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Protection against Oxidative Damage Properties of *Hibiscus sabdariffa* Drink (Zobo) and Added Natural Food Additives

Oxidative Damage Properties of Hibiscus Sabdariffa Drink and Additives

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Abstract

The study aimed at determining the biochemical property of Hibiscus sabdariffa drinkandhow it is been affected by ginger and cloves. H. sabdariffa drink containing different concentrations of cloves, ginger and their mixture was producedrespectively along with the control. Antioxidant activity was evaluated at the beginning and at theend of storage. Possible DNA damage- protection of the drink was also determined. Results were analyzed using Analysis of Variance and means were separated using Duncan Multiple Range Test. There was significant($p<0.05$) difference in the antioxidant activities, with H. sabdariffa drink produced with cloves having highest antioxidant activity. The phenolic, DPPH % scavenging activity and total reducing power being 405±0.5mg/L GAE,82.9±4.37 % and1.717±0.03 respectively.The phenolic content decreased on storage while the antioxidant activities of zobo containing spices increased. The protective effect of H. sabdariffa drink on DNA against damage was reduced by ginger and cloves.

Keywords: Hibiscus sabdariffa Ginger Cloves DNA Damage Antioxidant

1. Introduction

Prevalence of diseases like cancer has aroused great interest in the effect of free radicals on health in recent times. Free radicals are reactive oxygen species produced during metabolic process in the body which adversely affects the human body under certain circumstances. The uncontrolled production of reactive oxygen species and the unbalanced mechanism of antioxidant protection adversely alter lipids, proteins, and deoxyribonucleic acid (DNA) and trigger a number of human diseases which are associated with aging, such as cancer, cardiovascular disease, cognitive impairment, Alzheimer's disease, immune dysfunction, cataracts, and muscular degeneration (Aslan &Ozben, 2003; Meyer &Sekundo, 2005). It has also been well established that free radicals affect food quality, reducing its nutritional content and promoting the development of food deterioration (Puertas*et al*., 2005; Ozgen*et al.,* 2006).

However, application of external source of antioxidants can assist in coping with oxidative stress but the safety of synthetic antioxidants has recently been questioned due to their toxicity and possible carcinogenicity. Hence, in recent years, the restricted use of synthetic antioxidants has caused increased interest in natural antioxidant substances (Baardseth, 1989; Gülçin*et al*., 2005).

Antioxidants have been found to be present in foods as vitamins, minerals, carotenoids, and polyphenols, among others and they are often identified in food by their distinctive colours (Liyana-Pathirana&Shahidi, 2006). A number of studies have reported that phenolic compounds in spices and herbs contribute significantly to their antioxidant properties (Wu *et al*., 1990).

H.sabdariffa (sorrel) drink called zobo in the Northern part of Nigeria is known and popular in every part of the country. It is a non-alcoholic drink produced from the red calyces of *H. sabdariffa*. There are published data on its antioxidant properties and some of the additives currently being employed in its production among which are ginger and cloves (Mazza&Miniati, 2000; Kikuzaki&Nakatani, 1993; Ziauddin*et al.*, 1995). However, in order to make reasonable contribution towards standardization of *H. sabdariffa*. drink (zobo) processing for optimum antioxidant properties, it is imperative to study both the individual and combined anti-oxidant properties of *H. sabdariffa,* ginger and cloves as their individual functions as well as their combined additives or synergistic effects are crucial to their beneficial effects (Calsen*et al.,*2010).

2.0. Materials and Methods

2.1. Materials

Hibiscus sabdariffa (the dark red variety), granulated sugar, dried cloves and fresh gingerwere obtained from open market in Ilishan – Remo Ogun State, Nigeria. Plasmid DNA was supplied by Roche Diagnostics GmbH, Roche Applied Science 68298 Mann Heim, Germany. Chemicals used in biochemical analysis were all of analytical grade.

