# Nutrition Composition and Analysis of Medicinal Herbal Potential of *Horsfieldia glabra* Warb. Seeds

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**Abstract**
*Horsfieldia glabra* Warb. is a locally consumed plant indigenous to Chiang Rai Province, Thailand and of late, there has been considerable interest in evaluating its health benefits. The aim of this study was to investigate *H. glabra* Warb. seeds in order to assess their nutritional properties and its potential as a medicinal herb. In terms of nutritional assessment, the experiment found that *H. glabra* Warb. seed extract contained 68.24 g per 100 g dry weight of lipids, 7.80 g per 100 g dry weight of protein, 14.20 g per 100 g dry weight of carbohydrates, 5.04 g per 100 g dry weight of crude fiber, 1.41 g per 100 g dry weight of ash and 6.827 kcal per 1 g dry weight of energy. The analysis of its potential as a medicinal herbal determined the antioxidant activity and Minimum Inhibitory Concentration (MIC) of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The results revealed that *H. glabra* Warb. seed extraction displayed antioxidant activity (62.01 ± 3.99% DPPH inhibition activity) with a IC50 value of 0.358 ± 0.02 mg/ml. Experiments investigating MIC determined that the extract exhibited the minimum inhibitory concentration of *S. aureus* (15.625 mg/ml) with 8.00 ± 1.73 mm clear zone. Whereas Streptomycin concentration of 30 mg/ml inhibited 9.00 ± 2.00 mm clear zone. GC-MS analysis of *H. glabra* Warb. seeds determined that the major compounds were $\alpha$-resorcinol (40.769%) and 4-vinylphenol (23.761%).

**Keywords**: *Horsfieldia glabra* Warb., Proximate analysis, Antioxidant activity, MIC, $\alpha$-resorcinol

## 1 Introduction

During recent years, many plants throughout the world have been studied for their medicinal value. The aim of the studies was to determine the potent pharmacological activity, low toxicity and economic viability of the plants. Among infectious diseases, scientists face challenges in searching for new antimicrobial sources from plants in order to develop commercial antimicrobial drugs [1], [2]. Many plants provide useful medicinal compounds and most of these are secondary metabolites. The antimicrobial compounds acquired from Finnish plant materials have been inspected. Nine microbial species (*Aspergillus niger*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) were studied in this experiment. It was found that the most effective plant extract against *C. albicans* was *Lythrum salicaria* L. Whereas, the other extracts such as Betula pubescens Ehrh., *Pinus sylvestris* L. and *Solanum tuberosum*. L. were found to inhibit *S. aureus* [3]. Moreover, the antibacterial properties of certain other plant extracts (*Entada Africana*, *Terminalia avicennoides*, *Mitragyna stipulosa* and *Lannae acida*) were investigated. The extracts were able to inhibit *E. coli* with the minimum inhibitory concentration ranging from 1.56 mg/ml to 50.00 mg/ml [4].

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The free radicals caused oxidative damage, which has been associated with various diseases such as Alzheimer's disease, liver injury, diabetes and general inflammation. Therefore, the researchers in this study have tried to identify natural antioxidants (such as polyphenols, flavonoids or related compounds) that can inhibit the development of free radicals for human disease prevention [5]. The antioxidant activity of selected Algerian medicinal plant extracts has been evaluated. There are many plants that have displayed antioxidant activity using the DPPH method with an IC50 value range from 4.30 $\mu$g/mL to 486.6 $\mu$g/mL. The results revealed that *Pistacia lentiscus* exhibited the highest antioxidant capacity (4.30 $\mu$g/mL) [2]. Similarly, the antioxidant activity of certain fruits grown in northern Greece was also determined. Many fruit specimens displayed antioxidant activity e.g. *Cornus mas* (80.15 ± 19.78 $\mu$mol AAE (ascorbic acid equivalent)/g FW), *Zizyphus jujube* (69.55 ± 0.35 $\mu$mol AAE/g FW), *Prunus avium* (32.60 ± 10.30 $\mu$mol AAE/g FW) and *Pyrus communis* (20.57 ± 5.18 $\mu$mol AAE/g FW) [6] Plants have proven to be beneficial to humans both as a functional food for human consumption and for their nutritional properties. For example, Quinoa, *Chenopodium quinoa* Willd., was found to be composed of minerals, vitamins, fatty acids and antioxidants that offer advantages in the way of providing nutrition to humans [7]. Furthermore, the nutritional composition of some wild plant foods has been examined. Some fruits were clearly higher in crude protein, carbohydrates and energy [8]. In addition, the chemical composition of the Bamboo (*Phyllostachys pubescens*) was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). Thus plants are commonly used in Asian dishes and contains several beneficial compounds such as phenolic compounds and aromatic hydrocarbons [9].

