



Secondary Metabolites in *Haplophyllum tuberculatum* (Forssk.) A. Juss and Their Biological Significance: A Comprehensive Review

Hemal Rana¹ and P. K. Patel²

¹Research Scholar, Department of Botany, S.P.T. Arts & Science College, Godhra, Gujarat.

²Department of Botany, S.P.T. Arts & Science College, Godhra, Gujarat, India

Email: ranahemal1311@gmail.com

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ABSTRACT

Haplophyllum tuberculatum (Forssk.) A. Juss, a perennial herb of the family Rutaceae, is a medicinally important plant widely distributed across North Africa, the Middle East, and Southwest Asia. It has been extensively used in traditional medicine systems for the treatment of malaria, arthritis, headaches, parasitic infections, and gynecological disorders. The plant is phytochemically rich, producing a diverse array of secondary metabolites including quinoline alkaloids, furoquinoline alkaloids, isoquinoline alkaloids, acridone alkaloids, aryl naphthalene lignans, furanocoumarins, flavonoids, phenolic compounds, terpenoids, saponins, amides, and volatile terpenoids in its essential oils. These secondary metabolites exhibit a broad spectrum of pharmacological activities encompassing antioxidant, antimicrobial, antifungal, antiprotozoal, anti-inflammatory, antidiabetic (α -glucosidase inhibition), anticancer, antiviral, and insecticidal effects. Recent studies have isolated three novel alkaloids from Omani *H. tuberculatum* as competitive α -glucosidase inhibitors with IC_{50} values as low as 2.28 μ M, significantly exceeding the standard drug acarbose. Lignans such as justicidin A, justicidin B, and tuberculatin have shown potent antiprotozoal activity against *Leishmania donovani*, *Trypanosoma brucei*, and *Plasmodium falciparum*. Essential oils characterized by GC-MS show β -caryophyllene, β -phellandrene, limonene, and myrcene as dominant volatiles with confirmed antifungal and insecticidal properties. This review compiles and critically analyses the current state of knowledge on secondary metabolites of *H. tuberculatum* and their documented biological significance, highlighting research gaps and future prospects for drug discovery.

Keywords: *Haplophyllum tuberculatum*, secondary metabolites, alkaloids, lignans, flavonoids, antioxidant, antimicrobial, anticancer, α -glucosidase inhibition, essential oils, Rutaceae



1. INTRODUCTION

Plants are the most prolific producers of structurally diverse secondary metabolites — natural compounds not directly involved in primary metabolic processes but playing crucial roles in plant defence, signalling, and ecological interactions. These metabolites also constitute the primary source of leads for modern drug discovery. The family Rutaceae, comprising approximately 2,100 species distributed across tropical and subtropical regions, is known for its chemical richness in alkaloids, coumarins, flavonoids, and essential oil constituents with wide pharmacological relevance (Tasci & Kaya, 2021).

The genus *Haplophyllum* A. Juss. (Rutaceae) encompasses approximately 70–133 species of low shrubs and perennial herbs distributed across the subtropical and tropical regions of the Old World, markedly in Iran, Turkey, North Africa, and Central Asia (Raissi et al., 2016). Among these, *Haplophyllum tuberculatum* (Forssk.) A. Juss. stands out as one of the most extensively studied and medicinally utilised species. It is a gland-covered perennial herb that flourishes in sandy hills and rocky slopes of arid regions, known locally as "Harmal" or "Shajarat al-Rutha" across its range (Al-Rawahi et al., 2024).

Ethnopharmacologically, *H. tuberculatum* has been used in Oman for the treatment of headaches, arthritis, warts, freckles, skin discolouration, parasitic diseases, and infections; in Saudi Arabia for malaria, rheumatoid arthritis, and gynaecological disorders; and in Tunisia as an anti-parasitic against *Enterobius vermicularis* and as a tea flavouring (Al-Rawahi et al., 2024; Hamdi et al., 2017). These diverse traditional uses are consistent with the broad-spectrum pharmacological activities documented in contemporary scientific literature.

