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Spectroscopic screening of assorted pigmented vegetables and fruits common in metropolitan Kampala culinary recipes for anthocyanins

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Abstract
Pigmented ingredients of Kampala cookery recipes: beet root (*Beta vulgaris*), red onions (*Allium cepa*), red cabbage (*Brassica oleracea* var. capitata f. rubra), eggplant (*Solanum melongena* L.) and red dodo (*Amaranthaceae*) were screened for anthocyanins. Sliced 5.0±0.1g of leaves, soft parts and/or peelings of the samples were extracted with acidified methanol for 24 hours. Filtered extracts were concentrated to dryness and aqueous diluted 1ml of the extracts were analyzed by UV spectrophotometry. A scan from 200-600nm generated characteristic absorption spectra of each extract. Results showed that all the samples had anthocyanins except beetroot extract that had balantins with absorption peaks at 210, 220, 235, 255, 290, 480, 535 and 550nm. Red cabbage had maxima at 225, 240, 255, 290 and 555nm. The maximum absorbances of eggplant were observed at 210, 220, 245, 265 and 290nm while red dodo had maximum absorbances at 205, 215. 240, 250, 290 and 540nm. Red onions had absorbances at 220, 250, 270 and 290nm. Beet root is a rich nutritious source of balantins. Further research to identify and characterize the anthocyanins should be done.

Keywords: pigmented, vegetables, eateries, fruits, culinary

1. Introduction
Kampala, the capital city of Uganda has many hotels, cafes, resorts, cafeteria and restaurants serving up to half of the estimated 1.53 million populace [1]. These eateries serve tossed salads, snacks and quick foods in containment of pigmented fruits and vegetables such as carrots, beetroot, red onions, eggplants (ebiringanya), red dodo (embuga), cabbages, jack fruits (ffene), water melons, jambul, mangoes (emuyeme), red and purple fleshed potatoes (lumonde). Most pigmented fruits and vegetables contain health enhancive anthocyanins which are ubiquitous polar vacuolar family of pigments that contigent on their pH confers red, blue, purple and black coloration to tissues of higher plants such as leaves, stems, roots, flowers, tubers, fruits and bracts [2, 3].

Anthocyanins accumulate in the vacuoles of epidermal and/or subepidermal cells as an aqueous solution and have gained wide acceptance as man have consumed pigmented plants and plant parts from the beginning of time without any reported detrimental results. Thus, they are popularized substitutes for synthetic colorants which obviously are limitedly comestible. Anthocyanins are chemically polar glycosides of polyhydroxyl and polymethoxyl derivatives of 2-phenylbenzopyrylium or flavylium salts [4] with family members differing only in the number of hydroxyl groups present in the molecule, the degree of methylation of the hydroxyl groups, the nature, number and location of sugars conjugated to the molecule and the number and nature of aliphatic or aromatic acids attached to the sugars in the molecule [2]. They share a basic carbon skeleton in which hydrogen, hydroxyl or methoxyl groups can be in six distinct positions.

In kingdom plantae, 17 anthocyanins are known though only 6 predominate in higher plants [4] and these are differentiable by the number of hydroxyl groups present on the carbon ring and in the degree of methylation of these hydroxyl groups. The identity, number and position of the sugars attached to the carbon skeleton are rationally variable, with the common sugars linked to carbon-3, 5 and 7 being glucose, arabinose and galactose. Subsequently, monosides, biosides and triosides can be subtly differentiated. The chromophore for anthocyanins is anthocyanidin, sometimes called flavylium cation or aglycone (Fig. 1). Anthocyanins are biosynthetic equivalents of the other over 4,000 natural phenolic flavonoid compounds reported in teas, honeys, wines, fruits, vegetables, nuts, olive oil, cocoa and cereals, structurally characterized by a carbon skeleton of a $C_6C_3C_6$ (C15) aromatic unit with multipatterned substitutions. Anthocyanins are in essence sugar derivatives of anthocyanidins [5] synthesized via the phenylpropanoid pathway. The six predominant anthocyanins in higher plants are glycosides of cyanidin (Cy), pelargonidin (Pg), petunidin (Pt), delphinidin (Dp), malvidin (Mv) and peonidin (Pn) [5] (Table 1). Their structural configurations differ by glycosidic substitutions by either glucose, galactose, rhamnose, xylose, or arabinose at the 3 and 5 positions on the

