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ORIGINAL RESEARCH

Qualitative phytochemical screening and antimicrobial evaluation of the total alkaloids of the hydroalcoholic extracts of the leaves and stem bark of *Musanga cercropioides* (Urticaceae)

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Abstract

Background: *Musanga cercropioides* R.Br. ex Tedlie (Urticaceae) is a plant that is widely used in Nigerian traditional medicine for the treatment of various infections such as cough, diarrhoea, and vaginal candidiasis.

Objective: To study the antimicrobial potency of the total alkaloids of the hydroalcoholic extracts (HAE) of the stem bark (ST) and leaves of *Musanga cercropioides*.

Method: The antimicrobial activities of the total alkaloidal constituent of the HAE of the ST and leaves were screened using agar diffusion method using *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Escherichia coli*, and *Candida albicans* as test organisms. The preliminary phytochemical screening of the ST and leaves were performed following the conventional standard procedures of Trease and Evans.

Results: Phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, tannins, sterols, saponins and terpenoids. The HAE of both ST and leaves exhibited notable activities against the tested microorganisms at different concentrations (500, 250, 125, 62.5 mg/ml) with zones of inhibition ranging from 8.6±0.5mm to 20.0±1.0mm. The highest activity against bacteria had a zone of inhibition of 18.6±1.1mm and 20.0±1.0mm for fungus. The zone of inhibition for antibacterial control drug (Ciprofloxacin) was 42.3±2.0mm while that of antifungal control drug (Griseofulvin) was 32.0±0.8mm. The total alkaloid yield for the ST was 1.200%w/w and 1.332%w/w for the leaves.

Conclusion: *Musanga cercropioides* may be a valuable source of a therapeutic agent for potent clinical antimicrobials.

Keywords: Antimicrobial, Phytochemical screening, *Musanga cercropioides*, Total alkaloids.

Introduction

In many parts of rural Africa, as well as other regions of the world, traditional health care practices are observed to be largely dependent

on the use of medicinal plant parts and extracts. The effects of these medicinal plants on animal and human health are reportedly diverse and largely scientifically linked to their bioactive components. [1,2] Nevertheless,

there is a paucity of information on the chemical composition of many of these medicinal plant materials.^[3]

Alkaloids are widely known as organic heterocyclic nitrogen compounds that are basic-forming water-soluble salts. Alkaloids have been observed to possess the ability to intercalate with DNA, thereby resulting in impaired cell division and cell death. The mechanism of action of alkaloids such as harmane and berberine is linked to this property.^[4] The chemical evaluation of medicinal plants and their isolates is of global research interest due to the increasing microbial resistance to conventional antibiotics. With each passing decade, the incidence of multi drug-resistant bacteria keeps rising, thus, leading to an increase in morbidity, mortality, and cost of health care.

Musanga cecropioides is a common tree found in the tropical African forest. It is reported to be a native of Angola, Cameroon, Cote d'Ivoire, Democratic Republic of Congo, Ethiopia, Ghana, Liberia, Sierra Leone, Sudan, Togo, Uganda, and Nigeria.^[5] The plant is abundant in the swamp forest, river or lakeside and has an altitude of 700-1200m, with large adventitious roots. The fruits are dispersed by elephants and other animals. ^[6, 7] Many researchers have reported on the medical uses of different parts of *Musanga cecropioides* in different countries of tropical Africa. The uterotonic effect of the leaves of *M. cecropioides* has been reported.^[8] *Musanga cecropioides* R.Br. ex Tedlie (Cecropiaceae) is a medicinal plant widely used in the treatment of many diseases, among which are constipation, schizophrenia, chest infections, rheumatism, leprosy, trypanosomiasis, hypertension, dental infections, malaria, wounds, and jaundice.^[8-10] Some scientific works have shown the hypotensive, hypoglycaemic, antidiabetic, antidiarrhoeal and antibacterial properties of *M. cecropioides* extracts and extractives.^[10-13] In addition, various parts of the plant have been reported

to contain some specific triterpenoid acids like kelaic, musangic and cecropioic acid, and a host of other biochemical compounds.^[14-22]

