A Comparative study of the Antioxidant activity (DPPH), Total flavonoid, Total Tannin, Total polyphenol levels in plant extracts of the *Annona muricata, Ribes nigrum and Manilkara zapota.*

Joneshia Bryan-Thomas

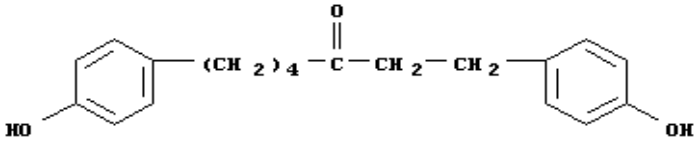
Department of Medical Technology, Northern Caribbean University, Mandeville, Jamaica W.I.,

Abstract- The research aimed at unearthing the total levels of selected phytochemicals and the antioxidant (DPPH) in *Annona muricata, Ribes nigrum and manilkara zapota* plant extracts, which are widely known for their medicinal properties and locally grown in Manchester Jamaica West Indies. The leaves, bark and stem were collected and identified by an agronomist. They were aseptically treated then dried at room temperature for 4 days after which they were milled by the use of dry blend commercial heavy duty blender. The pulverise samples were stored at room temperature in sealed plastic screw top sterile cups until processed. The plants were tested for the presence of Phytochemicals (total flavonoids, total poly-phenols, and total tannins). DPPH assay was conducted to determine the antioxidant activity of *Annona muricata, Ribes nigrum and Manilkara zapota* plants respectively. *M. zapota* had the highest flavonoid content of 2.51 mg/g which was three times that of the *A. muricata* and more than that from *R.nigrum.* The highest poly-phenol content was obtained from the *R.nigrumwhich produced values of 5.72 mg/g*, followed by *M. zapota*, with *A. muricata* having the lowest poly phenol content. The lowest total tannins was obtained from *A. muricata* with the highest content being observed from *R.nigrum.M. zapota*showed the highest antioxidantactivities, with the lowest being obtained from*A. muricata.*Samples were processed in duplicates.

Index Terms- DPPH assay, phytochemicals (total flavonoid, total tannin and total polyphenol levels),*Annona muricata, Ribes nigrum, Manilkara zapota*

I. INTRODUCTION

Antioxidants are substances that help protect the body from oxidative damage caused by free radicals. Antioxidants are a very important part of our diet as they help to prevent oxidative cell damage by acting as free radical scavengers and thereby preventing oxidation and repairing the damage caused by free radicals (Godic&Dahmane 2014). According to Thenmozhi A, et al 2011, in the last few decades, several epidemiological studies have shown that a dietary intake of foods rich in natural antioxidants correlates with reduced risk of coronary heart disease particularly a negative association between consumption of polyphenols-rich foods and cardiovascular diseases has been demonstrated. This association has been partially explained on the basis of the fact that polyphenols interrupt lipid per oxidation induced by reactive oxygen species (ROS). Based on Birasuren B et al 2012 who coted from several experts, "generally, plants contain a variety of phytochemicals, the consumption of which has been correlated with positive regulatory effects in human health, the oxidative stress occurs from an imbalance between the antioxidant defense systems and the formation of reactive oxygen species (ROS), and may damage essential biomolecules such as proteins, DNA, and lipids. Oxidative damage may cause cellular injuries and death, and exacerbate several degenerative diseases associated with aging, including cancer, cardiovascular disease, atherosclerosis, and neurodegeneration". The sour-sop leaf contains phytochemicals called *Annonaceousacetogenins* which are also found in the bark and twigs of the sour-sop tree. Research studies have shown that sour-sop extracts are active *against Leishmaniabraziliensis*, *L. Panamensis promastigotes*, cancer cell line U 937 and hepatoma cell lines in vitro (Cassileth et al 2010). Recent studies reported the isolated compounds found in sour-sop to be effective anti-tumor agents. Sour-sop compounds which are anti-cancer are safe enough to protect healthy cells unlike chemotheraphy which causes the compromise of healthy cells along with the cancerous cells, accompanied with hair loss, nausea and weight loss (Richardson, L 2011).



Acetogenin-G, (C19 H22O3).

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol (Huang DJ et al 2005). This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colourless ethanol solution. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometry (Huang DJ et al 2005), so it can be useful to assess various products at a time

The aim of this research was to evaluate the comparative analysis of the total percentage of antioxidant activity along with selected phytochemicals (total flavonoid, total tannin and total

polyphenol levels) of the plant materials. This study will contribute to further knowledge relating to the screening of antioxidant compounds present in these plant extracts that were locally grown in Jamaica. The prospects of the findings gives insight to the authentic nature of these plants as future medicinal product and prospective nutraceutical remedy. The increased interest in biological compounds such as plant polyphenols, flavonoids and tannins is the motivation for thisstudy.