A and C rings. Additional structural variations are possible through sugar group acylation with acids, usually acetic, *p*-coumaric, caffeic, malonic, sinapic, ferulic, oxalic and succinic acids. Pg, Cy and Dp are unmethylated and are most prevalent in Kingdom plantae with 80% in leaves, 69% in fruits and 50% in flowers [4].

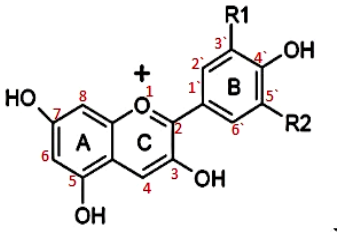

Fig 1: General structure of anthocyanins

In Fig.1, R_1, R_2 are usually OCH_3, H or OH, position 3 from oxygen on heterocyclic aromatized ring C is usually H or a glycosol, while position 7 in the biphenyl ring is usually a glycosol or OH. The molecules are typically more stable when complexed with metals [5].

Table 1: The 17 aglycones present in nature*

Aglycone	Substitutions around A, B and C aromatic rings							Colouration
	3	5	6	7	3` (R_1)	4`	5(R_2)	
Apigeninidin (Ap)	H	OH	H	OH	H	OH	H	Orange
Hirsutidin (Hs)	OH	OH	H	+OMe	OMe	OH	OMe	Bluish-red
6-Hydroxycyanidin (6OHCy)	OH	OH	OH	OH	OH	OH	H	Red
Luteolinidin (Lt)	H	OH	H	OH	OH	OH	H	Orange
Europinidin (Eu)	OH	OMe	H	OH	OMe	OH	OH	Bluish-red
Aurantinidin (Au)	OH	OH	OH	OH	H	OH	H	Orange
Tricetinidin (Tr)	H	OH	H	OH	OH	OH	OH	Red
Petunidin (Pt)	OH	OH	H	OH	OMe	OH	OH	Bluish-red
Capensinidin (Cp)	OH	OMe	H	OH	OMe	OH	OMe	Bluish red
Rosinidin (Rs)	OH	OH	H	OMe	OMe	OH	H	Red
Peonidin (Pn)	OH	OH	H	OH	OMe	OH	H	Orange-red
Pulchellidin (Pl)	OH	OMe	H	OH	OH	OH	OH	Bluish-red
Cyanidin (Cy)	OH	OH	H	OH	OH	OH	H	Orange-red
Pelargonidin (Pg)	OH	OH	H	OH	H	OH	H	Orange
5-Methylcyanidin (5-MCy)	OH	OMe	H	OH	OH	OH	H	Orange-red
Delphinidin (Dp)	OH	OH	H	OH	OH	OH	OH	Bluish-red
Malvidin (Mv)	OH	OH	H	OH	OMe	OH	OMe	Bluish-red

*Partly excerpted from [4], +OMe is chemically OCH_3.

Anthocyanins are pH indicators as their color changes with pH variations. They attract pollinators, disperse seeds and protect plant tissues from photoinhibition and oxidation from photosynthesis. With other flavonoids, they offer resistance to plant insect incursions [6] as demonstrated by protection of cotton leaves against tobacco budworm by cyanidin 3-glucoside [7] as well as deterring of aphids [8]. In combination, anthocyanins and 3-deoxyanthocyanidins play pivotal roles in flowering of angiosperms [4].

