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Comparison of Antioxidant, Flavonoid and Polyphenol Content of Three Selected Solanaceae Genera from Kigezi, Southwest Uganda

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Abstract

Solanaceae is family of plants widely used in food, sauce and herbal medicine because its members are rich in carbohydrates, proteins, fats, vitamins, mineral salts and other trace and essential nutrients like phenols and flavonoids that retard degenerative diseases and stress. Knowledge on purposeful use of solanaceae in food and medicine in Uganda is low. Aqueous extracts of dry leaves of three selected solanum genera growing in Kabale were compared for their polyphenol and flavonoid contents and antioxidant properties. Total polyphenol (TPC) and total flavonoid (TFC) contents were determined by the Folin-Ciocalteu and aluminium chloride colorimetric methods respectively. Antioxidant properties and radical scavenging were determined using DPPH, hydrogen peroxide, thiocyanate-iron (III) complex and iron (II)/(III)-linoleic acid systems. The polyphenol content of dry leaves of *S. anguivi* was 1750 ± 0.70, that for *S. macrocarpon* was 104 ± 0.45 and *S. nigrum* was 97.80 ± 0.15 GAE/g yet the flavonoid content was 7.40 ± 0.30, 35.00 ± 0.60 and 16.40 ± 0.40 mg/QE/g for *S. anguivi*, *S. macrocarpon* and *S. nigrum* respectively. The DPPH scavenging at IC_{50} were 7.80 ± 0.25; 45.60 ± 0.30 and 42.90 ± 0.20 respectively yet hydrogen peroxide scavenging at IC_{50} stood at 6.89 ± 0.15; 27.00 ± 0.35 and 17 .90 ± 0.20 µg/mL in the respective order for *S. anguivi*, *S. macrocarpon* and *S. nigrum*. The available data suggests the plants are very good food supplements of high nutritive and chemotherapeutic values. However, there is need to perform in vivo and vitro experiments to deduce their efficacy on mammals.

Keywords: Antioxidants, Scavenging, *S.anguivi*, *S. macrocarpon*, *S. nigrum*, Polyphenols, Flavonoids, Free radicals, Gallic acid, Quercetin, Hexacyanoferrate (III), Thiocyanate, Absorbance.

INTRODUCTION

Surviving on this planet earth requires overcoming diseases like cancer, stroke, and cardiovascular disease. This can only be achieved by tapping resources from nature like fruits, leaves and roots of plants in form of alternative medicines. By late 1990's many scientists had isolated bioactive compounds from barks, fruits, leaves and roots of herbs, shrubs and trees making plants very valuable for life of humans and essential on daily basis in human deity [1, 2].

Quantitative determination of curative compounds in the bark, fruits, leaves and roots may help alleviate potency of diseases. Presence of polyphenols, terpenes, vitamins, flavonoids, saponins, minerals salts and tannins in barks, fruits, leaves and roots of various herbs, shrubs and trees provides chance to provide alternative nutrients, food complements and supplements. Knowledge about dietetics indicates that good human diet requires varying quantities of fruits, grains, legumes, vegetables and this provides chance not to suffer cancer, diabetes, obesity and heart diseases [3].

The study on solanum genera has been on going in many parts of the world because of their food, nutrient, medicinal and anti-nutrient values. In Uganda, three Solanum genera. *S. terminate Forssk., S. incanum L. and S. nigrum* L was cited in literature by the 1970's [4] and a few years later some data was produced on the three species S. anguivi, S. aethiopicum L. and S.

nigrum [5] and later classification on the entire solanum family was documented [6, 7] Leaves of Solanum macrocarpon, gboma eggplant were reported to have high nutritional value because they contain fat, crude fiber, protein, calcium, and zinc[8] and are source of methionine [9, 10].

