



EVALUATION OF ANTIOXIDATIVE AND ANTIMICROBIAL PROPERTIES OF NATURALLY FERMENTED *ANANAS COMOSUS* SOLD IN IGBOORA, IBARAPA CENTRAL LOCAL GOVERNMENT AREA OF OYO STATE

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Abstract

Fermented fruit extracts of pineapple (Ananas comosus) has been employed in folkloric medicine to manage diseases such as the typhoid fever in tropical Africa. The present study was carried out to evaluate the phenolic content, ability to scavenge DPPH free radicals and examination of antimicrobial activities of fermented extract of ripe flesh (RF), ripe skin (RS), unripe flesh (URF) and unripe skin (URS). The epicarp (skin) and the flesh of the ripe and unripe fruits were separated. 200g of both the skin and fruit were homogenized in 400ml of water and allowed to ferment at room temperature. The phenolic content were determine spectrophotometrically and the result of percentage phenolic content showed that Day 7 had the highest value of ripe flesh (RF) ($6.82 \pm 0.02\%$), ripe skin (RS) ($5.48 \pm 0.1\%$), unripe flesh (URF) ($3.95 \pm 0.01\%$) and unripe skin (URS) ($5.07 \pm 0.02\%$) while the lowest percentage value recorded at Day 1 for the extracts. However, the diphenylpicrylhydrazyl (DPPH) had the highest percentage value at Day 1 for RF ($62.00 \pm 0.2\%$), RS ($91.00 \pm 0.003\%$) and URF ($8.00 \pm 0.3\%$) while URS had the highest value ($12.00 \pm 0.2\%$) at Day 7. Also the antimicrobial activities of the fermented extracts of Ananas comosus on the 7th day of fermentation showed that the E.coli has effect on fermented extract of RF with 5mm diameter zone of inhibition, Streptococcus faecalis has effect on RF (2.0mm), RS (3.0mm) and URS (3.0mm), Salmonella spp. Has effect on RF (2.0mm), RS (3.0mm) and Klebsiella pneumonia has effect on RF (6.0mm) and RS (4.0mm) respectively. The results showed that fermentation enhanced the antioxidative and antimicrobial properties of Ananas comosus. Hence the present study serves as a report to provide a possible scientific basis for the use of extract of Ananas comosus in the management of gastrointestinal diseases in traditional medicine.

Keywords: Phenolic contents; Free radical; Extract; E.coli; *Ananas comosus*; Inhibition; Antioxidant

Introduction

In the earliest part of the century, plants were vital sources of raw material for medicines. Later, techniques were developed to produce synthetic replacement for many of the medicines that had been derived from the forest. But recently, problems with drug resistant microorganisms, side effects of modern drugs, and emerging diseases where no medicines are available, have encouraged an interest in plants once again as significant sources of new medicines. Modern-day researchers are coming to appreciate fully the vast medicinal knowledge of the indigenous people (Valko *et al.*, 2007). Drug experts from developed countries are now researching on plants, some of which are known to have been used for medicinal purposes and others which offer potential. There is emerging interest in the use of naturally occurring antioxidants as an ingredients in dietary

supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease (Evelson *et al.*, 2001; Ventuani *et al.*, 2004). Although, oxidation reactions are crucial for life, they can also be damaging, hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases (Cook *et al.*, 2007). 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 (Wolf, 2005). Moreover, restrictions over the use of synthetic antioxidants such as terbutylhydroxyanisole (BHA) or tert – butylhydroxytoluene (BHT) in food industries further strengthen the concept of using naturally

occurring compounds as antioxidants (Schneider, 2005).

Phenolic compounds or polyphenol constitute one of the most numerous and ubiquitously distributed group of plant secondary metabolites, with more than 8,000 phenolic structures currently known. Natural polyphenol can range from simple molecules (phenolic acids, phenylpropanoids and flavonoids) to a highly polymerized compound (melanin tannins) with flavonoids representing the most common and are widely distributed in the plant kingdom and are therefore an integral part of the diet, with significant amounts being reported in vegetables, fruits and beverages (Coppens *et al.*, 2003). Diets high in fruits and vegetables, which are high in antioxidants, promote health and reduce the effects of aging process (Thomas, 2004). Although the dietary intake of phenolics varies considerably among geographic regions, it is estimated that daily intake range from about 20mg to 1g, which is higher than that of vitamin E (Schnieder, 2005). Polyphenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory, anti-allergic, hepatoprotective, antiviral, anti-carcinogenic and vasodilatory actions (Evelson *et al.*, 2001); many of these biological functions have been attributed to their free radical scavenging and antioxidant activity many medicinal role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Coppens, 1993). Many antioxidant compounds which are responsible for such antioxidant activity could be isolated and then used as antioxidants for the prevention and treatment of free radical – related disorders (Valko *et al.*, 2007). Brome lain is a crude extract from pineapple that contains among other components, various closely related proteinases, demonstrating its safety and lack of undesired side effect which earned it growing acceptance and compliance among patients as a phytotherapeutical drug (Maurer, 2001).

