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Research

Determination of the Proximate and Phytochemical Constituents of a Recipe Produced by Fermenting Matured Unripe Pawpaw Fruit (*Carica papaya* Linn.) Recommended for Healing Peptic Ulcer

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Abstract

Many herbs are rich in phytochemically active compounds that have a beneficial effect on certain human tissues and organs and therefore can be used as medicines to treat, cure or prevent diseases. Pawpaw (Carica papaya Linn.) is a global fruit that serves as food and medicinal herb. This study was aimed at determining the bioactive agents in medicinally potent product derived from fermented unripe pawpaw (C. papaya) which is claimed to cure peptic ulcer. Ulcer formation involves breaking the mucosal barrier and exposing the underlying tissue of the stomach or duodenal lining to the corrosive action of acid and pepsin. The proximate values, phytochemical and mineral analysis of the fermented unripe mature pawpaw solution was determined for a period of six days and repeated thrice. Average values were used for the analysis. The result showed that fermented unripe pawpaw (C. papaya) contains bioactive substances which were probably responsible for the treatment of peptic ulcer in humans. These included alkaloids, beta carotene, flavonoids, lycopene, malic acid, phytate, phenol, tannin and papain. These bioactive agents increased with ageing. In the proximate analysis, there was a decrease in the moisture content of the fruit solution while other parameters increased with ageing. Crude fibre was absent in the sample. In conclusion, the cytoprotective and antimicrobial properties of the solution may account for the anti-ulcer property of the fermented unripe pawpaw (C. papaya) fruit and this recommendation is justifiable because it contains considerable amount of bioactive agents that function against the disorder.

KEY WORDS: Herbs, Phytochemicals, Bio-active agents, Unripe pawpaw.

Introduction

The medicinal benefits of herbs have been known for centuries. Records of Native American. Egyptian, and Hebrew medical practices show that herbs were used extensively to cure practically all known illness. Many herbs contain powerful ingredients that if used correctly can heal the body (Balch and Balch, 2000). Herbalists however contended that nature provides other ingredients in the same herbs to balance powerful ingredients (Balch and Balch, 2000). These other components though they may be less potent, may help to act as buffers, synergies, or counterbalances working in harmony with the powerful ingredients. Today however, scientists are taking a second look at herbal remedies particularly in the past years, a growing body of research has pointed to the therapeutic potential of numerous herbs, but a lot of work remains to be done; only about 15% of the estimated plant species has been investigated for possible medicinal uses (Balch and Balch, 2000).

Nature's pharmacy is an abundant one. Many herbs are rich in compounds that have a beneficial on certain tissues and organs and therefore can be used as medicines to treat, cure or prevent disease. Herbal remedies can help nourish the immune system, stimulate the ration of damaged liver tissue, build the strength of the adrenal glands, counter the adverse effects of chemotherapy and balance the endocrine system, stimulate milk production and improve night vision among other things (Balch and Balch, 2000). The papaya is a short-lived, fast-growing, woody, large herb to 10 or 12 feet. It generally branches probably only when injured. All parts contain latex. The hollow green or deep trunk is straight and cylindrical with prominent leaf scars. Its diameter may be from inches to over a foot at the base (Kudada, 2000). The leaves emerge directly from the upper part of the stem in a spiral on nearly petioles 1 to 3.5 feet long. The blade, deeply divided into 5 to 9 main segments varies 2 feet in width and has prominent yellowish ribs and veins. The life of a leaf is 4 to 6 months (Kudada, 2000).

Papayas prefer to be warm with both direct sunshine and reflected heat, so the hottest place against the house where nothing else seems happening in an ideal location. They also like to be free from wind as much as possible, although this is not as critical as their need for sun. Papayas can be grown successfully in shade, but the fruit is rarely sweet. They are best planted in moulds or against the foundation of a building where water can be controlled (Marys *et al.,* 2000). Papayas need a light, well-drained soil. They are easily killed by excess moisture. The soil needs to be moist in hot weather and dry in cold weather. Papayas do not tolerate salty water or soils (Marys *et al*., 2000). Watering is the most critical aspect in raising papayas. The plants should be kept on to the dry side to avoid root rot, but also need enough water to support their large leaves. In winter the plant prefers to remain as dry as possible. A plant that has been injured by frost is particularly susceptible to root rot (Marys *et al.,* 2000).Papayas do not need to be pruned, but some growers pinch the seedlings or cut back established plants to encourage multiple trunks (Marys *et al.,* 2000). The green fruit is reported to contain protein, fat, carbohydrate, fibre, ash, Ca, P, Fe, 7Mg, Na. K, Beta -carotene equivalent, thiamine, riboflavin, niacin, ascorbic acid as well as vitamin E (Okwu, 2004).

