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Phytochemical screening, UPLC analysis, evaluation of synergistic antioxidant and antibacterial efficacy of three medicinal plants used in Kinshasa, D.R. Congo

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Plant extracts are used worldwide due to their biologically active compounds, which support food preservation and help combat various diseases through their antimicrobial and antioxidant properties. In the capital city of the Democratic Republic of the Congo, an ethnobotanical survey revealed the use of *Dysphania ambrosioides* (L.) *Mosyakin & Clemants*, *Ocimum gratissimum* L. and *Tetradenia riparia* (Hochst.) Codd, often in combination, for treating oral microbial diseases. While these plants have been widely studied individually; their combined potential has not been investigated. The present research aims to explore the phytochemical composition, the synergistic antimicrobial, and antioxidant potential of different extracts from these three mentioned plants. Phytochemical composition of the decocted and percolated extracts from the three plants was determined using qualitative analysis and the ultra-performance liquid chromatography quadrupole time of flight tandem mass spectrometry (UPLC-QTOF-MS). Antimicrobial activity was assessed using the broth dilution method, while antioxidant activity was evaluated using the DPPH method. For the antimicrobial studies, the decocted and percolated extracts were tested against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Qualitative phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, iridoids, and anthraquinones in all the plant extracts. The methanolic extract of *T. riparia* exhibited the highest phenolic content (299.146 ± 0.143 mg GAE/g extract), while *O. gratissimum* had the highest flavonoid content (138.256 ± 0.277 mg QE/g extract). UPLC analysis identified several metabolites in the plant extracts including rosmarinic acid, cirsimaritin, xanthomicrol and kaempferol derivatives. Rosmarinic acid was consistently identified across all the plant extract combinations, while other flavonoids such as apigenin 7-glycosides, kaempferitin and luteolin 7-O-glucoside, were detected in specific plant extract combinations. The decocted plant extracts exhibited higher antioxidant activity than the percolated extracts, with *O. gratissimum* showing the highest antioxidant activity (11.744 ± 0.584 µg/mL), followed by *T. riparia* (12.916 ± 0.972 µg/mL). The extract combinations from *O. gratissimum* and *T. riparia* demonstrated synergistic antioxidant activity (CI = 0.57). Amongst all extracts, the highest antibacterial activity was

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observed in the decocted extracts of *O. gratissimum* and *T. riparia* against *S. aureus* (MIC = 500 µg/mL), with their combination showing additive antibacterial activity (FICI = 1). The aim of this study was primarily to evaluate the combinatory potential of these three plants as reliable sources of antimicrobials and antioxidants for the treatment of various microbial diseases in the future. The study provides evidence of the synergistic antioxidant and antibacterial potential of *O. gratissimum* and *T. riparia*. These results suggest that these plant extracts are promising sources of natural antimicrobial and antioxidant agents, with potential application in the pharmaceutical and food industries for combating several infectious diseases.

Keywords Bio-active compounds, Antioxidant activity, Antibacterial activity, *Dysphania ambrosioides*, *Ocimum gratissimum*, *Tetradenia riparia*

Despite substantial advancements in modern medicine, microbial diseases persist as significant global threats[1]. Among these diseases, acute respiratory infections are one of the leading causes of medical consultations and main cause of mortality for children. The prevalence and severity of acute respiratory infections are rising steadily, constituting a major public health problem. They are the sixth leading cause of all-age mortality, and the leading cause of death for children under five[2]. In addition, microbial resistance to synthetic drugs is increasing, while the development of new antimicrobial treatments is slowing down. This shift had led to a greater focus on finding new, effective, and affordable drugs to treat infections, particularly in developing countries where infectious diseases contribute to 50% of the mortality rate[3–5].

Historically, various antimicrobial compounds derived from both natural and synthetic sources have been identified for treating infections. However, only a limited number of these agents are globally affordable. The increase in multi-drug resistance bacteria has exacerbated the challenges related to both the affordability and accessibility of these treatments. As a result, the rates of morbidity, mortality, and healthcare costs have escalated. Therefore, there is a growing focus in modern medicine on developing antibiotics from non-synthetic sources to address the socio-economic and health challenges posed by the multi-drug resistant microbes[6,7].

In response, the World Health Organization emphasizes the importance of herbal medicines as a primary healthcare modality in numerous low-income countries. Globally, approximately 80% of the world population relies on medicinal plants for disease treatment, with a significantly higher prevalence in African nations[8]. The Democratic Republic of the Congo (DRC), known for its vast equatorial forests, rich biodiversity and profound traditional knowledges of medicinal plants, incorporates these resources extensively into its healthcare system. A large proportion of the Congolese population uses traditional medicine, placing medicinal plants at the core of these practices and emphasizing their role in the treatment of various infectious and non-communicable diseases. Indeed, plants are vital resources for drug discovery due to their extensive use in traditional medicine[9]. Natural compounds from plants have historically been used as medicine, and numerous studies demonstrate their biological activities against various infectious agents. The Food and Agriculture Organization report indicates that at least 25% of pharmaceutical drugs in modern pharmacopoeia are plant-derived, with many of these being synthetic analogues derived from plant prototype compounds[10]. Fundamental methods for assessing bioactive properties and identifying compounds from plant extracts include phytochemical screening and antibacterial activity testing[11–13].

Free radicals play a key role in the development of various diseases. Reactive oxygen species (ROS), generated by redox enzymes in the body, are continuously produced as they react with foreign chemicals in a suitable environment.

Under normal circumstances, antioxidants neutralize reactive oxygen species, maintaining a balance between their production and the available antioxidants. However, when ROS production exceeds the body's antioxidant capacity, oxidative stress occurs. This can lead to various health issues such as diabetes, inflammation, abnormal cell division, ulcers and accelerated aging[14].

