

## Bioactive Compounds of *Stelechocarpus burahol* (Kepel) from Indonesia for Future Perspective of Health: A Literature Review

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### ABSTRACT

*Stelechocarpus burahol* (Kepel) is widely distributed in Indonesia, where its fruit has traditionally been consumed and used as a natural body fragrance from ancient times to the present. In recent decades, this species has attracted growing interest in the health sector due to its rich content of bioactive compounds. However, the natural population of *S. burahol* has declined over time and is currently regarded as a conservation species. This article presents an overview of the therapeutic potential of *S. burahol* and highlights *in vitro* cultivation strategies aimed at optimizing the production of bioactive metabolites. The review is based on an extensive analysis of scientific journals, conference proceedings, theses, and reference books, using keywords such as *S. burahol*, Kepel, bioactive compounds, and *in vitro* culture. Available evidence indicates that *S. burahol* represents a promising target for bioprospecting in the health sector, particularly due to its reported anti-inflammatory, antioxidant, antibacterial, anticancer, anti-acne, antidiabetic, antihyperuricemic, and anti-implantation activities. Furthermore, *in vitro* cultivation approaches offer a viable alternative to conventional field cultivation, which remains limited, for the enhanced production of bioactive compounds from this species.

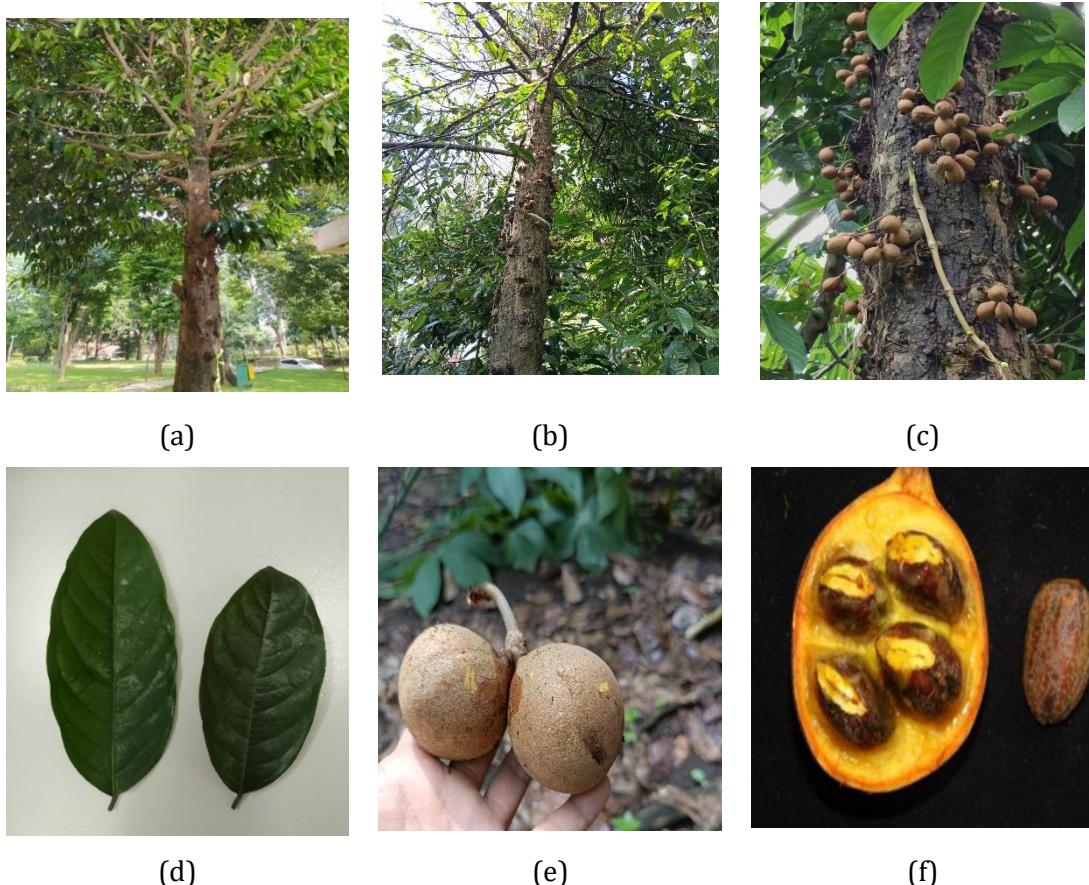
## INTRODUCTION

*Stelechocarpus burahol* or Kepel, part of the *S. burahol* species (Blume), *Stelechocarpus* genus, *Annonaceae* family, *Magnoliales* order, *Magnoliopsida* class, *Magnoliophyta* division, and *Plantae* kingdom (Soeroto et al., 2018). The type of *Annonaceae* plants such as shrubs, trees, or lianas (Lestari et al., 2017; Lestari & Ningrum, 2021; Chatrou et al., 2012; Chaowasku et al., 2018; Probojati et al., 2023). The *Annonaceae* family has different local names in each region in Indonesia. Other names *S. burahol* are turalak (in Sunda) or simpol, kecindul, and cinful (Probojati et al., 2023). *S. burahol* and *S. cauliflory* are *Annonaceae* that are native plants of Indonesia, especially in Java, and Kalimantan (Sundari et al., 2023; Chatrou et al., 2012). *Annona muricata*, *Annona squamosa*, *Annona montana*, and *S. burahol*, as members of the *Annonaceae* family,

