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Ficus religiosa L.: Callus, Suspension Culture and Lectin Activity in Fruits and *In vitro* Regenerated Tissues

Varsha Parasharami[1*], Priya Yadav[2], Shilpa Mandkulkar[1] and Sushama Gaikwad[2]

[1]Plant Tissue Culture Division, National Chemical Laboratory, Pune, India.
[2]Biochemical Science Division, National Chemical Laboratory, Pune, India.

Authors' contributions

The work was carried out in collaboration by two senior authors VP and SG and their students. Authors SM and PY performed the experimental work. Authors VP and SG planned, executed and analyzed the data and made the final draft. All the four authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Ficus religiosa L. is a tree of immense cultural heritage in Asian countries. It is respected by followers of many religions and faiths. Fruits of *Ficus religiosa* L. are the 'figs' and possess many medicinal properties reported in ethnomedicinal and pharmacological studies. These medicinal properties range from antidiabetic, anticancer, anticonvulsant, antimicrobial, antiviral and antioxidant activities. In the figs, pollination takes place with 'wasp'. Till date no work on 'fruit tissue culture' has been reported in this species. For the first time the callus cultures have been developed using 'fig fruits'. Fruit callus was multiplied on solid medium using 2 mm to 3 mm diameter fruits. During the present study, lectin/hemagglutinin activity was detected in fruits and fruit callus extracts for the first time. Both *In vivo* fruits (figs) and *In vitro* fig callus were used to assay the hemagglutinin activity using pronase treated and untreated rabbit blood erythrocytes. Fruit extract showed 4-8 times more hemagglutination activity in presence of pronase treated erythrocytes. It is the first report of callus/suspension culture and detection of thermostable (up to 70ºC) hemagglutinin/lectin from fruits in this species. Preliminary biochemical characterization of the lectin activity e.g. metal ion requirement, EDTA, pH and temperature stability was carried out during the present study.

**Corresponding author: Email: va.parasharami@ncl.res.in;*

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1. INTRODUCTION

F. religiosa L. tree (Family Moraceae) is unrivalled in its antiquity and religious significance. It is also known as the '*Bodhi tree*', the tree of enlightenment which is probably why many people use its decoction as a brain tonic. This plant has been used in traditional Indian medicine for various ranges of ailments [1]. Many pharmacological and ethno medicinal uses for curing more than 50 ailments have been reported which include antioxidant, antiulcer, and antiamnesia [2-6]. Recently human cervical cancer cell lines treated with aqueous extracts of *Ficus religiosa* showed cell cycle arrest and apoptosis [7]. Biotechnological investigations are fewer and recent. Callus induction from leaf and nodal, inter nodal segments and shoot apices has been reported recently [8,9]. No callus and suspension culture studies using inflorescence/ fruit (which is a fig) has been reported till date. These figs are grown on axillary leafy branchlets, green when young, paired or solitary, red to brown when mature, globose to depressed and 1–1.5 cm in diameter. Male, gall (sterile female) and female flowers occur within the same fig. All figs require pollinator wasp for fertilization. The pollinator wasp of *F. religiosa* is *Blastophaga quadraticeps* [10,11]. Hence, the fruits as explants for tissue culture were not tried till now as contamination problems increases due to this pollinator wasp and other insects inside the fig fruit/ inflorescence.

Plant cell culture is considered as a promising alternative for producing bioactive compounds that are difficult to be obtained by chemical synthesis or plant extraction. Cell culture systems are useful in large-scale culturing of plant cells, which form a continuous and reliable source of secondary metabolites and can be purified easily due to the absence of significant amounts of pigments. This method removes all seasonal constraints and eliminates the geographic barriers for production of secondary metabolites [12]. Plant cells are biosynthetically potent; each cell in culture retains complete genetic information and is able to produce complete range of chemicals found in parent plants. Novel compounds that are not produced by parent plants can also be produced by plant cell culture. Plant cell culture facilitates easy downstream processing, recovery and rapid production of secondary metabolites.

Cell suspension culture has been widely used for cell proliferation in the research field and has extended to commercial use for production of secondary metabolites. These cell suspension cultures follow a growth cycle with lag, exponential, linear and stationary phase. To make the best use of this technique, it is essential to maintain cell quality under liquid culture which has to be kept on shaker at 90 rpm.