Antioxidants play a key role in scavenging free radicals, preventing oxidation in easily oxidizable substrates, and thus inhibiting the formation of new free radicals[15]. These compounds are commonly abundant in plants in the form of polyphenols, ascorbate, terpenoids, and tocopherols[16–19]. They help protect the body against the harmful effects of free radicals, that contribute to oxidative stress. Although the human body has its own effective defensive mechanisms, these can weaken with age, making it essential to consume foods rich in antioxidants. Due to the presence of free radicals, biomolecules such as amino acids, fatty acids and deoxyribonucleic acids can suffer significant damage[20].

In our previous ethnobotanical survey conducted on the use and preparation of the medicinal plants, several plants were reported as frequently used for treating oral microbial diseases in the capital city of DRC[21]. Among them, *Dysphania ambrosioides* (L.) *Mosyakin & Clemants*, *Ocimum gratissimum* L. and *Tetradenia riparia* (Hochst.) Codd, were the most commonly cited (Table 1), often used in combination for various indications. Previous research has explored the phytochemical properties and antibacterial activity of oils from these three plants[22–29]. *O. gratissimum*, for example, is widely available and commonly used in African communities for the treatment of a range ailments, including diarrhea, ear infections, dermatoses, ophthalmias, headaches, fevers, cold, cough, skin problems, pneumonitis, hematuria, purulent urethritis, dystopias, pelvic aches, candidoses, digestive dysmenorrhea, emesis and hemorrhoids (pile)[30–32]. Several studies have highlighted its antimicrobial, anesthetic, anti-stress, anti-inflammatory, anthelminthic, antidiarrheal, antipyretic, anti-mutagenic, anti-

Scientific name	Family name	Local names	Parts used	Locally used in the study area to treat	Frequencies of citations
O. gratissimum	Lamiaceae	Dinsusu nsusu, Mazulu	Leaves, roots	Pneumonia, cough, diarrhea, skin pathology, fever, headaches, conjunctivitis, dysentery, rheumatism, menorrhagia	103
T. riparia	Lamiaceae	Mutuzo, Mutubya	Leaves, roots	Headaches, cough, stomachache, diarrhea, fever, dengue, respiratory problems	53
D. ambrosioides	Amaranthaceae	Lukunga-nioka, Lukunga bakishi, Kepamekusu, Diniioka nioka, Nkasi	Leaves	Worms (hookworms, roundworms, threadworms, ringworms), lice, stomachache, fever, cough, asthma, headaches, dysmenorrhea, eczema, itches	67

Table 1. Ethnobotanical data on medicinal plants selected for phytochemical analysis and antibacterial activity.

Phytochemical compounds	*O. gratissimum*	*T. riparia*	*D. ambrosioides*
Alkaloids	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Iridoids	+	+	+
Anthraquinones	+	+	+
Coumarins	+	–	–
Terpenes	+	+	–

Table 2. Phytochemical screening of methanolic extracts of *O. gratissimum*, *T. riparia*, and *D. ambrosioides*. + indicates presence, – indicates absence.

ulcerative, antiseptic, gastro-protective, hepatoprotective, sedative and fungicidal properties[33–37]. The plant extract has also been used for treating gastrointestinal helminths in both animals and humans[38].

D. ambrosioides has been used for thousands of years in traditional medicine to treat various conditions[39]. Infused and decocted extracts of *D. ambrosioides* are commonly used for treating gastrointestinal disorders, typhoid, dysentery, galactogen, oral abscesses, ulcers, purulent wounds, and diabetes[40–42]. Studies have shown that *D. ambrosioides* exhibits inhibitory activity against a wide range of pathogenic bacteria, including *Bacillus cereus*, *Micrococcus luteus*, *Helicobacter pylori*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*[42–44]. Additionally, its essential oils have demonstrated antiviral activity against the Coxsackie B4 virus (CVB4)[45], antioxidant properties[46], anticancer effects[47], and anti-leishmanial activity against amastigotes and promastigotes of *Leishmania amazonensis*[48].

T. riparia is used for the treatment of various health problems, including pains, stomachaches, coughs, headaches, toothaches, ulcers, dyspepsia, rheumatism, malaria, bilharzia, skin diseases, wounds, several kind of fevers, back problems, throat infections, chest infections, snake bites and muscle-skeletal pain[49–52]. Extracts and essential oils of *T. riparia* have shown antibacterial and antifungal activities[52–54], bioacaricidal activity[55,56], antiviral activity[57], larvicidal, antimalarial, insecticidal, antiproliferative and antioxidant activities[49,58].

Despite all the investigations done on *D. ambrosioides*, *O. gratissimum* and *T. riparia*, there is no research available on the bioactivity of their combinations. Therefore, the current study aims to evaluate the phytochemical composition, antioxidant, and antibacterial activities of these three plants, both individually and in combination.

Results and discussion

Qualitative phytochemical screening

Phytochemical screening is used to evaluate the constituents of plant extracts, and their predominance. This preliminary step is crucial in the search for bioactive compounds and plays a key role in the development of therapeutic drugs[59]. In this study, a qualitative phytochemical analysis of methanolic extracts from *D. ambrosioides*, *O. gratissimum* and *T. riparia* was conducted, as shown in Table 2. Alkaloids, saponins, flavonoids, iridoids and anthraquinones were detected in all the three plant extracts. Coumarins were found only in in *O. gratissimum* while terpenes were present in both *O. gratissimum* and *T. riparia*. Our results are consistent with those reported by Ahmed et al. (2019) and Olamilosoye et al. (2018)[60,61]. The therapeutic potential of these plants is likely attributed to the presence of these phytochemicals.

Flavonoids are known for their antioxidant activities, inhibiting the initiation, promotion and progression of tumors[62]. Alkaloids obtained from the beta-carboline group are recognized for their strong antimicrobial, anti-HIV and antiparasitic properties[63]. Terpenoids, on the other hand, exhibit a wide range of pharmacological activities, including anti-inflammatory, anticancer, antioxidant and antibacterial properties[62,64,65].

