Original Research Article

Vitamin, Bioactive compound Contents and Antioxidant Activity of *Ficus capensis* Fruit.

Abstract

Ficus capensis is a tropical, evergreen tree with more than 800 species. Virtually all the parts are locally used for the treatment of various illnesses. This study investigated the Vitamin, Bioactive compound contents and the Antioxidant activity of the fruits of *Ficus capensis*. The analyses were conducted using standard biochemical methods. Various concentrations of 20, 40, 60, 80 and 100 µg/ml of the sample were subjected to 2,2-diphenyl-1-pycrylhydrazyl (DPPH) radical scavenging, Reducing Power and inhibition of lipid peroxidation in-vitro antioxidant tests and compared with standard antioxidant (Butylated hydroxyl anisole (BHA). From the results of the vitamin analysis, the fruit contained Vitamins A (1.15 ± 0.18 mg/g), B_1 (0.14 ± 0.00 µg/g), B_2 (0.12 ± 0.01 µg/g), B_3 (0.26 ± 0.02 µg/g), C (8.12 ± 0.16 mg/100g), E (1.44 ± 0.45 mg/g) and Folic acid (81.23 ± 6.29 µg/g). The bioactive compound evaluated were Total Phenol (13.97 ± 1.40 mgGAE/100g), Flavonoids (11.03 ± 0.16 mgCE/100g), β-carotene (0.34 ± 0.001mg/100g) and Lycopene (0.14 ± 0.002). The antioxidant activity assays showed that the fruits of *Ficus capensis* exhibited scavenging activity on DPPH with EC_{50} of 30 µg/ml while that of the standard was 28µg/ml. It inhibited lipid peroxidation with EC_{50} of 80 µg/ml while that of the standard was 62 µg/ml and exhibited reducing power activity with $OD_{0.5}$ of 38 µg/ml while that of the standard was34 µg/ml. Results from this study revealed that fruits of *Ficus capensis* is a potential antioxidant agent due to its free radical scavenging and reducing power activities which were comparable with that of the standard antioxidant. The presence of antioxidant vitamins and bioactive compounds makes it a natural source of antioxidants.

Keywords: Ficus, capensis, fruits, vitamin, bioactive, antioxidant

Introduction

Greater percentage of the world's population still depend on herbal medicines for their health needs which includes management, cure and treatment of diseases. Herbal medicines usually play roles in first-line and basic health services, both to people living in remote areas where it is the only available health service and to people living in poor areas where it offers the only affordable remedy (Adesina, 2014). Even in areas where modern medicine is available, the interest in herbal medicines and their utilization have been increasing rapidly in recent years. Herbal medicines account for a significant percentage of the pharmaceutical market (Adesina, 2014). Plants which include fruits and vegetables contain many bioactive compounds. They serve as herbal medicines and used in traditional medicine.Bioactive compounds are present in small quantities in foods, mainly in fruits, vegetables and wholes grains and provide health benefits beyond the basic nutritional value (Gökmen, 2016). Bioactive compounds are

molecules that can present therapeutic potential with influence on energy intake, while reducing pro-inflammatory state, oxidative stress, and metabolic disorders (Siriwardhana et al., 2013). Studies have shown that high consumption of food rich in bioactive compounds such as flavonoids and carotenoid, has a positive effect on human health and could diminish the risk of numerous diseases such as cancer, heart diseases, stroke, Alzheimer's, diabetes, cataracts and age related functional decadence(Hassimotto et al.,2015).Plants are rich in natural antioxidants of which the best known arecarotenoids, tocopherols, vitamin C, flavonoids, and different other phenolic compounds (Iqbal andBhanger, 2006). In recent times, flavonoids, amongst other natural antioxidantshave received increasing attention. In comparison to vitamin C and E, dietary flavonoids are considered to be more powerful antioxidants (Sultana and Anwar, 2008).Flavonoids are known antioxidants which are highly effective in scavenging oxygen radicals. They exhibit anti-cancer, anti-ageing, hypolipidemic, and anti-inflammatory activities (Braca et al., 2002). More so, the protective effects of flavonoids in biological systems are attributed to their capacity to scavenge free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidases (Heim et al., 2002).

