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Study of Phytobioactive Constituents and Antioxidant Potential of Different Fractions of Leaves Extract of *Boswellia serrata*

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Authors' contributions

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

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ABSTRACT

Many of the current treatments that we use to treat our various ailments are derived from plants or plant-based therapies. The herb is particularly important in traditional medicine because of its ethnomedicinal properties. Boswellia serrata, often known as Indian frankincense, is a plant extract that has been utilised in Ayurvedic medicine for thousands of years. Because *Boswellia serrata* has a strong anti-inflammatory effect, Western medicine has verified its traditional use in the treatment of osteoarthritis, as it has with many other herbs. The goal of this study was to find out what phytochemicals were present in *Boswellia serrata* leaf extracts, as well as their antioxidant activities. The MeOH (85 percent) extract of *Boswellia serrata* leaves exhibited the highest total phenolic content and antioxidant activity, according to the findings of this study. Furthermore, the EtOAc and n-BuOH fractions (85 percent) generated from MeOH extract had a high total phenolic content and antioxidant activity. Total phenolics and antioxidants have a strong positive relationship. For further separation and identification of their chemical components, sophisticated chromatographic and spectroscopic technologies are advised due to the significant quantity of total phenolics and antioxidant capacity of EtOAc and n-BuOH fractions. The leaf of *Boswellia serrata* is a great source of natural antioxidants, according to this study. Furthermore, overall phenolic content and antioxidant activity have a close association.

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1. INTRODUCTION

Humans have employed medicinal plants as pharmaceuticals to treat a range of ailments since ancient times, and they have had a significant impact. The Indian Frankincense tree is also known as Boswellia serrata. It's a medium-sized deciduous tree that's native to India, Asia, and Africa [1]. The leaves of *Boswellia serrata* are imparipinnate and alternating. Tree bark is typically papery and thin. The sepals and petals of the flowers are tiny and white. Fruit that is trifled is divided into three valves. Fruits have heart-shaped seeds linked to the inner angle of the fruit [2]. Cough, asthma, and bronchitis are among the disorders for which it is prescribed. The majority of gum resins are utilised in medicine [3].

The hydroxyl radical (OH), hydrogen peroxide (H2O2), peroxynitrite (ONOO-), nitric oxide (NO), and hypochlorous acid (HOCl) are highly reactive oxidants generated naturally in the human body or as a result of external stressors such as ionising radiation, pollution, stress, or even a poor diet [4]. If not neutralised, these RONs induce bimolecular damage such as protein, lipid, DNA, and carbohydrate damage, as well as the production of harmful consequences such as lipid peroxides, enzyme performance loss, mutagenesis, and carcinogenesis [5-7]. Antioxidants are substances that scavenge free radicals and minimise oxidative stress, preventing or slowing the oxidation of oxidizable products. Endogenous enzymatic antioxidant defences in humans include catalase, superoxide dismutase, and glutathione peroxidase, to name a few. Cells are protected from oxidative damage by endogenous enzymatic antioxidant defences [8]. Cellular ageing, carcinogenesis, coronary heart disease, diabetes, and neurodegenerative infections are among the illnesses. As a result, exogenous antioxidants, especially those derived from plants, play a critical role in reducing the risk of free radical damage [7]. As a result, consuming more antioxidants in the diet may be beneficial to human health [8].

Flavonoids, glycosides, saponins, terpenes, sterols, tannins, alkaloids, and other beneficial secondary metabolites are abundant in plants. The bulk of these categories, according to study, exhibit antioxidant action. The Salix genus (Family Salicaceae) has 400 species and is well-known for its therapeutic capabilities. Thunb. Salix mucronata Salix species have a wide range of phytochemical constituents, notably salicin (natural aspirin), flavonoids, terpenoids, lignans, and phenolic acids, according to several studies. (Syn. Salix safsaf or Salix subserrata) is found across Egypt's Nile River [9]. The vast majority of these substances have pharmacological and biological effects. Salicin and salicylic acid, two isolated chemicals from the Salix genus, are used to treat fever, pain, and inflammation [10]. The goal of this study was to determine the phytochemical components and antioxidant activity of different *Boswellia serrata* leaf extracts.

