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Antioxidant Capacities of Hot Water Extracts and Endopolysaccharides of Selected Chinese Medicinal Fruits

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Abstract: Fruits are a rich source of antioxidants and traditional Chinese fruits have been studied for their chemopreventive and chemotherapeutic properties against cancers and other diseases. The total phenol and flavonoid contents of eleven Chinese fruits extracts were determined. Total phenolic and flavonoid contents were estimated by both the Folin-Ciocalteau and aluminium chloride methods. The antioxidant activities were evaluated by four assays: a biological assay using Saccharomyces cerevisiae, DPPH radical scavenging activity, chelating ability for ferrous ions and ferric reducing antioxidant power (FRAP). The phenols and flavonoids contents of the hot water extracts were in the range of 17.7 to 94.7 mg/g and 12.3 to 295.4 mg/g, whereas the endopolysaccharides lie in the range of 4.5 to 77.4 mg/g and 22.7 to 230.0 mg/g. Significant amounts of phenols and flavonoids were present in the majority of the fruit extracts and showed strong antioxidant activities. The antioxidant properties of the fruit extracts of Crataegus pinnatifida, Illicium verum, Ligustrum lucidum, Momordica grosvenori and Psoralea corylifolia as determined by the DPPH and FRAP methods, were significant compared to other fruit extracts. In the present study, we found that significant amounts of phenolic and flavonoid compounds were present in these fruit extracts and may contribute to in vitro antioxidant activities.

Keywords: fruits; phenols; flavonoids; polysaccharides; anti-oxidant activity

1. Introduction

Fruits are a rich source of antioxidants (polyphenols, vitamin C, carotenoids etc.) that can prevent the formation of free radicals. Free radicals and non-free radical compounds are constantly produced in the human body during cell metabolism [1] and these molecules contain oxygen. These reactive molecules are called reactive oxygen species (ROS). Free radicals play an important role in cell metabolism [2] and excess production of these free radicals is toxic and may cause oxidative damage to biological molecules in the human body [3,4]. There is an evidence to suggest that reactive oxygen species are involved in the pathogenesis of many diseases including age-related disorders, cancer, atherosclerosis, neurodegenerative diseases and inflammation [5–9]. The antioxidant compounds [9,10] can inhibit the production of ROS in the human body as well as prevent the damage of the cells caused...
by oxidative stress [11,12]. There is strong evidence from studies in epidemiology, animal tumor models and in vitro studies demonstrating that a significant number of natural compounds found in fruit and vegetables could lower the incidence of cancer [13–15]. Several papers have reported that phenolics and flavonoids of plants and fruits [4,9,16–18] show different antioxidant activities depending on the availability of their phenol and flavonoid contents. The phenolics and flavonoids have also been shown to inhibit the inflammation process and are expected to have anti-inflammatory properties. [19].

The main objectives of the present study was to (i) determine the phenolic and flavonoid contents, (ii) systematically evaluate the antioxidant activities by using Saccharomyces cerevisiae, DPPH radical scavenging activity, chelating ability for ferrous ions and ferric reducing antioxidant power (FRAP) and (iii) correlate their relationship with total phenolic and flavonoid content of 11 Chinese medicinal fruits. The reported medicinal uses of these fruits are given in Table 1.

