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SCANNING OF ANTIOXIDANT ACTIVITIES OF PHENOLIC CONSTITUENTS IN COLD SAUCES AND IT'S DNA PROTECTION POTENTIAL STUDY

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ABSTRACT

The sauces or chutneys are an important part of Indian food. In this research, the antioxidant activity of various aqueous sauces (chutneys) made from a combination of phenolic ingredients was investigated. These ingredients included Dates, Jaggery, Tamarind, Mint and coriander leaves, green chilies, Garlic, Ginger, lemon juice, coconut kernel, curry leaves, roasted chick peas (Chana), roasted Ground nuts, onion, tomato, white sesame seeds, red chilly powder, salt. The The total phenolic contents of all the sauces was determined by the Folin-Ciocalteu (FC) spectrophotometric method. The Antioxidant Capacity was evaluated using DPPH and DNA Damage Assay.The sauce made from Date, Jaggery, Tamarind, Mint, Coriander leaves, green chilies, Garlic, Ginger, lemon juice, and salt had a high TPC but did not show strong DNA protection despite its high antioxidant capacity in the DPPH assay. Sauces where coconut and groundnut were the main ingredients did not demonstrate notable antioxidant activity.The result showed that when the ingredients were combined, they exhibited both antagonistic and synergistic effects.

Keywords: *Antioxidants, DNA Damage, DPPH, TPC.*

Introduction

Free radicals are the unstable molecules in the body produced due to basic metabolic process. The change in life style, changes in diet and exposure to hazardous substance also results in the formation of free radicals. They play an important role in the defense system of the body but an excess of highly reactive free radicals can damage important cellular components such as DNA, proteins and lipids[1].

Antioxidants are defined as molecules that prevent free radicals from taking electrons and causing damage [2]. Antioxidants play an important rolein the maintenance of good health and protection from coronary heart disease and cancer[3]. Antioxidants are formed in human body on its own, but in an insufficient amount. Oxidative stress occurs when body has too many free radicals and very few antioxidants.

Due to toxicity and side effects of synthetic drugs, a high cost of treatment and an inadequate supply of drugs; the demand for plant material, as a source of drug, has increased in the developing countries. This has raised the interest among scientists, consumers and food manufacturers to develop food with specific health effects and potent antioxidant properties. It is important to know the antioxidant capacity of the food we eat, vegetables spices we consume.

Chutney is a classic Indian food. Chutney originated over 2,000 years ago on the Indian subcontinent in the form of a paste made from fresh ingredients. Research has reported that most of the ingredients such as Mint (Mentha spicata L.), Coriander leaves (*Coriandrum sativum* L.) ,Tamarind (*Tamarindus indica*), Garlic (*Allium sativum* L.), Lemon (*Citrus limon* L.), Sesame (*Sesamum indicum* L.), Chilli (*Capsicum annuum*L.), fresh Coconut *(arecaceae)* used to prepare chutneys possess high phenolic content, a characteristic associated with high antioxidant activity . Not much study is carried

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out to know whether the combination of these ingredients causes synergistic or antagonist effect on antioxidant capacity. As sauce or chutneys are an important part of Indian food, it is necessary to know the composition and health benefit in terms of its antioxidant content. The objective of present research was to investigate antioxidant activity of aqueous sauces (Chutneys) prepared using different combination of phenolic ingredients and to identify best ingredients and composition of cold sauce (chutney) on the basis of enhanced antioxidant activity.

Materials and Methods

Materials

Dates, Jaggery, Tamarind, Mint and coriander leaves, green chilies, Garlic, Ginger, lemon juice, coconut kernel, curry leaves, roasted chick peas (Chana), roasted Ground nuts, onion, tomato, white sesame seeds, red chilly powder, salt.

Preparation of Aqueous Cold Sauces:

Different aqueous cold sauces (8 types) were prepared using these ingredients.

Type 1 : Date, Jaggery, Tamarind

Type 2 : Mint and Coriander leaves, green chilies, Garlic, Ginger, lemon juice and salt

Type 3 : Coconut kernel, Mint and coriander leaves (small quantity) , green chilies, Garlic, Ginger, salt, roasted chick peas (Chana), curry leaves

Type 4 : Roasted Ground nuts & chick peas, onion, tomato, Garlic, Ginger, Tamarind, Red chilly powder and salt

Type 5 : Coriander leaves, green chilies, Garlic, white sesame seeds, lemon juice and salt.

