Annals of Plant and Soil Research 26(3): 481-486 (2024)
https://doi.org/10.47815/apsr.2024.10387

Enzymatic activities of *Drosera indica* L. (A carnivorous plant) and toxicity to *Artemia salina* L.

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Received, Febuary, 2024; Revised accepted, May, 2024

ABSTRACT

A lot of deaths have been noted in the last few decades due to various health care factors, including antimicrobial resistance (AMR) and other anthropogenic activities. The sources of novel pharmaceuticals are needed globally, and researchers are searching unexplored plant groups. Drosera indica is a plant belonging to the carnivorous plant group and unexplored. Enzymatic activities and cytotoxicity of Artemia salina L were evaluated, and excellent activities of lipase were found, followed by amylase and protease. It was noticed that non-polar extracts are more effective to A. salina (500 mg/ml) than polar extracts of D. indica. Results showed the pharmacological potential of D. indica in viral infections, which should be explored at an advanced level.

Keywords: Anticancer, antimicrobial, carnivorous plants, enzyme activities

INTRODUCTION

Several health problems are emerging globally which are lethal and difficult to treat easily (Kumar *et al.,* 2013; Chuskit *et al.,* 2024; Rani and Gupta, 2024). Many factors are associated with this situation like malpractices of drugs, overdose, excess use of antibiotics, metallic life style, chemical fertilizers, junk foods, pollution, poor immunity, and climatic changes (Devi *et al.,* 2023). Every year lot of deaths are recorded due to various health care issues (Murthy and Ranjitha, 2023). Deaths due to various health problems are categorized in three groups. Infectious diseases, maternal, perinatal, and nutritional conditions come under Group-1. Non-communicable diseases come under Group-2 and injuries come under Group-3. About 7.7 million deaths are linked with bacterial infections which is 13.6 % of global deaths. About 3.4 million deaths due to respiratory infections, about 2.6 million deaths due to HIV/AIDS, about 1.8 million deaths about diarrheal infections, about 1.6 million deaths due to tuberculosis and about 1.1 million deaths are observed due to malaria (Michaud, 2009). For the above problems, research is going in pharmaceutical industry but main problem is antimicrobial resistance (AMR). Therefore, researchers globally searching new source to extract antimicrobial and other pharmaceutical to fight against above mentioned health problems (Garaniya *et al.,* 2023; Chhabra and Sharma, 2023) as well as against AMR. In this aspect, carnivorous plants are suitable due to less works on bioactivity are reported, having different types of enzymes and defence mechanisms. Keeping this in view, an attempt has been made to evaluate enzymatic activities of *Drosera indica* and its toxicity to *Artemia salina*. *D. indica* is a carnivorous plant and usually grows in poor quality of soil (Rivadavia *et al.,* 2003) and have medicinal potential (Kottapalli, 2006). Present study highlights the importance of unexplored group of plants like carnivorous plants to extract some active constituents against contemporary health problems.

METHODOLOGY

Collection of plant parts

Plant parts of *Drosera indica* was collected from the Chandrabhaga, Puri district of Odisha state, India. Experimental plant was identified by the authors followed flora book and an herbarium sheet (APRFH-019) has submitted to Herbarium Unit, Ambika Prasad Research Foundation, Odisha, India.

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Test for enzyme activity

The experimental plant was tested for the presence and the activity of three selected enzymes (amylase, protease, and lipase).

Test for Amylase: To test the presence of amylase, starch (a polymeric carbohydrate) was taken as positive control which breakdown to give two polymers of carbohydrate amylose and amylopectin in the presence of enzyme amylase. The solution is prepared using the experimental plant with starch and distilled water. The filtrate of the sample was mixed with 2-5 drops of Fehling's A & B and kept in water bath. Appearance of this orange or red precipitate indicated the presence of carbohydrate. Means, there was some appearance of enzyme which broke the starch in to carbohydrates. The initial & final time and temperature was observed and recorded in 10 mins, 30 mins, 1h, 6h, 18h and in 24h. Distilled water was taken as negative control for this activity (Jahangirpuria *et al.,* 2017).

