Efficacy Of *Ficus religiosa* And *Curcuma longa* Leaf Extracts On *Heterotermes indicola* Under Laboratory Conditions

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Abstract
Termite infestations can cause severe damage to crops, resulting in yield loss and contamination of products. Using synthetic insecticides to manage termites often leads to environmental pollution and the development of resistance in termites. Since many plants are known to have insecticidal properties, this study aimed to evaluate the effectiveness of locally available plants at various concentrations for termite control. The current study was performed to evaluate the efficacy of ethanolic leaf extracts of *Ficus religiosa*, and *Curcuma longa* against *Heterotermes indicola* under laboratory conditions. Mortality of termites increased when feed on *F. religiosa* leaf extracts with minimum feeding rate at the maximum concentration (30%). The results showed that *F. religiosa* was more potent than *C. longa*, with LC_{50} values of 8.71 and 22.74, respectively. Chemical composition of plants extracts by Gas chromatography-mass spectrometry (GC-MS) revealed 14 and 18 compounds in selected ethanolic leaf extracts respectively. The largest chemical components based on percentage of sample identified from *F. religiosa* were n-Hexadecanoic acid, 1-Hexacoosene, 9,12,15-Octadecatrienal, Phytol, and dl-a-Tocopherol. The main components of *C. longa* were identified as, Benzenmethanol, a, a,4-trimethyl, 1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl) cyclohex-3, Cyclohexene, 1-methyl-4-1 (1-methylethylidene)- and 2-Methoxy-4-vinylphenol.

Key words: *Ficus religiosa, Curcuma longa, Heterotermes indicola,*

Introduction

Termites, which are classified under the order Isoptera, are recognized as major pests causing considerable issues in agriculture and households across many countries (Sileshi et al., 2013; Thomas et al 2023). *Heterotermes indicola* (Wasmann), a subterranean termite belonging to the Rhinotermitidae family and order Isoptera, feeds exclusively on wood (Matsui et al., 2009). This lower termite is found in tropical, subtropical, and warm temperate regions of Pakistan, Sri Lanka, and India (Mani et al., 2012). It is known for causing significant timber damage in both agricultural and urban settings. *H. indicola* has a sophisticated nesting system with dispersed colonies and earthen tunnels or galleries that target timber (Manzoor and Mir, 2010). It can spread over distances of 100 meters or more by forming satellite nests in wood and rafters and can also create hanging tubes to search for moisture and food (Salihah et al., 2012; Azhar et al., 2024)

Because termite infestations are becoming more widespread there is a growing demand for the discovery of termite treatments that are safe for both humans and the environment (Meepagala et al. 2006). Many attempts both in the field and the lab have been attempted to take advantage of the termite activity that is caused by plant extracts. In the past a few different plant species were investigated for their potential termite fighting abilities (Adams et al. 1988). Plants have a defense mechanism that involves the production of secondary metabolites such as coumarins, alkaloids, terpenoids and chromenes particularly monoterpenoids. These all are examples of secondary metabolites. These secondary metabolites have been tested to see whether or not they have the ability to control household pests. In the 1990s oil saw a renaissance in popularity as a result of a growing awareness of the fumigant and contact insecticidal properties that they possess against a wide variety of pest insects (Isman 2000). The negative effect of phytochemicals on insects can be seen in a variety of ways including an inhibition of calling behavior (Khan and Saxena 1986), a delay in growth, toxicity (Hiremath et al. 1997), an avoidance of oviposition (Zhao et al. 1998).
Tabassum et al. (2023) investigated the termiticidal effects of ethanolic leaf extracts from Calotropis gigantea and *Morus alba* against *H. indicola*. They found that both plants caused significant mortality in *H. indicola* and exhibited repellent properties. Tabassum and Aihetasham (2024) find out the efficacy of *Piper nigrum* and *Tamarindus indica* against *H. indicola* and found that both plant leaf extracts were toxic, causing 88% and 84.33% mortality respectively at highest concentration of 30%.

The current study was undertaken to assess the toxical potential of the leaf extracts of *F. religiosa* and *C. longa* against *H. indicola*. It includes the

- Ethanolic extraction of selected plant leaf using soxhlet extractor.
- Collection of *Heterotermes indicola* (Wasmann) to determine the feeding bioactivity in extracts under laboratory conditions.
- Structural characterization of compounds in leaf extracts through GC-MS.

