International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

# In Vivo: Anti-Larvicidal (Anthelmintic) Activity of Spondias Pinnata Against Fasciola Gigantica Larvae

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**ABSTRACT**

The aim of the present study is to evaluate in vivo anti-larvicidal (anthelmintic) activities of Spondias pinnata plant against sporocyst, redia, and cercaria (Fasciola gigantica) larvae. The liver fluke species of F. gigantica are caused zoonotic diseases in cattle and human populations. The common intermediate host snail Lymnaeids (Lymnaea acuminata) are carriers of the Fasciola. It can be controlled by the break of the life cycle in the intermediate host snail. The in vivo exposure of leaf powder and, various organic extracts, of S. pinnata, was observed up to 8h at various concentrations against sporocyst, redia, and cercaria. The anti-larvicidal effect of different preparations was observed at 2, 4, 6, and 8h of the exposure period. The exposure of leaf powder of S. pinnata in vivo was more effective against the sporocyst (2h $LC_{50}$ 68.64mg/L and 8h $LC_{50}$ 72.13mg/L). The column extract of leaf powder (CELP) of S. pinnata against sporocyst, redia, and cercaria in 2h $LC_{50}$ was 66.38, 69.63, and 68.33 mg/L and 8h $LC_{50}$ was 63.10, 66.03, and 65.86 mg/L, respectively. The ethanolic column extract of the S. pinnata leaf powder have better anti-larvicidal activities than the other organic extract.

**Keywords:** Anti-larvicidal; Anthelmintic; Spondias pinnata; Lymnaea acuminata; Fasciola gigantica.

## INTRODUCTION

Fasciola causes liver flukes in both humans and cattle's. This fluke was limited to a particular part of the geographical division, but it is now widely distributed throughout the world, and infect have been observed in America, Europe, Africa, and Asia (Soliman, 2008). This disease is spreading worldwide due to global exchanges and the movement of infected host animals (Soliman, 2013; Cabada et al., 2014; Bless et al., 2015). Fasciola hepatica and F. gigantica causes fascioliasis and completes its life cycle in the freshwater host snail Lymnaea acuminata (Mas-Coma et al., 2009; Kumar et al., 2018; Kumar et al., 2020; Vishwakarma and Kumar, 2021). Liver fluke infection can occur in humans and cattle due to consumption of Fasciola larvae contaminated water, leaves of aquatic plants, aquatic vegetables (Mas-Coma et al., 2018; Kumar et al., 2020). This disease damages the liver of the animals and causes liver cirrhosis which decreases their growth, development, and body weight, higher mortality, and low production of milk, meat, and wool (Kumar and Singh, 2006; Vishwakarma and Kumar, 2021). The host snails are infected through a free-swimming miracidium larva that develops sporocyst, redia, and cercaria in the snail body. These larval stages transform into a parasite stage, followed by asexual

reproduction. Therefore, one of the strategic techniques to control fluke could be to kill the larval stages early in the life cycle within the host.

Anthelmintic synthetic larvicides are often used to control Fasciola larvae, but their use leads to the development of resistance in the larvae and also has adverse effects on the environment and non-target organisms (Sunita et al., 2013a). Synthetic drugs/chemicals can be replaced by the use of phytochemicals which are easily available, biodegradable, eco-friendly, and low cost. Medicinal plants play the most important role in traditional medicine in various developing countries. A common traditional Indian medicinal plant Spondias pinnata are uses against various human diseases (Kalase and Jadhav, 2013). This plant is mainly found in India, Sri Lanka, Bangladesh, and Southeast Asian countries (Jain et al., 2014). This plant traditionally used in treatment of infectious disease like dysentery and ulcer (Hout et al., 2006). The leaf of S. pinnata is aromatic, acidic, and astringent in nature which is used in dysentery. The ethanolic extracts of S. pinnata pulp have antimicrobial activities (Keawsaard and Liawruangrath, 2009). In the present study, dried leaf powder, various organic extracts and S. Pinnata column extract of F. gigantica has been subjected to determination of in vivo anthelmintic larvicidal activity against sporocysts, redia and cercariae larvae.