In the traditional treatment of ulcer, a big unripe pawpaw fruit is cut into pieces. The peel or seeds are not removed. The whole fruit is simply cut into cubes, and then soaked in a bowl containing 1200mls of water for four days .The pawpaw solution is sieved and half glass of the solution is taken three times daily for two weeks. This has been recommended as a very good remedy for the treatment of any type of ulcer (Adodo, 2006). The major objective of this study therefore was to monitor changes that occurred during the period of the experiment and consumption by ulcer patients bearing in mind that it would involve metabolic changes of the raw materials that are characteristic of the unripe pawpaw during the period of hydrolysis and fermentation, including P^H values of the sieved solution produced.

Materials and Method

Materials: Test tubes, separating funnel, beakers, pipettes, crucible, conical flask, filter paper, Buchner funnel, plastic bowls, unripe pawpaw fruit (*carica papaya).*

Equipment: 6405 Jenway UV-visible spectrophotometer, water bath,, Gulfex medical & scientific DHG 9101.15A oven, Gallenkomp 9082A incubators, weighing balance, muffle furnace, soxhlet heating mantle, Eyela SB-100 rotary evaporator, water bath. Equitron autoclave, refrigerator, atomic absorption spectrophotometer (AAS), micro kehjahl apparatus, digestion block heaters, fume cupboard, Markham Distillation Apparatus, Jenway : 'meter or colorimeter, Buck 211GP Atomic Absorption Spectrophotometer (AAS),Jenway digital Flame Photometer (PFP7 Model).

Reagents: Vanadate- Molybdate yellow solution, HNO3, Cone. H2SO4, NaOH, acetone, n-Hexane, L. 2% Boric Acid Solution, Methyl Red - Bromocresol green mixed indicator, Kjeldahl catalyst table, sulphuric acid, glacial acetic acid, magnesium oxide, Zinc acetate, HCL, ferrocyanide, isobutylalcohol, magnesium carbonate, ammonium thiocyanate, selenium tablet, sulphuric acid.

Collection of plant material: Samples of pawpaw fruits were collected from the Faculty of Science and Technology quadrangle, Babcock University. The collection, sampling and experimentation were done over a period of three weeks.

Proximate Analysis: This involves the partitioning of compounds in food into six categories based on the categories properties of the compounds. These categories are: Moisture content, ash content, protein, crude fibre, crude fat and carbohydrate. Samples of the material were analyzed chemically according to the official methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C., 1990).

Moisture Content evaluation: Traces of water and volatile components were removed initially by air-drying and later by oven drying the samples.

Crude Protein determination: The crude protein and total nitrogen were determined using the Kjeidahl method **(**Kjeidhal, 1883**)**.

Crude Fibre determination: Crude fiber is determined gravimetrically after chemical digestion and solubilization of other materials present. The starch and the protein are dissolved by boiling the sample with acid then with sodium hydroxide. The residue of cellulose and lignin is washed, dried and weighed.

Crude Fat determination: This was done using soxhlet method **(**Kjeidhal, J., 1883**).**

Carbohydrate evaluation: This component is not determined analytically but it is calculated by difference in the overall proximate analysis.

Carbohydrates (%) = 100 - (% Fibre + % Moisture + % Protein + % Fat + % Ash)

Phytochemical tests: Qualitative and quantitative phytochemical analysis of samples was conducted following the standard procedures (Dhirya and Manimegalai, 2013; Brinda *et al*., 1981; Trease *et al*., 1989).

Mineral analysis: The mineral analysis of fermented unripe pawpaw solution was determined three times using the Atomic Absorption Spectrophotometer (AAS).

Results and Discussion

Result of the proximate analysis of the unripe pawpaw fruit extract is shown in Table 1. The moisture content of the solution decreased with increasing period. It was highest on day 1 (97.40%) and lowest on day 6 (89.70%). Moisture content was also higher than all other proximate parameters: ash content, fat content, protein content, carbohydrate and crude fibre. There was however absence of crude fibre in the solution. Ash content of the solution increased with ageing. It was highest on day 6 (0.10%) and lowest on day 1 (0.02%). Fat content of the solution was lowest on day 1 (0.03%) and was highest at both days 4 and 6 (0.05%). It was however higher than ash content but lower that protein, carbohydrate moisture content. Protein content increase gradually from 0.14% on day 1 to 0.24% on day 6. Carbohydrate content also increased with increased age from day 1 (2.41%) to day 6 (9.91%). It was thus higher than the ash conent, protein content and fat content.