2. MATERIAL AND METHODS

2.1 Collection of *H. indicola*

The workers of *H. indicola* species were collected from old trees of *Populous euramericiana* in Lahore. The collected specimens were then maintained on water soaked filter paper and 5g oven dried soil in each petri plate for at least one week.

2.2 Leaf collection

Leaves from selected plants were gathered from trees in the Botanical Garden of the University of the Punjab. The leaves were shade-dried for three days and then stored in polythene bags for later experimental use

2.3 Extract preparation

The leaves of medicinal plants were ground into fine powder using a grinder. For extraction 20 grams of each leaf powder was taken separately with 200ml of ethanol in a Soxhlet extractor. The following concentrations (30%, 20%, 10% and 5%) of the extracts were prepared to perform experiment. Extraction was performed by following protocol of Vogel and Zieve (1964).

2.4 Gas chromatography/ mass spectrometry (GC-MS)

For GC-MS analysis extracts from Soxhlet extractor underwent hydro distillation to determine their constituent parts. Samples were distilled at a temperature below 200°C and filtered through filter paper with pores of 0.20μm. The Gas Chromatography temperature ranged from 50 to 250°C at a rate of 4°C per minute, with a 5-minute hold for the solvent. The injector was heated to 250°C. Helium gas, serving as the inert gas flowed at a rate of 1.0 ml/min. in the split less mode and 2 μl of samples were injected.

The sample percentage composition of each sample was computed and a qualitative analysis was performed based on the percent area of each peak of the sample chemicals. The mass spectrum of each compounds was compared to the mass spectrum from the NIST 98 spectra collection (USA National Institute of Science and Technology software).

2.5 Anti-termite assay

A no-choice bioassay was employed to assess the efficacy of plant extracts against *H. indicola*, following the protocols of Elango et al. (2012) and Ashraf et al. (2020). Petri dishes were cleaned, washed, and oven-dried for a full day. Filter paper circles were cut out, soaked in 1 ml of the extract solutions, and then dried. Termites were divided into experimental and control groups. The experimental groups were treated with different concentrations of extract solutions, while the control group was treated with distilled water. One hundred termite workers were released into each Petri dish. To maintain constant humidity, a cotton plug soaked in water was placed in each Petri dish. All experimental Petri dishes were kept in the dark at 26°C, with the humidity maintained between 75-80%. All experiments were conducted in replicates, with the control treated with distilled water only. Mortality percentages were recorded every 24 hours for ten days using the following formula:

Mortality rate= ODP ÷ TP × 100

where ODP is the observed dead population of workers, and TP is the total population.

2.6 Repellency assay

For the repellency test, filter papers were halved, with one half treated with plant extracts at various concentrations and the other half with distilled water. Ten termites were placed in the center, and their distribution was recorded every fifteen minutes over two hours. Each concentration was tested in triplicate. A treatment was deemed repellent if 21 out of 30 termites consistently preferred the untreated half in three replicates.

3. STATISTICAL ANALYSIS

The percentage mortality of termites was measured and evaluated using one-way ANOVA with a P = 0.05 considered statistically significant (P<0.05). LC_{50} and LC_{90} were calculated using 'probit analysis' in Minitab version 21.

4. RESULTS

When workers of *H. indicola* exposed to different concentrations of *F. religiosa* the highest mortality rate of 100% was observed at an extract concentration of 30% and the mortality rate of 38% was at an extract concentration of 5%. In case of *C. longa* highest mortality rate of 68.33% was observed at an extract concentration of 30% and the lowest mortality rate of 9% at an extract concentration of 5% as shown in **Fig 1.** Probit analysis was performed to calculate median lethal concentration LC_{50} and LC_{90} of each plant leaf extract and termite treatment. All leaf extracts of plants *C. longa* and *F.*

religiosa gives LC_{50} values of 22.74 and 8.71, and LC_{90} 39.89 and 17.08 values respectively as shown in **Table 1.** Probability plot for both plants are shown in **Fig 3 & 4.**

