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ANALYSIS OF THE PHYTOCHEMICAL COMPOSITION OF THREE SELECTED FRUITS: *MUSA ACUMINATA, MALUS DOMESTICA* AND *CITRUS PARADISI*

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ABSTRACT: *Phytochemicals are gaining interests globally in recent times due to their wide range of medicinal importance. The main objective of this study was to evaluate the phytochemical contents of fruits; Musa acuminata, Malus domestica and Citrus paradisi. Preliminary screening involved the use of qualitative methods to detect the presence of alkaloids, saponins, steroidal saponins, phenols, terpenoids, flavonoids, tannins, carbohydrates, proteins, deoxy sugars, reducing sugars. Quantitative estimation of the phytochemicals was done using standard procedures. Qualitative analysis carried out showed that; alkaloids, tannins, phenols, saponins and flavonoids were present in all the three fruits. Highest quantities of alkaloids were obtained in Musa acuminata (70.49 mg/g) and Citrus paradisi (43.19 mg/g). Citrus paradisi (0.45 mg/g) and Musa acuminata (0.42 mg/g) contained more saponins than Malus domestica (0.38 mg/g). Flavonoid was more in Citrus paradisi (10.02 mg/g) and Musa acuminata (6.51 mg/g). Citrus paradisi showed the highest content of phenol (7.11 mg/g) while Musa acuminata and Malus domestica recorded more tannin content of 6.03 mg/g and 2.28 mg/g respectively. Relatively, highest percentage occurrences of phytochemicals were as follows; alkaloids (82.35%), flavonoids (7.61%) and tannins (7.04%) in Musa acuminata, alkaloids (53.44%), phenols (24.52%) and flavonoids (10.85%) in Malus domestica and alkaloids (70.02%), flavonoids (16.25%) and phenols (11.52%) in Citrus paradisi. This study shows that these fruits could be sources for the exploitation of phytochemicals beneficial in food, cosme-ceutical, pharmaceutical and alternative medicine industries.*

KEYWORDS: Citrus Paradisi, Malus Domestica, Musa Acuminata, Phytochemicals, Qualitative Analysis, Quantitative Analysis.

INTRODUCTION AND LITERATURE REVIEW

Phytochemicals are bioactive chemicals of plant origin which contribute their beneficial characteristics to humans' health and disease prevention. Phytochemicals are called secondary metabolites as they are manufactured during the stationary growth process of plants and they have little need for them. They are naturally synthesized in different parts of the plant; stem, root, flower, bark, fruits, peel, leaves etc. in differing quantities and qualities (Pallavi et al., 2017).

Phytochemicals are regarded as non-nutritive but bioactive plant chemicals possessing varying degrees of disease preventive properties. They contain antioxidants e.g. polyphenols and

carotenoids which protect cells from damage by free radicals (Omoregiem and Osagie, 2012). Studies have shown that antioxidants improve the health of the immune system and are also associated with lowered risk in coronary disease (Devasagayam et al., 2004; Mathew et al., 2012). Phytochemicals also exert anti-tumour, hormonal stimulation and antibacterial effects in diseases prevention and therapy (Umamaheswari et al., 2017).

Antimicrobial agents in the market are becoming less potent daily in the treatment of infectious diseases, no thanks to the emergence/ re-emergence of antimicrobial resistance. More microbial pathogens are acquiring multi-drug resistance leading to a drastic fall in the survival rate of both hospitalized and non-hospitalized patients. To overcome this menace, it is necessary to discover new and potent antimicrobial prototypes, thus, attention is shifting to phyto-medicinal alternatives. Phytomedicines derived from herbal plants are widely used in many parts of the world; India, China, Japan, Korea Cameroun, Nigeria etc. due to the presence of diverse bioactive compounds which possess useful potentials to meet the current therapeutic requirements (Sheikhlar *et al*., 2013).

