Antiplasmodial Properties of Medicinal Plants From East Singhbhum District of Jharkhand in India

ABSTRACT

It has been reported that malaria is one of the most important vector-borne diseases in Jharkhand with significant morbidity and mortality. The National Vector Borne Disease Control Programme has prepared new treatment guidelines for malaria that recommend phytochemical constituents-based therapy as the first-line drug for malaria. It is being observed in the treatment of malaria that the cases of drug resistance are increasing day by day. Finding new antiplasmodial or antimalarial plant species to develop effective malarial drugs is the necessity of time to avoid the problem of drug resistance. The tribal people have been using traditional medicinal plants since ancient times. They are using the medicinal plants found in their area for the treatment of fever, loss of appetite, digestive disorders, diabetes, jaundice, joint pain, skin and various other diseases. A community-level study was conducted to document the traditional knowledge and identify the antimalarial plant species with the help of traditional healers (vaidya/Hakeem) as well as by involving Sabar, Santal, Ho tribes and other communities of the area. The present study is focused on the exploration of antiplasmodial and antimalarial medicinal plants which are traditionally used by the ethnic or tribal people of East Singhbhum district in Jharkhand. Further, the review of scientific literature was conducted to validate the documented knowledge and related antimalarial plant species. A total of 28 medicinal plants of East Singhbhum have been identified and reviewed which have good antiplasmodial properties. These medicinal plants are rich in bioactive compounds such as flavonoids, alkaloids, terpenoids, tannins, essential oils, anthraquinones pyrroles etc. The present study deals with the details of those active compounds and concludes the best drug for control of *Plasmodium vivax* and *P. falciparum*. However, more advanced research is needed to establish the effectiveness of bioactive compounds in vitro and in vivo, understand their basic mechanisms of action and begin the process of clinical research.

KEYWORDS: Medicinal Plants, Traditional Medicines, Antimalarial, Antiplasmodial Properties, Bioactive Compounds, East Singhbhum, Jharkhand, India

1. INTRODUCTION

India has been the center of a varied range of medicinal plants for centuries which are used by the tribal and local communities in the treatment of various ailments. These plants are not only used in human diseases but are also applied in veterinary treatment and even insect-pest management of different agricultural crops. There are about 18000 flowering plant species in India of which around 7000 are medicinally important. These plants are used in folk or traditional and documented systems of medicines including Ayurveda, Unani, Siddha and Homeopathy. These documented plants and their associated traditional knowledge are key resource bases for traditional healing systems [1]. Jharkhand is one of the biodiversity-rich states of India because of its diverse geographical and ecological conditions. The state is a land of different tribes, tropical dry deciduous forests, flora and fauna and hence called cultural and herbal heritage and

natural sanctuary of the country. The total forest cover in Jharkhand is 23,605 km² which is 29.62% of the geographical area of Jharkhand [2]. The rich biodiversity of Jharkhand has more than 600 species of medicinally important plants. About 70% of the state population relies on traditional and herbal systems of medicine. East Singhbhum, the focused area of the present study is one of the biodiversity-rich districts of Jharkhand. The district is dominated by ethnic and tribal populations (28.5%), which include Santal, Ho, Bhumij, Munda and Oraon [3]. These people have a treasure of traditional knowledge in ethno-medicines and herbal formulations. Forest cover and biodiversity of the district are important sources of livelihood, food and health of the local communities. The community used medicinal plants available in their area as folk medicines for centuries. These medicinal plants contain various bioactive compounds, mainly secondary metabolites and essential oils which are applied as the potential cure to different diseases or disorders including malaria in human beings. Malaria is a vector-borne disease widely observed in different climatic zones of Jharkhand. Changing climatic conditions is one of the major factors spreading malaria. The warm and humid conditions favor the breeding of vectors on a large scale. Hence, the transmission of malaria is a dynamic process influenced by the changes in ecological and meteorological conditions [4, 5]. It has been reported that at present the number of malarial patients in Jharkhand is about 31,600 [6]. Malaria has been a major health problem in the East Singhbhum region of Jharkhand, particularly in the rainy season. [7]. The cases of malaria in the East Singhbum region of Jharkhand are about 3000. Most of the tribal peoples and vulnerable tribal groups (PVTG) of the region are suffering from malaria. These communities are living in inaccessible forests, hills, valleys, and perennial streams. This topography and environment are favorable for rapid vector growth and the propagation of the malaria parasite which results in a strong transmission of a fatal form of malaria parasite *Plasmodium falciparum*. The disease is a big cause of death, disability and financial loss for the rural and tribal peoples of the area. The community has limited access to timely treatment and social security. Experts found that malaria is the reason for maternal death, stillbirth, low birth weight in infants and weak immunity [8, 9]. The government of India has adopted a framework for the elimination of malaria covering the period 2016 to 2030 as per the Global Technical Strategy. The aim set by the government is to reach zero Malaria cases by 2027 before the certification for malaria-free status by WHO [4]. Medicinal plants traditionally used by tribal people can play an effective role in achieving the aim set by the government to eliminate Malaria. The rural and tribal people of Jharkhand use different plant-based traditional medicines in the treatment of Malaria and high fever. It was found in the reviewing of different scientific literature that there are 29 medicinal plants which are mostly used by the people of East Singhbhum and other regions of Jharkhand in the treatment of Malaria and intermittent fever. These medicinal plants are rich in bioactive compounds such as flavonoids, alkaloids, terpenoids, tannins, essential oils, anthraquinones pyrroles etc. The present study deals with the review of all 28 plant species to know about the antimalarial properties of the plants and their active compounds for control of *Plasmodium vivax* and *P. falciparum*. This review work will provide updated information for further research on the effectiveness of bioactive compounds and the development of antiplasmodial drugs.