Lectins are carbohydrate binding proteins, occurring ubiquitously in nature; in plants, animals and microbes. They are involved in vital cellular activities such as cell to cell recognition and signalling, cell differentiation, immune response and cancer metastasis. A wealth of literature is available on lectins reflecting the tremendous research being carried out in this field [13-15]. Lectins in plants have been studied since the advent of lectinology in 1888. The proposed biological role of lectins in plants is in defence mechanism; some important cellular biochemical activities are also reported. They are commercially available and widely used in analytical and clinical industry. In spite of the tremendous medicinal use of *Ficus religiosa*, there is no report on the lectin activity from any of its plant part.

For preliminary studies of biochemical characterization, purification and other studies on the lectins the parameters such as metal ion requirement, pH and temperature stability are essential to be checked therefore; during the present study we have studied effect of EDTA and many other metal ions with *F. religiosa* lectin.

2. MATERIALS AND METHODS

2.1 Plant Materials and Explants Preparations

2.1.1 Selection of figs

The fruits of *F. religiosa* L. were collected from more than 60 year old tree of *F. religiosa* L. present in the campus of National Chemical Laboratory, Pune and identified and authenticated by Botanical Survey of India. (No- BSI/WRC/Tech./2012/ LS-1 Dated 16-5-2012). The fruiting season was between January to May. After collection of twigs with fruits (Fig. 1A) the fruits were separated carefully from the twigs and selected according to the size for sterilization for callus induction as shown in (Fig. 1B). The twigs with fruits were stored at -20ºC. The fresh fruits showing (2-3 mm diameter) the presence of hemagglutinin activity against rabbit erythrocytes were used for callus induction.

Fig. 1A. Twig with small sized green fruits of *F. religiosa* L

Fig. 1B. Different size freshly collected fruits of *F. religiosa* L. separated according to size

2.1.2 Sterilization treatment of fruits for callus induction

Small fruits sized 1-3 of Fig. 1B were sterilized together while 4-6 sized fruits were sterilized in separate flasks. After initial experiments size 4-6 fruits were not used for initiating callus cultures. The H_2O_2 and NaOCl alone or in combination have been used for the sterilization standardization experiments. Details of sterilization treatments have been given in Table 1. 0.5% (v/v)Tween 20 as detergent (Merck, India), Bavistin (BV- BAIF-India), Sodium

hypochlorite (NaOCl-Merck, India) and Cefotaxime (TXM- ALKEM) were used for explants sterilization and/ or as media additives.

2.1.3 Callus Culture

MS (Murashige and Skoog) [16] was used to initiate callus from the fruit explants (media details in section 2.2.4). The callus induction medium contained 3.0% sucrose (w/v), antifungal agent BV and anti bacterial agent TXM. BV was added to the medium before autoclaving. The pH of the medium was adjusted to 5.8±0.2. Media were solidified using Bacto^{TM} Agar 0.7% (w/v). Medium autoclaving was done at 15 psi pressure for 20 minutes. TXM was added aseptically after filter sterilization to autoclaved medium. Solid medium was poured in disposable petriplates (90 mm diameter-Tarson, India). Each petriplate contained cut Figs (20 explants/ petriplate). More than 100 explants were used for each experiment. After 20-25 days of dark incubation, callus growth was observed from cut ends of the fruits (wound callus). Initially, shifting of explants within 5 to 10 days of incubation was important to reduce oozing of brown phenolics from cut ends which otherwise hampers callus growth. Several media compositions were tried (data not shown), finally two media showing optimum callus growth were selected for further experiments. The media which gave healthy callus were WPM [17] and MS basal medium along with combination of BA (6-benzyl amino purine) 2.22 to 4.44 µM and 2,4-D (2,4-dichlorophenoxy acetic acid) 4.52 to 22.62 µM. Each experiment was repeated three times and data was scored. Table 2 (2nd column) shows the two media compositions which were used to develop the callus from the fruit explants.