Following such leads, plant parts are often assayed for phytochemicals that may be present. The phytochemicals are then subjected to further isolation, purification and characterization and used as the basis for new pharmaceutical products. Vegetables and fruits such as; *Musa acuminata*, *Malus domestica* and *Citrus paradisi* are excellent sources of phytochemicals. *Musa acuminata*, commonly called banana (English), is a herbaceous plant of the genus Musa and family Musaceae. It is a tropical plant grown all over the world for food and income. Various parts of the banana plant are rich in tannins, terpenoids, saponins, flavonoids, and phenols with antidiarrhoeal, antidiabetic, hypocholesterolemic, antilithiatic, antitumoral, antimutagenic, hepatoprotective, antifungal and antibacterial properties (Aina et al., 2019).

Malus domestica (apple) is an edible fruit of the genus Malus and genus Rosaceae, widely cultivated because of its numerous benefits. Apple, especially its peels have been found to possess potent antioxidant activity and can hinder the growth of liver, lung and colon cancer cells. Apple is high in antioxidants; procyanidin, epicatechin, quercetin-3-rhamnoside, quercetin-3-galactoside, quercetin-3-glucoside, catechin, cyanidin-3-galactoside, chlorogenic acid, phloridzin gallic acid and coumaric acid that help in the prevention and repair of oxidation damage and inflammation on the cellular level, increased production of essential neurotransmitter acetylcholine in the brain, and protection of neuron cells against oxidative stress-induced neurotoxicity (Patel et al., 2012; Avrin et al., 2018). Also, apple consumption has been reported to improve pulmonary health as it has been inversely linked with asthma, bronchial hypersensitivity and other cardiovascular diseases (Lee *et al*., 2013).

Citrus paradisi (grapefruit) is a rich source of flavonoids. Interest in the possible health benefits of flavonoids has increased largely due to their oxidant and free-radical scavenging activities. The antioxidant activity of flavonoid has been related to their protection against vitamin C oxidation (Middleton *et al*., 2000). Grape seed oil contains tocopherols (Vitamin E) and high contents of phytosterols and polyunsaturated fatty acids such as linoleic acid, oleic acid and alpha-linoleic acid. Grape seed oil from crushed seeds is used in cosme-ceuticals and skin care products for perceived health benefits (Soto et al., 2015). The main objective of this study is to do a qualitative and quantitative analysis of the aqueous extracts of *Musa acuminata*, *Malus domestica* and *Citrus paradisi* for phytochemicals present.

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METHODOLOGY

Collection of Plants: The ripe fruit samples; *Musa acuminata*, *Malus domestica* and *Citrus paradisi* were obtained at the Anatomy Shopping Complex, University of Benin, Benin City on March 28, 2017. At the time of collection, the physical conditions of the fruit were intact.

Organoleptic evaluation of the samples: organoleptic properties; colour, shape, odour, taste and texture of the fruit samples were tested by a panel of 10 students and were certified to be in good conditions.

Preparation of extracts: The fruit samples were washed thoroughly and weighed individually. The outer surfaces of the fruits were peeled off, the pulp cut into smaller pieces, transferred separately into an electric blender and blended thoroughly to paste. The paste was transferred into a jar of known weight and filtered. Extracts obtained (filtrates) were stored in the fridge at 4°C for further analysis. An aliquot (1/3) of each extract was used for the phytochemical screening.

Phytochemical screening of the extracts: The phytochemical screening of the extracts was conducted using standard procedures described by Kokate et al. (2011) and Sumathy et al. (2011). Qualitative and quantitative assays were carried out for alkaloids, carbohydrates, reducing sugars, deoxy-sugars, saponins, phenolics, terpenoids, flavonoids, proteins, steroidal saponins and tannins.

- General tests for alkaloids: Dragendorff's reagent (2 drops) was added to 2 ml of the fruit extract. Again, 2 drops of Wagner's reagent was added to 2 ml of the extract. Formation of reddish brown precipitates indicated the presence of alkaloids. Also, 2 drops of Hager's reagent was added to 2 ml of the extract, formation of yellow precipitate connoted a positive result. Lastly, 2 drops of Mayer's reagent was added to 2 ml of the extract. Formation of milky precipitates indicated a positive result. The above tests were carried out differently on the aqueous extracts of the three fruits.

