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Total Phenolic Content and In Vitro Comparative Antibacterial Activities of Aqueous Of Unripe Pawpaw Peel Against Gram Positive and Gram Negative Bacteria

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Abstract: *The total phenolic contents and in vitro comparative anti bacterial activities of aqueous of unripe pawpaw peel against gram positive and gram negative bacteria were investigated using standard methods of analysis. Unripe papaya peels has a long history and proof of being an effective medicinal fruit used to treat some human ailments. The study examined the aqueous extracts of both oven air dried and sun dried unripe papaya peels for the possible anti oxidants and anti microbial properties. The result revealed that extract of oven air dried unripe pawpaw peel was richer in total phenol (23.949mgGAE/g) than extracts of sun dried unripe papaya peel with total phenolic contents of 21.095 mgGAE/g. Also, the oven air dried papaya peel extract shows maximum zone of inhibition of 12mm against Escherichia coli in the anti bacterial assay. However, sun dried unripe peel extract showed no zone of inhibition against Bacillus spp. Therefore, unripe papaya peels can be therapeutically used in the management and treatment of some infectious diseases as well as oxidative stress induce human ailment because of their anti microbial potency due to their high total phenolic contents.*

Keywords: *Unripe pawpaw, phenolic content, anti bacterial activities, Gram positive organisms, Gram negative organism*

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I. Introduction

The papaya or pawpaw is the fruits of the tree Carica papaya. It has structure similar to that of melon and could be green or yellow colour on the outside with a golden – yellow or orange – coloured pulp (1). Pawpaw is consumed locally often as a breakfast fruit or in mixed fruit salad with other fruits, acting as a mild laxative. Papaya fruits belongs to the family of Caricaceae and reports has shown the use of several species of caricaceae as remedy against a variety of diseases (2). Ripe papaya is most commonly consumed as fresh fruit whereas green papaya as vegetable usually after cooking or boiling (3).

Clinical and epidemiological researches have revealed recently that vegetables and fruits contains bio active compounds with anti microbial capacity (4). They have been shown to help prevent cardiovascular diseases, atherosclerosis and decrease the risk of some types of cancers among many other health benefits (5). Papaya peels (not only fruits are also a good source of polyphenols and they contain anti microbial properties (6). According to (7), papaya is known for its hydrogen peroxide and hydroxyl radical scavenging activity along with guava, water melon, grape fruit and kiwi fruis.

Several researches have been carried out on fruits and reported in literature. (8) reported on the anti microbial activity and phyto chemical analysis of citrus fruit peels – utilization of fruit waste; (6) investigated the in vitro anti bacterial activities and composition of Carica papaya while (9) reported on the phyto chemical and anti oxidant analysis of aqueous extracts of unripe pawpaw (Carica papaya) Linn). Also (10) investigated the anti bacterial activities of different solvent extracts of Carica papaya fruits parts on some gram positive and gram negative organisms. Therefore, to further elucidate the inclusion of unripe pawpaw peels into folks medicine, this present study is aimed at investigating the total phenolic contents of differently prepared aqueous extracts and also comparing their anti bacterial activities with those of standard drugs against gram positive and gram negative bacteria.

II. Materials And Methods

Unripe matured pawpaw fruits were obtained from the botanical garden of the Department of Science Laboratory Technology of the Federal Polytechnic Ilaro, Ogun State, Nigeria and transported in clean dry

polyethylene sacs to the laboratories of the Department of Food Technology and Science Laboratory Technology for subsequent processing and analyses.

The unripe matured pawpaw fruits were washed with clean potable water to remove sands, dirts, insects fragments and other adhering materials. The peels were removed using stainless table knife. The peel were diced with the aid of stainless dicers into small pieces and then washed again with distilled water. A portion of the peels was oven dried air dried at 50^{o}C for 24 hours while another portion was sun dried for 120 hours (5 days). A portable electric blender was used to blend the dried peels into fine powder.

Preparation of Extracts

About 25g of the oven air dried unripe matured pawpaw peels was put into a 250ml beaker containing 100ml distilled water. The peels were boiled in a water bath at 60oC for 30 minutes. The aqueous extracts were separated by filteration with whatman no 1 filter paper (with pore size of 0.45mm) and then centrifuged at 100rpm for 10 minutes. The same procedure was repeated for the powdered peels (11).

Analytical Procedures

Analysis of Total Phenol Content

The total phenolic contents of both sun dried and oven air dried extracts were determined according to the method described by (12). Appropriate dilution of extract was mixed with 2.0ml of 10% folic concentration reagent (v/v) and neutralized by 20ml of 75% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45% and absorbance measured at 755nm. The total phenol content was calculated using gallic acid as standard and the result expressed as milligram of gallic acid per gram dry weight of extracts.

Assessment of Anti bacterial Extracts

Anti bacterial activities of oven air dried and sun dried peel extracts synthesized were tested against Escherichia coli (Gram negative bacteria) and Bacillus spp (Gram positive bacteria). The bacteria strains employed in this work were obtained from the Molecular Biology Laboratory of Covenant University, Ota, Ogun State, Nigeria after proper identification by a certified Molecular Microbilogist.

Preparation of Samples for Anti microbial Assays.