Phytochemical investigations have established *H. tuberculatum* as a rich source of secondary metabolites spanning multiple chemical classes. Scientific interest in this species has grown substantially in recent years, driven by the discovery of novel alkaloids with potent antidiabetic activity, the isolation of antiprotozoal lignans, the characterisation of bioactive essential oils, and growing evidence for anticancer potential. Despite this progress, systematic reviews integrating phytochemistry with biological activity data remain limited (Raissi et al., 2016; Tasci & Kaya, 2021).

The present review aims to provide a comprehensive and updated synthesis of current knowledge on the secondary metabolite profile of *H. tuberculatum* and the biological significance of its constituent phytochemicals, addressing their pharmacological potential from both traditional use and experimental perspectives, and identifying research gaps warranting future investigation.

2. BOTANICAL DESCRIPTION AND DISTRIBUTION

Haplophyllum tuberculatum (Forssk.) A. Juss. is a perennial herbaceous plant belonging to the tribe Ruteae, family Rutaceae. The plant typically grows to 20–60 cm in height, characterised by densely glandular-punctate leaves, yellowish flowers borne in terminal corymbs, and tuberculate fruits — the latter giving rise to the



species epithet "tuberculatum" (Raissi et al., 2016). The entire plant surface is covered with oil-secreting glands, which account for the high content of essential oils.

The species is distributed across a wide geographic range encompassing Morocco to western Pakistan, including Algeria, Tunisia, Libya, Egypt, Sudan, Saudi Arabia, Yemen, Oman, Iran, and parts of Central Asia (Hamdi et al., 2017). It typically colonises arid and semi-arid habitats including sandy plains, rocky slopes, gravel plains, and disturbed land. Within the genus *Haplophyllum*, *H. tuberculatum* represents the sole species present in the Sultanate of Oman (Al-Rawahi et al., 2024).

3. SECONDARY METABOLITE PROFILE

H. tuberculatum elaborates an exceptionally diverse range of secondary metabolites. These can be broadly categorised into nitrogen-containing compounds (alkaloids, amides), oxygen heterocycles (coumarins, flavonoids), phenylpropanoids (lignans), terpenoids (essential oils, sterols), and polyphenolics (tannins, phenolic acids). The following sections review each class in detail.

3.1 Alkaloids

Alkaloids represent the most chemically diverse and pharmacologically significant class of secondary metabolites in *H. tuberculatum*. Multiple classes have been identified: quinoline alkaloids, furoquinoline alkaloids, isoquinoline alkaloids, acridone alkaloids, amide alkaloids, and quinolone alkaloids (Tasci & Kaya, 2021; Raissi et al., 2016).

3.1.1 Quinoline and Furoquinoline Alkaloids

Furoquinoline alkaloids are characteristic of the family Rutaceae and have been consistently reported from *H. tuberculatum*. Notable compounds include dictamine, γ -fagarine, skimmianine, and flindersine. Dictamine and skimmianine have been reported to exhibit significant antimicrobial and antiprotozoal activities (Al-Nour et al., 2021, cited in Agour et al., 2024). γ -Fagarine has demonstrated muscle relaxant and vasodilatory effects. Al-Rehaily et al. (2001) reported the isolation of several alkaloids from the aerial parts of Saudi Arabian *H. tuberculatum*, including (+)-dihydroperfamine, providing early structural characterisation of these compounds.

3.1.2 Novel Alkaloids — Antidiabetic Significance

A landmark 2024 study by Al-Rawahi et al. isolated three structurally novel alkaloids from Omani *H. tuberculatum* and evaluated them as competitive α -glucosidase inhibitors. These alkaloids demonstrated remarkably potent antidiabetic activity, with IC_{50} values as low as 2.28 μ M — substantially lower than the reference standard acarbose — representing among the most potent plant-derived α -glucosidase inhibitors



reported to date (Al-Rawahi et al., 2024). This discovery significantly elevates the pharmacological relevance of the alkaloid fraction.

