Characterization of antioxidant from daunkemangi (*Ocimum sanctum*) extracted using ultrasonic bath

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Abstract. The antioxidant properties of *Ocimum sanctum* extract were characterized. The extract was prepared by extraction using ultrasonic bath at 50 °C for 30 minutes. The characterization was performed using FT-IR analysis. The phytochemical assays were carried out using various reagents. The results confirmed the presence of phenolics, flavanones, flavonoids, steroids and terpenoids in the extract. The DPPH assay also showed the antioxidant radical scavenging activity by percentage of inhibition of 57.95%.

1. Introduction

Antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate [1]. Antioxidants can be classified into two major types based on their source, i.e., natural and synthetic antioxidants [2]. Natural antioxidants can be found in all plant parts and they include carotenoids, vitamins, phenols, and flavonoid [3]. Various researches have reported that natural antioxidants can be obtained from teas [4], fruits such as orange [5], grapes [6], berries [7], as well as herbs such as peppermint [8], thyme [9], sage [9], parsley [10], rosemary [11], coriander[10], etc. Spices and herbs are rich sources of antioxidants [12,13,14]. Plant phenolic compounds are secondary metabolites possessing high antioxidant activity and are widespread in the species of *Lamiaceae*[15,16,17]. *Ocimum sanctum*, commonly known as the white holy basil herb belonging to *Lamiaceae* family is one of the oldest and popular medicinal plant rich in various phytoneutrients and antioxidants. Kemangi (holy basil) is cultivated in Southeast Asia but is also abundantly grown in Australia, West Africa, and some Arab countries [18]. The leaf of the plant owes a stronger, somewhat pungent taste than other basils due to a sesquiterpenoid beta-caryophyllene, and a phenylpropanoid eugenol. The chemical composition of Tulsi is highly complex, containing many biologically active phytochemicals with variable proportions among varieties or even plants within the same field [19]. Kemangi is known to possess total phenolic content of 41.90 mg GAE/g dry basis of plant[20]. In this paper, the characterization, phytochemical screening, and antioxidant activity of holy basil extract were presented.
2. Materials and Methods

2.1. Materials

2.1.1. Plant Material. The leaves of *Ocimum sanctum* were purchased from a local market.

2.1.2. Chemicals. Ethanol, ferric chloride, sodium hydroxide, acetic acid, sulfuric acid, chloroform, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH•), were used as received.

2.2. Preparation of Plant Extract

The fresh leaves of *Ocimum sanctum* were washed with clean and flowing water and then dried using dryer at 40 °C for 24 hours. The dried leaves were ground into powder and 10 grams of ground leaves were mixed with 100 ml of 96% ethanol in a 250 ml erlenmeyer. Extraction were conducted in an ultrasonic bath (Elmasonic S 300 H) at 50 °C for 30 minutes. After extraction, the mixture was filtered using No. 1 Whatman paper, and the filtrate was concentrated using rotary evaporator. The obtained extract was stored at−20 °C until further analysis.

2.3. Fourier Transform-Infra Red (FT-IR) Analysis

Fourier Transform-Infra Red analysis of the plant extract was performed using Bruker in the region of 4000-500 cm⁻¹.

2.4. Phytochemical Analysis

2.4.1. Phenolics. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate occurrence of a blue, violet, purple, green, or red-brown color indicates the presence of phenolics.

2.4.2. Flavonones. To the substance, 10% sodium hydroxide was added; yellow to orange color shows the presence of flavanones.

2.4.3. Flavonoids. To the substance, 2 ml of the 10% aqueous sodium hydroxide was added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

2.4.4. Steroids (Liebermann-Burchard test). To 0.2 g of each portion, 2 ml of acetic acid was added, the solution was cooled well in ice followed by the addition of conc. H₂SO₄ carefully. Color development from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. aglycone portion of cardiac glycoside.

2.4.5. Terpenoids (Salkowski test). To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

2.5. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay.

The antioxidant activity of ethanolic extract of *Ocimum sanctum* was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH•) as a free radical, according to the method described by Brand-Williams et al. (1995) []. 1000 ppm of extract stock solution was prepared. To the 100 µL of system containing the extract, 3.9 ml of DPPH• (25 mg DPPH•/L of ethanol) was added and let sit for 45 minutes. The absorbance was determined using a spectrophotometer at 517 nm. Ascorbic acid was used as a standard. The percentage of inhibition was calculated as

\[
\text{Percentage of inhibition} = \left( \frac{(A_0 - A_1)}{A_0} \right) \times 100
\]

where \(A_0\) was the absorbance of the control (blank) and \(A_1\) was the absorbance in the presence of the extract.
3. Results and Discussions

3.1. Fourier Transform-Infra Red (FT-IR) Analysis

From Figure 1, it can be observed that band at 3379.05 cm\(^{-1}\) is related to OH or hydroxyl group [21]. The band at 2924.35 cm\(^{-1}\) could be related to C-H stretching vibration of alkane [22]. At 1640.44 cm\(^{-1}\), it could be related to stretching vibration of aromatic C=C; and to the vibration of N-H of amines, C=O of amides and carboxylic groups [23]. The band at 1374.57 cm\(^{-1}\) could be due to CH\(_3\) bend. There was a non-identified band at 1163.57 cm\(^{-1}\). The band at 1042.79 cm\(^{-1}\) could be related to C-O of alcohols.

![Figure 1. FT-IR Bands of Ocimum sanctum Extract](image-url)
3.2. Phytochemical Analysis

Table 1. The results of the phytochemical screening of leaves extracts of *Ocimum sanctum*

| Chemical groups | Inference |
|-----------------|-----------|
| Phenolics       | +         |
| Flavonones      | +         |
| Flavonoids      | +         |
| Steroids        | +         |
| Terpenoids      | +         |

−= absence, += presence.