## MATERIAL AND METHODS

### Collection of infected host snails

Adult infected snail L. acuminata (2.5±0.21 cm in length) were collected locally from ponds and low-lying areas. Infected snails have often followed the allocation of more resources to growth with the result the infected snails can grow large than uninfected snails (Sunita and Singh, 2011), locomotion is very slow than uninfected ones, and it appeared yellowish spots, foots are swollen and shedding cercaria were appeared at the mouth of snails and shell morphology is changed in infected snails (Hay et al., 2005; Lagrue et al., 2007; Sunita et al., 2013b).

### Leaves crude powder

The leaves of Spondias pinnata were collected from the college campus. The leaves were washed with water, and completely dried in shade, after drying the leaf material was grind in an electric grinder machine and the raw powder thus obtained was sieved with the help of a fine mesh cloth. The collected crude powder was stored in a small glass container in laboratory condition.

### Organic extracts of crude powder

Ten gram dried leaves powder S. pinnata was extracted with 200 mL of 98% acetone, 99.7% chloroform, 95% ethanol, 98% ether, and 98% methanol at laboratory condition ($26C^0$) for 48 hours. Each preparation was filtered separately through sterilized Whatman No-1 filter paper and the filtered extracts were subsequently evaporated under a vacuum machine. The residues, thus obtained, were used for the determination of larvicidal activity. The stem powder of S. pinnata yielded 130 mg acetone, 140 mg chloroform, 125 mg ethanol, 135 mg ether, and 133 mg methanol extracts.

### Preparation of column extracts

Five hundred milliliter of ethanol extract purification of dried leave powder of S. pinnata was subjected to silica gel (60-120 mesh, Qualigens Glass, Precious Electrochemidus Private Limited, Bombay, India) chromatography through a 5×45 cm glass column. Five-milliliter fractions eluted with ethanol (95%) were collected in a small glass bottle. Organic solvent was evaporated under a vacuum machine and the remaining solids extracts obtained were used for the experiment.

### Experimental assay

In vivo larvicidal activities of S. pinnata leaf and their various preparations at different concentrations were determined in the body of host snail L. acuminata against sporocyst, redia, and cercaria larvae of F. gigantica. After exposure 2h, 4h, 6h, and 8h, various preparations of S. pinnata leaf powder in different concentrations against larvae and treated snails were dissected under a light microscope and dead and live larvae (sporocyst, redia, and cercaria) was counted for data analysis. The larval death was confirmed by seizing the movement/locomotion. The movement/locomotion was continuously observed for up to 48h in treated groups of the experiment to ensure larval death (Vishwakarma and Kumar, 2021). The larval mortalities data were analyzed to determination of lethal concentration ($LC_{50}$) value, Lower confidence limit (LCL), upper confidence limit (LCL), slope value (s-value), t-ratio, g-value, and heterogeneity factors (HF-value) with the help of a POLO computer program (Robertson et al., 2007).

## RESULTS

In this study, we have observed that in vivo toxic properties of dried leaf powder of S. pinnata, their various organic extract, and column extract against sporocyst, redia, and cercaria larvae was time and concentration-dependent (Table-1). The exposure of dried leaf powder in vivo of the S. pinnata was more effective against the sporocyst (2h $LC_{50}$ 74.86mg/L and 8h $LC_{50}$ 72.13mg/L) (Table-1). Therefore, the 8h $LC_{50}$ of ethanol extract against sporocyst, redia, and cercaria was 68.64, 68.44, and 71.11 mg/L, respectively (Table-1). However, a higher effect was observed against redia larvae. The exposure of column extract of leaf powder (CELP) of S. pinnata against sporocyst, redia, and cercaria in 2h $LC_{50}$ was 66.38, 69.63, and 68.33 mg/L and 8h $LC_{50}$ was 63.10, 66.03, and 65.86 mg/mL, respectively (Table-1&Fig-1). The high effects of column extract were observed against sporocyst larva (8h $LC_{50}$ 63.10 mg/L) of Fasciola. Therefore, no mortality was observed in the control group.