2.2. Production of H. Sabdariffa Drinksvwith Ginger, Cloves and Mixture of Ginger and Cloves

Tostudy the antioxidant properties of *H. sabdariffa* drink and, ginger and cloves used in its production. *H. sabdariffa* drink (with 5% concentration of *Hibiscus sabdariffa* calyx) was produced. The drink was produced by soaking *Hibiscus sabdariffa* calyx in hot (100 ^{o}C) water for 1 h (Ilondu&Iloh, 2007)with the spices (ginger and cloves) and was filtered with already sterilized sieve. Spices added separately were fresh ginger, cloves and, mixture of ginger and cloves. Five different concentrations of each were added separately and the concentrations were; 0.05 %, 0.15 %, 0.25 %, 0.35 % and 0.45 % while the control contained no spice. Percentage concentration of sugar added to the drink was 9% (Appel, 2003). Plastic bottles and sieve were sterilized by soaking in 1.0% sodium hypochlorite (10ml in 25litres of water) for 30 min and rinsing with distilled water.Different samples of the drink were dispensed into the sterilizedplastic bottlesand were pasteurized in a batch process at 72^{o}C for 5 min (Perry & Staley, 1997; Ukwuru & Uzodinma, 2010;Braide, 2012). They were stored at ambient temperature of 28± 2 ^{o}C.The phenolic content and antioxidant activity were determined at the beginning and at the end of 16 days storage.

2.3. Analyses of H. Sabdariffa Drink with Ginger, Cloves and Mixture of Ginger and Cloves

2.3.1. Total Phenol Content

The amount of total phenolic compounds was determined according to the method of Singleton and Rossi (1965). Different samples (0.2 ml) were made up to 1ml with methanol and0.5ml of folin–Ciocalteu's reagent and 7.5ml of distilled water were added. This was kept at room temperature for 5min after which 10 ml of 7% Sodium Carbonate was added to the mixture and then incubated at 90 min at room temperature. After the incubation absorbance was read at 760 nm and that of gallic acid standard solutions prepared at a concentration ranging from 0 to 500 mg l^{-1} (Li*et al*., 2009). Total phenol values were expressed in terms of gallic acid equivalent (mg per 1 litre of zobo) which is a common reference compound.

2.3.2. DPPH Scavenging Activity

The procedure was according to the 2-2- diphenyl- 2 – Picrylhydrazyl, DPPH assay of Mensor*et al.,* (2001). One millilitre of 0.3mM of methanolic DPPH solution was added to 0.05ml of the different samples, made up to 2.5ml with methanol and allowed to stand at room temperature for 30 min (to ensure proper reaction). The absorbance of the mixture was read at 518 nm and converted to % Antioxidant Activity using the formula,

$$\text{A A}\,\% = \frac{\text{Abs}_{\text{Blank}} - \text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Blank}}} \times 100$$

2.3.3. Total Reducing Power

The total reducing power was carried out according to Yen and Duh (1993). The different samples (0.05 ml) made up to 1ml with methanol was mixed with 0.5ml phosphate buffer (0.5%, 20Mm, pH 6.6) and 0.5ml of 1% Potassium Ferricyanide. It was incubated at 50 ^{o}C for 20 min, after which 0.5ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 2,500rpm for 10 min. The supernatant was diluted with 1.5 ml of distilled water and 0.1 % ferric chloride (0.3ml) was added. The absorbance was read at 700 nm. Increase in absorbance of the reaction mixtures indicated increase in the reducing power.

2.3.4. Plasmid DNA

To study the effect of *H. sabdariffa* drink, ginger and cloves on the integrity of PBr 322 plasmid DNA in the presence of Fenton's reagent.*H. sabdariffa* drink (Zobo) was produced with 5% concentration of *Hibiscus sabdariffa* calyx. The different samples were *H. sabdariffa* drink without spice (control), *H. sabdariffa* drink with 0.45 % cloves, *H. sabdariffa* drink with 0.45% ginger and *H. sabdariffa* drink with 0.45 % mixture of cloves and ginger. These different samples were concentrated in a rotary evaporator (model, eyela N- 1001) at 60 ^{o}C and later dried in the Gallekamp oven (model, DHG-9101.SA) at 60 ^{o}C. The samples were kept in the refrigerator for subsequent analysis.

