Assessment of antioxidant activity of rosemary (Rosmarinus officinalis) and Betel (Piper betel) leaves extract combination

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Abstract
In the present study several type of biochemical test (Antioxidant ability assays, Total phenol content (TPC), Total flavonoid content (TFC), Ferric reducing antioxidant power (FRAP) assay, Reducing power assay, DPPH free radical scavenging assay, Superoxide anion radical scavenging activity, Hydrogen peroxide scavenging assay, Nitric oxide radical scavenging assay and 2, 2’-azino-bis (3 ethylenbenzothiazoline-6-sulfonic acid (ABTS) were exercised to assess antioxidant activity of rosemary and betel leaves extract combination (RE+BE) in 1:1 ratio. The result indicated that combination of RE+BE exhibited high antioxidant ability assays (326.06±13.37 μg ascorbic acid), TPC (196.55±4.63 mg of gallic acid (GAE)/g), TFC (23.61±4.95 mg rutin/g), FRAP ranging from 68.20±2.25 to 8.58±1.79 μm Fe (II)/g. Reducing power assay was found from 4.44±0.04 to 8.44±0.05 mg AsAE/g and IC50 value of RE+BE combination for DPPH. Superoxide anion radical scavenging activity, Hydrogen peroxide scavenging assay, Nitric oxide radical scavenging assay and ABTS was found 28.39±2.17, 17.50±1.61, 28.54±3.35, 57.74±2.47 and 53.97±5.46 respectively. It can be concluded that combination of rosemary and betel leaves extract could be use as antioxidants in food industry to prevent the issue of lipid oxidation and rancidity.

Keywords: Antioxidant properties, betel leave, rosemary leave, DPPH, total phenolic content

Introduction
Lipid oxidation of food is major problem of any food industry. To overcome oxidative deterioration in food products, most effective approach to is to integrate antioxidants into formulations. Antioxidants either synthetic or natural have become an indispensable group of food additives mainly because of their unique properties of enhancing the shelf life of food products without any damage to sensory or nutritional qualities (Nanditha and Prabhasankar, 2008) [17]. In industrial processing, mainly synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are used in food industry. However, increasing concerns over the safety of synthetic food additives has resulted in a trend towards “natural products”. Plants are persistently the liberal source to furnish man with valuable bioactive substances (Tayel and El-Tras, 2012) [11] and thus different plant products are being evaluated as natural antioxidants to preserve and improve the food quality. Natural antioxidants extracted from herbs and spices exhibit various degrees of efficacy when used in different food applications (Bowser et al., 2014) [07]. Among natural antioxidant sources, rosemary is more potent source of natural antioxidants, belonging to the Lamiaceae family, is a pleasant-smelling perennial shrub that grow in several regions all over the world (Ozacan et al., 2008) [21]. The antioxidant activity of rosemary is due high phenolic content such as carnosic acid, rosmarinic acid, carnosol, rosmanol, rosmariniquinone and rosmarydiphenol reported by naveeva et al. (2013) [18] and Riznar et al. (2006) [20]. Rosemary leaves extract incorporated fried chicken snacks have improved physicochemical, microbiological and sensory score than the control observed by Saini et al. (2019) [21]. Similarly betel belongs to the Piperaceae family and mainly originates from South East Asia. Antioxidant activity of betel is due to its biochemical compound such as catechol, hydroxy-chavicol, chavibetol, allylpyrocatechol, chavibetol acetate and allylpyrocatechol diacetate in betel as reported by Dasgupta et al. (2014) [08] and Rintu et al. (2015) [25]. Therefore, the present study has been undertaken to explore antioxidant potential of rosemary and betel leaves extract in combination in-vitro.
Material and Methods

Extract preparation
The rosemary and betel leaves in 1:1 ratio, were oven dried at 50°C for 12 hrs followed by grinding and sieving. Preweighed powdered leaves were extracted with 70% Ethanol for 24 hrs at 40°C. The extract was collected and concentrated under reduced pressure in a rotary vacuum evaporator (Labconco Corporation, USA) until semi solid consistency. The semi solid mass was oven dried at 50°C at overnight to obtain dried extract. The extract were reconstituted with the same solvent as used for extraction to obtain 5% solutions and stored at 4°C.