*Horsfieldia glabra* Warb. (Myristicaceae family) is usually found in Chiang Rai Province, Thailand. A description of *H. glabra* Warb. is as follows: Evergreen tree (height: 20–25 m) with brown bark. Leaves are elliptic to obovate (long: 13–20 cm, wide: 3.5–8 cm), leathery, obtuse at the base, acute apex and petioles that are 10–25 mm long. Flowers are unisexual (dioecious), males with 3 tepals and stamens 6–12, grouped in axillary racemes (long: 6–19 cm), solitary or grouped in the female axillary racemes which are pauciflorous. Ovoid fruit (diameter: 20–35 mm), yellow and smooth.

**Figure 1**: Fruit and seed of *H. glabra* Warb.

The fruits are comprised of dehiscent berries with ellipsoid seeds completely surrounded by an aril (Figure 1). There has been success in isolating the compounds from *H. glabra* Warb. arylalkanones (1-(2,4,6-trihydroxyphenyl)-9-phenylnonan-1-one and 1-(2,6-dihydroxy-phenyl)-4-methyl-4-tridecen-1-one. The known compound 1-(2,6-dihydroxyphenyl)-11-phenylundecan-1-one, (+)-asarinin, (−)-dihydrocubebin and trimyristin) was found from the methanol extract of arils of *H. glabra* Warb. [10]. It is of considerable interest to study the potential health benefits of consuming of *H. glabra* Warb. seeds. The purpose of the study is to analyze the nutrition composition, antioxidant activity, antimicrobial activity of *H. glabra* Warb. seeds.

## 2 Materials and Methods

### 2.1 *Nutritional information of H. glabra Warb. seeds*

*H. glabra* Warb. seeds were harvested from Wiang Chai District, Chiang Rai Province, Thailand. The seeds were dried, weighed (100 g) and proximate analysis was performed (the composition of fat, protein, carbohydrates, crude fiber and ash) following AOAC methods [11]. Bomb calories method was used for energy quantification. The analysis was examined in triplicate.

### 2.2 *Antioxidant activity of H. glabra Warb. seeds*

*H. glabra* Warb. seeds were ground and extracted 3 times with methanol (Merck, HPLC grade, Germany).

The solution was filtered and evaporated to a crude extract. The seed extract was used for determination of DPPH (1,1-diphenyl-2-hydrazy) radical scavenging activity. The extract of each different concentration was added in equal volumes to the methanolic solution of DPPH. The absorbance was recorded at 517 nm after being placed at room temperature for 30 minutes. Butylated hydroxytoluene (BHT) was used as the standard control. The experiment was performed in triplicate. The percent inhibition of antioxidant activity was examined [12], [13]. The percent inhibition was calculated by the following formula:

$$\% \text{ inhibition} = \frac{[\text{Absorbance (control)} - \text{Absorbance (sample)}] \times 100}{\text{Absorbance (control)}}$$

The plotted graph of percent inhibition against the different concentrations was used to determine the IC50 value (total antioxidant presence necessary to decrease the initial DPPH radical concentration by 50%).

### 2.3 *Minimum Inhibitory Concentration (MIC) of H. glabra Warb. seeds*

The test organisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) from Thailand Institute of Scientific and Technological Research, Pathumthani, Thailand were adjusted to equal the turbidity of 0.5 McFarland standard giving a final inoculum of $1.0 \times 10^8$ CFU/mL. Each of the bacterial inoculum of 100 $\mu$L was uniformly spread using sterile cotton swabs on a sterile petri dish with the nutrient agar (NA) and the specimens were kept at 37ºC for 24 hours. *H. glabra* Warb. seed extract was diluted in four concentrations of 125, 62.5, 31.25 and 15.625 mg/mL. Seven mm filter paper discs loaded with each concentration were placed onto the surface of the agar and incubated for 24 h at 37ºC ± 1ºC, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm of inhibition zone [14]. Thirty mg commercial streptomycin (purchased from Fluka) discs were used as a positive control. Streptomycin inhibited gram negative bacterial and some gram positive bacterial such as *S. aureus* [15]. Tests were performed in triplicate.

