



## Bioprospecting Two Underutilized Seeds: Phytochemical and Biological Insights into *Pongamia pinnata* and *Benincasa hispida*

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### Abstract

**Background:** *Pongamia pinnata* (ganuga) and *Benincasa hispida* (ash gourd) seeds are rich in bioactive compounds despite often being underutilized. This study compares their phytochemical composition, extraction methods, and biological activities.

**Methods:** Literature review and qualitative phytochemical screening (e.g., Shinoda, Mayer's, and foam tests) were performed. Fixed oils were extracted using maceration with non-polar solvents, and yield and physicochemical properties were analyzed.

**Results:** Both seeds showed comparable extraction yields with distinct phytochemical diversity through maceration. *P. pinnata* showed a broad range of phytochemicals, including flavonoids, alkaloids, phenolics, tannins, saponins, and terpenoids, associated with multiple pharmacological activities. *B. hispida* contained flavonoids, phenolics, saponins, sterols, and terpenoids but lacked alkaloids and volatile oils, showing mainly antioxidant and anti-inflammatory effects. The absence of certain compounds is likely due to metabolic and genetic factors.

**Conclusion:** Ganuga and ash gourd seeds exhibit distinct yet complementary phytochemical profiles with significant therapeutic potential. Their safety and bioactivity make them promising candidates for nutraceutical and pharmaceutical applications, though further studies are needed.

**Keywords:** *Pongamia pinnata*, *Benincasa hispida*, Ganuga seeds, Ash gourd seeds, Phytochemical screening, Maceration, Fixed oil, Karanjin, Pharmacological activities.

### Introduction

Seeds from medicinal plants are increasingly recognized as valuable sources of bioactive compounds with significant therapeutic potential. *Pongamia pinnata* (L.) Pierre (family Fabaceae), commonly known as Ganuga or Karanja, and *Benincasa hispida* (Thunb.) Cogn. (family Cucurbitaceae), known as ash gourd or winter melon, are two such plants whose seeds are gaining attention beyond their traditional uses.