In line with the search for new and more potent antimicrobials, the present study aimed to achieve, the hypotensive effects of both the water extract of the leaf and stem bark have already been observed. ^[9] The bark-macerate is known to be used as a decoction for pulmonary disorders, while the bark scrapings are used as a blood purifier, galactagogue, analgesic, and antipyretics.^[6] The isolation of isovitexin, vitexin, chlorogenic acid and procyanidins from the leaves have been documented. Phytochemical studies on the leaves and stem bark revealed the presence of triterpenoids, alkaloids, tannins, flavonoids, saponins, and glycosides.^[10] The aerial roots and the young branches are also popular for their capacity to produce sap for drinking among several tribes in south-east Nigeria and Western Cameroon. The present study investigated the antimicrobial potency of the total alkaloids of the hydroalcoholic extracts of the leaves and stem bark of *Musanga cecropioides*. The extracts were studied for the possible development of a plant-based antimicrobial agent which may combat drug resistance menace.

Methods

Collection and authentication of samples

The leaves and stem bark of the plant, *Musanga cecropioides* were collected at Oko Ojoo, Owonowen village, Ijebu-Igbo, Ogun State, on the 21st March 2019. Contaminants were removed by handpicking from the bulk while pest-infested specimens were also removed.

The plant was authenticated at the Federal Research Institute of Nigeria (FRIN), Ibadan, with voucher number FH106429.

Drying procedures

The leaves and stem bark of *Musanga cercropioides* were air-dried under the shade at room temperature for three weeks and then properly blended, using a manual grinder. It was stored in an airtight container that was kept in a cool dry place to prevent degradation from moisture until extraction.

Preliminary phytochemical screening

Little amounts of powdered samples of leaf and stem bark were used for the subsequent tests, adopting the conventional standard Trease and Evans method.

1. Test for Sterols

Salkowski test: In 2ml of plant extract, 2ml of chloroform and 2ml of concentrated sulphuric acid were added and shaken well. The mixture was observed for colour change.

Liebermann-Burchard Test: 2ml of methanolic plant extract was mixed with chloroform. Between 1ml and 2ml of acetic anhydride and 2 drops of concentrated sulphuric acid from the side of the test tube were added to the mixture and it was observed for color change.

2. Test for Terpenoids

Salkowski test: The extract was mixed with 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. The color change was observed.

3. Test for Alkaloids

The methanolic plant extract was warmed with 2% sulphuric acid solution for two minutes. It was filtered and few drops of Mayer's and Wagner's reagents were added separately and the color changes were observed.

4. Test for Flavonoids

A small quantity of the extract was heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture was filtered and the filtrate was used for the following tests:

A. Ammonium Test: The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. The color change was observed.

B. Aluminum Chloride Test: The filtrate was shaken with 1 ml of 1% aluminum chloride solution and observed for the light yellow color, which remained, indicating the presence of flavonoids, dilute NaOH and HCl were added and the color change was observed.

5. Test for tannins

A small quantity of the extract was boiled with 5 ml of 45% solution of ethanol for 5 minutes. Each of the mixtures was cooled and filtered. The different filtrate was used for the following test:

a. Ferric Chloride Test: 1ml each of filtrate diluted with distilled water and two drops of ferric chloride added. The color change was noted.

b. Lead Sub-acetate Test: 1ml of the different filtrate was added with three drops of lead subacetate solution. The color change was noted.

6. Test for phenols

Ellagic Acid Test: The test solution was treated with a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) sodium nitrous solution. The color change was noted.

7. Test for saponin

Foam Test: The extract was diluted with 20 ml of distilled water and agitated in a graduated cylinder for 15 minutes. The mixture was observed for frothing.

Haemolysis Tests: Leaf extract was added to one drop of human blood placed on a glass slide. This was observed for the appearance of the haemolytic zone. The color change was noted.

