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Phytochemical constituents in edible parts of anchote (*Coccinia abyssinica* (lam.) (cogn.)) accessions from Ethiopia

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YA conceived idea, designed the study, collected data, prepared the manuscript. NR designed the study, supervised research. AM supervised research. KSK conceived idea, designed the study, prepared the manuscript. RKL supervised research, prepared the manuscript. GDH conceived idea, designed the study, supervised research, prepared the manuscript.

ABSTRACT

Anchote (*Coccinia abyssinica*) accessions from Ethiopia were tested for the presence of major phytochemicals using qualitative and quantitative methods. The qualitative tests were performed for 12 phytochemical compounds with 6 solvent extracts and water using standard methods. Quantitative analysis for important secondary metabolites included total phenols, total flavonoids, crude saponins and beta-carotene. Of the 12 phytochemicals tested, five compounds showed positive results for all the seven extracts in the tubers, whereas only two phytochemicals responded positively in the leaves. The water extract of anchote showed positive results for 11 of the phytochemicals while n-butanol showed positive results for six in both the tuber and leaf parts. The water extract also had the highest phytochemicals in both the tuber and leaf parts when compared to the other solvent extracts. Anchote leaf had the highest total phenol and flavonoid contents followed by the fruit and the least concentration was in the tuber for all the accessions. The leaf contained the highest percentage of saponins (27.65%) while tuber had the lowest (14.65%). The β-carotene content in five accessions of anchote leaf ranged from 25.9±0.03 to 35.2±0.16 µg/g. Anchote is popular in the Oromo and non-Oromo tribes in Ethiopia, because of its medicinal role. Owing to the various phytochemical compounds present in the plant further screening and identification of its active compounds is recommended.

Key words: *Coccinia abyssinica*, phytochemicals, qualitative test, quantitative analysis

INTRODUCTION

Plants are valuable sources of food and medicine for treating common and infectious diseases in a safe, effective and economic ways (Aliyu et al., 2008; Hossain et al., 2014). In addition to the nutritional value, the protective effects of plant species like fruits, vegetables, herbs, spices, pulses and minimally processed cereals and starchy plant foods provide some protection against the development of chronic illnesses such as cancer, cardiovascular diseases, type II diabetes, hypertension, cataract, and impaired cognitive function (Halvorsen et al., 2002). Because of their therapeutic effects plants are used to synthesize many useful drugs and about 80% of medicines are directly or indirectly obtained from the so called medicinal plants (Yadav et al., 2010). Natural products have long been a thriving source for the discovery of new drugs because of their chemical diversity (Selvam et al., 2014).

Metal chelation, free-radical scavenging, inhibition of cellular proliferation, modulation of enzymatic activity, antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, anti-mutagenic, and anticarcinogenic potentials are the basic functions which can be recognized in plant materials rich in phenolics (Aaby et al., 2004). Thus, their demand is increasing worldwide because of their protective nature against diseases and their ability to reduce oxidative degradation of lipids and thereby improve food flavour, colour and nutritional properties (Edeoga et al., 2005). To identify the bioactive chemical constituents belonging to different classes, it is essential to conduct a preliminary test to screen out different phytochemical constituents derived from plant sources.

Anchote [(*Coccinia abyssinica* (Lam.) Cogn.] is the only tuberous cucurbit belonging to the family *Cucurbitaceae* in the genus *Coccinia* (Girma and Hailu, 2007). It is an indigenous root and tuber crop widely produced in the south and southwestern parts of Ethiopia. It is also regarded as a medicinal plant due to the fact that it is used traditionally on people with bone-fracture, joint dislocation as well as on women during birth or lactation (Habtamu and Kelbessa, 1997; Girma and Dereje, 2015). Anchote is commonly produced for its tuber as food in the south and southwestern Ethiopia (Amare, 1973; Abera, 1995). The high medicinal value of the anchote tuber is possibly because of its high calcium and protein contents (Amare, 1973). A report by Dawit and Estifanos (1991) which suggested that the juice prepared from anchote tuber can be used to treat gonorrhea, tuberculosis and cancer with the assumption that the juice contains saponins as the active ingredient. However, a comprehensive study is required to prove such claims. In view of all the merits that anchote is used as a traditional medicine, it is imperative to investigate its phytochemical constituents for possible future commercial use.

