Evaluation of Total Phenolic and Flavonoid Content and Free Radical Induced DNA Damage Preventive Potential of Piper nigrum (Linn) and Cinnamomum cassia (Blume)

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Abstract - **Reactive oxygen species (ROS) contributes to the development of various degenerative diseases. Antioxidants play an important role in the biological system by suppressing ROS. Scientific research has indicated a credible antioxidant potential for traditional ethnomedicinal use of spices. The present study was designed to evaluate the free radical scavenging potential and oxidative DNA damage preventive activity of spices Piper nigrum seed and Cinnamomum cassia bark commonly used in Indian cuisines. By using the soxhlet extraction procedure the aqueous, 50% acetonic, and 50% methanolic extracts of P. nigrum seed and C. cassia bark were obtained. Their antioxidant activity was investigated by using standard methods namely Reducing power assay and DPPH [2-Diphenyl-1-picrylhydrazyl] assay and also tested for H2O2 mediated DNA damage. Their total phenolic content (TPC) and total flavonoid content (TFC) were also determined. At 250 µg/ml concentration highest reducing ability of PNME (70.43%) and CCME (76.26%) were found. Similarly at the same concentration DPPH radical scavenging activity of methanolic extracts of both the spices PNME (73.42%) and CCME (79.45%) were highest when compared to standard ascorbic acid. The results indicate that methanolic extracts of both the spices (PNME and CCME) possessed a comparatively high number of phenolic contents whereas acetonic extracts of spices (PNAcE and CCAcE) were found to have a higher number of flavonoids. Various extract of sample spices has shown moderate to significant protection towards damage induced by Fenton reaction on calf-thymus DNA. In addition, the total free radical scavenging activity of the methanolic extract was remarked to be higher than that of its acetonic counterpart. All these effects were found to be related to the total phenolics and flavonoid content of the extracts. Concluding that these spices should be recommended in our daily nutritional needs because of their therapeutical and nutraceutical property.**

keywords - **Antioxidant activity, DNA damage, Phenolic content and Flavonoid content**

I. Introduction

Major recent studies in the past decade have been remained seen an explosion of interest in the field of free radicals and reactive oxygen species (ROS) in biology thus producing a medical revolution that promises a new age of health and disease management [3,38,39]. Excess free radical production originating from endogenous and exogenous sources [20,41] induces cellular damage by starting chemical chain reactions such as lipid peroxidation or by oxidizing protein or DNA [10,33,34]. Thus, these progressive changes are manifested as many disease conditions including atherosclerosis, inflammatory conditions, certain cancer aging, and neurological disorders viz. Alzheimer's disease and Parkinson's disease [3,38]. Many experiments provide evidence that DNA and RNA are susceptible to oxidative damage [39,40]. Oxidative nucleotide as glycol, dTG, and 8- hydroxy-2-deoxy-guanosine is found to be increased during oxidative stress [11].

The revival of interest in natural drugs especially those derived from plants started in the last two decades mainly because of the widespread belief that green medicines are healthier and safer than synthetic ones. A large number of indigenous drugs available in the market, prescribed by physicians, which on clinical trials are found to be effective. Most of the drug plants are wild medicinal plants and they have their value in chemical substances present in various plant tissues [2,36,42]. These chemical substances can produce a specific physiochemical action in the human body. Recent studies have shown that plant-derived bioactive principles such as phenolic acids, polyphenols, alkaloids, flavonoids, and terpenes possess high redox power which enables them to scavenge free radical, peroxides, superoxide anions (O2-), hydroxyl radical (OH**.**) or lipid peroxyl thereby inhibits the oxidative mechanism that leads to degenerative diseases [5,15,32].

The use of spices has a long history and the antioxidant character of spice ingredients have been the subject of considerable study as noted by a substantial number of publications in many journals [13,22]. One reason for the continued interest in examining spices is the desire to find natural antioxidants that have minimal impact on the sensory characteristics of the food [7,4]. The most widely used synthetic antioxidant compounds were butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which have been restricted from usage because of their liver damage and carcinogenic potential. In the present scenario, strong emphasis has been given to explore novel and natural antioxidants from dietary plants because they can buffer the human body from various diseases caused by oxidative damage of lipid, protein, and nucleic acid [13, 35].

Among the spices investigated to date one belongs to the Lauraceae family is *Cinnamomum cassia (Blume)* and the other from the Piperaceae family is *Piper nigrum (Linn)*. Cinnamon is a tropical evergreen tree original habitat to Srilanka, South-West India, and Burma. Part used in this study is cinnamon bark which yields 1-2% volatile oil whose chief constituents are cinnamic aldehyde, cinnamyl acetate, phenyl propyl acetate, orthocumaric aldehyde, tannic acid. It is generally used in medicine as a cardiac stimulant, dyspepsia, diarrhoea, vomiting, and dysentery [6]. It is stomachic, carminative and its tincture is useful in uterine haemorrhage and menorrhagia [23] and also found to be effective against hyperglycemia [14]. *Piper nigrum* is a climbing perennial shrub, mostly found cultivated in Southern states of India, Srilanka, Bangladesh, and other tropical countries. Peppercorn are composed of many health-benefiting constituents. Volatile oil having sesquiterpenes, alkaloids viz. piperine, piperlongumine [23] monoterpenes hydrocarbons including α-pinene,myrecene, β-pinene, limnonene, eugenol, linalool, α-phellendrene [23]. In Asian countries, it is used for the treatment of respiratory tract diseases like cough, bronchitis, asthma, cold, as counter-irritant and analgesic [21] in menstrual pain and increasing the flow of urine [35]. It possesses antibacterial, anti-tumourigenic activity, anti-inflammatory, carminative and anti-flatulence properties [4].
Although these spices are widely used in traditional medicines, only a few studies have been conducted on their pharmacological activities. Phenolic and flavonoid compounds and their various derivatives are widespread in the plant kingdom, acting as free radical scavengers. Therefore, the present study was designed to comparatively evaluate and report i) the total phenolic and flavonoid content in aqueous, acetonic and methanolic extracts of spices understudy ii) the antioxidant activities using *in-vitro* assay models which are 2, 2 Diphenyl-1, picryl hydrazyl (DPPH) assay and Reducing power assay along with their iii) protective effect on calf- thymus DNA damage induced by Fenton's reaction.