3.1.3 Amide Alkaloids

Amide alkaloids, including tuberine, tubacetine, and tubasenicine, are structurally distinctive compounds reported from *H. tuberculatum*. These compounds bear cinnamoyl and feruloyl moieties and have been implicated in the anti-inflammatory and analgesic activities of the plant (Raissi et al., 2016). Djouahra Fahem et al. (2023) confirmed the presence of alkaloids in Algerian *H. tuberculatum* and demonstrated their significant antibacterial activity against both Gram-positive and Gram-negative pathogens using TLC characterisation and disc diffusion methods.

3.2 Lignans and Arylnaphthalene Lignans

Lignans constitute another major and biologically significant class of secondary metabolites in *H. tuberculatum*. Arylnaphthalene lignans — justicidin A, justicidin B, diphyllin, and tuberculatin — have been consistently isolated from this species (Al-Rehaily et al., 2001; Sheriha & Abou Amer, 1984).

Tuberculatin, a novel lignan apioside, was first isolated from *H. tuberculatum* and its structure established as 4-O-(β -D-apiofuranosyl)-6,7-dimethoxy-1-(3',4'-methylenedioxyphenyl)-3-hydroxymethylnaphthalene-2-carboxylic acid lactone. It co-occurs with justicidin A, justicidin B, and diphyllin (Sheriha & Abou Amer, 1984). Justicidin B, in particular, has been characterised as a potent cytotoxic lignan active against chronic myeloid and chronic lymphoid leukaemia cell lines, and also demonstrates antiprotozoal, antifungal, antiviral, and antiplatelet properties (Hemmati & Seradj, 2016).

In a landmark antiprotozoal study, Mukherjee et al. (2020) tracked the antileishmanial activity of *H. tuberculatum* root extracts using HPLC-based activity profiling, isolating eight bioactive compounds from the chloroform fraction. Among these, lignans tetrahydrofuroguaiacin B, nectandrin B, furoguaiaoxidin, and 3,3'-dimethoxy-4,4'-dihydroxylignan-9-ol demonstrated significant activity against *Leishmania donovani* axenic amastigotes, *Trypanosoma brucei rhodesiense*, and *Plasmodium falciparum* (Mukherjee et al., 2020). Compounds 1, 2, and 5–13 were reported for the first time from *Haplophyllum* species and the Rutaceae family, underscoring the novelty of this plant's lignan chemistry.

3.3 Flavonoids and Phenolic Compounds

Polyphenolic compounds, particularly flavonoids and phenolic acids, are abundantly present in *H. tuberculatum* and are primarily responsible for its antioxidant and anti-inflammatory activities.

Hamdi et al. (2017) conducted a detailed UPLC-ESI-MS analysis of *H. tuberculatum* leaf extracts and demonstrated that glycosylated flavonols dominate the polyphenolic profile. The ethyl acetate extract was found



to be richest in phenolics, with a total phenolic content (TPC) of 262 mg gallic acid equivalents/g and total flavonoid content (TFC) of 99.1 mg quercetin equivalents/g of dry weight — exceptionally high values indicative of strong antioxidant potential. Individual compounds identified include rutin, kaempferol, quercetin, myricetin, and resveratrol.

Sellami et al. (2022) performed LC-MS analysis of both aqueous and ethanolic whole plant extracts and reported that glycosylated flavonols constituted 65.37% and 68.77% of the total identified compounds in the ethanolic and aqueous extracts, respectively. This dominance of flavonol glycosides was correlated with the significant antioxidant, analgesic, anti-inflammatory, and wound healing activities observed in vivo (Sellami et al., 2022).

Al-Saeghi et al. (2022, cited in Agour et al., 2024) reported antibacterial activity of multiple fractions (methanolic, hexanic, chloroformic, ethyl acetate, butanolic, and aqueous) against both Gram-positive and Gram-negative bacterial strains, with the phenolic-rich fractions showing strongest activity.