2.1.4 Suspension culture

Both MS basal medium along with BA (4.44µM) and 2,4-D (10.95 µM) / or 2,4-D (10.95 µM) were used for raising suspension culture. Liquid medium was prepared and 40 mL was poured in 250 mL pre autoclaved conical flasks (Borosil make). Two months old callus were used to initiate fruit suspension cultures. Subculture and the growth curve studies were regularly performed by harvesting the cells from suspension cultures after every 15 days. Wet weight was noted down after filtering the callus through Whatman® filter paper no #1. For dry weight studies the callus on filter paper were dried in hot air oven set at 56ºC temperatures. Once the dry weight was stabilized after 2-3 days the final dry weight was noted down. Each experiment was repeated 3 times.

2.1.5 Culture condition

Fruit cultures in solid medium were incubated in dark in culture rooms at 25±2ºC temperature. Callus started growing within 20-25 days of incubation but one or two changes of liquid medium was required to avoid phenolic browning at cut end of the explants. Fruit suspension cultures were maintained at 90 rpm in rotator shaker (Steel Mate, India). The shaker room temperature was also maintained at 25±2ºC. Complete darkness was not maintained for shaker room.

2.1.6 Statistical Analysis

All the experiments were repeated three times. More than 100 *F. religiosa* (2-3 mm diameter) sized fruits were used during each experiment. Data were measured after 15-30 days for callus induction and for suspension culture; it was measured after every 15 days to 70 days to obtain the growth curve and the remaining suspension culture was harvested after 70

days of incubation and data was analyzed. The standard deviation (S.D) was recorded for % sterile and % responsive explants.

2.2 Hemagglutination Assay from *In vivo* Fruits and *In vitro* Fruit Callus

2.2.1 Hemagglutination assay

The hemagglutination assay [18] was carried out for the detection of hemagglutinin in the extract from different sizes of *in vivo* fruits and *in vitro* fruit callus from *F. religiosa*. One gm of freshly collected fruits from each size range (Fig. 1B) were crushed in 3 ml PBS (Phosphate buffer 20 mM, pH 7.2, containing 150 mM NaCl), in mortar and pestle. The extract was centrifuged at 3355 g for 5 min. and supernatant (1.5 mL) was collected and tested for hemagglutination assay.

The rabbit blood was collected from National toxicology Centre, Pune India. After centrifugation of blood at 301.9 g for 5 min, serum was removed. Rabbit erythrocytes (RBCs) were washed thoroughly with PBS (Phosphate buffer 20 mM, pH 7.2, containing 150 mM NaCl) and 3% suspension of these erythrocytes was prepared in PBS. Pronase enzyme (10 mg/ml) was added to the 3% erythrocyte suspension and incubated at 37ºC for 1 h. The treated erythrocytes were further washed thrice with PBS and used for the assay [19].

Hemagglutination assays were performed in standard U-shaped microtitre plates (Tarson India) by the two-fold serial dilution method. A 50 µL aliquot of the erythrocyte suspension was mixed with 50 µL of serially diluted hemagglutinin. The hemagglutination was examined visually after incubation for 30 min at room temperature. The unit of hemagglutination activity (U) was expressed as the reciprocal of the highest dilution (titre) of the hemagglutinin that showed the complete hemagglutination. The activity of the hemagglutinin is defined as the units of the hemagglutinin per g weight of fruits or callus (U x 10^3/g).

2.2.2 Effect of EDTA, Ca^{2+}, Mg^{2+} and Zn^{2+}, temp. on hemagglutinin activity of *F. religiosa*

The effect of EDTA (Ethylenediaminetetraacetic acid) and metal ions- Ca^{2+}, Mg^{2+} and Mn^{2+} on hemagglutinin activity was studied. The solutions $CaCl_2$ (Sigma-Aldrich, USA), $MgSO_4 \cdot 7H_2O$ (Qualigens, India), $ZnSO_4 \cdot H_2O$ (sd fine chem, India) and EDTA (Sigma-Aldrich, USA) were prepared separately to get the final concentration of 1 mM of each metal ion in PBS. The PBS with metal ions was used for the extraction of the fruits, size- 2-3 mm diameter. The supernatant was collected and tested for hemagglutination assay and the effect of each metal ion was recorded.

2.2.3 Thermostability Studies of the Lectin

The supernatant from 2-3 mm diameter sized fruit extract was treated to the temperatures of 50ºC, 60ºC and 70ºC in water bath (Quality-R, India). The hemagglutinin activity of heat treated samples was carried out with the pronase treated erythrocytes.

