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PHYTOCONSTITUENTS AND *IN VITRO* ANTIOXIDANT STUDIES IN SOME COMMON FRUITS AVAILABLE IN MAIDUGURI, NIGERIA

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ABSTRACT
The study was carried out to investigate the phytoconstituents, reducing power and total antioxidant capacity of some common fruits viz. *Citrulis vulgaris, Cucumis melo, Cucumis sativus, Malus domestica, Ananas comosus, Musa paradisica, Psidium guajava, solanum melongena* and *citrus senensis in vitro* by spectrophotometric method. The preliminary qualitative phytochemical analysis showed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins, and polyphenols in all the nine methanol fruit extracts. *Psidium guajava, Ananas comosus, Citrus senensis, Malus domestica, Solanum melongena* revealed the presence of Alkaloid whereas Alkaloid is not detected in the other fruits viz; *Citrulis vulgaris, Cucumis melo, Cucumis sativus, Musa paradisiacal.* Saponins is detected in *Cucumis sativus* and *Cucumis melo* only. The reducing power studies showed *Psidium guajava* to have the highest activity followed by *Malus domestica>Citrus senensis >Solanum melongena>Citrulis vulgaris>Ananas comosus>Cucumis melo>Cucumissativus>Musa paradisiaca.* The antioxidant capacity studies showed *Citrulis vulgaris* to have the highest antioxidant capacity followed by *Cucumis melo>Cucumissativus >Psidium guajava>Citrus senensis>Musa paradisiacal > Malus domestica >solanum melongena>Ananas comosus.* This study revealed the potentials of these common fruits as natural antioxidants sources.

KEYWORDS: fruits, antioxidant, phytoconstituents,

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INTRODUCTION

It is widely accepted that fruits and vegetables have many healthful benefits. Consumption of fruits is beneficial to health and contributes to decrease in mortality rate of cardiovascular and other diseases (Vinson *et al,* 2001). This potential is attributed to some natural antioxidant phytonutrients (Rice Evans *et al*, 1996,). The majority of the antioxidant capacity of a fruit may be from polyphenols, flavonoids tannins, along with vitamins A, B C, and E and Carotenoid (Frei and Higdon 2003)

Antioxidants are molecules with the potential to inhibit the oxidation of other molecules. Oxidation reactions produce free radicals which mediate chain reactions. The occurrence of these reactions leads to cell damage or death. Antioxidants terminate these chain reactions by removing free radicals intermediates and inhibiting other oxidation reactions. Antioxidants are oxidized at the end of the reaction (Valko *et al* 2007).

Oxidation reaction though crucial to life can also be damaging. Plant and animals maintain a complex systems of multiple type of antioxidants, such as glutathione, vitamin C, E, as well as enzymes such as catalase, superoxide dismutase, and various peroxidases (Valko *et al* 2004). Low level of antioxidant or inhibition of the antioxidant enzymes causes oxidative stress and may lead to cell damage or death.(BJelakovic *et al* 2007). Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart diseases and even altitude sickness.

Terrestrial plants began producing non marine antioxidants such as ascorbic acid, polyphenols, and tocopherols as part of their adaptation from marine life. The evolution of angiosperm plants about 50-200 million years ago

resulted in the development of many antioxidant pigments as chemical defenses against reactive oxygen species that are by product of photosynthesis(Benzie 2003, Valentao *et al* 2002)

The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with antioxidative activity is likely to be the one that is readily oxidized. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells (Wong et al, 2006)

In recent times, there is an increasing interest in the role of free-radical-mediated damage in the etiology of human diseases. In the status of normal metabolism, the levels of oxidants and antioxidants in humans are maintained in balance, which is important for sustaining optimal physiological conditions. (Temple 2000). Overproduction of oxidants in certain conditions can cause an imbalance, leading to oxidative damage to large biomolecules such as lipids, DNA, and proteins (Liu, 2002). Oxidative damage to body cells and molecules has been widely postulated to be involved in the causation and progression of a range of chronic diseases, such as cardiovascular diseases, neuronal diseases, cataracts and several forms of cancer (Haliwell 1997). Human metabolism counts on an antioxidant defensive system involving enzymes and proteins to prevent these effects. However the defenses can be overwhelmed in certain circumstances so that harmful effects occur. It is accepted that the intake of antioxidant substances reinforces defenses against free radicals. The use of synthetic antioxidants has been limited because of their toxicity. Therefore, it is of great significant and necessity that research focuses on discovering potential natural effective antioxidants to replace the synthetic ones.