## DISCUSSION

The medicinal plant Spondias pinnata have antioxidant, anti-inflammatory, anti-dermentia, anti-arthritis, anti-pyretic, anti-hypertension, antifertility, anti-microbial, anti-helmintic, and analgesic properties (Sameh et al., 2018). In vivo exposure of dried leaf powder and various organic extracts of S. pinnata have toxic properties against sporocyst, redia, and cercaria larvae of the F. gigantica. The treatment of dried leaf powder of S. pinnata in vivo against sporocyst, redia, and cercaria in 2h $LC_{50}$ was 68.64, 69.22, and 70.36 mg/L and 8h $LC_{50}$ was 72.13, 73.18, and 76.63 mg/mL, respectively. Sameh et al., (2018) has been reported the leaf extract of S. pinnata have antibacterial, and anti-viral properties. Whereas, the ethanolic extract of S. pinnata bark powder exhibits antipyretic activities at 200 and 400 mg/Kg oral dose (Panda et al., 2014). The root and bark extract of S. pinnata are also uses in the treatment of menstruation and gonorrhea disease (Sameh et al., 2018). The aqueous and methanolic bark extract of S. pinnata exhibits antibacterial properties against S. typhimurium, E. coli, and V. cholera (Arif et al., 2008; Chetia and Gogoi, 2011). The leaf of S. pinnata has alkaloids, flavonoids, steroid, tannins, triterpenoids, saponin, and resins (Samesh et al., 2018).

The active phytochemicals of S. pinnata may be enter in the host snail and gradually diffused in the larval body and progressively increases along with concentration and exposure period in the larvae. The presence of flavonoids, tannins, steroid, triterpenoids, alkaloids, resins, and saponin active components in S. pinnata may be responsible for toxicity of sporocyst, redia, and cercaria larvae. The results section indicates that S. pinnata leaf extracts have a significant amount of toxic larvicidal active phytochemicals which easily diffused in larvae and caused in vivo mortality. The maximum toxic effect was observed in

the ethanol extract among other organic extracts that indicate the active phytochemicals of S. pinnata leaf are more soluble in ethanol. After the in vivo exposure of S. pinnata leaf extracts in water, it may be possible that the active phytochemicals interact with larval enzyme and cause moiety effect.

The values of lower confidence limits (LCL), upper confidence limits (UCL), and $LC_{50}$ are significantly variable in all the exposure periods (Table-1). The t-ratio value is greater than 1.96 which indicates that the regression is significant in all groups of the experiment along with various concentrations of extracts.

## CONCLUSION

The results of the present study reveal that leaf powder of S. pinnata and their various organic extract have toxic effect against F. gigantica larvae of sporocyst, redia, and cercaria. The various preparations of S. pinnata leaf can be used to control of Fasciola larvae. It also can be use for the treatment of infected host snails. Therefore, the ethanolic extract and their column extract of the S. pinnata leaf powder exhibits better anthelmintic larvicidal properties than the other organic extract. It also encourages further research to find out how the active components of S. pinnata produce their toxic effects at the molecular level in Fasciola larvae.

**Conflict of Interest:**

The authors have no conflict of interest.

**Table-1: In vivo toxicity of dried leaf powder (LP), various organic extracts, and column extract of Spondias pinnata was observed against sporocyst, redia, and cercaria larva.**

