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Plant part substitution as a sustainable conservation strategy for some medicinal plants based on phytochemical equivalence

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Abstract
Medicinal flora represents an essential source of bioactive substances for both conventional and contemporary medicine; nevertheless, excessive extraction of non-renewable parts such as roots, bark, and heartwood has led to significant reductions in wild populations, especially among endemic and slow-growing species. This review proposes and evaluates the substitution of plant parts by replacing vulnerable bark and roots with renewable aerial parts, using published evidence of phytochemical equivalence for sustainable conservation of medicinal plants. This review provides an outline of the published evidence for five representative medicinal plants *Myrica esculenta*, *Pelargonium sidoides*, *Warburgia salutaris*, *Litsea chinensis* and *Aegle marmelos* for screening phytochemical similarities between traditional and renewable plant parts. The available studies report the occurrence of shared bioactive constituents including gallic acid, protocatechuic acid salicylic acid, warburganal, polygodial, flavonoids and phenolic compounds although quantitative similarities vary among species. Evidence exists that renewable aerial parts may be possible alternatives to destructively harvested bark and roots, where phytochemical equivalence is supported by analytical techniques such as HPTLC, HPLC or LC–MS/MS. The review also discusses opportunities, limitations and future research required for the implementation of plant part substitution in the conservation of medicinal plants, sustainable harvesting practices and policy frameworks aligned with the Convention on Biological Diversity, Nagoya Protocol, WHO-IUCN-WWF policy and the United Nations Sustainable Development Goals (SDGs 3, 13 and 15).

Keywords Conservation, Plant part substitution, Sustainable, Environment, Ethnopharmacology

1 Introduction

Most medicinal species employed in the Indian systems of medicine including Ayurveda, Siddha, Unani and Traditional Chinese Medicine (TCM) are slow-growing perennials whose most valued components (heartwood, bark and subterranean organs) are obtained through destructive harvesting practices. This intensive exploitation has made

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these taxa highly vulnerable, threatening their persistence in natural habitats as well as the ecological integrity of the ecosystems in which they occur. Systematic phytochemical studies on the therapeutic potential of these plants are based on the ethnobotanical knowledge from antiquity to the present day, which has led to the discovery of many bioactive compounds and the development of modern pharmaceuticals.

The constant over-exploitation caused by population growth, urbanisation, and industrialisation still reduces the wild populations, despite the well-known value of herbal medicines, which are preferred owing to their wide availability and relatively fewer side effects. In addition, indiscriminate collection methods and illegal trade further aggravate declines in species richness and genetic diversity [1]. Hence, these medicinal resources need to be preserved and managed on a sustainable framework.

The following are some of the approaches that can be used to conserve these valuable plant populations:

i. Establishment and expansion of special conservation areas; enactment of strict laws to prevent the unsustainable collection of bark and roots.
ii. Develop targeted education and outreach programmes to raise awareness on medicinal plant diversity, their ecological importance and their role in health care and sustainable livelihoods.
iii. Development and optimisation of large-scale cultivation systems particularly for slow-growing taxa including ex situ propagation protocols and controlled environment agriculture.
iv. Prioritisation of plant-part substitution, replacing non-renewable parts (e.g., bark, roots, rhizomes) with renewable vegetative tissues (e.g., leaves, young stems) wherever phytochemically and therapeutically feasible [2, 3].

1.1 Strategic insights into plant part substitution for conservation and sustainability

The natural regeneration of plant species is steadily declining due to increasing demand, indiscriminate collection, and overexploitation. Globally, the growing number of herbal and nutraceutical industries has significantly intensified the demand for medicinal and aromatic Plants (MAPs). Notably, plant roots and bark constitute a major portion of the raw materials used accounting for approximately 30% and 13% respectively. Harvesting these parts typically involves uprooting or felling the entire plant, leading to irreversible destruction and threatening the survival of many valuable species from the environment [4].

Exploitation of plant parts in an unsustainable way can lead to the decline of important species and ultimately a loss in biodiversity. Stem bark is among the most frequently harvested plant parts and its removal poses significant risks to plant survival. The extent of damage depends on the quantity and method of bark extraction; excessive or improper harvesting can severely disrupt physiological functions, often leading to the eventual death of the plant. This highlights the urgent need for studies focused on understanding the ecological consequences of bark removal and developing sustainable harvesting practices and if possible, employ plant part substitution studies to fulfil the purpose [5].

However, plant-part substitution cannot be assumed solely on the basis of traditional use or the general presence of similar phytochemical classes. The biosynthesis, transport and storage of secondary metabolites are often organ-specific and dependent on tissue development. Hence, the qualitative and quantitative distribution of characteristic metabolites in roots or bark may differ considerably from that in renewable aerial parts. These variations may also be influenced by plant age, growth stage, season, genotype, and environmental conditions. Hence, the suitability of an alternative plant part should be established by a systematic comparative assessment of relevant marker compounds, supported by qualitative and quantitative phytochemical data, to ensure that the benefits of conservation are realised without compromising phytochemical and therapeutic significance [4, 5].

2 Studies on plant part substitution as a conservation strategy for medicinal plants: a global perspective

In response to escalating anthropogenic pressures on wild medicinal plant populations including overharvesting, habitat degradation and surging demand for herbal formulations researchers and conservationists have increasingly evaluated plant-part substitution as a sustainable management strategy.

Phytochemical profiling and bioactivity assays conducted across diverse geographic regions have provided robust validation of such substitutions, demonstrating that renewable organs (e.g. leaves, young stems, fruits) can match or approximate the therapeutic constituents of traditionally harvested plant parts. Together, these multidisciplinary initiatives confirm that plant-part substitution constitutes not merely a conceptual framework but a pragmatic, evidence-based approach that reconciles biodiversity conservation with the continued provision of safe and effective traditional healthcare (Fig. 1; Table 1).