### 2.4 *Gas Chromatography-Mass Spectrometry (GC-MS) analysis of H. glabra Warb. seeds*

The extracts were analyzed in a GC-MS system with GC 7890A Agilent Technology machine. A 30 m DB5-MS column (0.25 mm I.D., 0.25-$\mu$m film Thickness) was used. The inlet temperature was 250°C and the oven temperature was programmed to be 60°C then raised at a rate of 3°C/min to 240°C. Injection volume was 1 $\mu$L and solvent delay was 4 min with a total runtime of 60 min. Mass spectra scan ranged from 50 to 550 amu. MS spectra of separated components were identified based on WILEY and NIST Libraries for botanical compounds.

## 3 Results and Discussion

### 3.1 *Nutritional information of H. glabra Warb.*

The nutritional information of *H. glabra* Warb. seeds is presented in Table 1. Fat was recorded at the highest level of composition (68.24 g per 100 g dry weight). Carbohydrate composition was 14.20 g per 100 g dry weight. Protein, crude fiber and ash were 7.80, 5.04 and 1.41 g per 100 g dry weight, respectively. Nutritional evaluation of *Myristica fragrans* (Myristicaceae family) revealed that fat, carbohydrate, protein, crude fiber and ash were 13.34, 41.57, 11.50, 12.52 and 9.84 g per 100 g dry weight, respectively [16]. It is indicated that *H. glabra* Warb. presented higher fat composition than *Myristica fragrans*. The earlier research had investigated the nutritional value of tropical plant seeds. There are some tropical plant seeds that have been found to have high fat composition such as *Jatropha curcas* L. (50.33 g per 100 g dry weight), *Pentaclethra macrophylla* Benth.(52.07 g per 100 g dry weight), *Telfairia occidentalis* Hook. f. (51.4 g per 100 g dry weight), *Citrullus vulgaris* Schrader (55.4 g per 100 g dry weight), *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. (62.8 g per 100 g dry weight) and *Entandrophragma angolensis* (Welw.) C. DC. (61.08 g per 100 g dry weight) [17]. It was found that *H. glabra* Warb seeds exhibited higher fat composition over the previously listed tropical plant seeds. The energy content of the *H. glabra* Warb. seeds was found to be 6.827 kcal/g. These seeds were found to provide high levels of energy upon consumption over many other studied plants, e.g. *Lonchocarpus*

*sericeus* (Poir.) Kunth ex DC. (6.164 kcal/g), *Sesbania pachycarpa* DC. (4.787 kcal/g), *Albizia zygia* (DC.) Macbr. (4.536 kcal/g), *Entada scandens* (L.) Benth. (3.446 kcal/g) and *Acacia leucophloea* (Roxb.) Willd. (0.382 kcal/g) [17]. It is of significant interest that *H. glabra* Warb. seeds possess high fat content with high energy consumption and may contain essential oil. Further studies should investigate the chemical components and examine the essential oil of these seeds for consumption or for other possible beneficial applications. In a previous experiment on *Myristica fragrans* Houtt. (Myristicaceae family), it was found chemical composition of essential oil e.g. sabinene (21.37%), 4-terpineol (13.92%) and myristicin (13.57%) [18]. Morover, the other plant species such as Euterpe oleraceae was found to be composed of saturated and unsaturated fatty acids. The two dominant compounds were oleic acid (53.9%) and plamitic acid (26.7%) [19]. Furthermore, the essential oils were extracted from *Origanum scabrum* and *Origaum microphyllum* presenting carvacrol, terpinen-4-ol, linalool, sabinene, $\alpha$-terpinene, and $\gamma$-terpinene [20].

**Table 1**: Nutritional information of *H. glabra* Warb. seeds

| Nutritional Facts | Composition |
|---|---|
| Fat (g per 100 g dry weight) | $68.24 \pm 0.232^{a}$ |
| Protein (g per 100 g dry weight) | $7.80 \pm 0.061^{c}$ |
| Carbohydrates (g per 100 g dry weight) | $14.20 \pm 0.284^{b}$ |
| Crude fiber (g per 100 g dry weight) | $5.04 \pm 0.096^{d}$ |
| Ash (g per 100 g dry weight) | $1.41 \pm 0.061^{e}$ |
| Energy (kcal/g) | $6.827 \pm 0.026$ |

Statistical significance was determined by analysis of variance (ANOVA) with adjustments for multiple comparisons with Turkey's test. Values are means ± standard deviation of triplicate determinations. Values on the same column with different superscripts are significantly different ($P \leq 0.05$).