In-vitro antioxidant assays analysis of RE+BE combination
(1) Antioxidant ability assays, total phenolic and flavonoid content of RE+BE combination
An antioxidant ability assay of the RE+BE combination was evaluated by the phosphomolybdenum method of Prieto et al. (1999) [22]. The Total phenol content was determined by using spectrophotometric methods of Singleton et al. (1999) [29]. Total flavonoid content was determined by the aluminum chloride colometric assay by Meda et al. (2005) [16].

(2) Ferric reducing antioxidant power (FRAP) and reducing power assay of RE+BE combination
Ferric reducing antioxidant power (FRAP) assay in the RE+BE combination was carried out by modified method of Benzie and Strain (1996) [5]. Reducing power assay of the RE+BE was followed by the method of Oyaizu (1986) [20].

(3) In-vitro free radical-scavenging activities of RE+BE combination
DPPH free radical scavenging assay of the RE+BE combination was measured by Blios (1958) [60]. Superoxide anion radical scavenging activity of RE+BE combination was followed by the method of Nishimiki et al. (1972) [19].

Hydrogen peroxide scavenging assay of the RE+BE combination was determined by the method of Jayaprakasha et al. (2004) [14]. The method of Garrat (1964) [11] with slight modification was used to determine the nitric oxide radical scavenging activity of the RE+BE combination. The method of Re et al. (1999) [24] was followed to analyze ABTS free radical scavenging activity of the test sample.

Statistical analysis
All experiments were conducted in triplicate and data expressed as mean ± SD.

Result and Discussion
(1) Antioxidant ability assays, total phenolic and flavonoid content of RE+BE combination
Antioxidant ability assays, total phenolic and flavonoid content of RE+BE combination are presented in table 1. Antioxidant ability assays of RE+BE combination was found 326.06±13.37 μg ascorbic acid equivalents at 100 μg/ml. Similarly Albayrak et al. (2013) [30] and Prayitno et al. (2016) [31] reported antioxidant ability assay for rosemary and betel leaves to extract to be 229.03 and 127.35 μg ascorbic acid equivalents respectively. TPC for test sample was detected 196.55±4.63 mg of gallic acid (GAE) per g. In this way Erkan et al. (2008) [10], Tavassoli and Djomeh, (2011) [30], Abramovic et al. (2012) [62], Albayrak et al. (2013) [63], Teruel et al. (2015) [12] and Hendel et al. (2016) [12] reported TPC of rosemary extract was 162, 4.99, 31.8, 67.23 and 128.97 mg GAE/g respectively. Alam et al. (2012) reported that the total phenolic content of betel extract was 136 mg GAE/g. The total flavonoid content of RE+BE combination was detected 23.61±4.95 mg rutin/g. Hendel et al. (2016) [12] and Abrahim et al. (2012) [60] observed total flavonoid content in rosemary and betel extract was 38.1 and 19.82 mg rutin/g equivalents. Flavonoid with a certain structure and particular hydroxyl position in the molecule can act as a proton donating and show radical scavenging activity (Hou et al., 2003) [13].

Table 1: Antioxidant ability assays, TPC and flavonoid content of combination of RE+BE

<table>
<thead>
<tr>
<th>Sample</th>
<th>Antioxidant ability assays (μg ascorbic acid)</th>
<th>Total phenolic content (mg of gallic acid/g)</th>
<th>Total flavonoid content (mg rutin/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE+BE combination</td>
<td>326.06±13.37</td>
<td>196.55±4.63</td>
<td>23.61±4.95</td>
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<tr>
<td>Mean ± SD, (n=3)</td>
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</table>

(2) Ferric reducing antioxidant power (FRAP) and reducing power assay of RE+BE combination
The ferric reducing ability and reducing power assay of the RE+BE combination ranging from 68.20±2.25 to 8.58±1.79 μm Fe (II)/g and 44.72±0.04 to 8.44±0.05 mg AscAE/gm respectively (showed in figure 1 and 2). The absorbance of RE+BE combination increased due to the formation of the [Fe²⁺-TPTZ] complex with increasing concentration. FRAP assay measures the reducing potential of an antioxidant reacting with a ferric triprydiltriazine [Fe³⁺-TPTZ] complex and producing a colored ferrous tripyridyltriazine [Fe²⁺-TPTZ] reported by Benzie and strain, (1996) [5]. Normally, the reducing properties are associated with the existence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom (Duh et al., 1999) [9]. Teruel et al. (2015) [12] found FRAP assay of rosemary extract was 1186.54 μm Fe (II)/g. Abrahim et al. (2012) [60] and Manigauha et al. (2009) [19] found similar finding in betel leaves.