Total phenolic and flavonoid content

Phenolic and flavonoid contents are important criteria for evaluating plant extracts both quantitatively and in terms of their biological potency, as they play crucial roles in various physiological processes[62,63]. The total phenolic and flavonoid contents of the methanolic extracts from *D. ambrosioides*, *O. gratissimum*, and *T. riparia* are shown in Table 3. The results revealed that the methanolic extract from *T. riparia* had the highest

Extracts	Phenol content (mg GAE/g extract)	Flavonoid content (mg quercitin/g extract)
T. riparia	299.146 ± 0.143	112.843 ± 0.255
O. gratissimum	153.597 ± 5.277	138.256 ± 0.277
D. ambrosioides	155.602 ± 1.712	63.925 ± 0.494

Table 3. Total phenolics and flavonoid content of the methanolic extracts of *O. gratissimum*, *T. riparia*, and *D. ambrosioides*.

total phenolic content (299.146 ± 0.143 mg GAE/g extract), followed by *D. ambrosioides* (155.602 ± 1.712 mg GAE/g extract) and *O. gratissimum* (153.597 ± 5.277 mg GAE/g extract). In contrast, the methanolic extract from *O. gratissimum* had the highest flavonoid content (138.256 ± 0.277 mg QE/g extract), followed by *T. riparia* (112.843 ± 0.255 mg QE/ g extract) and *D. ambrosioides* (63.925 ± 0.494 mg QE/ g extract).

Our results are slightly higher than those reported by Panda et al. (2022) and Olamilosoye et al. (2018)[22,61]. The discrepancy could be attributed to several factors, including the plants' harvesting season, soil pH, drying conditions, geographical location, chemotype or subspecies, the specific plant part or genotype used, and the extraction method employed. Phenolics are known to possess antioxidant, antimutagenic, and anti-carcinogenic activities, as well as the ability to modulate gene expression. Flavonoids are active constituents that perform various biological activities, such as resisting microbial infections, ulcer, arthritis, angiogenic, and cancerous diseases along with inhibiting the formation of mitochondrial adhesion[60].

UPLC analysis

Ultra-performance liquid chromatography-quadrupole time of flight-mass spectrometry (UPLC-QTOF-MS) analysis is one of the most widely used techniques for detecting various metabolites present in plants. This technique was employed to tentatively identify the metabolites present in the decocted and percolated extracts of *O. gratissimum*, *T. riparia* and *D. ambrosioides*, as well as in their combination extracts : *O. gratissimum* + *T. riparia* (OT), *O. gratissimum* + *D. ambrosioides* (OD), *T. riparia* + *D. ambrosioides* (TD) and *O. gratissimum* + *T. riparia* + *D. ambrosioides* (OTD). The chromatograms for the percolated extracts are shown in Fig. 1, while those for the decocted extracts are provided in the supplementary data file (Fig. S1). The compounds tentatively identified in the percolated extracts are listed in Table 4, while those in the decocted extracts are shown in Table S1.

The major compounds identified in the percolated extract of *O. gratissimum* included rosmarinic acid, cirsimaritin and xanthomicrol (Figs. S2, S3). Rosmarinic acid was also detected in the percolated extract of *T. riparia* (Fig. S2), while several flavonoids such as apigenin 7-glycosides, kaempferitrin (a 3,7-dirhamnoside of kaempferol) and kaempferol 3,7-diglycosides, were identified in *D. ambrosioides* (Figs. S4, S7). Additionally, rosmaniric acid and xanthomicrol were detected in the extract combination of *O. gratissimum* + *T. riparia* (Figs. S2 and S3). Moreover, rosmarinic acid, kaempferitrin, vitexin and xanthomicrol were identified in the percolated extract combination of *O. gratissimum* + *D. ambrosioides* (Figs. S2, S3, S4 and S5). For the percolated extract combination of *T. riparia* + *D. ambrosioides*, luteolin 7-O-glucoside, rosmarinic acid, apigenin 7-glycosides and kaempferitin were putatively identified (Figs. S2, S4, S7 and S8). Finally, in the percolated extract combination containing all three plants, epigallocatechin 3-gallate, 3-galloylcatechin epicatechin 5-gallate, rosmarinic acid, xanthomicrol and apigenin 7-glycosides were identified (Figs. S2, S3, S7, S9 and S10).

Rosmarinic acid had previously been identified as one of major compounds in the methanolic, hydromethanolic and ethanolic extracts of *O. gratissimum*[66]. Similarly, cirsimaritin and xanthomicrol had also been detected in *O. gratissimum*[67]. Additionally, several quercetins and kaempferol derivatives have been identified in *D. ambrosioides*[68].

Antioxidant activity

The scavenging of free radicals through the DPPH is a widely accepted technique for assessing the antioxidant activity of plant extracts. This method is favored for its efficiency, requiring less time for analysis, making it a popular choice for evaluating the antioxidant potential of plant extracts. DPPH is considered as an effective antioxidant due to its high hydrogen-donating ability. The removal of free radicals is crucial in preventing their toxic effects, which contribute to various diseases, including cancer and bacterial diseases[59,69–71].

The results of the antioxidant activities of the percolated and decocted extracts of *D. ambrosioides*, *O. gratissimum* and *T. riparia* as well as of their combinations using the DPPH method are shown in Tables 5 and 6, respectively. The decoction-based extracts demonstrated higher antioxidant activity compared to the percolation-based extracts.

When considering the extracts obtained through decoction, the extract from *O. gratissimum* exhibited the highest antioxidant activity (11.744 ± 0.584 µg/mL), followed by that from *T. riparia* (12.916 ± 0.972 µg/mL). The extract from *D. ambrosioides* displayed the lowest antioxidant activity (203.492 ± 9.285 µg/mL). However, among the extracts obtained through percolation, the extract from *T. riparia* exhibited the highest antioxidant activity (20.157 ± 3.125 µg/mL), followed by that from *O. gratissimum* (22.747 ± 1.139 µg/mL). In contrast, the extract from *D. ambrosioides* displayed the lowest antioxidant activity (275.053 ± 13.20 µg/mL).