Mean values of the fermented unripe pawpaw which was screened for phytochemicals are presented in Table 2. Alkaloid was highest on the sixth day (0.125%). It was higher than flavonoid, lycopene, oxalate,phenol and tannin. Flavonoid increased with age. It was higher than tannin only but lower than alkaloid, lycopene,oxalate,phenol,phytates and saponin. Lycopene increased from 0.05% on day 1 to 0.034% on day 6. Oxalate gradually increased with age and generally higher than the value of alkaloid, flavonoid,lycopene, phenol, saponin, and tannin. Phenol was highest on sixth day. It had greater value than flavonoid,lycopene and tannin. Phytates had the highest value compared to all the other phytochemicals. It was highest on day 6 (0.335%). Saponin increased progressively with age from 0.110% to 0.132%. Tannin had the lowest value compared to other phytochemicals. It was lowest on day 1 (0.003%) and highest on day 6 (0.022%).Results of the organic acids evaluated for a period of four days are presented in table 3. Beta carotene increased with ageing. It had greater values than malic acid and methyl salicylate. Malic acid was highest on the sixth day (0.275%) and lowest on the first day (0.014%). Methyl salicylate had the lowest value compared to other organic acids that were determined. It increased gradually with age.

Papain which is a protein digestive enzyme increased substantially from day 1 (2.42%) to day 6 (17.80%).

Mean values of results from mineral analysis are also presented in table 4. Sodium had the highest value compared to other minerals. It was highest on the sixth day (**3**.60%). Potassium, calcium, magnesium, manganese and zinc increased with age. Zinc had the lowest value compared to all other minerals that was analyzed. Iron was lowest on day 1(0.014%), highest on day 4 (0.71%) and decreased to 0.534% on day 6.

Herbs, fruits and vegetables are used principally as food and medication. Some are consumed fresh while some are processed to varying degree. Pawpaw (*Carica papaya* Linn.) is a global fruit that has satisfied these areas of utilization. Infact, no part of the pawpaw plant is not

useful. This study aimed at determining the bioactive agents in medicinally potent product derived from fermented unripe pawpaw (*Carica mtaya* Linn.) which is claimed to cure peptic ulcer (Adodo, 2006). The study was carried out to compare the proximate, phytochemical and mineral compositions of fermented unripe mature pawpaw solution for a period of six days (1, 2, 4, 6 days). The results presented showed that the crude protein content of the solution increased with ageing which may be indicative of a higher rate in enzymatic activity due to the process of fermentation (Onwuka, 2005). The crude fat initially increased but later became constant. Wax forms protective coatings on the tissues of plants, outer covering of fruits and it is a chemical agent that maintains or create emulsion in both plant and animal. They are the structural elements of biological membranes as phospholipid and sterols (Nelson and Cox, 2008). Prostaglandin, a derivative of fatty acid helps to protect the lining of the alimentary canal, that is, the entire digestive passage, including the lining of the stomach and intestines. It also blocks the production of gastric acid which causes peptic, ulcer indicating effectiveness in this medically used herb (Nielson, 2002).

Crudie fibre was not appreciably present in the sample. It was in trace amount. It is largely a digestible plant matter considered to play a role in the prevention of many diseases of the digestive tract (Mensah *et al.,* 2008). The ash content of the sample increased rapidly and was highest on the 6th day of the experiment. Ash content is a measure of the mineral element or inorganic matter essential for the maintenance of life in food samples. They are essential for proper functioning of tissues and act as second messengers in some biochemical cascade mechanisms (James, 2004).