The ability of different *H. sabdariffa* drink samples to protect supercoiled pBR322 DNA from devastating effects of hydroxyl radicals generated by Fenton's reagent was assessed by the DNA nicking assay described by Lee *et al.* (2002). The reaction mixture was initiated with 0.5 µl of plasmid DNA (pBR 322) in eppendorf tubes and 10 µl of Fenton reagent (30 mM H_2O_2 + 50µM ascorbic acid + 80 µM $FeCl_3$). To this mixture, 5 µl of different concentrations (10 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml) of samples of *H. sabdariffa* drink without spice*,H. sabdariffa* drink with cloves, *H. sabdariffa* drink with ginger, *H. sabdariffa,* drink with mixture of cloves and ginger were added respectively and final volume of the mixture was brought up to 20 µl using ddH_2O (double distilled water). A control contained only pBR322 DNA and ddH_2O and another control had only pBR 322 DNA and Fenton's reagent while the test samples contained the samples with pBR 322 DNA and Fenton's reagent. Samples were incubated for 30 min at 37 ^{o}C after which 2.5µl of loading buffer (0.25% bromophenol blue 50 % glycerol) was added and the tubes centrifuged in a micro centrifuge at 10,000 rpm for I min.

The DNA samples were electrophoresed on 1% agarose gel in TBE buffer, at 50V (1.5 – 2 V/cm) for 2 h. After the electrophoresis, the gel was observedfor fragmentation of DNA under an UV lamp.

2.3.5. Statistical Analysis

All measurements were replicated and data were analysed using analysis of variance (ANOVA). Duncan multiple range testswas used to separate means (SAS version 9.3) and experimental results were expressed as means ± SD.

3.0. Results and Discussion

3.1. Phenolic Content, DPPH% Scavenging Antioxidant Activity and Total Reducing Power

Phenolic content, DPPH% scavenging antioxidant activity and total reducing power are assays used for determining antioxidant activities of compounds (Singleton & Rossi, 1965; Yen & Chen, 1995; Brand-Williams *et al.,* 1995; Oktay*et al*., 2003;Wrolstad*et al.,* 2005; Huang *et al.,* 2005;Nabavi*et al.,* 2009a). Phenolic content of the *H. sabdariffa* drink samples was comparable to what was reported by Li *et al.* (2009) for white and rose wines and Di Majo*et al.* (2008) for wine from different varieties of grapes. The phenolic content of *H. sabdariffa* drink containing cloves was higher than the corresponding drink with ginger or mixture of cloves and ginger. The results are as presented in Table 1.

Table 1: Phenolic content (mg/L GAE) of *H. sabdariffa* drink produced with spices stored at ambient (28 ± 2°C) storage condition

Samples	Spice Conc. (%)	Days of Storage	
		0 day	16th day
H. sabdariffa drink (control)	0.0	306.7± 1.24^{c}	261.9±1.00^{c}
H. sabdariffa drink + cloves	0.05	369 ± 0.58^{ab}	331.6±1.07^{b}
	0.15	370.7± 1.52^{ab}	338.1± 0.58^{ab}
	0.25	373±1.00^{ab}	341.8±1.53^{ab}
	0.35	379.9±1.06^{ab}	356.7± 0.47^{a}
	0.45	405±0.5^{a}	359.6± 0.5^{a}
H. sabdariffa drink + ginger	0.05	316.7±1.00^{c}	303.6±1.05^{bc}
	0.15	328.1±0.58^{bc}	317.3± 1.16^{a}
	0.25	339± 0.12^{ab}	320.1± 2.00^{b}
	0.35	348.7± 1.05^{b}	343± 0.57^{ab}
	0.45	361.9± 1.32^{ab}	348.4 ±0.76^{a}
H. sabdariffa drink + Mixture of cloves & ginger	0.05	366.4± 1.53^{ab}	325.6±1.25^{b}
	0.15	367.6± 1.42^{ab}	327±1.00^{b}
	0.25	369.9±2.08^{ab}	327.6±3.24^{b}
	0.35	371±1.00^{ab}	353.9± 1.33^{a}
	0.45	373.3±1.51^{ab}	359.6 ±1.37^{a}

Means with the same superscript in the same column are not significantly different ($p< 0.05$).