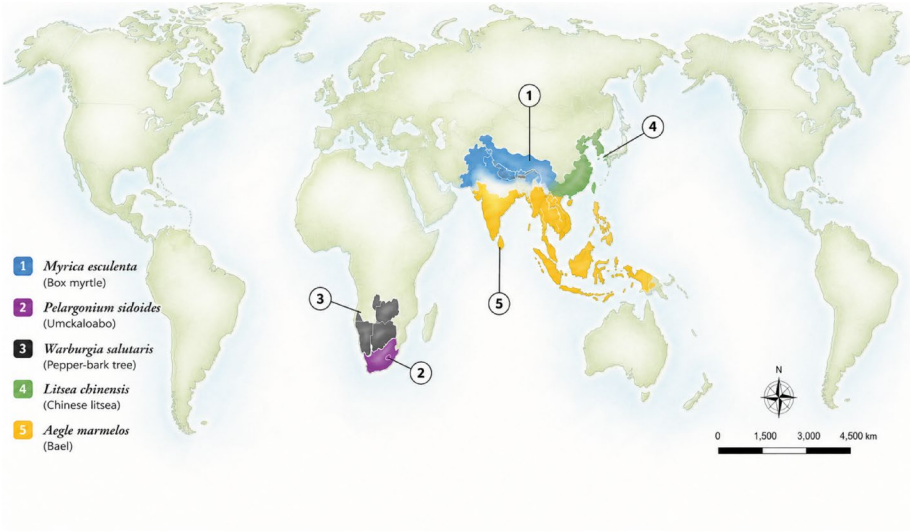


Fig. 1 Native geographical distribution of the five medicinal plant species reviewed for evaluating plant part substitution based on phytochemical equivalence: (1) *Myrica esculenta* – Himalayas (India, Nepal, Bhutan, SW China); (2) *Pelargonium sidoides* – South Africa and Lesotho; (3) *Warburgia salutaris* – Southern Africa; (4) *Litsea chinensis* – China, India, Nepal, Myanmar; (5) *Aegle marmelos* – Indian subcontinent and Southeast Asia

Table 1 Some of the studies carried out on plant parts substitution-based approach as a viable conservation strategy for medicinal plants

S. no.	Name of the plant	Plant parts traditionally used	Plant parts as substitution	Active compounds that can be substituted	References
i.	*Myrica esculenta* (Myricaceae)	Stem bark	Small branches	Flavonoids & Phenols	[6]
ii.	*Pelargonium sidoides* (Geraniaceae)	Underground parts	Aerial parts	Gallic acid, Protocatechuic acid & Salicylic acid	[7]
iii.	*Litsea chinensis* (Lauraceae)	Heartwood	Small branches	Phenols	[8]
iv.	*Aegle marmelos* (Rutaceae)	Roots	Stem	Flavonoids & Phenols	[9]
v.	*Warburgia salutaris* (Canellaceae)	Bark	Leaves	Warburganal, Polygodial	[10]

2.1 Myrica esculenta (Morella esculenta, Ascarina rubra)

The plant species, *Myrica esculenta* is commonly known as Himalayan Bayberry, is used extensively in many medicinal systems throughout the Asia. Organoleptic and macroscopical studies revealed that the leaves are dark green in colour, thin, spirally organized, astringent to the taste, and have an acute apex. Cuticularized epidermis with polygonal cells was discovered in the transverse section. Major phytoconstituents includes alkaloids, sugars, flavonoids, glycosides, phenolic compounds, and tannins [11].

2.1.1 Geographical distribution

Approximately 675 wild edible fruits are known to exist in the Indian Himalayan Region (IHR). Among them, the Myricaceae family includes *Myrica esculenta* Buch–Ham. ex D. Don. popularly known as Kaphal, is among the most prized wild fruits. Trees grows at an elevation of 900–2100 m higher above the sea level. The species has spread eastward from Ravi river to Malaya, Assam, Khasi, Jaintia, Naga, and the Lushi highlands in India, Singapore, China, Japan [12].

2.1.2 Ethnomedicinal appraisal of Myrica esculenta

A significant portion of the population in Uttarakhand's rural areas uses stem bark to treat headaches, asthma, and chronic coughs [13, 14]. Bark powder is also inhaled to treat ulcers [15]. The Sub-Himalayan region's natives apply bark paste to wounds, joint problems, paralysis, and headaches, and they employ bark decoction as a mouth freshener and toothache remedy [16, 17]. Various ethnic groups living in the rural Orissa region also use bark to treat mental illness. Fruit is consumed raw or used to make cool drinks, and tribal groups in Meghalaya utilize its juice to treat bacterial dysentery and bark is chewed to treat wounds and ease toothaches [17, 18].

2.1.3 Comparative phytoconstituent analysis for sustainable plant part substitution in Myrica esculenta

Myrica esculenta is a rich source of various secondary metabolites like phenolic compounds, flavonoids, diarylheptanoids, steroids and triterpenoids. The stem bark has important bioactive compounds such as gallic acid, catechin, proanthocyanidins, castalagin, quercetin, myricanol, myricanone, β-sitosterol and taraxerol which are responsible for the antioxidant, anti-inflammatory and antimicrobial activities. These

phytoconstituents form the phytochemical basis for the assessment of plant part substitution and comparative phytochemical equivalence [6].

Total phenolic content was quantified by the Folin–Ciocalteu assay. Aliquots of each extract or gallic acid standard (GAE) (20–100 mg mL^{-1}) were mixed with Folin–Ciocalteu reagent, deionized water, and 7% Na_2CO_3, then incubated at room temperature for 90 min. Absorbance was measured at 550 nm using a UV–Vis spectrophotometer. Stem-bark extracts exhibited a significantly higher total phenolic concentration (276.78 ± 5.36 mg GAE g^{-1} dry weight) compared to small-branch extracts (31.24 ± 2.57 mg GAE g^{-1} dry weight) [6].

Total flavonoid content was determined via aluminum-chloride colorimetry. Extracts or quercetin standards (QUE) (20–100 mg mL^{-1}) were combined in a 10 mL volumetric flask with distilled water, 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH, then brought to volume with distilled water. Following mixing, absorbance was recorded at 510 nm. The stem bark contained substantially more flavonoids (121.68 ± 6.81 mg QUE g^{-1} dry weight) than the small branches (12.94 ± 1.12 mg QUE g^{-1} dry weight) [6].

Qualitative HPTLC profiling of both stem-bark and small-branch extracts revealed congruent phytochemical bands, indicating the presence of analogous secondary metabolites in each plant part. These results support the feasibility of substituting small branches for bark within *Myrica esculenta*, consistent with traditional healer practices and contributing to the sustainable utilization of this species [6].

