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Comparative study of cytotoxic potential and phytochemical screening of *Xanthosoma sagittifolium* rhizome and *Syngonium podophylam* leaf

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Abstract
Brine shrimp lethality bioassay is a simple technique used to identify bioactive compounds having cytotoxic, antitumor, and pesticidal activity from plant sources. In our study we determined cytotoxic potential and phytochemical screening of *S. podophyllum* leaves and *X. sagittifolium* rhizomes. Cytotoxicity was evaluated in terms of LD_{50} (lethal dose 50%). Considerable cytotoxic activity was found in all extracts of our present investigation. Among all extracts NHFSPL showed the highest cytotoxic potential with LD_{50} value of 16.67 µg/ml and the lowest activity found in CMEXSR having LD_{50} value of 59.21 µg/ml. In phytochemical screening we found qualitative presence of bioactive compounds in different extracts. The results of our study indicated that bioactive components presented in these plants could be accounted for its biological activity.

Keywords: *Xanthosoma sagittifolium, Syngonium podophyllum,* Brine shrimp, Cytotoxic, phytochemical analysis, LD_{50}

1. Introduction
Since ancient civilization different plants are used in the society as medicine for their novel therapeutic activities. In modern age, most of the medicines are derived from plants that are known as medicinal plants. According to the WHO (World Health Organization) - a medicinal plant is any plants which, in one or more of its organ contain substance that can be used for therapeutic purpose or which are a precursor for synthesis of useful drugsand nowadays, about 80% of world's population depend on plants for their primary health care. [1], [2] *Syngonium Podophyllum* and *Xanthosoma sagittifolium* belong to the family Araceae. Leaves of *S. podophyllum* are traditionally used against fungal infection, itching, rashes, dry skin etc. It is also traditionally applied as local folk medicine to treat rheumatism, arthritis, pain, and swelling. [3, 4] *Xanthosoma sagittifolium* species is used to prevent osteoporosis in Brazilian traditional medicine [5].

Brine shrimp lethality bioassay (BSLA) is a general bioassay which appears capable of detecting a broad spectrum of bioactivity that is present in plant extracts or samples. It is a very useful tool to isolate bioactive compounds from plant extract. [5, 6] Since its introduction by Meyer *et al.,* 1982, BSLA is successfully used as an indicator for the bioassay guided isolation of active anti-tumor and pesticidal compounds which is accepted internationally. [7, 8] The assay has advantages of being rapid (24 hours), low cost, easy of performing and commercially available and doesn't need animal serum as is needed for cytotoxicities, which make it a very useful bench top method [9].

In the present study, crude methanolic extract of *X. sagittifolium* and *S. podophyllum* including its different fractions were tested in vivo for evaluating their cytotoxic effect against brine shrimp napulii and phytochemical profile. The in vivo lethality test has been performed as a preliminary study of cytotoxic and anti-tumor agents [10]. So, the base line information of the therapeutic effect on these plants that was found will be helpful for further research processes for the development of new drugs of great therapeutic importance.

2. Materials and Methods

2.1. Collection of Plant Materials: The fresh leaf of *Syngonium podophyllum* Schott. (Family: Araceae) and the rhizome of *Xanthosoma sagittifolium* Schott. (Family: Araceae) were collected from the area of Dhaka city and Bogra city, Bangladesh respectively during May-June, 2013. The identity of these plant parts has authenticated by Bangladesh National Herbarium, Dhaka where a voucher specimen was deposited having accession number: *Syngonium podophyllum* Schott. (DACB 38722) and *Xanthosoma sagittifolium* Schott. (DACB 41076).

2.2. Preparation of plant extract: After collection plant materials were thoroughly washed under running tap water to remove dirty materials and shade dried for several days with occasional sun drying. The fresh sun-dried whole plant material was then dried in an oven at 50 °C followed by grinding in mechanical grinder into coarse powder. Powdered plant materials (500 gm) were macerated with 90%methanol and extracted for 72 h before filtration (three times). We obtained 28.87 gm of *S. podophyllum* Crude methanol extract (CMESPL) and 8gm of *X. sagittifolium* Crude methanol extract (CMEXSR) by concentrating using rotary evaporator (60 rpm at 50 °C) after filtration of plant sample. Only the crude methanol extract of *S. podophyllum* (CMESPL) (10 mg) was then fractionated, initially with n-hexane named as NHFSPL followed by chloroform, ethyl acetate and water which were represented as CLFSPL, EAFSPL and AQFSPL in our study. The methanol extract and its fractions (n-hexane, chloroform, ethyl acetate and aqueous) were re-filtered and evaporated at low pressure (60 rpm at 37 °C) to remove excess solvent. Concentrated extract and different fractions were stored until further use and yield value of these were recorded.