Therefore, the aim of this study was to quantify the antioxidant compounds, namely total phenolics, total flavonoids and β-carotene contents, and to screen out major biochemical constituents including alkaloids, carbohydrates, glycosides, cardiac glycosides, flavonoids, steroids, coumarins, phenols, oxalate and saponins from different organic solvents extracts of anchote tuber, leaf and fruit.

MATERIALS AND METHODS

Sample collection and preparation

Different edible portions (leaves, fruits and tubers) of anchote were collected from the experimental field of Deberezeit Agricultural Research Center from November 2011 to January 2012 in Debrezeit, Ethiopia at their maturity stage and when ready for consumption. The samples were thoroughly washed with running tap water and rinsed with distilled water. The leaves were then air dried under a shade while the tubers and fruits were cut into small pieces and dried in an oven (DHG- 9055A, Memment, Germany) set at 105^{o}C until a constant mass was obtained. The dried samples were ground to a fine powder using an electric grinder (FW 100, Yusung Industrial Ltd, China) and sieved to pass through a 0.425 mm mesh. Finally, each powder sample was stored in a sealed airtight plastic bags, at 4^{o}C until analysis.

Extraction procedures
Extraction for qualitative test

The extraction for phytochemicals was executed according to the procedures described by Ugochukwu et al. (2013). The tuber and leaf samples of anchote were treated with various organic solvents for extracting and qualitative screening for phytochemicals in the Department of Food and Nutrition Laboratory, Chosun University, Republic of Korea. A 5-g powder sample was taken from 10 randomly selected accessions of the leaf and tuber and was dispersed in 50 ml of solvents, namely n-hexane, n-butanol, acetone, methanol, ethyl acetate, ethanol and water. The solutions were left to stand for two hours at room temperature, then boiled at 60^{o}C for 30 minutes and the supernatant was filtered with Whatman filter paper No. 1. Finally, the filtrate was centrifuged at 2500 revolution per minute (rpm) for 15 minutes, and the filtrates were used for the phytochemical screening.

Extraction for quantitative tests

Anchote fruit, leaf and tuber powder were extracted according to Barros et al. (2007) and Ferreira et al. (2007) with some modification. Each plant material (0.1g) was separately extracted in 20 ml of 75% methanol by stirring at 150 rpm at room temperature for 24 h using a temperature shaker incubator (ZHWY-103B). The supernatant was recovered and the residue was extracted two more times as described above. The resulting extracts were combined and filtered through a Whatman No. 2 filter paper. The combined methanolic extracts were evaporated at 40°C to dryness using a rotary evaporator (Rikakikai Co. Ltd., Tokyo, Japan), and adjusting the volume with methanol to 20 ml. The final extract was kept in a refrigerator at 4^{o}C until used for the determination of total phenol and flavonoid content.

Qualitative test of phytochemicals

For preliminary identification of phytochemical constituents, crude extracts of n-hexane, n-butanol, acetone, methanol, ethyl acetate, ethanol and water were screened for the detection of alkaloids, carbohydrate, coumarins, fatty acids, flavonoids, glycosides, oxalate, phenolic compounds, saponins, steroids, tannins and terpenoids according to standard procedures as follows:

Terpenoids (Salkowski's test)

Five ml of each extract was mixed in 2 ml of chloroform, and concentrated 3 ml of sulphuric acid was carefully added to form a layer. The formation of a reddish-brown coloration at the interface was considered a positive indicator for the presence of terpenoids (Edeoga et al., 2005).

Steriods

Steriods test was conducted according to Kumar et al. (2009). One ml of the extract was dissolved in 10 ml of chloroform, and an equal volume of concentrated H_2SO_4 was carefully added down the side of the test tube. The presence of steroids was confirmed by the change of the upper layer to a red color and H_2SO_4 layer to a yellow colour with green fluorescence.