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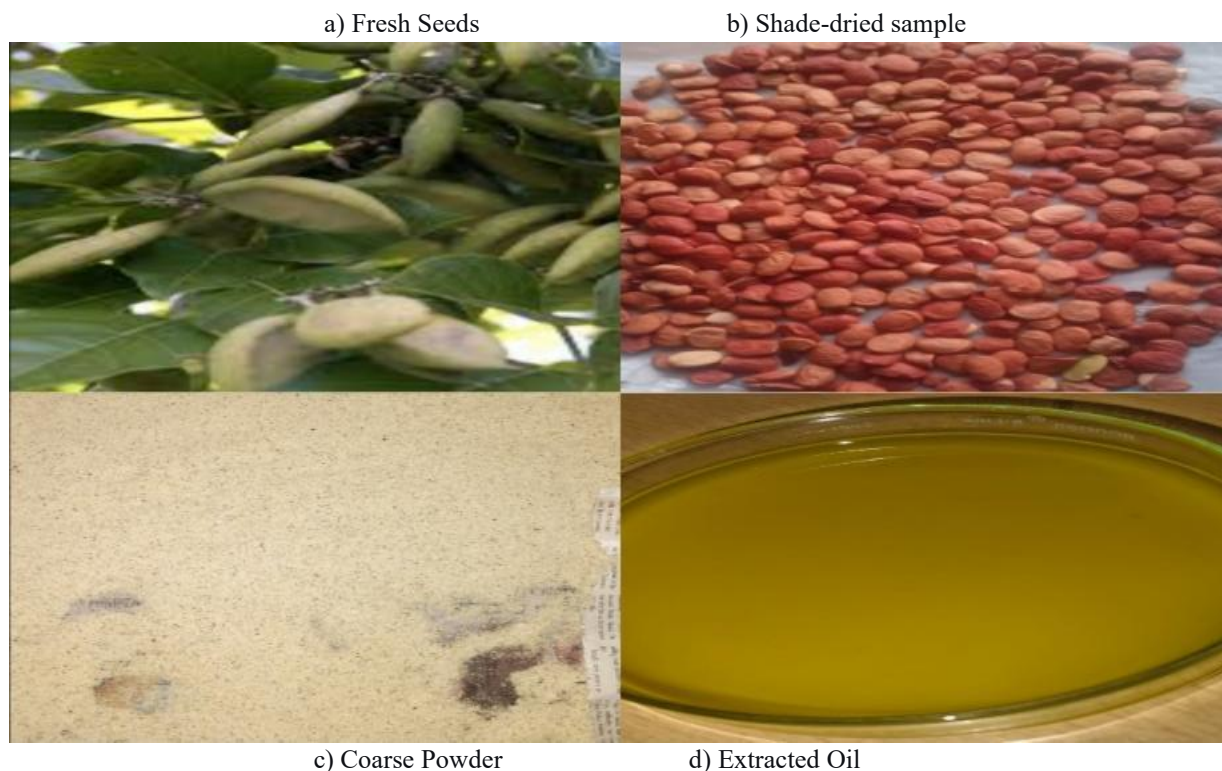
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*P. pinnata* is extensively used in traditional medicine systems. Its seeds are rich in bioactive compounds, including flavonoids (karanjin, pongamol), alkaloids, glycosides, and fixed oils, which contribute to a wide array of biological activities such as antioxidant, antimicrobial, anti-inflammatory, and antidiabetic effects [1]. The seed oil is also recognized for its industrial applications in biodiesel production [2]. Similarly, *B. hispida* seeds, a byproduct of the widely cultivated vegetable, are an important source of phytochemicals like flavonoids, phenolic compounds, saponins, and tannins. These constituents are linked to pharmacological activities including antioxidant, anti-ulcer, anti-inflammatory, and cardioprotective effects [3,4]. The effective utilization of these seeds requires a thorough understanding of their phytochemical composition and the development of efficient extraction methods. The maceration method, a simple and effective technique, is particularly suitable for extracting thermolabile bioactive compounds without the need for sophisticated equipment [5,6]. This study aims to present a comparative analysis of *P. pinnata* and *B. hispida* seeds, focusing on their extraction processes, detailed phytochemical profiles, associated biological activities, and the physicochemical properties of their fixed oils. In recent years, there has been a growing interest in plant-derived bioactive compounds due to their potential therapeutic applications and lower side effects compared to synthetic drugs. Medicinal plants serve as an important source of novel drug candidates, particularly due to the presence of diverse secondary metabolites such as flavonoids, alkaloids, tannins, and phenolic compounds [13,33]. These phytochemicals play a crucial role in protecting plants against environmental stress and pathogens, while also exhibiting significant pharmacological activities in humans.

Underutilized plant seeds, such as those of *Pongamia pinnata* and *Benincasa hispida*, have gained attention as sustainable sources of nutraceuticals and functional ingredients. *Pongamia pinnata* is widely recognized for its medicinal and industrial applications, including its use in traditional medicine and biofuel production [1,2]. Similarly, *Benincasa hispida* has been extensively used in traditional systems of medicine for its antioxidant, anti-inflammatory, and neuroprotective properties [3,23]. The exploration of such plant resources is essential for the development of safe and effective natural therapeutics [27].



**Figure 1:** Processing of *Pongamia Pinnata* (Ganuga)



a) Fresh sample                      b) Shade-dried seeds                      c) Coarse powder

**Figure 2:** Processing of *Benincasa hispida* (Ash gourd)

## 2. Materials and Methods

### 2.1. Plant Material

The study utilized seeds from *Pongamia pinnata* (ganuga) and *Benincasa hispida* (ash gourd). The seeds were collected, cleaned, and dried in shade to preserve the bioactive constituents. The dried seeds were ground into a coarse powder using a mechanical grinder and stored in airtight containers until further use.

### 2.2. Extraction Method: Maceration

The maceration method was employed for the extraction of fixed oils from both seed powders.

- **Preparation:** 50 g of the powdered seed material was placed in a clean, dry conical flask.
- **Solvent Addition:** 250 mL of a non-polar organic solvent (n-hexane or petroleum ether) was added to completely immerse the sample.
- **Maceration:** The flask was sealed and kept at room temperature for 48-72 hours. The mixture was shaken intermittently to enhance solvent penetration.
- **Filtration:** After maceration, the mixture was filtered using Whatman filter paper to separate the liquid extract from the solid residue.
- **Evaporation:** The filtrate was subjected to evaporation using a water bath maintained below 50°C to remove the solvent.
- **Collection:** The remaining viscous residue was collected as the crude oil extract.
- **Storage:** The extracted oil was weighed to determine the yield and stored in amber-colored airtight containers under refrigerated conditions.