2. MATERIALS AND METHODS

2.1 Plant Materials

The Minor Forest Produce Processing & Research Centre in Bhopal provided fresh *Boswellia serrata* leaves (MFP-PARC). For the extraction procedure, the plant's leaves were dried in the shade, ground into a fine powder with an electric mill, and stored under dry conditions.

2.2 Chemicals

N.S scientific, Bhopal M.P. provided DPPH (1, 1diphenyl-2-picryl hydrazyl radical), ABTS (3-ethylbenzthiazoline-6-sulphonic acid), Folin Ciocalteu reagent, potassium persulphate, and disodium hydrogen phosphate (India). Ammoniummolybdate, sodium carbonate, sodium nitrite, sodium hydroxide, and aluminium chloride were supplied by Merck (Dolphin pharmacy Pvt Ltd, Mumbai) (India). Rutin, gallic acid, BHT (butylatedhydroxy toluene), and ascorbic acid were provided by N.S Scientific, Bhopal M.P. (India). -tocopherol (Vitamin E) was also sold by N.S Scientific in Bhopal, Madhya Pradesh (India).

2.3 Extraction Process

A total of 800 grammes of dried *Boswellia serrata* leaf powder was divided into four equal halves. Each part (200 g) was extracted three times, in that order, with pure methanol, MeOH (85%), MeOH (70%), and distilled water. Each extract was vacuum evaporated until dry using a rotatory evaporator. The chemical components, as well as total phenolic and flavonoid content, of the dried extracts were determined by storing them

in dry vials. The antioxidant activity of these extracts was also investigated.

2.4 Process of Fractionation

The methanolic extract was treated with petroleum ether (85 percent). Organic solvents such as chloroform (CHCl3), ethyl acetate (EtOAc), and n-butanol (n-BuOH) were used to fractionate the defatted methanolic extract, which was subsequently evaporated under reduced pressure until dry.

2.5 Phytochemical Screening

Flavonoids (Shinoda test), alkaloids (Wagner's and Dragendorff's tests), sterols (Salkowski test), tannins (10 percent Lead acetate test), triterpenoids (Libermann Burchard test), saponins (Frothing test), and cardiac glycosides (NaOH and Molisch tests) were detected in the phytochemical screening of *Boswellia serrata* different extracts.

2.6 Total Phenolic Content

By measuring the intensity of the produced blue hue, the total phenolic content was estimated using the FolinCiocalteu procedure [11]. 0.5ml of plant extract dissolved in methanol (200g/ml) was combined with 2.5ml of 10 fold diluted Folin Ciocalteu reagent and 2ml sodium carbonate. After 30 min incubation in dark with steady shaking. The absorbance was measured at 760 nm against a gallic acid standard solution. The total phenolic content (TPC) of the various plant extracts was calculated as the average of three independent assays and reported as mg gallic acid equivalent/g dry weight extract (mg GAE /g extract).

2.7 Total Flavonoid Content

The total flavonoid concentration was determined using Barku et al., (2013) [12]'s aluminium chloride colorimetric approach. The hydroxyl groups in flavonoids combine with aluminium chloride to form a compound (AlCl3). A pink tint was created by the reaction with sodium nitrite. 250 litres of plant extract in methanol (500 g/ml) were mixed with 75 litres of NaNO2 (5%), and 1.3 litres of distilled water. After 5 minutes, 150 litres of 10% AlCl3 were added. After 6 minutes, the reaction mixture was diluted with 275 l distilled H2O and 0.5ml of 1M NaOH was added. The absorbance at 510nm was measured after 15 minutes and compared to a reference rutin solution. All studies were done in triplicate and the total flavonoid content (TFC) was represented as mg rutin equivalent per gramme extract (mg RE /g extract).