H. sabdariffa drink without spice (control) consistently had lowest phenolic content both before and after storage. The phenolic content of the *H. sabdariffa* drink samples containing spices increased with increasing spice concentration indicating that there was a kind of additive or synergistic effect in the phenolic content of the spices and *H. sabdariffa* used in the drink production (Graversen*et al*.,2008; Liu*et al.*,2008; Altunkaya*etal*., 2009; Romano *et al.,*2009). There was significant difference in the phenolic content of the samples and at the end of 16 days storage there was a reduction in the phenolic content of all the samples.

The antioxidant action of phenolic compounds is being determined by their free radical scavenging capacity and iron reducing ability. With an increase in the concentration of the spices, there was an increase in the free radical scavenging capacity (Table 2) and in iron-polyphenol complex determined in reducing powerassay (Table 3) which has being demonstrated to inhibit the formation of oxygen radicals associated with many pathological conditions (Yoshino and Murakami, 1998).

Table 2: DPPH % Scavenging antioxidant activity of *H. sabdariffa* drink produced with spices stored at ambient (28 ± 2°C) storage condition

Samples	Spice Conc. (%)	Days of Storage	
		0 day	16th day
H. sabdariffa drink (control)	0	73.2± 4.59[a]	69.8± 4.95[b]
H. sabdariffa drink + cloves	0.05	77.5±4.82[a]	83.6 ± 3.96[a]
	0.15	78.2± 3.22[a]	84.0± 4.53[a]
	0.25	78.8± 3.8[a]	85.8±3.38[a]
	0.35	81.0± 4.39[a]	87.3± 3.51[a]
	0.45	82.9± 4.37[a]	88.7± 3.3[a]
H. sabdariffa drink + ginger	0.05	73.7± 3.65[a]	76.9± 2.93[ab]
	0.15	75± 4.8[a]	77.8± 3.27[ab]
	0.25	76.9 ± 4.31[a]	80.7±4.59[a]
	0.35	78.3± 4.4[a]	81.2±4.48[a]
	0.45	78.8± 3.87[a]	82.2± 4.1[a]
H. sabdariffa drink + mixture of cloves and ginger	0.05	75.7± 4.16[a]	78.3± 3.16[ab]
	0.15	76.1± 4.84[a]	81.7± 3.88[a]
	0.25	77.3± 4.33[a]	82.5± 3.82[a]
	0.35	79.5± 3.07[a]	85± 2.49[a]
	0.45	80.1± 3.17[a]	86.7± 4.29[a]

Means with the same superscript in the same column are not significantly different ($p < 0.05$).

Table 3: Total reducing power of *H. sabdariffa* drink produced with spices stored at ambient (28 ± 2°C) storage condition

Samples	Spice Conc. (%)	Days of Storage	
		0 day	16th day
H. sabdariffa drink (control)	0.0	1.628± 0.04[a]	1.445± 0.11[b]
H. sabdariffa drink + cloves	0.05	1.674± 0.13[a]	2.316±0.04[a]
	0.15	1.679±0.05[a]	2.337±0.03[a]
	0.25	1.694± 0.04[a]	2.345±0.04[a]
	0.35	1.703± 0.05[a]	2.371± 0.05[a]
	0.45	1.717± 0.03[a]	2.374± 0.05[a]
H. sabdariffa drink + ginger	0.05	1.655± 0.07[a]	2.278± 0.03[ab]
	0.15	1.662± 0.07[a]	2.289± 0.05[ab]
	0.25	1.668± 0.07[a]	2.317± 0.02[a]
	0.35	1.670± 0.04[a]	2.331± 0.04[a]
	0.45	1.674± 0.06[a]	2.348± 0.02[a]
H. sabdariffa drink + mixture of cloves & ginger	0.05	1.656±0.03[a]	2.313± 0.03[a]
	0.15	1.672± 0.02[a]	2.332± 0.05[a]
	0.25	1.681± 0.06[a]	2.357± 0.03[a]
	0.35	1.689± 0.01[a]	2.361± 0.07[a]
	0.45	1.662± 0.07[a]	2.369± 0.04[a]

Means with the same superscript in the same column are not significantly different ($p < 0.05$)