Fatty acids

A sample of crude extract (0.5 ml) was mixed with 5 ml of ether and a filter paper was immersed into the mixture. The soaked filter paper was then evaporated and the

appearance of transparency on the filter paper was taken as an indicator for the presence of fatty acids (Savithramma et al., 2011).

Flavonoids

Two ml of extract was filtered using filter paper, then 5 ml dilute ammonia and 1 ml concentrated H_2SO_4 were added slowly and the development of a yellow color that disappears on standing was considered as an indicator for the presence of flavonoids (Ayoola et al., 2008; Alagesaboopathi and Sivakumar, 2011).

Tannins

To a 2-ml extract, 3 drops of 1% lead acetate were added and the formation of a yellowish precipitate was taken as an indication for the presence of tannins (Savithramma et al., 2011).

Alkaloids

A crude extract (2 ml) was treated with 3-5 drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and the formation of a reddish-brown precipitate or colouration was an indicator of the presence of alkaloids (Ugochukwu et al., 2013).

Carbohydrates (Molisch's test)

A few drops of Molisch's reagent were added to 2 ml of the extract followed by the addition of 2 ml of concentrated H_2SO_4 down the side of the test tube. The mixture was then allowed to stand for 2-3 minutes and checked for the formation of a red or dull violet colour at the inter-phase to indicate the presence of carbohydrates (Ugochukwu et al., 2013).

Phenols (ferric chloride test)

Two ml of the extract was treated with 5% aqueous ferric chloride and observed for formation of deep blue or black colour to confirm the presence of phenols (Ugochukwu et al., 2013).

Glycosides (Salkowski's test)

A 3-ml crude extract sample was mixed with 2 ml of chloroform, and then 2 ml of concentrated H_2SO_4 was added carefully and the mixture was shaken gently. The appearance of a reddish-brown colour was taken as an indicator for the presence of steroidal ring, i.e., glycone portion of the glycoside (Yadav and Agarwala, 2011).

Oxalate

To 3 ml of the extract, a few drops of glacial ethanoic acid was added. A greenish black colouration was checked to prove the presence of oxalates (Ugochukwu et al., 2013).

Saponins (foam test)

To 2 ml of the extract, 5 ml of distilled water was added. The mixture was shaken vigorously and observed for the appearance of a stable persistent froth on warming, as preliminary evidence for the presence of saponins (Abba et al., 2009).

Coumarins

To 2 ml of the extract, 3 ml of 10% NaOH was added and the formation of a yellow colour indicated the presence of coumarins (Savithramma et al., 2011).

Quantitative determination of phytochemicals
Determination of total phenols

Total phenol content of anchote leaf, tuber and fruit were determined by the Folin–Ciocalteu method (Singleton and Rossi, 1965). Diluted extract (40 µl) was added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 µl of saturated sodium carbonate (75 gm/l) was added. After 2 h of incubation at room temperature, the absorbance at 765 nm was measured. Gallic acid (G7384; Sigma-Aldrich) with the concentration range of 0, 1, 10, 25, 50, 100 and 250 mg/l were used for the standard calibration curve (Absorbance = 0.0024 catechin µg - 0.0010, R^2 = 0.9989). The results were expressed as gallic acid equivalent (GAE)/gm sample on dry weight basis and calculated as mean value ± SD (n = 3).

Determination of total flavonoids

Total flavonoid was determined by a colourimetric method as described in Xu and Chang (2007). A 0.25 ml of the extract was mixed with 1.25 ml of deionized water and 75 µl of a 5% $NaNO_2$ solution was added. After 6 min, 150 µl of a 10% $AlCl_3.6H_2O$ solution was added to the mixture. The mixture was incubated at room temperature for 5 min, after which 0.5 ml of 1 M NaOH and 2.5 ml of deionized water were added. The mixture was then thoroughly vortexed and the absorbance of the pink colour was measured at 510 nm against the blank. For the calibration curve (+)-catechin was used with concentrations of 10, 20, 40, 60, 80 and 100 mg/l (absorbance = 0.0037 catechin mg - 0.0008, R^2 = 0.9984). Results were expressed as mg (+)-catechin equivalent (CE)/g of extract.