The antioxidant potential of the extracts from *O. gratissimum* and *T. riparia* correlates with the higher flavonoid content found in these extracts compared to those from *D. ambrosioides* (Table 3). Flavonoids possess excellent antioxidant properties and exhibit their antioxidant activity by scavenging free radicals and ROS, chelating metals, and preventing the oxidation of low-density lipoproteins[72,73]. Additionally, flavonoids such as

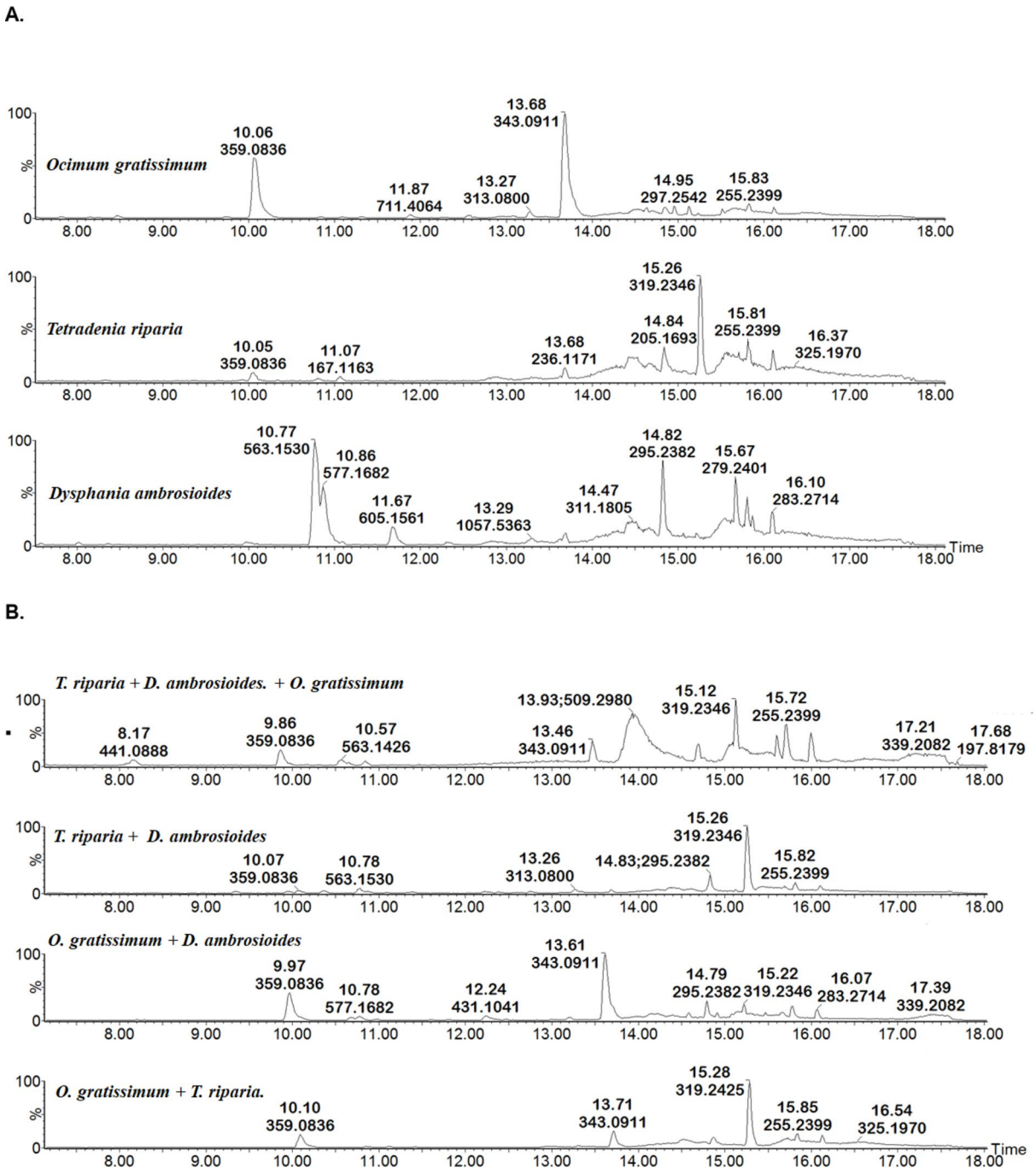

Fig. 1. ESI negative mode BPI chromatograms of percolated extracts of the tested plant species (**A**) and their combinations (**B**). The tentatively identified compounds are shown in Table 4.

rosmarinic acid, xanthomicrol and derivates of kaempferol found in these plant extracts have been reported to have higher antioxidant properties[72,74–76]. Another contributing factor could be the presence of terpenes, which were found in *T. riparia* and *O. gratissimum* extracts (Table 1), and not in *D. ambrosioides*. Terpenes are known for their effective antioxidant activity[64,65].

The antioxidant combination index (CI) revealed that the decocted extract of *O. gratissimum* + *T. riparia* combination exhibited synergistic effects (CI = 0.57), showing the highest antioxidant among all combinations tested. In contrast the combination of *O. gratissimum* + *D. ambrosioides* displayed additive effects (CI = 1.17). All other combinations resulted in antagonistic effects (as shown in Tables 5 and 6). These findings suggest that the proton-donating ability of the decocted extract of *O. gratissimum* + *T. riparia* combination was greater than that of the individual extracts.

This observed synergy may be attributed to various mechanisms, such as the formation of stable intermolecular complexes between the antioxidants, which can enhance their collective antioxidant activity beyond that of