3. RESULTS AND DISCUSSION

Cell cultures have been developed from different sized fruits of *F. religiosa* L. during present study. At initial studies it was detected that fruits when induced for callus, contaminated

within 15 days of culture. Detailed study was carried out to find out proper fruit size and sterilization treatment for callus induction. Sterile callus after second subculture (S_2) onwards was used to develop suspension cultures. During present study both cell and suspension culture have been developed for the first time from *F. religiosa* fruits and established them for phytochemical analysis in future. *In vivo* immature fruits were used for detection of hemagglutinin activity with hemagglutination assay. Only 2-3 mm diameter sized fruits showed the maximum activity, hence attempts were made to grow callus from same sized fruits. The hemagglutinin activity was detected from the fruit callus too. This is the first report of detection of hemagglutinin activity directly from fruits and fruit callus cultures of *F. religiosa* L.

3.1 Sterilization Treatment

As shown in Fig. 1B, size 2 - 3 mm and size 4 - 6 mm diameter fruits were sterilized separately. Only 2 -3 mm diameter sized fruits showed 12 well hemagglutinin activity. Therefore, throughout the study only 2 - 3 mm size fruits were used for callus development. Each fruit was cut into 2 halves and was inoculated with cut surfaces touching the medium (Figs. 2 a, b). As shown in Fig. 2c within 3-5 days when phenolic extrudes was observed on medium and the positions of the explants were changed so that the phenolics will not affect the growing callus.

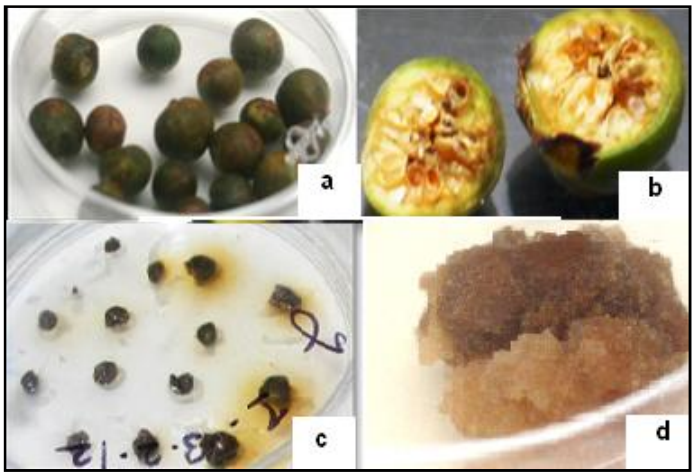

Fig. 2. Fruits (a) after sterilization each fruit was cut into two halves (b) and inoculated facing cut ends touching the medium (c) fruit callus in petriplate after 60 days (d)

In many tree species callus growth is shown to be adversely affected by the leaching of phenolics in the medium from mature explants, hence shifting of explants within 3-5 days was done to avoid this problem.

Table 1 describes the different sterilization treatments applied for small size fruits (1-3 size of Fig. 1B). The optimum treatment standardized (TR No 2) gave almost 80% sterile fruits after 15 days of inoculation. Hence treatment No 2 was chosen during further experiments. Use of BV both during sterilization treatment, its addition in media and 2% NaOCl treatments seems to be the deciding factors for optimum results for sterilization during the preset study. Small

sized *Ficus religiosa* fruits were collected during March to May in summer time when atmospheric temperature was between 30ºC to 40ºC. This also was deciding factor for getting optimum sterile explants. Getting sterile explants from such mature trees is a difficult task, hence to obtain callus from fruits was a challenge.