- Tests for carbohydrates: The Molisch test was used. To 2 ml of the extract, 2 drops of 1% alcoholic naphthol was added followed by the addition of 2 ml of concentrated sulphuric acid (conc. H_2SO_4) down the side of the test tube. A positive result was based on the formation of a purple ring at the interface of two layers of alcoholic naphthol and conc. H_2SO_4.

- Tests for reducing sugars: Fehling and Tollens tests were used to detect the presence of reducing sugars. About 2 ml of the extract was placed in a test tube and 4 ml of Fehling's A/B solution previously mixed were added. The resulting solution was boiled in a hot water bath for 3 minutes and observed for formation of a reddish brown precipitate.

  For Tollen's test, 2 ml of the extract was placed in a test tube, 2 drops of Sodium hydroxide (NaOH) solution was added in a drop-wise manner till the precipitate dissolves. Approximately, 2 drops of the extract was then added to the resulting solution. Formation of precipitate of silver mirror indicated a positive result.

- Tests for deoxy-sugars: Keller-kiliani test was used. To 2 ml of the extract, 2 drops of dilute acetic acid, 5% ferric chloride and conc. H_2SO_4 were added. Formation of violet rings at the interface of two liquids indicated a positive result.

- Test for saponins; Frothing test was conducted. The extract (1 ml) was diluted with 10 ml of distilled water and shaken vigorously for 1 minute. Observation of positive results was based on formation of persistent frothing that remains on standing.

- Test for steroidal saponins: The presence of steroidal saponins was evaluated via Lieberman-Burchard's test. The extract (2 ml) was placed in a test tube to which 1 ml of chloroform was added. Acetic anhydride (2 drops) was added to the mixture, followed by 2 drops of conc. H_2SO_4. Formation of purple rings in the chloroform layer indicates the presence of confirmed steroidal saponins.

- Test for tannins: Gelatin test was conducted. Here, 2 ml of 1% gelatin solution in 10% sodium chloride (NaCl) was added to 2 ml of the extract and observed for the formation of precipitates.

- Test for phenolic compounds: This was done via Ferric chloride and Folinciocalteu's tests. For the ferric chloride test, distilled water (5 ml) was added to 2 ml of the extract, ferric chloride (2 drop) was added to the resulting solution which was then observed for the formation of intense coloration. Folinciocalteu's test was conducted by adding 2 ml of folinciocalteu's phenol reagent to 5 ml of the extract, followed by the addition of 5 ml of 7% sodium carbonate (Na_2CO_3) to the resulting solution. Formation of intense purple colour indicated a positive result.

- Tests for flavonoids. Alkaline reagent, lead acetate and aluminium chloride tests were carried out to detect the presence of flavonoids. In an alkaline reagent test, 20% sodium hydroxide was added to 2 ml of the extract, followed by a few drops of dilute hydrogen chloride (HCl). Formation of an intense yellow precipitate which dissolves on adding dilute HCl confirmed a positive outcome. Lead acetate test was conducted by adding few drops of lead acetate to 2 ml of the extract and observed for the formation of a milky precipitate. Lastly, aluminium chloride test was performed by adding 0.1 ml of 1% aluminium trichloride and 1M potassium acetate to 3 ml of the extract and allowed to stand for 30 minutes. Formation of a yellow solution on standing indicated a positive result.

- Test for proteins: This was done via Xanthoproteic and Ninhydrin assays. For the first, 2 drops of concentrated nitric acid (conc. HNO_3) was added to 2ml of extract and observed for the development of yellow precipitates. In the latter, 2 drops of ninhydrin solution was added to 2 ml of extract and observed for the formation of a clear, yellow solution.

Quantitative analysis of phytochemicals: The amount of alkaloids in the fruit extracts was determined using the method of Harborne (1973). About 200 ml of 10% acetic acid in ethanol was added to 5 ml of the extract in a beaker and left to stand for 4 hours. The mixture was then filtered and the extract concentrated in a water bath to about 1/4 of its original volume. Concentrated ammonium hydroxide was added until precipitation was complete. The resulting solution was left to settle then the precipitate collected, washed properly with dilute ammonium hydroxide and filtered. The resulting residue is alkaloid which was then dried and weighed.