### Table 1. List of medicinal fruits used in this study.

| Chinese Fruits        | Family Name | Medicinal Uses                                                                 |
|-----------------------|-------------|-------------------------------------------------------------------------------|
| 1. Prunus mume Siebold & Zucc. | Rosaceae     | Fever, cough and intestinal disorders [20]                                     |
| 2. Lycium barbarum L.  | Solanaceae   | Cardiovascular and inflammatory diseases [21], vision-related diseases [22], such as age-related macular degeneration and glaucoma [23] or from neuroprotective [24], anticancer [25] or immunomodulatory activity. |
| 3. Amomum villosum Lour. | Zingiberaceae | Digestion disorder [26]                                                       |
| 4. Amomum kravanh Pirre ex Gagnep. | Zingiberaceae | Anti-asthmatic [27]                                                            |
| 5. Craegaus pinnatifida Bunge | Rosaceae     | Anticancer [28]                                                                |
| 6. Ilicium verum Hook. f. | Iliciaceae   | Antibacterial [29], neurotropic, hypothemic and analgesic [30]                 |
| 7. Amomun tsao-ko Crevost & Lemarié | Zingiberaceae | Malaria, throat infections, abdominal pain, stomach disorders, dyspepsia, nausea, vomiting and diarrhea [31] |
| 8. Ligustrum lucidum W.T.Aiton | Oleaceae    | Immunomodulatory, anti-inflammatory, hepatoprotective, anti-tumor and anti-aging act [32] |
| 9. Momordica grosvenori Swingle | Curcubitaceae | Anti-diabetic [33], anticancer [34], anti-inflammatory, antioxidant, anti-diabetic, and nephroprotective properties [35] |
| 10. Psoralea corylifolia L. | Fabaceae     | Anticancer [36,37]                                                             |
| 11. Schisandra chinensis Turcz. Baill. | Schisandraceae | Anti-inflammatory [38], antiviral, anticancer and neuroprotective effects [39] |

2. Results and Discussion

2.1. The Yields, Total Phenolic and Flavonoid Content of HWEs and ENPs

Table 2 shows the yield of hot water extracts (HWEs) and endopolysaccharides (ENPs). The yields of the HWEs are in the range 478.8 to 4580.6 mg/10 g dry fruit, whereas the ENPs lie in the range of 116.3 to 761.5 mg/10 g. The total phenolic and flavonoid content of the HWEs and ENPs were measured using the F-C reagent and aluminium chloride methods respectively. The results obtained for the HWEs and ENPs are presented in Table 3. Among the eleven Chinese medicinal fruits studied, L. lucidum HWE has the largest phenolic content (94 mg/g) followed by A. tsao-ko (76 mg/g). The ENP of L. lucidum contained significant amount of phenolic compounds (77 mg/g) followed by M. grosvenori (52 mg/g) and P. corylifolia (53 mg/g) ENPs. Other fruits ENPs contained appreciable amounts of phenolic compounds in the range of 21–37 mg except A. tsao-ko (4.5 mg/g). The amounts of flavonoid contents in majority of the fruit extracts (HWEs and ENPs) have the largest values compare to phenolic contents (Table 3). The proportional relation (%) of flavonoid contents to phenolic contents in the present study fruit extract were shown in Figure 1. The flavonoid contents were the chief constituents (75%) in most of the fruit except Lycium barbarum and Momordica grosvenori fruits (Figure 1) where the phenolic contents were present more than 25%.
Table 2. Yields of hot water extracts and endopolysaccharides of fruits studied.

| Chinese Fruits               | Hot Water Extracts (mg/10 g Dry Fruit) | Endopolysaccharides (mg/10 g Dry Fruit) |
|-----------------------------|--------------------------------------|----------------------------------------|
| 1. Prunus mume              | 2864.40                              | 500.18                                 |
| 2. Lycium barbarum         | 4580.86                              | 200.50                                 |
| 3. Amomum villosum          | 887.07                               | 446.86                                 |
| 4. Amomum kravanh           | 478.81                               | 240.99                                 |
| 5. Crataegus pinnatifida    | 3239.41                              | 761.56                                 |
| 6. Illicium verum           | 1440.41                              | 387.42                                 |
| 7. Amomum tsao-ko           | 514.61                               | 116.34                                 |
| 8. Ligustrum lucidum        | 1601.63                              | 397.34                                 |
| 9. Momordica grosvenori     | 2737.64                              | 365.29                                 |
| 10. Psoralea corylifolia    | 1404.95                              | 263.80                                 |
| 11. Schisandra chinensis    | 3250.40                              | 474.40                                 |

Figure 1. Proportional relation (%) of flavonoids content to phenolic content in the fruit extracts. (A) Hot water extracts; (B) Endopolysaccharides.