II. *In-vitro* antioxidant activity determination

Both the type of spice extracts was tested for *in-vitro* antioxidant activity using standard methods of DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay [6,29,30] and Reducing power assay [17,29,30]. The final concentration of 50, 100, 150, 200 & 250 µg/ml were used for the standard solution as well as for sample spice extracts. The absorbance was colorimetrically analysed against the corresponding blank solution. The percent inhibition was calculated by using the standard formula as suggested by Nikhat *et al.*, Jinesh V.K *et al* [13, 25].

DPPH (2, 2-Diphenyl-1-picrylhydrazyl) assay

The antioxidizing capacity of the sample extracts was determined with 2, 2-Diphenyl-1-picrylhydrazyl assay. To be reduced by the antioxidants present in the extracts. The 2, 2-Diphenyl-1-picrylhydrazyl free-radical, serve as the model oxidizing agent. On reaction with hydrogen donors, the DPPH radical is reduced to corresponding hydrazine. The purple colour of the DPPH radical turns yellow when reacts with hydrogen donating chemical compound. This discoloration assay is determined by the addition of an antioxidant to methanolic DPPH solution. The degree of discoloration shows the free radical scavenging potential of an antioxidant compound in terms of hydrogen donating ability. 0.1mM solution of DPPH (39.4 mg of DPPH in 1000ml of analytical grade methanol) was prepared and then 1ml of this freshly prepared solution was added into 3ml of (aqueous and methanolic powder extract) extract solution of different concentrations (50-250µg/ml) prepared with distilled water. The mixture was shaken strenuously and kept in dark for 10 min. Later using a colorimeter (ELICO Ltd. SL. No.07051, Hyderabad, India) the decrease in absorbance of the resulting solution was measured at 517nm and compared with the standard solution of 1% ascorbic acid. The lower degree of absorbance of the reaction mixture stipulates higher free radical scavenging activity. The tests were conducted in triplicates. The DPPH radical scavenging activity was calculated by the following formula: -

DPPH radical scavenging activity (%) = $[A_0- (A_1- As)]/A_0 \times 100$

Where A_0 is the absorbance of the control solution containing only DPPH after incubation, A_1 is the absorbance in the presence of spice extract in DPPH solution after incubation, andAs is the sample extract solution absorbance without DPPH for baseline correction which comes from a different colour of sample solution (optical blank for A_1).

Reducing power assay

The free-radical scavenging activity of reducing the power of aqueous and methanolic extract of spices was evaluated by the method of Oyaizu1986 and Oktay 2003 [26, 27]. Chemical compounds with free-radical reducing capability form potassium ferrocyanide (Fe^{2+}) on reaction with potassium ferricyanide (Fe^{3+}). Later, in the reaction mixture on the addition of ferric-chloride ($FeCl_2$), potassium ferrocyanide (Fe^{2+}) reacts with it and forms a blue colour ferric-ferrous complex which can be colorimetrically determined at 700nm [39]. In a separate series of various concentrations (50-250ug/ml) of the aqueous, acetonic, and methanolic extracts powder was mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) followed by the addition of 2.5ml of potassium ferricyanide (1%w/v). The mixture was mixed thoroughly and incubated for 20 minutes at 50˚C. After incubation 1.5ml of 10%, TCA was added and centrifuged at 3000g for 10 minutes, furthermore out of it 0.5 ml of the supernatant was mixed with 1ml of distilled water as well as 0.5 ml of ferric chloride (0.1% w/v). the solution was mixed properly and the absorbance was measured at 700nm using a colorimeter (ELICO Ltd., Hyderabad, India), against a blank which was prepared without adding extract. The higher value of the reaction mixture stipulates an increase in reduction potential. 1% ascorbic acid was used as standard. All the tests were conducted in triplicates. The percent increase in reducing power was calculated by using the following formula: -

% Increase in reducing power = $[(A_{test} / A_{Blank})-1] \times 100$

Where, A test is an optical density of test solution and A Blank is the optical density of the blank solution.