Quantity of tannins was determined by using a spectrophotometer as follows; 1 ml of the extract was mixed with 100 ml of distilled water in a beaker and stirred for 1 hour. The sample was filtered into a 50 ml volumetric flask and made up to mark, after which 5 ml of the filtered sample was pipetted out into a test tube and mixed with 2 ml of 0.1 M iron(III) chloride in 0.1 M HCl and 0.008 M potassium ferricyanide trihydrate (K4Fe(CN)6.3H2O). The absorbance of the sample was measured at 395 nm wavelength within 10 min via a spectrophotometer.

Determination of the amount of saponins was done as described by Sofowora, (1993). Exactly 20 ml of fruit extracts in a conical flask was mixed with 100 ml of 20% ethanol and in a water bath (about 55^{o}C) for 4 hours with continuous stirring. The mixture is then filtered and the residue re-extracted with another 200 ml of 20% ethyl alcohol. The combined extracts were heated in a water bath at 90^{o}C to reduce its volume to about 40 ml. Afterwards, the concentrate was transferred into a 250 ml separating funnel with the addition of 20 ml diethyl ether and vigorously shaken. The aqueous layer was recovered while discarding the diethyl ether layer and the purification process was repeated. 60 ml of n-butanol was then added and extracted twice with 10 ml of 5% sodium chloride. The solution left after extraction was evaporated in a water bath and the samples were oven-dried to constant weight.

For flavonoids, the method of Boham and Kocipai (1994) was used. 50 ml of 80% aqueous methanol was added to 2.5 ml of the extract in a beaker which was then covered and left to stand for 24 hours at room temperature. Afterwards, the supernatant obtained was discarded leaving the residues. Residue was extracted by washing three times in 50 ml of ethanol and filtered. The filtrate was then transferred into a water bath until the solution is evaporated into dryness. The sample is then weighed until a constant weight was gotten.

Statistical analysis: The quantitative data obtained were subjected to statistical analysis using one way Analysis of Variance (ANOVA). Percentage plots were done using graphpad software.

RESULTS/FINDINGS

Organoleptic properties: The result for the evaluation of the organoleptic properties of *Musa acuminata*, *Malus domestica* and *Citrus paradisi* is presented in Table 1.1.

Table 1.1. Organoleptic properties of *Musa acuminata*, *Citrus paradisi* and *Malus domestica.*

Properties	*Musa acuminata*	*Citrus paradisi*	*Malus domestica*
Colour	Milky	Yellow	Brown
Taste	Sweet	Sour	Sweet
Odour	Aromatic	Tangy	Fruity
Shape	Curved	Ellipsoid	Oblong
Texture	-	-	-

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Phytochemical screening

The result of preliminary phytochemical analysis is shown in Table 1.2. With the exception of reducing sugars, deoxy sugars and proteins, all other phytochemicals tested were detected in at least, one of the fruit samples.

Table 1.2. Phytochemical screening of the fruit extracts.

Phytochemical	*Musa acuminata*	*Citrus paradisi*	*Malus domestica*
Alkaloids	+	+	+
Carbohydrate	+	+	+
Reducing sugars	_	_	_
Deoxysugars	_	_	_
Saponins	+	+	+
Phenolics	+	+	+
Terpenoids	+	_	+
Flavonoids	+	+	+
Proteins	_	_	_
Steroidal saponin	_	_	_
Tannins	+	+	+

+ indicates presence of the component
- indicates absence of the component.