At the end of the storage period, the results of diphenyl-1-picrahydrazyl (DPPH) scavenging antioxidant activities and total reducing power showed an increase (with significant difference among the samples) in antioxidant activities of *H. sabdariffa* drink preserved with the spices. While *H. sabdariffa* drink without spice had a slightly reduced radical scavenging activities and total reducing power. The relationship between total phenol contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables (Klimczak*et al.,* 2001;Jayaprakasha, 2008; Anagnostopoulou*etal*., 2006) and there has being varying reports. The reduced phenolic content and increased antioxidant activities observed in *H. sabdariffa* drink samples containing spices is similar to the report of Anagnostopoulou*et al.* (2006) that there may not be a direct correlation between radical

scavenging activity and phenolic content and this is being corroborated by the report of Nickavar*et al. (*2007). Moreso, the extracts are very complex mixtures of many different compounds with distinct activities (Mensor, 2001;Hou, 2003). This observation could also be partly explained by the previous report of Tsai *et al.* (2002) that under different processing temperatures and storage periods, anthocyanin content declines. However, other phenolic compounds increase and in overall there is only a relatively small decrease in total phenolic compounds and antioxidant activity.

Reduction in the scavenging activityand total reducing power of *H. sabdariffa*drink without the spices (control) could be as a result of antioxidant activity of a food not depending only on the phenolic content but on other components like flavonoids and ascorbic acid. Flavonoids with a certain structure and particularly hydroxyl position in the molecule can act as proton donating and show radical scavenging activity (Mensor, 2001; Hou, 2003). Such kind of flavonoid could have been contributed by the spices in*H. sabdariffa* drink containing spices while the control did not contain spice that could have contributed flavonoid.

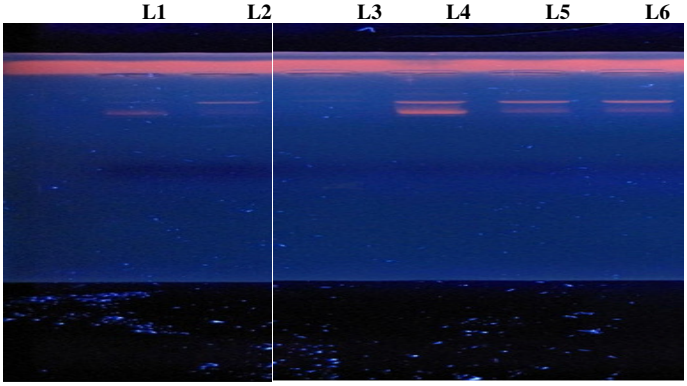
3.2. Plasmid DNA

The results of the effect of different concentrations (10 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml) of *H. sabdariffa*drinkwithout spice, *H. sabdariffa*drinkwith cloves, *H. sabdariffa*drink with ginger, and *H. sabdariffa*drink with mixture of cloves and ginger on the integrity of PBr 322 plasmid DNA in the presence of Fenton's reagent are presented in Fig. (1 – 4).

The plasmid DNA composed mainly the super coiled form (below band) as in lane 1 (pBR322 plasmid DNA with double distilled H_2O) and open circular form (above band) as in lane 2 (DNA and Fenton's reagent) with nicking caused by hydroxyl radicals (Kumar and Chattopadhyay, 2007).It was found that the addition of Fenton's reactionmixture (Fe^{3+}+H_2O_2+ ascorbic acid) to plasmid DNAresulted in increase of the open circular form.

Fig. 1 represents effect of *H. sabdariffa*drink (without spice)on protectionagainst Fenton reagent induced changes of pBR 322 DNA. Lane 1 (pBR322 DNA and ddH_2O) was mainly supercoiled plasmid pBR 322 while Lane 2 (pBR322 DNA and Fenton's reagent) was mainly open circular plasmid PBr 322. Lane 3- 6 shows the effect of the different concentrations (10 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml) of the drink on the plasmid DNA. Addition of *H. sabdariffa* drink inhibited the total formation of open circular form and this agrees with earlier findings of Tsai *et al*. (2004) and Jackman *et al.*(1987). The *H. sabdariffa* render protection either by neutralizing the H_2O_2 or by scavenging the OH generated from the Fenton's reaction (Ajith, 2010).

