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Allelopathic potential of *Alternanthera philoxeroides* (Mart.) Griseb on growth and development of *Eichhornia crassipes* (Mart) Solms

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ABSTRACT

We investigated the allelopathic effects of *A. philoxeroides* stem on *E. crassipes* and found reduction in plant height (32 to 61 %), number of leaves (13 to 38 %), number of branches (20 to 43%) and fresh weight (63 to 73 %). The *E. crassipes* plants got dried in cisterns treated with *A. philoxeroides.* The allelochemicals present in *A. philoxeroides* plants were inhibitory to water hyacinth. The allelopathic leachate of *A. philoxeroides* caused maximum decrease in growth of *E. crassipes*, due to presence of alkaloids and phenols. In control, there were no detrimental effects on growth of *E. crassipes*.

Key words: Allelopathy, alligator weed, *Alternanthera philoxeroides*, aquatic weeds, *Eichhornia crassipes*, growth, inhibitory effects, leachates, water hyacinth.

INTRODUCTION

Allelopathy is an interference mechanism, where plants released chemicals that affects the other plant's growth, population and communities (7,11). The allelopathic potential of plants depends on the solubility and adsorption of allelochemicals present in them (3). Allelochemicals present in plant residues, exudates and leachates of leaves, stem, roots, fruits and seeds interfere with growth of other plants. These chemicals affect the germination, seed emergence and seedling growth. Allelopathy plays an important role in agricultural ecosystems in crop-crop and crop-weed interactions. These interactions may be beneficial or detrimental.

The alligator weed *(Alternanthera philoxeroides* family Amaranthaceae) is important invasive aquatic weed found in water bodies, ponds, irrigation canals and anaerobic rice fields. *A. Philoxeroides* leaves and stem vary greatly in size and shape. Fleshy succulent stems grow horizontal and float on the water surface forming rafts or matted clumps, act as a substrate for other aquatic plants. Fibrous root arises at the stem nodes and hang free in water. It is cosmopolitan in distribution due to its invasive nature, high growth rate and the ability to produce several phytochemicals, which are inhibitory to field crops: mustard, rice and lettuce (8,12,13,14), affects soil properties, nutrients availability, population and community structure, and its invasion (4).

Eichhornia crassipes (Water hyacinth; family Pontederiaceae) a free-floating aquatic weed is quick growing aggressive invader, hence, gains advantage over other aquatic weeds, as it tolerates wide range of climatic conditions. Its erect stem grows profusely in aquatic

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bodies. It competes with cultivable fishes for space, nutrients and dissolved oxygen, harbors unwanted aquatic organisms and decreases fish yield.

In recent years, Aquatic weeds have spread very rapidly due to release of fertilizer nutrients from crop fields in water bodies (4). Allelopathy in aquatic ecosystems influences the competition between different aquatic plants due to their differential allelopathic potential (1,2). e.g., *Eichhornia crassipes* (water hyacinth*)* and *Alternanthera philoxeroides* (Alligator weed). *Alternanthera philoxeroides* and water hyacinth [*Eichhornia crassipes]* are invasive species worldwide, affecting aquatic ecosystems. *E. crassipes* is more aggressive invader than *A. philoxeroides*. Both of these weeds have harmed the aquatic ecology and spread quickly. Allelopathy in aquatic system plays an important role, as use of chemical is not suggested to safeguard aquatic flora and fauna. Investigations indicate that allelopathy potential of *A. philoxeroides* suppresses the water hyacinth.

It is hypothesized that *A. philoxeroides* hampers the growth of water hyacinth by releasing the water soluble allelochemicals. *A. philoxeroides* plants kills the water hyacinth plants (5) and thus successfully invades new areas (8,11,13). Hence, this study aimed (i). to evaluate the allelopathic effects of *A. philoxeroides* allelochemicals on growth and development of *E. crassipes,* (ii). to assess the phytotoxicity of *A. philoxeroides* on *E. crassipes* and (iii). the competitive interactions between water hyacinth (*Eichhornia crassipes)* and Alligator weed (*Alternanthera philoxeroides*) to find ecofriendly control of *Eichhornia crassipes*

MATERIALS AND METHODS

The study was conducted in a greenhouse during the year 2019 at AICRP Weed Management, University of Agricultural Sciences, Main Research Station, Hebbal, Bengaluru, India (latitude of 12º 50′ North, longitude of 77º 35′ East, altitude: 899 m, annual rainfall: 925 mm. Mean max and min temperature: 29 ºC and 13.1ºC respectively.