TPC and TFC determination methods (Folin–Ciocalteu,  $\text{AlCl}_3$ )

### 2.3. Yield Calculation

The percentage yield of the extracted oil was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{Weight of extracted oil (g)}}{\text{Weight of seed powder (g)}} \times 100$$

### 2.4. Phytochemical Screening

Qualitative phytochemical analysis was performed on the seed extracts using standard procedures to identify the presence of various secondary metabolites [19,20,25,26].

- **Flavonoids:** Shinoda test (magnesium turnings and conc. HCl).
- **Alkaloids:** Mayer's test (Mayer's reagent).
- **Glycosides:** Keller-Killiani test (glacial acetic acid,  $\text{FeCl}_3$ , conc.  $\text{H}_2\text{SO}_4$ ).
- **Saponins:** Foam test (vigorous shaking with distilled water).

- **Tannins:** Ferric chloride test (FeCl<sub>3</sub> solution).
- **Phenolic Compounds:** Ferric chloride test.
- **Fixed Oils:** Spot test (pressing extract between filter paper).
- **Terpenoids:** Salkowski test (chloroform and conc. H<sub>2</sub>SO<sub>4</sub>).

### 3. Results and Discussion

#### 3.1. Extraction Yield and Physicochemical Properties

The fatty acid composition of the extracted oils is consistent with previously reported studies, where *Pongamia pinnata* oil is rich in oleic and linoleic acids, contributing to its industrial and pharmacological importance [1,7,27]. Similarly, *Benincasa hispida* seed oil contains a high proportion of unsaturated fatty acids, which are associated with cardiovascular health benefits [29,30]. The presence of flavonoids such as karanjin and pongamol in *Pongamia pinnata* is strongly associated with its antioxidant and anti-inflammatory activities, primarily through free radical scavenging and inhibition of inflammatory mediators [12,27]. Alkaloids and other secondary metabolites contribute to its antimicrobial and antidiabetic properties, as reported in previous studies [13,28]. *Benincasa hispida* seeds are particularly rich in phenolic compounds and flavonoids, which are known to exert antioxidant effects by neutralizing reactive oxygen species (ROS) [29,30]. The presence of sterols further contributes to hypocholesterolemic activity by reducing intestinal absorption of cholesterol [33]. The observed differences in phytochemical composition between the two seeds can be attributed to variations in their biosynthetic pathways and genetic makeup.

*Pongamia pinnata* tends to accumulate nitrogen-containing compounds such as alkaloids, whereas *Benincasa hispida* predominantly synthesizes non-nitrogenous phytochemicals like phenolics and flavonoids [34]. Similar variations have been reported in other medicinal plant studies, highlighting the influence of metabolic diversity on phytochemical profiles [33].

#### Mechanism of action:

The pharmacological activities of phytochemicals are closely associated with their molecular mechanisms of action. Phenolic compounds and flavonoids act as potent antioxidants by scavenging reactive oxygen species (ROS) and inhibiting oxidative stress, which is a major contributor to chronic diseases such as cancer, diabetes, and cardiovascular disorders [32,33]. These compounds stabilize free radicals by donating electrons or hydrogen atoms, thereby preventing cellular damage. Alkaloids present in *Pongamia pinnata* exhibit antimicrobial activity by disrupting microbial cell membranes and inhibiting nucleic acid synthesis [17]. Additionally, tannins are known to precipitate microbial proteins, leading to inhibition of bacterial growth [18]. Anti-inflammatory activity of these phytochemicals is mediated through inhibition of cyclooxygenase (COX) and lipoxygenase (LOX) pathways, resulting in decreased production of inflammatory mediators [10]. These combined mechanisms contribute to the broad-spectrum therapeutic potential of both *Pongamia pinnata* and *Benincasa hispida* seeds.