Determination of crude saponins

Crude saponin determination was done according to Obadoni and Ochuko (2002) as cited by Edeoga et al. (2005). A 20g sample was mixed with 100 ml 20% aqueous ethanol in a conical flask. The mixture was then heated over a hot water bath at 55°C for 4 h with continuous stirring. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250-ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously until the aqueous and ether layers were separated. The aqueous layer was collected and the ether layer discarded. The purification process

was repeated until no more layer formation was observed. To the collected aqueous extract 60 ml n-butanol was added and washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was evaporated in a water bath and dried in a convection oven at 100^{o}C until constant weight. Finally, the saponin content was calculated as a percentage.

Determination of beta carotene

Beta carotene content of anchote leaf was determined following the method described by Zakaria et al. (1979). A 2-g sample of leaf was extracted repeatedly with petroleum ether until the residue was colourless. The extract was decanted into a separating funnel and washed repeatedly with distilled water to remove the aqueous-acetone phase. The upper petroleum ether layer was collected, dried over anhydrous sodium sulphate. The petroleum ether phase was transferred to a drying flask and evaporated to dryness in a rotary vacuum evaporator. The dried residue was then dissolved in 1 ml of petroleum ether and eluted using chromatographic column on neutral alumina. β-carotene eluted using petroleum ether was collected in a flask and the volume was measured using a measuring cylinder. The optical density (OD) was read using UV/visible spectrophotometer at 450 nm. Finally, β-carotene content was calculated with the following formula:

$$\beta \text{ carotene } (\mu g/g) = ((A \times V(ml) \times 104))/(A1\%_{1cm} \times W)$$

where:
A = Absorbance
V (ml) = Volume of the solution that gives an absorbance of A at a specified wavelength
$A1\%_{1cm}$ = Absorption coefficient of carotenoid in solvent used PE is 2592
W = Weight of sample in gram.

Statistical analysis

The average of triplicate measurements was analyzed using one-way analysis of variance (ANOVA). Means were compared by Duncan multiple range test (DMRT) with mean square error at 5% probability using SAS, 2004 version 9. Mean ± standard deviation was used to express the data.

Table 1. Phytochemical screening of leaf and tuber parts of anchote (*Coccinia abyssinica*) in different solvents

Leaf extracts (n=10)							
Phytochemicals	Water	Methanol	Ethanol	Acetone	Ethyl acetate	n-hexane	n-butanol
Alkaloids	+	-	-	+	+	+	-
Carbohydrate	+	+	+	+	+	-	-
Coumarins	+	+	+	+	+	+	+
Fatty acids	+	+	-	+	+	-	-
Flavonoids	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	-	+
Oxalate	-	-	-	+	+	+	+
Phenolic compounds	+	+	+	+	-	-	-
Saponins	+	+	+	-	-	-	-
Steriods	+	+	+	-	-	+	+
Tannins	+	+	+	+	+	+	-
Terpenoids	+	+	+	+	-	+	+
Tuber extracts (n=10)							
Alkaloids	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+
Coumarins	+	+	+	+	-	-	+
Fatty acids	+	-	-	+	+	+	-
Flavonoids	+	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+
Oxalate	-	-	-	-	-	-	-
Phenolic compounds	+	-	-	-	-	-	-
Saponins	+	+	+	-	-	-	-
Steriods	+	+	+	-	-	-	-
Tannins	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+	+

+ = present; – = absent; n = number of samples used in each test

RESULTS

Qualitative phytochemical screening

The qualitative test for phytochemicals in the leaf and tuber of Anchote are presented in Table 1. All the 12 tested phytochemicals were present in the leaf sample. Coumarins and flavonoids gave a positive result in all seven extracts, and glycosides, tannins and terpenoids were found in six of the extracts. Of all the tested phytochemicals, presence of saponins was positive only in water, methanol and ethanol extracts. In the case of the tuber, the presence of alkaloids, carbohydrate,

glycosides, tannins and terpenoids was observed in all seven extracts, whereas coumarins were present in five of the extracts, fatty acids in four and saponins and steroids were present in three of the seven extracts. Flavonoids and phenolic compounds were present only in the water extract and oxalate was absent in all the extracts.