The ethnomedical uses of anthocyanins is extensively documented, with broad-spectrum health enhancive properties such as treatment of liver malfunction, hypertension, visual acuity [9, 10] probably by augmentation of rhodopsin regeneration, microbial infections, diarrhea among others [12-14]. They are also important in cognitive decline and neural dysfunction. Fruit extracts rich in anthocyanins reversed senescence-related deficits in various neural and behavioural parameters [15], albeit are reportedly more therapeutic in assimilated mixtures [16-18]. Reports reveal anthocyanins have antiulcer potential [19] and shielding effect warding off UV radiation [20]. Koide and co-workers [21] reported anti-tumor potential *in vitro* and *in vivo* of red soybeans extracts, dominated by cyanin conjugated with glucose and rhamnose. In point of fact, Wang and Mazza [22] in their detailed investigation concluded that anthocyanins possess an inhibition potential on diatomic nitric oxide synthesis, an innocuous synthesis implicated for chronic inflammations. Anthocyanins are used as colorants in food industry as safe and effective food additives [23] as the aqueous extracts contain potent polyphenolic antioxidant [24].

The accentuating volume of novel polyacylated anthocyanins with marked stability have proven to be of exploitative importance to food scientists. Anthocyanins have found use in dye-sensitized organic solar cells owing to their demonstrated ability to convert light into electricity [25] which confers multifaceted advantages such as lower purity requirements, abundance of component materials and ability to be produced on flexible substrates [26] as compared to p-n junction silicon cells.

Several studies have investigated anthocyanins. Maira and others [27] reported total anthocyanin content in *C. uvifera* pulp fruit and strawberry to be of comparable amount of 24.0mg/100g. Eva *et al.* [28] analyzed eggplant (*Solanum melongena L.)* and violet pepper (*Capsicum annuum L.*) peel extracts in duplicate by UV-vis spectrophotometry and detected anthocyanins and other antioxidants in the peels. Cis- and trans-isomers of nasunin, an anthrocyanin were isolated by Ichiyanagi *et al.* [29] from eggplant peel.

Anthocyanins carrying hydroxycinnamoyl moieties have been found to predominantly occur in their trans-form in flower petals [30, 31] and fruits. Sharma and Seshardi (in Mazza and Miniati) [32] reported the presence of an unusual anthocyanin (petunidin diglucoside) in four cultivars of chilli peppers grown in India. The most common eggplant anthocyanin nasunin (delphinidin-3-*p*-coumaroylrutinoside-5-glucoside) and its presence was first reported by Kuroda and Wada (in Mazza and Miniati) [32]. Nasunin occurs as optical stereoisomers: cis- and trans-isomers, and the latter is reportedly more stable. Contrastingly, only delphinidin-3-rutinoside and negligible delphinidin-3-rutinoside-5-glucoside were reported in Bulgarian eggplant [33] devoid of acylglycosides. Albeit, mutual anthocyanins-carotenoids co-occurrence in Solanaceae fruits has been reported from Tamarillo (*Cyphomandra betaceace*) [34] and tomato (*Lycopersicon esculentum* L.) by Jones *et al.* [35]. Two anthocyanins were separated and identified from purple cultivar egg plants (Zi Chang) by Yanjie *et al.* [36] using high-performance liquid chromatography–electrospray ionization tandem mass spectrometry.

A team of Agricultural Research Service scientists identified 36 anthocyanins in red cabbage, including eight that had never before been detected in the cabbage [37]. Jing and Giusti [38] extracted and identified anthocyanin from purple corn (*Zea mays* L.). Screening factorial design experiments was carried

out to investigate the extraction process of anthocyanins from red grape pomace with CO_2, along with either methanol or water as cosolvent, at high pressure by Mantell and co-workers [39]. The quantification of the total anthocyanins achieved via colorimetry yielded study results indicating a stiff influence of the type and percentage of cosolvent. Excellent results was with 20 mol% of methanol, 100 bar, 60 °C and a flow-rate of 22 mmol/min in two hours. Alexandra and co-researchers [40] analyzed red cabbages for anthocyanins. Red cabbage heads were lyophilized and mixed into powder samples and concentrated with acidified methanol extraction and acidified water reconstitution for assaying. Appreciable variation was noted for anthocyanin content among the genotypes ranging from 0-10.50mg/g in green control cultivars (dry weight, Cyanidin 3,5-diglucoside equivalent). Three genotypes were identified with a concentration of anthocyanins in excess of 9mg/g that represented concentrations significantly higher than the enumerated in any of the hybrid cultivars. Acylated anthocyanins accounted for over 75% of those identified, representing types that are more reasonably more stable than non-acylated ones under variable pH, temperature and light conditions.