In view of the huge importance of fruits as antioxidant sources, this study compare the antioxidant property of commonly consumed fruits viz. *Ananas comosus Citrus senensis Citrulis vulgaris, Cucumis melo, Cucumis sativus, Malus domestica, Musa paradisica, Psidium guajava,* and *Solanum melongena in vitro* in order to evaluate their extent antioxidant capacity utilizing their methanol extract, reducing power(using Fe^{2+}/Fe^{3+} complex) and total antioxidant capacity (using phosphomolybdenum) by spectrophotometric method of Prieto *et al* 1999 .

MATERIAL AND METHODS

Plant materials

Nine different commonly consumed fruits were selected. Sample of fresh ripe fruits were purchased from Maiduguri fruits and vegitables market, Maiduguri, Borno State in the last quarter of 2011. The fruit comprises of *Ananas comosus*(Pineapple), *Citrus senensis(*Sweet orange), *Citrulis vulgaris* (Water melon), *Cucumis melo (*Sweet melon), *Cucumis sativus (*Cucumber*), Malus domestica*(Apple), *Musa paradisica(*Banana), *Psidium guajava*(Guava) and *Solanum melongena* (garden egg). The fruits were authenticated by a taxonomist in the Department of Biological Sciences, University of Maiduguri.

Extraction

Each fresh fruit was washed under running tap water followed by washing with distilled water to remove the surface debris. Exactly 250g of peeled fruit pulps were weighed and minced using a mixer grinder for fine maceration. The ground fruit then homogenized and extracted in 250ml methanol solvent for 7 days in dark at room temperature with intermittent shaking. After 7 days , the whole extracts are filtered using muslin cloth at first and then through a filter paper. The filtrate is then concentrated and stored in a desiccators for 3 days then preserved in a deep freezer at -4ºC. (Trease and Evans 1978)

Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in methanol extracts of each of the nine fruits used (Trease and Evans 1978)

Evaluation of *in vitro* Antioxidant Activity

General chemicals and instruments

All chemicals and solvents used in the study were of analytical grade; trichloro acetic acid(TCA), ascorbic acid, monobasic and dibasic sodium phosphate, potassium ferri cyanide, ferric chloride, sulphuric acid, sodium phosphate, ammonium molybdate.

UV Spectrophotometer, Centrifuge, rotator evaporator, weighing balance and pH meter were the instruments used for the study.

Reducing power assay:

The reducing power of the extract was evaluated according to Oyaizu, 1986. Different amount of methanol extract were patched in methanol solvent and diverse with 2.5ml of 0.2M phosphate buffer(pH 6.6) and 2.5 ml of 10% $K_3Fe(CN)_6$. This mixture was incubated at 50 ^oC for 20 min, 2.5ml of 10% TCA was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution was assorted with methanol (2.5ml) and $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increase in absorbance of the reaction mixture indicates increased reducing power. The experiment was conducted in triplicates and the reducing power was expressed as equivalent of ascorbic acid (µg) per mg of extract.

Total antioxidant capacity (phosphomolybdenum method)

The total antioxidant capacity was measured by spectrophotometric method of Prieto *et al* 1999. At different concentration, methanol extracts were prepared in their respective solvents and combined in an Eppendorf tube with 1ml of reagent solution (0.6M H_2SO_4, 28mM sodium phosphate, 4mM ammonium molybdate mixture). The tubes were incubated for 90min at 95^oC. The mixture was cooled to room temperature and the absorbance was read at 695nm against blank. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid (µg) per mg of extract.

RESULTS

Qualitative phytochemical analysis

The preliminary qualitative phytochemical analysis (Table 1) showed the presence of carbohydrates, proteins, amino acids, steroids, glycosides, flavonoids, tannins, and polyphenols in all the nine fruits methanol extract. *Psidium guajava, Ananas comosus, Citrus senensis, Malus domestica,Solanum melongena* revealed the presence of Alkaloid whereas Alkaloid is not detected in the other fruits viz; *Citrulis vulgaris, Cucumis melo, Cucumis sativus, Musa paradisiaca*. Saponins are detected in *Cucumis sativus* and *Cucumis melo* only.

Reducing power assay

The reducing power studies (Table 2) showed Psidium guajava to have the highest activity followed by Malus domestica > Citrus senensis > Solanum melongena > Citrulis vulgaris > Ananas comosus > Cucumis melo > Cucumis sativus > Musa paradisiaca

Total antioxidant capacity

*Total ant*ioxidant capacity studies showed *Citrulis vulgaris* to have the highest antioxidant capacity followed by *Cucumis melo>Cucumissativus >Psidium guajava>Citrus senensis>Musa paradisiacal > Malus domestica >Solanum melongena>Ananas comosus.* This study revealed the potentials of these common fruits as natural antioxidants sources.