**Table 1:** Physicochemical properties of extracted oils

Property	P. pinnata (Ganuga) Seed Oil	B. hispida (Ash Gourd) Seed Oil
Color	Yellow to brown viscous liquid	Pale yellow liquid
Odor	Characteristic	Mild
Solubility	Soluble in organic solvents	Soluble in organic solvents
Key Fatty Acids	Oleic, Linoleic, Palmitic	Linoleic, Oleic
Primary Use	Biodiesel, Pharmaceuticals	Nutritional, Nutraceuticals

#### 3.2. Phytochemical Profile and Biological Activities

The phytochemical screening revealed distinct profiles for the two seeds.

### 3.2.1. *Pongamia pinnata* (Ganuga) Seeds

Qualitative analysis confirmed the presence of flavonoids, alkaloids, glycosides, saponins, tannins, phenolics, terpenoids, and fixed oils (Table 2). The presence of flavonoids (karanjin, pongamol) and phenolics correlates with its strong antioxidant and anti-inflammatory activities [12,15]. Alkaloids and fixed oils contribute to its antimicrobial and wound healing properties [13,15]. The diverse array of compounds underpins its antidiabetic, insecticidal, and hepatoprotective effects [14]. The absence of certain compounds like anthocyanins, cyanogenic glycosides, and volatile essential oils is due to the plant's specific metabolic pathways, which favor the synthesis of non-volatile lipids and flavonoids [16,17].

**Table 2:** Phytochemical profile of *Pongamia pinnata* seeds

Phytochemical Group	Presence/absence (+/-)	Key Compounds/Bioactivity
Flavonoids	+	Karanjin, Pongamol (Antioxidant, Antimicrobial)
Alkaloids	+	Moderate amounts (Antimicrobial, Analgesic)
Glycosides	+	Cardioprotective, Antioxidant
Saponins	+	Small quantities (Anti-inflammatory, Immune booster)
Tannins	+	Antimicrobial, Astringent
Phenolic Compounds	+	High content (Antioxidant)
Fixed Oils	+	Oleic, Linoleic, Palmitic acids (Anti-inflammatory, Emollient)
Terpenoids	+	Anti-inflammatory, Antimicrobial
Anthocyanins	-	-
Cyanogenic	-	-

### 3.2.2. *Benincasa hispida* (Ash Gourd) Seeds

The screening showed that ash gourd seeds are rich in flavonoids, phenolics, saponins, tannins, glycosides, fixed oils, sterols, and terpenoids (Table 3). This composition directly supports its reported antioxidant, anti-inflammatory, and cardioprotective activities [21,22]. The notable absence of significant alkaloid content distinguishes it from many medicinal plants, while the lack of volatile essential oils aligns with its mild odor. The absence of cyanogenic glycosides adds to its safety profile for consumption [23,24].

**Table 3:** Phytochemical Profile of *Benincasa hispida* Seeds

Phytochemical	Presence/Absence(+/_)	Key Compounds/Bioactivity
Flavonoids	+	Antioxidant, Anti-inflammatory
Phenolic Compounds	+	High content (Antioxidant, Anti-aging)
Saponins	+	Anti-inflammatory, Antimicrobial, Immune modulation
Tannins	+	Astringent, Antimicrobial
Glycosides	+	Cardioprotective, Antioxidant
Fixed Oils	+	Linoleic, Oleic acids (Cardioprotective, Nourishing)
Sterols	+	Hypocholesterolemic
Terpenoids	+	Anti-inflammatory, Antimicrobial
Alkaloids	-	-
Volatile Oils	-	-

### 3.3. Quantitative Estimation of Bioactive Compounds:

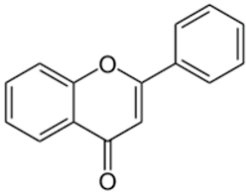
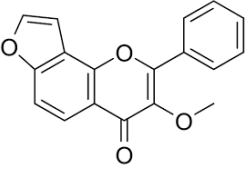
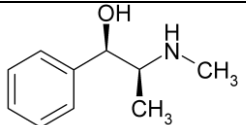
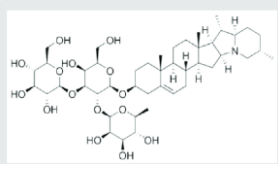
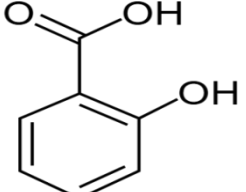
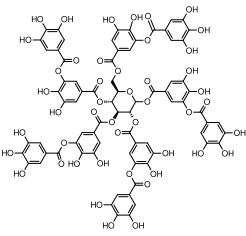
In addition to qualitative phytochemical screening, quantitative estimation of total phenolic content (TPC) and total flavonoid content (TFC) is essential to evaluate antioxidant potential. Phenolic and flavonoid compounds significantly contribute to free radical scavenging activity and therapeutic efficacy [29,30]. Previous studies have reported that *Pongamia pinnata* seeds possess moderate to high phenolic and flavonoid content, while *Benincasa hispida* seeds exhibit relatively higher phenolic content associated with strong antioxidant activity

