www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(5): 377-380

www.thepharmajournal.com
Received: 22-03-2020
Accepted: 24-04-2020

Ashish Saini
Department of LPT, PGIVER, Jaipur, Rajasthan, India

Anurag Pandey
Department of LPT, PGIVER, Jaipur, Rajasthan, India

Sanjita Sharma
Department of LPM, PGIVER, Jaipur, Rajasthan, India

Umesh S Suradkar
Department of LPT, PGIVER, Jaipur, Rajasthan, India

Yellamelli R Ambedkar
Department of LPT, PGIVER, Jaipur, Rajasthan, India

Priyanka Meena
Department of LPT, ACVM, Jaipur, Rajasthan, India

Ravi Raman
Department of LPT, CVAS, Bikaner, Rajasthan, India

Asman S Gurjar
Department of LPT, MJF, Jaipur, Rajasthan, India

Corresponding Author:
Ashish Saini
Department of LPT, PGIVER, Jaipur, Rajasthan, India

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

Ashish Saini, Anurag Pandey, Sanjita Sharma, Umesh S Suradkar, Yellamelli R, Ambedkar Priyanka Meena, Ravi Raman and Asman S Gurjar

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 ethylbenzothiazoline-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 44.72±0.04 to 8.44±0.05 mg AscAE/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) [31] 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, rosmariquinone and rosmaridiphenol reported by Naveena *et al.* (2013) [18] and Riznar *et al.* (2006) [26]. Rosemary leaves extract incorporated fried chicken snacks have improved physico-chemical, microbiological and sensory score than the control observed by Saini *et al.* (2019) [27]. 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 50ºC for 12 hrs followed by grinding and sieving. Pre-weighed powdered leaves were extracted with 70% Ethanol for 24 hrs at 40ºC. 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 50ºC 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) [05]. 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) [04]. 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) [03] and Prayitno *et al.* (2016) [22] reported antioxidant ability assay for rosemary and betel leave 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) [02], Albayrak *et al.* (2013) [03], Teruel *et al.* (2015) [32] and Hendel *et al.* (2016) [12] reported TPC of rosemary extract was 162, 4.99, 318, 64.71, 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) [01] 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

Sample	Antioxidant ability assays (µg ascorbic acid)	Total phenolic content (mg of gallic acid/g)	Total flavonoid content (mg rutin/g)
RE+BE combination	326.06±13.37	196.55±4.63	23.61±4.95

Mean ± SD, (n=3)

(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 [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) [05]. 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) [32] found FRAP assay of rosemary extract was 1186.54 µm Fe (II) /g. Abrahim *et al.* (2012) [01] and Manigauha *et al.* (2009) [15] found similer finding in betel leaves.