Ananas comosus is a fruit that is consumed in many part of the world and it is known for its medicinal value. To provide more evidence for the medicinal effect of the fruit and its health benefits, therefore, research to identify anti-oxidative compounds is an important issue that needs to be addressed. Polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and antimicrobial studies, are the

most crucial, though, a variety of plants are known to be source of phenolic compounds. But their antioxidative effects have not been carried out and evaluated. The fact still remain that, we should be a good vegetarian and let natural food be our drug which necessitated for the study.

This research work was aimed at evaluating antioxidant as well as antimicrobial properties of pineapple (*Ananas comosus*) obtained from Igboora, Ibarapa Central Local Government Area, Oyo State. It also attempted to determine free radical scavenging ability of fermented extract of ripe fruit, ripe fruit, unripe fruit and unripe skin of *Ananas comosus*

Materials and methods

Plant materials

Fruit of *Ananas comosus* (Ripe and Unripe) were purchased from local market in Igboora Ibarapa Central Local Government Area of Oyo State. The species of the fruit was authenticated by Botanist at University of Ibadan (Professor Ayodele).

Preparation of plants

The epicarp (skin) and flesh of ripe and unripe fruits of *Ananas comosus* were separated. The seeds were discarded. About 200g of both the skin and fruits were quickly homogenized on 400ml of water 1:2 (w/v). The homogenates were divided into four (4) parts and allowed to ferment at room temperature.

Biochemical analysis

Total phenolic determination:

The total phenolic content was determined by mixing 0.2ml of the extract of the Spices with 2.5) 10% Folin–Cioaltea's reagent (v/v) and 2.0ml of 7.5% sodium carbonate was subsequently added. The reaction mixture was incubated at 45°C for 40 mins, and the absorbance was measured at 760nm in the spectrophotometry. Gallic acid was used as standard phenol and was also treated like sample.

Free radical scavenging ability:

The free radical scavenging ability of the fermented extract against DPPH was also determined according to Melov *et al.* (2005). 0.5ml of sample extracts was added to 3ml of absolute ethanol and 0.3ml of DPPH (diphenyl-picryl-hyrazyl) radical solution, (0.5 mm in ethanol). The change in colour (from deep violet to light yellow) was measured spectrophotometrically at 517nm after 100 minutes of reaction in the dark.

Blank: the blank solution was prepared by mixing 3.3ml ethanol with 0.5ml of sample.

Control: The control was prepared by mixing 3.5ml ethanol with 0.3ml DPPH radical solution. The scavenging activity percentage (AA %) was determined according to Melov *et al.*, (2005).

$$AA \% = 100 - \frac{(\text{Absorbance sample} - \text{Absorbance blank} \times 100)}{\text{Absorbance control}}$$

Antimicrobial studies

The antimicrobial activities of the fermented extracts on the test organism were done using test organism using the agar diffusion method. About 1ml of 18 hr broth culture of each of the test organisms was introduced into separate sterile petri dishes and labeled accordingly. About 20ml of sterile molten nutrient agar was poured into each petri dish containing the test organism and 20ml of sterile molten Saboraud dextrose agar was poured into a petri dish and was mixed together gently by swirling the petri dish in one direction to ensure holes were bored into the plates using sterile cork borer of 7mm aseptically. The well bored on each plate was filled with extract. Petri dishes inoculated were incubated upright at 37°C for 18 – 24hrs. The relative susceptibility of the organisms to the extracts was indicated by clear zone of inhibition round the well. This was then examined, measured and recorded in millimeter (Mm) using ruler.

Result and discussion

Result

The results obtained from this study are presented in Table 1, 2 and 3. From the result in table 1, it can be observed that all fruits extracts were rich in phenolic content. The range of phenolic content is between 3.41 and 6.82. the highest total phenolic level has been observed in RF (Ripe flesh) and RS (Ripe skin) while the lowest total phenolic level was observed in URS (Unripe skin) and URF (Unripe flesh) as shown in table 1. Also, it was observed that phenolic content increased on fermentation days, from day 1 to day 7 except URS that dropped on day 3.