Neda *et al.* [41] separated and purified anthocyanins from red cabbage by High-Speed Countercurrent Chromatography. The team employed a biphasic mixture of tert-butyl methyl ether/n-butanol/acetonitrile/water (2:2:1:5) acidified in trifluoroacetic acid. Anthocyanins in six small Chilean berries (arrayán, chequén, murta, calafate, meli and *Chilean blueberry* var. Brigitta) were accurately detected and identified using HPLC with UV-visible detection and high-resolution time of flight mass spectrometry by Anghel *et al.* [42]. The 31 anthocyanins identified in the six berries were mainly 3-*O*-glycoside conjugates and their derivatives.

Giusti *et al.* [43] reported anthocyanins from red radish (*Raphanus sativus*) which included Pelargonidin 3-O-[2-O-(b-glucopyranosyl)-6-O-(trans-p-coumaroyl)-b-glucopyranoside] 5-O-(6-O-malonyl-b-glucopyranoside),pelargonidin3-O-[2-O-(b-glucopyranosyl)-6-O-(trans-feruloyl)-b-glucopyranoside]5-O-(6-O-malonyl-b-glucopyranoside); pelargonidin 3-O-[2-O-(b-glucopyranosyl)-6-O-(trans-pcoumaroyl)-b-d-glucopyranoside] 5-O-(b-glucopyranoside) and pelargonidin 3-O-[2-O-(b-glucopyranosyl)-6-O-(trans-feruloyl)-b-gluco-pyranoside]5-O-(b-glucopyranoside). Elucidations of the 3-dimensional molecular conformation of the molecules using NOESY techniques revealed the closeness of the proton from cinnamic acid acyl group and C-4 of pelargonidin [44]. This study reported the spectroscopic presence of anthocyanins in some common vegetables and fruits: beetroot, red onions, red cabbage, eggplant and red dodo common in Kampala culinary recipes.

2. Materials and methods

2.1 Sample collection

1Kg of beet root, red dodo, red cabbage, egg plants and red onions (Fig, 2) were bought from Nakasero market, Nakasero Hill, Market square road, Kampala and washed. The leaves, soft parts and/or peelings of the samples were cut into small pieces using a stainless-steel knife prior to extraction.

a) Beet root (b) Red cabbage

(c) Eggplant fruits d) Red onions

(e) Red dodo (f) Extracted samples

Fig 2: The samples used in the study

2.2 Extraction procedure

Methanol was acidified with 5% acetic acid. To 1600mls of methanol, 8mls of 0.5% acetic acid was added and agitated for three minutes. 5.0±0.1g of the sample slices of beet root, red dodo (leaves and parts of the stem), red cabbage (leaves), eggplant (peelings) and red onions (scale leaves) were weighed into labeled 250ml conical flasks and the flasks were flooded with acidified methanol until the slices were covered completely. The flasks were sealed off with Aluminium foil (Hotpack, Kampala) and macerated for 24 hours. The crude extracts were obtained by filtration through Whatmann No. 1 filter paper. The filtrate was concentrated almost to dryness under reduced pressure at 30ºC using a rotary evaporator. Little distilled water (1ml) was added to the extracts to reduce the acid concentration. The concentrates were transferred to labeled sample bottles and placed in a deep freezer where they were kept awaiting spectroscopic analysis.

2.3 Spectroanalytical procedure

1ml of the acidified concentrates were separately diluted to make 100ml of extracts using ultrapure distilled water. A portion of the extracts were introduced into cuvettes and analyzed using Double beam optimal geometry Genesys 10S

UV-Visible spectrophotometer (Thermo Scientific, USA). A scan from 200 to 600nm (Ultraviolet to near Infrared region) was performed to generate the characteristic absorption spectra of the extracts.