are widely used for health by the community. *S. burahol* is an uncommon species found in Indonesia (Pamungkas et al 2023). The leaves of *Annona muricata* and *S. burahol* used by local people in Yogyakarta for therapeutics for hypertension and gout (Santoso et al., 2024). This plant thrives in lowland secondary forests, typically at elevations of 600 meters, and blossoms during September and October (Pamungkas et al 2023). *S. burahol* is a plant with a trunk that can grow up to 25 m tall and has a diameter of about 40 cm (Figure 1) (Pamungkas et al 2023; Sundari et al., 2023). The fruit can begin to mature between six and eight years after it has been planted, appears brownish, and clusters grow directly on the trunk (Handayani et al. 2020). The size of the Kepel fruit has a length of around 8 cm, and has a diameter of approximately 5–6 cm, which is shaped like an

oval. It is high in vitamin C and consists of roughly 10% water. The leaves of the Kepel tree are elongated, glossy, and dark green in color (Handayani et al. 2021). The seeds in the fruit are estimated to be 3 cm long, oval, and each fruit consists of 4 or 6 seeds (Shadrina et al.,

2022). *S. burahol* fruit is edible and can help prevent body odor (Darusman et al., 2012). People have traditionally used *S. burahol* as a breath freshener and to make their bodies smell good (Poniewierka, et al 2022; Amin et al., 2017; Radji, 2022).



**Figure 1. Morphology of Kepel (*S. burahol*) plant (a) trees, (b) tree trunk, (c) tree trunk with fruit, (d) leaves, (e) fruit, and (f) flesh fruit and seed (Shadrina et al., 2022)**

## METHODS

A comprehensive literature search was conducted using major academic databases, theses, books, and institutional repositories, covering peer-reviewed journals and conference proceedings. In ancient times, *Stelechocarpus burahol* was used as a perfume ingredient, particularly by princesses in royal palaces. In recent decades, studies have demonstrated that *S. burahol* contains various bioactive compounds and has potential as a bioprospective therapeutic agent. However, the population of *S. burahol* has become increasingly rare and has declined over time (Handayani et al., 2020; Pamungkas et al., 2023). Therefore, studies on the cultivation of *S. burahol* (kepel) are of

significant interest and warrant further exploration. The search terms used included “*Stelechocarpus burahol*”, “kepel”, “bioactive compounds”, “secondary metabolites”, and “in vitro cultivation”. The databases were initially screened based on relevance to the research scope, followed by full-text evaluation. A total of eighteen eligible studies were included in this review. The inclusion criteria comprised studies focusing on *S. burahol*, kepel, or other medicinal plants that reported bioactive compounds, secondary metabolites, biological activities, or applications of in vitro callus culture. Studies unrelated to *S. burahol* or medicinal plants, or those lacking experimental data, were excluded. Data synthesis was conducted from January to April 2025.