Alkaloid extraction

The procedure of Cabezas N.J., *et al* (2009) was adopted with little modifications. Two hundred and fifty grams of powdered *Musanga cercropioides*, leaves and stem bark were weighed separately into an amber bottle, and 1200mls of methanol was added. The mixtures were occasionally agitated and left to remain at room temperature for 72 hours. The mixtures were filtered with Whatman filter paper and the extracts concentrated to 50mls

in each case using a rotary evaporator and evaporated to dryness to obtain a greenish-black solid in each case.

To the dried methanolic extracts, 100mls of 10% HCl was added, agitated for one hour and incubated for 12 hours at 10°C. It was filtered and the filtrate (60mls) was washed with 40mls of chloroform five times with the aid of a separating funnel. The chloroform layer yielding brown non-alkaloidal extract upon evaporation was not investigated. The aqueous layer was adjusted to pH 10 with ammonia and extracted five times with 40mls of chloroform. The washed basified extract was then evaporated to dryness to get the alkaloid extract. This extract was kept for the antimicrobial studies.

Chemical identification tests for alkaloids in the extract

A little amount of extract was dissolved in Dimethyl sulphoxide (DMSO) and used for the following tests:

Dragendorff's test: To the test solution, few drops of Potassium Bismuth Iodide were added and the colour change was observed.

Wagner's test: To the test solution, 1.3g of iodine and 2g of potassium iodide were added and the colour change was observed.

Hager's test: To the test solution, Saturated Picric acid was added and the colour change was observed.

Mayer's test: To the test solution, 1.36g of Mercuric chloride, 3g of Potassium iodide in 100 ml of water were added and the color change was observed.

Phytochemical screening of the alkaloidal extract of leaf and stem bark

A little amount of alkaloid extracts of leaf and stem bark were dissolved separately in Dimethyl sulphoxide (DMSO) and was used as a stock solution for the following tests in each case:

Test for Flavonoids

From the stock solution, 1ml was taken into separate test tubes and few drops of dilute

NaOH solution were added. The color changes were observed.

Test for Saponin

From the stock solution, 1ml was taken into a test tube and diluted with 20 ml of distilled water and shaken by hand for 15 minutes and was observed for frothing.

Test for Steroids

To 1ml of the stock solution in a test tube, was added chloroform (10ml), and an equal volume of concentrated sulphuric acid was added on the sides of the test tube. The color changes were observed.

Test for Tannins

From the stock solution, 3ml was taken into a test tube and this was diluted with chloroform, 1ml of acetic anhydride was added to this mixture. One milliliter of sulphuric acid was added carefully on the side of the test tube. The colour change was noted.

Test for Terpenoids

One milliliter of the stock solution was dissolved in 2mls of chloroform, and 1ml each of acetic anhydride and concentrated sulphuric acid was added. The colour change was observed.

Test for Anthraquinones

500mg each of dried plant leaves and stem was boiled separately in 10% HCl for 5 minutes and filtered and the filtrate was allowed to cool. Few drops of 10% NH₃ were added to an equal volume of chloroform and 2ml of the filtrate. The solution was observed for any colour formation.

Determination of the extract percentage yield

The yield of the different extracts from leaves and stem bark were weighed and the percentage yield was calculated using the formula below:

$$\text{Percentage yield} = \frac{qc}{qps} \times 100\%$$

Where:

qc = quantity of extract in gram

qps = quantity of powdered plant used

Antimicrobial screening

Preparation of Muller Hinton agar

The prepared Muller Hinton Agar was used for the direct sensitivity test for the bacterial organisms used. Briefly, the media was prepared and treated according to the manufacturer's guidelines. Nineteen grams of the medium was dissolved in one litre of distilled water enclosed in a screw cap container and autoclaved at 121°C for 15 minutes. After autoclaving, the media was left to cool around 42°C and then dispersed aseptically into the sterile petri dish and was left to set. The agar was incubated at 37°C for 24 hours to confirm their sterility. When no growth occurred after 24 hours, the plates were considered sterile.