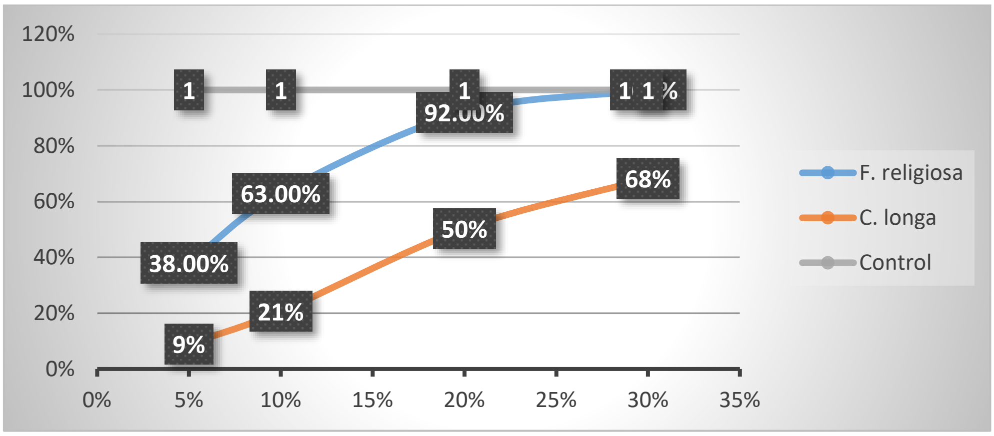


Fig 1. Percentage mortality of *H. indicola* at different concentrations of leaf extracts

Table 1. Median lethal concentration (LC_{50}) and (LC_{90}) of filter paper treated with four *F. religiosa* and *C. longa*

Sr No.	Plants name	LC_{50}	LC_{90}	Significance	95% confidence interval
1	*F. religiosa*	8.71	0.34	Significant	-9.9987-0.2409
3	*C. longa*	22.74	5.54	Significant	-1.0000-0.9092

Repellency test

Both plant extracts were repellent at highest concentrations, as most of the termites were present on untreated filter paper. Their presence on untreated filter paper indicating repellency as shown in **Fig 2.**