III. Determination of Total Phenolic Content

The total phenolics were determined by the modified Folin-ciocalteau method [24]. 1 ml of all the three type extracts was collected in three different 10 ml volumetric flasks separately. To each flask, 5 ml of Folin-ciocalteau reagent (1:10v/v distilled water) and 4 ml (75g/L) of sodium carbonate were added. The solution was vortexed for 15 seconds and allowed to stand for 30 min at 40º C.for colour development. The absorbance was measured against the blank in a double beam spectrophotometer (ELICO Ltd., Hyderabad, India) at absorption maxima 765 nm. Three reading were taken per solution to get reproducible results. The total phenolic content was determined and expressed as mg Gallic acid equivalents per gram of dry extract using the equation obtained from a standard Gallic acid calibration curve, y= 6.2548x – 0.0925, R2 = 0.9962

IV. Determination of Total Flavonoid Content

Aluminium chloride colorimetric method was used for the determination of the total flavonoid concentration of extracts [9, 24]. Individually 1 ml of the extracts were mixed with 0.1 ml of 1 M potassium acetate, 0.1 ml of 10% aluminium chloride, 1.5 ml of methanol, and 2.8 ml of distilled water. The solution was incubated for 30 min. at room temperature and the absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (ELICO Ltd., Hyderabad, India). The total flavonoid content was determined as mg of Quercetin equivalent per gram using the equation obtained from a standard Quercetin calibration curve, y= 4.7385x +0.0355; R2 = 0.9993

V. Protective assay of spice extracts against H2O2 – Induced DNA damage by electrophoresis

The DNA damage assay was quantified using the method described by Rivero et al 2005 and Nandini et al 2007 [24,31] with minor modifications. To study the protective effect of all the extract of spices *Cinnamomum cassia* and *Piper nigrum* against DNA damage induced by Fenton reaction [24]. The calf-thymus DNA (200µg) in the absence and presence of 100µl of each undiluted spice extracts were exposed to the action of hydroxyl radical generated by the mixture of 100µl of H2O2 (1mM) and10µl of CuSO4 (100 µM) [3,36] 50µl phosphate buffer (50mM). For 1 the reaction mixture was incubated at 37ºC. Finally, the mixture was subjected to 1% agarose gel electrophoresis at room temperature. Subsequently, the gel containing 15µl of ethidium bromide (10 mg/ml) was observed under ultraviolet light, using a trans-illuminator, and photographed [3].

Table 1: Reducing power assay of various extracts of *Piper nigrum* seeds and Ascorbic acid

Concentration of spice extract used in µg/ml	% Increase in reducing power (Mean ± SEM, n=3)			
	PNAE	PNAcE	PNME	Ascorbic acid
50 µg/ml	11.96 ± 0.54	16.03 ± 0.49	20.33 ± 0.36	38.62 ± 0.15
100 µg/ml	22.53 ± 0.41	25.42 ± 0.28	34.61 ± 0.64	58.07 ± 0.80
150 µg/ml	28.78 ± 0.47	36.48 ± 0.22	49.98 ± 0.58	65.36 ± 0.25
200 µg/ml	35.62 ± 0.71	44.13 ± 0.37	53.46 ± 0.49	76.41 ± 0.33
250 µg/ml	46.02 ± 0.32	57.22 ± 0.10	62.43 ± 0.28	83.12 ± 0.28

Spice extracts were compared with ascorbic acid control (Statistical analysis was done by one way ANOVA using Graph Pad Prism V.5.00)

Table 2: Reducing power assay of various extracts of *Cinnamomum cassia* bark and Ascorbic acid

Concentration of spice extract used in µg/ml	% Increase in reducing power (Mean ± SEM, n=3)			
	CCAE	CCAcE	CCME	Ascorbic acid
50 µg/ml	18.63 ± 0.38	22.01 ± 0.83	23.94 ± 0.34	38.62 ± 0.15
100 µg/ml	31.68 ± 0.30	35.68 ± 0.57	40.18 ± 0.52	58.07 ± 0.80
150 µg/ml	39.87 ± 0.48	41.29 ± 0.35	42.86 ± 0.17	65.36 ± 0.25
200 µg/ml	44.15 ± 0.52	48.53 ± 0.42	59.02 ± 0.22	76.41 ± 0.33
250 µg/ml	51.09 ± 0.43	63.25 ± 0.23	70.26 ± 0.16	83.12 ± 0.28

Spice extracts were compared with ascorbic acid control (Statistical analysis was done by one way ANOVA using Graph Pad Prism V.5.00)

Table 3: 2, 2-Diphenyl-1-Picryl-Hydrazyl assay of various extracts of *Piper nigrum* seeds and Ascorbic acid

Concentration of spice extract used in µg/ml	% Increase in reducing power (Mean ± SEM, n=3)			
	PNAE	PNAcE	PNME	Ascorbic acid
50 µg/ml	28.69 ± 0.08	35.16 ± 0.43	40.38 ± 0.72	53.59 ± 0.56
100 µg/ml	34.82 ± 0.22	43.68 ± 0.23	52.72 ± 0.59	68.10 ± 0.89

150 µg/ml	41.53 ± 0.10	51.09 ± 0.62	59.19 ± 0.83	75.18 ± 0.44
200 µg/ml	46.13 ± 0.32	59.61 ± 0.38	65.94 ± 0.52	81.22 ± 0.69
250 µg/ml	58.08 ± 0.37	66.57 ± 0.29	73.42 ± 0.96	89.48 ± 0.74