Antimicrobial testing of the extracts of the leaf and stem bark of Musanga cercropioides

The agar cup plate diffusion method was used to determine the antimicrobial activity of the extracts against bacteria. The following cultures of microorganisms provided by the Pharmaceutical Microbiology Department of Olabisi Onabanjo University (OOU) were used: *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Bacillus subtilis*, were all incubated for 4 hours before use. The tests were performed using stock solution concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml prepared by dissolving 1g of the plant extract in 2mls of Dimethyl sulphoxide (DMSO). The Muller Hinton agar was prepared and 20mls each was aseptically dispensed into sterile Petri dishes and were allowed to solidify. The bacteria were introduced into the solidified agar plates by a method called surface flooding. A sterile cork borer of 6mm diameter was used to make five equidistant holes per plates in the solidified agar. Thereafter, the wells were filled with the extracts at varying concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml respectively with the antibiotics standard (ciprofloxacin) placed centrally to serve as the control. This test was done in triplicate and incubated at 37°C for 24 hours. The antibacterial activity was observed

and the zones of inhibition were measured using a transparent meter rule and recorded.

Antifungal screening

The agar cup plate diffusion method using Sabouraud Dextrose agar was used to determine the antifungal activity of the extracts against fungus. The culture of microorganism provided by the Department of Pharmaceutical Microbiology, (OOU) was used: *Candida albicans* was incubated for 4 hours. The tests were carried out by using stock solution concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml prepared by dissolving 1g of the plant extract in 2mls of Dimethyl sulphoxide (DMSO). The Sabouraud Dextrose agar was prepared and 20mls each was aseptically dispensed into sterile Petri dishes and allowed to solidify. The organism was introduced into the solidified agar plates by a method called surface flooding. A sterile cork borer of 6mm diameter was used to make five equidistant holes per plates in the solidified agar. Thereafter, the wells were filled with the extracts at varying concentrations of 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml respectively with the antifungal standard (Griseofulvin) placed centrally to serve as the control. This test was, however, done in quadruplicate, and it was then kept at room temperature for 48 hours. The antifungal activity was observed and the zones of inhibition were measured using a transparent meter rule and recorded.

Results

1. Total alkaloid determination. The total alkaloid yield for the 250g/ dried plant sample for the stem bark and leaves of *Musanga cecropioides* was determined to be 3.00g (1.200%) and. 3.33g (1.332%) respectively.
2. Qualitative phytochemical tests on the powdered stem bark and leaves indicated the presence of alkaloids,

3. flavonoids, tannins, terpenoids sterols, phenols, and saponins. The same tests indicated only the presence of alkaloids on the total alkaloidal

extracts from the stem bark and leaves (without other detectable phytochemicals) as shown in Table I below.

Confirmatory chemical tests for total alkaloids

Table I: Chemical Identification tests for alkaloids on the extracts of *Musanga cecropioides*.

Test	Observation	Result
Mayer's	Cream-coloured precipitate	Alkaloid present
Dragendorff's	Orange precipitate	Alkaloid present
Wagner's	Red-brown precipitate	Alkaloid present
Hager's	Yellow precipitate	Alkaloid present

Antimicrobial screening

Table II: Antimicrobial activities of alkaloid extract of the leaves of *Musanga cecropioides* against different bacteria.

Bacterial strains	Inhibition zone (mm)					Standard (Ciprofloxacin)	Control (DMSO)
	62.5 (mg/ml)	125 (mg/ml)	250 (mg/ml)	500 (mg/ml)			
<i>Staphylococcus Aureus</i>	11.3±0.5	12.0±0.5	13.3±1.5	15.6±0.5		29.3±0.5	0
<i>Escherichia coli</i>	11.6±0.5	13.0±1.0	16.0±1.0	18.0±1.0		42.3±2.0	0
<i>Klebsiella pneumonia</i>	10.6±0.5	11.3±0.5	12.6±1.1	16.6±1.5		36.6±1.5	0
<i>Bacillus subtilis</i>	13.0±1.0	14.3±0.5	16.0±1.0	18.6±1.1		32.0±1.0	0

Values are expressed as mean ± SEM

Table III: Antibacterial activity of alkaloid fraction of the stem bark of *Musanga cecropioides* against different bacterial organisms.