3. Results

The absorbances of the extracts were read at the absorption maxima (Table 2).

Table 2: Absorption maxima of the methanolic extracts

Extract	UV-Visible absorption spectra													
	λmax_1	Abs_1	λmax_2	Abs_2	λmax_3	Abs_3	λmax_4	Abs_4	λmax_5	Abs_5	λmax_6	Abs_6	λmax_7	Abs_7
Beet root	210	0.087	220	0.066	235	0.074	255	0.088	290	0.088	480	0.129	535	0.141
Red cabbage	225	0.140	240	0.150	255	0.166	290	0.327	555	0.042	NA	NA	NA	NA
Eggplant	210	0.056	220	0.065	245	0.076	265	0.072	290	0.105	NA	NA	NA	NA
Red dodo	205	0.081	215	0.065	240	0.071	250	0.081	290	0.050	540	NA	NA	NA
Red onions	220	0.099	250	0.094	270	0.066	290	0.112	NA	NA	NA	NA	NA	NA

[+]NA-No Absorption peak, λmax = maximum absorption wavelength, Abs-Absorbance

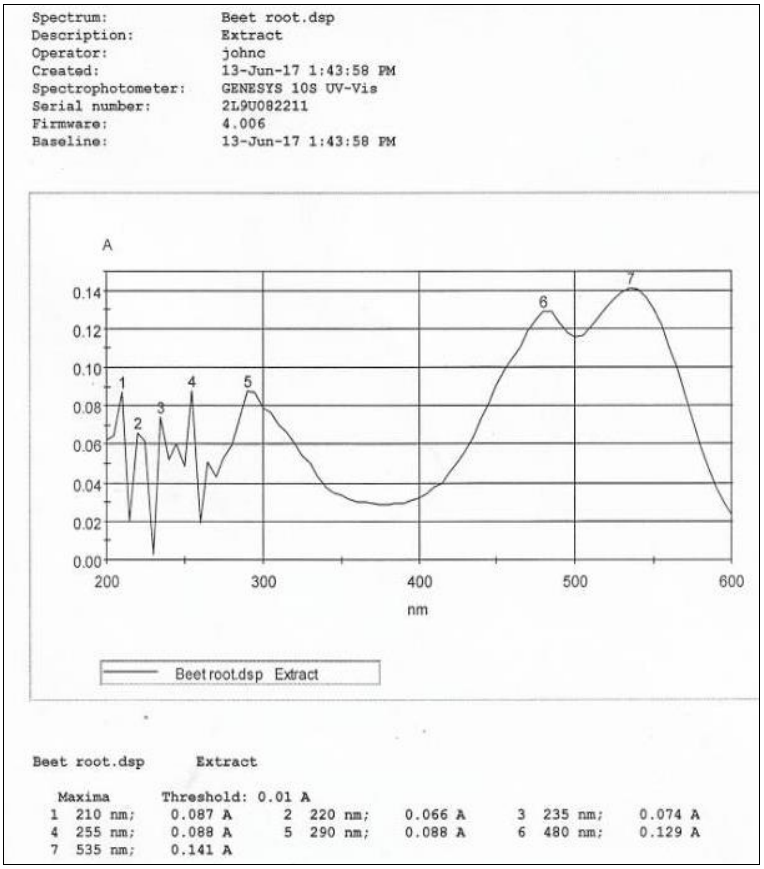

Fig. 3: Absorption spectrum for beet root extract

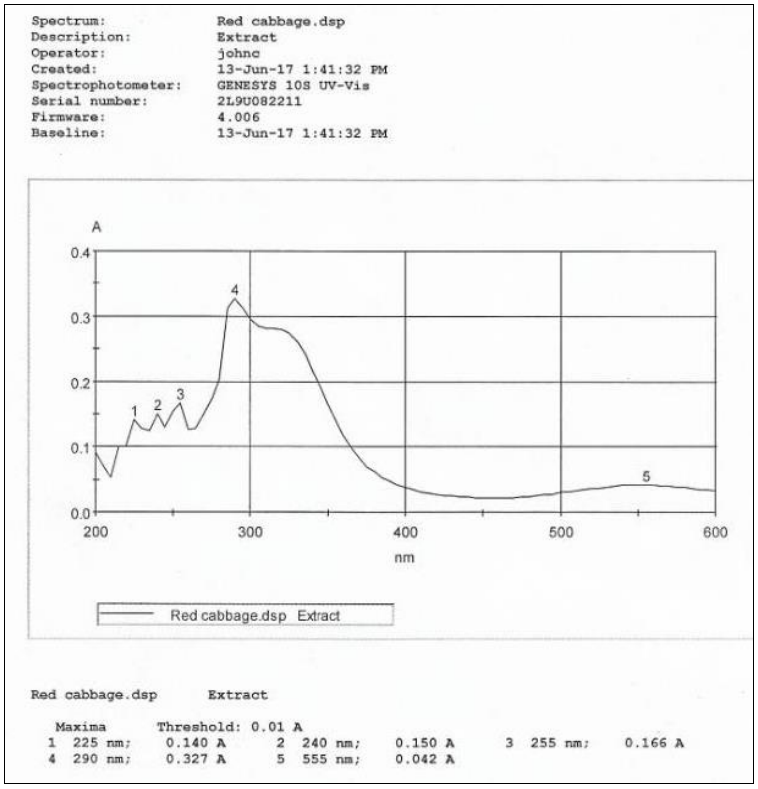

Fig 4: Absorption spectrum for red cabbage extract

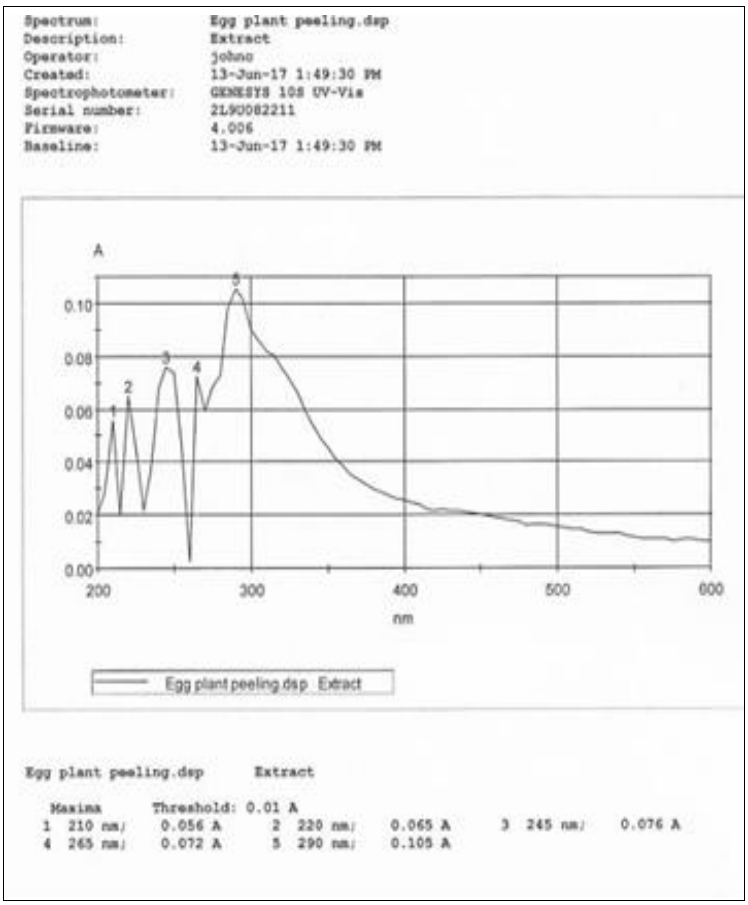

Fig 5: Absorption spectrum for egg plant