Quantification (in mg/g) of phytochemicals in the three fruits studied

The amounts of the most predominant phytochemicals (alkaloids, flavonoids, saponins, phenols and tannins) from the preliminary screening were further determined. Results show that alkaloid was lowest in *Malus domestica* with a concentration of 12.51 ± 0.12 mg/g and highest in *Musa acuminata* (70.49 ± 0.15 mg/g). Flavonoid was lowest in *Malus domestica* (2.54 ± 0.00 mg/g) but highest in *Citrus paradisi* (10.02 ± 0.01 mg/g). Saponin was lowest in *Malus domestica* (0.38 ± 0.01 mg/g) and highest in *Citrus paradisi* (0.45 ± 0.02 mg/g). Phenolic content was lowest in *Musa acuminata* (2.15 ± 0.01 mg/g) and highest in *Citrus paradisi* (7.11 ± 0.05 mg/g). Highest tannin content was obtained for *Musa acuminata* (6.03 ± 0.03 mg/g) and *Malus domestica* (2.28 ± 0.01 mg/g). Test of significance (2-tailed) showed that, only the differences in the amount of saponins obtained for the three fruit extracts were significant ($p \leq 0.05$) (Table 1.3). Furthermore, comparing the percentage occurrence of the phytochemicals in each fruit showed that, alkaloid was the highest occurring in *Musa acuminata* with 82.35% while saponins had the lowest with 0.49%. In *Malus domestica*, alkaloid was also the highest occurring with 53.44% while saponin was the least (1.62%). Similar result was obtained for *Citrus paradisi* (Fig. 1.1 to 1.3).

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Table 1.3. Quantitative phytochemical analysis of the fruit extracts (in mg/g)

Samples	Alkaloids	Flavonoids	Saponins	Phenols	Tannins
Musa acuminata	70.49 ± 0.15	6.51 ± 0.03	0.42 ± 0.02	2.15 ± 0.01	6.03 ± 0.03
Malus domestica	12.51 ± 0.12	2.54 ± 0.00	0.38 ± 0.01	5.74 ± 0.11	2.28 ± 0.01
Citrus paradisi	43.19 ± 0.10	10.02 ± 0.01	0.45 ± 0.02	7.11 ± 0.05	0.91 ± 0.00
F value	2.512	2.942	20.550	3.381	2.008
p	0.129	0.099	0.002	0.077	0.182

Results are expressed as mean value ± standard deviation

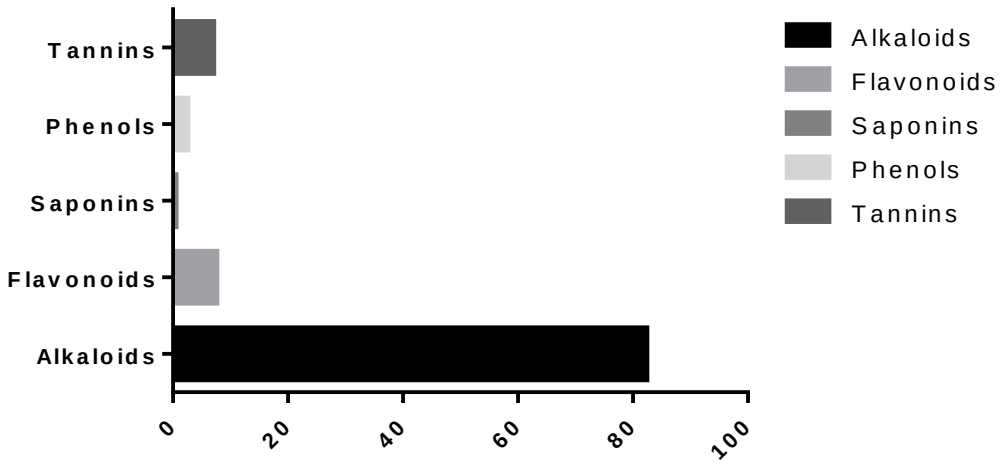

Fig. 1.1. Phytochemicals in *Musa acuminata* (expressed in %)

