EFFECT OF COOKING METHODS ON TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITY OF SELECTED WILD EDIBLE PLANTS

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ABSTRACT

Objective: The target of this appraisal was to explore the impacts of various cooking techniques, for example, boiling and microwave cooking on total phenolics and antioxidant activity of Zanthoxylum acanthopodium, Viburnum foetidum, Houttuynia cordata, Sonchus arvensis and Oenanthe linearis, widely consumed by the common individuals of the North-Eastern area of India.

Methods: The antioxidant activities of the plants were determined by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, ABTS radical scavenging ability, reducing power capacity, estimation of total phenolic content, flavonoid content and flavanol content.

Results: Total phenolics content (TPC) of fresh vegetables ranged from 108.28 to 253.99 mg/100 g (as gallic acid equivalent) on a dry weight basis. Total antioxidant capacity of fresh plants (IC50 mg dry extract) determined by DPPH and ABTS ranged from 0.37-1.23 and 0.29-0.89, respectively. Boiling caused the highest losses of TPC, resulting in a reduction of the TPC on dry weight (DW) basis ranging from 9.37% in O. linearis up to 25.97% in Z. acanthopodium whereas microwave cooking enhanced TPC ranging from 4.09% to 10.38%. Similarly, boiling treatment decreased the DPPH radical scavenging activities ranging from 10.65 to 29.77% and ABTS radical scavenging activities ranging from 5.88-16.35%, whereas microwave cooking increased the DPPH and ABTS radical scavenging activities ranging from 8.02-24.20% and 9.86 to 19.70% respectively, in the studied plants.

Conclusion: The results suggest that the best cooking method for increasing the concentration of polyphenols and antioxidants was microwave cooking while boiling was the least recommended method.

Keywords: Wild edible plants, Antioxidant activity, Cooking methods

INTRODUCTION

Various kinds of wild edible plants are used as food sources in developing nations. Some people commonly collect wild edible plants and other plants from their natural surroundings to fulfill their appropriate level of nutrition due to the rapid rise in population, lack of fertile land for cultivation, and high prices of accessible staples. Due to dietary restrictions, cultural usage, and taste preferences, rural residents typically grow a large variety of wild vegetables without cultivating them. The evaluation of diverse wild edible plants has received a lot of attention recently because they are an essential part of the human diet, supplying the body with protein, energy, and essential vitamins, minerals, and hormone precursors. The inclusion of protein, carbohydrates, and other macronutrients in wild edible plants helps to lower the chance of developing diseases like cancer, coronary heart attack, diabetes, etc. [1].

Phenolic compounds are a class of phytonutrients with potent antioxidant effects. They can be divided into simple phenols, phenolic acids, derivatives of hydroxycinnamic acid, and flavonoids.

Antioxidants play an important role to protect the human body against damage by reactive oxygen species [2]. Free radicals have a special affinity for proteins, lipids and nucleic acids and are recognised to play a significant role in the development of aging-related degenerative diseases [3, 4]. Although many studies have shown that synthetic antioxidants like butylated hydroxanisole (BHA) and butylated hydroxytoluene (BHT) are effective free radical scavengers, their usage is restricted as they could be carcinogenic [5, 6].

Recent reports suggest that fruits and vegetables are good sources of natural antioxidants such as vitamins, carotenoids, flavonoids and other phenolic compounds [7, 8]. The existence of several bioactive compounds in food with potential antioxidant action has increased consciousness about the relationship between antioxidants and the risk of diseases [9]. Dietary vegetables have a significant and consistent preventive effect against the risk of a variety of age-related disorders, including cancer, cardiovascular disease, cataracts, and macular degeneration [10].

Before consumption, the majority of the vegetables are either boiled in water or microwaved. These cooking techniques would alter the physical characteristics and chemical makeup of vegetables in a variety of ways [11]. Total phenolic content, lycopene content, and antioxidant activity of tomatoes were only marginally affected by boiling and baking [12] while frying significantly reduced the antioxidant activities of tomatoes. According to Zhang and Hamazu, cooking affects the antioxidant components and antioxidant activity of broccoli [8]. Ismail, Marjan, and Foong (2004) [13] found that thermal treatment reduced the total phenolic content in all vegetables, such as kale, spinach, cabbage, swamp cabbage and shallots and antioxidant activity in some of them. Green beans, peas, peppers, squash, broccoli, leeks, and spinach are common vegetables that are eaten cooked. Wild vegetables are also consumed this way. However, very little information is available in the literature regarding the effect of cooking on the antioxidant activity and total phenolics of these vegetables. Therefore, the present study was undertaken to investigate the effects of different cooking methods on the antioxidant activity and total phenolics of Zanthoxylum acanthopodium, Viburnum foetidum, Houttuynia cordata, Sonchus arvensis and Oenanthe linearis collected from different places in Meghalaya State, India.