2.8 Assays for Antioxidant

2.8.1 DPPH scavenging method

The DPPH radical (1,1diphenyl-2-picryl hydrazyl radical) is a stable violet radical that turns yellow when reduced. Using the technique described by Alam et al., [13], the optical density loss was measured spectrophotometrically at 517nm. 1.5 ml of a freshly prepared DPPH solution (DPPH was dissolved in methanol with an absorbance of 0.10.05) was added to 1.5 ml of a serial concentration of different plant extracts in methanol. The absorbance was measured at 517 nm against a blank sample after 30 minutes in the dark. All trials were done in triplicate and used ascorbic acid, vitamin E, and BHT as controls. The extracts' DPPH scavenging activity was evaluated, and the SC50 (Concentration of sample required to scavenge 50% of DPPH radicals) value was derived using the following equation:

$$\text{DPPH scavenging activity (SA) \%} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Where Asample is the absorbance of a sample solution, and Acontrol is the absorbance of the control solution (containing all of the reagents except the test sample).

2.8.2 ABTS assay

According to Kaur et al., various extracts have a higher capacity to quench the ABTS+ cationic radical (2-2azinobis (3-ethylbenzthiazoline-6-sulphonic acid) than Trolox® (the water soluble analogue of vitamin E) (2011). The ABTS+ was made by combining ABTS (7 mM) with potassium persulphate (2.45 mM) overnight and then storing it in the dark at 5 °C in a refrigerator. The highly coloured ABTS stock solution was diluted 1:70 with ethanol, and its absorbance at 734nm was adjusted to 0.70.01. Finally, in a micro cuvette, 100l (200g/ml) of each plant extract was mixed with 1ml of ABTS solution, and the absorbance was measured after 2.5 minutes against a blank sample. Trolox® standard methanol solution (final concentration 0-15M). The oxidised solutions' absorbance was compared to the Trolox® standard calibration

curve. The results were calculated as mmolTrolox® equivalents per 100 g dry weight of plant extract.

2.8.3 Total antioxidant capacity (TAC) assay

The total antioxidant capacity was calculated using the Phosphomolybdate assay. To convert Mo (VI) to Mo (V), this method relied on extracts forming a green phosphate Mo (V) complex under acidic conditions (V). According to Abdel-Gawad et al., [14], the procedure was conducted out. In a nutshell, 5ml of reagent was combined with 0.5ml of plant extract in MeOH (500g/ml) (0.6M sulphuric acid, 28mM disodium hydrogen phosphate and 4mM ammonium molybdate).

For 90 minutes, the tubes were sealed and immersed in a 95°C water bath. The tubes were cooled to room temperature after the incubation period and the absorbance at 695nm was measured against a blank (5ml reagent in addition to 0.5ml methanol under the same conditions). In milligrammes of ascorbic acid equivalents, the total antioxidant activity was assessed. All of the experiments were done in duplicate.

2.9 Statistical Analysis

The statistical analyses were performed using SPSS (16) software and Microsoft Excel program version 2010. All experimental studies were done in triplicate, and the data were provided as means standard deviation (SD).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Plant cells produce both primary and secondary metabolites (carbohydrates, lipids, and proteins) (alkaloids, phenolics, essential oils, terpenes, sterols, flavonoids, tannins, etc.). Natural chemicals play an important role in the treatment of a number of illnesses, according to a review of the literature [15-16]. Salix extracts also have a high concentration of phenolic and flavonoid components, according to research.

These natural clusters are used to treat a wide range of diseases. As a result, preliminary phytochemical screening of numerous *Boswellia serrata* extracts [MeOH (85%), MeOH (70%), and water] was carried out in the current study to evaluate the major chemical contents and the ability of these compounds to scavenge free radicals in the extracts studied.

According to the data in table1, the different extracts include large amounts of flavonoids and phenols, as well as modest amounts of tannins, sterols, triterpenoids, and cardiac glycosides. The findings also revealed that MeOH (85%) extract has high phenolic and flavonoid content, so this extract was defatted utilizing petroleum ether and segregated using multiple organic solvents, including CHCl3, EtOAc, and nBuOH. According to Table 1, the EtOAc and n-BuOH fractions included considerable amounts of flavonoids, tannins, phenols, cardiac glycosides, moderate amounts of sterols and saponins, and low amounts of alkaloids. Because phenolic compounds have a high ability to scavenge free radicals, the occurrence of these secondary metabolites in the tested plant implies that *Boswellia serrata* might be an efficient antioxidant. Which are associated with many diseases [17-19].