Antioxidants provide protection against degenerative diseases including cancer, coronary heart, and Alzheimer's diseases (Iqbal andBhanger, 2006). Reactive Oxygen Species (ROS), contribute to cellular aging, mutagenesis, carcinogenesis, and coronary heart disease, likely through destabilization of membranes, DNA and protein damage, and oxidation of low-density lipoprotein (LDL) (2). Mechanism of action of antioxidants includes the suppression of ROS formation, the inhibition of enzymes or chelating of elements involved in free-radical production. Furthermore, antioxidants scavengereactive species, and upregulate antioxidant defences (Montoro et al., 2005).

The plants of *Ficus* species are well known in the field of traditional medicine. *Ficus* species have been found to be rich source of phenolic acid and flavonoids making them capable of protecting against disorders of oxidative stress (Sirisha et al, 2010). *Ficus capensis* commonly known as bush fig is a fast growing deciduous or evergreen tree(Onyeleke et al., 2008). The fruits are often hairy, soft and edible but watery and tasteless, having many seeds. Traditionally,*Ficus capensis* has been used in gastrointestinal tract relaxation (Ayinde and Owolabi, 2009; Ayinde et al., 2013) as antioxidant (Sirisha et al., 2010), antimicrobial (Adebayo-Tayo and Odeniyi, 2012) immune booster(Daikwo et al., 2012).

Materials and methods

Collection and identification of plants material

Fresh fruits of *Ficus capensis* were collected from department of Applied Biology and Biotechnology garden of Enugu State University of Science and Technology and was authenticated in the Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu, Nigeria.

Sample Preparation

The fresh fruits of *Ficus capensis* were rinsed thoroughly with distilled water and were cut into halves. The fruits were ground with mortar and pestle.

Vitamin Analysis

Vitamins A and E were determined according to the method of Rutkoski *et al.* (2006), Vitamin C was determined according to the method of klein and Perry (1982), Vitamins B_1, B_2 and B_3 were determined using the method of Kirk and Sawyer (1991) while Vitamin B_9 was determined according to the method of Padmarajaiah*et al*. (2002).

Bioactive Compound Analysis.

Total phenol, flavonoids, β-carotene and Lycopene contents were determined by using the slightly modified colorimetry method describedby Barros et al. (2007).

Antioxidant Assay

The stable 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) was used for the determination of free radical scavenging activity of the sample. This was assayed using the method of Ebrahimzadem, et al., (2009). The reducing power and inhibition of lipid peroxidation were determined according to the method of Barros et al,(2007).

Statistical Analysis

Data was analyzed using Excel Statistical Package and presented as mean ± standard deviation of duplicate analysis

Results

Vitamin Composition

The result of the vitamin analysis is presented in Table 1. From the result, vitamin B_9(81.23 ± 6.29 µg/g) was the highest in composition while vitamin B_2(0.12 ± 0.01 µg/g) was the least.

Table 1: Vitamin Composition of fruit of *Ficus capensis*

Vitamins	Composition
A(mg/g)	1.15 ± 0.18
B_1 (µg/g)	0.14 ± 0.00
B_2(µg/g)	0.12 ± 0.01
B_3(µg/g)	0.26 ±0.02
C (mg/100g)	8.12 ± 0.16
E (mg/g)	1.44 ± 0.15
B_9 (µg/g)	81.23 ± 6.29

Values are mean ± standard deviation (n=2)

Bioactive Compound Content

Table 2 shows the result of the bioactive compound composition of fruit of *Ficus capensis.* Flavonoid content was higher (13.97 mgGAE/100) than other bioactive compounds evaluated.