II. Material and Methods

A) Plant materials

Annona muricata, *Ribes nigrum* and *Manilkara zapota* were theplant samples chosen for this study. They were all collected and identified.

B) Extraction procedure

An aqueous extraction was prepared using the equivalent of 1 gm of milled plant material to 100 ml of hot water and the mixture steeped for 5 minutes. Samples of the infusions were also lyophilized (Labconco Freeze Zone 4.5 Liter Freeze Dry System – Model 7750001) for DPPH antioxidant study.

C) Phytochemical Analysis

These studies were performed according to the standard methods as reported by Marinova , D et al 2005 and Rajpal, V. 2005.

D) Quantitative phytochemical analysis

Determination of Total Polyphenols content (Marinova, D et al 2005)

The Folin-Ciocalteu assay was used to determine the total phenol content of the plant extracts. 1ml of various concentrations of the extract or standard (Gallic acid) was added to a 25 ml volumetric flask containing 9ml of distilled water. A reagent blank consisting of distilled water was prepared. 1ml of Folin- Ciocalteu phenol reagent was added to the mixture and shaken. After 5 minutes, 10 ml of 7% sodium carbonate was added to the mixture. The volume was then made up to the mark. After incubation for 90 minutes at room temperature, the absorbance against the reagent blank was determined at 550 nm with a UV visible spectrophotometer. Total phenolic content was expressed as mg Gallic acid equivalent (GAE).

E) Determination of the Total flavonoid content (Marinova, D et al 2005)

The aluminum chloride colorimetric assay was used to measure the total flavonoid content of the plant extracts. 1ml of various concentrations of the extract or standard (quercetin) was added to a 10ml volumetric flask containing 4ml of distilled water. 0.30 ml of 5% sodium nitrate was added to the flask and after 5 minutes 0.3 ml of aluminum chloride was added. The volume was made up to the mark with distilled water. The solution was mixed and absorbance was measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalent (QE). Quercetin was used as the positive control. Samples were analyzed in duplicates.

F) Determination of the Total Tannin content **(**Rajpal, V. 2005)

Total tannin was measured by a titrimetric indigosulphonic acid assay according to the method from the 'Standardization of Botanicals by Dr. V. Rajpal 2005. In a 250 mL conical flask containing 1 mL of tea infusion, 2.5 mL of indigosulphonic acid solution and 75 mL of distilled water were added. This solution was titrated with constant stirring against a N/10 (0.1M) potassium permanganate solution until an end-point with a 'golden yellow' color was visible. 1mL of N/10 potassium permanganate is equivalent to 0.004157 g of tannin compounds calculated as tannic acid. A blank test was determined by titrating 2.5 mL of indigosulphonic acid in 75 mL of water against the N/10 (0.1M) potassium permanganate solution. Total tannins was expressed as mg per gram and mg per serving. All samples were analyzed in duplicates.

G) Determination of In-vitro Antioxidant analysis using 2, 2-diphenyl-1- picrylhdrazl (DPPH) **(**Williams, L.A.D 2006)

In vitro antioxidant activities of *Annona muricata*, *Ribes nigrum and manilkara zapota* extracts were evaluated using the DPPH assay.

H) DPPH radical scavenging assay

A stock solution of 5.0 mg/mL was prepared using the lyophilized infusion of *Annona muricata*, *Ribes nigrum and manilkara zapota* extracts. 20 µL of this solution was then added to 2.0 mL of methanol to produce a final testing concentration of 50 µg/mL. 400 µL of 0.02 % (w/v) stock solution of 2, 2-diphenyl-1- picrylhdrazl (DPPH) in methanol was then added to the solution and the mixture was incubated for 20 mins. Absorbance was determined at 517 nm against a methanol blank using a spectrophotometer. All samples were measured in duplicates the mean values with ± SEM were presented. The percentage anti-oxidant activity (% AA) was determined by the following equation:

$$\% AA = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

The percentage inhibition was determined by comparing the results of the test and the control.

I) Graphs and Statistical Analyses

Summary statistics and graphs were obtained using Microsoft Office Excel 2013. Means were compared using analysis of variance (ANOVA) at $p \leq 0.05$.

III. Results and Discussion

Table 1 Qualitative phytochemical analysis of *A.muricata, R.nigrum* and *M. zapota*

PLANT MATERIAL TESTED	POLY-PHENOLS	FLAVONOIDS	TANNINS
A.muricata,	+	+	+
R.nigrum	+	+	+
M. zapota	+	+	+

(+) The presence of the Phytochemical (-) The Absence of the Phytochemical

Table 2 Qualitative antioxidant analysis DPPH (2, 2-diphenyl-1-picrylhydrazyl.)