Spice extracts were compared with ascorbic acid control (Statistical analysis was done by one way ANOVA using Graph Pad Prism V.5.00)

Table 4: 2, 2-Diphenyl-1-Picryl-Hydrazyl assay of various extracts of *Cinnamomum cassia* bark and Ascorbic acid

Concentration of spice extract used in µg/ml	% Increase in DPPH radical scavenging activity (Mean ± SEM, n=3)			
	CCAE	CCAcE	CCME	Ascorbic acid
50 µg/ml	32.69 ± 0.08	39.56 ± 0.25	46.38 ± 0.72	53.59 ± 0.56
100 µg/ml	39.82 ± 0.22	48.12 ± 0.63	55.72 ± 0.59	68.10 ± 0.89
150 µg/ml	47.53 ± 0.10	56.37 ± 0.44	64.19 ± 0.83	75.18 ± 0.44
200 µg/ml	54.13 ± 0.32	63.78 ± 0.80	70.94 ± 0.52	81.22 ± 0.69
250 µg/ml	62.08 ± 0.37	71.15 ± 0.48	79.45 ± 0.96	89.48 ± 0.74

Spice extracts were compared with ascorbic acid control (Statistical analysis was done by one way ANOVA using Graph Pad Prism V.5.00)

Table 5: Total Phenolic and Flavonoid content of various extracts of *Piper nigrum* (seeds) and *Cinnamomum cassia* (Bark)

Spice extracts	*Cinnamomum cassia*			*Piper nigrum*		
	CCAE	CCAcE	CCME	PNAE	PNAcE	PNME
Total Phenolic content (TPC)	19.73 ± 0.24	30.28 ± 0.33	45.81 ± 0.14	14.53 ± 0.29	25.17 ± 0.41	37.87 ± 0.19
Total Flavonoid content (TFC)	15.62 ± 0.51	29.50 ± 0.72	38.12 ± 0.37	10.85 ± 0.48	21.93 ± 0.52	29.49 ± 0.62

a) mg gallic acid equivalent (GAE)/g dry weight
b) mg quercetin acid equivalent (QAE)/g dry weight

All the determinations are means of three biological replicates and expressed as ±SEM

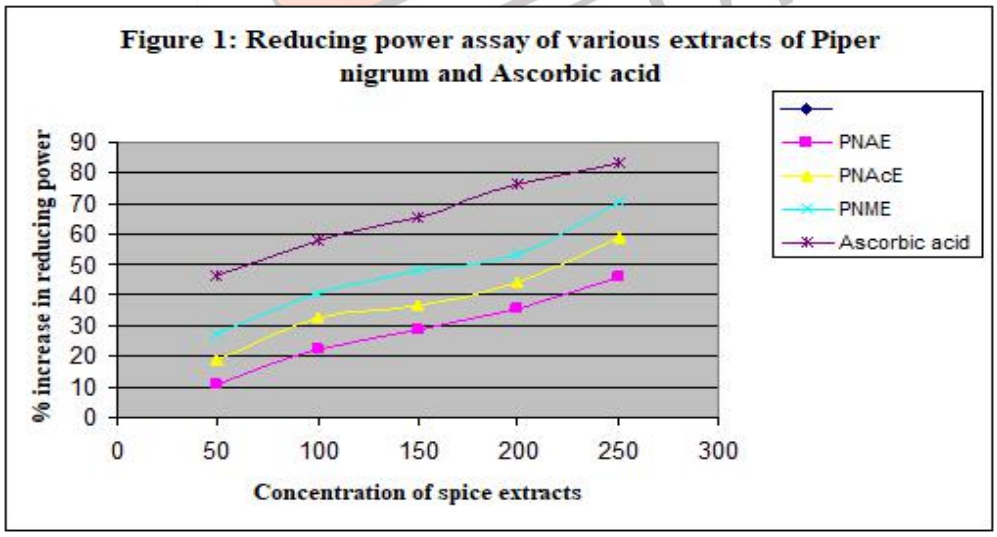

Figure 2: Reducing power assay of various extracts of Cinnamomum cassia and Ascorbic acid

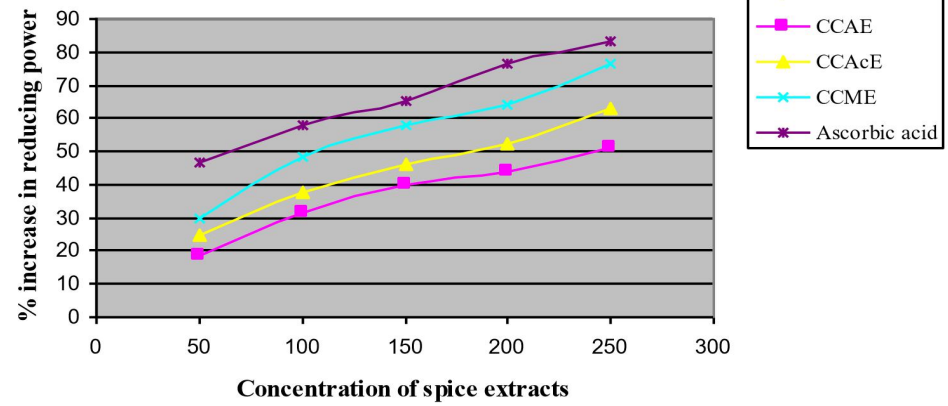

Figure 3: DPPH free radical scavenging activity of various extracts of Piper nigrum and Ascorbic acid