3.4 Essential Oils and Volatile Terpenoids

The essential oil of *H. tuberculatum* has been characterised from specimens collected across its geographic range — Oman, Saudi Arabia, Morocco, Egypt, Tunisia, and Iran — using GC-MS and GC-FID analysis following hydrodistillation. Despite some variation with geographic origin, common dominant constituents consistently emerge.

Al Yousuf et al. (2005) identified 30 compounds in Omani *H. tuberculatum* essential oil, constituting 99.7% of total oil, with β -phellandrene (23.3%), limonene (12.6%), (Z)- β -ocimene (12.3%), β -caryophyllene (11.6%), myrcene (11.3%), and α -phellandrene (10.9%) as dominant constituents. The essential oil exhibited antibacterial activity, partially inhibiting *E. coli*, *Salmonella choleraesuis*, and *Bacillus subtilis* growth at 25 mg.

Jaradat et al. (2023) characterised Moroccan *H. tuberculatum* essential oil (HTEO) and detected 34 compounds with β -caryophyllene as the major constituent (36.94%). This study demonstrated antifungal activity particularly against *Aspergillus flavus* (inhibition rate 76.50% $\pm$ 0.60%) with MIC values ranging from 20.53 to 76.26 mg/mL. In silico molecular docking confirmed the antifungal mechanism through interaction with FAD glucose dehydrogenase of *A. flavus* and β -1,4-endoglucanase of *A. niger*. The HTEO also displayed high total antioxidant capacity (758.34 mg AAE/g EO) and potent insecticidal activity against *Callosobruchus maculatus* with LC₅₀ values of 30.66 and 40.28 μ L/100g for contact and inhalation tests, respectively (Jaradat et al., 2023).

Sabry et al. (2016) demonstrated cytotoxic activity of Egyptian *H. tuberculatum* essential oils against lung carcinoma (H-1299) and liver carcinoma (HepG2) cell lines, with IC₅₀ values of 4.1 μ g/mL and 4.7 μ g/mL, respectively. The cytotoxic activity was attributed to the dominance of α - and γ -terpinene in the oil.



3.5 Coumarins and Furanocoumarins

Furanocoumarins and psoralens are UV-fluorescent constituents identified in *H. tuberculatum* and are characteristic of the Rutaceae family. These compounds are detectable by their blue-violet fluorescence at 365 nm under UV light. Furanocoumarins from Rutaceae species are known for their photosensitising properties and have demonstrated photoactivated antimicrobial, anticancer, and antiprotozoal activities in other species of the family. Their specific contribution to the biological activities of *H. tuberculatum* merits dedicated investigation using HPLC-based profiling coupled with bioassays (Raissi et al., 2016).

3.6 Saponins and Sterols

Steroid saponins have been isolated from the water fraction of *H. tuberculatum* root extracts (Mukherjee et al., 2020). These saponins are structurally related to molluscicidal saponins previously described from *Balanites aegyptiaca* and are implicated in the molluscicidal activity reported for *H. tuberculatum*. Qualitative phytochemical screening consistently confirms the presence of saponins (foam test) and sterols/triterpenoids (Salkowski and Liebermann-Burchard tests) across extracts of different polarities (Hamdi et al., 2017).

4. BIOLOGICAL SIGNIFICANCE — PHARMACOLOGICAL ACTIVITIES

4.1 Antioxidant Activity

Antioxidant activity represents one of the best-characterised pharmacological properties of *H. tuberculatum* and has been demonstrated consistently across multiple studies using DPPH, ABTS, FRAP, β -carotene bleaching, and phosphomolybdenum (TAC) assays.