Table 2: Bioactive Compound Composition of fruit of *Ficus capensis.*

Bioactive Compounds	Concentration
Total Phenol (mgGAE/100g)	13.97 ± 1.39
Flavonoids (mgCE/100g)	11.03 ± 0.16
Beta Carotene (mg/100g)	0.34 ± 0.001
Lycopene (mg/100g)	0.14 ± 0.002

Values are mean ± standard deviation (n=2)

Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of the fruit of *Ficus capensis* was dose-dependent. The highest activity was observed at the highest concentration tested (100 µg/ml) and gave 21.19% activity while the standard antioxidant (BHA) gave 89.96% activity as shown in Fig. 1. The effective concentration at 50% (EC_{50}) was 30 µg/ml and 28 µg/ml for the fruit of *Ficus capensis* and BHA respectively(Fig 2).

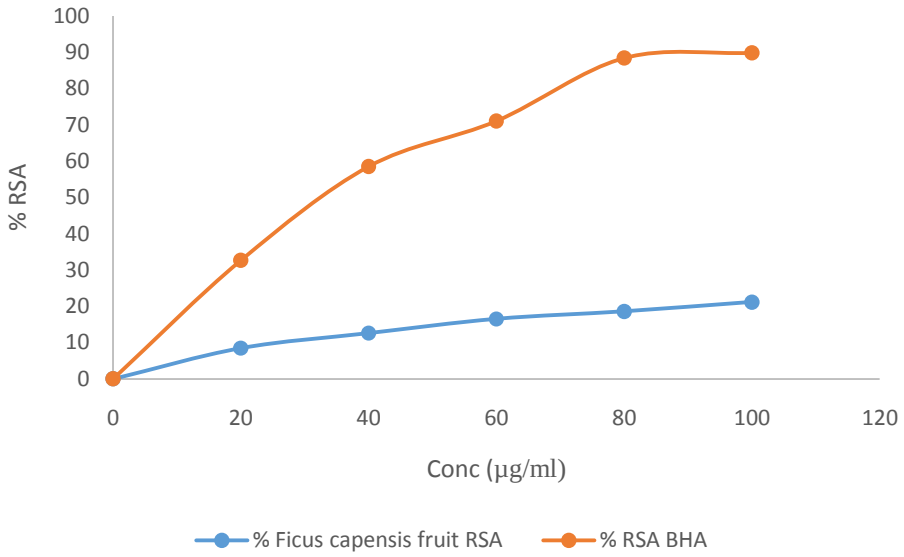

Fig. 1: Percentage DPPH Radical Scavenging Activity (RSA) of fruit of *Ficus capensis* and BHA

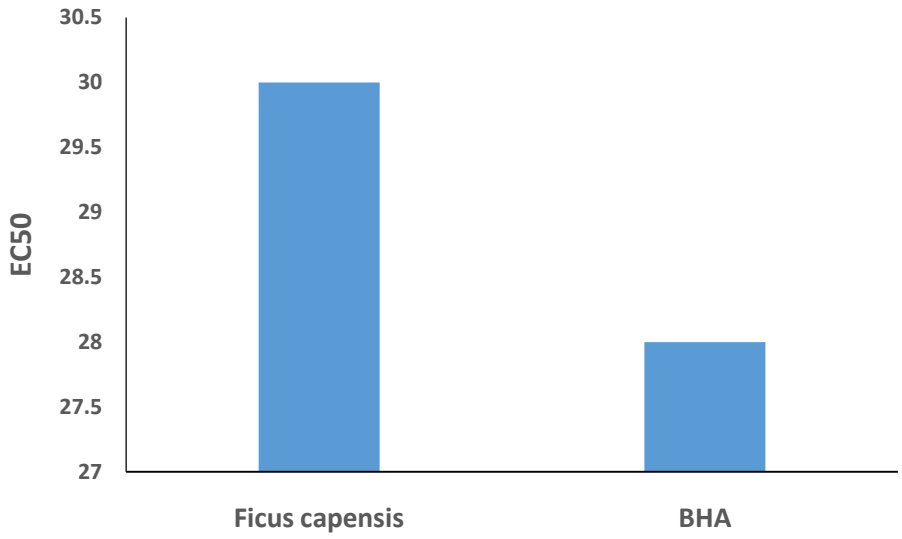

Fig. 2: The EC_{50} of fruit of *Ficus capensis* and BHA

Reducing Power Activity

The reducing power activity of fruit of *Ficus capensis* and BHA is shown in Fig. 3. As the concentration increased, the reducing power activity increased shown by the increase in the absorbance. At 100 µg/ml, the absorbance of fruit of *Ficus capensis* was 0.623 while that of BHA was 1.352. Fig. 4 shows the $OD_{0.5}$ of 38µg/ml and 34 µg/ml for fruit of *Ficus capensis* and BHA respectively.