2.3. Chemicals: Methanol (Sigma chemical company, USA), N-Hexane (Sigma chemical company, USA), Ethyl Acetate (Sigma chemical company, USA), Chloroform (Sigma chemical company, USA), Ninhydrin reagent (Merck, Germany), α-naphthol (Sigma chemical company, USA), Concentrated Sulphuric Acid (H_2SO_4) (Merck, Germany), Sodium Picrate Solution (Sigma chemical company, USA), Bromothymol Blue (Sigma chemical company, USA), Feric Chloride ($FeCl_3$) (Sigma chemical company, USA), Lead Acetate(Sigma chemical company, USA), Benzene(Merck, Germany), Ammonium hydroxide (NH_4OH) (Merck, Germany), Acetic Anhydride (Sigma chemical company, USA),Sea salt (non-ionized, NaCl) (Merck, Germany), Vincristine sulphate (Sigma chemical company, USA), Dimethyl Sulfoxide (DMSO) (Sigma chemical company, USA) and *Artemia salina* leach. (Brine shrimp eggs)

2.4. Phytochemical assays: Phytochemical screening tests were carried out on the plant samples according to the standard protocols in order to classify the types of organic constituents present in the plant samples. The organic constituents such as *α*-Amino acids, carbohydrates, cyanogenic glycosides, glycosides, organic acids, phenolic compounds, terpenoids and sterols contains are investigated in the plant crude extracts and different fractions [11, 12, 13, 14].

2.5. Assessment of Cytotoxic Activity: Brine shrimp lethality bioassay is the assay which is widely used in the bioassay for the bioactive compounds [7, 8]. Simple zoological organism (*Artemia salina*) was used here as a convenient monitor for the screening of bioactivity. The eggs of the brine shrimp, *Artemia salina*, were collected from an aquarium shop (Dhaka, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) at around 37 °C temperature with constant oxygen supply for 48 hr to mature shrimp called nauplii. The bioassay was performed to predict the cytotoxic activity [7, 15] of the crude methanolic extract of *S. podophyllum* (leaf) and its different fractions (n-hexane, chloroform, ethyl acetate and aqueous) and crude methanolic extract of *Xanthosoma sagittifolium* rhizome. 2 mg of each sample was dissolved in 400 µl dimethyl sulfoxide (DMSO) to get a concentration of 5 µg/µl for each of the sample, which were used as stock solutions for further serial dilution to make 5 different doses of lower concentrated solutions (20, 40, 60, 80 & 100 µg/ml) for each, and 3 replicates of each dose were used to carry out cytotoxicity assay. The concentration of DMSO in each vial should not exceed 50 µl/5 ml of brine as because above this concentration cytotoxicity due to DMSO may arise. Vincristine sulphate was used as the standard at 0.31, 0.63, 1.25, 2.5, 5 and 10 µg/ml of concentration respectively. 10 shrimp nauplii were taken in each vial of different extract contains various dose and incubated for 24 hrs. After 24 hrs, number of live nauplii was counted in all the vials. Percentage of mortality was calculated by following equation and standard deviation was also calculated.

$$\% \text{ Nauplii mortality} = \frac{N_t}{N_0} \times 100$$

Where, N_t = Number of died nauplii after 24 hrs of incubation, N_0 =Number of total nauplii transferred i.e. 10 nauplii.

3. Results and Discussion

3.1. Solvent extracts: The yield amount of crude methanolic extract of *S. podophyllum* leaves (CMESPL) was greater than crude methanolic extract of *X. sagittifolium* rhizome (CMEXSR) and values are 28.87gm (5.774%) and 8.00gm (1.60%) respectively. Among these four fractions of CMESPL, Aqueous fraction of *S. podophyllum* leaves (AQFSPL) was maximum as shown in Table-1 to the tune of 31.09% followed by N-hexane fraction of *S. podophyllum* leaves (NHFSPL) and Chloroform fraction of *S. podophyllum* leaves (CLFSPL) having value of 30.64% and 24.87% respectively and the least in Ethyl acetate fraction of *S. podophyllum* leaves (EAFSPL) which was 13.40%. It could be clearly stated there were more polar compounds in CMESPL than non-polar entities.

3.2. Phytochemical analysis: A preliminary phytochemical investigation was carried out according to the conventional method to identify the types of phytoorganic constituents that are present in *S. podophyllum* leaves and *X. sagittifolium* rhizomes and the results are summarized in Table-2. The Result of investigation shows that, Only Carbohydrates and Terpenoids are present in all samples of *S. podophyllum le*aves and *X. sagittifolium* rhizomes in different amounts. Other constituents such as *α*-Amino acids, cyanogenic glycosides, glycosides, organic acids, phenolic compounds, and sterols are also present in different sample in different amount. The presence of such chemical constituents in investigated samples of *S. podophyllum* leaves and *X. sagittifolium* rhizomes indicated their medicinal potentiality[16].