MATERIALS AND METHODS

Plant materials

The fresh edible parts of plant materials Zanthoxylum acanthopodium DC (Rutaceae), Viburnum foetidum Wall

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the calibration curve: $y = \frac{R^2}{\gamma}$, where $y$ was the absorbance and $x$ was the Rutin equivalent.

**Measurement of reducing power**

The ability of the extracts to reduce iron (II) was assessed by the method mentioned by Oyain [17]. Extracts (100 µl) of plant extracts were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50 °C for 20 min. Aliquots of 10% trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml 0.1%). The absorbance was measured at 700 nm. Reducing power is given in ascorbic acid equivalent (AAE) in milligrams per 100 gram (mg/100g) of dry material using the equation based on the calibration curve: $y = 0.0023x - 0.0063$, $R^2 = 0.9955$ where $y$ was the absorbance and $x$ was the ascorbic acid equivalent.

**Determination of DPPH free radical scavenging activity**

The free radical scavenging activity of the plant samples and butyrylhydroxyl tolune (BHT) as a positive control was determined using the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) [18]. Aliquots (20-100 µl) of the tested samples were placed in test tubes and 3.9 ml of freshly prepared DPPH solution (25 mg L⁻¹) in methanol was added to each test tube and mixed. After 30 min, the absorbance of the solution was measured at 517 nm (UV-visible spectrophotometer, Shimadzu UV 1800). The capability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH \text{ scavenged (\%)} = \left(\frac{A_c – A_t}{A_c}\right) \times 100$$

Where $A_c$ is the absorbance of the control reaction and $A_t$ is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as a percentage inhibition of DPPH radicals by the extract.

**Scavenging activity of ARBS radical cation**

The 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS⁺)-scavenging activity was measured according to the method described by Re et al. 1999 [19]. ABTS was dissolved in water to a 7 mmol concentration. The ABTS radicals were produced by adding 2.45 mmol potassium persulphate (final concentration). The completion of radical generation was obtained in the dark at room temperature for 12–16h. This solution was then diluted with ethanol to adjust its absorbance at 734 nm to 0.70±0.02. To determine the scavenging activity, 1 ml of diluted ABTS solution was added to 10 µl of plant extract (or water for the control), and the absorbance at 734 nm was measured 6 min after the initial mixing, using ethanol as the blank. The percentage of inhibition was calculated by the equation:

$$\text{ABTS scavenged (\%)} = \left(\frac{A_c – A_t}{A_c}\right) \times 100$$

Where $A_c$ and $A_t$ are the absorbencies of the control and of the test sample, respectively. The antioxidant activity of the extract was expressed as a percentage of inhibition of ABTS radicals by the extract.

Values are presented as the mean±standard error mean of three replicates. The total phenolic content, flavonoid content, reducing power and radical scavenging activities of each plant extract were calculated using linear regression analysis.

**RESULTS AND DISCUSSION**

The total phenolic content of vegetables is shown in table 1. The fresh vegetables contained 108.26±6.33–253.99±12.17 mg GAE/100g total phenolics and the rankings were V. foetidum>H. cordata>Z. acanthopodium>O. linearis>S. arvensis.
Table 1: Antioxidant properties of wild edible plants and effect of cooking