Test for Protease: Protease activity was seen by using casein, as positive control which breaks to give amino acids in the presence of protease. The experimental plant was mixed with casein and distilled water. To the filtrate of this solution 2-3 drops of Ninhydrin reagent was added and kept in water bath. Appearance of purple or bluish colour indicated the presence of amino acid. The initial & final time and temperature was observed and recorded in 10 mins, 30 mins, 1h, 6h, 18h and in 24h. Distilled water was taken as negative control for this activity (Friedman, 2014).

Test for Lipase: To carry out this experiment chicken fat was used as positive control and a solution was prepared with experimental plant, distilled water and with chicken fat. In the presence of lipase, fat get degrade in to fatty acid. To test the presence of fatty acid, 2-3drops of lead acetate was used in the filtrate sample. Appearance of whitish or yellowish precipitate indicated the presence of fatty acid and ultimately the lipase. This activity was observed in 10 mins, 30 mins, 1h, 6h, 18h and in 24h. Distilled water was taken as negative control for this activity.

Cytotoxicity test using Brine Shrimp (*Artemia salina*)

Test sample preparation for Brine shrimp assay: The DMSO (Dimethyl sulfoxide) was taken as standard solvent for dissolving different extract to obtain the stock solution. 5% DMSO was taken to observe the Brine shrimp assay and to prevent the own toxicity of DMSO. Five different concentrations namely 100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml, 500 mg/ ml were prepared. Pure DMSO and artificial sea water were used as negative control (Kumar *et al.,* 2012; Dash and Kumar, 2020).

Hatching of Brine shrimp cysts: Hatching of cysts was done using artificial seawater made through dissolving commercial marine salt (3.6 g) in distilled water (200 ml). The nauplii were hatched within 18-24 hours at 30-35ºC (Kumar *et al.,* 2012).

Brine shrimp lethality test: Ten larvae of Brine shrimps were transferred to each of prepared test tubes contain 100µl of drug of different concentration (100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml, 500 mg/ ml in sea water containing 5% DMSO) and 1ml of distilled water using a pipette and tip (Musa, 2012). Survivors were counted every hour up to 4 hours and later up to 24 hours. The movability was cited as +4 indicate highly motile, +3 indicate motile, +2 indicate paralyzed and +1 indicates slow (Kumar *et al.,* 2012). The total death and percentage mortality at each dose level and control were determined and recorded (Musa, 2012).

RESULTS AND DISCUSSION

The whole experimental plant species was used to study the presence and the activity of different enzymes like amylase, protease, and lipase. For amylase, the experiment was conducted by using starch and to test the presence of carbohydrates Fehling's A & B was used and the appearance of orange or red precipitate indicates the presence of carbohydrate which indicates the activity of amylase. The initial & final time and temperature was observed and recorded in 10 mins, 30 mins, 1h, 6 h, 18 h and in 24 h. The result showed the time duration and the temperature difference required to activate the enzyme. To test the presence of protease,

casein was taken which gives amino acids in the presence of enzyme protease. Ninhydrin reagent was used which gives a purple or bluish colour, indicated the presence of amino acid. The initial & final time and temperature was observed and recorded in 10 mins, 30 mins, 1h, 6h, 18h and in 24h. The result showed the time duration and the temperature difference required to activate the enzyme. To carry out this experiment chicken fat was used as positive control and a solution was prepared with experimental plant, distilled water and with chicken fat. In the presence of lipase, fat get degrade in to fatty acid. Appearance of whitish or yellowish precipitate indicated the presence of fatty acid and ultimately the lipase. This activity was observed in 10 mins, 30 mins, 1 h, 6 h, 18 h and in 24 h. Distilled water was taken as negative control for this activity (Figure 1).