Plate I: General view of the experiment, treatments imposed in Trapezium shaped cement cistern

Plate 2- Single plant- *Eichhornia crassipes*

Plate 3- T1- Control- *Eichhornia crassipes*

Plate 4- Only Alligator weed -Leachate Tank (Alternanthera *philoxeroides)*

Plate 5- In population of alligator and water hyacinth weeds

Plate 6: T2- *E. craipes* 20 Bulbs + 45 stem segments of *A. philoxeroides*

Plate 7: T3- *E. crassipes* 20 Bulbs + 80 stem segments of *A. philoxeroides*

Plate 8: T4- *E. crassipes* 20 Bulbs+ 20 days' old *A. philoxeroides* Leachate

Plate 9: T5- *E. crassipes* 20 Bulbs+ 40 days' old *A. philoxeroides* Leachate

Plate II: Effect of *Alternanthera philoxeroides* on water hyacinth

Cement Tank/Cistern: Trapezium shaped cement cisterns (Plate I) with breadth 60.2 cm. and Length 40.9 cm. height 40.2 cm filled with 105 liters of water was used to conduct the experiment. The total volume of water in each cistern was kept constant by adding water every 15 days, to compensate for water loss through plant evapotranspiration.

Plant material collection: The *Alternanthera philoxeroides* and *Eichhornia crassipes* (*w*ater hyacinth) plants were collected from Hebbal Lake, Bengaluru (Plate 2 to 9).

***Eichhornia crassipes (w*ater hyacinth):** water hyacinth plants of similar shape and weight (about 45 to 50 g/plant) with 12-cm shoot length, long rhizome segments of each with bulb and at least one stolon connection with 6- branches were selected and washed several times using tap water. The washed plant samples were placed in the trapezium cistern containing only water.

Preparation of *Alternanthera philoxeroides (Alligator* weed*) cuttings: A. philoxeroides* stems having 5-6 leaves with nodes were collected from Hebbal lake. The stem cuttings were washed with tap water to remove the impurities. At the start of the experiment 45 and 80 numbers of *A. philoxeroides* cuttings were added in T2 and T3 treatments respectively, in cistern with 20 bulbs of *E. crassipes*.

Preparation of leachates of *Alternanthera philoxeroides (Alligator* weed)*:* Eighty segments of *A. philoxeroides* having 5-6 leaves with nodes were collected. The stem was washed thoroughly to remove the impurities and were maintained in cistern /leachate tank for extraction of leachates (Plate 4). Ten liters of leachate of 20 and 40 days old from the leachate tanks was added to treatment T4 (Plate 8) and T5 (Plate 9) respectively as per the treatment protocol over control (Plate 3).

Allelopathic effects of *A. philoxeroides* on growth and development of *E. crassipes*

This experiment was done during 2019 *Kharif* season in complete randomized design (CRD) with four replications and 5-treatments.

T1- Control- *E. crassipes* 20 Bulbs alone (Plate 3)
T2- *E. crassipes* 20 Bulbs + 45 segments of *A. philoxeroides (*Plate 6)
T3- *E. crassipes* 20 Bulbs + 80 segments of *A. philoxeroides* (Plate 7)
T4- *E. crassipes* 20 Bulbs + 20 days' old *A. philoxeroides* Leachates was added from Leachate tank (Plate 8)
T5- *E. crassipes* 20 Bulbs + 40 days' old *A. philoxeroides* Leachate was added from Leachate tank (Plate 9)

(Ten liters of 20- and 40-days old leachate from the leachate tank was added to T4 and T5 treatment cement cisterns (Plate 4)

Observation on allelopathic effect of *A. philoxeroides* on growth of water hyacinth on plant height (cm), number of branches/plants, number of leaves/plant and fresh weight (g/plant) were recorded at 15, 30 and 45 days after imposition of treatments (DAIT).