<table>
<thead>
<tr>
<th>Plant</th>
<th>Total phenolic content (mg GAE/100 gm DPM)</th>
<th>Total flavonoid content (mg AAE/100 gm DPM)</th>
<th>Reducing power (mg RE/100 gm DPM)</th>
<th>DPPH Radical scavenging activity (% of inhibition)</th>
<th>ABTS Radical scavenging activity (% of inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. acanthopodium</td>
<td>Raw: 152.98±5.24*</td>
<td>Raw: 45.99±1.08*</td>
<td>Raw: 59.97±2.49*</td>
<td>Raw: 22.08±0.04*</td>
<td>Raw: 25.58±0.05*</td>
</tr>
<tr>
<td>Boiled</td>
<td>113.24±4.09(-25.97%)</td>
<td>36.44±1.12(-20.76%)</td>
<td>45.68±2.28(-23.83%)</td>
<td>17.05±1.08(-22.78%)</td>
<td>21.96±0.04(-14.15%)</td>
</tr>
<tr>
<td>Microwave cooking</td>
<td>167.15±4.22(+9.26%)</td>
<td>(+11.26%)</td>
<td>(+7.28%)</td>
<td>(+20.47%)</td>
<td>(+11.38%)</td>
</tr>
<tr>
<td>V. foetidum</td>
<td>Raw: 253.99±2.17</td>
<td>Raw: 43.89±1.07</td>
<td>Raw: 62.22±0.05</td>
<td>Raw: 54.73±0.05</td>
<td>Raw: 67.53±0.07</td>
</tr>
<tr>
<td>Boiled</td>
<td>264.85±3.38(+4.27%)</td>
<td>47.15±1.18(+7.42%)</td>
<td>67.14±3.24(+7.90%)</td>
<td>58.29±0.09(+6.50%)</td>
<td>71.25±0.09(+5.51%)</td>
</tr>
<tr>
<td>Microwave cooking</td>
<td>276.07±3.19(+8.69%)</td>
<td>(+20.64%)</td>
<td>(+12.92%)</td>
<td>(+11.89%)</td>
<td>(+9.86%)</td>
</tr>
<tr>
<td>H. cordata</td>
<td>Raw: 184.55±6.20</td>
<td>Raw: 44.81±1.06</td>
<td>Raw: 54.76±3.37</td>
<td>Raw: 31.28±0.09</td>
<td>Raw: 47.76±2.11</td>
</tr>
<tr>
<td>Boiled</td>
<td>165.91±3.45(-10.10%)</td>
<td>(-14.62%)</td>
<td>(-22.37%)</td>
<td>(-10.65%)</td>
<td>(-5.88%)</td>
</tr>
<tr>
<td>Microwave cooking</td>
<td>192.97±4.33(+4.56%)</td>
<td>(+11.23%)</td>
<td>(+9.07%)</td>
<td>(+8.02%)</td>
<td>(+7.50%)</td>
</tr>
<tr>
<td>S. arvensis</td>
<td>Raw: 108.28±6.33</td>
<td>Raw: 24.72±1.04</td>
<td>Raw: 34.18±2.45</td>
<td>Raw: 20.72±0.09</td>
<td>Raw: 29.53±0.05</td>
</tr>
<tr>
<td>Boiled</td>
<td>89.18±4.25(-17.63%)</td>
<td>16.95±2.14(-31.43%)</td>
<td>26.55±1.58(-31.43%)</td>
<td>17.87±0.04(-31.43%)</td>
<td>24.75±0.06(-31.43%)</td>
</tr>
<tr>
<td>Microwave cooking</td>
<td>119.52±5.53(+10.38%)</td>
<td>(+18.93%)</td>
<td>(+16.88%)</td>
<td>(+19.70%)</td>
<td>(+16.88%)</td>
</tr>
<tr>
<td>O. linearis</td>
<td>Raw: 142.22±3.20</td>
<td>Raw: 44.41±1.06</td>
<td>Raw: 47.29±1.53</td>
<td>Raw: 12.19±0.41</td>
<td>Raw: 16.98±0.46</td>
</tr>
<tr>
<td>Boiled</td>
<td>137.95±2.66(-9.37%)</td>
<td>38.07±2.44(-14.27%)</td>
<td>39.55±3.22(-14.27%)</td>
<td>8.56±0.35(-14.27%)</td>
<td>14.12±0.80(-14.27%)</td>
</tr>
<tr>
<td>Microwave cooking</td>
<td>148.05±1.32(+4.09%)</td>
<td>(+11.53%)</td>
<td>(+7.97%)</td>
<td>(+24.20%)</td>
<td>(+11.43%)</td>
</tr>
<tr>
<td>Range of Loss/Increase in %</td>
<td>Boiled: Loss</td>
<td>Loss</td>
<td>Loss</td>
<td>Decrease (10.65)</td>
<td>Decrease (5.88)</td>
</tr>
<tr>
<td>Range of Loss/Increase in %</td>
<td>Microwave cooking: Increase (11.23-20.64)</td>
<td>Increase (7.28)</td>
<td>Increase (8.02-24.20)</td>
<td>Increase (9.86-19.70)</td>
<td></td>
</tr>
</tbody>
</table>

Each value in the table was obtained by calculating the average of three experiments and data are presented as mean±standard error of the mean (SEM). Statistical analysis were carried out by Tukeys test at 95% confidence level and statistical significance were accepted at the p<0.05 level. The superscript letter a,b,c denotes the significant differences within raw and different cooking method of an individual plant. The negative value within bracket indicates percentage decrease and positive value within bracket indicates the percentage increase of the test parameters.