3.2 Total Phenolic Contents

The Folin Ciocalteu test was used to determine the total phenolic content; this spectro-photometric assay can measure all phenolics present in plant extracts. Table 2 reveals that MeOH (85%) extract has the highest total phenolic content (130.211.49 mg GAE/g ext.), followed by MeOH (70%) extract (128.120.644 mg GAE/g ext.), and water extract has the lowest total phenolic content (83.491.04 mg GAE/g ext.).

Table 3 reveals that the EtOAc and n-BuOH fractions produced from MeOH (85%) extract had the highest overall phenolic content (249.312.19 and 158.29 2.81 mg GAE/g ext.).

The CHCl3 fraction had the highest phenolic content (91.412.23 mg GAE/g ext.), whereas the rest of the sample had the lowest (64.250.54 mg GAE/g ext.).

Owing to the existence of phenolic hydroxyl groups, it has been observed that phenolic compounds derived from medicinal plants are particularly reactive in neutralising free radicals by donating an odd electron or hydrogen atom [20-21].

Table 1. Preliminary phytochemical screening of *Boswellia serrata* leaf extracts and fractions derived from MeOH (85%) extract

Phytochemical constituents	Tests	MeOH ext.	MeOH (85%) ext	MeOH (70%) ext.	Water ext.	Fractions of MeOH (85%) ext.			
						CHCl3 fraction	EtOAc fraction	n-BuOH fraction	Residue fraction
Flavonoids	Shinoda test	++	+++	++	+	+	+++	+++	+
Alkaloids	Wagner's test	+	+	--	--	--	+	+	-
	Dragendorff's test	+	+	--	--	--	+	+	-
Tannins	10%Pb acetate test	++	++ +	++	+	+	++ +	+++	--
Sterols	Salkowski test	+	+ +	+	--	++	++	++	--
Triterpenoids	Libermann-Burchard test	+	+ +	+	--	+	++	++	--
Cardiac glycosides	NaOH test	++	+ +	+	+	+	++	++	+
	Molisch test	++	++	++	++	+	++	+++	+
Phenols	FeCl3test	++	+++	++	+	++	+++	+++	+
Saponins	Frothing test	+	+	+	--	-	+	++	--

(+++): high amount, (++): moderate amount, (+): small amount, (-): Absent

Table 2. Yield, total phenolic and flavonoid contents of various leaf extracts of *Boswellia serrata*

Extract	Yield %	Total phenols (mg gallic acid equivalent (GAE)/ g ext.)	Total flavonoids (mg rutin equivalent (RE)/ g ext.)
MeOH ext.	19.04	127.08±0.63	44.81±0.9
MeOH (85%) ext.	17.76	130.21±1.49	63.49±1.33
MeOH (70%) ext.	15.3	128.12±0.64	60.45±0.88
water ext.	10.2	83.49±1.04	27.81±0.62

The results were expressed as the mean ± standard deviation (SD) of three independent experiments.

3.3 Total Flavonoid Content

Flavonoids are a kind of polyphenolic compound that comes in a variety of forms. They are particularly potent radical scavengers of most oxidising chemicals, particularly singlet oxygen and a range of free radicals associated in a wide range of diseases [22]. As a result, flavonoids in plant extracts boost their ability to scavenge or deactivated free radicals [23].

The total flavonoid contents of several *Boswellia serrata* leaf extracts were sorted in the following order, as shown in table 2: The maximum flavonoid content is found in MeOH (85%) extract (63.491.33 mg RE/g ext.), followed by MeOH (70%) extract (60.450.88 mg RE/g ext.), and MeOH extract (44.810.9 mg RE/g extract). RE is found in the least level in the water extract (27.810.62 mg RE/g ext.). The total flavonoid content of the different fractions of MeOH (85%) extract (Table 3) is organised as follows: The EtOAc fraction (118.71.72 mg RE/g ext.) has the highest content, followed by the n-BuOH fraction (51.552.74 mg RE/g ext.) and the CHCl3 fraction (35.122.55 mg RE/g ext.). Total flavonoid content was lowest in the residue fraction (18.421.29 mg RE/g).

