Asian Journal of Biotechnology and Bioresource Technology

Volume 10, Issue 4, Page 82-91, 2024; Article no.AJB2T.124928
ISSN: 2457-0125

Evaluation of Antifungal Properties and Phytochemical Composition of Selected Euphorbiaceae Species

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajb2t/2024/v10i4221

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124928

Original Research Article

Received: 11/08/2024
Accepted: 14/10/2024
Published: 19/10/2024

ABSTRACT

Aims: To study antifungal activity and phytochemical analysis of Euphorbiaceae members.
Study Design: Experimental.
Place and Duration of Study: The research was carried out at the Department of Botany, Maharashtra College of Arts, Science & Commerce, Mumbai, India, over a period spanning from April 2022 to December 2023.
Methodology: Six medicinal plants from the Euphorbiaceae family were tested for their antifungal activity in vitro and phytochemical profile. Plant extracts were prepared in different concentrations of methanolic extracts and tested against five plant pathogenic fungi *Fusarium oxysporum, Penicillium chrysogenum, Rhizopus stolonifer, Colletotrichum gloeosporioides* and *Alternaria alternata* for their antifungal activity. In preliminary screening, all the plant extracts were tested against fungi using the poisoned food technique.

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Cite as: *Nikhat, Shaikh Saiyada, and Zafar S. Khan. 2024. "Evaluation of Antifungal Properties and Phytochemical Composition of Selected Euphorbiaceae Species". Asian Journal of Biotechnology and Bioresource Technology 10 (4):82-91. https://doi.org/10.9734/ajb2t/2024/v10i4221.*

Results: The extract with 20% concentration was found to be most effective in inhibiting mycelia growth. *Phyllanthus emblica* exhibited 100% inhibition in the extract with 20% conc. Whereas, *Codiaeum variegatum* exhibited the lowest inhibition i.e. 10.74% in the extract with 5% conc. In preliminary phytochemical analyses, all the plants showed the presence of tannin, saponin, flavonoid, phenol, alkaloids, terpenoids, glycosides and protein except in *Jatropha pandurifolia* in which few secondary metabolites were present.

Keywords: Antifungal activity; Euphorbiaceae; plant pathogenic fungi and Phyllanthus emblica.

1. INTRODUCTION

Euphorbiaceae is an important family which contains numerous medicinal plants. It is one of the largest family of flowering plants comprising with over 300 genera and 8,000 species. Plants from this family contains various compounds like alkaloids, flavonoids, steroids, saponin, phenolic compounds, fatty acid, esters, minerals, etc. that have showed different activities in human beings and animals. Species of Euphorbiaceae are extensively used as remedies against several diseases such as cancer, diabetes, diarrhea, heart diseases, hemorrhages, hepatitis, jaundice, malaria, ophthalmic diseases, rheumatism and scabies, etc [1]. *Acalypha wilkesiana* is traditionally known to have remarkable medicinal values and nearly every organ of the plant, such as the stems, roots and leaves, are used as a therapeutic agent to treat and control a variety of diseases owing to their broad phytochemical constituents [2,3]. *Acalypha wilkesiana* is used in West Africa for the treatment of headache and cold and in Nigeria, the cold extract of the leaves is used to bath babies with skin infection [4].

The leaves extracts of crotons (*Codiaeum variegatum)* are reported to have many medicinal properties including purgative, sedative, antifungal, antiamoebic and anti-cancerous activities [5,6]. Anti-inflammatory, antifungal, antiamoebic and anticancerous activities [7]; also used to treat irregular menstruation [8], wound healing [9]. *Phyllanthus emblica* leave have an anti-neutrophilic activity. It reduces blood cholesterol, blood glucose as well as triglyceride levels [10].