Medicinal plants	RT min	Acquired $[M-H]^-$ m/z	Theoretical $[M-H]^-$ m/z	Molecular formula	Tentative identification
O. gratissimum	10.06	359.0836	359.0767	$C_{18}H_{16}O_8$	Rosmarinic acid
	13.27	313.0800	313.0712	$C_{17}H_{14}O_6$	Cirsimaritin
	13.68	343.0911	343.0818	$C_{18}H_{16}O_7$	Xanthomicrol
T. riparia	10.05	359.0836	359.0767	$C_{18}H_{16}O_8$	Rosmarinic acid
D. ambrosioides	10.77	563.1530	563.1401	$C_{26}H_{28}O_{14}$	Apigenin 7-glycosides
	10.86	577.1682	577.1557	$C_{27}H_{30}O_{14}$	Kaempferitrin
	11.67	605.1561	605.1506	$C_{28}H_{30}O_{15}$	Kaempferol 3,7-diglycosides
O. gratissimum + *T. riparia*	10.10	359.0836	359.0767	$C_{18}H_{16}O_8$	Rosmarinic acid
	13.71	343.0911	343.0818	$C_{18}H_{16}O_7$	Xanthomicrol
O. gratissimum + *D. ambrosioides*	9.97	359.0836	359.0767	$C_{18}H_{16}O_8$	Rosmarinic acid
	10.76	577.1578	577.1557	$C_{27}H_{30}O_{14}$	Kaempferitrin
	12.25	431.1033	431.0978	$C_{21}H_{20}O_{10}$	Vitexin
	13.61	343.0880	343.0818	$C_{18}H_{16}O_7$	Xanthomicrol
T. riparia + *D. ambrosioides*	9.94	447.0977	447.0927	$C_{21}H_{20}O_{11}$	Luteolin 7-O-glucoside
	10.07	359.0836	359.0767	$C_{18}H_{16}O_8$	Rosmarinic acid
	10.78	563.1015	563.1401	$C_{26}H_{28}O_{14}$	Apigenin 7-glycosides
	10.86	577.1578	577.1557	$C_{27}H_{30}O_{14}$	Kaempferitrin
	15.26	661.4565	661.4468	$C_{42}H_{62}0_6$	Ziziphorin B
T. riparia + *D. ambrosioides* + *O. gratissimum*	7.07	457.0755	457.0771	$C_{22}H_{18}O_{11}$	Epigallocatechin 3-gallate
	8.17	441.0869	441.0880	$C_{22}H_{18}O_{10}$	3-Galloylcatechin Epicatechin 5-gallate
	9.86	359.0823	359.0767	$C_{18}H_{16}O_8$	Rosmarinic acid
	10.57	563.1446	563.1401	$C_{26}H_{28}O_{14}$	Apigenin 7-glycosides
	13.46	343.0870	343.0818	$C_{18}H_{16}O_7$	Xanthomicrol

Table 4. Tentative identification of the major compounds in the percolated plant extracts.

Decocted extracts	IC50 (µg/mL)	CI_1	CI_2	CI_3	CI	Interpretation
O. gratissimum	11.744 ± 0.584	–	–	–	–	–
D. ambrosioides	203.492 ± 9.285	–	–	–	–	–
T. riparia	12.916 ± 0.972	–	–	–	–	–
O. gratissimum + *D. ambrosioides*	13.050 ± 0.239	1.11	0.06	–	1.17	Additive
O. gratissimum + *T. riparia*	3.529 ± 0.362	0.30	–	0.27	0.57	Synergistic
D. ambrosioides + *T. riparia*	45.450 ± 3.013	–	0.22	3.51	3.73	Antagonistic
O. gratissimum + *T. riparia* + *D. ambrosioides*	11.953 ± 0.183	1.02	0.92	0.06	1.99	Antagonistic
Quercetin	3.21 ± 1.0					

Table 5. Antioxidant combination effects of decocted extracts of *O. gratissimum*, *T. riparia* and *D. ambrosioides* using the DPPH method.

the individual compounds. Additionally, unpredictable interactions between the examined compounds may contribute to the synergestic effect[77].

On the other hand, the additive effect observed with the combination of *O. gratissimum* + *D. ambrosioides* might indicate that the antioxidants acted independently without interfering with each other's activity during oxidation. This suggests that the antioxidants in this combination do not interact, allowing each other to function in isolation[78].

Several factors could explain the antagonism observed in all the other combinations. For instance, antioxidant polymerization, the formation of complex and adduct between antioxidants, may contribute to the decrease in the overall antioxidant properties[77]. Further research is needed to fully understand the precise mechanisms of action underlying the observed synergistic, additive, and antagonistic effects in this study.

Antibacterial activity

The global rise of multi-drug resistant microorganisms has prompted researchers to explore new natural compounds to combat microbial diseases[79]. Plants have long been recognized as a rich source of bioactive compounds with significant medicinal value[69,70]. This study investigated the antimicrobial potential of percolated and decocted extracts from *O. gratissimum*, *T. riparia* and *D. ambrosioides* against gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

Percolated extracts	IC50 (µg/mL)	CI_1	CI_2	CI_3	CI	Interpretation
O. gratissimum	22.747 ± 1.139	–	–	–	–	–
D. ambrosioides	275.053 ± 13.20	–	–	–	–	–
T. riparia	20.157 ± 3.125	–	–	–	–	–
O. gratissimum + *D. ambrosioides*	48.134 ± 0.858	2.12	0.17	–	2.29	Antagonistic
O. gratissimum + *T. riparia*	49.068 ± 31.011	2.16	–	2.43	4.59	Antagonistic
D. ambrosioides + *T. riparia*	197.560 ± 2.079	–	9.80	0.72	10.52	Antagonistic
O. gratissimum + *T. riparia* + *D. ambrosioides*	43.287 ± 0.316	1.90	2.15	0.16	4.21	Antagonistic
Quercétine	3.21 ± 1.0					

Table 6. Antioxidant combination effects of percolated extracts of *O. gratissimum*, *T. riparia* and *D. ambrosioides* using the DPPH method. The CI index (CI) = $CI_1 + CI_2$, or ($CI_2 + CI_3$) or ($CI_1 + CI_3$) or ($CI_1 + CI_2 + CI_3$) where $CI_1 = (D)_1/(D_X)_1$ and $CI_2 = (D)_2/(D_X)_2$, $CI_3 = (D)_3/(D_X)_3$; $(D)_1$, $(D)_2$ and $(D)_3$ are the doses (IC_{50} values) of the plant extracts in commination; $(D_X)_1$, $(D_X)_2$ and $(D_X)_3$ are the doses (IC_{50} values) of the plant extracts taken individually. Results were interpreted as synergistic interaction (CI < 1), additive interaction (CI = 1), or antagonistic interaction (CI > 1).