| Prepared Larvicides (mg.L$^{-1}$) | Larvae | 2h Exposure | | | | 4h Exposure | | | | 6h Exposure | | | | 8h Exposure | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $LC_{50}$ value | LCL | UCL | 't'-ratio | $LC_{50}$ value | LCL | UCL | 't'-ratio | $LC_{50}$ value | LCL | UCL | 't'-ratio | $LC_{50}$ value | LCL | UCL | 't'-ratio |
| S. pinnata (LP) | Spo | 74.86 | 68.64 | 78.32 | 3.31 | 73.69 | 68.65 | 77.98 | 2.10 | 73.08 | 69.13 | 78.42 | 2.18 | 72.13 | 67.49 | 78.40 | 3.71 |
| | Red | 75.82 | 69.22 | 79.88 | 3.18 | 75.06 | 69.12 | 79.63 | 2.42 | 74.81 | 69.44 | 79.82 | 2.26 | 73.18 | 65.38 | 78.43 | 2.19 |
| | Cir | 77.92 | 70.36 | 81.82 | 3.10 | 77.55 | 68.62 | 82.13 | 3.15 | 77.18 | 67.65 | 80.03 | 2.26 | 76.63 | 70.82 | 81.66 | 2.13 |
| Acet-Ext | Spo | 73.32 | 69.13 | 77.92 | 2.40 | 72.82 | 67.33 | 76.22 | 2.02 | 71.16 | 68.63 | 77.18 | 3.51 | 70.43 | 66.96 | 75.91 | 2.23 |
| | Red | 73.18 | 68.13 | 76.18 | 2.24 | 72.44 | 67.42 | 78.43 | 3.15 | 72.10 | 68.14 | 77.08 | 2.18 | 71.04 | 64.82 | 77.38 | 3.42 |
| | Cir | 76.13 | 69.43 | 79.89 | 2.23 | 76.01 | 69.14 | 80.80 | 2.20 | 75.82 | 70.22 | 79.11 | 2.32 | 74.36 | 68.40 | 79.36 | 2.30 |
| Chlo-Ext | Spo | 73.66 | 69.64 | 76.82 | 2.17 | 73.06 | 68.13 | 77.82 | 3.51 | 72.89 | 67.92 | 78.64 | 3.42 | 72.13 | 67.34 | 77.73 | 2.37 |
| | Red | 72.62 | 69.82 | 75.66 | 2.22 | 71.40 | 66.63 | 75.18 | 2.23 | 70.23 | 60.82 | 74.66 | 3.23 | 70.11 | 66.81 | 79.12 | 2.18 |
| | Cir | 74. | 68. | 78. | 2.3 | 74. | 68. | 79. | 3.4 | 73. | 68. | 78. | 3.1 | 72. | 67. | 77. | 3.4 |

| | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 86 | 44 | 13 | 0 | 11 | 73 | 27 | 8 | 66 | 72 | 63 | 5 | 42 | 13 | 42 | 1 |
| Ethn-Ext | **Spo** | 71.43 | 66.09 | 74.62 | 3.30 | 70.85 | 65.96 | 76.63 | 2.41 | 70.10 | 66.92 | 79.13 | 2.35 | 69.64 | 61.42 | 73.63 | 3.41 |
| | **Red** | 70.82 | 64.11 | 76.85 | 3.14 | 70.34 | 65.82 | 76.95 | 2.28 | 70.10 | 65.80 | 76.96 | 2.27 | 68.44 | 63.85 | 73.88 | 3.21 |
| | **Cir** | 73.55 | 67.82 | 77.36 | 3.12 | 73.10 | 67.69 | 78.63 | 3.13 | 72.63 | 67.42 | 77.38 | 2.25 | 71.11 | 65.93 | 78.63 | 2.38 |
| Ethe-Ext | **Spo** | 73.11 | 65.92 | 77.38 | 2.35 | 72.96 | 64.82 | 78.91 | 3.26 | 71.63 | 64.83 | 78.63 | 3.26 | 70.63 | 64.12 | 78.63 | 2.16 |
| | **Red** | 74.66 | 69.82 | 77.80 | 3.19 | 73.82 | 68.18 | 77.63 | 3.16 | 72.10 | 68.13 | 75.11 | 3.31 | 71.65 | 61.77 | 76.42 | 2.16 |
| | **Cir** | 74.65 | 68.58 | 79.42 | 3.23 | 73.30 | 66.83 | 77.82 | 2.11 | 72.65 | 66.92 | 78.13 | 3.67 | 71.82 | 65.48 | 79.11 | 2.35 |
| Meth-Ext | **Spo** | 73.82 | 64.96 | 78.92 | 2.28 | 72.13 | 63.43 | 77.38 | 2.51 | 72.06 | 63.22 | 77.82 | 2.11 | 71.11 | 67.92 | 76.33 | 3.31 |
| | **Red** | 73.11 | 68.11 | 78.82 | 2.29 | 72.82 | 66.38 | 78.08 | 2.18 | 71.18 | 66.73 | 78.82 | 2.26 | 70.08 | 66.43 | 77.34 | 3.32 |
| | **Cir** | 73.11 | 69.33 | 78.81 | 2.42 | 72.68 | 68.18 | 76.80 | 3.40 | 72.11 | 67.56 | 79.63 | 3.13 | 71.92 | 66.71 | 78.70 | 3.24 |
| CELP | **Spo** | 66.38 | 60.68 | 72.92 | 2.25 | 65.11 | 61.96 | 72.36 | 3.13 | 64.33 | 58.42 | 73.42 | 2.33 | 63.10 | 58.12 | 68.11 | 2.54 |
| | **Red** | 69.63 | 63.44 | 73.42 | 2.41 | 68.86 | 61.80 | 74.18 | 3.15 | 67.18 | 64.82 | 74.33 | 3.36 | 66.03 | 60.69 | 73.22 | 2.16 |
| | **Cir** | 68.33 | 61.69 | 73.39 | 2.16 | 67.11 | 60.81 | 74.11 | 2.21 | 66.34 | 60.56 | 71.30 | 3.22 | 65.86 | 60.13 | 70.03 | 2.38 |