Quantitative analysis of phytochemicals

Total phenols

The total phenol (TP) contents of the anchote fruit, leaf and tuber were expressed as gallic acid equivalent (GAE) in mg/g. The results showed that average TP content of fruit, leaf and tuber of anchote in the tested accessions varied significantly ($p < 0.05$) with values ranging from 3.02±0.86 to 59.90±0.56 mg GAE/100 g sample in the tuber and leaf, respectively (Table 2). Mean fruit TP concentrations ranged from 14.50±0.06 mg GAE/g to 57.33±0.03 mg GAE/g in which the accession encoded 'NJ' presented the highest TP concentration, whereas accession '223093' was found to have the lowest TP content.

Total flavonoids

Total flavonoid contents of anchote fruit, leaf and tuber are shown in Table 3. The total flavonoid content of anchote leaf in the tested accessions was found to be superior to the fruit and tuber samples with the value ranging from 14.28±3.38 to 19.23±3.27 mg CE/g. This was followed by fruit samples ranging from 1.65±0.27 to 4.16±1.80 mg CE/g, and tuber samples with the least total flavonoids content ranging from 0.41±0.21 - 0.63±0.17 mg CE/g). From all tested accessions the highest concentration of total flavonoid was exhibited in the 'DIGGA-1' leaf sample, whereas the lowest concentration was observed in the 'NJ' tuber sample.

Table 2. Total phenol contents in anchote (*Coccinia abyssinica*) fruit, leaf and tuber extract (dry weight)

Accessions	Parts used	Total phenolic (mg GAE/g)
90802-1	Fruit	19.03±0.21^{b}
223093	Fruit	14.50±0.06^{c}
NJ	Fruit	57.33±0.03^{a}
229702	Fruit	15.06±0.37^{c}
223090-1	Leaf	26.42±0.03^{e}
223109-1	Leaf	58.04±0.13^{b}
DIGGA-1	Leaf	46.61±0.73^{c}
240407-1	Leaf	59.90±0.56^{a}
KICHI	Leaf	36.02±0.88^{d}
223085	Tuber	3.66±0.32^{d}
223086	Tuber	30.02±2.12^{a}
223087-1	Tuber	8.66±1.65^{c}
223097	Tuber	3.02±0.86^{d}
NJ	Tuber	19.71±0.32^{b}

Means followed by different superscript letters in the same column of the same plant part are significantly different ($p< 0.05$); Data are mean±SD of triplicate measurements (n=3); GAE: Gallic acid equivalent

Table 3. Total flavonoid contents in anchote (*Coccinia abyssinica*) fruit, leaf and tuber extract (dry weight)

Accessions	Parts used	Total flavonoid (mg CE/g)
90802-1	Fruit	1.65±0.27^{b}
223093	Fruit	4.16±1.80^{a}
NJ	Fruit	1.94±0.20^{b}
229702	Fruit	1.88±0.64^{b}
223090-1	Leaf	14.28±3.38^{a}
223109-1	Leaf	15.74±2.15^{a}
DIGGA-1	Leaf	19.23±3.27^{a}
240407-1	Leaf	17.99±3.45^{a}
KICHI	Leaf	18.87±2.45^{a}
223085	Tuber	0.63±0.17^{a}
223086	Tuber	0.47±0.20^{a}
223087-1	Tuber	0.61±0.16^{a}
223097	Tuber	0.59±0.09^{a}
NJ	Tuber	0.41±0.21^{a}

Means followed by different superscript letters in the same column of the same plant part are significantly different ($p< 0.05$); Data are mean±SD of triplicate measurements (n=3); CE: Catechin equivalent