Microwave cooking was found to increase the total phenolic content of Z. acanthopodium (9.26%), V. foetidum (6.69%), H. cordata (4.56%), S. arvensis (10.38%) and O. linearis (4.90%). Boiling treatment decreased the total phenolic content in the range 9.37-25.97% in all studied plants except for V. foetidum. Only Z. acanthopodium plant had the most important loss (25.97%) (Fig. 1).

Among the studied wild edible plants, Z. acanthopodium had the highest total flavonoid content of (45.99 mg RE/100g) followed by H. cordata (44.88 mg RE/100g), O. linearis (44.41 mg RE/100g), V. foetidum (43.89 mg RE/100g) and S. arvensis (24.72 mg RE/100g) respectively (table 1). Microwave cooking was found to increase the flavonoid content in Z. acanthopodium (51.17 mg RE/100g), H. cordata (49.52 mg RE/100g), O. linearis (49.53 mg RE/100g), V. foetidum (52.95 mg RE/100g), and S. arvensis (29.40 mg RE/100g), whereas boiling decreased the flavonoid content in Z. acanthopodium (36.44 mg RE/100g), H. cordata (38.32 mg RE/100g) and O. linearis (16.95 mg RE/100g). On the other hand, the total flavonoid content of V. foetidum considerably increased in varying amounts upon boiling (fig. 2).

![Fig. 1: Total phenolic content in wild edible plants and effect of cooking, Values are mean±SEM (n=3)](image)
Our results showed a significant increase \((p<0.05)\) in total phenolics and flavonoids with microwave cooking. This may be because microwave treatment causes plant cell walls to break down, increasing the extractability of polyphenols. As a result, bound polyphenols may be released more readily in the microwaved sample than in the corresponding fresh sample.

When vegetables are subjected to various cooking methods like pressure cooking, microwaving, baking, griddling, or deep frying, variations in their antioxidant activity or scavenger capacity occur. These variations also depend on the type of vegetables, the bioavailability of phenolic compounds, the way the vegetables are cut, and the reaction system used for the assay \([20-22]\).

The reducing power (RP) of the raw and cooked vegetables was evaluated as Ascorbic acid equivalent (AAE) and has been introduced in table 1. Among the studied wild edible plants, \(V\). foetidum had the highest reducing power (62.22 mg AAE/100g) followed by \(Z\). acanthopodium (59.97 mg AAE/100g), \(H\). cordata (54.76 mg AAE/100g), \(O\). linearis (47.29 mg AAE/100g) and \(S\). arvensis (34.18 mg AAE/100g) respectively. Microwave cooking was found to increase the reducing power of \(V\). foetidum (12.92%), \(Z\). acanthopodium (7.28%), \(H\). cordata (9.07%), \(O\). linearis (7.97%), and \(S\). arvensis (16.58%), whereas boiling had decreased the reducing ability of \(Z\). acanthopodium (23.83%), \(H\). cordata (22.37%), \(O\). linearis (16.37%), and \(S\). arvensis (22.32%) (fig. 3). Boiling may decrease the activity by decreasing ascorbic acid, while microwave heating retains the active components in the cooked tissue \([22]\). Our results for most of the vegetables analyzed agree with this. The activity of vegetables cooked in the microwave oven was generally higher than that of those cooked in boiling water, because microwave heating does not stimulate the release of ascorbic acid or other antioxidants from cooked tissue.