In each batch, 10 infected snails were treated in various concentrations in six replicates of the above preparations and treated snails dissect under light microscope for larval counting.

The larval mortality was observed every 2h up to 8h exposure.

Abbreviations: LP=Leaf powder; Acet-Ext=Acetone extract; Chlo-Ext=Chloroform extract; Ethn-Ext = Ethanol extract; Ethe-Ext =Ether extract; Meth-Ext=Methanol extract; CELP =Column extract leaf powder; Spo=Sporocyst; Red=Redia; Cir=Cercaria; LC=Lethal concentration; LCL=Lower confidence limits; UCL=Upper confidence limits.

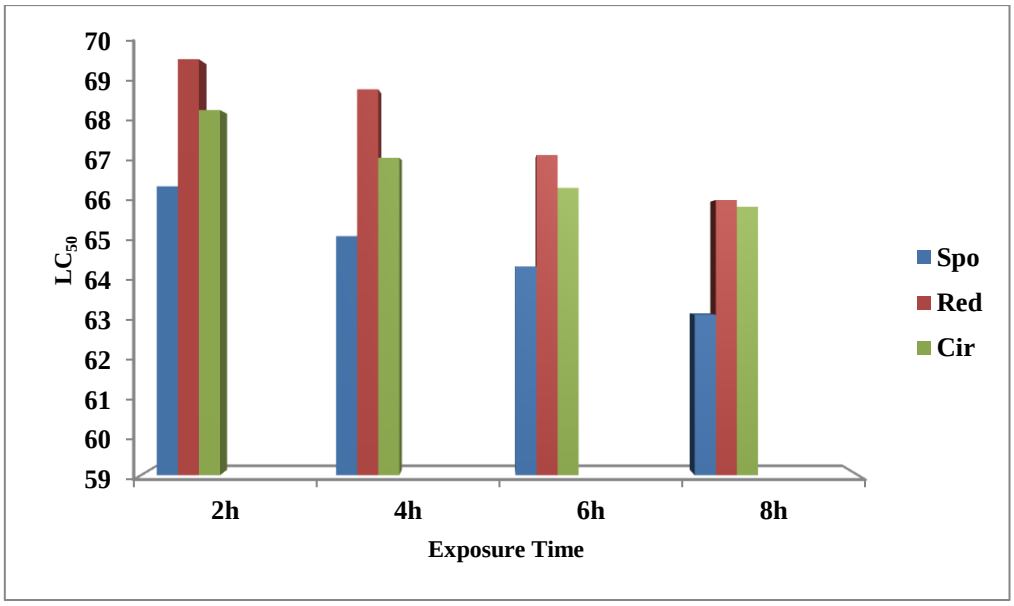


**Fig. 1: Histogram representing in vivo toxicity ($LC_{50}$) of column extract of leaf powder (CELP) of S. pinnata at 2h, 4h, 6h, and 8h against sporocyst (Spo), redia (Red), and cercaria (Cir), larva.**

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