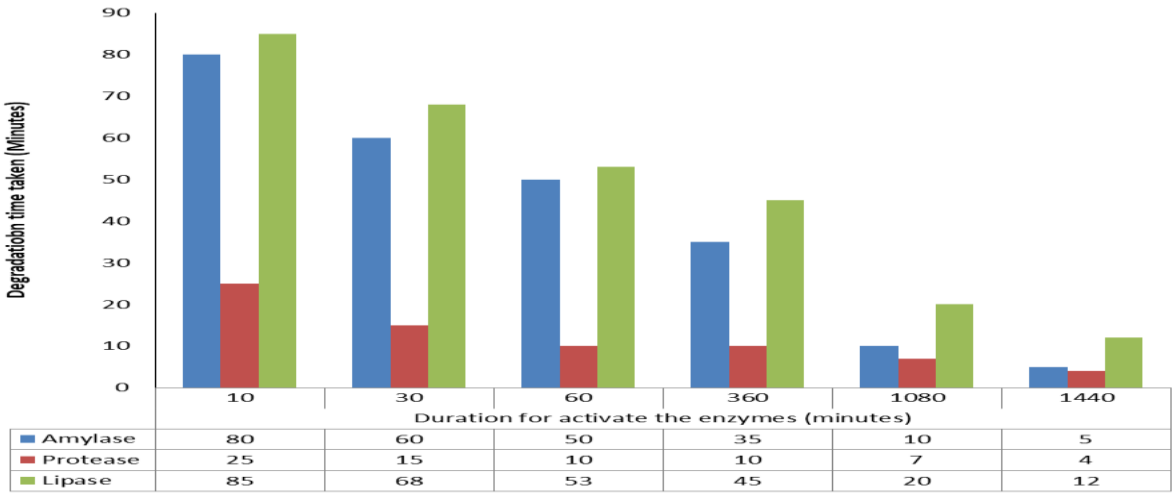

Figure 1: Enzymatic activity of *D. indica*

The cytotoxicity test of *D. indica* is carried out using four different solvents as per polarity index. The results revealed that extracts from non-polar solvents showed highest activity than extracts from polar solvents (Table 1-4). It was noted that extract of di-ethyl ether has potent activity followed by n-hexane, methanol and aqueous. The results indicate that the toxicity is due to some non-polar compounds which might be useful against in formulation of viral infections or non-infectious diseases like HIV, cancer, H1N1, Ebola etc.The result showed the excellent cytotoxicity activity against *Artemia salina*. The result of this test can be helped to understand the anti-cancer activity. Different extract (methanol, aqueous, n-hexane, di-ethyl ether) with different concentration (100 mg/ml, 200 mg/ml, 300 mg/ml, 400 mg/ml, 500 mg/ml) were tested. High concentration of di-ethyl ether showed highest cytotoxicity activity. Followed to this n-hexane extract also showed significant cytotoxicity against Brine shrimp. The other extract appeared a mild cytotoxicity activity (Table 1-4).

Table 1: Cytotoxicity against *A. salina* (n-hexane extract)

Solvent		Time [Hour(s)]					% of Inhibition
		1	2	3	4	24	
DMSO		+4	+4	+4	+4	+4	0
Distilled water		+4	+4	+4	+4	+4	0
Brine water		+4	+4	+4	+4	+4	0
n-hexane extract	100 mg/ml	+4	+4	+4	+4	+4	0
	200 mg/ml	+4	+4	+4	+4	+4	0
	300 mg/ml	+4	+4	+4	+4	+3	60
	400 mg/ml	+4	+4	+4	+4	+3	60
	500 mg/ml	+4	+4	+4	+4	+1	90

+4: live brine shrimp

Table 2: Cytotoxicity against *A. salina* (methanol extract)

Solvent		Time [Hour(s)]					% of Inhibition
		1	2	3	4	24	
DMSO		+4	+4	+4	+4	+4	0
Distilled water		+4	+4	+4	+4	+4	0
Brine water		+4	+4	+4	+4	+4	0
Methanol extract	100 mg/ml	+4	+4	+4	+4	+4	0
	200 mg/ml	+4	+4	+4	+4	+4	0
	300 mg/ml	+4	+4	+4	+4	+4	0
	400 mg/ml	+4	+4	+4	+4	+4	0
	500 mg/ml	+4	+4	+4	+4	+3	70

+4: live brine shrimp

Table 3: Cytotoxicity against *A. salina* (Aqueous extract)

Solvent		Time [Hour(s)]					% of Inhibition
		1	2	3	4	24	
DMSO		+4	+4	+4	+4	+4	0
Distilled water		+4	+4	+4	+4	+4	0
Brine water		+4	+4	+4	+4	+4	0
Aqueous extract	100 mg/ml	+4	+4	+4	+4	+3	10
	200 mg/ml	+4	+4	+4	+4	+2	60
	300 mg/ml	+4	+4	+4	+4	+2	60
	400 mg/ml	+4	+4	+4	+4	+2	70
	500 mg/ml	+4	+4	+4	+4	+2	70

