ANTIVIRAL ACTIVITY OF \textit{LATHAKARANJA} (\textit{CAESALPINIA CRISTA} \textit{L.}) CRUDE EXTRACTS ON SELECTED ANIMAL VIRUSES

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ABSTRACT

Viruses cause economically important diseases to animals and antiviral drugs are not commonly used in veterinary practice. Present study reports the antiviral effect of \textit{Caesalpinia crista} (\textit{Lathakaranja}) crude extracts the drug mentioned in Ayurvedic literature for \textit{krimighna} activity. The drug was evaluated using battery of phyto-chemical tests and crude extracts of aqueous, methanol, ethanol and chloroform was prepared. Antiviral activity against paramyxovirus and orthomyxovirus isolates recovered from disease outbreaks in poultry birds was tested. \textit{Aqueous, ethanol and methanolic extracts of lathakaranja showed complete inhibition on paramyxovirus while showing highly significant inhibitory activity on orthomyxovirus.} Results of the study conclude that the medicinal plant \textit{C.crista} might be useful against economically important viral pathogens of poultry birds.

KEY WORDS: Antiviral Activity, \textit{Lathakaranja}, \textit{Caesalpinia crista}, crude extracts, phyto-chemical study

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INTRODUCTION

Viruses are obligate intracellular parasites, which contain little more than bundles of gene strands of either RNA or DNA, and some are surrounded by a lipid-containing envelope. Unlike bacteria, fungus and parasites, viruses are not autonomous organisms and therefore, require living cells in which they replicate. Consequently, most of the steps in their replication involve normal cellular metabolic pathways, and this makes it difficult to design a treatment to attack the virion directly or its replication, without accompanying adverse effects on the infected cells (Wagner and Hewlett, 1999). Today viral diseases cause devastating endemic diseases both in animals and humans. Anti-virals are used clinically to limited extent in human medicine while these expensive antiviral drugs are not economically viable options in veterinary practice. The development of new medicinal plant products is vital in controlling the threats posed by pathogenic viruses. In this context various research groups in Asia, Far East, Europe and America have given particular attention to develop antiviral agents from their native traditional plant medicines.

Drugs acting on microbial agents have been mentioned in Ayurvedic texts as Krimighna Dravyas. The drug is known to act in Kushta (Sushruta Samhita Sthana 38/5, 38/10; Bhavaprakasha Nighantu; Saligrama Nighantu), Krimigna (Kayadeva Nighantu; Bhavaprakasha Nighantu). In addition, a special chapter has been mentioned about krimiprakarana in Charaka samhita, vimanasthana chapter 7. In Sushruta Samhita Uttaratantra chapter 54, krimi and its treatment protocol has been mentioned. Seed kernel of Lathakaranja with sour gruel elevates dysentery with mucous blood and gripping pain (Vrindha Madhava 6/6).

Many traditional medicinal plants have been reported to have strong antimicrobial activity (Makhloufi et al., 2012) and some of them have already been used to treat animals and people who suffer from viral infection (Hudson, 1990; Venkateswaran et al., 1987). Several hundred plant and herbs species that have potential as novel antiviral agents have been studied and wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulphides, polyphenolics, coumarins, saponins, furyl compounds, alkaloids, thiophenes, proteins and peptides have been identified. Some volatile essential oils of commonly used culinary herbs, spices and herbal teas have also exhibited a high level of antiviral activity (Cowan, 1999).

Lathakaranja (Caesalpinia crista L.) is one of the herbs mentioned in all Ayurvedic texts and has been generously used all over India since centuries. Traditionally, it is used in post partum period, as it is a uterine stimulant, to cleanse the uterus. It also alleviates fever, oedema and abdominal pain during this period. In the seeds of C. crista, bitter principles like bonducin and natin have been found in addition to other constituents like linolic acid, fatty acids, seta sitosterol, linolenic acids and 13 types of diterpenoids. Caesapin is the major constituent extensively studied for therapeutic effects. Percentage of nut-oil yield is 60–80%, kernel yield is 20%, bonducellin has been isolated and its structure established recently (Satnami and Yadava 2011). Studies have shown the antiascaridal (Javed et al., 1994), antimalarial (Linn et al., 2005) and anthelmintic (Jabbar et al., 2007) activity of crude extracts of C crista. However, literature is silent on antimicrobial activity especially the antiviral activity of the crude drug extracts.

Myxoviruses and paramyxoviruses cause economically important disease both in humans and animals (Beare, 1975). For the purpose of present study myxovirus isolates causing influenza and paramyxovirus isolates causing Newcastle disease in poultry birds have been selected to study the antiviral activity. The study results regarding the evaluation of antiviral activity of lathakaranja crude extract on orthomyxo and paramyxo viruses are presented here.
MATERIALS AND METHODS

Collection and identification of plant material

Based on the textual references in Ayurveda and the available recent literatures Lathakaranja was considered for its antimicrobial activity in the present study. Its authenticity was identified and confirmed using morphological and anatomical features by Professor N.P. Kaur, Department of Botany, College of Basic Sciences, Punjab Agriculture University, Ludhiana, Punjab. A voucher specimen was deposited at the Herbarium of the Babe Ke Ayurvedic College and Hospital, Daudhar, Moga, Punjab.