### 3.2 *Antioxidant activity of H. glabra Warb. seeds*

The highest antioxidant activity of *H. glabra* Warb. seed extract was 62.046% DPPH inhibition activity (0.5 mg/ml of concentration), while the IC50 value was 358 ± 0.02 $\mu$g/ml. The *H. glabra* Warb. seed extract showed weaker antioxidant activity than BHT (IC50 value of 49.75 $\mu$g/ml). The antioxidant activity of *H. glabra* Warb. seed extract, however, was less than the other plant from Myristicaceae family such as *Iryanthera ulei* Warb. [21], *Iryanthera lancifolia* [22], *Embelia ribes* Burm. f. [23] and *Myristica fragrans* [24]. Nevertheless, the *H. glabra* Warb. seed extract had a higher IC50 value than several plant e.g. *Lantana camara* in the different varieties of Chandigarh Purple (927.16 ± 2.88 $\mu$g/ml) and Yellow turning pink (475.33 ± 5.20 $\mu$g/ml) [5]. Moreover, the *H. glabra* Warb. seed extract presented higher antioxidant activity than certain parts of *Teucrium chamaerdys* L. var. *glanduliferum* Haussk, e.g. the petroleum ether extract of the flower (1165.75 ± 4.18 $\mu$g/ml) [25]. In addition, the *H. glabra* Warb. seed extract also exhibited greater antioxidant activity over the extracts of 13 commercially available citrus spp. (IC50 ranged from 0.6–3.8 mg/ml) [12].

### 3.3 *Minimum Inhibitory Concentration (MIC) of H. glabra Warb. seeds*

The inhibitory concentration of *S. aureus*, *E. coli*, and *P. aeruginosa* extracted from *H. glabra* Warb. seed extract is shown in Table 2. The results revealed that the extract inhibited *S. aureus* at a minimum concentration of 15.625 mg/ml. The inhibition zone of all concentrations was from 7.0 to 8.0 mm. However, the extract could not inhibit *E. coli* and *P. aeruginosa*, while streptomycin (antibiotic) inhibited *E. coli* with a clear zone area of 10.0 mm (Table 2). The results revealed that *H. glabra* Warb. seed extract was mostly effective against gram-positive bacteria (*S. aureus*) but could not inhibit gram-nagative bacteria (*E. coli* and *P. aeruginosa*). It may indicate that *H. glabra* Warb. seed extract acts specifically against the gram-positive cell wall. Gram-positive bacteria constituted a much thicker peptidoglycan outer membrane than gram-negative bacteria. While, the outer membrane of gram-nagative bacteria was composed of lipopolysaccharides that cause gram-negative bacteria extra resistance against penetration of antibiotics than gram-positive bacteria [26].

**Table 2**: Clear zone area of *H. glabra* Warb. seed extract

| Organism | Clear zone area (mm) ±SD of the extract concentration (mg/ml) | | | | |
|---|---|---|---|---|---|
| | 125 | 62.5 | 31.25 | 15.625 | Streptomycin |
| *S. aureus* | 8.0 ± 1.00 | 7.33 ± 0.58 | 7.00 ± 0.00 | 8.00 ± 1.73 | 9.00 ± 2.00 |
| *E. coli* | - | - | - | - | 10.67 ± 1.15 |
| *P. aeruginosa* | - | - | - | - | 7.33 ± 0.58 |