	MIC (µg/mL)		
	Bacterial strains		
Percolated extracts	Staphylococcus aureus	Escherichia coli	Pseudomonas aerogenes
T. riparia	500	2000	2000
O. gratissimum	500	2000	2000
D. ambrosioides	1000	2000	2000
Ciproflaxine	0.125	0.03	0.25

Table 7. Minimum inhibitory concentration (MIC) values of selected medicinal plants and control antibiotic against test bacterial strains.

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of an antimicrobial agent required to inhibit visible microbial growth after an overnight incubation. MIC is commonly used to assess the antimicrobial activity of new compounds or extracts, with lower MIC values indicating higher potency[63,70].

Our results demonstrated that the percolated extracts exhibited significant antimicrobial activity (Table 7) compared to the decocted extracts (Table S2). The decocted extracts were less effective against all tested microorganisms, despite the tentative identification of several potential flavonoids and polyphenols through UPLC (Table S1). Among the percolated extracts, *T. riparia* and *O. gratissimum* showed the lowest MIC (500 µg/mL) against *Staphylococcus aureus*, while *D. ambrosioides* followed at 1000 µg/mL. However, these extracts showed lower potency (2000 µg/mL) against gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). This reduced effectiveness against the gram-negative bacteria may be attributed to their extra outer membrane, which acts as a permeability barrier, limiting the entry of various substances, including antimicrobial compounds[80].

The antibacterial activity of our plant extracts may be linked to the phenolic high content and the flavonoids identified. Phenols, such as rosmarinic acid, are known to disrupt the cytoplasmic membrane function and the metabolism of energy, thus affecting the synthesis of nucleic acids. These compounds exhibit a broad spectrum of activities, including anticancer, antidiabetic, antiaging, antidepressant and antimicrobial activities[71,77]. Flavonoids such as cirsimaritin, xanthomicrol, kaemferitin, kaempferol 3,7-diglycosides, etc. have demonstrated inhibitory activity bacterial enzymes, including DNA polymerase, RNA polymerase, reverse transcriptase, telomerase, alongside antitumoral activities[78,79,81]. These findings suggest that the identified bioactive compounds contribute to the antimicrobial properties of the plant extracts tested in this study.

The combined use of plant extracts often leads to varying interactions due to the diverse range of compounds present in each extract. Previous studies have highlighted the enhanced antibacterial activity observed in certain plant combinations[82–84]. However, some interactions may reduce the effectiveness of the plant extract combinations, either by neutralizing each other, forming inactive complexes or by competing for the same molecular targets[85–87]. It is therefore crucial to confirm the effects of the plant extract combinations, especially as reported by local communities.

In our study, checkboard synergy assays was performed to evaluate the synergistic effects of the three selected medicinal plants commonly used in combinations in the capital city of DRC. The fractional inhibitory concentrations (FICs) and their interpretations are presented in Table 8. No strong synergistic interactions were observed. However, the percolated extracts of *O. gratissimum* (OG) and *T. riparia* (TR) showed additive effects (FICI = 1) against *S. aureus*; with their MIC decreased by 2-fold respectively.

This could be attributed to the presence of flavonoids, such as xanthomicrol and rosmaniric acid, identified in the UPLC chromatograms of *O. gratissimum* + *T. riparia* combination (Fig. 1, Figs. S2 and S3).

Bacterial strains	Medicinal plants			FIC values			FIC index	Interpretation
	A	B	C	FIC_A	FIC_B	FIC_C		
Staphylococcus aureus	OG	TR		**0.5**	**0.5**		**1**	Additive
	OG		DA	2		1	3	Indifferent
		TR	DA		2	1	3	Indifferent
	OG	TR	DA	1	1	0.5	2.5	Indifferent
Escherichia coli	OG	TR		1	1		2	Indifferent
	OG		DA	1		1	2	Indifferent
		TR	DA		1	1	2	Indifferent
	OG	TR	DA	1	1	1	3	Indifferent
Pseudomonas aeruginosa	OG	TR		1	1		2	Indifferent
	OG		DA	1		1	2	Indifferent
		TR	DA		1	1	2	Indifferent
	OG	TR	DA	1	1	1	3	Indifferent

Table 8. Fractional inhibitory concentration (FIC) values of percolated extracts from selected medicinal plants in combination against test bacterial strains. OG: *O. gratissimum*, TR: *T. riparia*, DA: *D. ambrosioides*. The FIC index (FICI) = $FIC_A + FIC_B$, or ($FIC_B + FIC_C$) or ($FIC_A + FIC_C$) or ($FIC_A+FIC_B+FIC_C$) where FIC_A was MIC of extract A in combination/MIC of extract A alone, FIC_B was MIC of extract B in combination/MIC of extract B alone and FIC_C was MIC of extract C in combination/MIC of extract C alone. FICI are interpreted as synergistic ($FICI \leq 0.5$), partial synergy ($0.5 < FICI \leq 0.75$), additive ($0.75 < FICI \leq 1.0$), indifferent ($1.0 < FICI \leq 4.0$), or antagonistic ($FICI > 4.0$).

All others combinations exhibited indifferent effects ($1.0 < FICI \leq 4.0$), suggesting that the combined effect was comparable to the activity of the most potent individual extract. Notably, no antagonistic effects were found in any of the plant extract combinations. Our results clearly indicate that the potent activity of one extract might not necessarily lead to a high synergy with another extract, but can potentially improve the overall antibacterial effect.

The antibacterial mechanisms of plant extract combinations are not fully understood; and further investigation is needed to explore the mode of action and underlying pathways of OG + TR combination.

Conclusion

The search for natural compounds with antimicrobial and antioxidant potential is growing daily due to the increasing prevalence of infectious diseases and the rise of antimicrobial resistance. This study highlights the promising therapeutic potential of *D. ambrosioides*, *O. gratissimum* and *T. riparia* and their combinations, owing to their rich phytochemical profiles, which include alkaloids, flavonoids, saponins and terpenes. The antioxidant activity of these plants, particularly *O. gratissimum* and *T. riparia* was shown to be significant, with decocted extracts demonstrating higher antioxidant activity compared to percolated extracts.