Table 1. Sterilization treatment for small size fruits (2-3 mm)

TR No	Details of the Sterilization Treatment	No of fruits	% Sterile explants ± SD
1	Running tap water wash + Soak in D/W (1 h) + 0.5% (v/v)Tween 20 wash (20 min)+ H_2O_2 10% (v/v) for (30 min) followed by distilled water wash + NaOCl 1%(w/v) for 30 min. Final wash with SDW (sterile distilled water) under laminar flow.	120	73.33±1.43
2	Soak in D/W (1 h) + 0.5% (v/v) Tween20 wash (10 min) + 1% BV w/v (30 min) 2 washes SDW + H_2O_2 10% (v/v) for 30 min followed by 2 SDW wash + NaOCl 2% (w/v) for 30 min. Final 3 washes with SDW under laminar flow.	120	80.33±5.20
3	Soak in sterile D/W (1 h) +0.5% (v/v) Tween20 (20 min) +)+ H_2O_2 10% (v/v) for 30 min followed by 2 SDW wash+ NaOCl 1% (w/v) for 30 min. Final wash with SDW under laminar flow.	120	75.50±2.50
4	Running tap water wash (1 h) + Soak in D/W (1 hr) +0.5% (v/v) Tween 20 (10 min) followed by 2 SDW washes + H_2O_2 20% (v/v) for 30 min followed by final wash with SDW under laminar flow.	120	51.66±1.43

TR No – Treatment Number

Table 1 show that with the treatment (TR No 2 (80.33 ± 5.2) percent sterile responsive fruit explants was obtained. Hence this treatment was used as the final treatment for 'Fig' sterilization throughout the study. Similarly aseptically added (TXM) between 100-300 mg (w/v) to autoclaved medium for fruit inoculation, also helped in controlling bacterial contamination.

In leaf explants of *F. religiosa* L. during call genesis study BV and TXM concentrations gave more than 70% sterile leaf explants [8]. The positive effect of BV and TXM has been earlier reported in *Pinus roxburghii* mature shoots and seed [20,21].

3.2 Induction of Callus on Solid Medium

For induction of callus MS basal medium with 2,4-D alone or in combination with BA as choice of medium was used. Table 2 shows results obtained with the two medium combinations of the two growth regulators, which were used for callus induction and suspension culture studies. Media1- MS basal medium and BA at 4.44 µM and 2,4-D at 10.95 µM gave responsive explants for callus induction 76.67±3.94. Significantly less sterile and responsive explants were obtained when above results were compared with Medium 2. Medium 2 had only 2,4-D as the growth regulator. This clearly indicated the role of BA for enhanced % responsive callus.

Table 2. Media comparison studies using fruit explants during initial experiments for induction of callus)

Media No.	Media (mg/L)	Sterile % ± S.D	contamination % ± S.D	Responsive % ± S.D
1	MS+ 4.44 µM BA, 10.95 µM 2,4-D+ 100 mg/L BV+ 300 mg/L TXM+ Suc. 3% + Bacto Agar 0. 7%	80.33±5.20	19.67±5.20	76.74±3.94
2	MS+ 10.95 µM 2,4D, 100 mg/L BV+ 100 mg/L TXM+ Suc. 3% + Bacto Agar 0. 7%	57.5±2.50	42.5±2.50	49.20±2.20

Experiments were repeated thrice and SD for percentage sterile and % responsive fruit explants has been recorded

Table 1 and 2 describes the overall results for callus induction in *F.religiosa* during the present study. Incorporation of BV and TXM in callus induction medium and during sterilization treatment use of BV improved the sterility of explants in culture. This is in agreement with our previous reports [8,13]. Fruit callus induction and further growth experiments have been carried out for the 1st time during the present studies in *F. religiosa* L. Fig. 3 shows different type of callus induced in solid medium from the fruits after 20, 30 and 45 days of incubation. Growth of friable callus from fruits was observed after 60 days of incubation. This friable callus was used for induction of suspension cultures which gave smooth callus without clustering of cells in shake flasks.

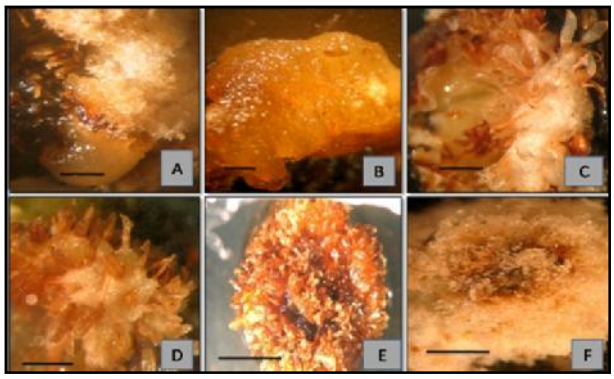

Fig. 3. Fruit callus *Leica* Stereo zoom microscope images A. Freshly initiated callus from fruit within 20 days, B. Callus after 30 days of induction, C D & E fully grown callus showing fibrous structures in 45 days, F. 60 days old friable callus used for induction of suspension cultures