Type 6 : Roasted Ground nuts, Garlic, Red chilly powder and salt

Type 7 : Tomato Ketchup i.e Tomato paste, sugar and salt,

Type 8 : Mint leaves, lemon juice and salt.

Extract Preparation

- ***Extract Preparation of Individual Ingredient:*** 1 g of each ingredient in powder form was dissolved in 20 ml distilled water. After thorough mixing, the filterate was used for DPPH Assay.
- ***Extract Preparation of Sauces:*** 5 g of each sauce was dissolved in 30 ml distilled water. Mixture was centrifuged and and clear solution was used to determine total phenolic content, antioxidant activity by DPPH and DNA Damage Assay.

Chemicals

DPPH extra pure purchased from SRL Pvt. Ltd. Gallic acid, Folin Ciocalteu phenol reagent and Ethidium bromide (EtBr) from Prerana Enterprises. Dipotassium hydrogen phosphate, potassium dihydrogen phosphate and hydrogen peroxide were purchased from SD Fine Chemicals Ltd. pBR322 was procured from Allianz BioInnovation. 0.25% Bromophenol blue, Xylene Cyanol FF 0.25%, Glycerol 30%, 7.5% sodium carbonate, Methanol . All reagents and chemical used were of Analytical grade.

Phytochemical Analysis

Total Phenolic Content

A volume of 0.5 mL of the plant extract (10 mg/mL) was mixed with 2.5 mL of the Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and were neutralized with 2.5 mL of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using UV-VIS spectrophotometer. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract.

Methods to evaluate Antioxidant Capacity

- **DPPH (2,2-Diphenyl-1-picrylhydrazyl) Ass**

The ability of the extracts to scavenge free radicals was measured using modified method described by Miliauskas et.al [4, 5]. In clean labeled glass tubes 3 ml of methanolic DPPH solution was mixed with 0.1ml of extract. After thorough mixing the tubes were and kept in dark for 30 minutes. The control was prepared by mixing 3ml DPPH with 0.1ml methanol. The absorbance at 520 nm was

measured using digital colorimeter. The experiment was performed in duplicate. The radical scavenging capacity was calculated using equation

$$\%\ Inhibition = \frac{\text{Absorbance of control- Absorbanceof sample}}{\text{Absorbanceof control}} \times 100$$

- **DNA Damage Assay**

The DNA damage assay was performed according to the method described by Ali and Sharma, (2014) [6]. The study was done using super coiled pBR322 plasmid as a standard DNA sample. For carrying out the assay, the plasmid (0.25µg) prepared in 100mM potassium phosphate buffer (pH7.4) was incubated with H_2O_2 (60mM) in the presence and absence of extracts (2 µL) shown in Table 1 . The tubes were incubated at 37°C for three hours. The pBR322 plasmid DNA (0.25µg) in 100mM potassium phosphate buffer (pH7.4)) along with distilled water was kept as a control. Reaction tubes were prepared using all 8 extracts of aqueous sauce.

Table 1: Protocol for DNA damage assay

		pBR322	H_2O_2	Phosphate Buffer	Extract	DW
1	pBR322 alone	1µL	0	1 µL	0	8 µL
2	pBR322+H_2O_2	1 µL	2 µL	1 µL	0	6 µL
3	pBR322+Extract	1 µL	0	2 µL	2 µL	5 µL
4	pBR322+H_20_2+ Extract	1 µL	2 µL	1 µL	2 µL	4 µL

Results and Discussion

- ***TPC of Sauce Extracts:*** The most important group of secondary metabolites present in ingredients of sauces are phenoilc compounds. Estimation of phenolic compounds in plant extract has gained attention because of their physiological function, including antioxidant activities**.** The mean absorbance obtained for generation of standard calibration curve is presented in Table 2 and Figure 1 is the calibration curve of Gallic acid.