2.1.4 Pharmacological activities

2.1.4.1 a. Anticancer activity: In the methylthiazolyltetrazolium (MTT) assay, the fruit's methanolic extract demonstrated moderate anticancer activity, resulting in 50, 48.29, and 46.19% inhibition of Hep G2, Hela, and MDA-MB-231 cancer cell lines at concentrations of 5 mg/mL. The extract's effect on the percent inhibition of cancer cells was found to increase with dose, which may be attributed to the presence of bioactive compounds like ferulic acid and gallic acid as determined by LC/MS analysis. Preclinical studies have demonstrated that the acetone and acid-methanol extracts of *M. esculenta* fruits exhibited strong anticancer proliferative activities that reduced the viability of C33A, SiHa, and HeLa cancer cells by 70–92% while displaying no cytotoxicity towards normal transformed cell lines [13].

2.1.4.2 b. Antidiabetic activity: Methanolic extracts of *M. esculenta* leaves caused a significant hypoglycaemic effect in a dose-dependent manner in streptozotocin-induced diabetic rats. Oral administration of the extract resulted in a substantial ($p < 0.05$) decrease in body weight, blood cholesterol, and blood glucose, as well as a favourable ($p < 0.05$) impact on the lipid profile of the extract-treated group relative to the positive vehicle-treated group [13].

2.1.4.3 c. Cardioprotective function: Although there is few particular research on *M. esculenta's* cardioprotective effects, its anti-inflammatory and antioxidant qualities point to possible advantages for cardiovascular health. The herb may improve heart health by reducing inflammation and oxidative stress [12].

2.1.4.4 d. Anti-inflammatory properties: Both in-vitro and in-silico investigations have shown that *M. esculenta* has strong anti-inflammatory properties. An aqueous extract of

the bark and root of *M. esculenta* was tested for its anti-inflammatory properties in vitro. The findings demonstrated that bark extract significantly inhibited 5- Lipoxygenase, 15-Lipoxygenase, and Hylurenadease, with respective values of 11.26 ± 3.93, 25.57 ± 8.94, and 21.61 ± 8.27 µg/mL. Rich in phenolic components as myricetin and arjunolic acid, the bark extract demonstrated strong inhibitory effect against TNF-α, COX-1, and COX-2, IL-10 which are inflammatory mediators. Strong binding affinities of these substances to inflammatory proteins were found by molecular docking simulations, compared with the reference standard, Celecoxib supporting the anti-inflammatory potential [19].

2.1.4.5 e. Antioxidants activity: *M. esculenta's* antioxidant capacity has been well investigated, especially in relation to its hydroalcoholic fruit extracts. Strong radical scavenging effect were correlated with high levels of total phenolic and flavonoid contents. The fruit extracts showed strong antioxidant activity in tests including DPPH, ABTS, and FRAP, suggesting that they may be used to treat illnesses linked to oxidative stress. Nonetheless, the correlation matrix indicated that the overall phenolic and flavonoid contents had a substantial ($P < 0.05$) beneficial effect on antioxidant activity [13].

2.1.4.6 f. Antimicrobial action: *M. esculenta* has antibacterial qualities that are broad-spectrum. Compounds like n-hexadecanol and eudesmol acetate, which are found in the essential oil extracted from its stem bark, have demonstrated efficacy against a number of infections, including *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*. The zone of inhibition of M. esculenta essential oil and standard were 20 and 17 mm for *B. pumilus*, and 18 mm and 15 mm for *S. aureus* respectively. These results support its traditional application in the treatment of infections [20].

2.2 Pelargonium sidoides (*Pelargonium rigidum, Pelargonium spectabile, Cortusina sidifolia, Geraniospermum sidifolium*)

2.2.1 Description

Pelargonium sidoides DC., commonly known as African geranium, is a perennial geophyte which belongs to the Geraniaceae family [7]. In addition to its distinctive dark, maroon-red petals, *P. sidoides* differs from the other members of the genus for its various traditional medicinal properties [21].

2.2.2 Geographical distribution

A perennial geophyte, *Pelargonium sidoides* is primarily found in the mountains of Lesotho and the Eastern Cape Province in South Africa. The Eastern Cape's near sea level to the Lesotho mountains' 2746 m are only two examples of the plant's broad altitudinal range to which it is adaptable [7].

2.2.3 Ethnomedicinal appraisal of Pelargonium sidoides

Pelargonium sidoides has been used traditionally in ethnoveterinary medicine for a variety of purposes, such as preventing purging in horses [21, 22], treating wounds with boiling leaves to keep maggots out, and treating calves with root decoctions as an anthelmintic cure [23–26]. Cattle with dysentery are treated orally with an extract made by soaking the roots in water. Extracts are used to treat diabetes, hypertension, respiratory, joint pains and inflammation [27–29].

2.2.4 Comparative phytoconstituent analysis for sustainable plant part substitution in Pelargonium sidoides

Pelargonium sidoides contains a diverse array of bioactive phytoconstituents, including highly oxygenated coumarins, phenolic acids (gallic, protocatechuic, and salicylic acids), flavonoids, and hydroxycinnamic acid derivatives, which form the phytochemical basis for evaluating plant part substitution [7].

Phenolic profiling of hydroalcoholic extracts from wild, acclimated, and tissue-cultured samples of *P. sidoides* were examined using UPLC-MS/MS (ACQUITY Ultra Performance LC™ linked to a Micromass Quattro micro™ API benchtop triple quadrupole mass spectrometer. The findings suggested that phenolic compounds in acclimatized (AA: acclimatized aerial parts; AU: acclimatized underground parts) and wild (WA: wild aerial parts; WU: wild underground parts) *P. sidoides* samples are identical. However, there was variation in the type and concentration of phenolic compounds between the different plant parts. For both acclimated and wild plants, the aerial parts had greater quantities of gallic acid, protocatechuic acid, and salicylic acid than the subterranean parts (Fig. 2). In wild plants, gallic acid was much greater in aerial parts (WA: 231.08 ± 31.5 µg/g DW) than in underground parts (WU: 72.7 ± 3.01 µg/g DW). In contrast, gallic acid levels in acclimatized plants were 101.77 ± 32.56 µg/g DW in the aerial parts (AA) and 110.8 ± 8.31 µg/g DW in the underground parts (AU) [7].