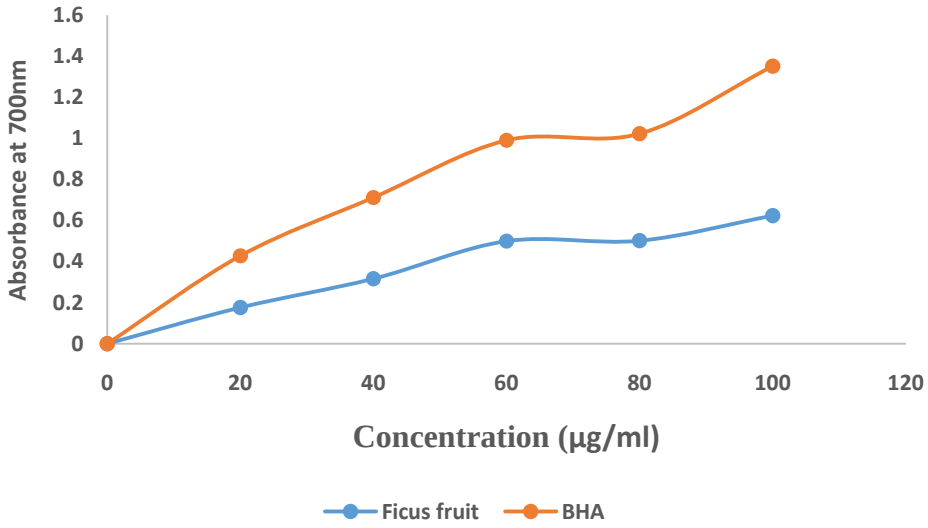

Fig. 3: Reducing Power Activity of fruit of *Ficus capensis* and BHA

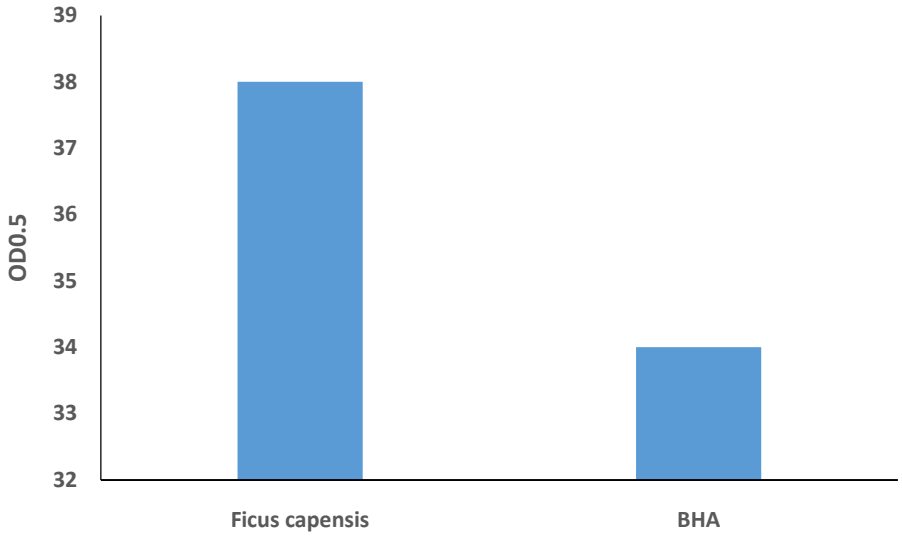

Fig. 4: $OD_{0.5}$ of *Ficus capensis* fruit and BHA

Inhibition of Lipid Peroxidation

The percentage inhibition of lipid peroxidation activity of fruit of *Ficus capensis* and BHA is presented in Fig.5. The activities were concentration-dependent with BHA exhibiting higher activity than *Ficus capensis* fruit. At 100 µg/ml, the activities were 29.294% and 72.703% for the fruit of *Ficus capensis* and BHA respectively. The EC_{50} for the fruit of *Ficus capensis* was 80 µg/ml while that of BHA was 62 µg/ml (Fig. 6).