## RESULT AND DISCUSSION

### Therapeutic properties of *S. burahol*

*S. burahol* (Kepel) demonstrates considerable potential as a novel source of bioactive compounds and as a candidate for drug discovery (Table 1). A toxicological assessment using the Brine Shrimp Lethality Test (BSLT) indicated that the ethanol extract of *S. burahol* fruit did not exhibit toxic effects (Rahmawati et al., 2024). The absence of lethality in *Artemia salina* larvae indicates that the extract possesses a high safety margin and low acute toxicity, supporting its potential for pharmaceutical applications. These findings are consistent with previous studies, which are generally recognized for their therapeutic benefits and low toxicity of natural products derived from *S. burahol*.

### Anti-halitosis activity

*S. burahol* is a promising plant species for potential anti-halitosis properties. Halitosis, or bad breath, commonly originates from the oral cavity and is primarily caused by anaerobic microorganisms that produce volatile sulfur compounds (VSCs), including methyl mercaptan ( $\text{CH}_3\text{SH}$ ), hydrogen sulfide ( $\text{H}_2\text{S}$ ), and dimethyl sulfide ( $(\text{CH}_3)_2\text{S}$ ) (Nakhleh et al., 2018; Amin et al., 2017). The water, methanol, ethanol, ethyl acetate, and butanol extracts of *S. burahol* fruit collected from Magelang, Central Java, contained flavonoids, polyphenols, and saponins (Amin et al. 2017). The ethanol extract exhibited the highest absorption capacity for the volatile sulfur compound methyl mercaptan, which is commonly produced by Gram-negative anaerobic oral bacteria. This suggests that *S. burahol* may serve as a natural agent for reducing halitosis through the inhibition or neutralization of sulfur compound production (Amin et al, 2017).

### Anti-inflammatory activity

The identification of multiple phenolic and iridoid compounds in the ethyl acetate extract of *S. burahol* fruit highlights its potential as a source of bioactive phytochemicals. Studied by Sundari et al. (2023) about the chemical composition of the Kepel plant (*S. burahol*), using the fruit flesh and peel obtained from Blitar, East Java. Notably, 1,5-dicaffeoylquinic acid (1,5-DCQA), also known as cynarin, and luteolin 7-O-

glucoside (cynaroside) are well-recognized for their strong antioxidant and anti-inflammatory activities, suggesting that these compounds may contribute significantly to the biological properties of the extract (De Stefano et al., 2021). In addition, the presence of iridoid derivatives, including the methyl hemiacetal form of 3,4-DHPEA-EA, further supports the potential anti-inflammatory effects of *S. burahol*, as iridoids are widely reported to exhibit anti-inflammatory activity across several plant families, such as *Rubiaceae*, *Plantaginaceae*, and *Scrophulariaceae* (Hussain et al., 2019). These findings suggest that *S. burahol* fruit peel has bioactive components that may contribute to its pharmacological potential, especially managing oxidative stress and inflammation.

### Antioxidant activity

Natural antioxidants encompass a wide range of compounds, including  $\beta$ -carotene, ascorbic acid, alkaloids, saponins, and tannins. Among these, phenolic constituents, such as flavonoids, cinnamic acid derivatives, coumarins, and tocopherols, are particularly recognized for their antioxidant capacities (Suryanti et al., 2016; Herlina et al., 2018; Suryanti et al., 2021; Suryanti et al., 2022). Flavonoids are among the major secondary metabolites responsible for the antioxidant activity of *S. burahol* (Kepel). Kepel, which were collected from Yogyakarta, have been reported to contain significant levels of flavonoids in their seeds. Rafiqoh et al. (2021) reported on the extracted and encapsulated flavonoids from Kepel seeds using various ethanol concentrations and extraction durations. The use of sodium tripolyphosphate (NaTPP) as a cross-linking agent yielded the optimal flavonoid encapsulation efficiency. Fourier Transform Infrared Spectroscopy (FT-IR) was used to characterize the chemical structure of the flavonoid compounds, while total flavonoid content (TFC) was quantified spectrophotometrically using UV-Vis analysis. The extract obtained with 50% ethanol produced the highest TFC after both one and two hours of extraction, indicating that moderate ethanol polarity facilitates optimal flavonoid solubility. The strength of antioxidant activity is commonly interpreted using  $\text{IC}_{50}$  values, which are categorized as weak (150–200 ppm), moderate (100–150 ppm), strong (50–100

ppm), and very strong (< 50 ppm) (Molyneux, 2003).