The objective of the research was to search new molecule or plants which having potential antifungal property. Antifungal activity of the plants from Euphorbiaceae family at various concentration was observed. To find out the active compound present in the plants, the evaluation of various phytochemicals presents in the methanolic as well as water extract of the plants were taken into considerations.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

A total six plants of family Euphorbiaceae were selected. Leaves of six plants namely *Phyllanthus emblica* L., *Phyllanthus acidus* L.*, Acalypha wilkesiana* Mosaica (Mull. Arg.)*, Jatropha pandurifolia* (Jacq.)*, Codiaeum variegatum* L. and *Drypetes roxburghii* (Wall.) were collected from Jijamata Udhyan, Byculla (East) Mumbai. Leaves of all six plants were washed thoroughly with tap water to remove debris, and shade dried at room temperature for eight to ten days. After complete drying, the leaves were ground to fine powder using an electric blender and stored in airtight bottles at 4^0C until further use.

2.2 Isolation, Purification and Identification of Fungi

Five fungi, namely *Fusarium oxysporum, Penicillium chrysogenum, Rhizopus stolonifer, Colletotrichum gloeosporioides* and *Alternaria alternata* were isolated by the procedures laid out by Baiyewu et al., in 2007 [11]. By using a sterile knife, segments (3 to 5 cm) of deteriorated fruit and vegetable tissues were cut and placed on the solidified potato dextrose agar (PDA) medium containing Streptomycin (to inhibit bacterial growth) in Petri plates, where it was incubated for seven days at room temperature. A pure culture was produced. The fungi were identified by studying the morphological characters, colony characters and the structure of the spores by referring to standard literature [12,13].

2.3 Preparation of Extracts

For extraction, 100 ml of 20% methanol and 10 gm of leaf powdered material were combined and heated for 30 minutes. The extract was filtered through a 4-fold muslin cloth and then through Whatman's No.1 filter paper [14]. For phytochemical analysis, water extract was also

prepared by the same method using distilled water.

2.4 Antifungal Assay

The antifungal activity of leaves extracts was determined by Poisoned Food Technique. PDA media amended with different concentrations of leaf extracts (5, 10, 15 and 20 % V/V) were sterilized by autoclaving and added to labeled Petri dishes [14].

Concentration (%)	Extract (ml)	PDA (ml)
5	0.75	14.25
10	1.50	13.50
15	2.25	12.75
20	3.00	12.00

In each Petri plate of 9 cm, 15 ml PDA media were poured. The Petri plates were inoculated with nine mm-diameter fungal discs from a seven-day-old culture placed at the center of solidified media plates, which were then incubated at 28^0C for seven days. The experiment was performed in triplicates, and for each treatment, a suitable control was maintained without extracts. The diameter of the radial mycelial growth of pathogens was measured in cm and recorded after seven days of incubation. Antifungal activity was recorded in terms of percent inhibition of mycelial growth (%) and calculated using the following formula [15].

Inhibition of mycelial growth (%) = (C – T/C) * 100

Where 'C' is the average diameter of the fungal colony in control plates and 'T' is the average diameter of the treated fungal colony in the plates with plant extracts.

2.5 Statistical Analysis of Antifungal Activity

The coefficient of correlation with respect to the concentration of extracts and the standard error of the mean of triplicates was calculated. For each fungus and plant extract, the coefficient of correlation was determined, with the percentage of mycelial inhibition being one component and plant extract concentration (i.e., 5%, 10%, 15%, and 20%) being another.

2.6 Qualitative Tests for Determining Phytochemicals Activity

2.6.1 Test for tannins

In 1ml of the extract solution, few drops of 10% ferric chloride solution was added. The appearance of blue-black colour indicated the presence of tannins [16].

2.6.2 Test for saponins

To 1 ml of extract solution, 10 ml of distilled water was added. The mixture was shaken vigorously. The formation of stable foam indicated the presence of saponins [16].

2.6.3 Test for flavonoids

A few drops of dilute NaOH solution were added to 1 ml of the extract solution. The addition of a few drops of dilute acid caused the solution to appear intense yellow and then become colourless, indicating the presence of flavonoids [17].

2.6.4 Test for alkaloids

The extract was dissolved in chloroform and the solution was extracted with dil. HCl or H_2SO_4 and acid layer was taken as test solution. To 1 ml of the acid layer, 1 ml of Mayer's reagent was added. Whitish of cream coloured precipitate indicated the presence of alkaloids [17].

