth instar larvae of *Spodoptera litura* by Summarwar and Pandey (2013). In all these studies, the various solvent extracts contain different phytochemicals, but more commonly the polyphenols. So, it was possible to suggest that the present insecticidal potential of *M. linearis* was due to the polyphenols in the extracts. Phenolic compounds have negative effects on insects and can decrease fertility and shortening their life span (Dawkar *et al.*, 2013). Chen *et al.*, (2014) isolated natural molecules like Flavones 5-hydroxyisoderricin, 7-methoxy-8-(3-methylbutadienyl)-flavanone and 5-methoxyisoronchocar-pin from *Tephrosia* species. These molecules showed deterrents against *Spodoptera exempta* and *Spodoptera littoralis*.

Johnson and Dowd (2004) noticed over expressing of transcription factor controlling flavonoid production in *Arabidopsis* to confer resistance against *Spodoptera frugiperda*. 100 ppm isoflavonoids isolated from the wild relatives of chickpea act as antifeedant against *Helicoverpa armigera* (Simmonds and Stevenson, 2001). Judaicin and maackiain were also found to be deterrent to *S. littoralis* and *S. frugiperda*, respectively. Cyanopropenyl glycoside and alliarinoside strongly inhibit feeding by *Pieris napi oleracea*, while a flavone glycoside, isovitexin-6''-D- β -glucopyranoside acts as direct feeding deterrent to the late instars (Simmonds and Stevenson (2001); Al Rashid *et al.*, (2012). Nevertheless, no literature was found considering the efficacy of *Marchantia* on *Spodoptera litura* but extracts of this bryophyta was found to cause significant mortality by affecting nutritional parameters and subsequently, development of this insect pests.

CONCLUSION

Bryophytes were distributed along the diverse ecological niche and possess rich source of magical phytochemicals. Bioprospecting of this group was essential to unravel this biowealth. In the present study, insecticidal assays served as an effective method to screen the potentiality of lower plant groups. The flavonoid extracts exhibited significant insecticidal potentialities against *S. litura* in terms of larvicidal, pupicidal and survival rates, which prove it as a potent insecticidal agent.

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