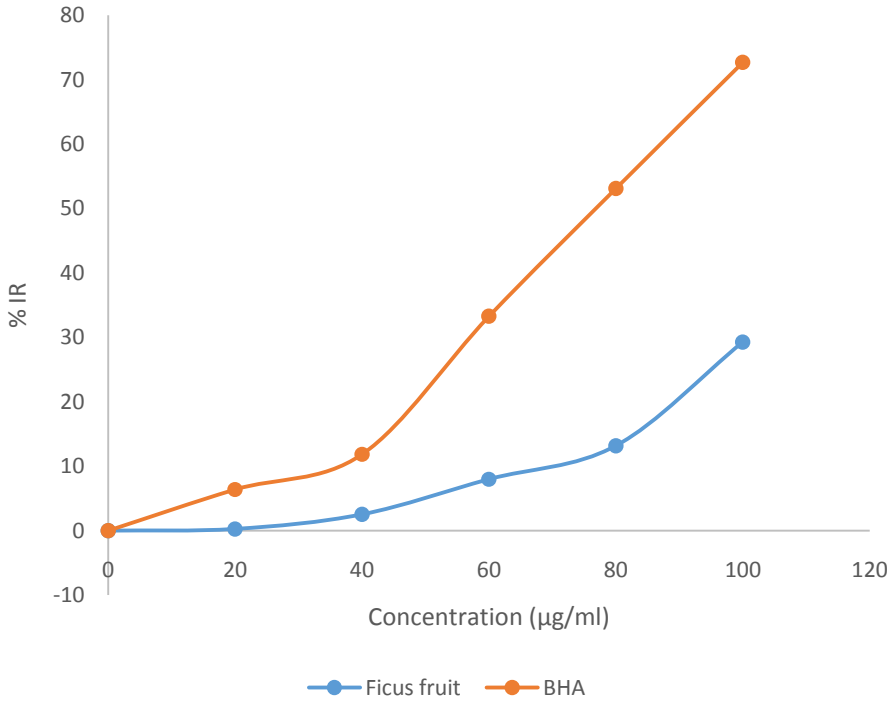

Fig. 5: Percentage Inhibitory ratio of fruit of *Ficus capensis* and BHA

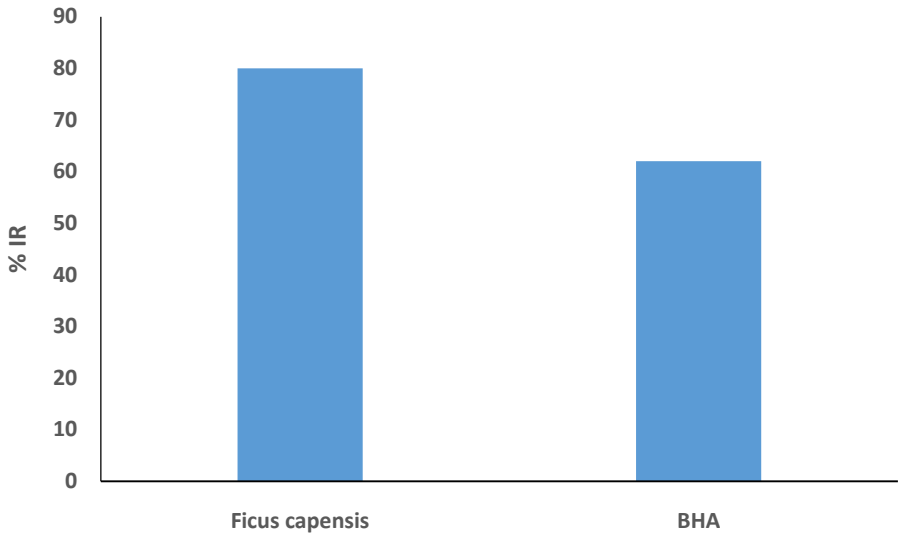

Fig 6**:** $EC_{50}$of fruit of *Ficus capensis* and BHA.