Similarly, aerial portions had larger quantities of protocatechuic acid (AA: 7.94 ± 4.19 µg/g DW; WA: 28.96 ± 10.38 µg/g DW) than subterranean parts (AU: 0.79 ± 0.16 µg/g DW; WU: 0.395 ± 0.10 µg/g DW). The content of salicylic acid was lower in underground parts (AU: 5.25 ± 1.99 µg/g DW; WU: 2.30 ± 0.41 µg/g DW) than in aerial parts (AA: 7.36 ± 1.53 µg/g DW and WA: 6.88 ± 3.27 µg/g DW). This difference was considerably more noticeable. In wild plants, the difference in concentration of these phenolic compounds between aerial and subterranean sections was significant, suggesting that environmental conditions may have an impact on the synthesis of secondary metabolites in these plants. In addition, the data supports the substitution of underground plant parts with that of aerial parts of *P. sidoides* [7].

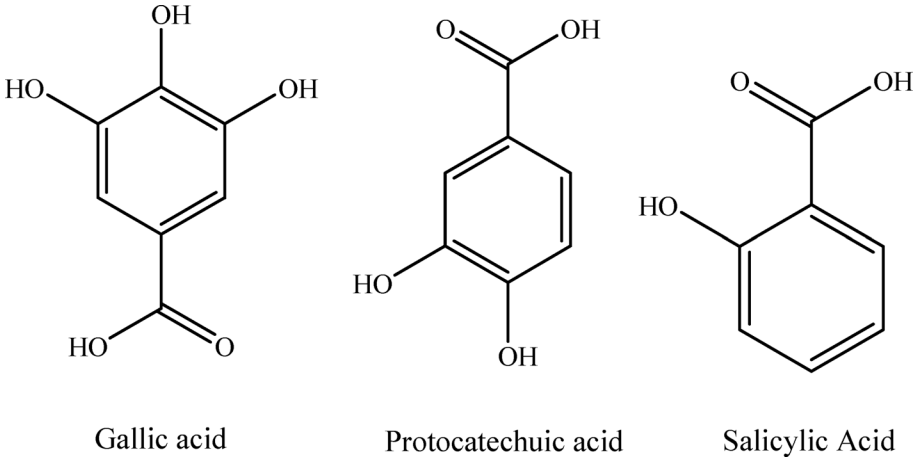


Fig. 2 Chemical structures of key bioactive compounds reported in *Pelargonium sidoides* & considered as phytochemical markers for plant part substitution

2.2.5 Pharmacological activities

There is a long history of using this well-known herbal remedy to treat respiratory tract infections, including tuberculosis, as well as gastrointestinal issues (Helmstädter, 1996). Reports of antitubercular activity and its potential as a treatment for problems of the ears, nose, and throat as well as respiratory tract infections, such as tonsillitis, bronchitis, sinusitis, and rhinopharyngitis, have increased interest in this traditional medication [30].

2.2.5.1 a. Antibacterial and antifungal property: Antibacterial activity of roots, leaves extracts and isolated constituents (scopoletin, umckalin, 5,6,7-trimethoxycoumarin, 6,8-dihydroxy5,7-dimethoxycoumarin, (+)-catechin, gallic acid, and its methyl ester) of *P. sidoides* against five gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Haemophilus influenzae*) and three gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus* 1451). The minimum inhibitory concentrations (MICs) of the other chemicals' antibacterial activity ranged from 200 to 1000 g/mL. MICs differed depending on the extracts and microbes examined [21].

2.2.5.2 b. Immunomodulatory property: In a series of bioassays, Kolodziej et al. (1997, 2001, 2003) examined the effects of *P. sidoides* preparations and isolated constituents on nonspecific immunological activities. Heparinized human whole blood samples were supplemented with Pelargonium sidoides extract (EPs® 7630) at dosages ranging from 0.3 to 30 g/mL. A substantial dose-dependent increase in the release of HNP 1–3 and BPI was found when plasma was analyzed for the amount of bactericidal/permeability-increasing protein (BPI) and human neutrophil peptides (HNP) 1–3 during a 5-hour incubation period. Therefore, it appears that EPs® 7630 promote host defense by increasing the release of antimicrobial peptides [21].

2.2.5.3 c. Effects on mucociliary system: In-vitro studies using EPs® 7630 to stimulate ciliary beat frequency which is a crucial defense mechanism of the mucociliary system in ciliated cell cultures of human nasal epithelium was carried out by Neugebauer et al. (2005). In a dose-dependent manner, three doses of EPs® 7630 (1, 30, and 100 g/mL) were examined and found to significantly increase ciliary beat frequency [21].

2.2.5.4 d. Support for respiratory health: The effectiveness of *Pelargonium sidoides* in treating respiratory tract infections, such as bronchitis, sinusitis, and the common cold, is well known. Its root extract, especially the standardized EPs® 7630, has been shown in clinical trials to significantly alleviate symptoms including cough and nasal congestion. The extract improves respiratory function by increasing the frequency of ciliary beats in respiratory cells, which facilitates mucus removal. While none of the pure compounds examined shown any antimycobacterial action under these experimental circumstances, the extracts demonstrated antimycobacterial activity against Mycobacterium TB (96% at 12.5 μg/mL) using the radiorespirometric BACTEC technique. In a broth microdilution alamar blue test, the same extract showed limited activity against *M. tuberculosis* (MIC of 100 μg/mL). The reference drug, rifampicin, demonstrated a MIC of 0.06 μg/mL in the experimental setup [31].

2.2.5.5 e. HIV-1 inhibition of entry: According to research, extracts from *Pelargonium sidoides* may operate as an entrance inhibitor by preventing HIV-1 from adhering to host cells. Its distinct mechanism sets it apart from other antiviral drugs and raises the possibility that it might be used in HIV-1 preventive measures. Using an HIV-1 reporter cell line, the EASY-HIT assay measures infection parameters linked to two temporally distinct steps of the HIV-1 replication cycle (i.e., release of infectious virions = step 2, resp., and expression of early viral proteins Tat and Rev = step 1) in order to assess antiviral effects. According to several tests, PS extract reduced infection parameters at both phases with comparable effectiveness; for step 1, the half-maximal inhibition (EC50) was 8.13 mg/mL (64.82; n = 19), and for step 2, it was 8.00 mg/mL (62.26; n = 2) [32].