2. ANTIPLASMODIAL PLANT SPECIES

East Singhbhum region of Jharkhand has a rich diversity of medicinally important plant species along with traditional healing knowledge. It has been reported in several studies conducted by ethnobotanists in and around the region that there are a large number of plant species used by

ethnic people and traditional healers in fever, malaria, body pain, shivering and headache. A total of 29 highly effective antiplasmodial or antimalarial plant species of the region identified and documented in the present study are mentioned below.

Table 1: Antiplasmodial Medicinal Plants of East Singhbhum

S. No.	Plant Species	Common Name	Part used	Traditional Uses
1.	*Aegle marmelos*	Wood Apple, Bael	Leaf, Seed	Antipyretic, Antidiabetic [10], Analgesic, Antiinflammatory, Antihepatotoxic, Antibacterial [11]
2.	*Andrographis paniculata*	King of Bitters, Kalmegh	Whole plant	Antimalarial, Antipyretic, Antiinflammatory, Antidiabetic, Antihypertensive, Antihepatotoxic [12, 13]
3.	*Aristolochia indica*	Birthwort, Snakeroot Ishwarmul	Leaf, Root	Snakebites, Fever, Malaria, Skin Diseases, Helminthes, Intestinal Disorders [14]
4.	*Artemisia indica*	Wormwood, Majtari	Whole plant	Malarial Fever, Tuberculosis, Lice, Wounds, Scabies, Dysentery [15]
5.	*Azadirachta indica*	Neem	Leaf, Stem Bark, Roots, Fruits	Fever, Malaria, Headache, Ulcers, Diabetes, Leprosy, Chickenpox, Skin Disease [16]
6.	*Berberis aristata*	Barberry, Daru Haldi	Root	Malaria, Skin Diseases, Diarrhea, Eye Broblem, Inflammation, Wound Healing, [17]
7.	*Cassia alata*	Ringworm Shrub, Senna,	Leaf, Seeds, Stem, Flower	Eczema, Ringworm, Malaria, Constipation [18]
8.	*Cassia occidentalis*	Coffee Senna, Chakod	Root bark	Fever, Laxative, Whooping Cough, Skin Diseases, Asthma, Joint pains [19]
9.	*Cissampelos pareira*	Velvetleaf, Harjori or Akanadi	Root, Stem	Malaria, Ulcer, Wound, Rheumatism, Asthma, Cholera, Diarrhoea, Snakebite, Rabies [20]
10.	*Curcuma longa*	Turmeric, Haldi	Rhizome	Malaria, Cancer, Skin Diseases, Joint Pain [21]
11.	*Cyclea barbata*	Hairy Cyclea, Raj Patha	Root, Leaf	Malarial Fever, Stomach Trouble [22, 23, 24]
12.	*Cyperus rotundus*	Purple Nutsedge, Motha	Root, Tuber	Anti-Malarial, Anti-Pyretic, Anti-Inflammatory, Hepatoprotective, Anti-Obesity, Anti-Diabetic [25, 26]
13.	*Datura metel*	Thornapple, Datura	Fruit	Malaria, Dandruff, Arthritis, Liver Problem, Viral Fever,