In addition, the antioxidant capacity of these extracts is commonly evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which is based on the reduction of the stable DPPH radical through the donation of an electron or hydrogen atom (Sunarni et al., 2007; Ibrahim et al., 2024). The presence of radical-scavenging antioxidants results in a color transition from violet to yellow, accompanied by a decrease in absorbance at 517 nm, signifying the formation of DPPH-H. This mechanism reflects the hydrogen-donating and radical-scavenging abilities of flavonoids, which are also known to neutralize superoxide radicals through similar pathways. Moreover, ethyl acetate extracts obtained from both the flesh and peel of *S. burahol* fruits have been reported to contain bioactive antioxidant compounds characterized by the presence of free hydroxyl groups on aromatic rings and conjugated double-bond systems (Sofyan et al., 2017). Among the major constituents identified, luteolin 7-O-glucoside (cynaroside) is a glycosyloxyflavone derived from luteolin, in which a  $\beta$ -D-glucopyranosyl moiety is linked at the C-7 position via a glycosidic bond. The deprotonated oxoanion form of luteolin 7-O- $\beta$ -D-glucoside has been shown to exhibit strong anti-inflammatory and antioxidant activities (Tian et al., 2021; De Stefano et al., 2021). These findings indicate that the antioxidant potential of *S. burahol* is closely related to its flavonoid composition, including luteolin derivatives, which play an important role in free radical scavenging and the modulation of cellular redox balance.

### Antibacterial activity

Phenolic compounds, alkaloids, saponins, tannins, and flavonoids are major classes of secondary metabolites that contribute significantly to the biological activities of medicinal plants. The ethyl acetate extract rich in phenolic compounds from *S. burahol* fruit peel exhibited inhibitory activity against *S. aureus*, but showed no inhibition against *E. coli* and *P. aeruginosa* (Sundari et al., 2023). The observed differences in antibacterial activity can be attributed to variations in the structural and functional characteristics of Gram-positive and Gram-negative bacterial cell walls, which related

in their metabolic pathways. In particular, phenolic compounds are generally more effective against Gram-positive bacteria, as they can interfere with the integrity of the peptidoglycan layer and increase membrane permeability, ultimately compromising bacterial cell viability (Aldulaim, 2017).

Oleuropein aglycone, a bioactive compound derived from *S. burahol* fruit peel extract, releases a methyl hemiacetal moiety from 3,4-DHPEA-EA and demonstrates potent antibacterial activity against *S. aureus* and *S. epidermidis* (Celano et al., 2018; Bisignano et al., 2014). The antibacterial mechanism of such phenolic derivatives is often associated with their capacity to induce oxidative stress in bacterial cells, leading to the disruption of vital cellular processes. Furthermore, *S. burahol* leaves contain hydroxyl groups within their flavone structures, which enhance their reactivity toward bacterial cell components and contribute to antibacterial effects (Sunarni et al., 2007). The methanol: water (7:3) extracts of *S. burahol* leaves, fractionated by isocratic elution using n-butanol: methanol: acetic acid (1:8:1) and purified through column chromatography, yielded a flavone-type compound identified via UV-Vis and FTIR spectroscopy with pronounced antibacterial activity (Indariani et al. 2017). Additionally, *S. burahol* exhibited inhibitory effects against *S. epidermidis*, as confirmed through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Shadrina et al., 2022). These findings suggest that the antibacterial activity of *S. burahol* is closely linked to its phenolic and flavonoid content, which play crucial roles in membrane disruption, radical scavenging, and the inhibition of microbial growth.