2.2. Anti-Oxidant Activities

The antioxidant activities of eleven selected medicinal fruits were evaluated by four methods, namely, DPPH free radical scavenging, ferrous ion-chelating capacity, ferric-reducing antioxidant power (FRAP) and yeast based antioxidant screening assay. The results are presented in Table 3. The majority of the hot water extracts (HWEs) and endopolysaccharides (ENPs) showed antioxidant activity against yeast model on the basis of inhibition ability against oxidative stress induced by H$_2$O$_2$ except I. verum and P. corylifolia fruits. The mechanism of antioxidant activity of fruit extracts possibly a direct inactivating effect on H$_2$O$_2$ by scavenging hydroxyl radicals or an activation of the cellular oxidative response, which would allow increased yeast growth [40].
Table 3. Antioxidant activities of endopolysaccharides (ENPs) and hot water extracts (HWEs) obtained for Chinese fruits studied.

| Chinese Fruits | Scavenging Activity on Yeast | Total Phenol Content (GAE mg/g ± SD) | Total Flavonoid Content (QE mg/g ± SD) | DPPH in Percent (I(%)$^a$) | Ferrous Ion-Chelating Ability % ± SD | EDTA Equivalent (ug/mL ± SD) | Ferric-Reducing Antioxidant Power (CPE $^b$ mg/g ± SD) |
|----------------|------------------------------|-------------------------------------|----------------------------------------|---------------------------|----------------------------------|-----------------------------|----------------------------------|
| 1-ENP $^d$     | + $^f$                       | 24.85 ± 0.52                       | 122.06 ± 3.39                         | 46.58 ± 0.00              | -                                | -                           | 30.48 ± 0.11                     |
| 2-ENP          | +                            | 21.24 ± 0.39                       | 29.76 ± 0.99                          | 19.86 ± 0.97              | 62.98 ± 0.37                     | 226.91 ± 1.93                | 34.73 ± 0.25                     |
| 3-ENP          | +                            | 23.65 ± 0.39                       | 116.66 ± 1.41                         | 37.33 ± 0.48              | 63.50 ± 2.34                     | 229.64 ± 12.21               | 24.91 ± 0.42                     |
| 4-ENP          | +                            | 24.85 ± 0.52                       | 100.06 ± 2.55                         | 27.40 ± 0.97              | 32.40 ± 5.42                     | 67.36 ± 28.28                | 13.63 ± 0.11                     |
| 5-ENP          | +                            | 37.61 ± 0.26                       | 227.06 ± 2.83                         | 46.92 ± 0.48              | -                                | -                           | 51.39 ± 0.21                     |
| 6-ENP          | -                            | 37.35 ± 0.13                       | 185.96 ± 0.42                         | 53.42 ± 0.97              | -                                | -                           | 36.98 ± 0.39                     |
| 7-ENP          | - $^g$                       | 4.51 ± 0.09                        | 22.66 ± 0.57                          | 20.89 ± 0.48              | 81.71 ± 1.72                     | 324.64 ± 9.00                | 1.16 ± 0.07                      |
| 8-ENP          | +                            | 77.44 ± 0.26                       | 230.06 ± 0.57                         | 76.37 ± 1.45              | 38.76 ± 2.09                     | 100.55 ± 10.93               | 54.21 ± 0.14                     |
| 9-ENP          | +                            | 52.07 ± 0.26                       | 96.66 ± 0.85                          | 68.15 ± 1.45              | -                                | -                           | 40.46 ± 0.35                     |
| 10-ENP         | -                            | 53.83 ± 0.39                       | 130.66 ± 2.55                         | 48.97 ± 1.45              | 48.08 ± 0.74                     | 149.18 ± 3.86                | 14.78 ± 0.32                     |
| 11-ENP         | +                            | 22.63 ± 0.26                       | 108.86 ± 0.57                         | 4.45 ± 27.61              | -                                | -                           | 11.68 ± 0.11                     |
| 1-HWE $^e$     | + $^f$                       | 25.22 ± 0.26                       | 47.10 ± 0.94                          | 39.38 ± 0.48              | -                                | -                           | 29.03 ± 0.25                     |
| 2-HWE          | +                            | 21.61 ± 0.13                       | 12.35 ± 0.82                          | 34.93 ± 0.97              | 64.11 ± 1.48                     | 232.82 ± 7.71                | 27.33 ± 0.53                     |
| 3-HWE          | +                            | 33.46 ± 0.39                       | 92.27 ± 1.18                          | 55.14 ± 1.45              | 25.35 ± 0.37                     | 30.55 ± 1.93                 | 36.71 ± 0.71                     |
| 4-HWE          | +                            | 29.11 ± 0.26                       | 70.52 ± 4.83                          | 32.22 ± 1.45              | 49.65 ± 1.23                     | 157.36 ± 6.43                | 15.61 ± 0.21                     |
| 5-HWE          | +                            | 49.48 ± 0.26                       | 182.40 ± 5.66                         | 50.00 ± 0.00              | -                                | -                           | 60.28 ± 1.31                     |
| 6-HWE          | -                            | 65.96 ± 1.31                       | 235.73 ± 0.94                         | 62.67 ± 0.48              | -                                | -                           | 67.71 ± 0.71                     |
| 7-HWE          | +                            | 76.33 ± 2.62                       | 295.40 ± 0.47                         | 69.52 ± 0.48              | 62.02 ± 5.17                     | 221.91 ± 27.00               | 76.78 ± 0.60                     |
| 8-HWE          | +                            | 94.67 ± 1.31                       | 221.07 ± 3.77                         | 77.40 ± 0.97              | 19.86 ± 9.12                     | 1.91 ± 47.57                 | 73.78 ± 0.18                     |
| 9-HWE          | +                            | 40.22 ± 1.31                       | 33.02 ± 0.12                          | 46.58 ± 0.00              | -                                | -                           | 35.56 ± 0.28                     |
| 10-HWE         | -                            | 57.72 ± 0.65                       | 56.85 ± 3.89                          | 50.34 ± 0.48              | 49.13 ± 2.96                     | 154.64 ± 15.43               | 19.76 ± 0.21                     |
| 11-HWE         | +                            | 17.72 ± 0.39                       | 33.43 ± 0.24                          | 26.37 ± 2.42              | -                                | -                           | 17.93 ± 0.39                     |