Several authors have reported anti-inflammatory, antiasthmatic, antiglaucoma, hypoglycemic, hypolipidemic, and weight loss effects of green leafy vegetables [8, 10, 11]. Such pharmacological properties resulted from presence of polyphenols [12], and flavonoids [10], and these act as strong antioxidants. It has been reported that consumers demand for items rich in antioxidants. Polyphenols and flavonoids present among solanum genera are on high demand as health supplements because when added to food they increase the nutritional value through providing natural antioxidants. The food processing industries sell fortified food stuff after addition of complements containing flavonoids and polyphenols extracted from plants. Consumption of food rich in antioxidants lowers rate of formation of free radicals in the body as well as overcoming peroxidation of lipids, this improves nutritional value of food and increases its quality. Synthetic antioxidants like butylated hydroxytoluene, butylated hydroxyanisole and propyl gallate are common food additives that increase shelf life, their use has increased the toxicity potential and carcinogenic effects, hence natural materials serve better purpose.

There is growing need for natural antioxidants in the food market as they have minimum side effects [10]. Good human health hinges on a balance of minerals, carbohydrates, fats, proteins and vitamins in diet. However, balancing the deity may not overcome effects of stress and degenerating diseases [13].

The finding of naturally occurring ingredients in plants that are curative prompted isolation and identification of compounds present in medicinal and non-medicinal herbs, shrubs and trees. The isolation of tannins, flavonoids, phenols, saponins, terpenes and cyanides have advanced the study on the chemotherapeutic nature of plants eaten as vegetables or/and used in herbal medicines While industrialists started manufacturing the equivalent compounds that were found curative and occur in plants, the natural forms still show better performance as they have minimum side effects.

Hence the study on plant materials is ongoing and is likely not to come to the end soon as longer as the human race continues to survive on Earth because herbal medicine is as old as the time man has lived. Among substances sought for from plant materials are flavonoids, steroids, mineral salts, phenols, vitamins, tannins, terpenes, are metabolites that make health better. Flavonoids are secondary plant metabolites with antioxidant activity.

The antioxidant tendency of flavonoids was reported to depend on the number and position of free hydroxyl groups [14]. Literature revealed that a total flavonoid content (TFC) for leaves of *Cassia* tora was 21.53 mg of quercetin equivalent per gram (QE/g) that was extracted to methanol [15]; that for *Solanum nigrum* was 0.64 mg catechin equivalents per gram fresh weight in the water extract [16] (Adebooye *et al*., 2012) and the TFC of 49.2 ± 3.4 mg rutin equivalents per gram dry weight in the methanol extract of *P. oleracea* [17]. It was further reported that genetic, biological, environmental, seasonal and year-to-year variations significantly affected the flavonoid content of vegetables [15].

Polyphenols are constituents present in plants that possess redox properties which are useful in antioxidant activity [18] and it was shown that the hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. Total phenol content (TPC) of many herbs and shrubs are reported in literature from different parts of the world. While TPC of 56.8 ± 5.9 mg GAE/g fresh weight of *A. sessilis* and 36.4 ± 6.1 mg GAE/g fresh weight of *I. aquatica* were observed in acetone–water–acetic acid extracts [19]; that for *C. tora* was 180.64 ± 6.51 mg GAE/g in water extract [20] and that in *P. oleracea* was 3.6 ± 0.089 mg GAE/g dry weight in the methanol extract. The concentration of phenols in plant extracts varies with amounts of sugars, carotenoids or ascorbic acid, or the duration, geographical variation and/or methods of extraction [21].

Free radicals are highly reactive species bearing uncompensated electron. Environmental pollution, X-radiation, toxins, physical stress, deep fried foods, exposure to chemicals have been linked to production of free radicals. Changes in gene expression, production of abnormal proteins and depletion of immune antioxidant system are some of the reported bad effects of free radicals in living systems. Asthma, arthritis, cancer, cell damage, atherosclerosis, cancer, stroke are some of the diseases caused by rapid rise in formation of free radicals in living systems [22]. However, the generated free radicals can be removed from the body through the antioxidant defense mechanisms.