3.3. Cytotoxic activity: All samples showed remarkable cytotoxic activity. From the table-3 and figure-1 it is showed that, N-hexane fraction of *S. podophyllum* leaves (NHFSPL) have maximum efficacy with minimum LD_{50} value 16.67 rather than other samples. Chloroform extract of *S.*

podophyllum leaves (CLFSPL) have the 2nd highest efficacy followed by AQFSPL, EAFSPL, CMESPL and CMEXSR and LD_{50} values are 40.00, 42.85, 52.94 and 59.21µg/ml respectively. So it can be concluding that, *S. podophyllum leaves* is more potential than *X. sagittifolium* rhizomes on cytotoxic activity.

Table 1: Yield of solvent extracts of *S. podophyllum* & *X. sagittifolium* and different fractions of CMESPL.

Extracts/ Fractions	Weight of container (A) (gm)	Weight of container with sample (B) (gm)	Weight of extract (gm)	Percentage yield of extract
CMESPL	87.53	116.4	28.87	5.77%
CMEXSR	88.25	96.25	8.00	1.60%
NHFSPL	87.88	90.94	3.06	30.64%
CLFSPL	85.05	87.53	2.48	24.87%
EAFSPL	87.39	88.73	1.34	13.40%
AQFSPL	85.12	88.23	3.11	31.09%

Here, CMESPL = Crude Methanol Extract of *S. podophyllum* Leaf, CMEXSR = Crude Methanol Extract of *X. sagittifolium* Rhizome, NHFSPL = N-hexane fraction of *S. podophyllum* leaf, CLFSPL = Chloroform fraction of *S. podophyllum* leaf, EAFSPL = Ethyl Acetate fraction of *S. podophyllum* leaf and AQFSPL = Aqueous fraction of *S. podophyllum* leaf.

Table 2: Results of chemical constituents of *S. podophyllum leaves* and *X. sagittifolium* rhizomes.

Case of compounds	Reagent's that are used	Observation					
		CMESPL	CMEXSR	NHFSPL	CLFSPL	EAFSPL	AQFSPL
α-amino acids	Ninhydrin reagent	+	+	+	-	-	-
Carbohydrates	10% α-naphthol and conc: H_2SO_4	++	++	+++	++	+	++
Cyanogenic glycosides	Sodium picrate solution	+	+	++	+	+	++
Glycosides	10% lead acetate	+	+	+	+	-	++
Organic acids	Bromothymol blue	+	+	+	-	-	+
Phenolic content	1% $FeCl_3$	+	++	++	-	-	-
Terpenoids	Acetic anhydride and conc: H_2SO_4	+	+	++	+	+	+
Sterols	Acetic anhydride and conc: H_2SO_4	+	+	-	+	+	-

Here, + = Present in mild amount, ++ = Present in moderate amount, +++ = Present in large amount, - = Not present.

Table 3: Cytotoxic activity of *S. podophyllum leaves* and *X. sagittifolium* rhizomes.

Tested materials	Concentration tested (µg/ml)	% of mortality	LD_{50}
Vincristine sulphate	0.0625, 0.125, 0.25, 0.5, 1, 2	25.67, 30.33, 43.67, 59.67, 73.33, 91.67	0.29
CMEXSR	20, 40, 60, 80, 100	30.33, 40.33, 50.67, 61.33, 74.33	59.21
CMESPL	20, 40, 60, 80, 100	36.67, 43.33, 56.67, 76.67, 90	52.94
NHFSPL	20, 40, 60, 80, 100	60, 70.33, 83.33, 90, 96.67	16.67
CLFSPL	20, 40, 60, 80, 100	50, 56.67, 70.21, 79.67, 93.33	20
EAFSPL	20, 40, 60, 80, 100	40, 46.67, 56.67, 73.33, 92.67	42.85
AQFSPL	20, 40, 60, 80, 100	41.33, 50, 58.67, 63.33, 86.67	40

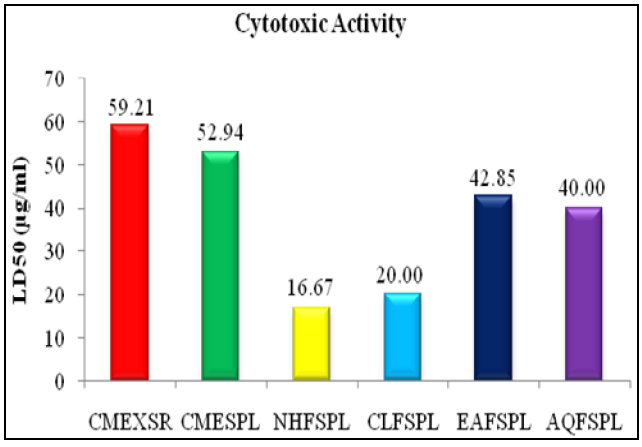

Fig 1: Comparison of LD_{50} among different extract of *S. podophyllum leaves* and *X. sagittifolium* rhizomes.

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