$^a$ The ratio of inhibition of free radical by DPPH in percent (I(%)$^a$) as follows; I (%) = [(A_{blank} − A_{sample})/A_{blank}] × 100; $^b$ Ferrous ion-chelating ability as follows; % = [A_{control} − (A_{sample} − A_{blank})]/A_{control} × 100; $^c$ Ferric reducing antioxidant power was expressed in g of chlorogenic acid power (CPE) per mg of dry weight; $^d$ ENP: endopolysaccharide; $^e$ HWE: hot water extract; $^f$ positive activity; $^g$ no activity; Yeast oxidative stress was measure on the basis of survival of yeast cells (yeast growth) after treatment of H$_2$O$_2$. 
Most of the fruits showed significant scavenging capacity and it was moderately varied between HWEs to ENPs. The DPPH radical absorbs at 515 nm and this absorption is inhibited in the presence of antioxidants such as phenolics and flavonoids. The percentage of DPPH inhibition in HWEs ranged from 26.37% to 77.40% while in ENPs it ranged from 4.45% to 76.37% (Table 3). The highest DPPH inhibition was shown by *L. lucidum* and the lowest by *S. chinensis*. The other fruits showed significant scavenging activity against DPPH in both HWEs and ENPs. The results in this study revealed that the extracts obtained from these fruits was free radical scavengers which reacted with DPPH radical by their electron donating ability [39] and their antioxidant activities due to a combination of their total phenolic and flavonoid content as reported in other studies [41,42].

The ability of the extracts to bind with Fe$^{2+}$ in the presence of ferrozine is compared with that of EDTA, which is a strong chelating agent that binds to metals via four carboxylate and two amine groups [43]. The ferrous ion-chelating ability results are summarised in Table 3. Among the eleven fruits studied, five of them (*Prunus mume*, *C. pinnatifida*, *I. verum*, *M. grosvenori* and *Schisandra chinensis*) do not show chelating capacity. The other fruit extracts showed significant capacity for Fe$^{2+}$ binding, suggesting their antioxidant ability. The HWEs and ENPs of *Lycium barbarum*, *A. tsao-ko* and *P. corylifolia* can be interpreted as having the highest ferrous ion chelating ability among the fruits studied. Flavonoid and phenolic compounds are known to act as antioxidants, radical scavengers and metal chelators [44].