Other documented bad effects of free radicals like damage to carbohydrates, proteins, lipids, nucleic acids, degradation of chemicals and making foods go rancid may have an advantage to alleviate pollution arising from overstay of materials dumped at a place [23]. The cardiovascular diseases, diabetes, cancer and inflammatory diseases are reported to be linked to free radicals in man (Gupta and Sharma, 2006) [24]. Free radicals can cause synthesis of abnormal proteins [25]. Exposure to ozone, cigarette smoke, air pollution,

chemical pollution significant increase free radicals in human bodies [26].

Reactive oxygen species (ROS) represents the major type of free radicals in the biological systems and are produced through the electrons transfer chains in the mitochondria [22].

Glutathione peroxidase (GPx)/oxidized glutathione reductase (GSSGRD), actions of non enzymatic substances like glutathione and vitamin E, and prooxidants are responsible for the generation of reactive oxygen and nitrogen species (ROS/RNS) such as superoxide anion (O_2^-), hydroxyl radical ($HO^\bullet$), hydrogen peroxide (H_2O_2), peroxyl radical ($ROO^\bullet$), singlet oxygen (O), nitric oxide ($NO^\bullet$), peroxynitrite ($ONOO^-$), and other free radicals [10, 11].

Natural or synthetic materials that remove uncompensated electrons from free radicals have been referred to as antioxidants. An antioxidant donates an electron to a free radical and counteracts its effects. Oxidative injury and the degenerative diseases can be overcome by scavenging for the free radicals by the antioxidants and this protects human body from injury [27].

Synthetic polyphenols bind to metals and retard oxidation process [28]. The challenges with feeding on synthetic polyphenols include the antinutritional and promotion of liver damage and carcinogenesis, so there is need to replace them with naturally occurring antioxidants in overcoming side effects of using synthetic antioxidants in treating oxidative stress [18, 28].

Consumption of food rich in antioxidants can overcome some degenerative diseases, oxidative damage and lipid peroxidation [10]. There is a lot of ongoing research on antioxidants for their use as dietary supplements and adjutants for use in therapeutic treatment of disorders caused by free radicals. Many medicinal plants used in traditional medicine are known as significant sources of natural antioxidants.

Different plants have attracted more attention for their efficiency against cancer, atherosclerosis, cerebral cardiovascular events, diabetes, hypertension and Alzheimer's disease [29]. Natural antioxidants are very efficient in blocking the process of tissue oxidation by free radicals and reactive oxygen species. The antioxidative and pharmacological properties of medicinal plants are related natural antioxidants such as flavonoids, tannins, coumarins, curcuminoids, xanthones, polyphenols, and terpenes.

They are found in various plant products such as herbs and spices like rosemary, thyme, oregano, sage, basil, pepper, clove, cinnamon, and nutmeg and plant extracts such as tea and grapeseed [30, 31]. Although the traditional use of plants in chemotherapeutics result from antioxidant potential of the plants, there is always no scientific evidence of the antioxidant property of the selected plants until scientists study them in detail.

The use of folk medicine at the primary health care level is widespread in Uganda. In most cases, folk medicine is usually the first choice for most patients suffering with cancers and various types of inflammations. Similarly, wild and home-grown vegetables are used in making sauce for food in many homes in the villages and towns of Uganda without knowledge on their effective contribution in the health system of the body. Most such beverages belong to the solanum genera like potatoes, tomatoes, eggplants and many herbs.

A balance between free radicals and the antioxidative defense system is important for proper physiological functions. However, the antioxidants that are produced naturally by the body may not be enough to prevent the ROS-induced damages. Therefore, antioxidant complements are important to boost the body's own capacity to reduce or counteract the oxidative damages [32].

Antioxidants are micronutrients that can either directly scavenge free radicals or prevent the generation of ROS and are present in fruits, roots and leaves. When eaten in suffice quantities they modulate metabolism, prevent degenerative and chronic diseases [33].