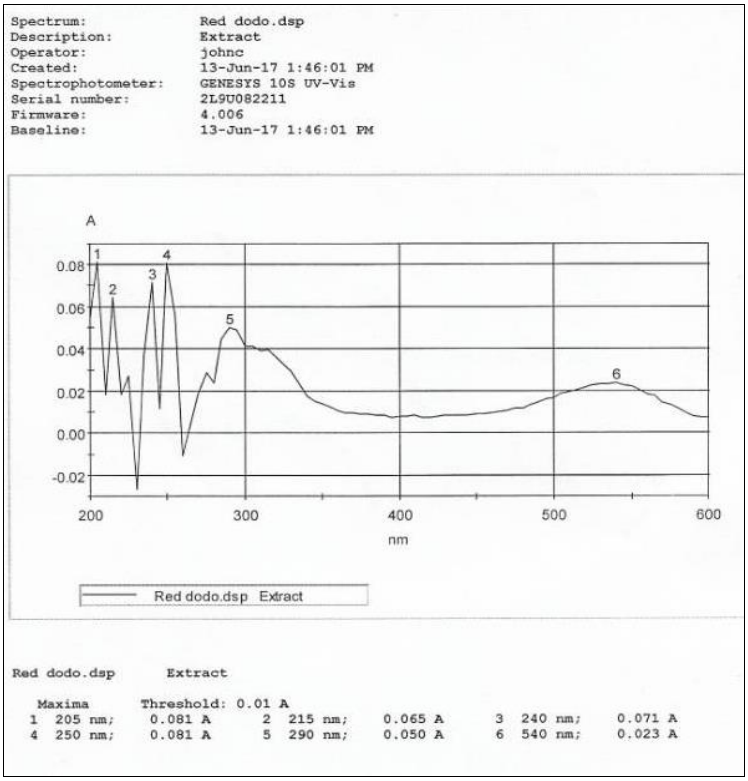

Fig 6: Absorption spectrum for red dodo extract

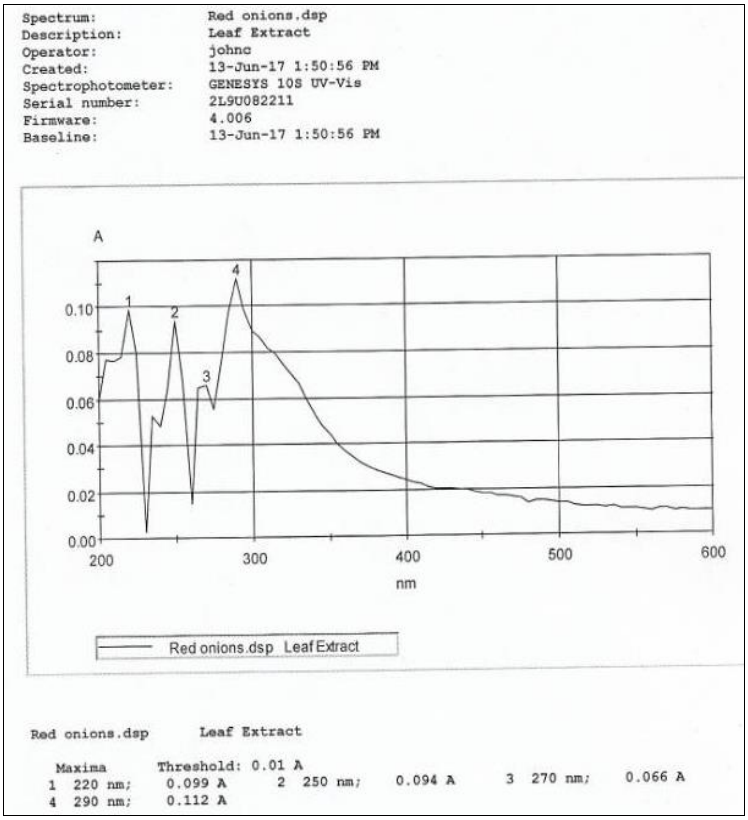

Fig. 7: Absorption spectrum for red onion extract

4. Discussion

Absorption spectra of anthocyanins from the vegetables and fruits were recorded between 200 and 600nm with a scanning UV-Visible spectrophotometer. Anthocyanins have two characteristic UV/Vis bands, band I in the 300 to 550nm range, arising from the B ring, and band II in the 240 to