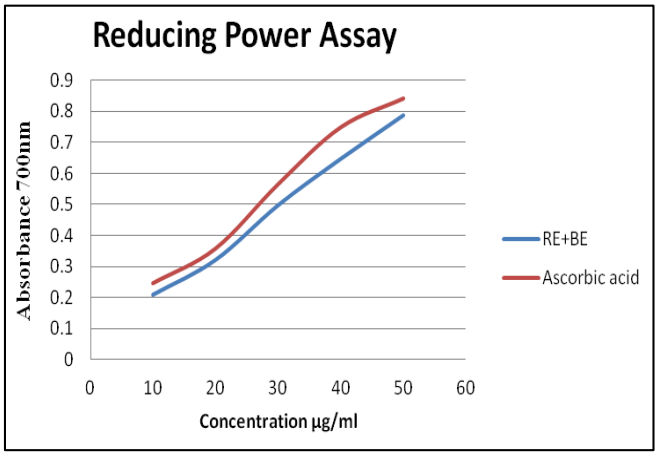

Fig 1: Reducing power assay of the RE+BE combination

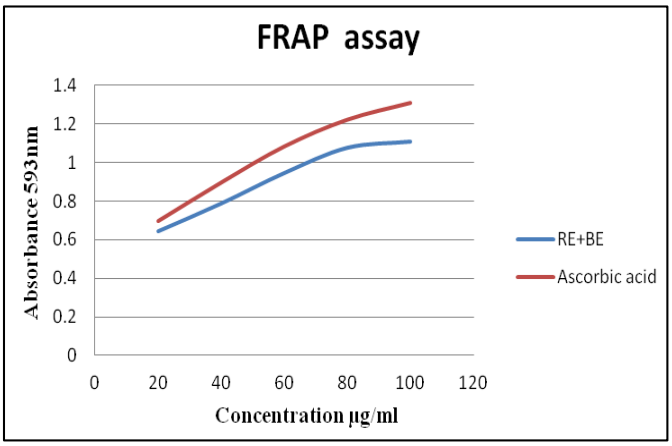

Fig 2: Ferric reducing antioxidant power (FRAP) assay of the RE+BE combination

(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) [06]. 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) [03] and Hendel *et al.* (2016) [12] reported IC_{50} 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 Abrahim *et al.* (2012) [01] reported the IC_{50} 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 H_2O_2 reduction by 74.56% and 77.78% respectively. In the nitric oxide radical scavenging assay RE+BE combination and ascorbic acid at concentration of 100µg/ml, inhibited nitric oxide radical by 69.41% and 89.45% respectively. Alam *et al.* (2012) [04] reported IC_{50} 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) [32] 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+BE and ascorbic acid are presented in table 2.

Table 2: IC_{50} value of *in- vitro* antioxidant activities of RE+BE

Samples	DPPH (µg/ml)	Superoxide anion (µg/ml)	H_2O_2 (µg/ml)	Nitric oxide (µg/ml)	ABTS (µg/ml)
RE+BE combination	28.39±2.17	17.50±1.61	28.54±3.35	57.74±2.47	53.97±5.46
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)

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

The first author thanks to the Rajasthan University of Veterinary and Animal Sciences (RAJUVAS) Bikaner (Rajasthan), India for providing financial support in the form of stipend and facilities for work.

Reference

1. Abrahim NN, Kanthimathi MS, Abdul-Aziz A. *Piper betel* shows antioxidant activities, inhibits MCF-7 cell proliferation and increases activities of catalase and superoxide dismutase. BMC Complementary and Alternative Medicine, 2012; 12(220):2-11.
2. Abramovic H, Terpinc P, Generalic I, Skroza D, Klancnik A, Katalinic V. Možina SS. Antioxidant and antimicrobial activity of extracts obtained from rosemary (*Rosmarinus officinalis*) and vine (*Vitis vinifera*) leaves. Croat. J. Food Sci. Tech. 2012; 4(1):1-8.
3. Albayrak S, Aksoy A, Albayrak S, Sagdic O. In vitro antioxidant and antimicrobial activity of some Lamiaceae species. Ira. J of Sci. and Tech. 2013; 37(A1):1-9.
4. Alam B, Akter F, Parvin N, Pia RS, Akter S, Choudhury J e tal. Antioxidant, analgesic and anti-inflammatory activities of the methanolic extract of *Piper betel* leaves. Avicenna Journal of Phytomedicine, 2012; 3(2):112-135.
5. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem. 1996; 239(1):70-76.
6. Blios MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 26:1199-1200.
7. Bowser TJ, Mwavita M, Al-Sakini A, McGlynn W, Maness NO. Quality and shelf life of fermented lamb meat sausage with rosemary extract. Food Sci. Jour. 2014; 8:22-31.
8. Dasgupta N, De B. Antioxidant activity of *Piper betel* L. leaf extract in vitro. Food chem, 2014; 88(2):219-224.
9. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of harng jyur (*Chrysanthemum moifolium* Ramat). Lebensm-Technol. 1999; 32:269-277.
10. Erkan N, Ayranci G, Ayranci E. Antioxidant activities of rosemary (*Rosmarinus Officinalis* L.) extract, blackseed (*Nigella sativa* L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chem. 2008; 110(1):76-82.
11. Garrat DC. The quantitative analysis of drugs. Chapman and Hall ltd, Japan. Biochem. Anal. Chem. 1964; 3:456-458.
12. Hanel HK, Dam H. Determination of small amount of total cholesterol by tschugaeff reaction with a note on the