Methods described by (13) was used for the preparation of samples for anti microbial assay. All the samples were kept in the oven at 60^{o}C until they were completely dried. The amounts of dried metabolites obtained from the samples were dissolved in a known volume of distilled water making extracts of different concentrations.

Anti bacteria Assay

The anti bacteria potency of the samples was checked using Mueller Hinton Agar well diffusion method. After solidification of nutrient agar plates, 20ml of standardized innoculum of the test organism was seeded on respective plates and wells of 5mm diameter were bored using a cork borer. About 50 ml of extract were loaded into the well. Plates were incubated at 37oC for 24 hours and were inspected for zone of inhibition. The zone of inhibition was compared with that formed by standard antibiotics: Gentamycin and Ampicillin (Gram negative antibacterial drugs) against E. coli, Penicillin and Augmentin (Gram positive anti bacteria drugs) against Bacillus.

III. Results And Discussion

Table 1: Concentrations of Extracts

S/N	Sample	Solvent	Weight of empty bottle (g)	Weight of empty bottle + extract (g)	1Xpbs (ML)	Extract (g)	Concentration (mg/ml
1	Sun dried	Water	120	146	5	26	5200
2	Oven dried	Water	120	223.5	5	23.5	4700

Table 11: Table showing Phenolic contents of both oven air dried and Sun dried extracts (mgGAE/g)

Extracts	Phenolic Contents (mg/GAE/g)
Oven air dried	23.949±0.568
Sun dried	21.095±1.717

Values are means of triplicates/(Expressed) as mean ±SD,A=3

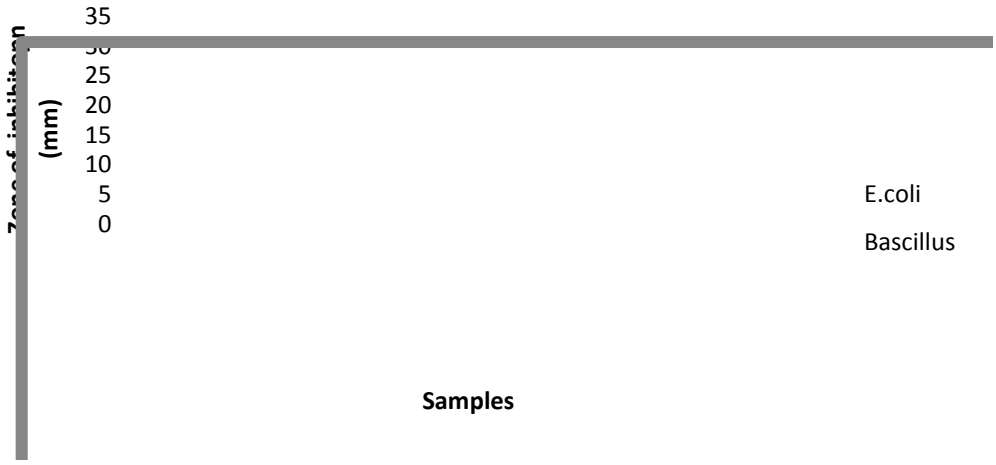

Chart 1: Showing the zone of inhibition of aqueous extract of unripe pawpaw peels and some standard antibiotic drugs.

IV. Discussion

Tables 1 and 2 showed the concentrations of extracts (9mg/ml) and the phenolic contents of the two extracts in mgGAE/g while Chart 1 revealed the zone of inhibition of aqueous extracts of unripe pawpaw peels with standard antibiotic drugs. From the results obtained, the aqueous extracts of unripe pawpaw peels contained high phenolic content that can provide good sources of dietary anti oxidants. The aqueous extracts of the oven air dried pawpaw peels are richer in total phenols (23.949±0.568) than the aqueous extracts of the sun dried unripe pawpaw peels (21.095±1.717). However fruit peels often thrown into the environment as agro waste can be utilized as sources of anti oxidants and anti microbes because they contain bio active compounds. The overall results for the TCP obtained in this present are higher than those reported for unripe pawpaw peel extracts compared on per 100g basis by (14). One of the readily available resources for primary health care system is herbal medicine with the contribution of phenolic compounds being one of the mechanism of the overall anti oxidant activities. Several authors according to (15) have concluded researches on the close relationship between total phenolic content and anti oxidant activities of fruits and vegetables.

The anti bacterial assay (Chart 1) revealed that the oven air dried unripe pawpaw peel extracts showing maximum zone of inhibition of 27mm, 30mm against Escherichia coli (E.coli) and Bacillus spp respectively while on the other hand, sun dried peels of pawpaw extracts showed maximum zone of 12mm against E. coli. However, no zone of inhibition was seen for Bacillus spp for sun dried unripe pawpaw peel extracts. It was also shown that nearly both extracts used in the study were effective against gram negative and gram positive bacteria with oven dried extracts more assertive against both organisms. Furthermore, the zone formed was comparable to the zone of inhibition formed by standard drugs i.e gentamycin, ampicillin, augmentin and penicillin. Bacillus spp, a gram negative bacteria was found to be resistant to augmentin and penicillin.

V. Conclusion

This study has revealed that extracts of unripe matured papaya peels can be used as both gram positive and gram negative antibacterial agents and could also serve as an effective drug against different diseases such as cardiovascular diseases, atherosclerosis, cancer etc after proper pharmacological evaluation.

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