3.3 Suspension Culture Studies

Both the MS and WPM media were used to initiate suspension culture from callus grown on agar solidified medium. Initial studies for 20-30 days showed that callus in WPM liquid culture was not homogeneous. It was observed that WPM suspension cultures produced very low biomass as compared to the higher biomass production in MS suspension cultures using same growth regulators (Comparative data not shown). Hence, for further experiments only MS medium as shown in Table 2 was used throughout the study. For growth curve studies,

the suspension cultures were routinely harvested after 15, 30, 45, 55 and 70 days. Fig. 3 shows the data with culture from Medium 1 as the biomass obtained was more compared to Medium 2. Wet weight was measured after harvesting the cells, while dry weight was measured after keeping the callus in dry air oven at 50°C (till constant dry weight was obtained).

The suspension culture was filtered through what man #1, 90 mm diameter filter paper to obtain the wet weight of the callus. It was then dried at 50ºC in hot air oven and dry weight was measured. For first 15 days no difference in cell mass was noticed (data not shown), hence biomass studies were started after 15 days of initiation of suspension. In the Fig. 4 wet wt callus showed the lag phase up to 25 days (no increase in cell growth), log phase up to 55-60 days and initiation of stationary phase after 70 days.

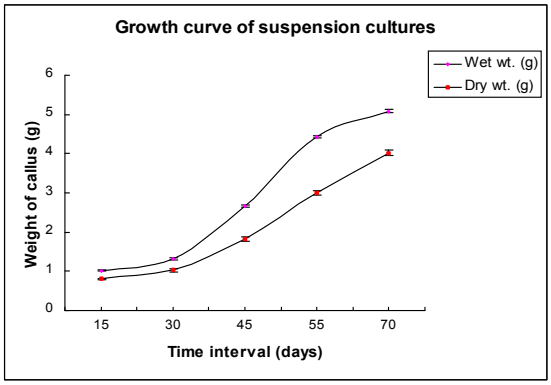

Fig. 4. Growth curve of *Ficus religiosa* callus from suspension for Wet weight and Dry weight Biomass in Medium No. 1

Table 4 shows the wet weight and dry weight results. The results are shown for two media compositions No 1 and 2. It is clearly indicated that even though both basal media compositions were same, the growth regulator BA in medium No.1 helped in getting more biomass for both wet and dry callus (5.08 g and 4.10 g) respectively, as compared to medium No.2 containing only 2,4-D.

Table 3. Fruit suspension culture after 70 days of incubation

Med. NO	Medium for Suspension Culture	Callus details used for suspension Culture	Wet Wt. of callus (g)	Dry Wt. of biomass (g)
1.	MS + 8.88 µM BA + 10.95 µM 2,4-D	2 months old Fruit callus from MS +8.88 µM BA+10.95 µM 2,4-D + 100mg/L BV + 300mg/L TXM	5.08	4.10
2.	MS + 10.95 µM 2,4-D	2months old Fruit callus from MS+4.44 µM BA + 100mg/LBV + 200mg/LTXM and MS+22.20 µM BA + 100mg/LBV + 200mg/LTXM	3.93	3.20

3.4 Hemagglutinin Activity Detection from *In vivo* Small Fruits and *In vitro* Fruit Callus

This is the first report of the detection of hemagglutinin activity from the fruits of *F. religiosa* Table 4 shows that the maximum hemagglutinin activity (U/g of fruits) was observed in *In vivo* fruits of 2-3 mm in diameter (7.68×10^3 U/g) followed by fruits of 5-10 mm diameter (3.84×10^3 U/g).The suspension culture from 70 days old dry fruit callus showed more hemagglutinin activity (2.88×10^3 U/g) as compared to wet callus. The liquid suspension filtrate without callus showed activity 1.92×10^3 U/g. The hemagglutinin activity was observed to be decreasing with increasing fruits' size (from 2-3 mm to 10 mm diameter). Pronase treatment is given to erythrocytes to enhance the exposure of oligosaccharide chains on the cell surface. Fig. 5 is showing enhanced hemagglutination with pronase treated erythrocytes (column no 5 and 6 - 12 well activity), column no 2-3-10 well activity without pronase treated erythrocytes), control have button formation i.e.no hemagglutinin activity.