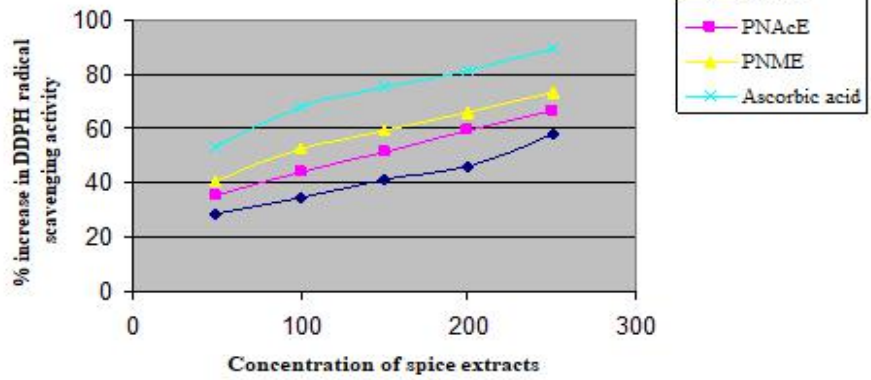

Figure 4: DPPH free radical scavenging activity of various extracts of Cinnamomum cassia and Ascorbic acid

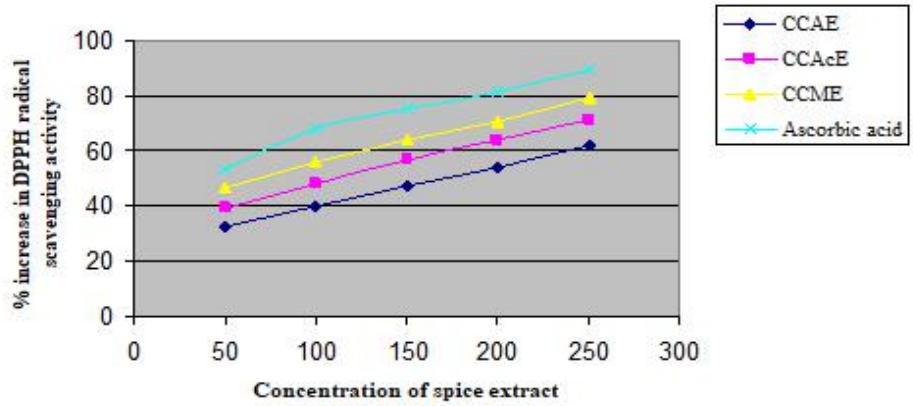

Figure 5: Protective assay of spice extracts against H_2O_2– Induced Calf-thymus DNA damage by electrophoresis

Lane 1: 200µg DNA + 100µM $CuSO_4$ +1mM H_2O_2 + 50mM phosphate buffer + PNME (100 µl)
Lane 2: 200µg DNA + 100µM $CuSO_4$ +1mM H_2O_2 + 50mM phosphate buffer + CCME (100 µl)
Lane 3: 200µg DNA + 100µM $CuSO_4$ +1mM H_2O_2 + 50mM phosphate buffer + PNAcE (100 µl)
Lane 4: 200µg DNA + 100µM $CuSO_4$ +1mM H_2O_2 + 50mM phosphate buffer + CCAcE (100 µl)
Lane 5: 200µg DNA + 100µM $CuSO_4$ +1mM H_2O_2 + 50mM phosphate buffer + PNME (100 µl)
Lane 6: 200µg DNA + 100µM $CuSO_4$ +1mM H_2O_2 + 50mM phosphate buffer + CCME (100 µl)
Lane 7: 200µg native calf-thymus DNA + 50mM phosphate buffer

VI. Result and Discussion

In the last decade, preventive medicine has undergone a great advancement, the increasing interest in the nutraceutical reflects the fact that consumers hear about epidemiological studies indicating that a specific diet or nutrients of diet are associated with a lower risk for a certain ailment. Many spices are known to have a beneficial therapeutic effect as prescribed in traditional Indian and other systems of medicine. In different studies, it has been reported previously by several researchers [1,18,29,37] that spices exhibited antioxidant properties when tested by standard methods. Antioxidants are secondary metabolites naturally found in plants. They are mainly phenolics viz. flavonoids and phenolic acids serving in plant defence mechanism to counteract reactive oxygen species (ROS) to ovoid oxidative damage and their potential to scavenge free radical are well correlated with the percentage of their phenolic compounds [19]. In this report different free radical generating systems were used to investigate the antioxidant and protective response of aqueous (PNAE, CCAE), 50% acetonic (PNAcE, CCAcE), and 50% methanolic (PNME, CCME) extracts. The reduction potential of various extracts of both the spices at different concentrations ranging from 50- 250 µg/ml is illustrated in Tables 1 & 2 and Figure 1 & 2 respectively. The reducing capacity of sample spices was compared with ascorbic acid as a control for the reduction of Fe3+ -Fe2+. The reducing capacity of a compound indicates its power to serve as an antioxidant. However different types of antioxidants possess a different mechanism of action to prevent oxidation of any compound viz. prevention of chain initiation, decomposition of peroxidases, binding of transition metal ion catalysts, inhibition of diene conjugates, prevention of continued hydrogen abstraction, and radical scavenging antioxidant activity.

