Original Article

Evaluation of Antioxidant Activity of Essential Oils of Some Indian Medicinal Plants by DPPH, FRAP and ABTS assay

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Abstract

Antioxidants are crucial for cell stability and protection against oxidative stress, which can cause conditions such as cancer, heart disease, and eye illnesses including age-related muscle degeneration. The present investigation was performed to investigate antioxidant activity of essential oils of Clove, basil, lemon grass, cinnamon and black pepper by DPPH, ABTS and FRAP assay. In this study essential oil were compared with methanol and chloroform extract. Results indicated that essential oils obtained showed significant antioxidant activity and clove oil showed maximum antioxidant activity than other. Clove oil, which has significant antioxidant properties, mostly contains eugenol.

Keywords: ABTS, antioxidant activity, Clove oil, DPPH, and FRAP.

INTRODUCTION

In the past thirty years, oxidative stress has become a popular notion in medical sciences. It actively participates in the physiology of many common disorders, including Alzheimer's, Parkinson's, high blood pressure, preeclampsia, atherosclerosis, and acute renal failure. In the pathophysiology of ageing and degenerative diseases like atherosclerosis, cardiovascular disease, diabetes, and cancer, oxidative stress is a major factor (Gutteridge, 1993). Reactive species of oxygen (ROS), which the cells produce by metabolising oxygen, have the potential to be hazardous. Under typical conditions, the rate of oxidant removal balances out the rate and amplitude of oxidant creation. Oxidative stress is brought on by a loss of pro- and antioxidant equilibrium. High ROS concentrations in biological cells have a significant impact on how well they operate, which can result in faulty cell activity, ageing, or disease (Rodrigo, 2009). Antioxidants are substances that, when present in very small amounts in foods or the human body, delay, limit, or stop oxidative processes that result in the degradation of food quality or the onset and spread of degenerative diseases in the organism. These antioxidant chemicals work to prevent oxidation through a variety of techniques and actions (Shahidi and Zhong, 2015). Exogenous antioxidants come mostly from plants used as food and medicine, including vegetables, fruits, fungi, cereals, drinks, spices, flowers, agricultural byproducts and traditional medicinal herbs (Cai et al., 2004; Shan et al., 2005; Fu et al., 2010; Fu et al., 2011; Deng et al., 2012, 2013; Guo et al., 2012; Li et al; 2013, 2014; Zhang et al., 2016). Polyphenols (anthocyanins, flavonoids, phenolic acids, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), and vitamins make up the majority of these naturally occurring antioxidants derived from plant sources (vitamin E and C). These natural antioxidants typically have a wide range of biological effects, including antibacterial, anti-inflammatory, anti-aging, antiviral, and anticancer properties, notably polyphenols and carotenoids (Peng et al., 2014; Manach et al., 2004; Jenab et al., 2006; Arathi et al., 2015; Zhang et al., 2015; Wojtunik-Kulesza et al., 2016; Balmus et al., 2016; Prasad, 2016; Salmone et al., 2016; Zhou et al., 2016). The present investigation was performed to investigate antioxidant activity of essential oils of Clove, basil, lemon grass, cinnamon and black pepper by DPPH, ABTS and FRAP assay.

Materials and Methods

All plant samples were purchased and collected from local market and local area and identified from Department of Botany, G.F. College, Shahjahanpur.

Extraction of essential oil

By employing Clevenger's equipment and hydro distillation, the E.O.A. was extracted. 50 g of the plant material were powdered and put into 1 L flasks with 250 ml of sterile water at 70 °C. After three hours, the oil was separated, dried over anhydrous sodium sulphate, and stored for later use in screw-top bottles at 4°C. For the experiment, varied concentrations of the oil (1.25–10 µl/ml) were dissolved in dimethyl sulfoxide. Methanol and chloroform extracts were prepared by Soxhlet extraction method using 100ml solvent with 10g powdered sampled, after evaporation of solvent the extract were collected and preserved.