Hamdi et al. (2017) established that the ethyl acetate extract of *H. tuberculatum* leaves exhibited strongest DPPH radical scavenging activity, consistent with its high TPC (262 mg GAE/g) and TFC (99.1 mg QE/g). Al-Burtamani et al. (2005) evaluated Omani *H. tuberculatum*: ethyl acetate extract showed highest antioxidant capacity — a pattern replicated across geographic populations (ethyl acetate > butanol > water > chloroform > hexane > methanol; Al-Nour et al., cited in Agour et al., 2024). Sellami et al. (2022) confirmed robust antioxidant capacity in both aqueous and ethanolic whole plant extracts correlated with high flavonol glycoside content.

Jaradat et al. (2023) reported a remarkably high total antioxidant capacity of 758.34 mg AAE/g EO for the essential oil of Moroccan *H. tuberculatum*, demonstrated by in silico docking of key volatile constituents against NAD(P)H oxidase. This dual (phytochemical and essential oil) antioxidant potential makes *H. tuberculatum* an attractive candidate for natural antioxidant applications.



4.2 Antimicrobial Activity

Antibacterial and antifungal activities of *H. tuberculatum* extracts and essential oils have been reported against a broad panel of clinically relevant microorganisms.

Djouahra Fahem et al. (2023) characterised alkaloid and polyphenol extracts from Algerian *H. tuberculatum* and demonstrated significant antibacterial activity using disc diffusion and agar dilution MIC determination. Al Yousuf et al. (2005) showed that 25 mg of essential oil inhibited *E. coli*, *S. choleraesuis*, and *B. subtilis* growth equivalently to 0.10 µg of gentamycin sulfate, suggesting potent broad-spectrum antibacterial effects. Hassan et al. (2020) provided the first report of antiviral properties in Egyptian *H. tuberculatum*, showing significant inhibition of Tobacco Mosaic Virus (TMV).

Antifungal activity has been documented against phytopathogenic fungi. Antimicrobial compounds isolated from *H. tuberculatum* aerial parts showed MIC values of 62.5 µg/mL against *Verticillium dahliae* and *Fusarium oxysporum*, and 125 µg/mL against *Alternaria solani* (ResearchGate, 2020). Jaradat et al. (2023) demonstrated 76.50% inhibition of *Aspergillus flavus* growth by HTEO.

4.3 Antiprotozoal Activity

The antiprotozoal potential of *H. tuberculatum* represents one of its most compelling pharmacological assets, validated through rigorous HPLC-based activity profiling.

Mukherjee et al. (2020) screened *H. tuberculatum* as part of a systematic evaluation of Sudanese medicinal plants and found significant bioactivity of the chloroform and water root fractions against *Leishmania donovani* at 10 µg/mL. Activity-guided fractionation isolated eight active compounds — four lignans (tetrahydrofuroguaiacin B, nectandrin B, furoguaiadoxidin, and 3,3'-dimethoxy-4,4'-dihydroxylignan-9-ol) and four cinnamoylphenethyl amides. These compounds exhibited IC₅₀ values in the µM range against *L. donovani* axenic amastigotes, *T. brucei rhodesiense*. Anti-malarial activity against *P. falciparum* (NF54 strain) was also confirmed (Mukherjee et al., 2020).

Justicidin B, an aryl-naphthalene lignan consistently isolated from *H. tuberculatum*, has separately been documented as a trypanocidal agent with activity against *Trypanosoma brucei*, making it a promising lead for treatment of neglected tropical diseases (Hemmati & Seradj, 2016).

4.4 Anti-inflammatory and Analgesic Activity

Sellami et al. (2022) investigated the anti-inflammatory and analgesic potential of aqueous (HTAE) and ethanolic (HTEE) whole plant extracts of *H. tuberculatum* in vivo. Both extracts demonstrated significant anti-inflammatory activity in carrageenan-induced paw oedema and analgesic activity in acetic acid-induced writhing tests in Wistar rats. The activity correlated with the high content of glycosylated flavonols (65–69% of identified compounds). Additionally, wound healing activity was confirmed by the excision wound model,



with the ethanolic extract promoting significantly faster wound contraction compared to control (Sellami et al., 2022).

