EVALUATION OF APOPTOTIC ACTIVITY OF BANGLADESHI PLANTS EXTRACT

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ABSTRACT

Objective: This study was carried out with four plants, *Ixora nigricans*, *Neolamarckia cadamba*, *Dalbergia stipulacea* and *Hymenidictyon excelsum*, extract and was intended to investigate as well as compare their activity on physiological model by studying in vitro. Method: The in vitro cytotoxic or apoptosis study was made using Brine shrimp lethality a bio-assay method, most known, simple and reliable method in this field. Result: Results of this study were obtained by following graphical analysis and expressed as median lethal concentration (LC₅₀). Among those plant sample the extract from *Ixora nigrican* was found with least value (LC₅₀ = 44.7µg/ml) at 95% confidence limit it ranges from 43.94 to 45.52µg/ml, the lower the value of LC₅₀, higher the toxicity. Conclusion: On the view point from this result, we can conclude by saying that the extracts we studied have some pharmacological property that can aid in the treatment of carcinoma. We need to identify the molecule that causing this effect and then we can move for clinical trial.

INTRODUCTION
Nature is a wide source of phytochemicals, most of which are useful for human beings. Men from the beginning of medicinal development have used plants for healing and curing diseases. Many of them are believed to have cancer chemotherapeutic agents. There are some infectious diseases that are being treated using different plant from the very beginning of medicinal era, such as, urinary tract infection, diarrhea, bronchitis, parasitic infection etc.[1-4] It is the study made for searching drugs useful in certain malignancy therapy. Genetically programmed cytotoxic activity inside the body is known as apoptosis. As cytotoxic agents are so called because they have a capacity to control the unwanted cell growth, especially those are rapid growing and malfunctioned, they are recommended for cancer chemotherapeutic agent for malformed cells carnage. There are many records, from past, of research for finding pharmaceutical hits as chemotherapeutic agent for more specific and effective, for example vincristine and vinblastine (1958) is approved for chemotherapy, whereas in 1992 Paclitaxel approved for breast, lung and prostate cancer and later in 2005 its semi-synthetic derivative Docetaxel was arrived, which is supposed to be more cytotoxic agent than previous one, all these drugs are tubulin inhibitor and synthesized from plant.[5,6] Until now researches are searching for more satisfactory lead molecule, which might be more effective and better from recent molecule.[7] [8] This study also aims to find a new pharmaceutical hit or lead plant extract, from which we can have new anti-carcinogenic agent in the future.

Plants that are chosen for present study have a good background in ethnomedicinal use, like N. caadamba extract is used as nematicidal agent and also in the relief of fever, its popular in Marma tribe Bangladesh. Among other three plant extracts, Hymendictyon excelsum is a well known fish poison, its root and wood is considered as emmenagogue and if taken in small amount acts as an abortion, it's also popular among the Marma tribe as fish poison.

Our main target in this following study is to gather information to find new molecule and to enrich our ethno-pharmacological knowledge about some common plant that are used for a long time in tribal society.

MATERIAL AND METHODS
COLLECTION AND IDENTIFICATION
These plants were collected from Chittagong hill tract, Bangladesh. Then they were identified by expert taxonomist, Dr. Sheikh Bokhtear Uddin, associate Professor, Department of Botany, University of Chittagong.
EXTRACTION\textsuperscript{[9]}

Extract from selected plant part was prepared following cold extraction process. Plant part was dried for several days (7 days) and then was macerated. The dried powder was then dissolved in about 500ml methanol for extraction and it took more 4-5 days. The well dissolved extract was then filtered and concentrated for further use.

CYTOTOXIC ASSAY

BRINE SHRIMP HATCHING

Simulated sea water was prepared for hatching brine shrimp\textsuperscript{[10]}, 38gm pure sea salt was added to 1000ml distilled water in this regard. Then shrimp eggs were given and the whole vessel was kept under proper lightened condition for hatching. After 24 hours incubation nauplii were collected for use.

PREPARATION OF SOLUTION AND EXPERIMENT

Mother solution was prepared using DMSO (dimethyl sulfoxide) and sea water as solvent (20% DMSO with rest portion water to make 1:1 solution). From the stock solution 7 other concentrations were prepared respectively. 7 test tubes were taken with 10 brine shrimp in each of them, filled with different conc. and sea water to make total volume 5ml. A test tube of DMSO was made as control. After 24 hour each test tubes were checked. From the % of morbidity of brine shrimp $LC_{50}$ (lethal concentration) was calculated by plotting against logarithm of concentration.

Statistical analysis\textsuperscript{[11]}: The probit analysis of the test along with their 95% CI for each different extract was calculated using “SPSS-17”.

RESULT AND DISCUSSION

Cytotoxic data contain the percent of mortality of brine shrimp. After 24 hour interval the number of nauplii was counted and the rate of mortality was estimated by dividing the number of dead by the initial number. Median lethal concentration was calculated by plotting a graph log concentration against the percent of mortality.\textsuperscript{[12]} We got $LC_{50}$ 3.04µg/ml of the standard vincristine sulfate (Table-1 and Figure-2), whereas $I. nigricans$ posse’s 44.73µg/ml. It is moderately good result in comparison to standard, from the view of the result it is clear that $I. nigrican$ has cytotoxic potent active substances. Other three plant extract also possessed cytotoxic property (Table-1 and Figure-1), $N. cadamba$ and $H. excelsum$ results also good.
Table 1: Cytotoxicity evaluation of four different Bangladeshi plants

<table>
<thead>
<tr>
<th>Group</th>
<th>LC₅₀ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard (Vincristine sulfate)</td>
<td>3.04</td>
</tr>
<tr>
<td>I. nigricans</td>
<td>44.73</td>
</tr>
<tr>
<td>N. cadamba</td>
<td>51.14</td>
</tr>
<tr>
<td>D. stipulacea</td>
<td>247.68</td>
</tr>
<tr>
<td>H. excelsum</td>
<td>64.67</td>
</tr>
</tbody>
</table>

Figure 1: Lethality bioassay of four studied plant and their comparison with each other

Figure 2: lethality bioassay of standard drug (vincristine sulfate)

CONCLUSION

The brine shrimp bio-assay is a rapid, cost effective and easy process to access bio-activity of test plants which represents the cytotoxicity or anti-tumor property. From many times
before herbal source is considered as safe in the treatment of different infectious diseases, this trend still continues among researchers to find active ingredients, pharmacologically active and has ability to cure infectious diseases, from plants.\textsuperscript{[14]}

These results suggest that these plants are quite toxic, but pharmacologically may be useful. Proper modification and phytochemical analysis and isolation may find some new molecule. Anti-oxidative property of these plants should be checked as well as anti-bacterial property, \textit{I. nigricans} and \textit{N. cadamba} expels most toxic result, it means they possibly have capacity to kill microbial invasion. We conclude by suggesting further investigation regarding these plant’s anti-cancer property.

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**Conflict of Interest**

The authors have declared that there no conflict of competing interest.

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