				Antifungal [27]
14.	*Diospyros melanoxylon*	Coromandel ebony, Tendu	Bark	Antiplasmodial, Antiprotozoal Antimicrobial, Anti-Inflammatory, Antidiabetic [28]
15.	*Eclipta alba*	False Daisy, Bhringaraj	Leaf	Malaria, Fever, Hair loss, Jaundice, Skin Diseases, [30, 31, 32]
16.	*Ficus sur*	Bush Fig	Stem Bark, Leaf	Malarial Fever, Leukoderma, Leprosy, Wounds, Respiratory Problems, Diarrhoea, Tuberculosis, Anaemia, Epilepsy [33]
17.	*Holarrhena pubescens*	Fever pod, Indrajau	Root, Bark, Seed	Malarial Fever, Diabetes, Piles, Leprosy, Diarrhea [34, 35]
18.	*Lantana camara*	Lantana, Putoos	Leaf	Fevers, Colds, Rheumatism, Asthma, Cuts, Intestinal Worms [36, 37, 38]
19.	*Moringa oleifera*	Drumstick Tree, Sahjan	Leaf, Bark, Flower, Fruit, Seed	Malaria, Malnutrition, Water Purifier, Joint Pain, Diabetes, Hypertension, Wound, Paralysis [39, 40, 41, 42, 43]
20.	*Ocimum sanctum*	Holy Basil, Tulsi	Bark, Leaves, Flowers, Roots, Fruits, Seeds	Malarial Fever, Cold, Headache, Stomach Disorders, Inflammation, Heart Disease [47, 48]
21.	*Picrorhiza kurroa*	Bitter-root, Kutki	Root, Rhizome	High Fever, Stomachache, Cough, Cold, Jaundice, Diarrhea, Dysentery [49, 50, 51]
22.	*Plumbago zeylanica*	Ceylon leadwort, Chitrak	Root, Aerial Part	Anti-Plasmodial, Anti-Tumour, Anti-Bacterial, Hepatoprotective, Anti-Fungal, Anti-Inflammatory [52, 53, 54]
23.	*Pongamia pinnata*	Indian Beech, Karanj	Leaf, stem, bark, flower, fruit, kernel, latex	Malaria, Fever, Wound, Skin Diseases, Diabetes, Head Lice [55, 56, 57, 58, 59]
24.	*Solanum incanum*	Bitter Apple, Jangli Bhata	Leaf, Fruit, Root	Malaria, Sore Throat, Stomach-ache, Head-ache, Painful Menstruation, Liver pain, Diabetes, Common Cold, Snake Bites [60, 61]
25.	*Solanum nigrum*	Black Nightshade, Makoi	Leaf, Fruit, Root	Malarial Fever, Eczema, Urinary Tract Infections, Dysentery, Prostate [62, 63]
26.	*Stachytarpheta cayennensis*	Snakeweed, Barahmasi	Leaf, Root	Malarial Fever, Eyewash, Laxative Inflammation, Hepatic and Renal Disorder, Helminthiasis, Diabetes [64,

				65]
27.	*Swertia chirayita*	Bitter stick, Chiraita	Whole Plant	Malarial Fever, Blood Purification, Laxative, Inflammation, Tuberculosis, Diabetes [66]
28.	*Swietenia mahagoni*	Mahogany	Seed, Bark	Malaria, Diabetes, Diarrhea, Wound [68, 69]

3. ANTIPLASMODIAL BIOACTIVE COMPOUNDS OF THE ENLISTED PLANT SPECIES

3.1 *Aegle marmelos*

Aegle marmelos is a valuable medicinal tree. All parts of the tree are used in the traditional system of medicine. The bioactive compounds extracted from the fruits, leaves, stem, stem bark, root, and root bark of *A. marmelos* have different pharmacological properties including antiplasmodial efficacy [69]. Terpenoids and Fagarine are two important bioactive compounds that have antimalarial or antiplasmodial properties [70]. *In vitro,* antimalarial activity of *A. marmelos* leaf methanol extract has shown the highest activity against *Plasmodium falciparum* [70, 71].