The aqueous aerial extract has also been reported to promote wound healing and display anti-inflammatory activity, validating its ethnopharmacological use (Jaradat et al., 2023). The sesquiterpene β -caryophyllene, a major essential oil component, is itself a known CB2 receptor agonist with potent anti-inflammatory activity, potentially contributing to these effects (Jaradat et al., 2023).

4.5 Antidiabetic Activity — α -Glucosidase Inhibition

Type 2 diabetes mellitus (T2DM) is a global health crisis characterised by postprandial hyperglycaemia, largely managed by α -glucosidase inhibitors that delay intestinal carbohydrate digestion. Plant-derived α -glucosidase inhibitors, particularly alkaloids and flavonoids, represent important leads for T2DM drug development.

Al-Rawahi et al. (2024) isolated three new quinoline alkaloids from Omani *H. tuberculatum* and demonstrated competitive α -glucosidase inhibition with IC_{50} values reaching as low as 2.28 μ M — a potency that substantially surpasses the clinical standard acarbose. Kinetic analysis confirmed competitive inhibition mode, and in silico molecular docking supported the binding mechanism at the active site of α -glucosidase. This discovery positions *H. tuberculatum* alkaloids among the most promising plant-derived antidiabetic compounds known, warranting advanced preclinical investigation (Al-Rawahi et al., 2024).

4.6 Anticancer Activity

Mounting evidence supports the anticancer potential of *H. tuberculatum* constituents across multiple tumour types.

Sabry et al. (2016) reported cytotoxic activity of Egyptian *H. tuberculatum* essential oil against lung carcinoma (H-1299) and liver carcinoma (HepG2) cell lines with IC_{50} values of 4.1 and 4.7 μ g/mL, respectively, attributing the activity to α - and γ -terpinene.

Al-Nour et al. (2021, cited in Agour et al., 2024) demonstrated anticancer potential of the aerial part ethanolic extract with cytotoxic activity linked to phytochemicals including polygamain, justicidin A, justicidin B, γ -fagarine, skimmianine, haplotubine, kusunokinin, and flindersine. Justicidin B, in particular, is a well-characterised cytotoxic lignan with potent activity against chronic myeloid and lymphoid leukaemia cell lines (Hemmati & Seradj, 2016).

Recent research has explored the anticancer mechanism of astragalin (ASG), a flavonoid isolated from *H. tuberculatum*. Studies demonstrated that ASG suppresses aerobic glycolysis in MDA-MB-231 breast cancer cells through the AMPK/mTOR signalling pathway, inhibiting glycolytic enzymes HK-2, LDH-A, and GLUT-1 expression, reducing glucose uptake and lactate production, and thereby inhibiting cell proliferation.



This mechanistic insight identifies a novel mode of anticancer action by a *H. tuberculatum*-derived flavonoid (ResearchGate, 2025).

Brine shrimp lethality (BSL) assays have confirmed cytotoxicity of hexane, chloroform, and ethyl acetate fractions of *H. tuberculatum* at 500 µg/mL concentration, with chloroform extract showing highest IC₅₀, consistent with its alkaloid and lignan content (Al-Burtamani et al., cited in Agour et al., 2024).

4.7 Insecticidal Activity

Jaradat et al. (2023) demonstrated potent insecticidal activity of *H. tuberculatum* essential oil against *Callosobruchus maculatus* (bean weevil), a major stored-grain pest. Contact and inhalation LC₅₀ values of 30.66 and 40.28 µL/100g were recorded after 24 h. A dose of 5 µL/100g significantly reduced oviposition (48.85%) and inhibited adult emergence (45.15%) compared to control groups. This insecticidal activity positions *H. tuberculatum* essential oil as a potential eco-friendly biopesticide alternative to synthetic insecticides (Jaradat et al., 2023).