DPPH (1,1-diphenyl-2-picrylhydrazyl) Free radical scavenging Assay

Utilizing the DPPH antioxidant technique, antioxidant activity was measured (Kubo et al., 1984). Ascorbic acid was utilised as the reference standard sample, and varied extract concentrations (12.5 to 500 µg/ml) were used for the test. 3 ml of DPPH (1 mM) were added to 0.1 ml of test material, and the mixture was incubated at 37 oC. The reaction's end product was seen on a spectrophotometer with a wavelength of 517 nm. The following formula was used to evaluate the anti-oxidant potential in terms of % inhibition:

% inhibition = [(Ac-As)] / Ac] x 100

Where: Ac is the absorption of the control sample, As is the absorption of the sample

ABTS radical scavenging Assay

Free radical scavenging activity of plant samples was determined by ABTS radical cation decolorization assay. ABTS·+ cation radical was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1), stored in the dark at room temperature for 12-16 h before use. ABTS·+ solution was then diluted with methanol to obtain an absorbance of 0.700 at 734 nm. After the addition of 5 µl of plant extract to 3.995 ml of diluted ABTS·+ solution, the absorbance was measured at 30 min after the initial mixing. An appropriate solvent blank was run in each assay.

FRAP (Ferric Reducing Antioxidant Potential) Assay

Benzie and Strain's FRAP test, developed in 1996, was used to examine the antioxidant activity. In order to conduct this test, freshly prepared sodium acetate buffer (300 mM, pH 3.6), 10 mM TPTZ solution (in 40 mM HCl), and 20 mM Fe(III) chloride solution were combined in the following ratios: 10:1:1 and 10:1:1, respectively. The 100 µl of extracts were combined with 3 ml of the FRAP reagent, and the mixture was incubated for 30 minutes before being examined at a 593 nm wavelength. By using FeSO4 solution, the standard curve was created. In terms of moles of Fe(II) per gramme of dry weight of the test sample, extracts' anti-oxidant capacity for ferric was expressed.

Statistical analysis

All information is presented as mean + S.D. Based on data collected from triplet trials carried out on different days, the mean values were determined.

Results and Discussion

Throughout the experiment, it was found that all of the plant samples exhibit strong free radical scavenging activity, which is a hopeful indicator of their potential as therapeutic agents. In the present studies Clove, Basil, Lemon grass, cinnamon and black pepper has been analyzed for the anti-oxidant determination by DPPH, ABTS and FRAPS assays. All the plant samples of herbs and plants were assessed by DPPH method and it was observed that all the extract posses moderate to maximum percent

free radical scavenging activity. Among all the samples the essential oil reported significant anti-oxidant activity followed by mathanol and chloroform extract (Table 1.1). The maximum free radical scavenging activity observed by Clove essential oil extract (75.80 ±5.50) followed by methanol extract of Clove (65.35% ±5.20) at the maximum concentration of 500 µg/ml. The IC50 value of all the sample extracts were also analyzed and represented in table 1.2. The minimum and significant IC50 value was estimated as 100.65±2.93 µg/ml by clove essential oil followed by other samples. In contrast of the results, standard ascorbic acid have shown minimum 72.88±2.89 µg/mlIC50 value against DPPH generated free radicals (Table 1.2). All plant samples were estimated to determine ferric reducing potential by FRAP assay and results were expressed in table 1.3. During the investigation it was observed that maximum ferric reducing potential was shown by clove essential oil (7.66 ±1.12 μmol Fe(II)/mg) followed by Lemon grass essential oil (7.22± 1.32 μmol Fe(II)/mg). All results are shown in the table 1.3. All the extracts were further examined to calculate the free radical scavenging activity obtained by the ABTS assay based on the prior free radical scavenging activity. For the assay, different concentrations of each extract were examined. In Table 1.4, the results were displayed. During the analysis, it was shown that clove essential oil (69.54± 4.33) and clove methanol extract (69.54± 4.65) at the highest concentration of 500 µg/ml both had the highest ABTS-generated free radical scavenging activity. There was further evidence of considerable free radical scavenging activity in other test samples (Table 1.4). To assess the total antioxidant power of food, dietary supplements, herbal extracts, or pure substances, numerous techniques have been devised. Few of them have, however, been widely utilised since it is challenging to determine total antioxidant capacity due to restrictions linked to methodological concerns and free radical sources (Prior et al., 2005; Schauss et al., 2006). Flowering-stage buds exhibited higher eugenol contents than the others, and as a result, had higher antioxidant levels than previous stages (Razafimamonjison et al., 2013). Other investigations revealed that the presence of eugenol and other phenolic components in clove oil gives it antibacterial and antioxidant properties (Radünz et al., 2018). Additionally, prior studies revealed that eugenol was a more potent antioxidant than artificial compounds like butylated hydroxyanisole (BHA) (Gülçin 2010) . One phenolic compound with an aromatic ring is eugenol. Due to its resonant structure, which enables it to stabilise itself, this structure enables phenolics to stabilise free radicals by transferring hydrogen atoms to radicals (Ogata 2000; Lee and Shibamoto, 2001; Gülçin 2009,2011; Zengin and Baysal, 2015; El-Maati et al., 2016).