Table 2: Mean Absorbance of Gallic Acid for Generation of Standard Curve

	I	II	Mean	I	II	Mean	SD
Blank	0	**0**	**0**	**0**	**0**	**0**	**0**
Extract 1	0.184	0.173	0.1785	0.141	0.135	0.138	0.028638
Extract 2	0.119	0.112	0.1155	0.092	0.09	0.091	0.017324
Extract 3	0.027	0.024	0.0255	0.021	0.02	0.0205	0.003536
Extract 4	0.134	0.14	0.137	0.121	0.116	0.1185	0.013081
Extract 5	0.058	0.055	0.0565	0.045	0.042	0.0435	0.009192
Extract 6	0.267	0.294	0.2805	0.233	0.221	0.227	0.03783
Extract 7	0.051	0.049	0.05	0.041	0.038	0.0395	0.007425
Extract 8	0.031	0.034	0.0325	0.026	0.027	0.0265	0.004243

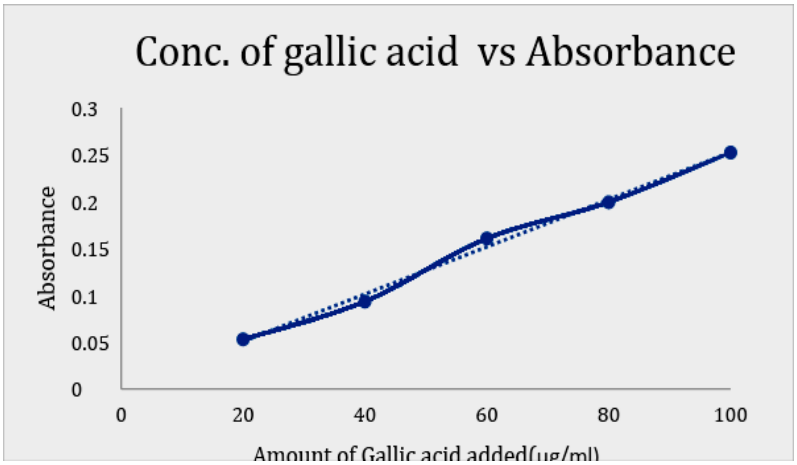


Figure 1: Calibration curve of Gallic acid

Calculations

From calibration curve, **y = 0.0025x and r^2 = 0.9989**

From the calibration curve the relation obtained is

y = 0.0025x - 0.0272 r^2 = 0.9989

'x' is calculated using Mean absorbance of each extract (y)

The value of 'x' obtained is in µg/mL converted to mg/ml.

Thus 'x' = c = Represents the concentration of gallic acid present in sample in mg/ml