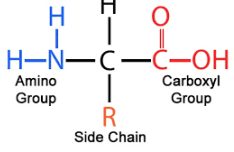
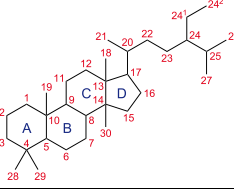
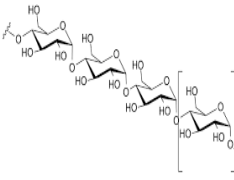
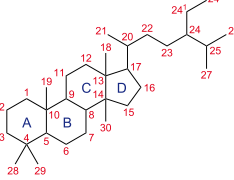
[29,30]. The total phenolic content is expressed as gallic acid equivalents (GAE), and total flavonoid content as quercetin equivalents (QE). Antioxidant activity is commonly assessed using DPPH radical scavenging assay, where IC<sub>50</sub> values indicate the concentration required to inhibit 50% of free radicals. Lower IC<sub>50</sub> values correspond to higher antioxidant activity [30].

### 3.4. Comparative Analysis:

This comparative study highlights that while both seeds are rich in valuable phytochemicals, their profiles are distinct. *P. pinnata* seeds offer a unique set of compounds like karanjin and a broader range of alkaloids, making them suitable for applications requiring potent antimicrobial and insecticidal properties. In contrast, *B. hispida* seeds, with their high saponin and sterol content, are more aligned with cardiovascular health and immune modulation. The absence of toxic phytochemicals in both seeds enhances their potential for development into safe, plant-based therapeutic agents and functional foods.

**Table 4:** Phytochemical Profile Table

Phytochemical Group	Basic Chemical Structure	Source (Ganuga Seeds / Ash Gourd Seeds)	Biological Activity	Mechanism of Action (MOA)	Medicinal Uses
<b>Flavonoids (e.g., Karanjin, Quercetin)</b>		Both (rich in Ganuga)	Antioxidant, anti-inflammatory	Scavenge free radicals, inhibit COX/LOX pathways	Anti-inflammatory disorders, cardioprotection
<b>Furanoflavonoids (Karanjin, Pongamol)</b>		Ganuga seeds	Antimicrobial, insecticidal	Disrupt microbial enzymes and membranes	Skin diseases, pest control
<b>Fixed Oils (Fatty acids: Oleic, Linoleic)</b>	Long-chain fatty acids (C16–C18)	Both	Nutritional, anti-inflammatory	Modulate lipid metabolism, reduce inflammation	Heart health, energy source
<b>Alkaloids</b>		Ganuga seeds	Antimicrobial, analgesic	Interfere with DNA replication, enzyme inhibition	Pain relief, antimicrobial therapy
<b>Saponins</b>		Both (more in Ash gourd)	Antioxidant, immune boosting	Increase membrane permeability, stimulate immunity	Immunomodulation, cholesterol reduction
<b>Phenolic Compounds</b>		Both	Antioxidant	Donate hydrogen to neutralize ROS	Anti-aging, chronic disease prevention
<b>Tannins</b>		Both	Astringent, antimicrobial	Protein precipitation, enzyme inhibition	Diarrhea, wound healing

<b>Proteins &amp; Amino acids</b>		Ash gourd seeds	Nutritional, tissue repair	Provide essential amino acids for metabolism	Growth, muscle repair
<b>Sterols (<math>\beta</math>-sitosterol)</b>		Both	Anti-inflammatory, hypocholesterolemic	Compete with cholesterol absorption	Cholesterol control
<b>Polysaccharides</b>		Ash gourd seeds	Immunomodulatory, antioxidant	Enhance macrophage activity	Immune support
<b>Terpenoids</b>		Ganuga seeds	Anti-inflammatory, antimicrobial	Inhibit inflammatory mediators (NF- $\kappa$ B)	Anti-inflammatory therapy
<b>Vitamins (Vitamin E, B-complex)</b>	Organic micronutrients	Ash gourd seeds	Antioxidant, metabolic support	Protect cell membranes from oxidative damage	Skin health, nerve function