+4: live brine shrimp

Table 4: Cytotoxicity against *A. salina* (Di-ethyl ether extract)

Solvent		Time [Hour(s)]					% of Inhibition
		1	2	3	4	24	
DMSO		+4	+4	+4	+4	+4	0
Distilled water		+4	+4	+4	+4	+4	0
Brine water		+4	+4	+4	+4	+4	0
Di-ehyl ether	100mg/ml	+3	+3	+3	+3	+3	0
extract	200 mg/ml	+4	+4	+4	+4	+2	40
	300 mg/ml	+4	+4	+4	+4	+2	60
	400 mg/ml	+4	+4	+4	+4	+2	60
	500mg/ml	+4	+4	+4	+4	+3	70

FUTURE ASPECTS

From literatures we get some knowledge about this wonderful flesh-eating plant species (Ellison and Gotelli, 2009), but it needs more survey and studies about its field data, diversity, habitat, phytochemical analysis, ethno-pharmacological values, trapping action and the benefits of this carnivory. These plants contain different medicinally important pharmacologically active compounds and having anti-oxidant, anti-bacterial & anti-cancer activities. Also, they contain diverse digestive enzymes like amylase, protease, lipase, esterase, phosphatase, chitinase, glucanase, nuclease, peroxidase etc (Ravee *et al.,* 2018).

This may be used in proper way in the field of medical science. Currently the pandemic coronavirus disease (COVID -19) creates negative impact on the society throughout the world. The coronavirus virion is an enveloped particle containing the spike (S), membrane (M), and envelope (E) proteins. In addition, some strains of coronaviruses, but not SCoV, express a hemagglutinin protein (HE) that is also incorporated in the virion. The genome of coronaviruses is a linear, single-stranded RNA molecule of positive (mRNA) polarity, and from 28 to 32 kb in length (Denison, 2004). The enzymes in *Drosera* species might have some significant activities which may leads to the inhibition of these viruses (Figure 2). The current studies may fulfil the gaps in the study of *Drosera* species and worthy for upcoming research.

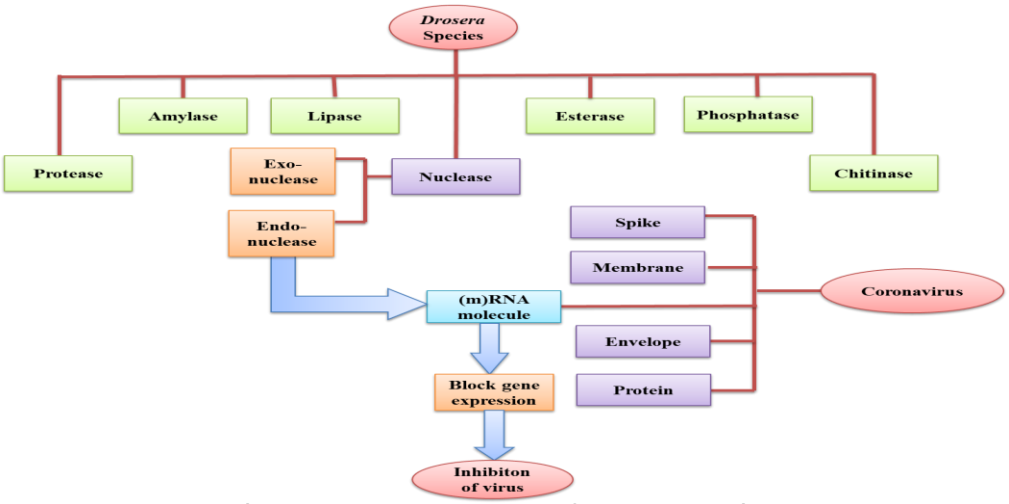

Figure 2: Future aspects of present study

CONCLUSION

Carnivore plants are unexplored in pharmacological aspects. *Drosera indica* showed the highest activity with lipase, followed by amylase and protease. Hence, it could be useful in primary metabolite disorders and viral infections. Non-polar extracts of *D. indica* showed excellent activity against *Artemia salina*. Therefore, it could be useful as an anti-cancer agent. There is a need for advanced research in pharmacological aspects.

ACKNOWLEDGEMENT

Authors are thankful to the local communities of Puri district for helping us to collect the plant specimen and thankful to the team members of APRF for helping us in laboratory for experimental works.