As a result, the overall flavonoid content in the ethyl acetate fraction was the highest. Table 3 illustrates the yield, total phenolic, and flavonoid content of various fractions derived from a *Boswellia serrata* 85 percent MeOH extract. Extract (mg gallic acid equivalent (GAE) / g ext.) total phenol yield (mg rutin equivalent (RE) / g ext.) total flavonoids 4.66 CHCl3 fraction 1.14n-BuOH fraction 6.88n-EtOAc fraction 1.14n-BuOH fraction 6.88n-BuOH fraction 6.88n-BuOH fraction 6.88n-BuOH fraction 6.88n-BuOH fraction 6.88n-BuOH fraction 6.88n-BuOH fraction 6.88n-BuOH 6.12 Fraction of residue The average and standard deviation (SD) of three independent experiments were used to calculate the results.

The results were expressed as the mean ± standard deviation (SD) of three independent experiments.

3.4 Assays for Antioxidant

3.4.1 DPPH scavenging method

DPPH (1, 1diphenyl-2-picryl hydrazyl radical) is a stable free radical with a maximum absorbance in methanol at 517 nm that changes the color from purple to yellow after acknowledging an electron or proton radical from antioxidant compounds (antioxidant extracts) to become a stable diamagnetic molecule [24]. Table 4 shows that MeOH (85%) extract is the most effective free radical scavenger extract (SC50= 97.440.39g/ml), followed by MeOH (70%) extract

Table 3. Yield, total phenolic and flavonoid contents of different fractions derived from MeOH(85%) extract of *Boswellia serrata*

Extract	Yield %	Total phenols (mg gallic acid equivalent (GAE)/ g ext.)	Total flavonoids (mg rutin equivalent (RE)/ g ext.)
$CHCl_3$ fraction	4.66	91.41±2.23	35.12±2.55
EtOAc fraction	1.14	249.31±2.19	118.7±1.72
n-BuOH fraction	6.88	158.29±2.81	51.55±2.74
Residue fraction	6.12	64.25±0.54	18.42±1.29

Table 4. DPPH scavenging activity, ABTS radical scavenging activity and total antioxidant capacity of various leaf extracts of *Boswellia serrata*

Extract	DPPH scavenging activity sc 50 (µg/ml)	ABTS radical scavenging activity (mm Trolox® eq. / 100 gm ext.)	total antioxidant capacity (mg equivalent of ascorbic acid / g ext.)
MeOH ext.	132.62±2.51	42.41±1.07	158.47±1.44
MeOH (85%) ext.	96.44±0.39	45.83±0.32	199.18±2.19
MeOH (70%) ext.	102.32±1.7	43.29±0.66	170.73±3.12
water ext.	200.10±2.04	27.69±0.64	111.74±2.59
Ascorbic acid	13.58±0.34	--	--
Vitamin E	22.12±0.21	--	--
BHT	18.74±0.076	--	--

(SC50= 101.321.7 g/ml). The water extract showed the lowest antioxidant activity (SC50= 200.102.04%/ml). Table 5 further revealed that the EtOAc and n-BuOH fractions (85 percent) obtained from the methanol extract had the highest antioxidant activity (SC50= 50.190.24 and 72.190.52 g/ml, respectively). Antioxidant activity was lowest in the residue fraction (SC50 = 213.681.17 g/ml).

According to the findings of this investigation, the EtOAc fraction is the most active fraction because it includes a high concentration of phenols. These findings are consistent with prior research on other plants, suggesting that plant phenolic chemicals are extremely significant due to their capacity to scavenge free radicals [25-26].

The results were expressed as the mean ± standard deviation (SD) of three independent experiments.