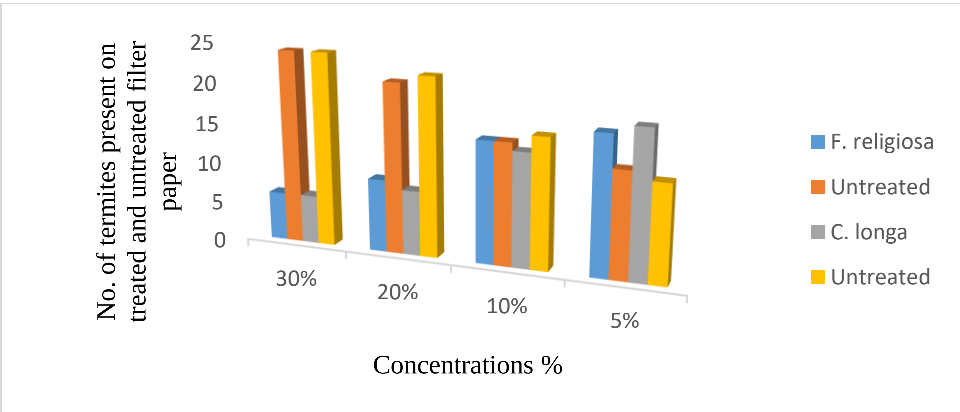


Fig. 2. Repellency test of selected plant leaf extracts against *H. indicola.*

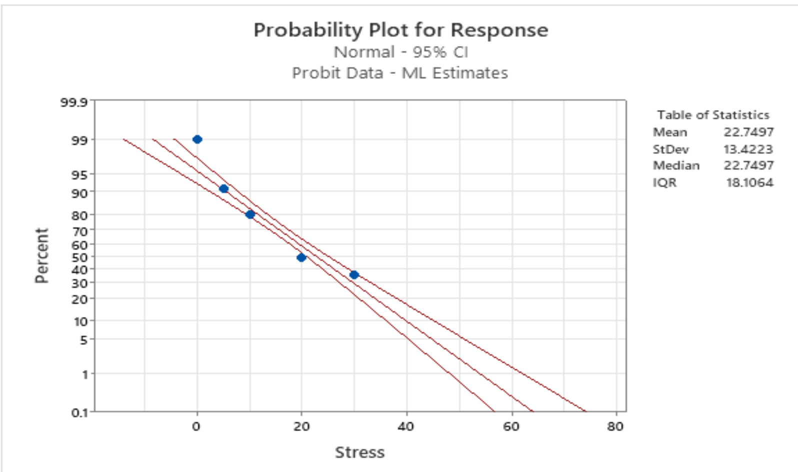


Fig. 3. Probability plot (r) of *C. longa*

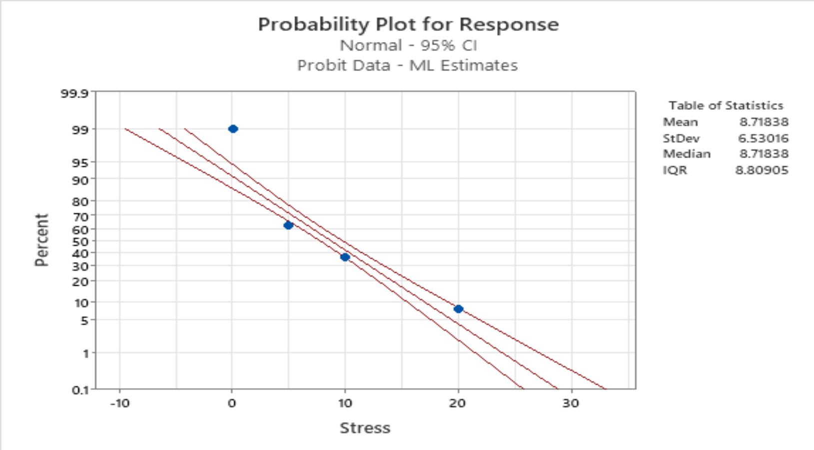


Fig. 4. Probability plot (r) of *F. religiosa*

Table.2 Two-way ANOVA for mortality of termites

Anova Table	SS (Type III)	Df	MS	F (DFn, DFd)	P value	P value summary	Significant?
Plants type	2009	3	669.7	F (3, 9) = 53.60	P<0.0001	****	Yes
Concentrations	9386	3	3129	F (3, 9) = 250.4	P<0.0001	****	Yes
Residual	112.4	9	12.49				

GC-MS ANALYSIS AND CHARACTERIZATION OF EXTRACTS

Table 3 shows the retention time, molecular formula, structural formula (based on NIST14 library used by the GCMS software**)** and percent composition of sample for chemical components identified from each of the tested leaf extract in our experiment. GC-MS analysis identified a total of 14 chemical compounds from F. religiosa and 18 from C. longa. Chromatograms from GC-MS analysis of solvent extracted leafs are shown in **Fig. 5 & 6.**