In table 2, the results showed that at day 1, the fruit extracts exhibited a very potent scavenging ability. The range of free radical scavenging was from 5.00 – 91.00 with the RS being the highest while the URF showed the lowest. This shows the remarkable reduction on the free radical by DPPH except URS. While Table 3, shows the antimicrobial activities. There was no zone of inhibition on day 1 and day 4, while 7 showed significant zone of inhibition here RF showed a clear zone of inhibition on culture plate grown with *Escherichia coli*, *Streptococcus faecalis*, *Salmonella spp*, *Pseudomonas aerogenosa* and *Klebsiella pneumonia*. RF shows clear zone of

inhibition on culture grown with *E.coli*, *streptococcus faecalis*, *Salmonella Spp*, *Pseudomonas aerogenosa* and *Klebsiella Pneumonia* while URF has no zone of inhibition at all on the organisms.

Discussion

Surveys from epidemiological studies have shown that those that are addicted to consumption of fruits, vegetables and medicinal plants (i.e. vegetarian) are associated with reduced risk of chronic diseases (Melov *et al.*, 1998). The consumption is being promoted in view of the various medicinal attribute associated with it. Polyphenols are capable of removing free radicals, chelate metal catalyst, activate antioxidant enzymes and reduce alpha tocopherol, radical and inhibit oxidases (Radimer *et al.*, 2004). The antioxidant capacities of the fruit extract was assessed by determining the antioxidant property of the fruit extracts.

The results of the study showed that all the fruit extract had significant amount of polyphenols, they are used as a protective and therapeutic effect essential for preventing content diseases and maintaining general wellbeing of man. Phenolic content inhibits auto oxidation of unsaturated lipids thus, preventing the formation of oxidized low density lipoprotein (LDL) which is considered to include cardiovascular disease (Radimer *et al.*, 2004).

The result reveals that the phenolic content of the fruit increase on fermentation from day 1, 3, 5 and 7 but URS dropped at day 3 and started increased from day 5 to day 7. RF has the highest phenolic content 6.82% at day 7. This might also indicate that the type phenol in RF may be different from others.

The free radical scavenging activities of the fruits extract indicates that the four extracts decreased in free radical scavenging in day 7 than in day 1. The highest percentage free radical scavenging was observed for RS in day 1 (99.00%) while URF has the least free radical scavenging activity (5.00%). This high free radical scavenging activity would account for the higher ability to reduce F^{3+} to Fe^{2+} as well as ability to scavenge DPPH free radical.

Antimicrobial studies revealed that at day 1 and day 4 the extract shows no inhibition zone while at day 7 there was significant zone of inhibition zone. The URF shows no zone of inhibition at all on day 7.

Conclusion and Recommendation

It can be concluded that the fermented *Ananas comosus* extract can be used to manage gastrointestinal diseases due to higher antioxidant property.

The fruit contain significant source of polyphenols and should be encouraged in everyday use because of its ability to prevent disease. People should be vegetarian and let natural fruit be their food and drug rather than junks food and synthetic drugs.

Recommendation

The Antioxidant analysis was based on fermentation of *Ananas comosus* extracted with water (aqueous extraction).

Results show that all the fruit extracts have antioxidant properties that increased with fermentation and contain one form of phenol or other. Therefore, people should embark on planting of fruits and appropriate standardization methods should be developed in order to solve the problem of dosage in the administration of plant fruits product in the prevention and cure of disease.

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Table 1: Percentage Phenolic Content of *Ananas comosus*

	DAY 1	DAY 3	DAY 5	DAY 7
RF	5.80 ± 0.01	6.08 ± 0.1	6.63 ± 0.1	6.82 ± 0.02
RS	4.58 ± 0.2	4.71 ± 0.1	5.13 ± 0.01	5.48 ± 0.1
URF	3.41 ± 0.02	3.71 ± 0.03	3.89 ± 0.002	3.95 ± 0.01
URS	4.27 ± 0.1	4.25 ± 0.1	4.97 ± 0.81	5.07 ± 0.02

Values were expressed as percentage standard deviation as total phenol of fruit.

**Table 2: Percentage DPPH decolorized**

Samples	Day 1	Day 7
RF	62.00 ± 0.2	54.00 ± 0.1
RS	91.00 ± 0.03	82.00 ± 0.04
URF	8.00 ± 0.3	5.00 ± 0.0
URS	6.00 ± 0.1	12.00 ± 0.2

Values were expressed as difference percentage standard deviation in relation to control.

RF: Ripe flesh; FS: Ripe skin; URF: Unripe flesh; URS: Unripe skin;

Note: - Dilution factor = 5 fold

Table 3: Antimicrobial effect of *Ananas comosus* on the 7th day of fermentation

MICROORGANISMS	RF	RS	URF	URS
<i>Escherichia coli</i>	5.0	-	-	-
<i>Streptococcus faecalis</i>	2.0	3.0	-	3.0
<i>Pseudomonas Aeruginosa</i>	-	-	-	-
<i>Salmonella Spp</i>	2.0	3.0	-	-
<i>Klebsiella Pneumonia</i>	6.0	4.0	-	-

Values represent diameter of zones of inhibition and were expressed in mm.