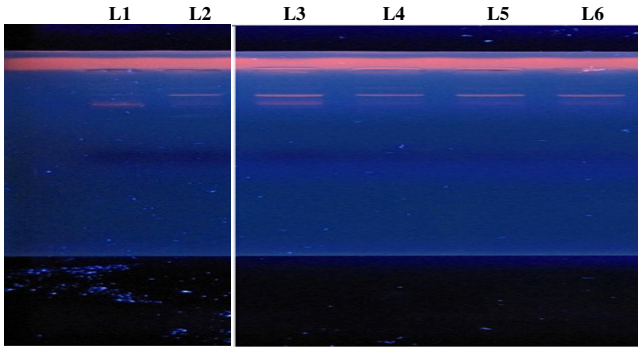
Figure 1: Effect of H. *sabdariffa* drink (Hs) against Fenton reagent (FR) induced changes of pBR 322 DNA



Lane 1: pBR322 DNA + H2O; Lane 2: pBR322 DNA + FR; Lane 3: pBR322 DNA + FR +10µg Hs; Lane 4: pBR322 DNA + FR + 50 µg Hs; Lane 5: pBR322 DNA + FR + 100 µg Hs; Lane 6: pBR322 DNA + FR + 200 µg Hs; Above bands: Open circular pBR322 DNA; Below bands: Sup *H. sabdariffa* drink *H. sabdariffa* drink *H. sabdariffa* drink ercoiled pBR322 DNA FR: Fenton Reagent; Hs : *Hibiscus sabdariffa*

Fig. 2 represents the effect of *H. sabdariffa*drink withclovesagainst Fenton reagent induced changes of pBR 322 DNA. Lane 1 (pBR322 DNA and ddH_2O) was mainly supercoiled plasmid pBR 322 while Lane 2 (pBR322 DNA and Fenton's reagent) wasmainly open circular plasmid PBr 322 as in Fig 1. With the addition of *H. sabdariffa*drinkwith cloves, there was reduction in the protective effect and the ability to prevent formation of open circular form decreased with increase in the concentration of the drink containing the spice (Lane 3 – 6). Though cloves had strong scavenging and reducing abilities, it however did not produce a synergy effect with *H. sabdariffa*in the protection of DNA against damage by the H_2O_2. This effect could have been as a result of possibilities of cloves also having prooxidant properties (Duke, 2002).

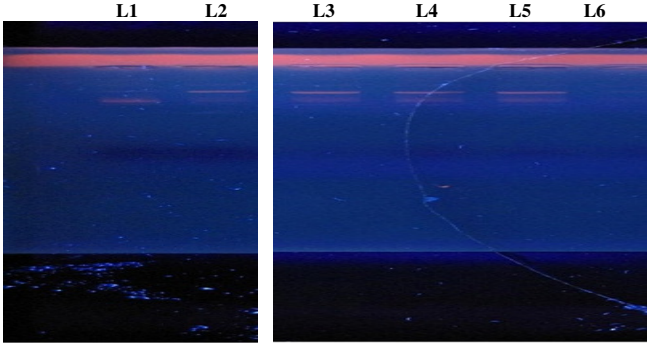
Figure 2: Effect of H. *sabdariffa* drink (Hs) with cloves(C) against Fenton reagent (FR) induced changes of pBR 322 DNA



Lane 1: pBR322 DNA + H2O; Lane 2: pBR322 DNA + FR; Lane 3: pBR322 DNA + FR + 10 µg Hs C; Lane 4: pBR322 DNA + FR + 50 µg Hs C; Lane 5: pBR322 DNA + FR + 100 µg Hs C; Lane 6: pBR322 DNA + FR + 200 µg Hs C; Above bands: Open circular pBR322 DNA; Below bands: Supercoiled pBR322 DNA.
FR: Fenton Reagent; Hs : *Hibiscus sabdariffa;* C: Cloves

The effect of *H. sabdariffa*drink with ginger against Fenton reagent induced changes of pBR 322 DNA is as presented in Fig. 3. *H. sabdariffa*drink with ginger offer better protection when compared to *H. sabdariffa*drink with cloves (Lane 3 – 6). There was a better retention of the supercoiled DNA though not as much as what was obtained with*H. sabdariffa*drinkalone. Thus, *H. sabdaiffa*drink with ginger could partially protect H_2O_2 -induced conversion of super coiled pBR 322 to open circular form (Ajith, 2010).