Based on this investigation, it was found that there was a concentration-dependent increase in the reduction potential of extracts. It was observed that the reduction potential of *Cinnamomum cassia* exhibits a higher reduction potential than *Piper nigrum* extract. It was also noticed that 50% methanolic extract of spices has a higher percentage of reduction potential as compared to 50% acetonic and aqueous extract of spices. The percent increase in reducing power of extract was in order CCME >CCAcE> CCAE and PNME >PNAcE> PNAE respectively. Both the spice extracts at different concentration shows moderate ($P<0.05$) to significant ($P<0.01$) activity but at higher concentration 250 µg/ml the values are more significant ($P<0.001$) for PNME (70.43%) and CCME (76.26%) while, that of ascorbic acid was 83.12%.

DPPH is a stable light-sensitive, nitrogen-centred free radical used as the model system to investigate the free radical scavenging activity of natural compounds. The assay is based on the measurement of the scavenging activity of antioxidants toward DPPH, which reacts with suitable reducing agents. In the reaction mixture, the electron become paired off tp DPPH, and the solution loses colour stoichiometrically depending upon the number of electron pair taken up, and when this reaction takes place the colour changes from purple to yellow which shows a characteristic absorbance at 517nm. The present result summarized in Figures 3 & 4 indicates that all the extracts of *P. nigrum* and *C. cassia* were found to interact with DPPH radical and transformed it into its corresponding hydrazine DPPH2 in a dose-dependent manner. The fall in extinction is correlated with the potential of antioxidants to scavenge free radicals. It is evident from the result that transformation of DPPH into its corresponding hydrazine DPPH2 by *P. nigrum* and *C. cassia* respectively at various concentrations follows nearly the same pattern of significance level ($P<0.01$) when compared to each other. In terms of inhibition percentage PNME (78.45%), CCAcE (71.15%), and CCME (79.45%) possess a more significant ($P< 0.001$) inhibition percentage at concentration 250µg/ml while that of control ascorbic acid was 89.48%. *P. nigrum* seed and *C. cassia* bark extracts in terms of DPPH radical activity were ranked as PNME >PNAcE>PNME and CCME>CCAcE> CCAE.

Polyphenols from plants are known to possess antioxidant activity. They can also block the action of an enzyme that cancer needs for growth and deactivate substances that promote the growth of cancer [22]. Evaluation of total phenolic content (TPC) and total flavonoid content
(TFC) proved that all the extracts of *P. nigrum* and *C. cassia* own a significant amount of total phenolics and flavonoids (Table 5). 50% methanolic extracts of both the spices were comparatively rich in phenolics (37.87mg/g GAE in PNME and 45.81mg/g GAE in CCME) whereas 50% acetonic extracts of *P. nigrum* and *C. cassia* results in the highest amount of flavonoid content (32.17mg/g QAE in PNAcE and 38.12mg/g QAE in CCAcE. TPC was expressed as mg/g gallic acid equivalent and TFC was expressed as mg/g quercetin. Aqua methanol has been generally found to be more efficient in the extraction of lower molecular weight polyphenols whereas aqua acetone is good for the extraction of high molecular weight flavonoids [23]. The high phenolic and flavonoid content is responsible for the bioactivity of these crude extracts. Several studies have shown that polyphenols present in the spices are responsible for the decrease in oxidative stress they are consumable micronutrients in our daily diet proof their role in the prevention of degenerative diseases with different kinetics and mode of action [28]. Flavonoids are effective scavengers of most oxidizing molecules, including singlet oxygen [16,22], suppresses reactive oxygen formation, chelates trace elements involved in free-radical production also up-regulates and protect antioxidant defences [6,22]. The presence of such active principles in these spices makes them suitable as pharmaceutical and nutraceutical products which to a certain extent protect against degenerative and chronic diseases.

The hydroxyl radical is an extremely reactive radical formed as a result of metabolism in a biological system and acts as a highly degenerative species in free radical pathology, capable of damaging almost every molecule found in living cells [12]. This radical can join the nucleotides in DNA and causes strand breakage, which results in carcinogenesis, mutagenesis, and cytotoxicity [3].
The agarose gel electrophoresis was presented in Figure 5 to show the protective effect of *P. nigrum* seeds and *C. cassia* bark extracts at concentration 1mg/ml against H2O2 – induced DNA damage in calf- thymus DNA. Fenton reaction generates hydroxyl radicals which were found to induce DNA strand break in calf-thymus DNA. The aqueous extracts of both the spices PNAE (Lane 5) and CCAE (Lane 6) at 1mg/ml concentration showed insignificant protection against DNA damage. However, both the aqua acetonic extract of PNAcE (Lane 3) and CCAcE (Lane 4) show moderate visible protection. Again, our result indicates that *C. cassia* methanolic extract, CCME (Lane 2) showed highly significant protection of DNA whereas PNME showed slight protection against DNA damage (Lane 1) when compared to a positive control (Lane 7).