3.4.2 ABTS assay

The ABTS (2-2azinobis [3-ethylbenzthiazoline-6-sulphonic acid] test) is a powerful tool for evaluating sequence-breaking antioxidants in lipid peroxidation as well as the antioxidant activity of hydrogen-donating antioxidants. In this test, the oxidation of ABTS produces a bright green coloured nitrogen centred ABTS. The maximum assimilation wavelength of these free radical cations is 734 nm, and they are stable over a wide pH range [17]. Table 4 demonstrates that the *Boswellia serrata* MeOH (85%) extract has the highest antioxidant activity (45.830.32 mm Trolox®eq. / 100 gm ext.). The antioxidant activity of the plant's water extract was low (27.690.64 mm Trolox® eq. / 100 gm ext.). Methanol (70%) and pure methanol extracts both had moderate activity (43.290.66 mm Trolox® eq. / 100 gm ext. and 41.411.07 mm Trolox® eq. / 100 gm ext., respectively). Table 5 shows that the dissimilar fraction derivative from MeOH (85%) has antioxidant activity, with the EtOAc portion having the highest antioxidant activity (76.221.61 mm Trolox® eq. / 100 gm ext.) followed by the n-BuOH and chloroform fractions (57.570.76 and 29.371.04 mm Trolox® eq./100 gm ext.). The antioxidant activity of the remaining fraction was modest (21.020.67 mm Trolox® eq./100 gm ext.).

3.4.3 Total antioxidant capacity (TAC) assay

The total antioxidant capacity of several *Boswellia serrata* extracts was calculated using the phosphomolybdenum procedure. Natural antioxidants convert Mo (IV) to Mo (V), resulting in green phosphate/Mo (V) complexes, according to a study of the literature.

These compounds have absorbance peaks at 695 nm [19].

According to the results of the current study (Table 4), MeOH (85%) extract has the highest total antioxidant capacity (199.18 2.19 mg equivalent of ascorbic acid/g ext.), followed by MeOH (70%) extract (170.733.12 mg equivalent of ascorbic acid/g ext.) and MeOH extract (158.471.44 mg equivalent of ascorbic acid/g ext.). Water extract had the lowest total antioxidant capacity (111.74 2.59 mg equivalent of ascorbic acid / g ext.).

Due to its highest phenolic content, the results in table 5 revealed that EtOAc has the highest total antioxidant capability (249.86 3.74 mg equivalent ascorbic acid / g ext.). The antioxidant capacity of the remaining fraction is modest (106.141.9 mg equivalent of ascorbic acid / g ext.).

The results were expressed as the mean ± standard deviation (SD) of three independent experiments.

Table 5. DPPH scavenging activity, ABTS radical scavenging activity and total antioxidant capacity of different fractions derived from MeOH (85%) extract of *Boswellia serrata*

Extract	DPPH scavenging activity sc 50 (µg/ml)	ABTS radical scavenging activity (mm Trolox® eq. / 100 gm ext.)	total antioxidant capacity (mg equivalent of ascorbic acid / g ext.)
$CHCl_3$ fraction	182.5±1.98	29.37±1.04	119.22±20
EtOAc fraction	50.19±0.24	76.22±1.61	249.86±3.74
n-BuOH fraction	72.19±0.52	57.57±0.76	233.45±1.57
Residue fraction	213.68±1.17	21.02±0.67	106.14±1.9
Ascorbic acid	13.58±0.34	--	--
Vitamin E	23.12±0.21	--	--
BHT	17.74±0.076	--	--

3.5 Relationship between Phenolic Content and Antioxidant Activity

The total phenolic content and antioxidant properties of *Boswellia serrata* extracts and different fractions derived from the MeOH (85%) extract demonstrated a favourable relationship, with relation coefficients (r2) = 0.76, 0.81, and 0.96 for DPPH, TAC, and ABTS, correspondingly. As a result, in this study, there is a linear and significant relationship between antioxidant potential and total phenolic content. These findings are similar with previous study on other plant extracts [27-33], which found that *Boswellia serrata* extracts might be effective sources of natural antioxidants.

4. CONCLUSION

The MeOH (85 percent) extract of *Boswellia serrata* leaves exhibited the highest total phenolic content and antioxidant activity, according to the findings of this study. Furthermore, EtOAc and n-BuOH fractions derived from MeOH extract (85%) displayed significant total phenolic content and antioxidant capacity. The antioxidant and total phenolics have a significant positive relationship. Because EtOAc and n-BuOH fractions contain a significant amount of total phenolics and have a strong antioxidant capacity, advanced chromatographic and spectroscopic methods are advised for further separation and determination of their chemical components.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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