Preliminary phytochemical analysis

Preliminary phytochemical analysis was done for qualitative assessment of phytoconstituents as per the standard protocols mentioned in Trease and Evans (1983). Grounded dried powder was subjected to tests for detection of tannins, alkaloids, saponins, cardiac glycosides, anthroquinone glycosides, steroids: (terpenoids and flavonoids), resins and volatile oils.

Preparation of crude drug extract from plant material

Plant extracts known to have antimicrobial activity was done as per the standard procedures published elsewhere. Aqueous extraction of the drug was done by subjecting 20 g of air dried powder for soxhlet extraction continuously for 12 h. The extract was air dried under mild heat of 50°C till moisture completely evaporated. Ethanol, methanol and chloroform extractions were accomplished, following the procedures reported elsewhere with minor modification suiting to the laboratory conditions. For each extraction, the ground powder was weighed 100 g and immersed in ten times of 80% ethanol or methanol or chloroform and allowed for cold percolation on magnetic stirrer for 24 h. The extracts were first filtered through double layer of muslin cloth and then with Whatman No.1 filter paper. The filtrate was air dried under low heat of 50°C and stored at −20°C till further use.

Cell culture, embryonated hen’s egg and virus isolates (Saif, 2003)

Bovine kidney cells (MDBK) cell line was grown as a monolayer culture in Eagles minimum essential medium (MEM) supplemented with 10% foetus bovine serum (FBS), 100 units/ml penicillin and100 µg/ml streptomycin, 20 mg/ml. The cultures were maintained at 37°C in a humidified 5% CO2 incubator. For the growth and culturing of viruses used in the study, embryonated hen’s eggs were obtained from a reputed poultry breeding company (Venkateswara Hatcheries Ltd. Ludhiana). Viruses were maintained in 10–11 days old embryos by inoculating via all allantoic route of inoculation. Standard laboratory and clinical viral isolates of Orthomyxovirus and Paramyxovirus were kindly provided by Department of Animal Biotechnology, Guru Anged Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana.

Cytotoxicity assay (Weyermann et al., 2005)

Cytotoxicity assays were conducted in vitro using cell cultures. The dry crude extracts were re-dissolved in dimethyl sulfoxide (DMSO) and 10 fold dilutions were made in cell culture medium. For tetrazolium-dye (MTT) cytotoxicity assay, adherent cell monolayers in cultures were trypsinized and washed with culture medium. The cells were plated at 15,000 cells/well in 96-well flat-bottomed plates. After a 24 h pre-incubation period, extract dilutions were added to the appropriate wells and the plates were incubated for 24 and 48 h at 37°C in a humidified CO2 incubator. Untreated cells were used as controls. After the incubation, the supernatants were removed from all the wells and 25 µl of MTT (2 mg/ml) solution in phosphate buffer saline (PBS) was added and the plates were incubated for 2 hr at 37°C. Then 125 µl of DMSO was added to the
wells to stabilize the MTT crystals. The plates were placed in shaker for 15 min and absorbance was read at 492 nm on multiwell spectrophotometer. Control cells lysed with DMSO 2 h previous to evaluation of cellular viability by the MTT assay were used as blank the spectrophotometer. The percentage of cytotoxicity was calculated as \((A−B)/A \times 100\), where A is the mean optical density of untreated wells and B is the optical density of the wells with plant extract.

Antiviral assay

To screen the antiviral activity, 10–11 days old embryonated hen’s eggs were used. Dilutions of the plant extracts were pre-incubated with the standard concentration of virus for one hr in shaker incubator at 37°C. After the incubation 0.1 ml of mixtures were inoculated to eggs through intra-allantoic route, holes were sealed and incubated for 48 h in humidified incubator of 37°C. Controls consisted of only virus, only plant extract and the DMSO. The observations for antiviral activity were recorded after 48 h by observing the survivability of embryos in the inoculated eggs and checking the embryo fluid by haemagglutination test using 1% chicken red blood cells.

Micro-haemagglutination assay (HA)

Haemagglutination assay was performed as per the standard procedure (Swayne et al., 1998). The test was conducted in 96-well V-bottomed plates; first 25 µl of PBS was added to all the wells. Allantoic fluids harvested from the inoculated embryonated hen’s eggs were serially diluted at 2 fold dilution. Equal quantity of 1% chicken RBCs were added to all the wells and incubated at room temperature for 20–30 min and reading was taken as mat formation and button formation. Titer of the virus was calculated as reciprocal of the highest dilution of the virus showing the haemagglutination.

RESULTS

Qualitative phytochemical analysis for tannins, alkaloids, saponins, cardiac glycosides, steroids: terpenoids and flavonoids were conducted to confirm the authenticity of the drug collected for the present study. All the results were in accordance with the previously published standard observations. Percentage of crude extracts in various solvents are presented in Table 1.