The antioxidant activity of the HWEs and ENPs measured by the FRAP assay are given in Table 3. The FRAP activity is significant in some of the analysed samples due to the presence of phenolic and flavonoid compounds and a similar trend is observed for many other plant extracts that have been studied [45]. The reducing power of a compound is dependents on its electron transfer ability [46]. The HWEs of *I. verum*, *A. tsao-ko* and *L. lucidum* exhibited high DPPH and FRAP values which can be interpreted as the highest antioxidant activities of the fruits studied. Also these three fruit extracts contained high content of phenolics and flavonoids. The ENPs of *L. lucidum* and *C. pinnatifida* showed significant DPPH and FRAP activities compare to other fruit ENPs. The fruit extracts of *L. lucidum* and *C. pinnatifida* had stronger DPPH radical scavenging activity and these fruit extracts are using for the treatment of different disorders [28,32]. The fruit extracts of *L. lucidum* showed protective effect against BHT-induced oxidative stress in rats [47]. The fruit extract of *C. pinnatifida* showed good antioxidant activity and neuroprotective effects [48].

### 2.3. Correlation between Antioxidant Capacities and Total Phenolic and Flavonoid Content

The correlation between the phenolic and flavonoid content, and their antioxidant activities were studied. The correlation results are presented in Table 4 and Figures 2–4. The antioxidant activities of the present study showed a significant correlation with phenolics and flavonoids except ferrous-chelating capacity. The correlation with hot water extracts were: $R^2 = 0.8797$ between DPPH and total phenolics, $R^2 = 0.6977$ between DPPH and flavonoids, $R^2 = 0.6835$ between FRAP and total phenolics and $R^2 = 0.8695$ between FRAP and flavonoids. The correlations with endopolysaccharides (ENPs) were: $R^2 = 0.6993$ between DPPH and total phenolics, $R^2 = 0.4051$ between DPPH and flavonoids, $R^2 = 0.4285$ between FRAP and phenolics and $R^2 = 0.4762$ between FRAP and flavonoids, respectively. The significant correlations between the antioxidant activities and phenolics & flavonoids showed that the phenolics and flavonoids largely contribute to the antioxidant activities of these fruit extracts, and could play an important role in biological activities. The antioxidant activities were significant in hot water extracts compare to endopolysaccharides. In addition, a good linear relationship was noticed in hot water extracts among antioxidant activities of DPPH and FRAP.
Table 4. Correlation R² values between the chemical compound and antioxidant activities of Chinese fruits.

| Compounds/Activities                  | Phenolic Compound | Flavonoids | DPPH         | Ferrous ion Chelating Ability | FRAP         |
|---------------------------------------|-------------------|------------|--------------|-------------------------------|--------------|
| Phenolic compound                     |                   | 0.6917     | 0.8798       | 0.1265                        | 0.6835       |
| Flavonoids compound                   |                   | 0.6993     | 0.4051       | 0.0238                        | 0.8695       |
| DPPH                                  |                   | 0.216      | 0.4767       | 0.2922                        | 0.7171       |
| Ferrous ion chelating ability         |                   | 0.2823     | 0.3354       | 0.4683                        | 0.0571       |

*a* Bold font values are correlation R² values of endopolysaccharides; *b* Normal font value are correlation R² values of hot water extracts.

Figure 2. Correlation R² values of phenolic and flavonoids contents, and DPPH and ferrous ion-chelating activities between the endopolysaccharides and hot water extracts.

Figure 3. Cont.
Figure 3. Correlation $R^2$ values of antioxidant activities between phenolic and flavonoid contents (hot water extracts).

Figure 4. Correlation $R^2$ values of antioxidant activities between phenolic and flavonoid contents (endopolysaccharides).

3. Experimental Section

3.1. Fruits

The dried fruits were obtained from Beijing Tong Ren Tang Chinese Herbal Medicine shop, Sydney, Australia. A voucher specimen of each sample has been deposited in the University laboratory. The scientific names and family names are given in Table 1. The samples were ground to a fine powder in a grinder before extraction.
3.2. Chemicals and Reagents

Gallic acid, quercetin, DPPH, DMSO, sodium carbonate, aluminium chloride, sodium nitrate, sodium hydroxide, hydrogen peroxide, Folin-Ciocalteu (F-C) reagent, ascorbic acid, 95% ethanol, bovine serum albumin (BSA), lipopolysaccharide (LPS: *E. coli* serotype 0127:B8), EDTA, N-(1-1-napthyl) ethylenediamine dihydrochloride, were purchased from Sigma (Sydney, Australia) and Lomb Scientific Pty Ltd (Sydney, Australia).