Statistical Analysis: The data was subjected to one-way analysis with multiple observations at different intervals.

GC-MS Analysis of Ethanolic extracts of *Alternanthera philoxeroides* (Mart.) Griseb

Plant materials and preparation of *A. philoxeroides* extract: Fully-grown plants of *A. philoxeroides* collected from Hebbal lake, Bengaluru were separated into roots, stem and leaves. Fresh plants were cut into small pieces (2-3 cms) with scissors, washed thoroughly 3-4 times with running tap water, then finally with sterile water followed by shade drying at room temperature for 20-30 days and thereafter powdered by electric blender and stored in airtight container. The protocol on extracts was followed as per Zhang and Fu method (17).

Preparation of *A. philoxeroides* ethanolic extracts for GCMS analysis: The extract of *A. philoxeroides* was prepared as under:

10 g of sample soaked for 24 h in 30 ml ethanol.

↓

Extract filtered by Whatman filter paper No. 1, evaporated to dryness and re-dissolved in DMSO (Dimethyl Sulphoxide).

↓

The extracts preserved in airtight container and kept at 4-5 °C for further use.

↓

The extracts analyzed by GCMS

The phytochemicals extracted in different plant parts of *A. philoxeroides* are present in Table 5.

RESULTS AND DISCUSSION

Plant height (cm)

The *E. crassipes* growth decreased with *A. philoxeroides* stem (8 to 10 %) and leachate (4 to 8 %). The percent decrease in plant height was commendable due to potential metabolites present in *A. philoxeroides* that would have been released in cistern (13). The inhibition of plant height increased with increasing leachate concentration and number of *Alternanthera philoxeroides* stems grown along with *E. crassipes* (Table 1 and Figure 1).

Table 1. Plant height (cm) as influenced by *A. philoxeroides* on *E. crassipes*

	Plant height	Days after Imposition of treatments (DAIT)				Inhibition/ Stimulation (%) (from 15 to 60 DAIT)
		15	30	45	60	
T1	Control	11.97[a]	13.19[a]	14.07[c]	14.56[c]	21.6
T2	20 EC +45 AP	13.76[ab]	13.03[a]	9.62[b]	6.86[a]	-50.1
T3	20 EC +80 AP	15.42[b]	13.06[a]	9.05[ab]	6.52[a]	-57.7
T4	20 EC+20 days old leachate	13.72[ab]	12.50[a]	9.27[ab]	8.12[ab]	-40.8
T5	20 EC+40days old leachate	14.94[b]	15.41[b]	7.76[a]	9.53[b]	-36.2
	SEm±	0.67	0.71	0.57	0.62	
	F cal	3.98	2.52	17.56	27.78	

EC - Eichhornea crassipes; AP- ***Alternanthera philoxeroides***
(-) sign indicates the % inhibition on the growth of *E. crassipes*

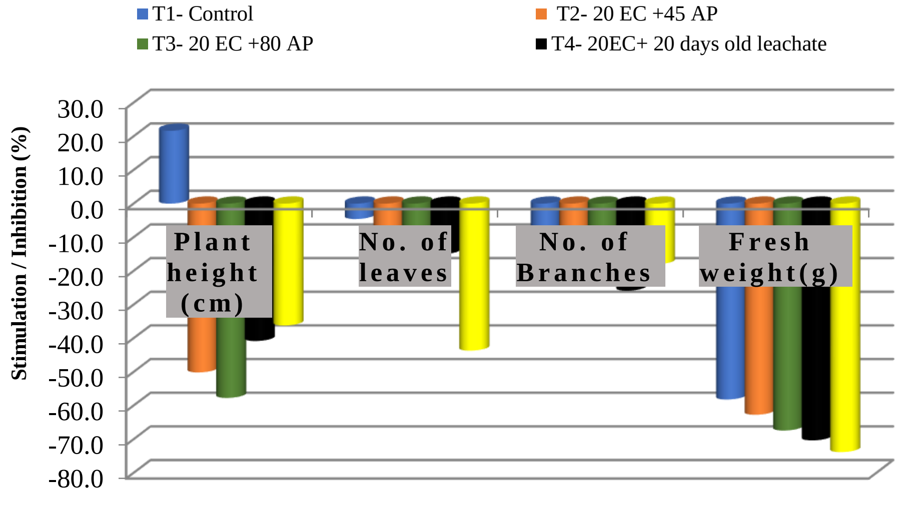

Figure 1. Inhibition/Stimulation (%) allelopathy effect on *E. crassipes* after imposition of treatments

