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Assessment of antioxidant activity of rosemary (*Rosmarinus officinalis*) leaves extract

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Abstract
Different kind 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 ethylbenzothiazoline-6-sulfonic acid (ABTS) were exercised to evaluate antioxidant activity of rosemary leaves extract (RE). The result showed that RE exhibit high Antioxidant ability assays (254.18±11.82 µg ascorbic acid), TPC (136.66±7.41 mg of gallic acid (GAE)/g), TFC (37.13±6.04 mg rutin/g), FRAP ranging from 32.17±1.12 to 1.28±1.56 µm Fe (II)/g, Reducing power assay was found from 33.09±0.09 to 6.42±0.05 mg AscAE/g and IC_{50} value of RE for DPPH, Superoxide anion radical scavenging activity, Hydrogen peroxide scavenging assay, Nitric oxide radical scavenging assay and ABTS was found 40.76±2.81, 31.62±1.43, 47.37±2.30, 70.48±2.13 and 70±4.67 respectively. It can be concluded that rosemary leaves could be use as antioxidants in food industry to overcome the problem of lipid oxidation and rancidity.

Keywords: Antioxidant properties, rosemary leave, DPPH, Total phenolic content

1. Introduction
The most common form of deterioration in any food products is oxidative rancidity; which leads to extensive flavor changes, structural damage to proteins leading to loss of freshness that discourages repeat purchases by consumers (Saini *et al*., 2019) [22]. The most effective approach to avoid oxidative deterioration in food products 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) [14]. 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) [26] 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) [5].
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) [18]. The antioxidant activity of rosemary is due high phenolic content such as carnosic acid, rosmarinic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol reported by Naveena *et al.* (2013) [15], Sebranek *et al.* (2005) [23] and Riznar *et al.* (2006) [21]. Rosemary leaves extract incorporated fried chicken snacks have improved physico-chemical, microbiological and sensory score than the control observed by Saini *et al.* (2019) [22]. Rosemary is a eminent beneficial medicinal herb that is commonly used in pharmaceutical products and traditional medicine as a digestive, tonic, astringent, diuretic, diaphoretic and useful for urinary ailments (Mahomoud *et al.*, 2005) [12]. Therefore, the present study has been undertaken to explore antioxidant activity of rosemary leaves *in-vitro.*

2. Material and Methods
2.1 Extract preparation: The rosemary leaves were oven dried at 50ºC for 12hrs followed by grinding and sieving.

Pre-weighed powdered leaves were extracted with 70% ethanol for 24 hrs at 40 ºC. The extract was collected and concentrated under reduced pressure in a rotary vacuum evaporator (Labconco Corporation, USA) until semi solid consistency. The semisolid mass was oven dried at 50 ºC 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.

2.2 *In-vitro* antioxidant assays analysis of rosemary leaves extract

2.2.1 Antioxidant ability assays, total phenolic and flavonoid content of rosemary extract

An antioxidant ability assay of the rosemary (*Rosmarinus officinalis*) leaves extract (RE) was evaluated by the phosphomolybdenum method of Prieto *et al.* (1999) [19]. The Total phenol content was determined by using spectrophotometric methods of Singleton *et al.* (1999) [24]. Total flavonoid content was determined by the aluminum chloride colometric assay by Meda *et al.* (2005) [13].

2.2.2 Ferric reducing antioxidant power (FRAP) and reducing power assay of rosemary extract

Ferric reducing antioxidant power (FRAP) assay in the RE was carried out by modified method of Benzie and Strain (1996) [3]. Reducing power assay of the RE was followed by the method of Oyaizu (1986) [17].

2.2.3 *In-vitro* free radical-scavenging activities of rosemary extract

DPPH free radical scavenging assay of the RE was measured by Blios (1958) [4]. Superoxide anion radical scavenging activity of RE was followed by the method of Nishimiki *et al.* (1972) [16]. Hydrogen peroxide scavenging assay of the RE was determined by the method of Jayaprakasha *et al.* (2004) [11]. The method of Garrat (1964) [8] with slight modification was used to determine the nitric oxide radical scavenging activity of the RE. The method of Re *et al.* (1999) [20] was followed to analyze ABTS free radical scavenging activity of the test sample.

2.3 Statistical analysis

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

3. Result and Discussion

3.1 Antioxidant ability assays, total phenolic and flavonoid content of RE

Antioxidant ability assays, total phenolic and flavonoid content of rosemary (*Rosmarinus officinalis*) leaves extract (RE) are presented in table 1. Antioxidant ability assays of RE was found 254.18±11.82 µg ascorbic acid equivalents at 100 µg/ml. Similarly Albayrak *et al.* (2013) [2] reported antioxidant ability assay for rosemary extract to be 229.03 µg ascorbic acid equivalents. TPC for test sample was detected 136.66±7.41 mg of gallic acid (GAE) per g. In this way Erkan *et al*. (2008) [7], Tavassoli and Djomeh, (2011) [25], Abramovic *et al*. (2012) [01], Albayrak *et al*. (2013) [2], Teruel *et al*. (2015) [27] and Hendel *et al*. (2016) [9] reported TPC of rosemary extract was 162, 4.99, 318, 64.71, 23 and 128.97 mg GAE/g respectively. The total flavonoid content of RE was detected 37.13±6.04 mg rutin/g. Hendel *et al.* (2016) [9] observed total flavonoid content in rosemary extract was 38.1 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) [10].