PLANT MATERIAL TESTED	Antioxidant analysis DPPH (2, 2-diphenyl-1-picrylhydrazyl.)
A.muricata,	+
R.nigrum	+
M.zapota	+

(+) The presence of the Phytochemical (-) The Absence of the Phytochemical

Table 3 Phytochemical profile of the plant materials (mean values with standard error± in mg/g)

	Total Flavonoids (mg/g)	Total Polyphenols (mg/g)	TotalTannins (mg/g)	DPPH Antioxidant Activitytest
A.muricatamurica	0.8±0.01	2.76±0.008	75.48 ±0.11	9.39±0.78
R.nigrum	1.56±0.03	5.72 ±0.02	104.74 ±0.58	40.75 ±1.09
Mzapata	2.51 ±0.04	5.48±0.02	101.7±0.88	46.24 ±1.59

Total Flavonoids

*M. zapata*had the highest flavonoid content (2.51 mg/g) which was about three times that of the *A. muricata* leaves and more than that from *R.nigrum.* See Table 3. The following table gives empirical evidence of the scope of literature supporting the results obtained. Table 4 reveals that According to Chanda S.V et al 2010, *M. zapota*which was obtained in india was seen having total Flavonoid contents of19.14 mg/g. The total content differered from locally obtained *M. zapota*which was collected at its peak of maturity and fruition. While literature presented information on the flavonoid contents of the *R.nigrum*plant to have total Flavonoid contents of 0.84 mg/g the present study revealed an increase of 1.56 mg/g. The plant sample was collected while the tree was un-fruited and was locally obtained.

Table 4 Literature evidence

R.nigrum	0.84mg/g	Diacones, Zorita et al 2015
Mzapota	19. 14mg/g	Chanda, S V et al 2010
A. muricata (matureleaves)	11.15mg/g	SRC
A. muricata (youngleaves)	41.25 gig	Green. C et al 2015

Total Poly-Phenol

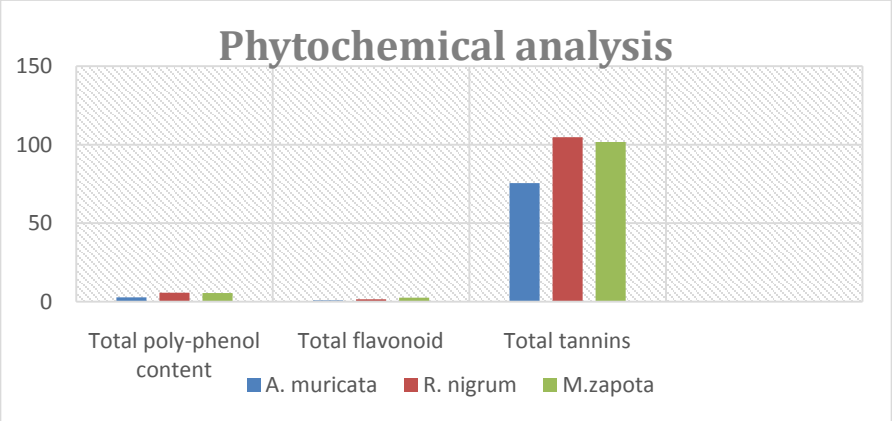
The highest poly-phenol content was obtainedfromthe *Ribesnigrum*extract of(5.72mg/g),which is in keeping with literature siting of 5.80 *mg/(Chanda S V et al 2010).*The result from the literature was significantly different from that of the *A.muricata s*ample. Whereas the mature leaves had a

valueof37.77gig and the youngleaves 50.45mg/g,thatofthesamplewasonly 2.76mg/g. The phenol content of 1.21 mg/g (literature) was given r *M zapota*which is almost 5 timeslessthantheresultsobtainedforthesampleplant material 5.48 mg/g (*Shanmugapriya, K et al 2011).*

TotalTannins

The total tannins ranged from 75.4 - 104.7 mg/g with the highest content obtained from the *M zapota*. See Table 3. There was sparse literature for the tannin content of the three plant materials tested. One literature review gave values of 23.59 mg/g for mature *A. muricata* leaves and 56.48 mg/g for the young leaves; which were less than that found from the *A. muricata* leaves (75.48mg/g)testedforthisreport(Green, C et al 2015).

Graph 1 Comparison of studies plant material with phytochemical results



Antioxidant Activity

The percentage (%) antioxidant activity ranged from a high of 46.24 to a low of 9.39 percent with the highest activity being seen in M. *zapota*(commonly known as naseberry); Chanda,S.V.etal 2010 revealed a high of 10.42%. This is comparably low when compared with the activity of the test sample plant. The % antioxidant activity for A. muricata (commonly known as soursop) sample was 9.39%. This was low when compared with results of other local studies done with the A. muricata showing percentages as high as (87.14%) unlike this present sample. No information for the % Antioxiadant Activities for the R. nigrum, (commoly known as blackcurrant), was found in the literature.