Fig 1: Reducing power assay of the RE+BE combination
FRAP assay

Fig 2: Ferric reducing antioxidant power (FRAP) assay of the RE+BE combination

(3) In-vitro free radical-scavenging activities of RE+BE combination

Increasing the concentration of the extract significantly increase the radical-scavenging activity. The RE+BE combination was capable of neutralizing the DPPH free radicals via hydrogen donating activity by 52.45%, 60.25%, 64.78%, 68.45%, 70.74% at concentrations of 20, 40, 60, 80, and 100 μg/ml respectively. In the DPPH radical scavenging assay, antioxidants react with DPPH (deep violet color) and convert it to yellow colored α, α-diphenyl-β-picryl hydrazine, degree of discoloration indicates the radical-scavenging potential of the antioxidant (Blois, 1958). The combination of RE+BE exhibited very strong superoxide anion scavenging activity. The extract of RE+BE combination and standard inhibited nitro blue tetrazolium (NBT) reduction for superoxide anion scavenging activity by 32.25%, 54.69%, 60.56%, 66.12% and 71.25% respectively at the concentration of 10 to 50 mg/ml. Erkan et al. (2008) [10], Tavassoli and Djomeh, (2011) [30], Albayrak et al. (2013) [33] and Hendel et al. (2016) [12] reported IC50 values for DPPH of rosemary extract 54, 24, 15.15 and 11 μg/ml respectively. While for betel extract Manigauha et al. (2009) [15] and Abraham et al. (2012) [10] reported the IC50 values of superoxide scavenging activity 20 and 288.3μg/ml respectively. Test sample (RE+BE combination) and ascorbic acid standard at concentration of 50μg/ml, inhibited H2O2 reduction by 74.56% and 77.78% respectively. In the nitric oxide radical scavenging assay RE+BE combination and ascorbic acid by concentration of 100μg/ml, inhibited nitric oxide radical by 69.41% and 89.45% respectively. Alam et al. (2012) [31] reported IC50 values of nitric oxide radical scavenging assay of betel extract 25 μg/ml. The combination of RE+BE inhibited ABTS radical but its scavenging activity is weaker than standard i.e. trolox. RE+BE combination and standard at a concentration of 50 μg/ml, inhibited ABTS radical by 88.75% and 94.95% respectively. Similarly Teruel et al. (2015) [12] demonstrated IC50 value of ABTS radical scavenging activity in rosemary extract. IC50 values for all in-vitro free radical-scavenging activities of RE+BE and ascorbic acid are presented in table 2.

Table 2: IC50 value of in-vitro antioxidant activities of RE+BE

<table>
<thead>
<tr>
<th>Samples</th>
<th>DPPH (μg/ml)</th>
<th>Superoxide anion (μg/ml)</th>
<th>H2O2 (μg/ml)</th>
<th>Nitric oxide (μg/ml)</th>
<th>ABTS (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE+BE combination</td>
<td>28.39±2.17</td>
<td>17.50±1.61</td>
<td>28.54±3.35</td>
<td>57.74±2.47</td>
<td>53.97±5.46</td>
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<tr>
<td>Ascorbic acid</td>
<td>9.44±1.41</td>
<td>14.48±1.80</td>
<td>19.45±3.66</td>
<td>32.93±2.42</td>
<td>-</td>
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<tr>
<td>Trolox</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>49.51±2.12</td>
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<tr>
<td>Mean ± SD, (n=3)</td>
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Conclusion

After the results interpretation of the current study, it can be concluded that plant based natural antioxidants like rosemary (Rosmarinus officinalis) and betel (Piper betel) leaves could be use as alternative to synthetic antioxidants in food industry to prevent the issue of lipid oxidation and rancidity.

Acknowledgments

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Reference

12. Hanell HK, Dam H. Determination of small amount of total cholesterol by tschugaef reaction with a note on the