2.3 Litsea chinensis (*Litsea undulata, Litsea cauliflora, Litsea caulostachys*)

2.3.1 Description

Litsea is among the most varied evergreen tree or shrub genera in the Lauraceae family. The *Litsea* species contain secondary metabolites with interesting chemical structures (alkaloids, lactones, sesquiterpenes, flavonoids, lignans, and essential oils) and notable bioactivities. Throughout the world, *litsea* species have been utilized in traditional medicine to treat a wide range of illnesses, such as the flu, gastrointestinal problems, diarrhea, diabetes, vomiting, bone pain, inflammation, central nervous system disorders, and other conditions [33].

2.3.2 Geographical distribution

Approximately 400 tree species belonging to the genus *L. chinensis* are found throughout tropical and subtropical Asia, North America, and South America. 72 (47 endemic) species of this family are found in China; they are primarily found growing on mountains that are at least 1500 m higher than sea level. Four species are found in Mexico and Central America, and twelve species are found in North America. 27 out of 46 species of *Listea*, found in India are endemic to Indian terrains [33, 34].

2.3.3 Ethnomedicinal appraisal of Litsea chinensis

For a long time, traditional medicine of India and indigenous Chinese medicines have used the genus *Litsea*, which is primarily found in tropical and subtropical regions, to treat conditions like diarrhoea, stomach aches, diabetes, edema, gastroenteritis, dyspepsia, colds, pain, severe injuries, asthma, arthritis, etc [35, 36].

2.3.4 Comparative phytoconstituent analysis for sustainable plant part substitution in Litsea chinensis

Litsea chinensis contains several phenolic and flavonoid constituents, including gallic acid, protocatechuic acid, caffeic acid, chlorogenic acid, quercetin, kaempferol, and rutin, contributing to its characteristic phytochemical profile [8].

A notable distinction between the heartwood and tiny branches is revealed by the measurement of active phytochemicals like total flavonoids and total phenolics in the ethanolic extracts of *Litsea chinensis*. It was found that, the heartwood had a higher flavonoid content (100.32 ± 3.19 µg QUE/g dry weight) than the small branches (41.74 ± 5.13 µg QUE/g dry weight), and the small branches had a higher total phenolic content (64.13 ± 0.34 µg GAE/g dry weight) than the heartwood (58.32 ± 0.41 µg GAE/g

dry weight). Although certain phytochemical groups differ, the high phenolic content of small branches supports their bioactivity to be comparable or even higher than that of heartwood. The prospective replacement of heartwood with small branches supports sustainable harvesting methods as a conservation strategy [8].

Furthermore, the HPTLC profiles of n-hexane, ethyl acetate, and ethanolic extracts of heartwood and small branches were subjected to comparative evaluation. Which revealed that numerous bands were similar to one another, further indicating the presence of numerous similar compounds in *L. chinensis* heartwood and small branches [8].

2.3.5 Pharmacological activities

2.3.5.1 a. Aphrodisiac activity: The effects of an ethanolic bark extract of *Litsea chinensis*, 500 mg/kg, body weight, P.O. on male sexual behaviour in rats were studied using a battery of tests including penile erection, homosexual mounting, stretching/yawning, aggressive behaviour, copulatory behaviour and orientational activities. The results showed a significant increase in ejaculation latency and a significant decrease in frequency of ejaculation [37].

2.3.5.2 b. Anti-inflammatory activity: In various test models, several extracts derived from species of Litsea have demonstrated anti-inflammatory properties. In herbal medicine, LC has long been used to treat inflammation, including rheumatoid arthritis, gastroenterologia, and edema. It has been documented that the bark of LC has anti-inflammatory properties. In LPS-activated RAW-264.7 macrophages, 0.01 mg/mL of the MeOH extract and its fractions dramatically reduced myeloperoxidase activity facilitated the conversion of chloride to HOCl and the production of O2. It also inhibited the synthesis of NO and PGE2 [34].

2.3.5.3 c. Activity of antioxidants: Several tests have been used to evaluate L. chinensis's antioxidant capacity. In the DPPH radical scavenging experiment, for instance, methanol extracts from the root and stem of L. elliptica and L. resinosa showed increased antioxidant activity, with EC_{50} values of 23.99 and 41.69 mg/L, respectively. These results suggest that the genus Litsea contains bioactive substances with antioxidant qualities [36].

2.3.5.4 d. Antimicrobial action: *Bacillus anthracis*, *Proteus vulgaris*, *Staphylococcus aureus*, and *Bacillus subtilis* are among the bacterial strains that have been demonstrated to be inhibited by ethanol extracts of L. chinensis leaves. Furthermore, total saponins from the closely related species L. coreana showed strong suppression against Proteus vulgaris, Staphylococcus aureus, and Enterobacter aerogenes. These antibacterial qualities point to possible uses in the management of infections [33].

2.4 Aegle marmelos (*Aegle marmelos var. mahurensis, Belou marmelos, Bilacus marmelos*)

2.4.1 Description

Indians have been using the plant *Aegle marmelos*, which is native to the subcontinent, for over 5000 years. The Indian traditional medical system, Ayurveda, and diverse forms of folk medicine make considerable use of leaves, bark, roots, fruits, and seeds to treat a wide range of illnesses [38, 39]. The pulp from bael fruits is used to make sweet treats like murabba, puddings, and juice. Bael fruits are utilized in food. Bael fruits are also

utilized in many traditional remedies as a laxative, to treat peptic ulcers, chronic diarrhea, dysentery, and respiratory ailments [40, 41].

2.4.2 Geographical distribution

Aegle marmelos (L.) Correa (Rutaceae) is a native of India and can be found in the wild throughout the central and South India's sub-Himalayan forests, as well as those in Sri Lanka, Bangladesh, Pakistan, Burma, Thailand, and much of Southeast Asia. It grows well in dry, semi-arid, and subhumid environments. It is grown all over India, but mostly in the gardens of temples, due to its spiritual importance [39].

2.4.3 Ethnomedicinal appraisal of Aegle marmelos

The plant is prized for its robust and long-lasting wood, delectable fruits, and several therapeutic qualities. In ethnomedicine, *A. marmelos's* leaves, fruits, stem, and roots have been utilized as an aphrodisiac, antipyretic, antiscourbutic, demulcent, antidiarrheal, antidysenteric, and hemostatic medication in addition to an antivenomous snake [42, 43]. It has a lot of riboflavin, vitamin C, and coumarins. Both the fruits and the bark are said to be effective Ayurvedic treatments for dysentery and other digestive issues [44, 45].