290nm range, arising from the A ring. The results indicated that the pigment from beet root *(B. vulgaris)* extract has seven absorption peaks. The absorption peaks corresponds to that reported for balantins. Betalain pigments are characterised by a maximum absorbance at about 535 nm (λmax) for the red-purple betacyanins (betanin with λmax = 535 nm and betanidin with λmax = 542 nm) and near 480 nm for the yellow betaxanthins (indicaxanthin, the common betaxanthin found in red beet root with λmax = 482 nm); for the betalamic acid λmax = 424 nm [45, 46].

The emergence of two absorption peaks obtained from beet root extract showed the presence of balantins [47]. As a group of the flavonoids, anthocyanin has two cluster groups which provide local absorption at UV Vis absorption that is the benzoyl and sinamoyl groups. Eggplant *(Solanum melongena)*, red cabbage (*Brassica oleracea* var. capitata f. rubra), red dodo *(Amaranthaceae)* and red onions (*Allium acepa*) showed the maximum wavelengths between 200-290nm which is the region in which anthocyanins absorb. The expected aglycone and absorbance for red cabbage are pelargonidin at 530nm and betanin at 536nm. According to available empirical data, maximum wavelength in the spectra of the 3, 7-*o*-diglucoside, 3,7,3'triglucoside and 3,7,3',5'-*o*-tetra glucoside of delphinidin have been observed at 537nm, 525nm, and 521nm respectively and anthocyanins 5-o and 7-*o* glucosides seem to exhibit their maximum wavelength at slightly longer wavelengths 5 to 9nm than the corresponding anthocyanin 3,5-O-diglycosides and anthocyanin 3,7-O-diglycosides. Therefore, eggplant *(Solanum melongena)*, red cabbage (*Brassica oleracea* var. capitata f. rubra), red onions (*Allium acepa*) and red dodo *(Amaranthaceae)* which showed their maximum wavelength at around 290 nm indicated the presence of 3, 7, 3'triglucoside.

At acidic pH, anthocyanins exist as flavylium cations which absorb maximally in the green wavelengths between 500 and 550nm with negligible absorption in the red region [6, 47, 48]. Anthocyanins also have an absorbance maximum between 267 and 275nm [6] and when hydroxycinnamic acids are covalently linked to anthocyanins in the UV-B and short wavelength in UV-A regions (310-330nm) [49]**.** The UV-visible spectral data on anthocyanins typically measured in acidified methanol give important information about the nature of the aglycone and aromatic acyl groups. Anthocyanins with 4-o-glycoslation have recently been identified and now UV-Visible spectral data for anthocyanins having moieties connected to all the hydroxyl positions have been reported [50]. Anthocyanins with sugar units on the B-ring connected to the 3'-4'- or 5'-hydroxyl seem to have the visible maximum wavelengths 4 to 14nm than the corresponding anthocyanin 3- glycosides. It is well known that absorption spectra of pelargonidin 3-o-glycosides show higher absorbances at wavelengths around 440nm than found in the corresponding spectra of the other common anthocyanidin 3-o-glycosides.

5. Conclusion

From the absorption spectra of the selected vegetables and fruits commonly utilized in Kampala city culinary, it can be concluded that balantins were confirmed present in beet root extract while the rest of the fruits had anthocyanins. Further research should be done to screen other vegetables and fruits used in metropolitan Kampala culinary recipes with purple, red and blue pigments such as red apple (*Malus domestica*), watermelon (*Citrullus lanatus*), Jambul (*Syzygium cumini*), blue and red fleshed potatoes for presence of anthocyanins.

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