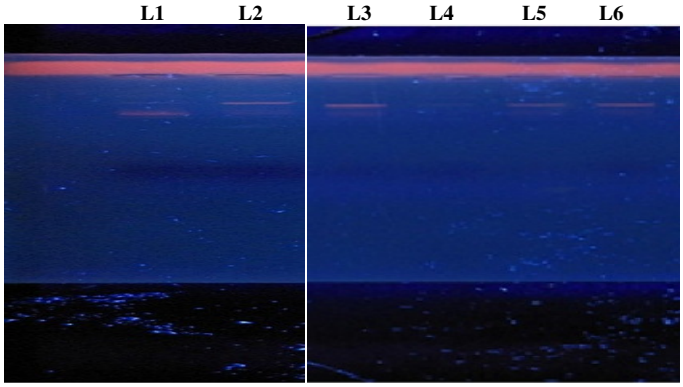
Figure 3: Effect of H. *sabdariffa* drink (Hs) with Ginger (G) against Fenton reagent (FR) induced changes of pBR 322 DNA



Lane 1: pBR322 DNA + H2O; Lane 2: pBR322 DNA + FR; Lane 3: pBR322 DNA + FR + 10 µg Hs G; Lane 4: pBR322 DNA + FR + 50 µg Hs G; Lane 5: pBR322 DNA + FR + 100 µg Hs G; Lane 6: pBR322 DNA + FR + 200 µg Hs G; Above bands: Open circular pBR322 DNA; Below bands: Supercoiled pBR322 DNA
FR: Fenton Reagent; Hs : *Hibiscus sabdariffa ;* G: Ginger;

In Fig. 4, Lane 3 – 6 shows the effect ofdifferent concentrations of *H. sabdariffa*drink with mixture of cloves and ginger on the plasmid DNA.Addition of *H. sabdariffa* drink with cloves and ginger, had the poorest protection of the plasmid DNA against Fenton induced oxidative damage. The action of the *H. sabdariffa*drink with mixture of*cloves* and ginger could be described as being antagonistic when it comes to protection of DNA against oxidative damage. This is in agreement with previous findings that under different conditions, combination of essential oils containing phenolic compounds has been found to either be synergistic or antagonistic in action (Burt, 2002; Randrianarivelo, 2009).

Figure 4: Effect of H. sabdariffa drink (Hs) with mixture of Cloves (C) and Ginger (G) against Fenton reagent (FR) induced changes of pBR 322 DNA



Lane 1: pBR322 DNA + H2O; Lane 2: pBR322 DNA + FR; Lane 3: pBR322 DNA + FR + 10 µg Hs CG; Lane 4: pBR322 DNA + FR + 50 µg Hs CG; Lane 5: pBR322 DNA + FR +100 µg Hs CG; Lane 6: pBR322 DNA + FR + 200 µg Hs CG; Above bands: Open circular pBR322 DNA; Below bands: Supercoiled pBR322 DNA
FR: Fenton Reagent; Hs : *Hibiscus sabdariffa ;* CG: Mixture of Cloves and Ginger

Similarly, Maizura,*et al.*(2011) reported that there was no synergistic effect observed for antioxidant activity in kesum(*Polygonum minus*), ginger (*Zingiberofficinale*) and turmeric (*Curcuma longa*) extract mixture.The antioxidant and prooxidant activity of phytophenolics depend on such factors as metal- reducing potential, chelating behaviour, pH and solubility characteristics (Decker, 1997). In addition the result of this finding is corroborated by the reports of Aruoma (1993) and Schildermam*et al.* (1995)that antioxidant may not protect target such as DNA and proteins against damage, and sometimes can even aggravate such damage in some systems.

Conclusion

Antioxidant properties o**f** *H. sabdariffa*drink are greatly influenced by ginger and cloves. However, they greatly reduced the ability of the drink to protect against induced oxidation damage by Fenton reagent.

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