'Total Phenolic Content' in mgGAE/g dry extract is calculated using formula

$$TPC\ \text{mg GAE/g dry extract} = \frac{c.V}{m}$$

where $V = \text{Volume of the extract used in ml}$

$m = \text{Mass of the extract used in grams}$

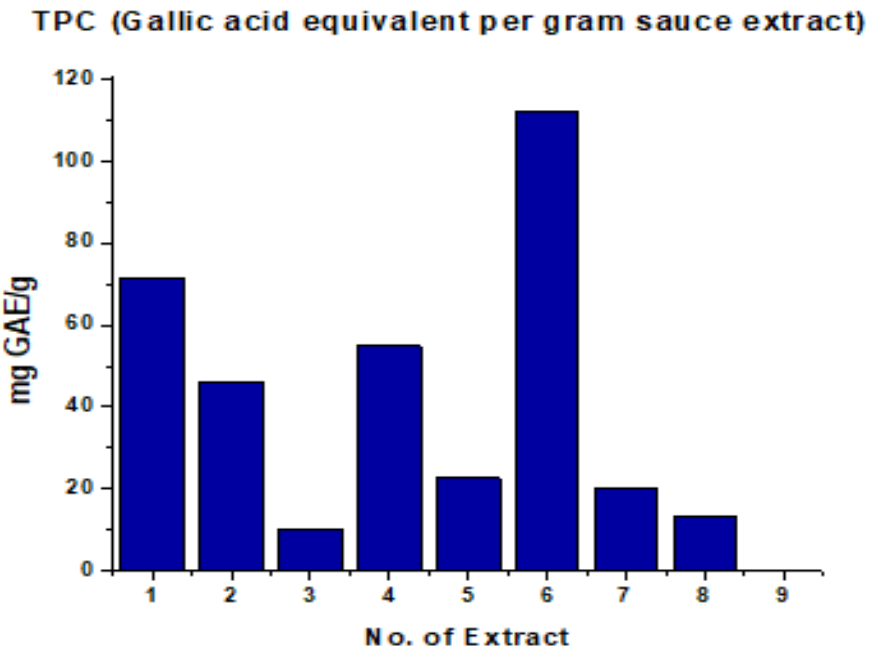


Figure 2: TPC of *Aqueous Sauce* Extract

The TPC values were higher in aqueous extracts of sauces of type 1, 2, 4, and 6. The sauce prepared using roasted Ground nuts, Garlic, Red chilly powder and salt showed maximum TPC followed by sauce prepared from dates, jaggery and tamarind. The TPC value was found to be minimum for sauce prepared by mixing Coconut kernel, Mint and coriander leaves (small quantity), green chilies, Garlic, Ginger, salt, roasted chick peas (Chana), curry leaves.

- ***DPPH Free- Radical Scavenging Activity***

It is believed that few ingredients of Indian cold sauces, are rich source of polyphenols and flavonoids hence exhibit high antioxidant activity mainly because of their ability to donate hydrogen atom or electrons and there by scavenge free radicals [7].

In present research work the antioxidant activity of all individual ingredients used to prepare aqueous sauces as well as all eight types of sauces i.e ingredients in combination were compared by DPPH radical scavenging assay. The violet colour methanolic solution of free radical, 2,2- Diphenyl-1-picrylhydrazyl (DPPH) with an odd electron gives a maximum absorption at 520 nm. When an antioxidant-rich extract is added to methanolic DPPH solution a gradual reduction in absorbance indicates that DPPH radicals are being scavenged. The more rapid the absorbance decreases, the more

potent the primary antioxidant activity. Therefore, the percentage DPPH radical inhibition by antioxidant from extract is a measure of DPPH radical scavenging capacity which in turn is directly proportional to antioxidant capacity.

Table 3: **DPPH Scavenging assay of *Aqueous* extracts of Ingredients**

Sr. No.	Ingredient	% Inhibition
1	Date	**16.43**
2	Jaggery	**9.58**
3	Tamarind	**6.84**
4	Ground Nut	**5.47**
5	Chick Peas	**1.3**
6	Sesame	**4.1**
7	Garlic	**0.0**
8	Ginger	**10.6**
9	Lemon	**0.0**
10	Onion	**2.81**
11	Green Chilli	**0.0**
12	Tomato	**2.81**
13	Mint Leaves	**27.39**
14	Coriander Leaves	**39.72**
15	Red Chilli	**6.8**
16	Coconut Kernel	**4.28**
17	Salt	**0.0**
18	Curry leaves	**20.54**

Table 3 shows the % inhibition of aqueous extracts of individual ingredients generally used for preparation of sauce. Amongst all extracts coriander leaves, mint leaves, curry leaves and date extract showed significant scavenging property. Jaggery and Ginger showed lower scavenging activity then leaves of coriander, mint and curry. Low inhibition was observed for aqueous extracts of tamarind, groundnuts, chick peas, sesame, onion, tomato, coconut kernel, red chilli. The results showed that all these extract have ability to reduce the radical to the corresponding hydrazine exceptgarlic, salt, lemon and green chillies.