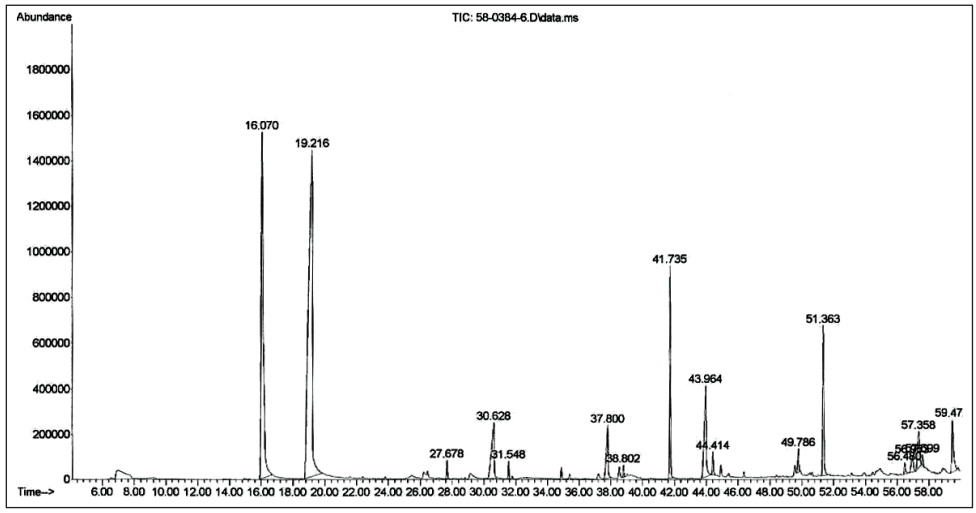


**Figure 2**: The GC-MS chromatogram of *H. glabra* Warb. seed extract.

The previous study of antimicrobial activity evaluation for ethanol extract of *Iryanthera ulei* Warb. (Myristicaceae family) related with the result of *H. glabra* Warb. seed extract. It also could inhibit *S. aureus* excepting *E. coli*, *P. aeruginosa* and *C. albican* [21]. *S. aureus* is associated with several diseases e.g. pneumonia, endocarditis, and gastroenteritis. The broad usage of antibiotics (penicillin, methicillin, vancomycin) to treat *S. aureus* has been found to damage the kidney. Therefore, plant extracts have come into use for the inhibition of *S. aureus* with minimum side effects, is easily available and is comparatively cost-effective. It was found that medicinal plant extracts (*Allium sativum*, *Cassia auriculata*, *Curcuma longa*, *Phyllanthus niruri* and *Piper betel*) exhibited effective properties against *S. aureus* [27]. Other plants that also have been found to inhibit *S. aureus* such as *Mindium Laevigatum* (Vent.) Rech. F. The minimal inhibitory concentration of 120 $\mu$g/ml was found against *S. aureus* while the other organisms (e.g. *E. coli*, *P. aeruginosa* and *C. albican*.) were not effective (>800 $\mu$g/ml) [28]. Moreover, the antimicrobial activities of certain plant extracts (*Cymbopogon citatrus*, *Vernonia amygdalina* and the seed extracts of *Garcinia kola*) were examined. It was found that the extract had the potential to inhibit *S. aureus*, *E. coli* and *C. albican*. The highest diameter zone of inhibition (26 ± 1.0 mm) was found in *Garcinia kola* extract against *S. aureus* [29]. In addition, in experiments, the pomegranate fruit (*Punica granatum* L.) was found to inhibit *S. aureus* growth and has been futher developed in antibacterial therapeutic drugs [30]. In an earlier study investigated the antimicrobial activities of selected medicinal plants from Algeria. Four bacteria species (*Bacillus subtilis*, *S. aureus*, *E. coli* and *P. aeruginosa*) and one yeast species (*C. albicans*) were used to test antimicrobial activities. It was found that the extract from many plant species such as *Sysimbrium officinalis*, *Rhamnus alaternus*, *Origanum glandulosum*, *Cupressus sempervirens*, *Pinus halipensis* and *Centaurea calcitrapa* displayed antimicrobial activities with an inhibition zone of 7.0 to 21.0 mm. On the other hand, some plants did not prove to inhibit any microorganisms [2].

### 3.4 *GC-MS analysis of H. glabra Warb. seeds*

GC-MS analysis was used to test the amount of active principles that exist in herbs for the cosmetic, drug, pharmaceutical or food industry. The GC-MS chromatogram of the chemical compound analysis is shown in Figure 2. It was found to be composed of 17 compounds with 4 unknown compounds. The compounds listed in Table 3 revealed that the highest content was 1, 3-benzenediol ($\alpha$-resorcinol) with 40.769% of the total extract. 4-Vinylphenol was found to be present at 23.761% of total extract. The extract was found to be comprised of other compounds such as dodecanoic acid, tetradecanoic acid, 2-tridecanone, n-hexadecanoic acid, 9-octadecenoic acid, $\alpha$-monoolein and farnesol.