### 3.5. Comparative Pharmacological Significance:

The comparative evaluation of *Pongamia pinnata* and *Benincasa hispida* seeds reveals distinct differences in their phytochemical composition and associated pharmacological activities. *Pongamia pinnata* seeds are rich in flavonoids such as karanjin and pongamol, along with alkaloids and fixed oils, which contribute to their potent antimicrobial, anti-inflammatory, and antidiabetic properties [1,8,9]. These bioactive compounds act through multiple mechanisms including enzyme inhibition, free radical scavenging, and modulation of metabolic pathways. In contrast, *Benincasa hispida* seeds are characterized by a higher content of phenolic compounds and flavonoids, which are primarily responsible for their strong antioxidant and cardioprotective effects [4,23,24]. The presence of plant sterols further enhances their ability to reduce cholesterol levels and support cardiovascular health. The variation in phytochemical profiles between the two plants can be attributed to differences in their genetic makeup and biosynthetic pathways, which influence the production of secondary metabolites [13,34]. Such diversity highlights the importance of plant selection in phytochemical and pharmacological studies. Overall, while *Pongamia pinnata* demonstrates stronger antimicrobial and anti-inflammatory potential, *Benincasa hispida* exhibits superior antioxidant activity. These complementary properties suggest that a combined use of both plant seeds may provide enhanced therapeutic benefits through synergistic effects [27].

### 3.6. Future Perspectives:

Future research should focus on the isolation and structural characterization of bioactive compounds using advanced analytical tools such as LC-MS and NMR spectroscopy [34]. Additionally, experimental validation through in vitro and in vivo studies is necessary to confirm the pharmacological activities of these compounds. The development of novel extraction techniques and formulation strategies may further enhance the therapeutic potential of these seeds [31,32]. Moreover, increasing interest in plant-based nutraceuticals emphasizes the importance of exploring underutilized seeds as sustainable sources of bioactive compounds [33].

### 3.7. Limitations:

This study has several limitations that should be considered. The phytochemical analysis was limited to qualitative screening methods, which do not provide precise quantification of bioactive compounds such as total phenolic and flavonoid content. Advanced analytical techniques such as HPLC and GC–MS, which are essential for compound identification and validation, were not employed [34]. Furthermore, the biological activities discussed were based on previously published literature rather than experimental validation through *in vitro* or *in vivo* studies. The use of a single extraction method (maceration) may also limit extraction efficiency compared to advanced techniques such as ultrasound-assisted or supercritical fluid extraction [31,32]. These limitations highlight the need for further detailed investigations.

### 3.8. Industrial & Nutraceutical Applications:

The bioactive compounds present in *Pongamia pinnata* and *Benincasa hispida* seeds have significant potential for industrial and nutraceutical applications. *Pongamia pinnata* oil is widely explored as a renewable source for biodiesel production due to its high lipid content and favorable physicochemical properties [2,7]. In addition, its medicinal properties make it a valuable component in herbal formulations. *Benincasa hispida*, on the other hand, is extensively used in functional foods and traditional medicine systems due to its antioxidant and health-promoting properties [23,27]. The incorporation of these seed extracts into nutraceutical products such as dietary supplements and functional beverages can enhance their therapeutic value. The increasing demand for natural and plant-based products further highlights the importance of exploring these underutilized seeds for commercial applications [33].

## 4. Conclusion

This study provides a comprehensive comparative analysis of *Pongamia pinnata* (ganuga) and *Benincasa hispida* (ash gourd) seeds. The maceration method proved effective for extracting fixed oils while preserving bioactive constituents. The detailed phytochemical screening elucidated the presence of diverse bioactive groups, directly correlating with their respective pharmacological activities. The findings confirm that both seeds are underutilized bioresources with significant potential for applications in herbal medicine, nutraceuticals, and functional food development. Their favorable safety profile, indicated by the absence of several toxic compounds, further supports their promise. Future research should focus on isolating individual active compounds, conducting *in vivo* efficacy studies, and developing standardized extraction protocols for large-scale industrial applications.

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