Thus the chemotherapeutic nature of plants is based on chemical substances present in its leaves, roots or/and stem that perform a specific physiological effect in man. Experimental results from solutions of fruit of Solanum macrocarpon revealed presence of very useful metabolites like flavonoids, saponins, alkaloids, phenols, phytates, tannins, cyanides, terpenoids and steroids in decreasing order of abundance [34, 35].

Hydrogen peroxide (H_2O_2) stimulates cellular proliferation [36], or differentiation [37]. It forms in biological systems by oxidizing enzymes such as superoxide dismutase [38]. However, aberrant accumulation of H_2O_2 is responsible for reactions, which may lead to pathological conditions like cancer, diabetes, and cardiovascular diseases [39, 40].

This happens because hydrogen peroxide decomposes rapidly generating hydroxyl radical (•OH) that initiates lipid peroxidation and damage of cell organelles [41] (Saed-Moucheshi *et al*., 2014). Retardation of production of hydrogen peroxide by plant antioxidants is of high interest in biological research. 2,2-diphenyl, 1-picryl hydrazyl (DPPH), is free radical which readily accepts electron or free radical. When DPPH takes up an electron the absorption band at wavelength of 515–528 nm

disappears. So concentration of DPPH can be followed by observing intensity of peak at wavelength of 517nm [42].

The DPPH was used to evaluate the radical scavenging capacity of plant extracts [43]. The antioxidants are the components of the plants which are capable of enacting the quenching of the stable purple-colored DPPH radical to the yellow-colored DPPH[44]. The ability to transform iron (III) ions in hexacyanoferrate (III) to iron (II) ions acts indicator for antioxidant activity [45].

In the ferric reducing antioxidant power (FRAP) assay, the yellow color test solution changes to green and blue [46, 47].The reaction taking place is:

$$Fe^{3+} + e \longrightarrow Fe^{2+}$$

This monitored through measuring color at 700 nm [48]. The FRAP is concentration-dependent [49]

Lipid peroxidation produces lipid hydroperoxides, which may transform to lipid alkoxyl or lipid peroxyl radicals. The radicals derived from fatty acids cause cellular damage and degenerative diseases [50]. Lipid hydro-peroxides are stable at room temperature, but they are decomposed to radicals by heat, UV light or by transition metals [51].

Although *Solanum nigrum* contains toxic solanine, it is also a reservoir of antioxidants having hepatoprotective, anti-tumor, cytostatic, anti-convulsant, anti-ulcerogenic and anti-inflammatory effects [52]. The glycoalkaloid, solanine in *Solanum nigrum* unripe berries make them too toxic [53].

The consumption of *Solanum macrocarpon* fruits may be a hazard because it was found to contain .hydrolysable tannins which in large doses decrease rate of growth in laboratory animals. Incidence of esophageal cancer, was associated with consumption of foods rich in tannins, suggesting that tannins may be carcinogenic [10]. The nutrients in the leaves suggests analgesic, anti-inflammatory properties and an increase in the potential for disease resistance and stress. S. macrocarpon contains solanidine and solasodine which are toxic alkaloids.Leaves treat cholesterol disorders [10]. Vegetables contain carbohydrates and reducing compounds so it is necessary to have a varied diet rich in vegetables. The leaves contain saponins which are important dietary and nutritional reserves and treat infections in the trachea.

Solanum anguivi Lam berries contain many pharmacological substances like gallic tannins and alkaloids, sterols and polyterpenes, polyphenols, flavonoids, catechin tannins, quinones, saponins and coumarins [54]. The chemical and nutritional analyses of plants have been studied in many parts of the world with fruits and vegetables taking the lead [55-59].