**Table 3**: Beneficial compounds of *H. glabra* Warb. seeds by GC-MS

| Retention time | Compound | % of total extract |
|---|---|---|
| 16.069 | 4-Vinylphenol | 23.761 |
| 19.216 | 1,3-Benzenediol ($\alpha$-resorcinol) | 40.769 |
| 27.678 | 2-Tridecanone | 0.508 |
| 30.625 | Dodecanoic acid | 4.831 |
| 31.547 | Dodecanoic acid, ethyl ester | 0.472 |
| 37.800 | Tetradecanoic acid | 3.084 |
| 38.802 | Tetradecanoic acid, ethyl ester | 0.336 |
| 44.415 | n-Hexadecanoic acid | 0.792 |
| 49.788 | 9-Octadecenoic acid | 0.791 |
| 56.477 | 5-Pentadecylresorcinol | 0.411 |
| 56.952 | $\beta$-Monolinolein | 1.108 |
| 57.358 | $\alpha$-Monoolein | 2.336 |
| 57.599 | Farnesol | 0.296 |

The major compounds of *H. glabra* Warb. seed extract were $\alpha$-resorcinol and 4-vinylphenol. Both compound presented antioxidant and antimicrobial activity as previous researches described [31]–[34]. Resorcinol was found to be present in other medicinal plants. The isolated compounds from *Patrinia villosa* extract include resorcinol. It was found that resorcinol exhibited a moderate level of DPPH radical-scavenging activity with IC50 of 171.2 ± 1.9 $\mu$g/ml [35]. In addition, the GC-MS analysis of *Foeniculum vulgare* var. Dulce revealed resorcinol as the major component, which exhibited antimycobacterial activity (MIC 100–200 $\mu$g/mL) [36]. The 4-vinylphenol content of *H. glabra* Warb. seed extract was found to be 23.761% and it has also been found to be present in other plants e.g. *Matricaria chamomilla* and *Urtica dioica* [37]. The methanolic extract acquired from the flowers of *Prunus mume* was analyzed by GC-MS. Several compounds were found to be present including 4-vinylphenol and the extract exhibited scavenging effects against DPPH radicals and superoxides [38]. In earlier study, many plants have been determined to contain similar compounds to *H. glabra* Warb. seeds and have also been determined to possess medicinal potential. The chemical constituents acquired from the stem extract of *Ficus religiosa* by GC-MS analysis revealed that 13 compounds were present including n-hexadecanoic acid and octadecanoic acid. Octadecanoic acid has shown hypocholesterolemic activity and n-hexadecanoic has shown antioxidant and hypercholesterolemic activity [39]. Furthermore, *Costus pictus* is a medicinal plant that possesses antihyperglycemic and insulin secretory activity. The study of the essential oil by GC-MS revealed certain compounds that were similarly found in *H. glabra* Warb. seed extract, such as hexadecanoic acid, dodecanoic acid, tetradecanoic acid and farnesyl acetone [40].

In further studies, it will be of interest to study the inhibition of other microorganisms such as *Aspergillus niger*, *Bacillus subtilis* and *Micrococcus luteus*, including a study of the secondary metabolites from *H. glabra* Warb. seeds that were found to inhibit important microorganisms and may have applications as antimicrobial drugs in the future. In this study, benefits were observed and recorded when people consumed *H. glabra* Warb. seeds. These seeds have been found to possess high levels of energy and fat. Besides, they also display antioxidant activity and possess essential compounds. This indicates that *H. glabra* Warb. seeds should be further studied for their potential to support local economies and in compliance with concepts of sustainable development.

## 4 Conclusions

*H. glabra* Warb. seeds have proven to be nutritionally beneficial. They were found to possess the high composition of fat (68.24 g per 100 g dry weight) and provided energy at a measurement of 6.827 kcal per 1 g dry weight. Moreover, these seeds exhibited antioxidant activity at a rate of 62.046% DPPH inhibition activity with IC50 value of 358 ± 0.02 $\mu$g/ml. In addition, the seeds found to inhibit *S. aureus* with a minimum concentration of 15.625 mg/ml and contained the highest compound of resorcinol (40.769%) from GC-MS analysis.

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