Table 1: Result of qualitative phytochemical analysis of nine methanol fruit extracts

TEST	Fruit extract								
	Ananas comosus	*Citrulis vulgaris*	*Citrus senensis*	*Cucumis melo*	*Cucumis sativus*	*Malus domestica*	*Musa paradisica*	*Psidium guajava*	*Solanum melongena*
Carbohydrate	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	+	+	+
Amino acids	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	+	+	-	-	-	-
Alkaloids	+	-	+	-	-	+	-	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Polyphenols	+	+	+	+	+	+	+	+	+

Key: + present, - not detected

Table 2: Reducing power assay of nine methanol fruit extracts (equivalent of ascorbic acid)

	Psidium guajava	*Malus domestica*	*Citrus senensis*	*Solanum melongena*	*Citrulis vulgaris*	*Ananas comosus*	*Cucumis melo*	*Cucumissativus*	*Musa paradisiaca.*
X	*123.6*	*112.8*	98.4	*98.2*	*70.3*	*56.2*	*50.3*	*48.5*	*32.8*

Key :X = µg of ascorbic acid per g of extract

Table 3: Total antioxidant capacity of nine methanol fruit extracts (equivalent of ascorbic acid)

	Citrulis vulgaris	*Cucumis melo*	*Cucumissativus*	*Psidium guajava*	*Citrus senensis*	*Musa paradisiaca*	*Malus domestica*	*Solanum melongena*	*Ananas comosus*
X	*142.2*	*128.8*	*120.4*	*112.8*	*89.5*	*85.5*	*76.4*	*76.5*	*50.4*

Key: X = µg of ascorbic acid per g of extract

DISCUSSION

Qualitative phytochemical screening

The result of the phytochemical analysis of the fruits extract revealed the presence of several bioactive compounds *viz* polyphenols, tannins, steroids, flavonoids and alkaloids. These therefore encourage antioxidant studies.

Reducing power assay

The reducing capacity of the extracts Fe^{3+} inferric cyanide complex to ferrous, Fe^{2+} form may serve as a significant indicator of its antioxidant capacity (Yildirim and Mavi, 2000). The existence of reductones are the key of the reducing power, which exhibit their antioxidant activity through the action of breaking the free radical chain by donating a hydrogen atom. The reduction of ferric complex to ferrous form occurs due to the presence of reductants in the solution. Absorbance of Fe^{3+} canbe observed by measuring the OD values at 700nm the reduction power of the extract increase with increase in concentration (Zou and Lu, 2004). In the presence study the methanolic fruit extracts were evaluated for the reducing power among the nine fruits extracts *Psidium guajava* had the highest activity and *Musa paradisiaca* had the least activity.

Total antioxidant capacity

Total antioxidant capacity by phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate, Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid (Prieto *et al*, 1999). In the present study the methanolic fruit extracts were evaluated for the total antioxidant capacity. Among the nine fruits total antioxidant capacity was found to be highest in *Citrulis vulgaris* and least in *Ananas comosus.*

Natural antioxidants, particularly in fruits, can be phenolic compounds (tannins, flavonoids, phenolic acids and tocopherols), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids, and amines), or carotenoids as well as ascorbic acid(Hall and Cuppett 1997).. Tannins are known to inhibit lipid peroxidation and lypoxigynases *invitro,* and information has been accumulated over the past few years demonstrating their ability to scavenge free radicals which are known to be important in cellular prooxidant states (Gyamfi and Aniya, 2002).. Several researchers have investigated the antioxidative activity of flavonoids compounds and have attempted to define the structural characteristics of flavonoids that contribute to their activity (Foote *et al,* 1996)..Phenolic acids, such as caffeic, chlorogenic, ferulic, sinapic, and p-coumaric acids, appear to be more active antioxidant than the hydroxyl derivative of the benzoic acid such as p-hydroxybenzoic, vanillic and syringic acids..α-tocopherol is one of the most active *in vitro* chain-breaking antioxidants (Burton and Ingold, 1981). Vitamin C is a hydrophilic antioxidant, and is considered to be a poor antioxidant within the lipophillic plasma membrane. Vitamin C plays a valuable role in the regeneration of vitamin E and thereby acts to reduce the rate of oxidative consumption of vitamin E (Sie and Stahl, 1997). Carotenoids also have a protective function against oxidative damage, and singlet oxygen is very powerfully quenched by β-carotene (Foote *et al*, 1996)

CONCLUSION

Considering the assays, it can be concluded that *Psidium guajava,Citrulis vulgaris,Cucumis melo*, are the best fruits followed by *Cucumissativus,Citrus senensis, Solanum melongena* in terms of the antioxidant potential of

the fruit tested. The present research programme establishes the antioxidant ability of the fruit extracts, even though extent potential varies from case to case. The result and inferences from different methods in other fruits under study differ substantially because each complex chemical reaction generates unique values. Hence, authors are of the opinion that an appropriate index needs to be developed which does not represent a specific antioxidant property but can rank the antioxidant capacity of most fruits.

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