Only *E. crassipes* (Plate 3) in separate tank as control continued to grow throughout the study period. Plant height increased as evident from the observation recorded from 15 to 60 Days after imposition of treatments (DAIT). The increase growth 21.6 per cent in *E. crassipes* under control due to favorable condition (12).

E. crassipes grows from seed and vegetative reproduction. Seeds are produced in capsules at the base of each flower. Daughter plants are produced by vegetative reproduction remain attached to the parent plant until they are broken off by wind or other physical damage. The increase in plant height under control has been observed. In treated cisterns, presence of *A. philoxeroides* affected the growth of *E. crassipes* (Plate 6 to 9). However, in control, increase in growth of plant height of E. *crassipes* was noted (Plate 3).

Number of leaves

A. philoxeroides plants and its leachates significantly inhibited the number of leaves. The decline in number of leaves due to release of allelochemicals from *A. philoxeroides*, reduced the production of new leaves. A variation in the effects of natural formulation type (plant material /leachates) was recorded. In case of leachates, the residue was more toxic than plant material (7). The percent reduction in number of leaves was remarkable with leachate, older the leachate, the effect is noteworthy and significant. This may be due to leaching of more allelochemicals from *A. philoxeroides* in the solution, thus inhibiting the new leaves. Allelopathic effect depends on the extract's concentration and the parts of the weed from which secondary metabolites were extracted (Table 2 and Fig. 1).

Plate 10 - Single plant- Eichhornia *crassipes*-Control

Plate 11 T2 -*E.craipes* 20 Bulbs + 45 stem segments of *A. philoxeroides*

Plate 12: T3- *E. crassipes* 20 Bulbs + 80 stem segments of *A. philoxeroides*

Plate 13: T4- *E. crassipes* 20 Bulbs+ 20 days' old *A. philoxeroides* Leachate

Plate 14: T5- *E. crassipes* 20 Bulbs+ 40 days' *philoxeroides* Leachate

Plate III: Effect of *A. Philoxeroides* on single plant of Water hyacinth in different treatments

Table 2. Number of leaves as influenced by *A. philoxeroides* on *E. crassipes*

	Leaves	**Days after Imposition of treatments**				**Inhibition/ Stimulation (%) (from 15 to 60 DAIT)**
		15	**30**	**45**	**60**	
T1	Control	5.03^{a}	5.27^{a}	5.40^{b}	4.80^{b}	-4.6
T2	20 EC +45 AP	6.37^{b}	5.92^{a}	5.72^{b}	5.60^{c}	-12.1
T3	20 EC +80 AP	5.60^{ab}	5.35^{a}	5.37^{b}	5.07^{bc}	-9.5
T4	20 EC+20 days old leachate	5.67^{ab}	5.20^{a}	5.05^{ab}	4.82^{b}	-15.0
T5	20 EC +40days old leachate	6.80^{b}	7.23^{b}	4.55^{a}	3.83^{a}	-43.7
	SEm$\pm$	0.41	0.36	0.27	0.25	
	F cal	2.95	5.38	2.61	6.73	

EC – Eichhornea crassipes; AP- ***Alternanthera philoxeroides***
(-) sign indicates the % inhibition on the growth of *E. crassipes*

Number of branches

A. philoxeroides stem and leachates also induced the allelopathic effects, but the results were different to interpret (Table 3, Fig.1). Number of branches was significantly suppressed with 20 days old leachate. Branches of *E. crassipes* was more sensitive to 20 days old leachate than older day leachates (40 days).