Table 1: Antioxidant ability assays, TPC and flavonoid content of rosemary leaves (RE) extract

Sample	Antioxidant ability assays (µg ascorbic acid)	Total phenolic content (mg of gallic acid/g)	Total flavonoid content (mg rutin/g)
RE	254.18±11.82	136.66±7.41	37.13±6.04

Mean ± SD, (n=3)

3.2 Ferric reducing antioxidant power (FRAP) and reducing power assay of RE

The ferric reducing ability and reducing power assay of the RE ranging from 32.17±1.12 to 1.28±1.56 µm Fe (II)/g and 33.09±0.09 to 6.42±0.05 mg AscAE/gm respectively (showed in figure 1 and 2). The absorbance of RE increased due to the formation of the [Fe^{2+}-TPTZ] complex with increasing concentration. FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine [Fe^{3+}-TPTZ] complex and producing a colored ferrous tripyridyltriazine [Fe^{2+}-TPTZ] reported by Benzie and strain, (1996) [3]. 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) [6]. Teruel *et al.* (2015) [27] found FRAP assay of rosemary extract was 1186.54 µm Fe (II) /g.

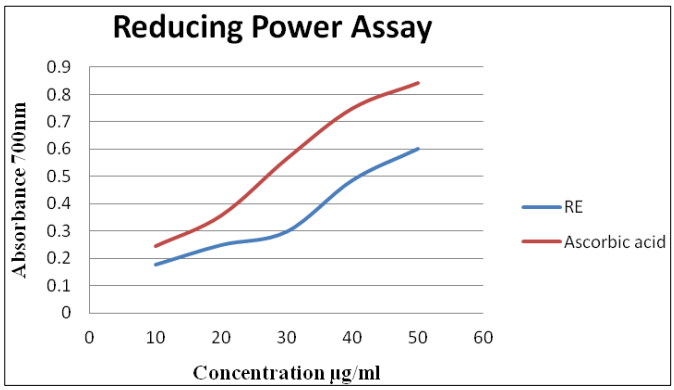

Fig 1: Reducing power assay of the rosemary (*Rosmarinus officinalis)* leaves extract

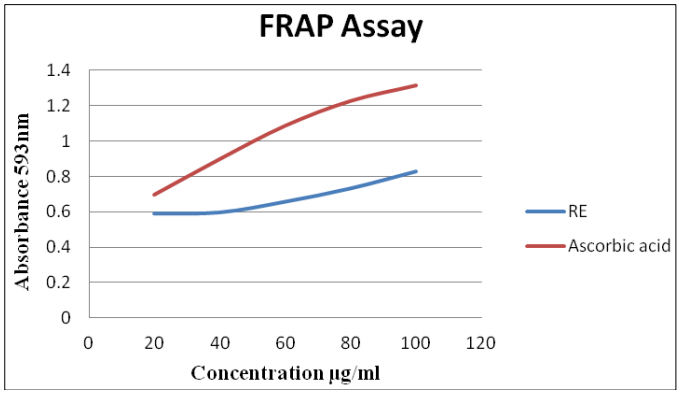

Fig 2: Ferric reducing antioxidant power (FRAP) assay of the rosemary (*Rosmarinus officinalis)* leaves extract

3.3 *In-vitro* free radical-scavenging activities of RE

Increasing the concentration of the extract significantly increase the radical-scavenging activity. The RE was capable of neutralizing the DPPH free radicals via hydrogen donating activity by 41.25%, 50.45%, 55.56%, 61.28%, 65.28% 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) [4]. The RE exhibited very strong superoxide anion scavenging activity. The extract of rosemary and standard inhibited nitro blue tetrazolium (NBT) reduction by 25.12%, 49.78%, 52.89%, 58.65%, 62.45% and for standard 38.45%, 59.23%, 65.68%, 70.56%, 78.12% respectively at the concentration of 10 to 50 mg/ml. Erkan *et al*. (2008) [7], Tavassoli and Djomeh, (2011) [25], Albayrak *et al*. (2013) [2] and Hendel *et al*. (2016) [9] reported IC_{50} values for DPPH of rosemary extract 54, 24, 15.15 and 11 µg/ml respectively. Test sample (RE) and ascorbic acid standard at concentration of 50µg/ml, inhibited H_2O_2 reduction by 68.45% and 77.78% respectively. In the nitric oxide radical scavenging assay RE and ascorbic acid at concentration of 100µg/ml, inhibited nitric oxide radical by 79.28% and 89.45% respectively. RE inhibited ABTS radical but its scavenging activity is weaker than standard i.e. trolox. RE and standard at a concentration of 50 µg/ml; inhibited ABTS radical by 70.96% and 94.95% respectively. Similarly Teruel *et al*. (2015) [27] demonstrated IC_{50} value of ABTS radical scavenging activity in rosemary extract. IC_{50} values for all *in-vitro* free radical-scavenging activities of RE and ascorbic acid are presented in table 2.

Table 2: IC_{50} value of *in- vitro* antioxidant activities of rosemary leaves (RE) extract

Samples	DPPH (µg/ml)	Superoxide anion (µg/ml)	H_2O_2 (µg/ml)	Nitric oxide (µg/ml)	ABTS (µg/ml)
RE	40.76±2.81	31.62±1.43	47.37±2.30	70.48±2.13	70±4.67
Ascorbic acid	9.44±1.41	14.48±1.80	19.45±3.66	32.93±2.42	-
Trolox	-	-	-	-	49.51±2.12

Mean ± SD, (n=3)

4. Conclusion

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

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