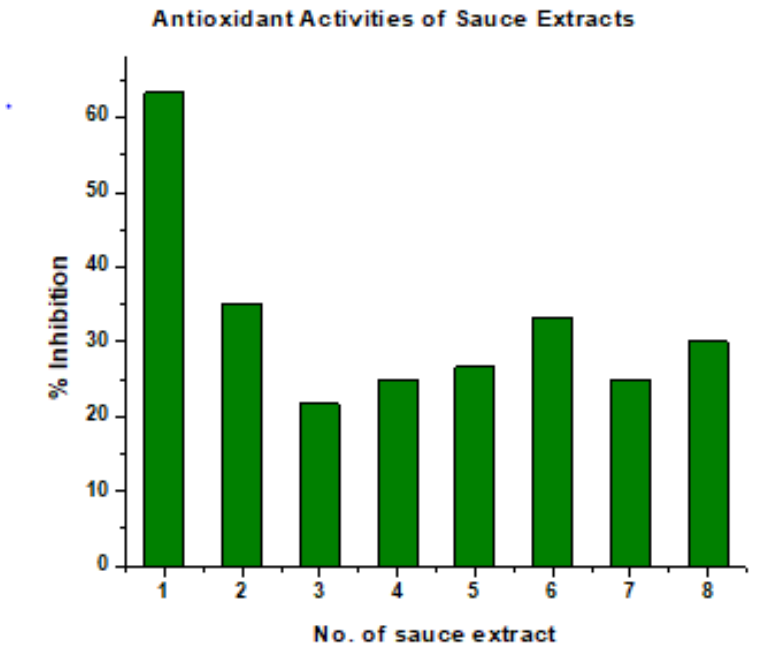


Figure 3: DPPH Scavenging assay of *Aqueous* Sauce Extracts

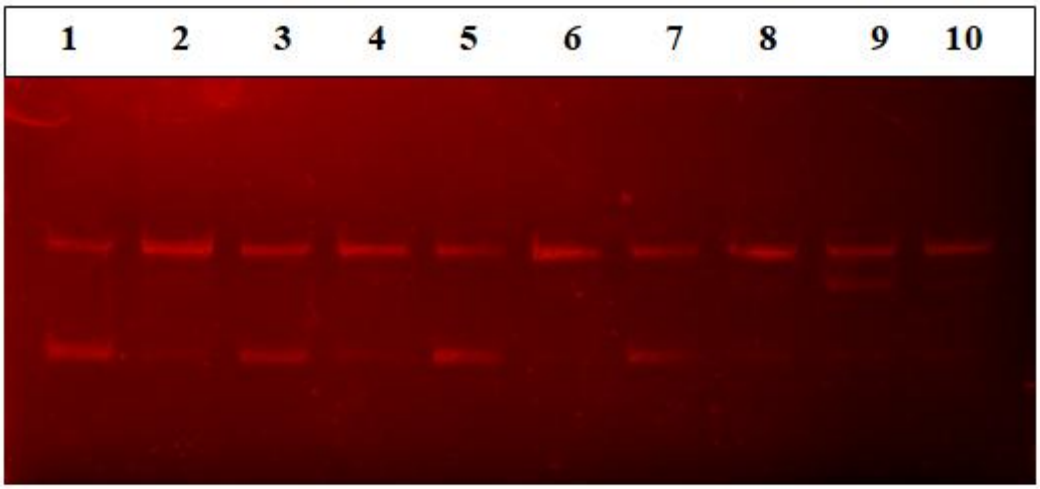
The DPPH inhibition of aqueous sauce extracts prepared by using different ingredients are presented in Figure 3. The extract of sauce Type -1 having ingredients date, jaggery and tamarind showed highest antioxidant activity. Extract 3 of sauce made up of ingredients coconut kernel, mint and coriander leaves (small quantity) , green chilies, Garlic, Ginger, salt, roasted chick peas (Chana), curry leaves showed least inhibition indicating antagonist effect. Sauces of Type 4 - 8 showed almost similar low antioxidant activity in DPPH assay.

- **DNA Damage Assay**

In the present study, the potential of each extract to prevent DNA damage is assessed by photolysing pBR322 DNA in the presence of H_2O_2 and performing agarose gel electrophoresis. pBR322 is a plasmid created in 1977 and named after the post doctoral researcher who constructed it. The p stands for plasmid and BR for Bolivar" and "Rodriguez." Hydroxyl radical generated from the photolysis of hydrogen peroxide, can react with almost all the components of DNA molecules, including purine and pyrimidine bases and the deoxyribose backbone[8,9].Also, end products such as malondialdehyde and unsaturated aldehydes, that can attach to DNA and produce mutagenic adducts which may generate due to oxidation of lipids induced by OH and other ROS[10].The damage caused to DNA when hydroxyl radical bound to it includes strand breakage, deoxysugar fragmentation and base modifications. As a result the supercoiled plasmid DNA can appear in three forms – supercoiled, open circular and linear. By comparing the nature of bands observed in agarose gel electrophoresis in presence or absence of antioxidant compounds DNA damage inhibition efficiency is evaluated.