Table 3. Number of branches as influenced by *A. philoxeroides* on *E. crassipes*

	Branches	**Days after Imposition of treatments**				**Inhibition/ Stimulation (%) (from 15 to 60 DAIT)**
		15	**30**	**45**	**60**	
T1	Control	5.40^{a}	5.47^{ab}	5.60^{a}	4.70^{ab}	-13.0
T2	20 EC +45 AP	6.30^{ab}	5.95^{ab}	5.77^{a}	5.60^{c}	-11.1
T3	20 EC +80 AP	6.70^{b}	6.07^{ab}	5.87^{a}	5.30^{bc}	-20.9
T4	20 EC+20 days old leachate	5.80^{ab}	5.25^{a}	5.30^{a}	4.30^{a}	-25.9
T5	20 EC+40 days old leachate	6.10^{ab}	6.25^{b}	5.82^{a}	5.00^{abc}	-18.0
	SEm$\pm$	0.31	0.29	0.24	0.28	
	F cal	2.58	2.03	NS	0.62	

EC – Eichhornea crassipes; AP- ***Alternanthera philoxeroides***
(-) sign indicates the % inhibition on the growth of *E. crassipes*

Fresh weight (g/plant)

The allelopathic potential of *A. philoxeroides* influenced fresh weight. The *E. crassipes* treated with *A. philoxeroides* stem or leachates were lower than control and at higher concentration induced greater phytotoxicity. The symptom of drying of *E. crassipes* was more predominant, inferring inhibitory effect by *A. philoxeroides* on growth of

E. crassipes. It is opined from the study that *A. philoxeroides* would seem to leach out an inhibitory chemical, resulted in root blackening, and chlorosis of leaf. Thus, suggesting the possibility of potential metabolites of *A. philoxeroides* killed *E. crassipes*. Studies conducted (5,9,10,11) revealed that *A. philoxeroides* contained water soluble phenolics namely, 4 hydroxy 3-methoxy benzoic acid (16.19 mg L^{-1}) and m-coumaric acid (1.48 mg L^{-1}), these chemicals would have caused phytotoxic to *E. crassipes.* The exact nature of the chemical, responsible for inhibitory allelopathic effect of *E. crassipes* is yet to be identified (Table 4, Fig. 1).

A. philoxeroides is regarded as an execrable weed that is widely distributed worldwide. In the present investigation, it was to be found have a detrimental effect on the growth of *E. crassipes* resulting in lower plant height, number of branches, leaves and fresh weight by invasion of *A. philoxeroides.* Earlier studies have shown that allelopathic compounds of *A. philoxeroides* inhibited the growth of *E. crassipes* compared to control. Findings showed that several allelopathic compounds are structurally similar to plant hormones (5,8), but the allelochemicals suppresses the activity of plant hormones. The overall effect of allelopathic compounds of *A. philoxeroides* to inhibit the growth of *E. crassipes* due to the presence of phenolic growth inhibitors as similar to the previous studies (14,15,16). Thus, the experiment suggests a clear potentiality for using *A. philoxeroides* as a biological agent to control *E. crassipes* in aquatic bodies.

The present study indicated *A. philoxeroides* exerted allelopathic effect on *E. crassipes* (Plate III). The inhibition increased with increase in number of stems grown with *E. crassipes* and older the leachates i.e., it was concentration dependent. Greater concentration of allelochemicals inhibited the growth by suppressing the synthesis of gibberellins and indole acetic acid (15). Our results are consistent with the reports on allelopathic effects *A. philoxeroides* in literature (5,8,11,13,16,18).

Table 4. Fresh weight (g/plant) as influenced by *A. philoxeroides* on *E. crassipes*

	Fresh weight	**Days after Imposition of treatments**				**Inhibition/ Stimulation (%) (from 15 to 60 DAIT)**
		15	**30**	**45**	**60**	
T1	Control	50.44[a]	51.46[b]	22.13[b]	21.09[c]	-58.2
T2	20 EC +45 AP	54.17[a]	49.64[b]	25.96[b]	20.19[c]	-62.7
T3	20 EC +80 AP	52.87[a]	51.09[b]	25.90[b]	17.23[b]	-67.4
T4	20 EC+20 days old leachate	42.52[a]	28.90[a]	13.02[a]	12.61[a]	-70.3
T5	20 EC+ 40 days old leachate	54.20[a]	56.12[b]	23.56[b]	14.19[ab]	-73.8
	SEm±	3.98	3.26	2.05	1.43	
	F cal	NS	10.69	2.41	1.98	