3.2 *Andrographis paniculata*

Andrographolide and diterpene lactone compounds are derived from *Andrographis paniculata* as potent antiplasmodial phytochemicals. These compounds are synergistic with curcumin and addictively interactive with artesunate. It has been reported that andrographolide-curcumin exhibited better antimalarial activity. These nontoxic compounds can be used as template molecules for designing new derivatives with improved antimalarial properties [72, 73].

3.3 *Aristolochia indica*

The antimalarial bioactive compounds isolated from different parts of *A. indica* are aristolochic acid, ceryl alcohol, -sitosterol, stigmast-4-en-3-one, friedelin, cycloeucalenol, and rutin [74]. Some of the compounds are extracted from the essential oil of the plants; these are trans-pinocarveol (24.2%), α-pinene (16.4%) and pinocarvone (14.2%) [75].

3.4 *Artemisia indica*

Some of the chemical constituents extracted from *Artemisia indica* are effective against *Plasmodium falciparum*. These are exiguaflavone A, exiguaflavone B, maackiain, and 2-(2, 4-dihydroxyphenyl)-5,6-methylenedioxybenzofuran. Exiguaflavones A and B exhibit in vitro antimalarial activities of 4.60 x 10(-6) and 7.05 x 10(-6) g/mL, respectively, against Plasmodium falciparum [76]. Artemisinin and polymethoxyflavonoid are also antiprotozoal potential extracted from essential oil [77].

3.5 *Azadirachta indica*

A. indica has been reported to be effective against *P. falciparum* in humans [78]. It has been reported that in vitro and in vivo models show that aqueous, methanolic, or ethanolic extracts of neem bark, stems and leaves have significant activity against the strains of *Plasmodium*

falciparum and *Plasmodium berghei* [79]. Some researchers have found that limonoid deacetylnimbin extracted from neem seeds is effective against early sporogony stages of *P. bergehi* [81]. Azadirachtin is one of the important phytochemicals isolated from A. indica is effective in the treatment of cerebral malaria [80].

3.6 *Berberis aristata*

The roots and other parts of *Berberis aristata* contain alkaloids such as berberine and berbamine, which have antimalarial properties. Berberine is an isoquinoline which is used as a substitute for quinine in the treatment of malaria. [81].

3.7 *Cassia alata*

The fresh leaf extract of *Cassia alata* was observed as antimalarial properties against one of the strains of *P. falciparum* parasites. This action was generated by some of the phytochemicals of the plant leaves, such as anthraquinones, alkaloids, tannins, flavonoids, terpenoids and steroids. The dichloromethane/methane (1:1, v/v) extract of *Cassia alata* was also reported effective against *P. falciparum* [82, 83].

3.8 Cassia occidentalis

The leaf extract of *Cassia occidentalis* has reported outstanding antiplasmodial activity *in vitro.* The important phytochemicals including Anthraquinone, Emodin, Rhein Crysophanol, Pinselin, Physcion and Aloe emodin are effective against malaria [84, 85].

3.9 Cissampelos pareira

The roots and stem of *Cissampelos pareira* contain alkaloids hayatinine and isoquinoline which are effective antimalarial agents. There is scientific evidence for the traditional use of this plant in the treatment of malaria [86].

3.10 Curcuma longa

Curcuma longa has different kinds of bioactive compounds effective against a large range of ailments. Curcumin is one of the most important phytochemicals of *C. longa* and has been in malaria, skin diseases and joint pains. It has been observed that curcumin, a polyphenolic organic molecule restrains chloroquine-resistant *P. falciparum* growth in culture in a dose-dependent mode with an IC_{50} of ~5 μM. Moreover, oral administration of curcumin to mice infected with the malaria parasite (*P. berghei*) reduces blood parasitemia by 80–90% and enhances their survival significantly. Therefore, curcumin may represent a novel treatment for malarial infection [87, 88].

3.11 Cyclea barbata

The fresh leaf of *Cyclea barbata* has been used traditionally in various ailments including intermittent fever, intestinal problems, bacterial diseases, inflammation and wound healing. It has been reported that the alkaloids and flavonoids of the plant species present in leaf, stem and root are effective in malaria [89].