4.8 Phytotoxic and Molluscicidal Activities

Crude extracts and isolated compounds from *H. tuberculatum* have demonstrated significant phytotoxic activity. In seed germination inhibition and seedling growth assays, crude extracts and alkaloid compound 4 significantly inhibited root and shoot growth of *Echinochloa crus-galli*, suggesting allelopathic potential relevant to weed management (ResearchGate, 2020). Molluscicidal activity, likely attributable to saponins and specific alkaloids, has also been documented and is consistent with the steroid saponin profile of the plant (Mukherjee et al., 2020).

5. DISCUSSION

The cumulative body of evidence reviewed herein establishes *H. tuberculatum* as a phytochemically exceptional plant with diverse and potent pharmacological activities. Several points warrant critical discussion.

The alkaloid fraction, particularly the furoquinolines and newly isolated novel quinoline alkaloids, emerges as most pharmacologically significant due to the unprecedented α-glucosidase inhibitory potency (IC₅₀ 2.28 µM; Al-Rawahi et al., 2024). This represents a major lead for T2DM drug development, though in vivo and toxicological studies are necessary before advancement to clinical trials.

The lignan fraction, led by justicidin A, justicidin B, and nectandrin B, demonstrates highly promising antiprotozoal and cytotoxic activities. The systematic HPLC-based activity profiling approach used by Mukherjee et al. (2020) is exemplary in linking chemical constituents to specific biological activities and



should be replicated for other fractions. Justicidin B's documented activity against *T. brucei* and leukaemia cell lines (Hemmati & Seradj, 2016) identifies it as a high-priority lead compound.

The polyphenolic fraction's antioxidant capacity — with TPC up to 262 mg GAE/g in the ethyl acetate extract (Hamdi et al., 2017) — is exceptional and supports the plant's use in treating inflammatory conditions. The dominance of glycosylic flavonols identified by Sellami et al. (2022) provides a mechanistic basis for observed anti-inflammatory and wound healing activities.

The essential oil, while showing some geographic variation in composition, consistently provides antimicrobial, antifungal, insecticidal, and cytotoxic activities. The β -caryophyllene-dominant oils (Jaradat et al., 2023) show particularly strong antifungal and antioxidant profiles. The proven cytotoxicity of essential oil fractions against H-1299 and HepG2 cancer lines (Sabry et al., 2016) merits further mechanistic investigation. Key research gaps identified include: (i) lack of systematic in vivo pharmacological studies for most activities; (ii) absence of comprehensive toxicity profiling (acute, subacute, and chronic); (iii) minimal mechanistic studies at the molecular level for most compound-activity pairs; (iv) limited studies on nanoparticle synthesis from *H. tuberculatum* material for enhanced biodelivery; (v) absence of published reports specifically addressing genetic fidelity of in vitro propagated material relative to phytochemical output.

6. CONCLUSION

Haplophyllum tuberculatum represents a pharmacognostically outstanding plant whose secondary metabolite repertoire spans alkaloids, lignans, flavonoids, furanocoumarins, terpenoids, saponins, and amides — each associated with distinct and often potent biological activities. The recent discovery of novel alkaloids as ultra-potent α -glucosidase inhibitors and the well-documented antiprotozoal lignans position this species as a high-priority target for drug discovery research. Its antioxidant, antimicrobial, anti-inflammatory, cytotoxic, insecticidal, and wound healing activities are scientifically validated across multiple independent studies. The essential oil, dominated by β -caryophyllene, β -phellandrene, and limonene, adds a volatile terpenoid dimension to its pharmacological potential.

Future research should prioritise: (i) in vivo validation of antidiabetic, anticancer, and antiprotozoal activities; (ii) mechanism-of-action studies at the molecular level; (iii) comprehensive toxicity evaluation; (iv) biosynthesis studies of nanoparticles from plant material with enhanced bioavailability; and (v) integrated phytochemical and genetic fidelity studies on tissue culture-derived material. Such multidisciplinary investigations will be pivotal in translating the rich secondary metabolite diversity of *H. tuberculatum* into validated therapeutic applications.



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