Moisture content of the sample decreased with ageing due to increase in the fat content and alcohol content of the sample. A decreased with ageing due to increase in the fat content and alcohol content of the sample. A decrease in the moisture content of a sample depletes a shorter shelf life of the sample. This indicates a long shelf life throughout the period of usage of the herbal solution (Onwuka, 2005). Fermented unripe pawpaw (Carica papaya Linn.) contains active substances responsible for the treatment of peptic ulcer in human such as, alkaloids, beta carotene, flavonoids, lycopene, oxalate, malic acid, methyl salicylate, phytates, phenol, saponin tannin, and papain (Balch and Balch, 2000). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effect on human and this has led to the development of powerful pain killer medication. Alkaloids play important roles in plants as endogenous biological barriers to protect against pathogens due to their strong antimicrobial, antifungi and anti – insect activities . the combination of belladonna alkaloids and Phenobarbital is used to treat irritable bowel syndrome and ulcers in the intestine. Fermented unripe pawpaw could also act as stimulant and analgesic due to the presence of alkaloid which increased with aging (Luch, 2009). Presence of alkaloid could actually reduce the risk of cancer. Beta carotene contributes to the orange colour of many different fuits and vegetables; it has a high tendency to oxidize that most food fat. It is a source of vitamin A when ingested in humans. It has antioxidant property, which helps to protect cells from damage. It also helps to protect the lining of the stomach and intestine thereby reducing the risk of ulcer (Mercadante *et al*., 1999). Flavonoids are antioxidant (preventing cellular damage brought on by free radicals- unstable oxygen molecules which are the natural byproducts of metabolism), antimicrobial and anti-inflammatory agents in humans and they are also responsible for color in many fruits and vegetables. The flavonoid content as analysed increases as fermentation proceeds (Middleton *et al*., 2000). They also have vasoconstriction effect on small superficial wounds or burns (Delle and Watermen,

1984). The healing effect of this herb can be enhanced by flavonoids. Lycopene has been found to posses antioxidant and antiproliferative properties in animal and laboratory studies, although activity in humans remains controversial. Numerous studies correlate high intake of lycopene- containing foods or high lycopene serum levels with reduced incidence of cancer and cardiovascular disease (Bunker *et al*., 2007). Malic acid is mostly found in unripe fruit and it occurs in living organisms as an intermediate metabolite in the krebs cycle. Malic acid is used as an antidulent as well as a flavoring agent in the processing of some foods (Jenson and William, 2007). Metyl salicylate serves as signal transducing molecule and pheromone in plant. It is used in human to treat joint and muscular pain (James, 2004). There was gradual increase as shown in the result. This has little or no effect for the treatment of ulcer. Oxalates (esters of oxalic acid) have physiological function both in plant and animal (Parivar, 1996). It affects calcium and magnesium metabolism and react with proteins to form complexes which have an inhibitory effect in peptic digestion. Oxalic acid binds calcium and forms calcium oxalate which is insoluble and adversely affects the absorption and utilization of calcium in the animal body. Phytic acid is a natural plant antioxidant. It has anti-inflammatory, anticancer, hypocholesterolemic and hypolipidemic effects (Salisbury and Ross, 2002). Presence of phytic acid has effect in the efficacy of fermented unripe pawpaw solution.

Phenols function as an antioxidant, anticarcinogenic, a precursor to a large collection of pain relieving drugs and also as an anesthetic. Dilute solutions of phenols are useful antiseptic but strong solutions are caustic and scarring to tissue. Phenol is widely used in the manufacture of resins, plastic insecticides, explosives, dyes, and detergents and as raw materials for the production of medicinal drugs such as aspirin (Weber *et al.*, 2004). Papain is a protein digesting enzyme found in pawpaw. There was a drastic increase in the amount of papain as fermentation progresses as shown in the result. It is the most active amount of papain as fermentation progresses as shown in the result. It is the most active substance against ulcer, inflammation, gas and sour stomach in human (Rawlings and Barett, 1994). Tannins act externally as water proof to the external layers of the exposed tissue since they precipitate proteins, thus protecting the underlying layers. They also have vasoconstriction effect on small superficial or exposed vessels, superficial wounds or burns (Delle and Waterman, 1984). This further strengthens the healing activity of the sample. Thus the healing effect of fermented unripe pawpaw could also be attributed to the above actions of flavonoids and tannins. Saponins are glucosides that are useful material for the synthesis of steroid hormone (Salisbury and Ross, 2002). Gradual increase was noticed in the amount of saponin as days increased.

Fermented unripe pawpaw also contained minerals like sodium, potassium, calcium, magnesium, zinc and iron in increasing amount as the number of days increased.