Phytochemicals are a large group of bioactives derived from plants which have potential protective effects against diseases[24]. The phytochemical screening revealed the presence of phenolics, flavanones, flavonoids, steroids, and terpenoids as shown in Table 1. The phenolics test resulted in
blackish-green color (Figure 1), in agreement with previous studies by Saptarini, et al., 2016 and Adhani, 2017 [25,26]. Phenolic compounds are well-known phytochemicals found in all plants. They consist of simple phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans and flavonoids [27]. Dried herbs and spices are generally a good source of phenolic compounds [28]. Most phenols react with iron (III) chloride \( \text{FeCl}_3 \) to form coloured complexes. The colours vary depending on various factors, e.g. the phenolic compound used, the solvent, concentration [29].

Flavonoids are biologically active low molecular weight secondary metabolites that are produced by plants, with over 10,000 structural variants now reported [30]. Flavonoids can be divided into a variety of classes such as flavones (e.g., flavone, apigenin, and luteolin), flavonols (e.g., quercetin, kaempferol, myricetin, and fisetin), flavanones (e.g., flavanone, hesperetin, and naringenin), and others [31]. Flavonanes are another important class which is generally present in all citrus fruits such as oranges, lemons and grapes [32]. The result of flavonones and flavonoids test were shown in Figure 3 & 4. Flavonoids generally dissolve in alkalis, giving a yellow solution (phenates) which on addition of acid becomes colourless. The flavonoids are generally yellow compounds and the intensity of their yellow colour increases with the number of OH groups and with increase of the pH of the medium [33].

Steroids are a group of cholesterol derived lipophilic, low-molecular weight compounds found in / derived from a variety of different marine, terrestrial, and synthetic sources. Plant steroids are of two broad categories: phytosterols and brassinosteroids [34]. Steroids are easily detected in a crude extract by a colored test obtained by the Liebermann-Burchard reaction, developed by two research groups, Liebermann in 1885 and Burchard in 1889. Steroids are revealed by a positive color which changes from violet to blue and grass green [35]. The result of steroid test in kemangi extract gave bluish green color (Figure 5), which is positive. This result is consistent with previous study by Inbaneson, 2012 [36].

Terpenoids are modified class of terpenes with different functional groups and oxidized methyl group moved or removed at various positions. Terpenoids are divided into monoterpenes, sesquiterpenes, diterpenes, sesterpenes, and triterpenes depending on its carbon units [37]. The result of terpenoids test showed the appearance of reddish-brown interface (Figure 6). Holy basil owes a stronger, somewhat pungent taste than other basils due to beta caryophyllene, a sesquiterpenoid, and eugenol [18]. This result confirmed the presence of terpenoids in the extract, which is also in agreement with previous study [36].

### 3.3. DPPH Radical Scavenging Assay

Antioxidant activity of kemangi extract was analyzed using 2,2–diphenyl–1–pycrylhydrazyl (DPPH) radical scavenging method. Ascorbic acid was used as a standard. The absorbance was measured at 517 nm. The extract and standard concentration were set as 1 mg/mL. The measurements were done in triplicates.

| Sample | Absorbance (nm) | % Inhibition |
|--------|----------------|--------------|
| Blank  | 0.581          |              |
| 1      | 0.238          |              |
| 2      | 0.253          |              |
| 3      | 0.242          |              |
| Mean   | 0.244          | 57.95        |
| Ascorbic acid | 0.243 | 58.18        |

DPPH is a stable and commercially available organic nitrogen radical, which reacts with hydrogen/electron donor compounds and has a maximum UV–Vis absorption within the range of 515–520 nm. DPPH assay is a reliable method to determine the antioxidant capacity of biological substrates. The DPPH radical scavenging activity is generally quantified in terms of inhibition percentage of the
pre-formed free radical by antioxidants [38]. The higher the number, the higher the antioxidant activity of the spice/herb [24]. From the result of DPPH assay, it showed that kemangi extract possess antioxidant activity. The inhibition percentage of kemangi extract obtained was close to that of ascorbic acid used as standard. Ascorbic acid or vitamin C is a naturally occurring organic compound with antioxidant properties [39].

The antioxidant effect of phenolic compounds is mainly due to their redox properties and their capacity to block the production of reactive oxygen species (ROS) such as hydrogen peroxide (H$_2$O$_2$) and the superoxide radical anion (O$_2^{•−}$)) formed in several in vitro and in vivo systems[14]. Phenolic compounds reduce or inhibit free radicals by transfer of a hydrogen atom, from its hydroxyl group. The reaction mechanism of a phenolic compound with a peroxyl radical (ROO•) involves a concerted transfer of the hydrogen cation from the phenol to the radical, forming a transition state of an H-O bond with one electron. The antioxidant capacity of the phenolic compounds is strongly reduced when the reaction medium consists of a solvent prone to the formation of hydrogen bonds with the phenolic compounds [40].

4. Conclusions
Kemangi or Ocimum sanctum extract possessed various phytochemicals such as phenolic compounds, flavonoids (flavonones), steroids, and terpenoids. Phenolic compounds found in plant extract contributed to the antioxidant activity of the extract in free radical scavenging, shown by the percentage of inhibition of free radical/ROS. Based on this study, kemangi (Ocimum sanctum) is a potential natural antioxidant source which can be incorporated in many products. One of the methods to produce antioxidant from kemangi is by extraction using ultrasonic bath, which has been conducted in this study.

5. References
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