3.3. Preparation of Hot Water Extracts and Endopolysaccharides

Approximately 30 g of each ground fruit was autoclaved with 50 mL of deionised water at 121 °C for 2 h. The extracted samples were centrifuged at 10,447 × g for 20 min and the supernatant transferred to a 50 mL volumetric flask. The residue was further rinsed two more times, and the pooled the extract adjusted to 100 mL. The preparation and isolation of hot water extracts and endopolysaccharides from the plants was as described previously [49–51] and is summarised in Figure 5. All hot water extracts and endopolysaccharides were filtered through a 0.45 µm nylon filter before analysis.

![Figure 5. Schematic diagram for the processing of hot water extracts and endopolysaccharides from medicinal fruits.](image)

3.4. Determination of Total Phenolic Content

The total phenolic content was determined using the Folin-Ciocalteu (F-C) colorimetric method [52]. Briefly, 50 µL of sample and 50 µL of F-C reagent were pipetted into an Eppendorf tube. The contents were vortexed for 10 sec and then left to stand at room temperature for 2 min before the reaction was stopped by adding 500 µL 5% (w/v) sodium carbonate solution and 400 µL distilled water and the volume adjusted to 1 mL. The mixture was then vortexed and incubated at 45 °C for 30 min before cooling rapidly with ice. The solution absorbance was measured at 760 nm. Gallic acid concentrations ranging from 25–300 µg/mL were prepared and the calibration curve was obtained using a linear fit ($R^2 = 0.9961$) [42]. The samples were analysed in duplicate.
3.5. Determination of Total Flavanoid Content

The total flavonoids were estimated by the aluminium chloride method [53]. Briefly, 0.5 mL of the sample and 300 µL of NaNO\textsubscript{2} (1:20 w/v) were pipetted into a test tube and the contents were vortexed for 10 s and left to stand at room temperature for 5 min. After standing, 300 µL of AlCl\textsubscript{3} (1:10 w/v), 2 mL of 1M NaOH and 1.9 mL of distilled water was added to the reaction mixture which was then vortexed for 10 sec and the absorbance measured at 510 nm. Quercetin concentrations ranging from 0 to 1200 µg/mL were prepared and the standard calibration curve obtained using a linear fit (R\textsuperscript{2} = 0.9980). The samples were analysed in duplicate.

3.6. Free Radical DPPH Scavenging Assay

The DPPH assay was carried out according to the procedure of Brand-Williams et al. [54], with minor modifications. In this study, different volumes (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µL) of endopolysaccharides and hot water extracts were mixed with a methanolic solution of DPPH radical (2.2 mg/L, 200 µL) in a 96 well microplate. The final volume of each well was made up to 300 µL by adding an appropriate amount of methanol. The mixture was shaken gently on a microplate reader, and the absorbance at 515 nm taken every 2 min for 30 min or until the absorbance reached its maximum value. The DPPH concentration in the reaction medium was calculated from a calibration curve derived from a serial dilution of the DPPH standard. The control (containing all reagents except the test compound) and standards were subject to the same procedure. The free radical scavenging activity is expressed as the inhibition percentage of free radicals by the sample and calculated using following the formula:

\[
\% \text{ of DPPH radical scavenging effect} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}}\right) \times 100
\]

where A\text{control} is the absorbance of control and A\text{sample} is the absorbance of sample at 515 nm. The samples were analysed in triplicate.