Bacterial strains	Inhibition zone (mm)					Standard (Ciprofloxacin)	Control (DMSO)
	62.5 (mg/ml)	125 (mg/ml)	250 (mg/ml)	500 (mg/ml)			
<i>Staphylococcus Aureus</i>	8.7±0.5	9.0±0.5	10.6±1.1	13.0±1.0		38.6±3.5	0
<i>Escherichia coli</i>	10.0±0.5	11.6±1.5	13.3±1.5	14.6±1.5		37.0±2.6	0
<i>Klebsiella pneumonia</i>	10.6±1.1	11.6±1.1	12.6±0.5	14.0±1.0		34.3±1.5	0
<i>Bacillus subtilis</i>	10.6±0.5	12.0±1.0	14.6±1.1	16.3±1.5		35.0±1.0	0

Values are expressed as mean ± SEM

Table IV: Antifungal activity of alkaloidal extract of the stem bark of *Musanga cecropioides* against *Candida albicans*.

Concentration (mg/ml)	Number of Trials (mm)					
	1	2	3	4	Mean	Standard deviation
500	17	18	16	17	17	0.707
250	15	17	16	15	15.7	0.828
125	14	15	13	14	14	0.707
62.5	13	13	15	14	13.7	0.909
Positive control (Griseofulvin)	30	30	32	30.5	31	0.866

Table V: Antifungal activity of total alkaloid extract of the leaves of *Musanga cecropioides* against *Candida albicans*.

Concentration (mg/ml)	Number of Trials (mm)					
	1	2	3	4	Mean	Standard deviation
500	20	18	18	17	18.2	1.089
250	18	17	16	16	16.7	0.8292
125	16	14	15	14	14.7	0.8292
62.5	15	13	12	13	13.2	1.0897
Positive control (Griseofulvin)	31	30	31	31	32	0.7071

The diameter of the cork borer used = 6mm

Mean zone of inhibition measured in millimetre

KEYS: 7mm indicates in activities; 8-12mm indicates weak activities; 12mm and above indicates strong activities

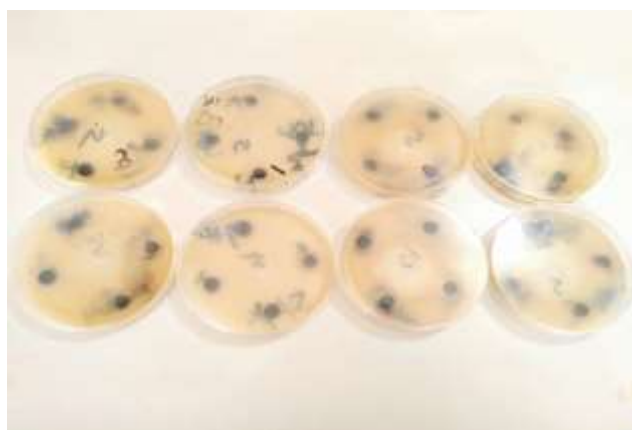


Figure 1 Image of agar plate of antibacterial testing of stem bark on *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Escherichia coli*.

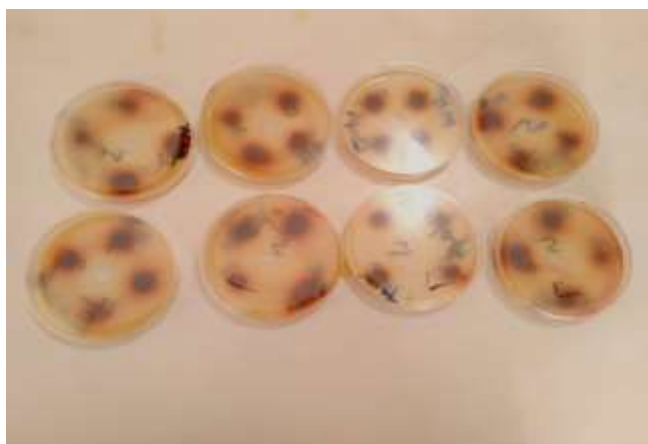


Figure 2: Image of agar plate of antibacterial testing of leaves on *Staphylococcus aureus*, *Klebsiella pneumonia*, *Bacillus subtilis*, and *Escherichia coli*.