2.4.4 Comparative phytoconstituent analysis for sustainable plant part substitution in Aegle marmelos

Aegle marmelos contains diverse bioactive phytoconstituents, including phenolic compounds such as gallic, chlorogenic, caffeic, ferulic, and ellagic acids; flavonoids including rutin, quercetin, kaempferol, and catechin; coumarins such as umbelliferone and scopoletin; and alkaloids including aegeline, skimmianine, dictamnine, γ-fagarine, and halfordinol, along with hydrolysable and condensed tannins. Both the stem and root extracts of *Aegle marmelos* exhibited analogous classes of phytochemicals, with phenolics, flavonoids, alkaloids, and tannins being quantified via spectrophotometric assays [9].

Total phenolic content (TPC), expressed as mg gallic acid equivalents (GAE), was 1.89 mg GAE/g in the stem extract versus 0.758 mg GAE/g in the root, indicating a 59.89% higher phenolic concentration in the stem. Total flavonoid content (TFC), determined by the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE), measured 1.021 mg QE/g in the stem and 0.800 mg QE/g in the root, a 21.64% increase in the stem that was not statistically significant. Alkaloid content, estimated against a caffeine standard and expressed as mg caffeine equivalents, differed by only 4.6% between stem and root. Total tannin content (TT) was likewise higher in the stem, showing a 13.58% increase relative to the root, though this variation was also not significant [9].

Thin-layer chromatographic analysis revealed comparable chemical profiles in both extracts, with Umbelliferone and Scopoletin present in each. High-performance liquid chromatography further demonstrated similar peak distributions and area percentages across stem and root extracts. Taken together, these qualitative and quantitative findings support the use of *A. marmelos* stem as a viable substitute for its roots [9].

2.4.5 Pharmacological activities

2.4.5.1 a. Antioxidant activity: Reactive oxygen species (ROS) have a well-established role in the advancement of certain illnesses, such as cancer and cardiovascular disease. Plants that contain antioxidants or polyphenol compounds can effectively neutralize reactive oxygen species (ROS) and reduce the progression of illness. The dry and ripe *A. marmelos* fruit was shown to have considerable lipoxygenase inhibitory action and free radical-scavenging activity at varied doses in an investigation into the fruit's potential medicinal uses [40].

2.4.5.2 b. Action against neurological diseases and cognitive function: According to a study examining the antioxidants in the leaf extract of *A. marmelos* potential for therapeutic use in Alzheimer's disease treatment, the ethyl acetate portion significantly reduced lipid peroxidation in the brain. Research that examined the routes of pain in mice by inducing writhing in them with acetic acid and using the tail-flick test revealed that methanol extract from *A. marmelos* leaves had a noteworthy analgesic effect that was dose-dependent. A study that examined the pharmacological justification for employing *A. marmelos* fruit as an analgesic found that the fruit's methanol extracts had both central and peripheral antinociceptive qualities [40].

2.4.5.3 c. Hepatoprotective activity: The degree of safety of *A. marmelos* was determined by a study assessing the harmful effects of the plant on Wistar rats. The extracts from *A. marmelos* demonstrated a broad therapeutic window and a high therapeutic index value, indicating that they are a medication with a large margin of safety. A research testing the hepatoprotective efficacy of *A. marmelos* against carbon tetrachloride-induced oxidative stress in male Wistar rats revealed lower levels of glutathione (GSH), GSH reductase, GPx, quinone reductase, and CAT. This indicated the restoration of hepatic antioxidant status at different concentrations. Out of all the extract concentrations evaluated, 25 mg/kg was shown to be the most therapeutically efficacious [40].

2.4.5.4 d. Effects of antidiabetes: Through a variety of routes, *A. marmelos* has demonstrated encouraging antidiabetic efficacy. Aqueous fruit extracts have been shown to improve insulin production and encourage partial regeneration of pancreatic β-cells in diabetic rat models, hence lowering blood glucose levels. Furthermore, leaf extracts have been shown to block the enzymes α-amylase and α-glucosidase, which aid in the digestion of carbohydrates, lowering postprandial blood glucose levels. With IC50 values of 46.21 and 42.07 µg/mL, respectively, the alcoholic extract of *A. marmelos* leaves markedly inhibited the enzymes α-amylase and α-glycosidase [46].

2.4.5.5 e. Antimicrobial characteristics: Antibacterial activity was evaluated against pathogenic bacterial strains. The aqueous extract showed its highest inhibitory effect against S. epidermidis, whereas the methanolic extract was most potent against *S. aureus* at a concentration of 40 mg/mL. In the hydroalcoholic extract, the strongest inhibition was observed against *E. aerogenes*, followed by *K. pneumonia* and *S. epidermidis*. The minimum inhibitory concentrations (MICs) for both the aqueous and methanol extracts of *Aegle marmelos* ranged from 10 mg/mL to 40 mg/mL, while those for the hydroalcoholic extract ranged from 40 mg/mL to 160 mg/mL [47].

2.4.5.6 f. Anti-inflammatory and analgesic effects: Experimental models have been used to assess *A. marmelos's* analgesic qualities. Research has shown that steam bark extracts have analgesic effects that are dose-dependent and on par with those of common analgesic medications. The tail flick test and acetic acid-induced writhes in rats and mice, respectively, were used to examine the analgesic effects of *A. marmelos's* steam bark extract at doses of 50, 100, and 200 mg/kg p.o. This implies that *A. marmelos* might be used as a natural pain reliever [48].

2.5 Warburgia salutaris (Warburgia breyeri, Chibaca salutaris) (Canellaceae)

2.5.1 Description

Other names for *Warburgia* include Peperbasboom, Isibhaha, and Pepperbark. For ages, the stem and leaves of this tree have been used to cure diseases caused by yeast, fungi, bacteria, and protozoa. It is considered one of the most valuable natural antimicrobials found in Africa. In traditional medicine, *Warburgia salutaris* bark is smoked for colds and coughs and used as an expectorant. It can also be applied topically to wounds and irritation. Previous research on *W. salutaris* revealed that it possessed promising antibacterial properties. This endangered tree species was then chosen for bioassay-guided purification to determine the active ingredients [49].