### Anti-acne activity

Acne is a prevalent inflammatory skin condition marked by increased sebum secretion, abnormal keratinization of the pilosebaceous follicle, microbial proliferation, and subsequent inflammatory responses (Nourin & Ballard, 2006). The primary causative agent is *Cutibacterium acnes*, a Gram-positive anaerobic bacterium commonly associated with pilosebaceous unit infections (Strauss et al., 2007). Phytochemical investigations of *S. burahol* leaves and fruits collected from Yogyakarta, Karang Anyar, Cilacap, and Nusa

Kambangan (Central Java) revealed the presence of tannins and flavonoids as major constituents (Rahmawati et al., 2010). The plant materials were extracted using ethyl acetate, methanol, and water for the leaves, while the fruits were sequentially extracted with hexane, ethyl acetate, and water. Interestingly, the leaf extract from the Nusa Kambangan sample contained only steroid compounds, indicating possible chemotypic variation influenced by environmental conditions. All extracts demonstrated the presence of vitamin C, suggesting additional antioxidant potential. However, antioxidant activity was generally low across the samples, and none of the extracts exhibited lipase inhibition activity (Rahminiati et al., 2010). The phytochemical profile rich in tannins, flavonoids, and vitamin C suggests potential utility of *S. burahol* extracts for acne therapeutics. These bioactive compounds are known to reduce oxidative stress, inhibit bacterial growth, and modulate inflammation, thereby addressing multiple pathogenic factors for acne therapeutics. Further investigations are warranted to isolate the bioactive constituents responsible for these effects and to substantiate their efficacy using both in vitro and in vivo experimental models.

### Anticancer activity

Phytochemical analyses have revealed that the flesh extract of *S. burahol* fruit contains anthocyanins, isoprenoids, epigallocatechin gallate (EGCG), and fatty aldehydes, whereas the peel extract predominantly contains flavonoids, tannins, luteolin 7-O-glucoside (cynaroside), and methyl hemiacetal in 3,4-DHPEA-EA (an iridoid compound) (Sundari et al., 2023). Among these constituents, EGCG is recognized as a potent antioxidant with well-documented anticancer and antiviral activities, including the inhibition of HIV replication (Abdallah et al., 2021; Pyrko et al., 2007). Furthermore, epigallocatechin gallate (EGCG) has been widely reported to exert protective effects against chronic conditions, including cardiovascular disease, diabetes, and osteoporosis, primarily by modulating oxidative stress levels and regulating inflammatory signaling pathways. Tannin group, and digalloyl glucose present in *S. burahol*, exhibits weak acidity and limited solubility in water (Bao et al., 2018). Tannins, as naturally occurring

polyphenols, are classified into four main categories: condensed tannins, hydrolyzable tannins, complex tannins, and phlorotannins. These compounds exert diverse biological effects, including antimicrobial, antioxidant, and anti-inflammatory activities, by interacting with proteins and metal ions. Iridoids, including methyl hemiacetal derivatives such as 3,4-DHPEA-EA, represent another important class of secondary metabolites identified in *S. burahol* fruit peel. Structurally, iridoids and their monoterpenoid derivatives consist of a cyclopentane ring fused to a pyran moiety or occasionally to an open-chain pentane structure. Iridoids isolated from various plant families, including *Scrophulariaceae*, *Rubiaceae*, and *Plantaginaceae*, have demonstrated notable anticancer and anti-inflammatory activities (Hussain et al., 2019). The presence of these iridoid and polyphenolic compounds in *S. burahol* supports its pharmacological potential as a multifunctional agent capable of mitigating oxidative stress, inflammation, and other pathological processes associated with chronic diseases.

### Antidiabetic and hypouricemic activity

*S. burahol* has long been used in traditional medicine to reduce uric acid levels and diuresis (Purwatiningsih et al., 2011; Diniatik et al., 2017). Hyperuricemia is a metabolic disorder in which uric acid accumulates in the blood and tissues due to impaired excretion (Johnson et al., 2018). Uric acid is the final product of purine metabolism, which occurs during the breakdown of nucleic acids (dinucleotide or ribonucleotide) via a series of enzymatic reactions (Mathews & Holde, 1990; Schunack et al., 1993). Excessive uric acid accumulation in the body, a condition known as hyperuricemia, can lead to gout, characterized by urate crystal deposition in joints and tissues (Mutschler, 1991; Purwatiningsih et al., 2011). The leaf extracts of *S. burahol* from Samigaluh, Yogyakarta, demonstrated significant hypouricemic effects in animal models. Both ethanol and hexane extracts were found to reduce serum uric acid concentrations, indicating potential inhibitory activity on uric acid biosynthesis (Purwatiningsih et al., 2011). In addition, flavonoid compounds isolated from the petroleum ether fraction of the methanol leaf

extract exhibited uric acid-lowering activity. The hypouricemic effect of flavonoids is attributed to their ability to inhibit xanthine oxidase, the key enzyme responsible for converting xanthine to uric acid during purine catabolism, and to their antioxidant capacity in scavenging superoxide radicals (Cos et al., 1998; Diniatik et al, 2017).