2.6.5 Test for proteins

In 1 ml of the extract, 1 ml of Millon's reagent was added and heated. Red precipitation or red colour solution indicates the presence of proteins [17].

2.6.6 Test for phenols

To 10 ml of the extract solution, 2 ml of distilled water and a few drops of 10% ferric chloride solution were added. The blue colour indicated the presence of phenols [18].

2.6.7 Test for terpenoids

To 0.5 ml of extract, 2 ml chloroform was added. 3 ml of concentrated Sulphuric acid was added to form a layer. A reddish-brown colouration of the interface indicated the presence of terpenoids [19].

2.6.8 Test for glycosides

1 ml of the extract, 1 ml glacial acetic acid, and drops of ferric chloride solution were added. 1 ml of conc. H_2SO_4 was added by the side of the test tube. A brown ring obtained at the interface indicated the presence of glycosides [20].

3. RESULTS AND DISCUSSION

3.1 Antifungal Activity

A total of six plant extracts were evaluated for antifungal activity against five plant pathogenic fungi at defined concentrations. Among these plants, *P. acidus* showed antifungal activity against all selected fungi at all defined concentrations (Fig. 2). While the remaining plants also inhibited mycelia growth of fungi except *P. chrysogenum*. The average colony diameter of the tested fungi in the poisoned food plates were significantly less than the colony diameter in the control plates, indicating the possible antifungal properties of the extracts. It indicates that the inhibition was concentration dependent. Among all six tested plants, *P. emblica* exhibited highest mycelial inhibition of *R. stolonifer*, followed by *A. wilkesiana* against *F. oxysporum*, *C. gloeosporoides* mycelia by 100, 74.81 and 74.81 percent respectively in the extracts with 20% plant extract concentration (Fig. 1 and Fig. 3). The lowest mycelial inhibition was observed by *P. acidus* and *C. variegatum* against *C. gloeosporoides* mycelia by 10.74 and 11.11 percent respectively in the extracts with 5% plant concentration (Figs. 2 and 5).

In the present study, statistical analysis of the coefficient of correlation between the plant concentration and percentage inhibition of mycelial growth, indicates that there is a direct correlation between the two components as all the results are positive and lie between 0.84 to 0.99 (Table 1).

Antifungal activity for the plant *Drypetes roxburghii* was also observed against different fungal species, namely *Fusarium oxysporium, Alternaria brassica, Aspergillus flavus,* etc [21]. The scientific evidence reviewed regarding *Drypetes roxburghii* attributes reveals that it is a rich source of nutrients and biologically active compounds. These substances are crucial in disease prevention and health maintenance [22]. The extracts exhibited growth inhibitory activity in a dose-dependent manner. The results show that *Phyllanthus emblica* extracts were found to be more effective against all the microbes tested [23]. The highest antifungal activity was demonstrated by the100 mg/100ml leaf methanol extracts, against the fungus *Rhizopus* spp. The inhibitory activity of the methanol extract of the leaf part of *Croton* spp was also considerably higher against the other fungi tested in comparison with other extracts [24]. Plant extracts (*Acalypha* spp) inhibited fungal growth in the ranges of 0.76–56.17% against *F. solani* and 2.02–69.07% against *A. alternate* [25].