Table 4. Percentage of saponin content from crude aqueous extracts of anchote leaves and tubers (dry weight)

Accessions	Parts used	Saponin (%)
90801	Leaf	27.65±0.27a
223087-1	Leaf	25.20±0.11b
223090-1	Leaf	24.95±0.08^{b}
223104	Leaf	16.54±0.06^{d}
DIGGA	Leaf	22.39±0.10^{c}
223110	Tuber	14.65±0.05^{c}
229702-1	Tuber	15.10±0.06^{c}
220563-1	Tuber	16.23±0.10^{b}
230565	Tuber	17.42±0.06^{a}
240407-1	Tuber	14.57±0.05^{c}

Means followed by different superscript letters in the same column are significantly different ($p< 0.05$); Data are mean±SD of triplicate measurements (n=3)

Crude saponins

The content of saponins in anchote leaf and tuber ranged from 16.54±0.06 to 27.65±0.27% and 14.65±0.05 to 17.42±0.06%, respectively (Table 4). Anchote leaf contained the highest percentage crude yield of saponins (27.65%) while the lowest yield was recorded in the tuber (14.65%).

Table 5. Content of beta-carotene in the leaf part of anchote accessions

Accessions	Parts used	β-carotene (µg/g)
223087-1	Leaf	33.1±0.19[a]
223090-1	Leaf	30.7±0.16[ab]
223109-1	Leaf	35.2 ±0.16[a]
DIGGA-1	Leaf	25.9±0.03[b]
KICHI	Leaf	34.9±0.36[a]

Means followed by different superscript letters in the same column are significantly different ($p < 0.05$); data are mean±SD of triplicate measurements (n=3)

Beta carotene

As shown in Table 5, the β-carotene content of anchote leaf in the tested five accessions ranged from 25.9±0.03 to 35.2±0.16 µg/g.

DISCUSSION

Qualitative phytochemical screening

In the qualitative screening test, diverse results were observed in different solvents for both the leaf and tuber. The presence or absence of phytochemicals in one or another solvent provides a very important clue in understanding their polarity and also helps in selection of appropriate solvent systems for separation of pure compounds (Gujjeti and Mamidala, 2013). According to the same authors, the selection of appropriate solvent systems for particular plant extracts could be known from the retention factor values of the compounds in different solvent systems using thin layer chromatographic studies. Tiwari et al. (2011) and Ugochukwu et al. (2013) also reported that successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure, and this observation was validated in the current study. According to Tiwari et al. (2011) there are factors affecting the choice of solvent such as quantity of phytochemicals to be extracted, rate of extraction, diversity of compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and potential health hazard of the extractant.

In the preliminary phytochemical evaluation of the current study, water extracts revealed a positive result in eleven of the twelve tested phytochemicals with the exception of oxalate which was absent in both leaf and tuber extract. This showed that water is more effective as a solvent than the other solvents used in this study. Comparing the general phytochemical composition of the leaf and tuber, a wider percentage of phytochemicals was observed in the leaf (73%) than that of the tuber (62%). Our result suggests that anchote leaf and tuber possess several known and unknown bioactive compounds, which may be a potential source of useful drugs. Thus, by isolating and identifying these bioactive compounds, useful products can be formulated to treat various diseases and disorders. In addition, the consumption of the leaf may also improve the health status of the consumers due to the presence of various compounds that have been reported to be vital for good health.