These findings suggest that the hypouricemic activity of *S. burahol* may result from the synergistic action of its bioactive components, especially flavonoids, which function both as enzyme inhibitors and as radical scavengers. The dual mechanism of xanthine oxidase inhibition and oxidative stress reduction highlights the therapeutic potential of *S. burahol* as a natural alternative for therapeutic hyperuricemia and gout-related conditions.

### Anti-implantation activity

An in vivo investigation of *S. burahol* collected from Krapyak, Yogyakarta, evaluated its potential antifertility activity in female Wistar rats with different doses of the ethanol extract. The treatment was given orally on a daily basis from the diestrus phase through the seventh day of gestation. Phytochemical analysis indicated that the extract contained a diverse range of secondary metabolites, including alkaloids, flavonoids, polyphenols, saponins, triterpenoids, and quinones (Sunardi et al., 2010). The extract showed a reduced number of offspring, suggesting that *S. burahol* exhibits antifertility properties by inhibiting implantation and inducing abortion (Sunardi et al., 2010). The evaluation of the anti-implantation activity of *S.*

*burahol* fruit flesh from Ambarawa, Semarang, Central Java, further supported these findings. The fruit flesh extract, prepared with dimethyl sulfoxide (DMSO), was tested in female mice to assess its effects on fetal mortality, ovarian weight, and endometrial thickness (Sunardi et al., 2010). The ethanol extract shows significant anti-implantation potential, indicating that Kepel fruit flesh may function as a natural antifertility agent.

The bioactive compounds present in *S. burahol*, including alkaloids, saponins, and tannins, are known to possess reproductive toxicity at specific concentrations. Alkaloids can interfere with embryonic development and inhibit fetal growth (Meiyanto et al., 2008). In addition, saponins may indirectly induce fetal death by decreasing follicle-stimulating hormone (FSH) secretion and disrupting pituitary function, leading to reduced estrogen levels. However, the absence of saponins, as indicated by the lack of embryo resorption, suggests that ovulation and early embryonic development were not adversely affected (Suparmi et al., 2015). These findings suggest that the antifertility activity of *S. burahol* is likely associated with its phytochemical constituents, such as alkaloids and flavonoids, which may act through anti-implantation and embryotoxic mechanisms. This highlights the potential of *S. burahol* extract as a candidate for natural contraceptive development, although further mechanistic and toxicological studies are necessary to clarify its safety and molecular mode of action.

**Table 1. Bioactive compound as therapeutics agent of *S. burahol***

Therapeutics agent	Bioactive Compound	Part of <i>S. burahol</i> plant	Source of <i>S. burahol</i>	Reference
Anti-halitosis	Flavonoid, saponin	polyphenol, and	Flesh of fruit	Magelang, Central Java
Anti-inflammatory	2,6,10,15,19,23-heksametil-2,6,10,14,18,22, tetrakosaheksaena (skualena) group (triterpene), Luteolin 7-O-glukosida (Cynaroside)	Peels/skin of fruit	Blitar, East Java	Sundari et al., 2023
Antioxidant	Flavon, flavonoid, 3,7,3',4' tetrahidroksi-5-metil	Leaves and seeds of a fruit	Patikraja District, Central Java; Blitar, East Java; Yogyakarta	Nurhidayah et al., 2022, Sundari et al.,