REFERENCES

Chhabra R. and Sharma R. (2023) Qualitative phytochemical screening of botanical extracts and their effect on yield parameters of rice against sheath blight. *Annals of Plant and Soil Research*, **25**(4): 672-680; https://doi.org/10.47815/apsr.2023.10321

Chuskit R., Singh R., Kauri S. and Batishi D.R. (2024) Antimicrobial potential of essential oil artemisia sieversiana: a medicinal plant from the high altitude nubra valley, Ladakh. *Annals of Plant and Soil Research,***26**(1): 64-73; https://doi.org/10.47815/apsr.2024.10334

Dash A.A. and Kumar S. (2020) Bioactive compounds and anti-microbial activity of ventilago denticulate fruits and its toxicity to *Artemia salina* L. *Acta Scientific Medical Sciences*, **4**(4): 93-96.

Denison M.R. (2004) Coronavirus research: Keys to diagnosis, treatment and prevention of SARS. National Academies Press (US).

Devi R.S., Bihari S.K. and Kumar S. (2023) Validation of tribal claims for formulation of future drugs through evaluation of ethno-pharmacological values of *Ludwigia adscendens*. *Medicinal Plants*, **15**(4): 691-697.

Ellison A.M. and Gotelli N.J. (2009) Energetics and the evolution of carnivorous plants-Darwin's 'most wonderful plants in the world', *Journal of Experimental Botany*, **60**(1)19–42.

Friedman M. (2014) Application of the Ninhyahin reaction for analysis of amino acids, peptides, and proteins to agricultural and biomedical sciences. *Journal of Agriculture and Food Chemistry*, **52**(3): 385-406.

Garaniya N.H., Bambhaneeya S.M., Patel S.G. and Bambharolia R.P. (2023) Comparing anti-nutritional factors in pigeon pea: seeds vs. Seed coats. *Annals of Plant and Soil Research*, **25**(4): 614-619; https://doi.org/10.47815/apsr.2023.10312

Jahangirpuria H.D., Makwana S.A. and Patel, C.G. (2017) Identification of carbohydrates. *The World Journal of Engineering and Applied Science*, **3**(6):1-17.

Kottapalli J. (2006). *Drosera indica* L. and *D. burmannii* Vahl., medicinally important insectivorous plants in Andra Pradesh - regional threats and conservation. *Current Science*, **91**(7): 943-946.

Kumar S., Behera S.P. and Jena P.K. (2013) Validation of tribal claims on *Dioscorea pentaphylla* through phytochemical screening and evaluation of antibacterial activity. *Plant Science Research*, **35**: 55-61.

Kumar S., Tripathy P.K. and Jena P.K. (2012) Ethnobotany and bioactive compounds in leaf of *Bixa orellana* L. and it's toxicity *Artemia salina* L. *Plant Science Research*, **34**(1&2): 93-96.

Michaud CM. (2009) Global Burden of Infectious Diseases. *Encyclopaedia of Microbiology*, 444-454. DOI: 10.1016/B978-012373944-5.00185-1.

Murthy F.S. and Ranjitha M.C. (2023) Insecticidal activity of the *Mentha arvensis* L. essential oil on stored grain pest *Callosobruchus maculatus*. *Annals of Plant and Soil Research*, **25**(1):145-151; https://doi.org/10.47815/apsr.2023.10248

Musa A.A. (2012) Cytotoxicity Activity and Phytochemical Screening of Cochlospermum tinctorium Perr Ex A. Rich Rhizome. *Journal of Applied Pharmaceutical Science*, **2**(7):155-159.

Rani M. and Gupta S. (2024) Ethnopharmacological implications of Argemone ochroleuca – A Critical Review. *Annals of Plant and Soil Research*, **26**(1):143-147; https://doi.org/10.47815/apsr.2024.10344

Ravee R., Salleh F.L.M. and Goh H.H. (2018) Discovery of digestive enzymes in carnivorous plants with focus in protease. *Peerj*, DOI:10.7717/peerj.4914.

Rivadavia F., Kondo K., Kato M. and Hasebe M. (2003) Phylogeny of the sundews, Drosera (Droseraceae), bassed on chloroplast rbcl and Nuclear 18S ribosomal DNA sequences. *American Journal of Botany*, **90**(1): 123-130.