Graphs showing average percent inhibition of fungal mycelia by plant extracts

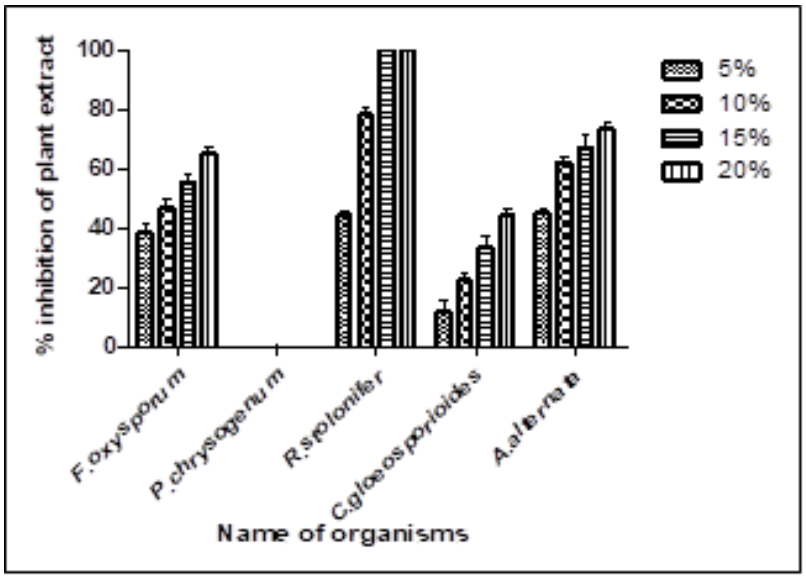

Fig. 1. *Phyllanthus emblica* showing inhibition of mycelium

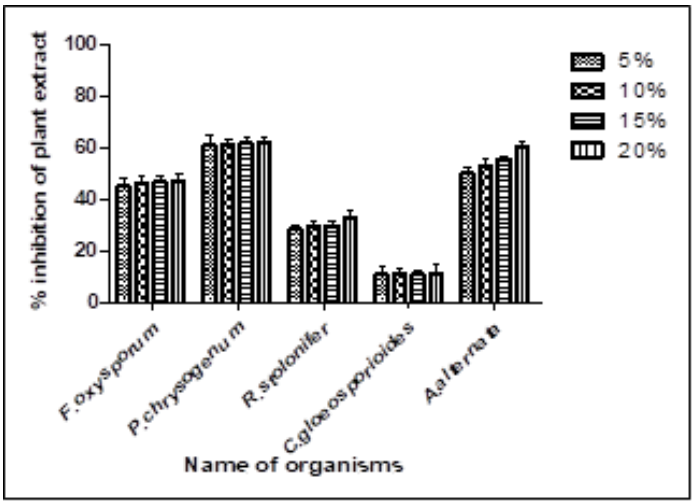

Fig. 2. *Phyllanthus acidus* showing inhibition of mycelium

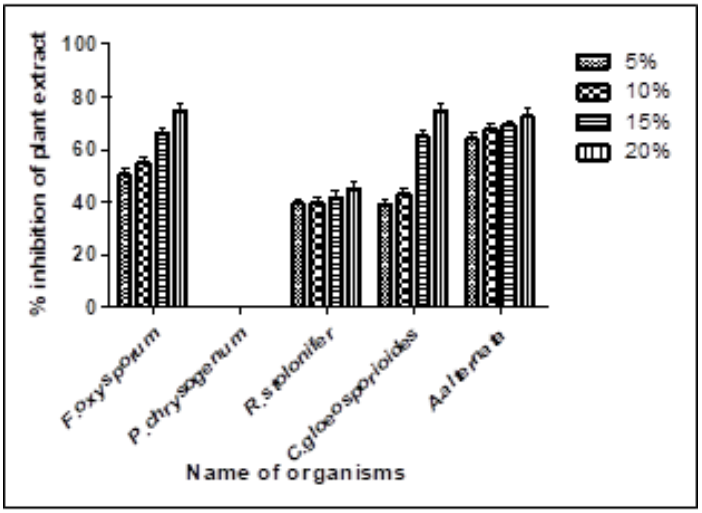

Fig. 3. *Acalypha wilkesiana* showing inhibition of mycellium

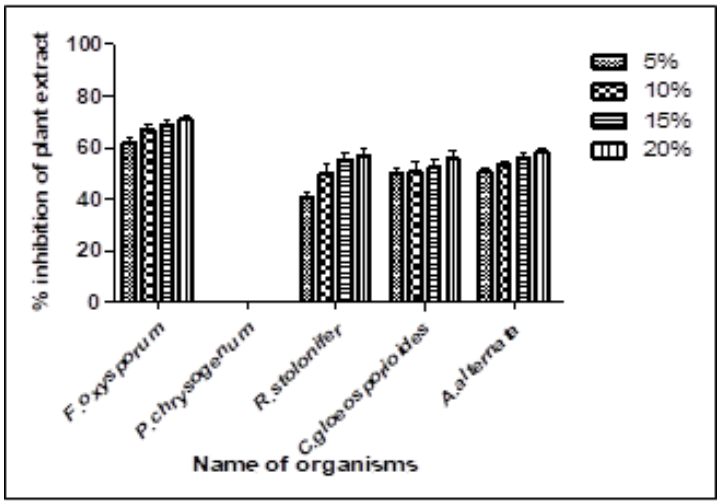

Fig. 4. *Drypetes roxburghii* showing inhibition of mycellium

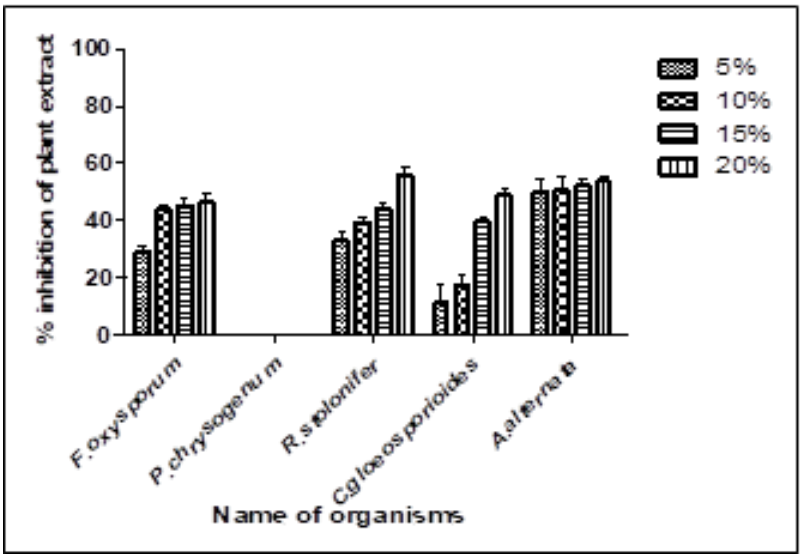

Fig. 5. *Codiaeum variegatum* showing inhibition of mycellium