determination of Inosterol. Acta Chem. Scand. 1995; 9:677-682.
13. Hou WC, Lin RD, Cheng KT, Hung YT, Cho CH, Chen CH, Hwang SY *et al*. Free radical scavenging activity of taiwanese native plants. Phytomed. 2003; 10:170-175.
14. Jayaprakasha GK, Jaganmohan RL, Sakariah KK. Antioxidant activities of flavidin in different in vitro model systems. Bioorg. Med. Chem. 2004; 12(19):5141-5146.
15. Manigauha A, Ali H, Maheshwari MU. Antioxidant activity of ethanolic extract of *Piper betel* leaves. Journal of Pharmacy Research, 2009; 2(3):491-494.
16. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 2005; 91:571-577.
17. Nanditha B, Prabhasankar P. Antioxidants in bakery products: a review. Critical Reviews in Food Sci. and Nut. 2008; 49(1):1-27.
18. Naveena BM, Vaithiyanathan S, Muthukumar M, Sen, AR, Kumar YP, Kiran M. Relationship between the solubility, dosage and antioxidant capacity of carnosic acid in raw and cooked ground buffalo meat patties and chicken patties. Meat Sci. 2013; 95(2):195-202.
19. Nishimiki M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem. Biophys. Res. Commun. 1972; 46(2):849-854.
20. Oyaizu M. Studies on products of browning reaction: Antioxidative activity of producing of browning reaction prepared from Glucosamine. Japan J. Nutr. 1986; 44:307-315.
21. Ozacan MM, Chalchat JC. Chemical composition and antifungal activity of rosemary (*Rosmarinus officinalis* L.) oil from Turkey. J Food Sci and Nutr. 2008; 59:691-698.
22. Prayitno SA, Kusnadi J, Murtini ES. Antioxidant activity of red *Betel leaves* extract (*Piper crocatum* Ruiz & Pav.) by difference concentration of solvents. Research Journal of Pharmaceutical, Biological and Chemical Sci, 2016; 7(5):1836-1843.
23. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 1999; 269(2):337-341.
24. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved abts radical cation decolorization assay. Free Radical Bio Med. 1998; 26:1231-1237.
25. Rintu D, Shinjini M, Kaustab M, Pramathadhip P, Umesh PS, Banerjee ER. Anti-oxidant and anti-inflammatory activities of different varieties of Piper leaf extracts (*Piper betel* L.) J of Nutri & Food Sci, 2015; 5(5):3-15.
26. Rinzar K, Celan S, Knez Z, Skerget M, Bauman D, Gleasar R. Antimicrobial and antioxidative activity of rosemary extract in chicken Frankfurters. Journal of Food Sci. 2006; 71(7):425-429.
27. Saini A, Pandey A, Sharma S, Ambedkar YR, Suradkar US, Meena P *et al.* Effect of rosemary (*Rosmarinus officinalis* L.) leaves extract on quality attributes of chicken powder incorporated fried chicken snacks J Anim. Res. 2019; 9(4):565-571.
28. Sebranek JG, Sewalt VJH, Robbins K, Houser TA. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. Meat Sci. 2005; 69(2):289-296.
29. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enz. 1999; 299:152-178.
30. Tavassoli S, Djomeh ZE. Total phenols, antioxidant potential and antimicrobial activity of methanol extract of rosemary (*Rosmarinus officinalis* L.). Global Vet. 2011; 7 (4):337-341.
31. Tayel AA, El-Tras WF. Plant extracts as potent biopreservatives for *Salmonella typhimurium* control and quality enhancement in ground beef. Journal of Food Saf. 2012; 32:115-121.
32. Teruel MR, Garrido MD, Espinosa MC, Linares MB. Effect of different format-solvent rosemary extracts (*Rosmarinus officinalis*) on frozen chicken nuggets quality. Food Chem. 2015; 172:40-46.