Table 1.1: DPPH free radical scavenging activity

Samples	**DPPH Percent Inhibition of extracts at various concentration (µg/ml)**					
	12.5	**25**	**50**	**100**	**250**	**500**
CiM	7.50±2.11	11.00±2.31	17.90±3.70	28.75±4.00	38.20±4.45	58.22±5.12
CiEO	8.02±2.83	12.80±3.51	19.77±4.00	29.25±4.21	39.82±3.80	60.65±5.18
CiC	6.10±1.97	8.45±3.01	13.88±3.86	26.95±4.20	37.44±4.10	57.82±3.76
ClM	9.00±3.34	11.20±4.86	20.15±3.84	32.22±4.12	45.10±5.24	65.35±5.20
ClEO	10.45±2.35	17.12±3.21	27.35±3.83	39.02±3.86	50.84±5.11	75.80±5.50*
ClC	7.51±1.23	10.00±2.21	16.13±2.71	24.22±3.11	36.02±4.25	56.24±4.26
BaM	8.10±4.21	11.84±3.40	18.78±3.20	31.60±3.67	38.66± 3.87	59.35±4.22
BaEO	8.22±3.31	13.72±2.74	21.90±3.83	33.33±3.02	41.44± 4.21	61.55±2.06
BaC	6.33±2.05	10.75±3.91	14.52±2.51	32.60±3.88	38.66± 3.87	59.02±3.22
LgM	8.32±2.91	11.20±3.41	17.74±3.66	30.45±3.22	37.35±4.13	58.66±4.70
LgEO	8.95±2.21	13.25±3.01	20.70±3.63	31.52±3.65	40.50±4.05	62.22±5.20
LgC	6.24±2.80	8.86±3.21	13.28±3.41	28.70± 4.69	36.25±3.95	55.90±3.05
BpM	7.10±3.11	10.71±3.02	15.84±4.23	23.02±3.55	35.28± 4.31	56.44±3.82
BpEO	8.11±2.93	12.20±3.54	18.24±3.01	30.07±3.75	39.10± 2.95	60.02±4.02
BpC	6.23±3.54	10.05±4.01	14.25±3.94	32.72±3.36	38.94± 4.22	58.62±4.10
Ascorbic Acid	16.65±2.55	23.95±3.69	34.20±3.54	63.80±3.05	71.22±3.50	85.95±3.11

(CiM= Cinnamon methanolic extract, CiEO= Cinnamon essential oil, CiC= Cinnamon chloroform extract, ClM=Clove methanolic extract, ClEO=Clove essential oil, ClC= Clove chloroform extract, BaM= Basil methanolic extract, BaEO= Basil essential oil, BaC= Basil chloroform extract, LgM=Lemmon grass methanolic extract, LgEO= Lemon grass essential oil, LgC=Lemon grass chloroform extract, BpM= Black pepper methanolic extract, BpEO= Black pepper essential oil, BpC= Black pepper chloroform extract)