2.5.2 Geographical distribution

Warburgia salutaris, the pepper-bark tree, or Chiov. (Canellaceae), is a highly sought-after protected medicinal plant in eastern and southern Africa (van Wyk and Wink 2004). The tree typically reaches a height of 5 to 10 m (m), though it can occasionally reach 20 m. The species lives in bushveld, wooded ravines, and evergreen forests [50].

2.5.3 Ethnomedicinal appraisal of Warburgia salutaris

With its bark, leaves, and roots being widely utilized in traditional medicine, *W. salutaris* is one of the most beneficial species of medicinal plants found in Southern Africa [51]. Many medical conditions, such as blood disorders, respiratory issues, rheumatism, sores, stomach ulcers, toothaches, fever, headaches, colds, coughs, feverish symptoms, influenza, inflammations, irritability, and malaria and respiratory issues, can be treated with the bark, leaves, stems, and roots [52, 53]. For millennia, people have employed *W. salutaris* leaves and bark to treat diseases caused by yeast, fungi, bacteria, and protozoa. *W. salutaris* is used to treat severe coughs that typically produce purulent sputum. *W. salutaris* powdered bark is frequently combined with fat and applied as an ointment or treatment for wounds [25]. To treat recurrent headaches, powdered bark powder may occasionally be applied to a head incision [54].

2.5.4 Comparative phytoconstituent analysis for sustainable plant part substitution in Warburgia salutaris

Warburgia salutaris contains diverse bioactive phytoconstituents, predominantly drimane-type sesquiterpenoids, including warburganal, polygodial, mukaadial, salutarisolide, and related drimane derivatives, together with phenolic and flavonoid constituents [10].

The current work shows that *Warburgia salutaris* leaves can be a good substitute for bark in the extraction of polygodial and warburganal, two important bioactive chemicals

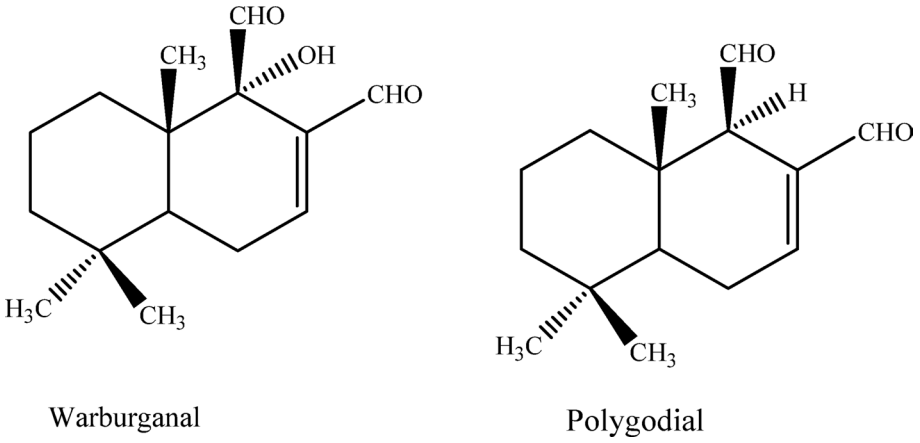


Fig. 3 Chemical structures of key bioactive compounds reported in *Warburgia salutaris* & considered for comparative phytochemical evaluation in plant part substitution studies

(Fig. 3). The leaves produce 0.05% polygodial and 0.06% warburganal, according to quantitative data, which are on par with or more than the equivalent bark yields of 0.03% and 0.06%, respectively. Additionally, 0.04% polygodial and 0.09% warburganal were produced by leaf samples from a Venda genotype, confirming their potential as a replacement. For the continuous use and commercial production of *W. salutaris*, replacing bark with leaves provides a sustainable and conservation-friendly method due to the simpler leaf matrix and the non-destructive nature of leaf harvesting [10].

Ethnomedicinal appraisal of Warburgia salutarisQ1Q2 Warburgia salutaris is native to eastern and southern Africa (e.g. Kenya, Tanzania, Mozambique, South Africa, Eswatini, Malawi, Zimbabwe) and is deeply embedded in local ethnomedicinal texts. Maasai healers in Kenya use decoctions of the bitter inner bark to treat malaria and pneumonia like chest infections. Likewise, rural healers in southern Africa (among Zulu, Venda, Shona and other groups) prepare boiled bark or leaf infusions as remedies for malaria, fever, colds, coughs, chest pains and gastrointestinal ailmeEthnomedicinal appraisal of Warburgia salutarisQ1Q2 Warburgia salutaris is native to eastern and southern Africa (e.g. Kenya, Tanzania, Mozambique, South Africa, Eswatini, Malawi, Zimbabwe) and is deeply embedded in local ethnomedicinal texts. Maasai healers in Kenya use decoctions of the bitter inner bark to treat malaria and pneumonia like chest infections. Likewise, rural healers in southern Africa (among Zulu, Venda, Shona and other groups) prepare boiled bark or leaf infusions as remedies for malaria, fever, colds, coughs, chest pains and gastrointestinal ailments. Bark is often dried and smoked or ground as a snuff to relieve congestion. Reported modes of administration therefore include oral decoctions and teas, inhalation of smoke, and topicals. W. salutaris bark and roots serve as a multipurpose traditional tonic/antiseptic across many African cultures, treating everything from malaria and respiratory infections to stomach aches, as documented in ethnobotanical and ethnomedical studies [52].

2.5.5 Pharmacological activities

W. salutaris bark extracts shown two significant biological activity. Both mycobacteria growth and recombinant mycobacterial NAT were suppressed by the extracts. Fr 30, the largely purified component that possessed the majority of these two properties, suppressed the growth of mycobacteria in a dose-dependent manner, but it had no effect on

the *M. bovis* BCG nat knockout strain, indicating that NAT might be the target within mycobacterial cells [55].

2.5.5.1 a. Bacteriostatic activity of *W. salutaris* extract and effect on cell morphology: Using the radiometric Bactec 460 TB system, the impact of 200 µg/mL of *W. salutaris* crude extract on the development of *M. tuberculosis* H37Rv was evaluated. During the course of the 11-day test period, the crude extract totally suppressed the development of *M. tuberculosis* H37Rv (data not shown). Anticipating this, treatment with isoniazid for the same amount of time at 0.05 µg/mL (MIC) totally suppressed the development of *M. tuberculosis* H37Rv (data not shown). The crude extract also suppressed *M. bovis* BCG growth in a concentration-dependent manner [55].