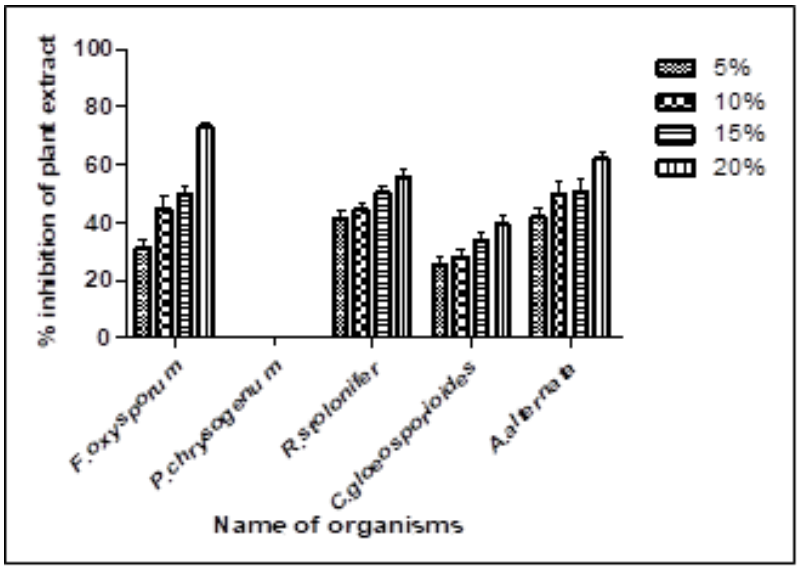

Fig. 6. *Jatropha pandurifolia* showing inhibition of mycelium

Table 1. Coefficient of correlation derived from average Percent inhibition of fungal mycelia by various concentration of plant extracts

Name of the plant	Name of the fungi*				
	F.o	P.c	R.s	C.g	A.a
Phyllathus emblica	0.99	-	0.92	0.99	0.95
Phyllathus acidus	0.99	0.99	0.91	0.90	0.98
Acalypha wilkesiana	0.98	-	0.94	0.96	0.99
Drypetes roxburghii	0.96	-	0.96	0.94	0.99
Codiaeum variegatum	0.84	-	0.97	0.97	0.97
Jatropha pandurifolia	0.96	-	0.99	0.98	0.94