Figure 3: Image of agar plate of antifungal testing of leaves and stem bark on *Candida albicans*

Discussion

Musanga cercropioides is a plant that is known to be commonly used among the rural dwellers in Ijebu land (Ogun State, South-west Nigeria) for the treatment of various ailments and infections. It is reported to be used in the treatment of ailments such as gonorrhoea and upper respiratory tract infection. [10] It has also been observed to be useful in the treatment of vaginal candidiasis. [11,12] Several other plants have also been studied to be of various medicinal benefits; the alkaloidal fractions from plants such as *Eclipta alba* and *Berberismicrophylla*, [13,14] have been reported to

exhibit significant antimicrobial properties., However, there are no reported work on the antimicrobial activity of the total alkaloids of the plant *Musanga cercropioides*, hence, the motivation to study and evaluate the antimicrobial activity of the total alkaloidal fractions of the leaves and stem bark of *Musanga cercropioides*.

The determination of the total alkaloid fractions of the leaves and stem bark of *Musanga cercropioides* using the maceration technique yielded 1.332%w/w for leaves and 1.20%w/w for bark. The presence of alkaloid was confirmed in the phytochemical screening

of the powdered leaves and stem bark of the plant as well as in the extracts. Various phytochemicals such as flavonoids, tannins, terpenoids, sterols, phenols, saponins, and anthraquinones were also detected as previously reported by other researchers. [15]

The presence of bioactive substances in plants has been reported to confer resistance against bacterial and fungal infection in such plants and this could explain the demonstration of the antibacterial and antifungal activity of the plant extract used in the present study. [16] From the results of the antibacterial susceptibility test carried out on the leaves and stem bark of *Musanga cercropioides*, the range of zone of inhibition was within 11.3 ± 0.5 mm to 18.6 ± 1.1 mm. The highest activity of the leaf extract against bacteria was observed to be the zone of inhibition of 18.6 ± 1.1 mm against *Bacillus subtilis* while the highest for the stem bark extract was 16.3 ± 1.5 mm against *Bacillus subtilis*. From the findings, it can be seen that the zones of inhibition of the bacteria reduced as the concentration of the plant extract decreased for both the leaves and the stem bark. This may be due to the variations in the concentrations of the extract showing that the observed antimicrobial activity is dose-dependent. It was observed that the results of the zone of inhibition of the leaves were higher than that of the stem bark and this may be due to the higher total alkaloid contents detected in the leaves.

For the antifungal activity, the highest activity had a zone of inhibition of 20.0 ± 1.0 mm for the leaf extract and 18.0 ± 0.7 mm for the stem bark extract. The highest zone of inhibition for the antifungal control drug (Griseofulvin) was 32 mm. These observations suggest that the total alkaloids fraction has marked antifungal activity.

Studies have shown that several factors may predispose bacteria to antibacterial agents such as previous encounters with the agents or the nature of the medium used, which may

affect the ability of the agent to diffuse. The observed activities of the extracts from *Musanga cercropioides* possibly provide a scientific basis for the local usage of this plant in the treatment of various ailments and diseases. The fact that the fraction was active against both Gram-negative and Gram-positive bacteria on which it was tested, may also indicate a broad spectrum of activity. Although the precise mechanism of action of the antimicrobial activities observed in the extracts studied is not known, literature reports suggest that alkaloids act through the following mechanisms: inhibition of nucleic acid synthesis, as they inhibit the enzyme dihydrofolate reductase in cell-free assays, [17] inhibition of cell division, induction of cell elongation without affecting DNA replication, nucleoid segregation, or distortion of membrane structures. [18]

Conclusion

This study has shown that *Musanga cecropioides* contains alkaloids which elicit antibacterial and antifungal properties. Further studies on the total alkaloids fractions may reveal the specific alkaloids responsible for the observed activities. The isolation, characterization and eventual development of these alkaloids to clinical drug(s) may be of great value to human health.

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Authors' Contributions: ISF designed the study. ISF and UG did the literature search. ISF and RAS participated in data collection and analysis while UG did the review of the draft of the manuscript. All the authors approved the final version of the manuscript.

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