The in vitro cytotoxicity of the crude plant extract was evaluated using MDBK cells and embryonated hens eggs thorough allantoic route of inoculation. The IC\textsubscript{50}s to the mammalian cells indicate the general cytotoxic effect of these compounds. In the present study antiviral drugs evaluated did not show any cytotoxicity to MDBK cells and embryonated hens egg. Hence might be safe for use in vitro studies (Table 2)

<table>
<thead>
<tr>
<th>Solvent used for drug extract</th>
<th>Yield of crude extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>6.9</td>
</tr>
<tr>
<td>Ethanol</td>
<td>4.5</td>
</tr>
<tr>
<td>Methanol</td>
<td>5.8</td>
</tr>
<tr>
<td>Chloroform</td>
<td>3.8</td>
</tr>
</tbody>
</table>
Table 2. Cytoxicity assay results (percentage toxicity) of different crude extracts of *Lathakaranja (Caesalpinia crista)* on MDBK cells.

<table>
<thead>
<tr>
<th>Plant crude extract</th>
<th>10&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>10&lt;sup&gt;-2&lt;/sup&gt;</th>
<th>10&lt;sup&gt;-3&lt;/sup&gt;</th>
<th>10&lt;sup&gt;-4&lt;/sup&gt;</th>
<th>10&lt;sup&gt;-5&lt;/sup&gt;</th>
<th>10&lt;sup&gt;-6&lt;/sup&gt;</th>
<th>10&lt;sup&gt;-7&lt;/sup&gt;</th>
<th>10&lt;sup&gt;-8&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>36</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>31</td>
</tr>
<tr>
<td>Ethanol</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>33</td>
<td>33</td>
<td>32</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Methanol</td>
<td>36</td>
<td>36</td>
<td>36</td>
<td>34</td>
<td>34</td>
<td>33</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Chloroform</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>34</td>
<td>34</td>
<td>32</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>Virus control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>DMSO control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Antiviral activity (percentage inhibition) of different crude extracts of *Lathakaranja (Caesalpinia crista)* on some standard animal viruses (values are means of five observations)

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Paramyxovirus</th>
<th>Orthomyxovirus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>100%</td>
<td>87.5%</td>
</tr>
<tr>
<td>Ethanol</td>
<td>100%</td>
<td>87.5%</td>
</tr>
<tr>
<td>Methanol</td>
<td>75%</td>
<td>96.25%</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0%</td>
<td>50%</td>
</tr>
<tr>
<td>Virus control</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>DMSO control</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Aqueous extract of *lathakaranja* showed complete inhibition on paramyxovirus while showing highly significant inhibitory activity on orthomyxovirus tested in the present study. Similarly, ethanol extract showed complete inhibitory activity on paramyxovirus while showing highly significant inhibitory activity on orthomyxovirus. Methanol extract showed significant inhibition of paramyxovirus while showing highly significant inhibition of orthomyxovirus. Chloroform extract showed no inhibition paramyxovirus while moderate inhibition was observed on orthomyxovirus (Table 3).

**DISCUSSION**

In the present study plant *Lathakaranja (Caesalpinia crista)* mentioned in Ayurvedic literatures was selected for evaluating antiviral activity. Their authenticity was established by phytochemical analysis and crude extracts of drug was prepared with different solvents using standard established extraction protocols. Their activity against economically important veterinary viral pathogens was evaluated.

It has been reported that the methanol extract of *C. crista* seed and seed kernel possess antifeedant and anthelmintic (Javed *et al.*, 1994; Hördegen *et al.*, 2006; Jabbar *et al.*, 2008).
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CONCLUSION

In the present study Orthomyxovirus and Paramyxovirus candidate animal viruses were selected since, they represent important group of viruses causing economically important and zoonotic diseases of domestic animals and poultry. Crude extracts of antiviral drugs were confirmed safe by testing for their cytotoxicity in MDBK cell lines and by inoculating embryonated chicken eggs. Many of the plants are being used individually or in formulations for treatment of infectious diseases and as germicidal agents. One of the major problems with these herbal formulations is that the active ingredients are not well defined (Cowan 1999). It is important to know the active component and their molecular interaction, which will help to analyse therapeutic efficacy of the product and also to standardize the product. Efforts need to focus on investigating mechanism of action of some of these plants using model systems.

Despite the lack of scientific understanding behind the mechanism of action of medicinal plant extracts, present investigation showed that the Ayurvedic medicinal plant C. crista has immense potential to be developed as antiviral agent. Ayurvedic medicines do have antiviral drugs for use in animal and human practice as to date no antiviral therapy is effectively followed for the reasons inherent to the biology of the virus. However it should be noted that these in vitro results may not translate into clinical effectiveness. Further, studies are needed to understand the phyto-constituents responsible for the observed pharmacological activity and to standardize the dosage and administration schedules for successful clinical applications both in medical and veterinary practice.
REFERENCES


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Conflict of Interest: None Declared