Table 1.2: IC50 Value of all extracts against DPPH assay

S. No.	Plant Name	IC_{50} values of DPPH Assay (in µg/ml)		
		Methanol	Essential oil	Chloroform
1	Cinnamon	190.83±3.60	103.20± 2.80	260.86±5.78
2	Clove	164.80±5.41	100.65±2.93**	236.20±6.69
3	Basil	173.90±4.60	101.82±2.74	241.92±6.33
4	Lemon grass	181.10± 4.83	102.05± 2.32*	252.46±6.11
5	Black Pepper	197.88± 4.21	106.75± 2.88	284.56±6.42
6.	Ascorbic acid	72.88±2.89*		

All the DPPH test was performed in triplicate on separate days and the results were expressed with the mean value of ±S.D. *p<0.05, **p<0.01

Table 1.3: Ferric reducing assay

S. No.	Plant Name	FRAP Value(µmol Fe(II)/mg)		
		Methanol	Essential oil	Chloroform
1	Cinnamon	5.24±1.58	7.00± 1.81	5.53± 1.38
2	Clove	5.90±1.39	7.66 ±1.12***	5.93± 1.58
3	Basil	5.10±1.58	7.18± 1.35*	5.28± 1.13
4	Lemon grass	5. 35± 1.75	7.22± 1.32**	5.51± 1.34
5	Black Pepper	5.53± 1.56	6.98± 2.11	5.65± 1.34
6.	Ascorbic acid	10.76± 1.19		

All the data expressed in above Table are the results of FRAP assay which was performed in triplicate at separate days and data expressed as ±S.D. value. *p<0.05, **p<0.01, ***p<0.005

Table 1.4:- ABTS free radical scavenging activity

Samples	Percent Inhibition of different accessions at various concentration (µg/ml)				
	12.5	25	100	250	500
CiM	7.44± 3.07	9.86± 3.86	22.72± 3.60	43.80± 4.11	50.40± 5.44
CiEO	7.77± 2.44	10.20±3.23	25.90 ± 3.20	46.24± 3.20	63.25± 5.11
CiC	5.92± 2.29	8.25±3.21	10.84 ± 3.33	27.74± 4.00	38.80± 3.48
ClM	8.22±3.45	13.33±4.49	28.36±4.21	49.65± 4.65	66.97±4.02

ClEO	8.90±3.86	15. 30± 4.15	34.95 ±4.46	54.33± 4.21	69.54± 4.33
ClC	7.33±3.00	11.66±3.89	26.43±4.07	43.20±4.11	63.55±4.22
BaM	8.05±2.79	12.75± 3.35	26.66 ± 4.01	47.90± 4.45	64.77± 5.12
BaEO	8.40±4.39	12.92±3.32	30.66± 4.32	51.11± 3.76	67.77± 5.11
BaC	7.27± 3.79	10.44± 3.56	23.88± 3.78	40.90± 4.11	60.38± 5.67
LgM	7.65±2.02	10.10±3.01	25.82±3.20	45.56±3.10	61.88±3.01
LgEO	7.90± 2.75	11.10±3.90	27.55±3.76	47.68± 4.10	65.75± 4.82
LgC	7.80±3.21	7.95± 4.10	23.25±4.47	44.28± 4.70	58.70± 4.25
BpM	8.57± 4.43	9.45± 3.65	20.20± 3.98	32.75± 3.92	48.96± 3.97
BpEO	8.10± 3.28	10.70± 4.12	22.20 ± 4.30	36.24± 4.00	55.42± 4.02
BpC	6.28± 4.10	8.92± 3.89	17.45 ± 4.05	28.62± 4.10	42.90± 5.02
Ascorbic Acid	12.78±2.28	19.22±2.97	49.20±4.22	61.74±5.19	78.90±6.38

Conclusion

The present investigation concluded that all the samples of Clove, basil, lemongrass, cinnamon and black pepper have significant compounds which can scavenge the free radicals generated during DPPH, ABTS and FRAP assay. This investigation prompts the further studies for the isolation of potential bioactive compounds.

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