EC – *Eichhornea crassipes*; AP- *Alternanthera philoxeroides*

(-) sign indicates the % inhibition on the growth of *E. crassipes*

According to our findings, allelochemicals contained in the donor plant *A. philoxeroides* were identified and quantified using GCMS in order to evaluate the bioactivity of these substances. Phytochemicals in different plant parts of *A. philoxeroides* extracted using ethanol is presented in Table 5. The preliminary phytochemical analysis showed the presence alkaloids, carbohydrates, saponins, phenols, flavonoids, aminoacids, diterpenes, quinone in the extracts of *A. philoxeroides.* The presence of these phytoconstituents justifies the use of these plants as natural bioherbicde. Identification and quantification of active constituents in plant material may be useful for proper standardization and formulations.

Table 5. Phytochemicals detected in ethanol extracts of different parts of *Alternanthera philoxeroides* by GCMS

Root	**Leaf**	**Stem**
2,6,10,14,18-pentamethyl-2,6,10,14,18-eicosapentaene	-	-
Neophytadiene	Neophytadiene	Neophytadiene
2-pentadecanone 6,10,14,-trimethyl	2-Pentadecanone, 6,10,14-trimethyl	2-Pentadecanone, 6,10,14-trimethyl
1,2-Benzenedicarboxylic acid bis(2-methylpropy) ester	-	-
Hexadecanoic acid, methyl ester	Hexadecanoic acid, methyl ester	Hexadecanoic acid, methyl ester
Dibutyl phthalate	Dibutyl phthalate	Dibutyl phthalate
n-Hexadecanoic acid	n-Hexadecanoic acid	n-Hexadecanoic acid
Hexadecanoic acid, ethyl ester	Hexadecanoic acid, ethyl ester	Hexadecanoic acid, ethyl ester
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
8-Octadecenoic acid, methyl ester	8-Octadecenoic acid, methyl ester	8-Octadecenoic acid, methyl ester
12(Z)-Conjugated linoleic acid	9(E),11(E)-Conjugated linoleic acid	9(E),11(E)-Conjugated linoleic acid
6-Octadecenoic acid, (Z)-	-	6-Octadecenoic acid, (Z)-
9-Octadecenoic acid, methyl ester, 10(E)-	9-Octadecenoic acid, methyl ester, (E)-	9-Octadecenoic acid, methyl ester, (E)-
Phthalic acid, butyl 2-pentyl ester	Phthalic acid, butyl 2-pentyl ester	Phthalic acid, butyl 2-pentyl ester
Methyl stearate	Methyl stearate	Methyl stearate
-	Hexadecanoic acid, methyl ester	----
-	Phytol	Phytol
-	Hexadecanoic acid, 2-hydroxy (hydroxymethyl)ethyl ester	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
-	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a tetrahydrobenzofuran-2(4H)-one	-
-	Octadecanoic acid	-
-	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)	-
-	Phenol, 4,4'-(1-methylethylidene)	-
-	-	Phthalic acid, decyl isobutyl ester
-	-	Phthalic acid, butyl tridecyl ester
-	-	Phthalic acid, butyl hept-3-yl ester

CONCLUSIONS

Allelopathy plays important role in agriculture. The plant part and leachates of *Alternanthera philoxeroides* proved inhibitory to growth and development of *E. crassipes*. All treatments inhibited the growth, number of leaves, branches and fresh weight of *E. crassipes* over control. Thus, applying the leachates of *A. philoxeroides* reduced the growth and development of *E. crassipes*, which is an invasive weed in the universe. Further research is required to identify the allelochemicals in donor plant. Bioactivity of major compound on water hyacinth. The allelochemicals which show greater bioactivity i.e., potential allelochemicals at various doses/rates causing the inhibitory effects helps in development of new ecofriendly weedicide.

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DECLARATION

We declare that all authors of this manuscript made a significant contribution, and we have not excluded any author that substantially contributed. We have followed the ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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