Therapeutics agent	Bioactive Compound	Part of <i>S. burahol</i> plant	Source of <i>S. burahol</i>	Reference
				2023 Rafiqoh et al., 2021
	Phenolic, and flavonoid	Fruit pulp	Yogyakarta	Herlina et al., 2018
	Antosianin (Pelargonidin-malonylrhamnoside); Isoprenoid (8-epiiridodial glukosida tetraasetat); Katekin (epigallocatechin gallate); and Aldehyda (5-oktadecenal)	Flesh of fruit;	Blitar, East Java	Sundari et al., 2023
	Flavonoid (Acid 1,5-dikafoikuinat); Tannins (1,6-di-o-galoilglukosa); Sianosida (luteolin 7-O-glukosa); Iridoid (metil hemiasetal 3,4-DHPEA-EA)	Peels/skin of fruit	Blitar, East Java	Sundari et al., 2023
Antibacterial	Phenolic, and methyl hemiacetal compounds	Peels/skin of fruit	Blitar, East Java	Sundari et al., 2023
	Flavonoid	Flesh of fruit and leaves	Cilacap, Central Java	Indariani et al., 2017
Anticancer	Epigallocatechin gallate, Digalloyl glukosa or 1,6-di-o-galloylglucose (tannins), and metil hemiasetal 3,4 DHPEA-EA (iridoids)	Flesh, and peel/skin of fruit	Blitar, East Java	Sundari et al., 2023
Anti-acne	Tanin, Flavonoid	Leaves	Cilacap, Nusakambangan, and Karang Anyar, Central Java; Yogyakarta	Rahminiwati et al., 2010
Antidiabetic and hypouricemic	Flavonoid; Kaempferol	Leaves	Yogyakarta	Purwatiningsih et al., 2011; Diniatik et al., 2017
Anti-implantation	Alkaloid compounds	Flesh of fruit	Yogyakarta; Semarang, Central Java	Sunardi et al., 2010; Suparmi et al., 2015

## Optimization of Bioactive Compound

The *S. burahol* or Kepel plant has long been recognized for its diverse pharmacological and health properties. Despite these benefits, its cultivation remains limited. The primary

constraint lies in its seeds, which exhibit prolonged dormancy and low germination rates, typically requiring 4–6 months to sprout (Handayani et al., 2020; Hutabarat et al., 2022). Moreover, the species is susceptible to

leafhopper (*Sanurus indecora*) infestation, has low economic value due to limited fruit yield relative to seed size, and shows poor vegetative propagation through cuttings or grafting (Sibarani et al., 2024; Saputra et al., 2024). These biological and agronomic limitations have contributed to a decline in natural populations, which their classification

as a species requiring conservation (Handayani et al., 2020; Pamungkas et al., 2023). Recent approaches have increasingly emphasized extraction techniques, including *in vitro* plant culture as in vitro propagation for maximizing strategies for the production of bioactive compounds (Table 2).

**Table 2. Treatment for producing bioactive compounds from *S. burahol* (Kepel)**

Treatments	Methods	Reference
Extraction	Used DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method	(Sunarni et al. 2007; Ibrahim et al. 2024)
	Used ethyl acetate, hexane, methanol, and water	(Rahminiwati et al. 2010)
	Used methanol, ethanol, and butanol	(Amin et al 2017)
	Used phenolic & flavonoid assay methods	(Handayani et al. 2016)
<i>In vitro</i> plant culture: callus culture and cell suspension culture	<ul style="list-style-type: none"> <li>Callus Culture using optimization factors</li> </ul> <p>Plant growth regulators (PGRs) such as high auxin levels for inducing friable callus in Kepel. Auxin and cytokinin ratio.</p> <p>Culture age: An 8-week-old callus shows optimal flavonoid accumulation</p> <p>Nutrient medium: Murashige and Skoog (MS) medium supports robust callus growth</p> <p>Stress induction: Mild abiotic stress can enhance phenolic and flavonoid biosynthesis</p> <ul style="list-style-type: none"> <li>Cell suspension culture using optimization factors</li> </ul> <p>Inoculum density: optimal cell density that synchronizes growth and metabolite accumulation</p> <p>Harvest time: maximum flavonoid production in Kepel cell suspensions is observed around day 12—15</p> <p>Agitation speed: ensures homogenous cell distribution and prevents aggregation</p> <p>Elicitation: addition of elicitors such as methyl jasmonate and salicylic acid for increasing secondary metabolite content</p>	(Isah, 2019), (Junairah et al. 2021) & (Semiarti et al. 2012)

The successful establishment of *S. burahol* *in vitro* cultures is highly dependent on effective sterilization and the elimination of endophytic contaminants. This studies reported that a rigorous multistep washing and surface-sterilization protocol is essential for obtaining axenic explants, given the high prevalence of endophytic fungi associated with *S. burahol*. The additional use of systemic

fungicide treatment on the plant appears to be a necessary pre-treatment strategy to further reduce internal contamination. These findings are consistent with reports on other tree and medicinal plant species, where endophytic microorganisms have a significant challenge in plant tissue culture establishment and can limit explant survival and growth [Habibah et al 2016].