3.7. Assay for Screening of Scavenging Activity in a 96-well Microplate Using Saccharomyces Cerevisiae

The antioxidant capacity of the fruit extracts was also measured using a Saccharomyces cerevisiae-based high throughput assay [4,55]. Yeast is the most characterized eukaryote at the molecular level; it serves as a paradigm for higher eukaryotes like plants and animals in fundamental cellular studies. The yeast based assay utilises the important physiological phenomenon of oxidative stress-mediated cell cycle arrest to screen the oxidant scavenging and intracellular antioxidant activity for a chemically diverse range of compounds. Saccharomyces cerevisiae BY4743 was cultured overnight in a 50 mL volume by inoculation of a single colony. The culture was then diluted to an optical density at 600 nm (OD\textsubscript{600}) of 0.2 in media, and 180 µL of the strain was added to each well in a 96-well microplate where 10 µL per well of each herbal extract had been added in duplicate. 10 µL of 32% v/v hydrogen peroxide of (H\textsubscript{2}O\textsubscript{2}) was added to give a final concentration of 4 mM. The initial OD\textsubscript{600} reading was taken using a microplate reader (Multiskan EX, Thermo Electron, Waltham, MA, USA), and the plates were then incubated in a 30 °C incubator with shaking at 750 rpm. Yeast growth was monitored by reading the OD\textsubscript{600} until 20 h. Ascorbate was used as the positive control and samples were analysed in duplicate.

3.8. Ferrous Ion-Chelating Effect

The ferrous ion-chelating effect of the sample was estimated by the method of Chua et al. [56]. Briefly, 740 µL of methanol and 200 µL of sample were incubated with 20 µL of 2 mM FeCl\textsubscript{2} solution. The reaction was initiated by adding 40 µL of 5 mM ferrozine into the mixture which was then allowed to stand at ambient temperature for 10 min. The absorbance of the reaction mixture was measured at 562 nm. Distilled water instead of ferrozine solution was used as a blank, to correct for any unequal
colour of the sample solution. EDTA-Na₂ was used as a reference standard. The ferrous ion-chelating ability was calculated as follows:

\[
\text{Ferrous ion – chelating ability (\%)} = \left( \frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})}{A_{\text{control}}} \right) \times 100
\]

where \(A_{\text{control}}\) is the absorbance of the control, \(A_{\text{sample}}\) the absorbance of sample or standard and \(A_{\text{blank}}\) the absorbance of the blank.

3.9. Ferric-Reducing Antioxidant Power Assay (FRAP)

The ferric-reducing antioxidant power (FRAP) of the extracts was tested using the assay of Oyaizu [57]. One mL of different concentrations of the samples as well as the reference chlorogenic acid was added to 2.5 mL phosphate buffer (0.1 M, pH 6.6) and 2.5 mL potassium ferricyanide (1.0%, w/v). Later, each mixture was incubated at 50 °C for 20 min and then 2.5 mL 10% trichloroacetic acid added. After the mixture was shaken vigorously, 2.5 mL of the solution was mixed with 2.5 mL distilled water and 0.5 mL of FeCl₃ (0.1%, w/v). After 30 min incubation, absorbances were read at 700 nm. Each analysis was carried out in duplicate. Increased absorbance of the reaction mixture indicates increased reducing power.

3.10. Data Presentation and Analysis

The results are expressed as mean ± standard deviation, calculated from the duplicate determinations. The linear relationships and significance tests of these data sets were also calculated. GraphPad Prism 5.01 (GraphPad Software, Inc., La Jolla, CA, USA) was used for the growth curve analysis of the dose-dependent experiments.

4. Conclusions

In general, fruits contain several nutrients such as fibres, micronutrients and vitamins. Moreover, secondary metabolites were identified in fruits, especially phenolic compounds. The phenolic compounds in fruits are the chief bioactive compounds and known for several health benefits. The antioxidant activities and antioxidant contents (phenolics and flavonoids) of 11 selected medicinal fruits were evaluated. The hot water extracts and endopolysaccharides obtained from the fruits in present study showed significant antioxidant properties. Some of the fruit extracts showed a strong correlation between DPPH and FRAP value which suggests that antioxidants in these HWEs and ENPs possess free radical scavenging activity and oxidant reducing power. The present study results show further evidence about the importance of phenolic compounds and their antioxidant activities. The fruit extracts in the present study showed moderate antioxidant activities and could be used in herbal formulations for the prevention of diseases caused by free radicals in biological systems like cancer. These fruits could be considered as antioxidant and cancer therapeutic agents. Further research is however needed to establish health benefits of these fruit extracts.

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Conflicts of Interest: The authors declare no conflict of interest.

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