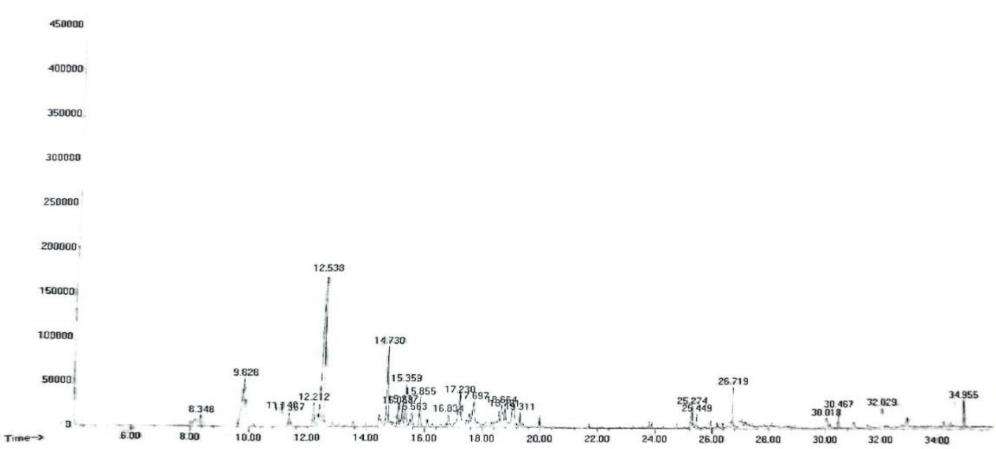


Fig 5. GC-MS chromatogram of leaf extract obtained from *C. longa*

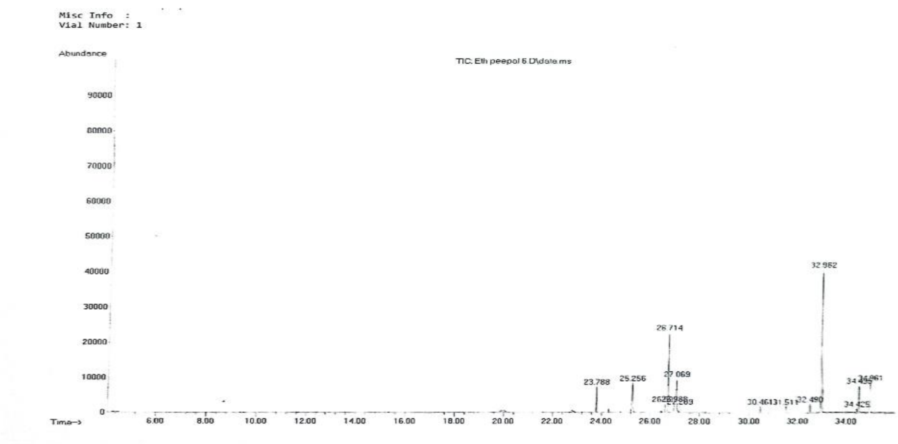
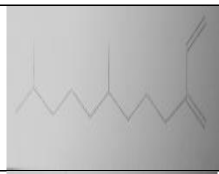
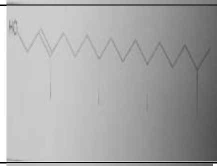
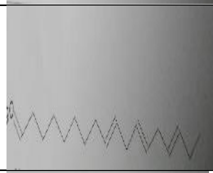
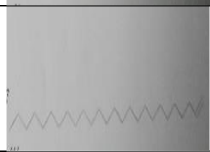
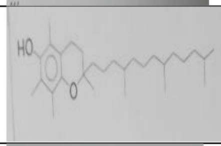
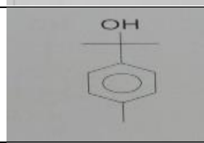
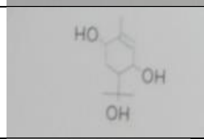
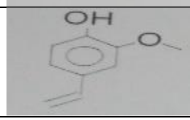


Fig 6. GC-MS chromatogram of leaf extract obtained from *F. religiosa*

Table 3. Largest components from GC-MS analysis of solvent extracted leaf of tested species.

Plant spp.	Compound name	Retention time	Relative %age composition	Molecular formula	Structural formula
Ficus religiosa	n-Hexadecanoic acid	25.256	10.098%	$C_{16}H_{32}O_2$	
	Phytol	26.714	15.947%	$C_{20}H_{40}O$	
	9,12,15-Octadecatrienal	27.069	8.397%	$C_{18}H_{30}O_2$	
	1-Hexacoosene	32.962	35.572%	$C_{26}H_{52}$	
	dl-a-Tocopherol	34.961	9.462%	$C_{28}H_{48}O_2$	
Curcuma longa	Benzenmethanol,a,a,4-trimethyl	12.538	28.455%	$C_{10}H_{14}O$	
	1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohex-3	14.730	11.287%	• $C_{10}H_{18}O_3$	
	2-Methoxy-4-vinylphenol	15.359	5.072%	• $C_9H_{10}O_2$	

5. DISCUSSION

Plants produced a variety of secondary metabolites like flavonoids, glycosides, alkaloids, sitosterols, phenols and tannins which are easily degradable when applied in a crop system. These phytochemicals which are present in plant parts helps in protecting them from the attack of insect pests. However, their production varies from plant to plant. Further parameters like plant age, plant parts (flower, leaf, fruit, bark, stem, root and seed) have been found to affect the production of phytochemicals (Prabhu et al. 2018). Agriculture perennial and annual crop plants, historical places, wildlife and tree plantation in forests are worsely destroyed or damaged by termites causing a great loss of economy (verma et al. 2009. The present research is conducted to evaluate the response of *H. indicola* against ethanolic leaf extracts of *Curcuma longa* and *Ficus religiosa*. Control setup was established using filter papers with distilled water to compare the termiticidal properties of extracts with distilled water. Controls had no effect on termite life as they were active and lived for long duration. Different concentrations of leaf extracts were prepared and applied on termites to observe mortality and repellency. As a result, plant extracts of F. religiosa showed maximum mortality and repellency against *H. indicola*.