VII. Conclusion

Damage due to freeradicals contributes to the etiology of many chronic health problems such as neurological diseases, cardiovascular, cancer, and inflammatory conditions. Various studies proved that antioxidants can prevent free radical-induced tissue or molecular damages. Synthetic antioxidants are reported to be hazardous to human health. Therefore, in recent years search for efficacious non-toxic natural compounds with antioxidant capability has been intensified.
It is concluded from the present studies that, medically presence of the biodynamic substances provides a scientific basis for the spices understudy in ethnomedicine for the treatment of various free radical-induced disease conditions and thus supports the hypothesis that the dietary intake of these spices can be useful to cope up with the oxidative stress generated diseases.

REFERENCES

[1]. Ajay S., Rahul S., Sumit G., Paras M., Mishra A., Gaurav A., 'Comprehensive review*: Murrayakoenigii Linn'*, *Asian Journal of Pharmacy and Life Science*, 2011; Vol. 1 (4); pp. 417-425.
[2]. Beecher C.W., Fornsworth, N.R. and Gyllenhaal, C., '*Natural products of woody palnts*(ed. Rowe, J.W.)', Speringer, Berlin, 1989; Vol 2; 1059.
[3]. BoukhiraSmahane et al, Phytochemical Studies, antioxidant activity and protective effect on DNA damage and deoxyribose of *Silene Vulgaris* extract from Morocco', International Journal of Pharmacognosy and Phytochemical Research, 2015; Vol. 7(6); 1172-1178
[4]. Campanella L., Bonanni A., Favero G. and Tomassetti M., 'Determination of antioxidant properties of aromatic herbs, olives and fresh fruits using an enzymatic sensor', *Analytical and Bioanalytical Chemistry*, 2003; Vol. 375(8), pp.1011 – 1016.
[5]. Chan L.W., Emily L.C., Chech. L., Saw L., Weng W., and Paul W. S. 'Antimicrobial and antioxidant activity of *Cortex magnolia officinalis* and some other medicinal plants commonly used in South-East Asia', *Chinese Medicine*, 2008; Vol.3, pp.15. Research article: Available from: http://www.cmjournal.
[6]. Dutta, A.C. and Dutta, T.C., '*Botany for degree students 6th Edition'*, Oxford University Press; 2007.
[7]. E.Choi and J.Hwang, 'Antiinflamatory, analgesic and antioxidant activity of the fruit of *Foeniculum vulgare*,'Fitoterphia , 2004;Vol.75(6);557-565
[8]. G. Agati, E. Azzarello, S. pollastri, M. Tattini, 'Flavonoids as antioxidants in plants; location and functional significance', Plant Science; 2012; Vol. 196; pp. 67-76
[9]. Geissman T. A., '*Flavonoid compounds, tannin, lignin and related compounds. Pyrolle pigments, Isoprenoid compounds and phenolic plant constituents*', New York, USA, Elsevier Press, 1963; pp 265.
[10]. Gilgun-Sherki Y., Melamed E., and Offen D., 'Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier', *Neuropharmacology*, 2001; Vol. 40(8), pp. 959–975.
[11]. Hattori Y, Nishigori C, Tanaka T, Ushida K, Nikaido O, Osawa T. '8- Hydroxy-2-deoxyguanosine is increased in epidermal cells of hairless mice after chronic ultraviolet B exposure'. J Invest Dermatol. 1997; 89:10405-9. [Pubmed] [Google Scholar]

[12]. Hochestein P, Atallah AS, 'The nature of oxidant and antioxidant systemsin the inhibition of mutation and cancer,' Mutation Research; 1988; 202; 363-375
[13]. Jinesh V. K, Jayashree V., 'Comparative evaluation of antioxidant properties of edible and non-edible leaves of *Anethumgraveolens.*Linn.*' Indian Journal of Natural Products and Resources*, 2010; Vol. 1(2), pp.168-173.
[14]. K.A. Steinmetzand J.D. Potter, 'Vegetables, fruit and cancer prevention: a review ', Journal of American Dietetic Association, 1996; Vol. 96, pp.1027-1039
[15]. Karsha P. V. and Bhagya Lakshmi O., 'Antibacterial activity of Black pepper (*Piper nigrum* Linn.) with special reference to its mode of action on bacteria', *Indian Journal of Natural Products and Resources*, 2010; Vol. 1(2), pp. 213-215.
[16]. Kumar R., Singh N.P., 'Effect of tannin in Pala leaves (*Zizypusnummularia*) on ruminal proteolysis', *Indian Journal of Anim Sci*, 1984; Vol.54, pp. 881 – 884.
[17]. Laxmi Devi S., Kannappan S., Anuradha C. V., 'Evaluation of *in-vitro* antioxidant activity of Indian bay leaf, *Cinnamomumtamala* (Buch-Ham) T.neer and Ebern using rat brain synaptosome as model system', *Indian J. Exp. Biol*., 2007;Vol. 45, pp. 77-82.
[18]. L. Bravo, 'Polyphenols, chemistry, dietary sources, metabolism and nutritional significance' Nutr.Rev; 1998; Vol.56; 317-333
[19]. Misba Khan, Showkat Ahmad Ganie et al, 'Free radical scavenging activity of *Elsholtziadensa'*, Journal of Acupuncture and Meridian Studies; 2012;5(3): 104-111
[20].Moein S., Moein R. M., 'Relationship between antioxidant properties and phenolics in *Zhumeriamajdae*', *J. Med. Plants Res*., 2010; Vol. 4(7), pp. 517-521.
[21].Moreau and Defraisse, *ComptesRendus des séances et memories de la Societe de Biologie*, 1922; Vol. 86, pp.321.