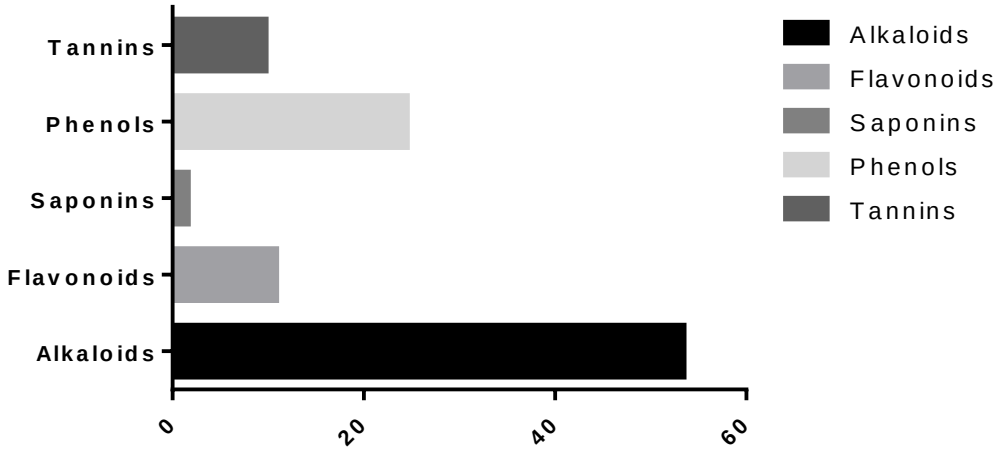

Fig.1.2. Phytochemicals in *Malus domestica* (expressed in %)

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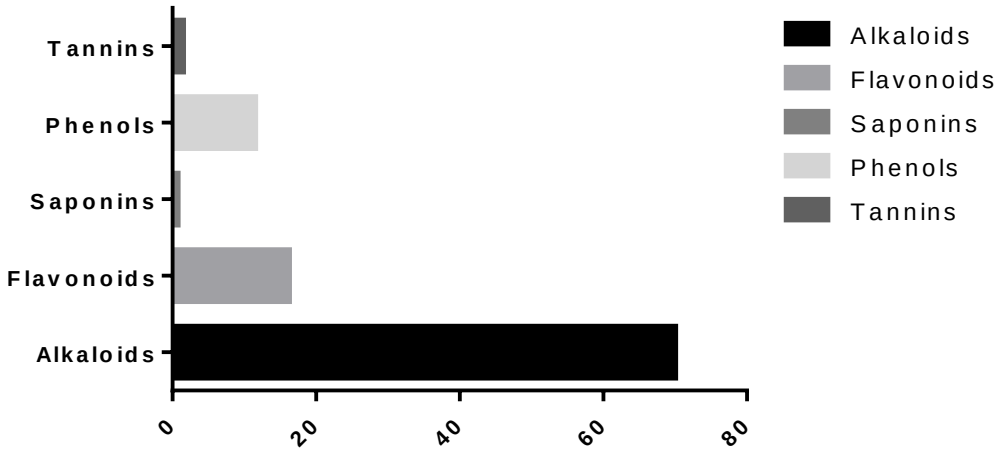

Fig.1.3. Phytochemicals in *Citrus paradisi* (expressed in %)

NB: Results reported are for the phytochemicals evaluated in this study

DISCUSSION

Many kinds of fruits have been reported to be good sources of phytochemicals. The qualitative phytochemical analysis conducted on aqueous extract of *Musa acuminata* revealed the presence of alkaloids, carbohydrate, saponins, phenolics, terpenoids, flavonoids and tannins. In general, secondary metabolites present in plants have been reported by Unuigbe et al. (2015) to be responsible for their therapeutic activity. Wang et al. (2017) reported that flavonoids are responsible for the antimicrobial activity associated with some ethno-medicinal plants.

The phytochemical analysis conducted on aqueous extract of *Malus domestica* revealed the presence of alkaloids, carbohydrate, saponins, phenolics, terpenoids, flavonoids and tannins. The medicinal value of the secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these substances include alkaloids, glucosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium for cell growth, replacement and bodybuilding (Kubmarawa et al., 2008).

The phytochemical screening conducted on aqueous extract of *Citrus paradisi* revealed the presence of alkaloids, carbohydrates, saponins, phenolics, flavonoids and tannins. These phytochemicals present in grape extract have been found to play protective roles against chronic degenerative diseases (Yang and Xiao, 2013). The phytochemicals including polyphenols, flavonoids and vitamins were found to be receiving more attention for research due to their beneficial impacts on human health (Onyema et al., 2016). Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention by acting as natural anticancer agents (Yildirim and Kutlu, 2015). Flavonoids serve as health promoting compounds and results in anion radicals (Wang et al., 2017). Phenols have been found to be useful in the preparation of some compounds such as dettol and cresol.