Boiling or pressure cooking causes lixiviation, which reduces the amount of total phenolics and carotenoids by 49% and 64%, respectively \([23]\). This is due to the fact that, on boiling, phenolic compounds enter the cooking water and complex phenol proteins are produced, reducing the antioxidant activities \([24, 25]\). The concentration of phenolic acids is highest in the outer layers of some vegetables \([10]\) and these are extremely exposed to water \([26]\) and as a result of which, the antioxidant properties of some vegetables are reduced \([20]\). On the other hand, microwave heating retains the active components in the cooked tissue \([22]\). Our results for most of the vegetables analyzed agree with this.
whereas *O. linearis* had the lowest activity with 12.19%. DPPH radical scavenging antioxidant activity of all vegetables under investigation significantly (*p*<0.05) increased during microwave cooking procedures of *V. foetidum* (11.89%), *Z. acanthopodium* (20.47%), *H. cordata* (8.02%), *O. linearis* (24.20%), and *S. arvensis* (18.43%), compared to the values for the fresh ones. Boiled cooking decreased the DPPH radical scavenging activity of *Z. acanthopodium* (22.78%), *H. cordata* (10.65%), *O. linearis* (29.77%), and *S. arvensis* (13.75%), whereas radical scavenging activity increased in the case of *V. foetidum* by 6.50% as compared to its raw form (fig. 4).

**Fig. 4: DPPH radical scavenging activities of wild edible plants and effect of cooking, values are mean±SEM (n=3)**

Antioxidant activity of wild vegetables, as determined by the ABTS radical scavenging method, decreased in the following order: *V. foetidum*-*H. cordata*-*S. arvensis*-*Z. acanthopodium*-*O. linearis* (table 1). Among all these test vegetables, fruits of *V. foetidum* showed the highest ABTS scavenging activity with inhibition of 67.53%, whereas *O. linearis* had the lowest activity with 16.88%. Boiled cooking decreased the ABTS radical scavenging activity of *Z. acanthopodium* (14.15%), *H. cordata* (5.88%), *O. linearis* (16.35%), and *S. arvensis* (16.18%), whereas radical scavenging activity increased in the case of *V. foetidum* by 5.51% as compared to its fresh form. On the contrary, ABTS radical scavenging antioxidant activity of all vegetables under investigation significantly (*p*<0.05) increased during microwave cooking procedures of *V. foetidum* (9.86%), *Z. acanthopodium* (11.30%), *H. cordata* (5.75%), *O. linearis* (11.43%), and *S. arvensis* (19.70%), compared to the values for the fresh ones (fig. 5).

It was shown that there was a statistically significant variation in the total antioxidant activity from raw to cooked edible plants. Similar results were observed by other researchers in a study on antioxidants in raw and cooked green leafy vegetables [27, 28].

**Fig. 5: ABTS radical scavenging activities of wild edible plants and effect of cooking, values are mean±SEM (n=3)**

The degree of antioxidant activity lost depends on the surface area between vegetables as well as the duration of time the vegetables are cooked for. The longer the cooking time, the greater the loss. Moreover, when compared to microwave cooking, boiled cooking reduces total antioxidant activity due to the leaching of antioxidants to the boiling medium [27]. It was reported that the antioxidant activity of vegetables was enhanced by microwave cooking. This suggests that the pro-oxidant activity was due to peroxidases which were inactivated during microwave cooking. Another study indicated that microwave cooking caused no change to the antioxidant potential of vegetables or enhanced it due to the improvement of antioxidant properties of naturally occurring compounds or the formation of novel compounds such as Maillard reaction products having antioxidant activity [29, 30].

The idea that unprocessed or raw foods are healthier, especially vegetables, is prevalent, but India is the country where preparing vegetables before eating them is most common. Understanding the ideal cooking technique is crucial if you want to preserve the vegetables’ healthy components. According to the current study, microwave cooking increases the antioxidant activity of green leafy vegetables and other vegetables through phenolics and flavonoids more effectively than other methods.
CONCLUSION

The results showed that V. foetidum was the only vegetable that maintained its high scavenging radical capacity in all the cooking methods. Among the vegetables that increased their total phenolic content, total flavonoid content, reducing power and radical scavenging activities in microwave cooking methods. On the other side, boiled cooking significantly reduced the polyphenol content, their radical-scavenging activity and antioxidant activity and their health-related properties. The results of the study serve as a database providing information on the effects of different cooking methods on the antioxidant potential of vegetables and might encourage the food industry to recommend particular cooking methods to help maintain the antioxidant properties of the vegetables that we eat. However, further research about cooking's impact on chemical efficacy in vivo should be carried out in the future.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

We state unequivocally that we have no competing interests.

REFERENCES