Callus induction was successfully achieved using Murashige and Skoog (MS) medium supplemented with picloram, confirming the strong auxinic effect of this growth regulator in cellular dedifferentiation [Habibah et al. 2016; Isah, 2019]. The formation of an unorganized, proliferative callus mass indicates that the applied hormonal balance was effective in differentiating plant cells into a totipotent state. The observation that picloram supported optimal callus formation suggests that a 7.5 mg/L concentration is a suitable hormonal threshold for stimulating cell division without inducing excessive tissue browning or necrosis. The effectiveness of synthetic auxins in inducing callogenesis in recalcitrant or slow-growing species.

The alternative platforms for secondary metabolite production are clearly supported by the present findings, the culture of callus and cell suspension. The callus tissue functioned as a compact biosynthetic unit capable of producing flavonoids, a major group of bioactive compounds in *S. burahol* [Habibah et al. 2019]. The observed increase in flavonoid content at eight weeks of callus culture and on day 15 in suspension cultures suggests that metabolite accumulation is closely related to the growth phase and cellular differentiation of the cultures. During these periods, cells maintain an optimal balance between proliferation and metabolic specialization, leading to enhanced secondary metabolite biosynthesis. Furthermore, the *in vitro* culture approach offers a sustainable approach for the controlled production of bioactive compounds from *S. burahol*, unlike conventional cultivation. In addition, *in vitro* cultures minimize dependence on seasonal variation, plant age, and limited natural resources, thereby enabling consistent metabolite yield. This strategy is not only for the large-scale production of flavonoids but also for the conservation of pharmacologically important species. Overall, the findings of this review highlight the potential of plant tissue culture techniques as a reliable and efficient method for optimizing the production of bioactive compounds in *S. burahol*. In addition, supporting their prospective application in pharmaceutical and nutraceutical development (Habibah et al., 2016; Habibah et

al., 2017; Habibah et al., 2019). Based on the reviewed evidence, *S. burahol* exhibits considerable potential as a bioprospective resource for the production of bioactive compounds, including flavonoids, tannins, phenolics, kaempferol, alkaloids, and methyl hemiacetal derivatives. These compounds have been associated with diverse biological activities, such as anti-inflammatory, antioxidant, antibacterial, anticancer, anti-acne, antidiabetic, antihyperuricemic, and anti-implantation effects, suggesting significant relevance for future health-related applications.

## CONCLUSIONS

*S. burahol* or Kepel has shown considerable potential as a bioprospective plant for health applications. The studies showed its medicinal properties, with bioactive compounds exhibiting anti-inflammatory, antioxidant, antibacterial, anticancer, anti-acne, antidiabetic, antihyperuricemic, and anti-implantation activities. These findings indicate that *S. burahol* is a promising candidate for the development of natural therapeutic agents. *In vitro* cultivation has been successfully applied as part of conservation and propagation efforts to ensure its sustainable utilization. Further comprehensive studies are recommended to explore its pharmacological potential and support its wider application in the health sector.

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## AUTHORS' CONTRIBUTIONS

Conceptualization, E.W.Z and I.N.A.; methodology, D.M. and I.N.A.; investigation, E.W.Z and I.N.A.; writing original draft preparation, E.W.Z., D.M., and I.N.A.; writing review and editing, E.W.Z., and I.N.A.; supervision, E.W.Z.; project administration,

E.W.Z. All authors have read and agreed to the published version of the manuscript.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

## ETHICAL CONSIDERATION

This manuscript did not require Institutional Review Board approval because it used publicly available data and did not involve human or animal participants directly or identifiable private data.

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