M.zapatahad the highest flavonoid content (2.51 mg/g) which was about three times that of the A. muricata leaves and more than that from R.nigrum. See Table 3.

IV. Summary

The flavonoid, the phenol and tannin content of most plants are at their maximum peak in young plants whilst the maturity of plants result in decline of secondary metabolites as biosynthesis slows down during plant growth. The unexpected low bioactivity in some of the planting material could be due to the plant maturity as well as, to the length of time of storage of plant material as there could be loss of plant potency during lengthy or unsuitablestorage.

Acknowledgment

This research was made possible through the funding from the Graduate Research and Grants affiliated with Northern Caribbean University, Mandeville, Jamaica W.I. and also Mr. Orlando Hamilton who suggested the use of *Ribes nigrum*in the study.

References

[1] Chanda,S.V.*etal*.AntioxidantCapacityof*Manilkarazapota*L.LeavesExtracts EvaluatedbyFourinvitroMethods,*NatureandScience*,2010;8(10)p.264

[2] ProductsUnitoftheScientificResearchCouncil

[3] Diaconeasa, Zorita, et al. Antiproliferative and Antioxidant Properties of Anthocyanin Rich Extracts from Blueberry and Blackcurrant Juice, *Int J. MolScL,2015*,16,p.2355

[4] Green, Cheryl *et al.* In-vitro Bioassays and Chromatographic Analyses Used to Screen Natural Products from Jamaica in the 21st Century, Research Signpost, Trivandrum, India, 2015, p.19-23

[5] Shanmugapriya, K., et al., Antioxidant Activity, Total Phenolic and Flavonoid Contents of ArtocarpusHeterophyllus and ManilkaraZapota Seeds and its Reduction Potential, *Int J. Pharmacy Pharmaceu. Sci.* Vol. 3, Suppl. 5, 2011, p.258

[6] Godic,A.,&Dahmane, R., (2014) The role of Antioxidants in Skin Cancer prevention and treatment. Oxid Med Cell Longev.

[7] Thenmozhi A, Nagalakshmi K, Mahadeva Rao U S. Qualitative analysis of phytochemicals, and comparative superoxide radical scavenging along with reducing potency of *Solanum nigrum* using various solvent extracts. Int J Green Pharm [serial online] 2011 [cited 2015 May 18];5:318-24.Available from: http://www.greenpharmacy.info/text.asp?2011/5/4/318/94355

[8] Sodipo OA, Akanji MA, Kolawole FB, Odutuga A. A Saponin is the active antifungal principle in Garcinia Kola, heckle seed. Biosci. Res. 1991; 3:171.

[9] Birasuren B, Oh HL, Kim CR, Kim NY, Jeon HL, Kim MR. Antioxidant Activities of Ribesdiacanthum Pall Extracts in the Northern Region of Mongolia. PrevNutr Food Sci. 2012 Dec;17(4):261-8. doi: 10.3746/pnf.2012.17.4.261. PMID:24471094

[10] Cassileth, Barrie R., Yeung, K. Simon, Gubili, Jyothirmai. Herb-Drug Interactions in Oncology, People's Medical Publishing House-USA, Shelton, CT, 2010, p 336

[11] Richardson, Lorna. The Cocktail: That is what cured my colon cancer, Author House, Bloomington, IN, USA, 2011, p 10.

[12] Huang DJ, Ou BX, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005;53:1841-1856

[13] Marinova, D., Ribarova, Atanassova, M. (2005). Total Phenolics and Total Flavonoids in Bulgarian Fruits and Vegetables. Journal of the University of Chemical Technology and Metallurgy 40: 255-260.

NPU WI#SRC-QSI-PRD-N-028E

[14] Rajpal, V. (2005). Testing Procedure of Tannins (by U.S.S.R.P. Method) in Terminalia Chebula fruit (Harir) extract, p 342-343. In: Standardization of Botanicals V2. Eastern Publishers, New Delhi, India, pp. 361.

NPU WI#SRC-QSI-PRD-N-030E

[15] Williams, L.A.D., Hibbert, S.L., Porter, R.B.R., Bailey-Shaw, Y.A., Green, C.E. (2006). Jamaican Plants with *in vitro* Anti-Oxidant Activity, p 1-12. In: Biologically Active natural Products for the 21st Century. L.A.D. Williams (Ed.) Research Signpost, Trivandrum, India.

NPU WI#SRC-QSI-PRD-N-032E

AUTHORS

First Author – Joneshia Bryan-Thomas, Department of Medical Technology, Northern Caribbean University, Mandeville, Jamaica W.I.,