* = Coefficient of correlation - = no mycelial growth, no coefficient of correlation
*(*Fo-*Fusarium oxysporum,* Pc-*Penicillium chrysogenum,* Rs *-Rhizopus stolonifer,* Cg-*Colletotrichum gloeosporioides,* Aa *-Alternaria alternata)*

Table 2. Phytochemical analysis of plant extracts

Name of the	*P. emblica*		*P. acidus*		*A. wilkesiana*		*D. roxburghii*		*C.variegatum*		*J. pandurifolia*	
Phytochemical	**W.ext**	**M.ext**	**W.ext**	**M.ext**	**W.ext**	**M.ext**	**W.ext**	**M.ext**	**W.ext**	**M.ext**	**W.ext**	**M.ext**
Tannins	+	+	+	+	+	+	+	+	+	+	-	-
Saponins	+	+	-	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
Alkalloids	+	+	-	+	+	+	-	+	-	+	-	+
Proteins	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	-	-
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+

(W.ext - water extract, M.ext -methanolic ext, + = presence, - = absence)

3.2 Phytochemical Analysis

In preliminary phytochemical analysis, *Phyllanthus emblica, Phyllanthus acidus, Drypetes roxburghii, Acalypha wilkesiana, Codiaeum variegatum* and *Jatropha pandurifolia* showed the presence of flavonoid, terpenoids, glycosides and protein in both extracts (methanolic and water extracts). In both the extracts of *Phyllanthus emblica* and *Acalypha wilkesiana* all the secondary metabolites were present. Water extracts of *Phyllanthus acidus, Drypetes roxburghii, Codiaeum variegatum* and *Jatropha pandurifolia* did not show alkaloids. Also both the extracts of *Jatropha pandurifolia* did not show tannins and phenols. As compared to the methanolic extract, water extract exhibited less solubility of secondary metabolites. Hence, it can be concluded that methanolic extract is more efficient than water extract in detecting phytochemicals (Table 2).

Methanolic *Phyllanthus emblica* extracts have shown tannin, saponin, flavonoids, phenol, alkaloids, terpenoids, glycosides, etc., [26]. Phytochemicals in the plant extract offer protection against cellular damage due to their ability of inhibiting lipo-oxygenases [27]. Phenolic compounds and flavonoids were reported to be associated with antioxidant properties, acting as scavengers of singlet oxygen and free radicals [28,29]. Gum, tannins and saponins containing plants are also rich sources of antioxidants [30,31]. Tannins aid in the repair of underlying tissue during wound healing [32]. Flavonoids have anti-inflammatory, anti-oxidative, anti-carcinogenic and anti-mutagenic effects as well as their ability to modify essential cellular enzyme activity [33]. The Phytochemical analysis obtained from the aqueous leave extract of *A. wilkesiania* indicated that carbohydrates, tannins and flavonoids were highly present in the extract. Phlobatanins, cardiac glycosides, saponins and alkaloids were also present [34]. The leave of A. *wilkesiana* showed a very high concentration of chloride, sodium and potassium ions. Calcium, iron, magnesium and zinc were in medium concentration while copper and manganese were in minute concentration. Lead and cadmium were not detected [34]. Phenols, flavonoids, and tannins have been found to be active on pathogenic microorganisms [35-37].

Phyllanthus emblica enhanced the effectiveness of the immunomodulatory system by raising blood levels of CD4, CD8, CD16, CD19, IgM, and IgG as well as albumin and globulin levels in the serum. In comparison to all experimental groups, the P. *emblica* group at a dose of 250 mg/kg b.wt. exhibited the most appreciable outcomes to increase immunity [38].

4. CONCLUSION

Among the fungi examined, extract susceptibility demonstrated that the fungus mycelial growth was inhibited as plant extract concentration was increased. It was proved by performing the statistical analysis for the coefficient of correlation, which showed a positive relation, hence a direct correlation. In the present work, *P. emblica* is found to be a potent plant, possibly due to the presence of secondary metabolites. Further studies will be conducted in order to find out new compounds.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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