Although sodium is not considered an essential micronutrient in most plants, it is necessary in the metabolism of some C4 plants. Within these C4 plants, sodium is used in the regeneration of phosphoenolpyruvate (PEP) and the synthesis of chlorophyll. In addition, the presence of sodium can offset potassium requirements in many plants by substituting in several roles, such as maintaining tugor pressure, serving as an accompanying cation in long distance transport, and aiding in stomata opening and closing. Sodium is classified as a dietary inorganic macromineral for animals maintaining body fluid and electrical potential in animal tissues (Subbarao, 2003). Potassium is an essential mineral micronutrient in human nutrition; it serves the same function as sodium in plant. It is the major cation (positive ion) inside animal cells, and it is thus important in maintaining fluid and electrolyte balance in the body. It is also important in preventing muscle contraction and the sending of all nerve impulses in animals through action potentials (Delia *et al*,. 2011). Calcium plays an important role in building stronger, denser bones early in life and keeping bones, strong and healthy later in life. It also has important uses such as some exocytosis, especially neurotransmitter release, and muscle contraction. It is used in the production of antacids for the treatment of peptic ulcer (Straub, 2007). The presence of calcium could have relieving effect on ulcer. Magnesium compounds are used medicinally as common laxatives, antacids (i.e., milk of magnesia), and in a number of situations where stabilization of abnormal nerve excitation and blood vessels spasm is required. Hundred of enzymes require magnesium ions to function (Wester, 1987). Presence of Magnesium could serve to reduce the effect of ulcer. Manganese is also important in photosynthetic oxygen evolution in chloroplasts in plants. The oxygen evolving complex (OEC) is a part of Photosystem II contained in the thylakoid membranes of chloroplasts; it is responsible for the terminal photo oxidation of water during the light reactions of photosynthesis and has a metalloenzyme core containing four atoms of manganese. It functions as an antioxidant in human (Emsley, 2001). Zinc is an essential trace element, necessary for plants & animals. In humans, zinc plays ubiquitous biological roles". It interacts with "a wide range of organic ligands" and has roles in the metabolism of RNA and DNA, signal transduction, and gene expression. It also regulates apoptosis. In plants, zinc help fight against diseases (Hambidge and Kreb, 2007). Iron is an essential part of hemoglobin; the red colouring agent of the blood that transports oxygen through our bodies.

Conclusion

This work attempted to identify the relative amount of bioactive agents in the fermented whole unripe (green) pawpaw (*Carica papaya* Linn.) fruit and observe the metabolic changes knat occurred during the process of hydrolysis and fermentation. Based on the findings fermented whole unripe (green) pawpaw (*Carica papaya* Linn.) fruit solution recommended for the treatment of peptic ulcer is

justifiable because it contains considerable amount of bioactive agents that function against the disease. It is recommended however that further study and research should be carried on the accurate dosage of the fermented whole unripe (green) pawpaw (*Carica papaya* Linn.) fruit solution, and proper efforts should be made for the control of pathogen for ensuring health safety in the herbal preparation of the cure for peptic ulcer.

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Table 1: Proximate composition of unripe pawpaw fruit extract.

	DAY 1	**DAY 2**	**DAY 4**	**DAY6**
Moisture Content (%)	97.40	95.60	91.80	89.70
CrudeFibre Content (%)	NIL	NIL	NIL	NIL
Ash Content (%)	0.02	0.03	0.04	0.10
Fat Content (%)	0.03	0.04	0.05	0.05
Protein Content (%)	0.14	0.17	0.21	0.24
Carbohydrate (%)	2.41	4.12	7.89	9.91

Table 2: Phytoehemical composition of unripe pawpaw fruit extract.

	DAY 1	**DAY 2**	**DAY 4**	**DAY 6**
Alkaloid (%)	0.073	0.089	0.110	0.125
Flavonoid (%)	0.004	0.014	0.018	0.030
Lycopene (%)	0.005	0.016	0.027	0.034
Oxalate (%)	0.121	0.137	0.141	0.155
Phenol (%)	0.010	0.018	0.027	0.038
Phytate (%)	0.107	0.113	0.240	0.335
Saponin (%)	0.110	0.116	0.127	0.132
Tannin (%)	0.003	0.011	0.019	0.022

Table 3: Organic acid composition of unripe pawpaw fruit extract.

	DAY 1	**DAY 2**	**DAY 4**	**DAY 6**
BetaCarotene 1 (%)	4.79	6.01	12.31	16.55
MalicAcid (%)	0.014	0.017	0.124	0.275
Metyl Salicylate (%)	0.007	0.010	0.018	0.024
Papain (%)	2.42	3.86	10.64	17.80

Table 4: Mineral composition of fermented unripe pawpaw solution.

	DAY 1	**DAY 2**	**DAY 4**	**DAY 6**
Sodium (mg/lOOg)	4.40	6.00	6.56	8.60
Potassium (mg/lOOg)	0.80	1.56	1.73	2.84
Calcium (mg/lOOg)	0.24	1.00	1.84	2.64
Magnesium (mg/lOOg)	0.86	0.90	0.93	0.98
Manganese (mg/lOOg)	0.20	0.52	0.59	0.67
Zinc (mg/lOOg)	0.05	0.09	0.12	0.17
Iron (mg/lOOg)	0.014	0.034	0.71	0.534