The results obtained through gel electrophoresis for the effect of aqueous sauce extracts on DNA damage are shown in figure 4.

pBR322 with H_2O_2 results in strand breakage by converting the supercoiled form into circular form while different sauce extracts have reversed the damage of DNA which has been induced by H_2O_2(Figure 6 : Lane 4,6, 8,10 with extracts 5- 8).The pBR322 resulted in the strand breakage by converting the supercoiled form into circular form. The extract5 - 8 have shown maximum antioxidant activity excluding extract 1-4. The results presented in figure 6 indicated that extracts 1 – 4 were not very effective to prevent DNA damage.



LANE 1 - DNA alone

LANE 2 - DNA+ H_2O_2

LANE 3 - DNA+ Extract 1

LANE 4 - DNA+ H_2O_2+ Extract 1

LANE 5 - DNA+ Extract 1

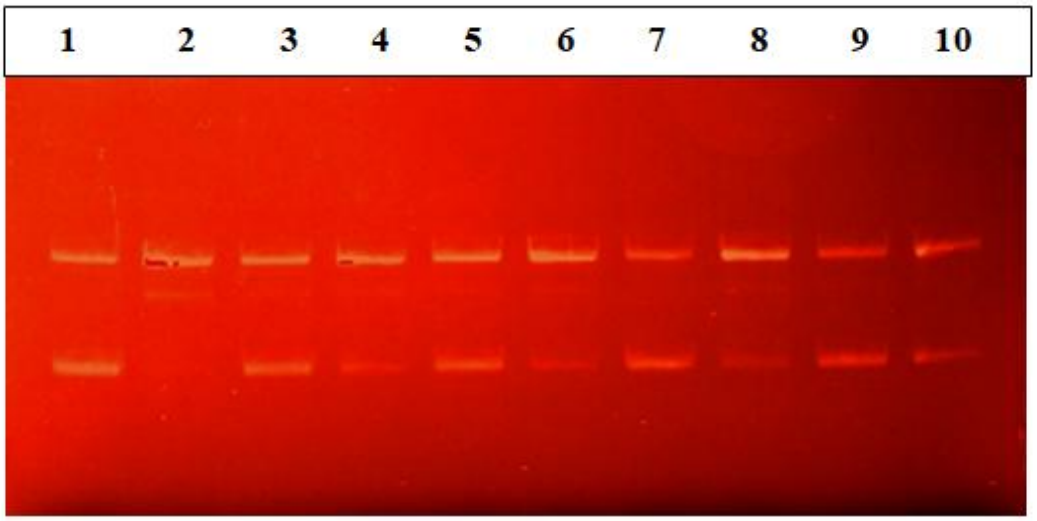
LANE 6 DNA+ H_2O_2+ Extract 2

LANE 7- DNA+ Extract 3

LANE 8- DNA+ H_2O_2+ Extract 3

LANE 9- DNA+ Extract 4

LANE 10- DNA+ H_2O_2 + Extract 4



LANE 1 - DNA alone

LANE 2 - DNA+ H_2O_2

LANE 3 - DNA+ Extract 5

LANE 4 - DNA+ H_2O_2+Extract 5

LANE 5 - DNA+ Extract 6

LANE 6 DNA+ H_2O_2+ Extract 6

LANE 7- DNA+ Extract 7

LANE 8- DNA+ H_2O_2+ Extract 7

LANE 9- DNA+ Extract 8

LANE 10- DNA+ H_2O_2 + Extract 8

Figure 4: Effect of *Aqueous Sauce* extract on glycated DNA

Conclusion

The DPPH assay showed that when the ingredients were combined, they exhibited both antagonistic and synergistic effects. The sauces prepared using combination of ingredients showed high TPC but DNA damage protection showed least antioxidant capacity. The sauces where coconut and ground nut was main ingredient did not show better antioxidant activity.

The Date, Jaggery and Tamarind combination, which showed high antioxidant activity in the DPPH assay demonstrated the least DNA protection. This indicates that antioxidant activity measured by DPPH does not always correlate with DNA protection potential. This indicates that antioxidant activity measured by DPPH does not always correlate with DNA protection potential.

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