In the present investigation the phytochemical composition of the leaf and tuber may be indicative of possible medicinal properties in these anchote extracts. Alkaloids, one of the tested phytochemicals represent a class which affects the central nervous system, reduces appetite and behaves as a diuretic; carbohydrates, coumarins and glycosides are known to exert a beneficial action on the immune system by increasing body strength, hence are considered valuable dietary supplements (Yadav et al., 2014). Coumarins may be beneficial for hyperproliferative skin diseases on the basis of their antimicrobial and anti-inflammatory effects (Theis and Lerdau, 2003). Glycosides also have vast therapeutic efficacy as they are found in almost every medicinal plant. Tannins have amazing stringent properties and are known to hasten the healing of wounds and inflamed mucous membranes. Flavonoids are used as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity (Salah et al., 1995; Benavente-García et al., 1997). In addition, it helps in managing diabetes induced oxidative stress (Yadav et al., 2014). Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess anti-microbial, anti-fungal, anti-parasitic, anti-viral, anti-allergenic, anti-spasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties (Wagner and Elmadfa, 2003; Rabi and Bishayee, 2009;). Moreover, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well (Sultana and Ata, 2008). Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells (Okwu, 2004). Steroids are responsible for cholesterol-reducing properties and also help in regulating the immune response (Shah et al., 2009).

Quantitative analysis of phytochemicals
Total phenols

Our values are higher than fruit TP content reported by Petridis et al. (2012) for some olive cultivars which ranged from 8.03 to 17.96 mg/g FW. On the other hand, the values of TP content reported by Kähkönen et al. (1999) for berries which were in the range of 12.4±0.6 - 50.8±1.0 mg/g GAE were found to be within the range of our result. Accession '240407-1' (59.90±0.56 mg GAE/g DW) was found to have the highest, and '223090-1' (26.42±0.03 mg GAE/g DW) the lowest TP content in leaf. The value in anchote leaf were comparably lower than the TP content in leaf extracts of *Adhatoda vasica* which varied from 63.95±2.1 to 92.4±0.14 mg/g (Maurya and Singh, 2010) but higher than different herb extracts with values ranging from 9.1±0.8 to 23.1±0.8 mg/g GAE (Kähkönen et al., 1999). The TP content in *Moringa oleifera* leaf extracts were found to be within the range of the result obtained in

leaf (Sreelatha and Padma, 2009). In the tuber the highest level of TP was found in accession '223086' (30.02±2.12 mg GAE/100 g), while the lowest was in '223097' (3.02±0.86 mg GAE/100 g). The TP content in anchote tuber was higher than the sweet potato genotypes with distinctive flesh color (Teow et al., 2007). In general, the TP content in anchote was found to be higher than the TP content reported for cereals (0.2±0.0 - 1.3±0.1 mg/g GAE) and vegetables (0.4±0.0 - 6.6±0.1mg/g GAE) (Kähkönen et al., 1999). In this study anchote leaves revealed higher TP concentration followed by fruits in all the tested accessions. This is in agreement with the scientific evidence that stated groups of secondary plant metabolites, antioxidant phenolics, and flavonoids are commonly found in various fruits, vegetables and herbs and they have been shown to provide a fruitful defense against oxidative stress from oxidizing agents and free radicals (Hossain and Shah, 2015). Phenolic compounds are a class of antioxidant agents which act as free radical terminators and primarily responsible for the hydrophilic antioxidant activity (Teow et al., 2007).

Total flavonoids

Flavonoids are the most important group of secondary metabolites and bioactive compounds in plants that may function as effective natural antioxidants in human diet (Kim et al., 2003; Ndhlala et al., 2010). Some of the proven biological activities of flavonoids include antimicrobial, antiviral, anti-inflammatory, anti-allergic, vasodilatory effects and inhibition of lipid peroxidation (Cook and Samman, 1996).

The highest concentration of total flavonoid compared to anchote leaf in this study was observed in mature leaf extract of *Moringa oleifera* (27±0.03 mg CE/g), but the tender leaf extract of *Moringa oleifera* (15±0.02 mg CE/g) was found to be in agreement with the result obtained in our study (15.74±2.15 mg CE/g) in leaf of accession '223109-1' (Sreelatha and Padma, 2009). On other hand Atanassova et al. (2011) had reported lesser content of total flavonoid for the medicinal herbs of lemon balm (0.45 mg CE/g), sage (0.28 mg CE/g) and mint (0.25 mg CE/g) compared to the leaf samples in our study. The reported values of total flavonoids by Marinova et al. (2005) for blue berries fruit (190.3 mg CE/100g = 1.903 mg CE/g) were in close agreement with the result obtained for anchote fruit of accessions 'NJ' (1.94±0.20) and '229702' (1.88±0.64) in the present study. As discussed by the author blue berries were the richest source of flavonoids. However, all the other fruit species tested by same authors have lesser flavonoid concentration compared to anchote fruits in the tested accessions.