The combination of decocted extracts from *O. gratissimum* and *T. riparia* displayed synergistic antioxidant activity, while the combination of *O. gratissimum* and *D. ambrosioides* showed additive effects. Additionally, antibacterial activity was stronger in the percolated extracts, with *O. gratissimum* and *T. riparia*, demonstrating the highest potency against *Staphylococcus aureus* and their combination exhibiting additive antibacterial effects. The identified bioactive compounds, such as rosmarinic acid, xanthomicrol and various flavonoids, are likely responsible for these effects.

The results from this study suggest that these plants, individually or in combination, hold significant therapeutic potential for treating oxidative stress-related diseases and microbial infections, warranting further research into their mechanisms and possible applications in drug development.

Materials and methods

Collection and Preparation of plant material

In a recent ethnobotanical survey, we identified *D. ambrosioides*, *O. gratissimum* and *T. riparia* as key medicinal plants used in multi-species recipes for treating oral microbial infections in the district of Mont-Ngafula, Kinshasa, DRC[21]. Based on this foundational research, fresh leaves of *O. gratissimum* and *T. riparia* as well as the stems and leaves of *D. ambrosioides*, were collected between January and February 2023 in the Ngansele district of the Mont-Ngafula, Kinshasa, for further analysis. The geographical coordinates are as follows: for *D. ambrosioides* 4°26′20″S 15°17′14″E; for *T. riparia* 4°26′20″S 15°17′13″E; and for *O. gratissimum* 4°26′20″S 15°17′13″E. The plant collection adhered to all ethical guidelines, and permission was obtained from the Faculty of Sciences and Technologies at the University of Kinshasa. Voucher specimens N° 001/23; N° 002/23, and N° 003/23 were deposited at the Herbarium of Kinshasa (IUK), which is part of the National Institute for Agronomic Research (INERA-RDC).

The collected plant material was air-dried at room temperature, then milled into powder using an electric grinder (Blinder Butterfly B-592). The powder was accurately weighed using an electronic weighing balance, then packed into polyethylene bags to prevent air entry and contamination. The bags were then stored in a

securely closed container, appropriately labeled, to facilitate further extraction processes. For combinations of different specimens, a ratio of 1:1 (m/m) was used for the mixture containing *O. gratissimum* and *T. riparia* (OT); *O. gratissimum* and *D. ambrosioides* (OD), as well as *T. riparia and D. ambrosioides* (TD). The mixture containing all the three plant materials (OTD) was prepared using the ratio 1:1:1 (m/m/m).

Preparation of extracts

The powder obtained from each dried plant material and their combination was extracted by decoction and percolation methods, as described by Milan[88] with slight modifications. For decoction, 10 g of powder was mixed with 100 mL of water. After heating the mixture at 100 °C for 5 min, it was then cooled to room temperature and filtered through Whatman paper. This method is used to extract water soluble and heat stable constituents from crude plant materials, as practiced by local communities in the capital city of DRC[21,88,89]. For percolation, 10 g of powder was allowed to stand in a mixture of methanol and ethyl acetate (50:50, v/v) for 48 h and then allowed to drip slowly through the percolator to maximize the extraction of both polar and apolar compounds[90]. The decocted solutions and percolates obtained were then evaporated, and the dried extracts thus obtained were stored in sterile vials and stored in a refrigerator (4 °C) for subsequent analysis. The extraction yield (*R*) was calculated as the percentage of the plant dry matter (DM) according to the formula:

$$\mathrm{R}\ (\%) = (\mathrm{Mex}/\mathrm{Mmv}) \times 100$$

where Mex is the dry residue following the percolation or decoction extraction and Mmv is the mass of the starting dry plant matter[25].

Microorganisms

The antibacterial activity of plant extracts was evaluated against selected standard bacterial strains of the American Type Culture Collection (ATCC). The bacterial strains chosen represent both Gram-positive and Gram-negative bacteria. The tested gram-positive bacterial strain was *Staphylococcus aureus* (ATCC 25923), and the tested gram-negative bacterial strain were *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). The microorganisms were maintained in cryotubes on Triptosoya + 20% glycerol broth at − 80 °C in the Microbial Unit of the Faculty of Pharmaceutical Sciences at the University of Kinshasa. The selected bacterial strains were cultivated using Mueller-Hinton broth[91].

Phytochemical screening

Phytochemical screening was conducted on the extracts using the methods outlined by Kilonzo and Munisi (2021), Shaikh and Patil (2020), María et al. (2018), and Wagner et al. (2018)[92–95] to determine the presence of secondary metabolites including phenolics, flavonoids, coumarins, anthraquinones, terpenoid, saponins and alkaloids. These metabolites were selected based on their known antibacterial potency and the availability of reagents in our laboratory. The results were denoted as (+) for the presence and (−) for the absence of phytochemicals. The total polyphenol content was determined using the Folin-Ciocalteu method[95,96], with gallic acid as the standard for the calibration curve. Flavonoids in the extracts were quantified and determined using the aluminum trichloride colorimetric method as described in Bahorun et al. (2016)[97]. Aluminum trichloride ($AlCl_3$) forms a yellow complex with flavonoids that absorbs light at 415 nm[98].

Ultra-performance liquid chromatography-quadrupole time of flight-mass spectrometry (UPLC-QTOF-MS) analysis

The dry extracts (1 mg) of each plant and of their combinations were dissolved in 1 mL of methanol: water (1:1) and then filtered through a 0.22 µm nylon syringe filter (13 mm diameter). A Waters acquity UPLC System equipped with a binary solvent delivery system and an autosampler was used for analysis. The chromatographic separation was achieved on a Waters BEH C_{18} (2.1 mm × 100 mm, 1.7 µm) column with a gradient elution of solvents A (water + 0.1% formic acid) and B (methanol + 0.1% formic acid), applied as follow: 0 min 3% B, 0.10 min 3% B, 14 min 100% B, 16 min 100% B, 16.5 min 3% B, 20 min 3% B. The flow rate was set at 0.3 ml/min. The injection volume was 5 µl. The separated compounds were analyzed by a Waters Synapt G2 high definition QTOF mass spectrometer in electrospray ionization negative mode. The following MS source parameters were used for negative mode: source temperature 120 °C, sampling cone 20 V, extraction cone 4.0 V, desolvation temperature 300 °C, cone gas flow 10.0 L/h, desolvation gas flow 600 L/h, capillary 2.6 kV.