Discussion

Plants have proven to be effective in the treatment and management of diseases. Hence, there is increase in efforts at identifying and exploiting potentially beneficial plants. Other parts of *Ficus capensis* has been reported to possess several potential health benefits, little is known about health benefits of the fruits. In view of this, this work intends to unravel the vitamin and bioactive compound contents of the fruits as well as the *in-vitro* antioxidant mechanism.

Vitamin Composition

The result of the vitamin composition showed the presence of vitamins A, B_1, B_2, B_3, B_9, C and E (Table 1). Vitamin B_9 was the highest in concentration (81.23 µg/g). These vitamins are molecules required in small amount and play important roles in cellular metabolism where they act as coenzymes as well as in health and well-being. Their deficiencies have been linked to some diseases (Traber and Stevens, 2011). The B vitamins majorly function as coenzymes in metabolic pathways of carbohydrate, proteins and amino acids. Vitamin C and E are well-known antioxidants that play vital roles in combating oxidative stress in cells such as in cancer cells (Risvi et al., 2014; Montecinos et al., 2014). Vitamin Cis involved in many body functions including formation of collagen, absorption of iron, the proper functioning of the immune system, wound healing and maintenance of cartilage, bones and teeth (Haworth et al., 1993). Derivatives of vitamin A such as all-trans- retinoic acid are used in the management of acute promyelocytic leukaemia (Sanz et al., 2010).Some of these vitamins and their derivatives have been suggested as chemo-preventive agents (Freemantle et al., 2003). Therefore, the discovery of more sources of these vitamins and their derivatives like *Ficus capensis* fruit can be a way to manage chronic diseases.

Bioactive Compounds Composition

Bioactive compounds are active medicinal chemical constituents.The presence of bioactivephytochemical constituents present in different plants parts gives them their medicinal value andbiological activities (Thilakarathna and Rupasinghe, 2013). Important bioactive compounds such as phenol, flavonoids, beta carotene and lycopene were present in the fruit of *Ficus capensis* (Table 2). *Ficus capensis* fruits showed reasonable amount of phenol at the concentration of 13.97± 1.39mgGAE/100g which have been reported to possess some medicinal properties. It has a defense role against free radicals and exhibits several properties beneficial to humans and its antioxidant property are important in determining their role as protecting agents against free radical-mediated diseases process (Cai et al., 2006). The presence of phenol may indicate that fruit of *Ficus capensis* have antioxidant activity. Flavonoids was present in the concentration of 11.03± 0.16mg/g. Flavonoids possess many medicinal benefits such as anti-cancer, anti-oxidant, antiviral properties and anti-inflammatory as well ascardio- protective and neuroprotective effects (Zhao et al., 2019; Zhao et al., 2018; Camero et al., 2018; Patel et al., 2018; Mazidi et al., 2019).The concentration of lycopene (0.14 ± 0.002 mg/100g) was very low.Lycopene is a carotenoid that naturally gives foods their pink/red colour. It is said to be good for bone, heart and vascular health (Russo et al., 2020; Li and Xu, 2014) and, as a potent antioxidant especially effective at scavenging singlet oxygen (Sies and Stahl, 1995).Beta carotene was slightly present and was found in the concentration of (0.34 ± 0.001 mg/g). ß- Carotene is regarded as the major carotenoid present in human diet (Johnson, 2002) and the main source of vitamin A in humans. It plays a crucial role in prevention of degenerative diseases such as cardiovascular diseases, diabetes and several types of cancers (Singh and Goyal, 2008).