Both plant extracts were toxic to *H. indicola* workers in a dose dependent manner and their efficiency varied depending on exposure time period and was a significant difference in the mortality as concentration increased from 5-30%. It was concluded that ethanolic extracts of tested plants have capacity for controlling termite population. Both extracts were effective in causing mortality of *H. indicola*. In the same manner these plant extracts must be applied over different bionomic zones of Pakistan on different termite species for effective command and control of diverse termite species.

Chetri et al. (2022) evaluate the larvicidal efficacy of *Curcuma longa* on larvae of *A. culex* and found that ethanolic extracts of turmeric after 48 hours shows 90% mortality of larvae at 1000ppm concentration. Ali et al. (2014) reported that the mortality of red flour beetle increased by increasing concentration of *A. sativum* and *C. longa* extracts. Acetone leaf extracts of turmeric (*Curcuma longa*) and garlic (*Allium sativum*) were used to evaluate their antifedant, toxicant, and growth regulatory effects against adults of *T. castaneum*. According to his findings percent mortality was directly proportional to increasing concentration of extracts. Both *A. sativum* and *C. longa* significantly reduced the larval, pupal and adult emergence as well as percent weight loss but A. sativum performed better as compared to the *C. longa*. Chander et al. (2000) also supported current findings who reported that turmeric (*Curcuma longa*) extract has repellent action against *T. castaneum, Oryzaephilus surinamensis, Cryptolestes ferrugineus, Sitophilus oryzae,* and *Corcyra cephalonica* even after 3 months under laboratory conditions.

Subash et al. (2019) determine the mosquitocidal activity of *F. religiosa* leaf extracts in ethanol, acetone hexane and benzene, against three mosquitoes. The ovicidal activity of *F. religiosa* leaf extracts at different concentrations find out. The petroleum ether extract of *F. religiosa* establish to more repellent than the additional extracts. According to their results ovicidal and repellent activity was dose dependent. From the results it can be concluded the petroleum ether extract of *F. religiosa* was an outstanding potential for controlling the vector mosquito *Cx. quinquefasciatus*. Repellent activity of F. religiosa ethanol, acetone, benzene and hexane extracts was tested against Cx. quinquefasciatus, An. stephensi and Ae. aegypti. In *F. religiosa* ethanol extract showed highest repellent activity was found to be 4.0 mg/cm with protection time up to 120, 160 and 200 minutes against *Cx. quinquefasciatus*, *An. stephensi* and *Ae. aegypti*.

In GCMS analysis of all selected plants different compounds were identified. i.e. Benzenmethanol, a, a,4-trimethyl, Cyclohexene,1-methyl-4-1(1-methylethylidene)-, Lupeol and 2-Methoxy-4-vinylphenol were the main components that were present in *C. longa*. The main components identified from *Ficus religiosa* were n-Hexadecanoic acid, Phytol, 1-Hexacosene, 9,12,15-octadecatriental and dl-a-Tocopherol. n-Hexadecanoic acid commonly called fatty acid effects against *P. falciparum* and displayed activity against intracellular amastigotes of *L. major*. Fatty acids also showed antimicrobial mechanism through cell membrane damage, by disrupting electron transport chain and oxidative phosphorylation of microbes (Desboise and Smith, 2010). Mary and Giri in 2018 showed the antimicrobial, anti-inflammatory and anti-cancer property of phytol and squalene.

6. CONCLUSION

The study concludes that plant extracts can be an effective alternative to synthetic insecticides for controlling termites. These plants contain bioactive phytochemicals that can be refined to create preservatives for protecting against termite infestation and damage. Additionally, these plants are locally available and affordable in Pakistan. High concentrations of the extracts proved lethal to *H. indicola*. Further research is needed to evaluate the effectiveness of these plant products against other termite species for improved and safer termite control.

DATA AVALIABILITY STATEMENT

The data associated with this study will be provided by the corresponding author upon request.

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