A constant infusion rate of 3 µl/min through a separate orthogonal ESI probe was used to compensate for experimental drift in mass accuracy. The trap collision energy was set to 28 V. Compounds were tentatively identified by generating their respective molecular formula from MassLynx V 4 and comparing their fragmentation patterns with those of matching compounds in various search databases, including Dictionary of Natural Products and MetFrag.

Antioxidant activity

The free radical scavenging ability of our extracts and their combination was tested by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay as described by Bahorun et al. (1996)[97].

Determination of antioxidant combination index (CI)

To investigate the possible synergistic antioxidant activity between the different plants used, an isobologram analysis based on the median effect principle (IC_{50}) was performed. The classical isobologram-combination index equation (CI) was used for analyzing the data[99]. The formula is as follows:

$$CI = CI_1 + CI_2; \quad \text{with } CI_1 = (D)_1/(D_X)_1 \quad \text{and} \quad CI_2 = (D)_2/(D_X)_2;$$

where $(D)_1$ and $(D)_2$ are the doses (IC_{50} values) of two plants in combination; $(D_X)_1$ and $(D_X)_2$ are the doses (IC_{50} values) of two plants taken individually.

Results were interpreted as synergistic interaction (CI < 1), additive interaction (CI = 1), or antagonistic interaction (CI > 1).

Antibacterial assay

The antibacterial activity test to determine the minimum inhibitory concentration (MIC) of organic and aqueous extracts was carried out using the broth dilution method on 96-well polyester microplates. The MIC of plant extracts was determined according to the protocol established by Golus et al. (2016)[100].

To prepare stock solutions of plant extracts, 20 mg of each extract was dissolved in 250 µL of 5% DMSO, then 9.75 mL Mueller Hinton broth was added, resulting in a concentration of 2 mg/mL for each solution. Triplicate wells were prepared in 96-well microplates for each bacterial strain, with 900 µL of Muller Hinton broth (MHB) added to each well. Next, 100 µL of extract was added to the first row, and serial dilutions were made using multichannel micropipettes from column 1 to column 9. The dilution series ranged from 2000 µg/mL to 7.8125 µg/mL. Column 10 was left empty, while 200 µL of MHB was placed in column 11 as a growth control, and 200 µL of 2 mg/mL extract was placed in the last column as a sterility control for the culture medium.

Reference strains were stored in cryotubes in a freezer at − 20 °C. To obtain the active culture, the stored strains were sub-cultured overnight at 37 °C on MacConkey for *Escherichia coli* ATCC 25,922, Mannitol Salt Agar (MSA) for *Staphylococcus aureus* ATCC 25,922. and Ketrimide agar for *Pseudomonas aeruginosa* ATCC 9027. A few bacterial colonies were transferred from subculture tubes to tubes containing sterile Mueller Hinton agar using a platinum loop. After incubation for 24 h, bacterial colonies were diluted to 10^8 CFU/mL, which correspond to an optical density (OD) of 0.5 MacFarland. The bacterial suspension was adjusted to approximately 1.0×10^6 CFU/mL and was added to the microdilution plate. The plate was then incubated at 37 °C for 18–24 h. After incubation, 2 µL of resazurin (1%) was added to each well as an indicator of microbial growth, and the place was incubated at 37 °C for an additional 4 h. The MIC was recorded as the lowest concentration of each extract that showed no visible pink color, indicating the absence of microbial growth. Each measurement was done in triplicate. For each strain, one sterility control well and one growth control well were included. To assess bacterial sensitivity, parallel experiments were carried out using ciprofloxacin as a positive control, starting at a concentration of 64 µg/mL in sterile water (range 64 µg/mL to 0.0009765625 µg/mL).

Synergistic effect: fractional inhibitory concentration (FIC)

Synergistic effects of plant extract combinations were evaluated using a checkerboard synergy assay to obtain fractional inhibitory concentration (FIC) values as published by Cicco et al. (2009)[101]. One extract was serially diluted along the abscissa, while another extract was serially diluted along the ordinate. The total volume of the combination was 100 µL per well. Then 100 µL of each bacterial suspension (1×10^6 CFU/mL) was added to each well of a 96-well microplate. The microplate was sealed and incubated at 37 °C for 18 h. Then, a second incubation was conducted for 4 h at 37 °C after adding 2 µL of resazurin to each well. Experiments were conducted in triplicates. The FIC index (FICI) was calculated using the following equation:

$$FICI = FIC_A + FIC_B$$

where FIC_A is the MIC of extract A in combination divided by the MIC of extract A alone and FIC_B is MIC of extract B in combination divided by the MIC of extract B alone.

Results were interpreted as synergistic interaction (FICI ≤ 0.5), partial synergy (0.5 < FICI ≤ 0.75), additive interaction (0.75 < FICI ≤ 1.0), indifferent (1.0 < FICI ≤ 4.0), or antagonistic interaction (FICI > 4.0).

Statistical analysis

All tests were carried out in triplicates, and data were presented as mean ± standard deviation. The statistical significance of differences between experimental parameters was established by analysis of variance (ANOVA) using the *t*-test, with P values < 0.05 considered statistically significant. GraphPad Prism 9.5.1.733 was used for all statistical analysis.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

LMN and TBT were responsible for conceptualisation, data curation and funding acquisition for the project. LMN, FMF, SMM and TBT were involved in investigation, visualisation and methodology. TBT, OKN and PMK supervised the work. TBT coordinated the project, wrote the first draft of the paper. TBT, LMN and SMM were involved in subsequent editing and review of the paper.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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