Alkaloids were detected in all the three fruit samples at a concentration ranging from 12.51 to 70.49 mg/g. However, highest concentration was recorded for *Musa acuminata* while *Malus domestica* had the lowest concentration. Alkaloids include; β-carboline, indole, oxindole, pyridine, piperidine, aporphine, lycopodium, methylxanthene, isoquinoline and by products of erythrine which function as: anti-oxidants; inhibitors of α-synuclein aggregation, acetylcholinestearase, butyrylcholinestearase, anti-amyloid and monoamine oxidase (MAO); agonists of dopamine, nicotine, n-methyl-d-aspartate (NMDA), muscarinic and adenosine receptors to ameliorate neurodegenerative disorders such as; Alzheimer's disease, Parkinson's disease, Huntington's disease, schizophrenia, epilepsy and stroke (Hussain et al., 2018).

Flavonoids possess great antioxidant effects and an inverse relationship with coronary heart disease has been reportedly associated with flavonoid intakes. Also, flavonoids have been shown to inhibit the growth of tumor and cancer cells (Ezeonu and Ejikeme, 2016). This research has shown that; *Musa acuminata, Malus domestica* and *Citrus paradisi* contain appreciable amounts of flavonoids. Flavonoids also play important roles as anti-bacterial, anti-viral, anti-inflammatory, anti-allergenic and hepatoprotective agents (Wang et al., 2017).

Tannin range was between 0.91 to 6.03 mg/g in the study. Tannins generally have antioxidant and antimicrobial activities in which they inhibit bacterial growth at low concentrations and at higher concentrations, inhibit fungal growth and activities (Sumathy et al., 2011).

Also, saponins ranged between 0.38 - 0.45 mg/g. Lowest concentration was for *Malus domestica* as against *Citrus paradisi*. This yield is observed to be higher compared to saponin yield of 0.075-0.25 mg/g in similar fruits studied by Aina et al. (2019), but much lower than yield of 8.6 to 19.90 mg/g reported by Koomson et al. (2018). Saponin is useful in treating fungal and yeasts infections, and also protects plants against microbial attacks (Sheik et al., 2013).

Quantitative analysis gave a range of 2.15 to 7.11 mg/g for phenolic content. Phenols are powerful antioxidants in humans and plants. Dietary intake of phenols has been reported to improve overall health status by fighting against diseases (Suarez et al., 2010). Comparative analysis of percentage phytochemicals in the fruit samples showed that; alkaloids, flavonoids and phenols were the most dominant in *Musa acuminata* and *Citrus paradisi* while alkaloids, phenols and flavonoids had the highest percentages in decreasing order in *Malus domestica*.

CONCLUSION

Secondary metabolites are present in various fruits extracts. While qualitative analysis indicates their presence or absence, quantitative analysis gives an approximate idea of the amount or concentration present. The outcome of the present study shows that, aqueous extracts of *Musa acuminata, Malus domestica* and *Citrus paradisi* comprises diverse phytochemicals at concentrations that offer various protective and therapeutic effects to the body. Hence, they can serve as alternative medicines to the synthetic ones or may be used as the bases of modern drugs in industrial (especially for pharmaceutical) applications in curing various diseases.

Future Research

Musa acuminata, *Malus domestica* and *Citrus paradisi* are widely consumed, and are rich sources of phytochemicals. The field of phyto-medicine has emerged as a new science with applications in pharmaceuticals as alternatives to synthetic medicines. Phytochemicals have been found to possess very strong antioxidant activity, decrease lipid oxidation, inhibit cancer cell proliferation and lower cholesterol; hence, they are being widely used in prophylactic and therapeutic regimens for cardiovascular disease, asthma, obesity, pulmonary dysfunction, cancer, diabetes, etc. While several studies have been done on the efficacy of phytochemicals in the prevention/ treatment especially of non-infectious diseases, more studies should be focused on their mechanisms of action and efficacies against infectious (bacterial, fungal, viral and parasitic) diseases.

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