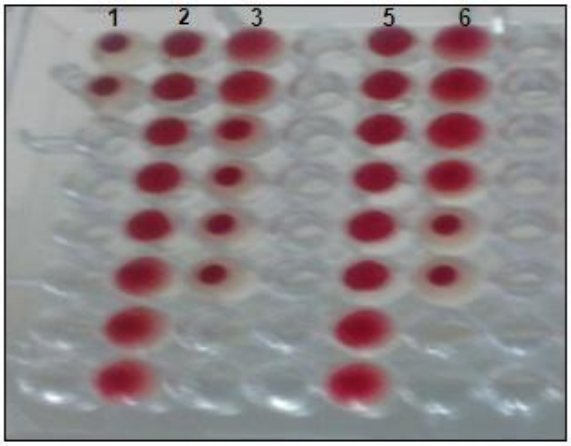

Fig. 5. Hemagglutination assay for the lectin activity. Button formation indicates no activity. Column 1: Negative control (PBS + 3% RBCs), Column 2 and 3: PBS + *F. religiosa* fruit extract (serially diluted) + 3% RBCs-10 well activity, Column 5 and 6: PBS + *F. religiosa* fruit extract (serially diluted) + 3% RBCs (Pronase treated)-12 well activity

Table 4. Hemagglutinin activity in fruits (different sizes) and 70 days old wet & dry callus U/g (10^3)

Sample	U/g (x 10^3) of fruits
2-3 mm small green fruits	7.68
5-10mm big green fruits	3.84
70 days old wet callus	1.92
70 days old dry callus	2.88
Suspension filtrate without callus	1.92

Hemagglutinin activity was not affected by the presence of EDTA, Ca^{2+}, Mg^{2+}, and Zn^{2+} indicating it is neither metalloprotein nor metal requiring hemagglutinin. Several lectins e.g. Con A have been reported to have absolute metal requirement for activity; so as a part of characterization, effect of metal ions on the present lectin was studied.

The activity of the above lectin was stable up to 70°C indicating it to be a thermostable protein. Thermos table lectins have also been reported from *Trichosanthes dioica*, *Moringa oliefera*, *Aracea* lectin [18,22,23,24].

Lectins have numerous applications in glycoprotein separation and purification, in biochemistry, cell biology and cancer cell studies [13] etc. The saccharide specificity of the present lectin can reveal its potential application in analytical as well as cancer cell studies. Also, examination of antimicrobial and anti insecticidal activity of the pure lectin can be performed. The purification, characterization and investigation of biochemical, biophysical properties would be the aspects of further studies with this lectin.

Further optimization of cultural conditions would definitely help in increasing the hemagglutinin activity in callus and suspension cultures, which will be a step towards drug discovery from *F. religiosa* under *in vitro* conditions, which has not been done so far despite numerous reports of its pharmacological and ethnomedicinal studies.

4. CONCLUSIONS

Callus cultures were raised from the fruits of *F. religiosa* for the first time which was possible only because of efficient sterilization treatments followed throughout the study. The cells from suspension culture were smooth/ evenly distributed when grown in MS basal medium. These cells were harvested for biomass and hemagglutinin activity detection. However, the cells from WPM basal medium had less biomass and formed clumps under suspension culture conditions. So, those cells were not used for further hemagglutinin activity detection.

Different sized fresh fruits were used for detection of hemagglutinin activity using rabbit erythrocytes. Only the fruit size (2-3 mm diameter) which were showing maximum activity were used for raising the callus. Hence, when the 70 days old wet and dry callus was analyzed for hemagglutinin activity, the callus also showed the activity during the present study. Even the suspension filtrate without callus showed the presence of hemagglutinin.

Biosynthesis of lectin in *F. religiosa* fruits seems to occur at early growth stage. The reason for growth dependant lectin activity is to be investigated. Also the structural studies and sugar specificity will throw some light on the novel properties of this lectin.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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