3.12 Cyperus rotundus

It was investigated that the crude hexane extracts of the air-dried tubers of Cyperus Rotundus have high potency in the in vitro test against Plasmodium falciparum (ECso = 0.66 pg ml- 1); the result showed significant anti-malarial activity of Cyperus Rotundus. The anti-malarial activity of C. rotundus. Among tested compounds, 10, 12-peroxycalamenene had a maximum effect with an EC50 of 2.33 × 10−6 M and dichloromethane extract with an IC50 between 10 and 50 µg/mL [90, 91, 92].

3.13 Datura metal

Datura metal is one of the plant species used in the traditional and modern systems of medicines for different diseases. The leaf and seed of D. metel contain alkaloids, flavonoids, glycosides, steroids and saponins which could be effective against larvicidal activities [93, 94].

3.14 Diospyros melanoxylon

The bark of *Diospyros melanoxylon* is used in the treatment of malarial fever. Plumbagin and diospyrin are the two significant bioactive compounds of *D. melanoxylon* which have potent activities against *P. falciparum* [95].

3.15 Eclipta alba

Eclipta alba is one of the highly valuable medicinal plants which has different important pharmacological activities such as antimalarial, analgesic, hair growth promoting, hepatoprotective, antibacterial and antidiabetic [98]. The fresh tender leaf extract of the species is against *P. berghei* as it contains various phytoconstituents such as β-amirin, phytosterols, flavones (luteolin), coumarin (wedelolactone) and triterpenes (ecalbatin, ursolic acid and echinocystic acid), Sesquiterpene lactones. Other biochemicals present in the plant parts are 5-hydroxymethyl-(2,2':5',2")-terthienyl tiglate, 5-hydroxymethyl-(2,2':5',2")-terthienyl agelate, 5-hydroxymethyl-(2,2':5',2")-terthienyl acetate [96].

3.16 Ficus sur

The review of the current status of *Ficus sur* has shown that the plant species has antiplasmodial activity. *F. sur* contains pentacyclic triterpenoids of oleanane and ursine structures as well as flavonoids and coumarins which are effective against antiplasmodial activities [97].

3.17 Holarrhena pubescens

Holarrhena pubescens is one of the important medicinal plant species of the Indian subcontinent. The species is used in traditional systems of medicine by the tribal people. The bark, seed and root of the plant are used in diabetes and malarial fever. The species contains several bioactive compounds including holarrhimine, conarrhimine, conessine, isoconnessine, antidysentericine, conessimine, conimine, conessidine, mokluangin A-D and conkurchicine. Chloroform extracts of the plant bark reported significant in vitro and in vivo anti-malarial activity [98, 99].

3.18 Lantana camara

Lantana camara is a common plant species in the East Singbhum region of Jharkhand which is used by the tribal communities for various uses. The aqueous and ethanolic leaf extracts of the plant leaf have shown antimalarial activity close to that of the standard drug chloroquine. The leaf extracts of *L. camara* contain alkaloids, flavonoids, terpenoids, saponins, carbohydrates, steroids and tannins in the different extracts [100].

3.19 Moringa oleifera

Moringa oleifera is a very common plant in India including Jharkhand. The leaves and seeds of this plant are the main parts used in the formulation of medicine for the treatment of different ailments including malaria. It has been confirmed that the bioactive compounds extracted from the leaves and seeds are effective against *P. falciparum, P. berghei, P. chabaudi and P. yoelii*. Flavonoids such as apigenin, kaempferol, rutin, and quercetin extracted from the leaf and seeds have shown antimalarial properties [101, 102, 103].

3.20 Ocimum sanctum

Extracts of leaves and roots of *Ocimum sanctum* Linn are used as antimalarial against *Plasmodium berghei* in vivo. The antiplasmodial bioactive compounds of *O. sanctum* include eugenol, apigenin, ursolic acid, rosmarinic acid [104. 105]

3.21 Picrorhiza kurroa

The dried roots of Picrorhiza kurro are traditionally used in the treatment of malaria and intermittent fever. Kutkin is the important bioactive compound of the plant species which includes kutkin is the active principle of P. kurroa and is comprised of kutkoside and the iridoid glycoside picrosides. Other bioactive compounds are apocynin, drosin, and nine cucurbitacin glycosides [106, 107].