Antioxidant Activity

DPPH scavenging activity:

One of the in-vitro antioxidant parameters used for testing the potency of plants is their ability to scavenge free radicals. DPPH radical scavenging assay is the most common method used in the study of antioxidant activity of plant extracts.This mechanism involves the protonation of the 2,2-diphenyl-1-picryhydrazl (DPPH) radicals turning it to stable free radical which is visually noticeable as a decoloration from purple to golden yellow and detected by spectrophotometric technique. Decrease in absorbance shows the more efficient antioxidant activity of the extract in terms of hydrogen atom donating capacity. *Ficus capensis* fruit demonstrated radical scavenging activity in a dose-dependent manner. Antioxidant activity of *Ficus capensis* fruit as measured by ability to scavenge (DPPH) free radicals was compared with the standard antioxidant; butylated hydroxyl toluene (BHT). The standard however gave a higher activity.The presence of phenolic compounds in the fruit of *Ficus capensis* may contribute directly to the observed antioxidant effect as phenolic compounds have been reported to have antioxidant activities (Afari et al., 2014).

The effective concentration of the extract at half maximum DPPH scavenging activity (EC_{50}) was calculated from graph of percentage scavenging activity against concentration of the extract was 30 µg/ml and that of BHA was 28 µg/ml. The EC_{50} was used to evaluate the antioxidant effectiveness of the sample. The lower the EC_{50} value, the higher is the

scavenging potential. This shows that BHA was the stronger DPPH scavenger having the lower EC_{50} value.

Reducing Power Assay

In reducing power assay, the yellow colour of the testsolution changes to different shades of green depending on the reducingpower of the test specimen. The presence of the reductants in the solution causes the reduction of the $Fe^{3+/}$ferricyanide complex to the ferrous form. Therefore, Fe^{2+}can be monitored by absorbance measurement at 700 nm. Previous studieshave reported and suggested that the reducing propertieshave been shown to exert antioxidant action by donatingof a hydrogen atom to break the free radical chain (Gordon, 1990). In this study, the observed increasing absorbance at 700 nm as the concentration of the extract increased indicates an increasein reducing ability. The antioxidants present in the fruit of *Ficus capensis* caused the reduction of Fe^{3+}/ferricyanide complex to the ferrous form, and thus provedthe reducing power.

Inhibition of lipid peroxidation

Thethiobarbituric acid reactive substances (TBARS) method has been used widely in determination of the extent of lipid peroxidation via formation of malondialdehyde (MDA) compound from lipid peroxidation of polyunsaturated fatty acids. Using brain homogenate as a medium of peroxidation in this study, percentage lipid peroxidation inhibition by the extract of fruit of *Ficus capensis* increased with increase in concentration. Antioxidant potential of extractcorrelates directlyto the scavenging of hydroxyl radical and consequently the inhibition of lipid peroxidation (Orabi et al., 2001). Lipid hydroperoxides generation can cause damage to molecules of the bio-system and have the ability to bind with DNA leading to strand breaks, mutation and carcinogenesis (Sharififaret al., 2009). Intrinsically, erythrocytes are highly susceptible to peroxidation due to the heavy buildup of polyunsaturated fatty acids and haemoglobin.During respiration erythrocytes are frequently exposed to high tension of oxygen, which can induce oxidative damage (Babu et al., 2001).

In this study, fruit of *Ficus capensis* exhibitedscavenging of radicals indicating the presence of primary antioxidantswhich possess anti-lipid peroxidation potential. Phenolic compounds have been reported to delay oxidativedegradation of lipids as well as enhance the quality and nutritional value of food (Kähkönen et al., 1994).

Conclusion

Plant sources with antioxidant abilities is of great interest to industries and researchers because of their medicinal properties in the supportive and definitive care of debilitating diseases. This study investigated the vitamin content, bioactive compound composition and antioxidant properties of fruit of *Ficus capensis*.The study showed that vitamins and bioactive compounds were present in varying concentrations.The antioxidant activity exhibited in different *in-vitro* antioxidant assays carried out in this study may be linked to the antioxidant constituents such as phenolic, flavonoid, ascorbic acid, lycopene and beta-carotene found in the fruit of *Ficus capensis*. Theseresults suggest that fruit of *Ficus capensis* may be a potentsource of natural antioxidants.

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