2.5.5.2 b. Impact of *W. salutaris* extracts on the action of recombinant NAT: In the presence of an arylamine or hydrazine substrate, NAT catalyzes the hydrolysis of AcCoA to produce free thiol, or CoA, which may be quantified by spectroscopy-photometry. For the enzymatic tests, 19 pure recombinant NAT was made, and isoniazid served as the substrate. Over the course of ten minutes, the starting velocity of the enzyme activity was monitored in the linear range. The acetylation reaction was suppressed by Fr 30 and *W. salutaris* crude extract [55].

2.5.5.3 c. Antimalarial action: The antimalarial action of *W. salutaris* is among its most noteworthy pharmacological characteristics. A strong anti-malarial agent has been found in iso-mukaadial acetate, a drimane sesquiterpene that was extracted from the stem bark. In vivo investigations employing a chloroquine-sensitive *P. berghei* model revealed a significant decrease in parasitemia and increased survival rates in treated rats, whereas in vitro investigations indicated an IC_{50} value of 0.44 µg/mL against Plasmodium falciparum. The chemical may be used as a lead compound for the development of antimalarial drugs because it showed little cytotoxicity in human cell lines [56].

2.5.5.4 d. Research on cytotoxicity and cancer extracts from *W. salutaris*: have been tested for their cytotoxic effects on a variety of cancer cell lines. The leaves' acetone extracts demonstrated strong anti-proliferative properties against MCF-7 cells, with an IC_{50} value of 34.15 µg/mL. These results imply that *W. salutaris* may have promise as a cancer treatment adjuvant, indicating the need for more investigation into its effectiveness and mechanisms of action [54].

2.5.5.5 e. Effects of antidiabetes: By evaluating iso-mukaadial acetate, the antidiabetic potential of *W. salutaris* has been investigated. Studies conducted in vitro showed that this substance improves glucose absorption in L6 rat myoblast cells, indicating that it may help increase insulin sensitivity. In L6 cells, iso-mukaadial acetate was shown to be non-toxic at 3 µg/mL. This discovery validates the plant's historic usage in diabetes treatment and calls for more research into its medicinal properties [57].

2.5.5.6 f. Antifungal and antibacterial properties: It has been shown that *W. salutaris* possesses broad-spectrum antibacterial action. Muzigadial, a sesquiterpenoid with strong antibacterial properties against Gram-positive bacteria, including Staphylococ-

cus aureus, was obtained from an ethyl acetate preparation of the stem bark. A minimal inhibitory concentration (MIC) of 12.5 to 100 μg/mL was identified. Furthermore, acetone extracts of the leaves shown antibacterial activity against S. aureus with a MIC of 0.16 mg/mL and anti-proliferative activities against cancer cell lines like MCF-7, with an IC_{50} value of 34.15 μg/mL [58].

3 Conclusion

This study demonstrates that targeted substitution of non-renewable organs (roots, bark, heartwood) with renewable aerial parts (leaves, young stems, small branches, fruits) offers a scientifically validated and ecologically sound strategy for conserving slow-growing, perennial medicinal species. Our review of five exemplar taxa *Myrica esculenta*, *Pelargonium sidoides*, *Litsea chinensis*, *Aegle marmelos* and *Warburgia salutaris* reveals that their aerial tissues possess profiles of alkaloids, flavonoids, glycosides, tannins and phenolic compounds comparable to those in traditionally harvested organs. For instance, small branches of *M. esculenta* match stem bark in total flavonoid and phenolic content, while leaves and stems of *P. sidoides* and *A. marmelos* yield gallic, salicylic and other bioactive acids at levels equivalent to underground parts. By maintaining therapeutic efficacy, plant-part substitution can substantially mitigate the overexploitation of wild populations, curb biodiversity loss and preserve ecosystem integrity. The National Medicinal Plants Board (NMPB and CCRAS under the Ministry of AYUSH, Government of India) promote the identification and scientific validation of substitutes for medicinal plants, especially threatened and high-demand species, as a means for sustainable resource utilisation and conservation.

Furthermore, this approach aligns with national and international conservation frameworks including India's Biological Diversity Act, the Nagoya Protocol, WHO-IUCN-WWF policy, UN SDGs 3, 13 and 15 thereby offering a replicable model for sustainable traditional-medicine systems.

4 Future perspectives

i. Policy integration

   Advocate for the formal incorporation of plant-part substitution guidelines into national and international biodiversity and pharmaceutical regulations to ensure long-term safeguarding of medicinal plant resources.

ii. Pharmacological validation

   Undertake rigorous in vivo and in vitro pharmacodynamic and toxicological studies to confirm therapeutic equivalence between substituted aerial parts and conventionally used organs across a wider range of disease models.

iii. Awareness and capacity building

   Develop targeted outreach programmes for traditional healers, cultivators and industry stakeholders highlighting ecological benefits, standardized harvesting

protocols and quality-control measures to facilitate broad acceptance of substitution practices.

iv. Sustainable harvesting and cultivation models

Design and implement best-practice guidelines and ex situ cultivation systems (e.g., controlled-environment agriculture, micropropagation) that prioritize renewable plant parts and optimize biomass yield without compromising genetic diversity.

v. Extension to additional species and ecosystems

Expand substitution research to other high-value medicinal taxa and diverse ecological regions, integrating ecological mapping, community-based monitoring and metadata on harvest methods to refine conservation management plans.

vi. Industry collaboration and product development

Partner with pharmaceutical, nutraceutical and herbal-product manufacturers to translate substitution strategies into scalable product pipelines, ensuring both market viability and resource sustainability.

vii. Integration with national and global goals

Continually align research outputs with the objectives of India's AYUSH mission, the IUCN's conservation priorities and relevant UN Sustainable Development Goals thereby contributing to resilient, validated and environmentally responsible healthcare systems.

Supplementary Information

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Supplementary Material 1

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Author contributions
M.M.: Material preparation, Writing—original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. A.D.: Visualization, Investigation, Formal analysis, Data curation. AG.: Literature review, Formal analysis, Data curation. SM.: Literature review, Data curation. S.P.: Writing—review & editing, Methodology, Investigation, Data curation, Conceptualization. P.I.: Writing—review & editing, Supervision, Methodology, Investigation, Data curation, Conceptualization. All authors read and approved the final manuscript.

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