Crude saponins

Saponins are present in different plant parts such as root, tuber, bark, leaf, seed, and fruit. Anchote leaf contained the highest percentage crude yield of saponins (27.65%) and this is comparable with leaves of *Anredera cordifolia* (Binahong) (28.14±0.22%) (Astuti, 2011). Minimum yield was recorded in tuber (14.65%) and was similar to the reported value by Unekwu et al. (2014) for wild edible Nigerian mushroom species known as *Cantharelle cibarius* (150.41±0.50 mg/g = 15.04%).

The result obtained in this study was higher than the reported values for medicinal plants from Nigeria (1.12±0.22 - 3.92±0.11%) (Edeoga et al., 2005), leaf and stem of *Andrographis neesiana* (1.05±0.10 - 3.40±0.80%) (Alagesaboopathi and Sivakumar, 2011), seeds of selected weed plants (0.48±0.06 - 1.29±0.03%) (Abbas et al., 2012), and stems of *Anredera cordifolia* (Binahong) (3.65±011%) (Astuti, 2011). However, it is lower than tuber saponin content of *Anredera cordifolia* (Binahong) (43.15±0.10%) (Astuti, 2011). Saponins comprise a large family of structurally related compounds containing a steroid or tri-terpernoid glycoside (Wina et al., 2005). Saponin were reported to have a wide range of beneficial pharmacological properties, such as producing inhibitory effect on inflammation, precipitating and coagulating red blood cells, having anti-diabetic, anti-tumorigenic and antiviral activities (Just et al., 1998). Saponins also have characteristic such as foam formation in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Okwu, 2004). According to Dini et al. (2009) the qualitative and quantitative saponin composition of food plants can vary considerably due to factors such as variety/cultivar, geographic effects/climate, season, stage of maturity and plant part used.

Beta carotene

Carotenes contain mainly β-carotene, the main precursor of vitamin A, which constitutes about 80% of carotenoid and it has antioxidant potential and anti-cancer activity and other health benefits including the protection against cardiovascular disease or cataract prevention (Bohm et al., 2002). β-carotene is also involved in cell differentiation, synthesis of glycoprotein, reproduction and overall growth and development (Vimala et al., 2011).

The result of the present study revealed that accession 'DIGGA-1' had the least β-carotene content which varied significantly ($p < 0.05$) from those of three accessions ('223087-1', '223109-1', 'KICHI') while no significant difference was observed with accession '223090-1'. Our result is higher than the reported β-carotene content for white-fleshed sweet potato (0.18 µg/g), pumpkin (578 µg/100 g) and tomato (365 µg/100 g) but lower than that found in carrot (6769 µg/100 g) and dark orange-coloured sweet potato clones (167 and 226 µg /g fw) (Teow et al., 2007; Tee and Lim, 1991). β-carotene is ubiquitously present in green leafy and yellow-orange fruits and vegetables and its content can be influenced by the growing conditions, maturity index, post-harvest handling conditions, as well as variety or cultivar.

CONCLUSION

Phytochemicals screened in anchote plant parts in this study pointed to the potential of the plant as a

pharmaceutical raw material. Therefore, an in-depth investigation is vital to provide concrete information through further isolation, identification, and characterization of the phytochemicals. Phytochemicals screened in the different plant parts of anchote showed the potential of the plant to be used as an ingredient in pharmaceutical industries. In addition, this study has confirmed the importance of choosing the solvents in the extraction procedure has great effect on successful extraction (quantity, rate, diversity and easiness) of the biologically active compounds from different parts of the plant material. Further study is required to identify the inhibitory compounds and toxicity of the solvent in the bioassay process and potential health hazard of the extractant.

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CONFLICT OF INTEREST

None.

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