3.22 Plumbago zeylanica

The root extract of Plumbago zeylanica contains plumbagin which was evaluated for chemical constituent and antimalarial effect against Anopheles stephensi Liston (Diptera). Plumbagin compound of P. zeylanica can be considered a new source of natural larvicide for the control of malarial vectors [108].

3.23 Pongamia pinnata

Pongamia pinnata has been reported to have antiplasmodial or antimalarial properties. The methanol extract of the bark of the tree contains alkaloids, flavonoids, triterpenes, tannins, carbohydrates, phenols, coumarins, saponins, phlobatannins and steroids. The isolated bioactive compounds are effective against Plasmodium falciparum and Plasmodium berghei parasites [109].

3.24 Solanum incanum

The leaves of Solanum incanum have prophylactic antimalarial properties. The ethyl acetate fraction of the leaves contains an alkane compound which plays a significant role in the inhibition of enzymatic activity [110].

3.25 Solanum nigrum

Solanum nigrum is an important medicinal plant used in the treatment of malaria in traditional medicine. The bioactive compounds of the herb are alkaloids, flavonoids, phenolic, deterpines and saponin isolated from the leaf ethanol extract of the plant used in malaria and other diseases [111].

3.26 Stachytarpheta cayennensis

The ethanolic leaf extract of *Stachytarpheta cayennensis* contains antimalarial properties as observed in its characteristic to suppress *Plasmodium berghei infection.* The fresh leaf extract of *S. cayennensis* constitutes antiplasmidial bioactive compounds such as alkaloids, terpenes and flavonoids [112].

3.27 Swertia chirayita

Swertia chirayita) is an important herb used traditionally in the treatment of several ailments including malaria, diabetes and liver problems. The herb is a rich source of flavonoids, xanthones, terpenoids, iridoid and alkaloids. The chemical components such as swerchirin, swertinin, decussatin, 1-hydroxy-3,5,8- trimethoxyxanthen-9-one and chiratol of *S. chirayita* have effective binding interactions to control malaria [113].

3.28 Swietenia mahagoni

In traditional system of medicine, Swietenia mahagoni is an important medicinal plant used in the treatment of malaria, diarrhea and diabetes. Laef, bark and seed of the plant consist of secondary metabolites which are effective in malarial fever, and bacterial and fungal infection. The methanol extract of the seed of the plant was reported with antimalarial properties [115, 116].

4. SAFETY OF THE DRUDGS

The above-mentioned medicinal plants and bioactive compounds are valuable sources for the development of novel drugs. The extracts of medicinal plants used in the treatment of malaria are common as an alternative to modern antimalarial drugs. The recommended doses of the antiplasmodial bioactive compounds were found safe and non-toxic for human beings. However, the efficacy and safety of most of the herbal medicines has not yet been established.

5. CONCLUSION

Malaria is one of the life-threatening parasitic diseases affecting the whole developing and poor countries causing huge social and economic loss. The situation is harsh as the malarial parasites have developed resistance against the majority of modern drugs. To control and eradicate the devastating disease and overcome drug resistance, novel medicinal plant-based bioactive compounds are required. There are many plant species in the East Singbhum region of Jharkhand which have been left unexplored with their properties to cure malaria. Further studies are required to find out the phytochemical constituents effective in the treatment of malarial.

6. FUTURE REMARKS

Development of new antiplasmodial drugs using the bioactive compounds extracted from medicinal plants will play a big role in solving the problems of developing countries to combat this formidable disease. The pace of research progress must be high to develop novel antiplasmodial medicines.

Plate 1: Photographs of the plants

Aegle marmelos

Andrographis paniculata

Aristolochia indica

Artemisia indica

Azadirachta indica

Berberis aristata

Cassia alata

Cassia occidentalis

Cissampelos pareira

Curcuma longa

Cyclea barbata

Cyperus rotundus

Datura metel

Diospyros melanoxylon

Eclipta alba

Ficus sur

Holarrhena pubescens

Lantana camara

Moringa oleifera

Ocimum sanctum

Picrorhiza kurroa

Plumbago zeylanica

Pongamia pinnata

Solanum incanum

Solanum nigrum

Stachytarpheta cayennensis

Swertia chirayita

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

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