[22].M. Shabbir, M. R. Khan and N. Saeed, 'Assesment of phytochemicals, antioxidant, anti-lipid peroxidation and antiheamolytic activity of extract and various fractions of *Maytenusroyleanus* leaves,' BMC Complementary and Alternative Medicine; 2013; Vol. 13; Article 143
[23].Nanasombat S. and Lohasuptawee, 'Antimicrobial activity of crude ethanolic extract and essential oils of spices against Salmonella and other enterobacteria', *KMITL. Sci. Tech.J*., 2005; Vol. 5(3), pp. 527538.
[24]. Nandini Goswami and Sreemoyee Chatterjee, 'Assesment of free radical scavenging potential and oxidative DNA damage preventive activity of *Trachyspermumammi* L. (Carom) and *Foeniculum vulgare* Mill. (Fennel) sed extracts' Hindwai Publishing Corporation BioMed Research International, 2014; Vol. 2014; Article ID 582767, 8 pages
[25]. Nikhat F, Satyanarayan D.and Subramanyam E.V.S., 'Isolation, Characterization and screening of antioxidant activity of the roots of *Syzygiumcumminii*(L) Skeel', Asian *J. Reacher Chem*., 2009; Vol. 2(2), pp.218-221.
[26]. Oktay M. Gulein I, Kufrevioglu, 'Determination of in-vitro antioxidant ativity of fennel (*Foeniculumvalgare*) seed extracs', *Labenson- Wiss V. Technol.,* 2003;Vol.36, pp.263-71.
[27]. Oyaizu M. 'Studies on product of browning reaction prepared from glucosamine', *Jap. J. Nutr,* 1986; Vol. 44, pp. 778-84.
[28]. Quy Diem Do and Yi- Hsu Ju, 'Effect of extraction solvent on total phenol content, total flavonoid content and antioxidant activity of *Limnoplilaaromatica'* , Journal of Food and Drug Analysis; 2014;Vol. 22(3) pp.296- 302
[29]. R. A. Khan, M.R. Khan and Sahreen,'Evaluation of *Launaea procumbens* use in renal disorders: a rat model', Journal of Ethanopharmacology, 2010; Vol.128 (2); pp. 452-461
[30]. Ramsevak, Russel, Nair, Muraleedharan G., Stommel, Manfre, '*In-vitro* antagonistic activity of monoterpenes and their mixtures against Toe-nail fungus pathogens', *Phytotherapy Research*, 2003; Vol. 17(4), pp.376-379.
[31]. Rivero D, Perez-Magarino et al, 'Inhibition of induced oxidative DNA damage by beers: corelation with the content of polyphenols and melanodins, J. Agric.Food Chemistry,2007; Vol.53; pp 3637-3642
[32]. Saxena S.N., Agarwal D., Saxena R. and Rathor S, 'Analysis of anti-oxidant properties of ajwain*Trachyspermumammi*L. seed extract', *International J. Seed Spices*, 2012; Vol. 2(1), pp.50-55.
[33]. Scalbert A., 'Antimicrobial properties of tannin', *Journal of Phytochemistry*, 1991; Vol. 30, pp. 3875 – 3883.
[34]. Shoib A. Baba and shahid A. Malik, 'Determination of total phenolic and flavonoid content, antimicrobial and and antioxidant of root extract of *Arisaema jacquemontii*Blume' Science Direct, Journal of Taibah University for Science, 2014; Vol.9; pp. 449-454
[35]. Sies H., 'Oxidative stress: oxidants and antioxidants', *Exp Physiol*., 1997; Vol. 82(2), pp.291- 295.
[36]. Singh R., Singh N., Saini B. S., 'In-vitro antioxidant activities of pet. Ether extract of black pepper', *Indian Journal of Pharmacol*., 2008; Vol.4, pp.147-151. Available from: http://www. Ijp-online.com
[37]. Sukh Dev., '*Plants and society'* (eds Swaminathan, M.S. and Kochhar, S.L.), Macmillan, Landon, 1989; p.267.
[38].Tachibana Y., Kikuzaki H., Lajis N.H., Nakatani N., 'Antioxidant activity of carbazole from *Murrayakoenigii* leaves', *J. Agric. Food Chem*, 2001; Vol.49(11), pp. 5589-5594.
[39]. V. Jaishree and V. Shabna, 'A comparative study of *in vitro* antioxidant and DNA damage protection of soxhlet vs microwave assisted extracts of *Micheliachampaca* Linn. Flowers', Indian Journal of Natural Product and Resources; 2011; Vol. 2(3); 330-334
[40]. V. Lobo, A. Patil, A. Pathak and N. Chandra, 'Free radicals, antioxidants and functional foods: Impact on human health', Pharmacognosy Review, 2010; Vol.4(8), pp.118-126
[41]. Woo RA, Melure KG, Lee PW. 'DNA dependent protein kinase acts upstrem of p53 in response to DNA damage', Nature., 1998;394:700-4 [PubMed] [Google Scholar]
[42]. Young I. S., Woodside J. V., 'Antioxidants in health and disease, *J Clin Pathol*., 2001; Vol.54, pp.176– 186.