In West Africa, the fruits of *Solanum anguivi* are served to nursing mothers, the young, the aged and anemic patients [60]. The roots are used as carminative and cough expectorant, nasal ulcers, asthma, difficult parturition, toothache, cardiac disorder, worm expeller, nervous disorder and fever [61]. The phytochemical properties of *S anguivi* was reported [62, 63] and cholesterol lowering properties of saponin extracted from the fruits of *S. anguivi* has been documented [64]. The phytochemical composition of *S. anguivi* grown in Nigeria was investigated and showed presence compounds which are bioactive secondary metabolites supporting its medicinal values [65]. Traditionally *Solanum anguivi*, Gnagnan treats diarrhea, malaria and prostate diseases.

S, anguivi L berries are characterized by bitterness due to presence of various phelolic compounds like polyphenols, flavonoids, tannins [66] and *S. anguivi*.berries were reported to have the highest content in polyphenols compared to some edible plants in Iran and India [67]. Common phenols in plant extracts are flavonoids and phenolic acids [68].

The interest of this study is to quantify and compare content of flavonoids and phenols in leaves of *S.anguivi, S. macrocarpon* and *S. nigrum* extracted in water and also determine antioxidant potential for these vegetables growing in Kigezi since such work does not appear anywhere in literature.

MATERIALS AND METHODS

Geographical location of Kabale

Kabale is located the south western part of Uganda in the Rift valley region near the border with Rwanda.

Sampling

Leaves were picked from the plants whose photographs are shown below.

The appearance of the selected solanum species in their habitat is shown in Figure 1 below.

Figure 1a: Appearance of S. *macrocarpon*

Figure 1b Appearance of *S. nigrum*

Figure 1c: Appearance of *S. anguivi*

The chemicals used in the present study were ascorbic acid (ASC), 2, 2-diphenyl, 1-picryl hydrazyl (DPPH), sodium nitroprusside, sulphanilic acid, sodium chloride, ferric chloride, disodium hydrogen orthophosphate, potassium dihydrogen phosphate, and Folin-Ciocalteu reagent. All chemicals and solvents used were of AnalaR grade.

EXPERIMENTAL

Preparing extracts

The leaves from the three genera were dried at room temperature separately and coarsely ground before extraction. A known amount of powder (250 g) was extracted at room temperature for 24 h by percolation using water (600 ml, 70/30 v/v). The extract was separated from the residue by filtration through Whatman number 1 filter paper. This procedure was repeated thrice and the resultant extract was concentrated on a rotary evaporator under high vacuum and the residue was then freeze-dried for complete solvent removal. The final extract was stored at 4 °C in an airtight container for future use.

Determining polyphenol content

The total polyphenol content was determined for individual extracts using the Folin–Ciocalteu method [19].

The final extract (1mL) was mixed with 10% (w/v) Folin–Ciocalteu reagent and left to stand for 5 minutes. 75% sodium carbonate solution (2.0mL) was added and mixture incubated at 50 °C for 10 minutes with intermittent agitation. After which the absorbance of the mixture was determined wavelength of 765 nm against pure methanol as blank using ultra violet spectrophotometer. The experiment was repeated thrice. The data obtained was used to calculate mg/g of gallic acid equivalents in milligrams per gram (mg GAE/g) of dry extract.

Determining flavonoid content

The flavonoid contents of individual extracts were measured as per the colorimetric Dowd method [69].

Portion of final extract (1.0 ml) was mixed with 10% aluminium chloride solution (0.2 ml) in methanol together with 1M potassium ethanoate solution (0.2 mL) and distilled water (5.6 mL). Then the mixture was incubated for 30 minutes at room temperature. At end of 30 minutes the absorbance of the mixture was read off from a spectrophotometer at wavelength 415 nm against the blank. The experiment was repeated thrice.

The results were used to compute mg/g of quercetin equivalents in milligrams per gram (mg QE/g) of dry extract.

DPPH radical scavenging activity

The radical scavenging activity of the crude extracts was adopted to measure antioxidant activity using the colorimetric DPPH method at wavelength of 517 nm [70, 71].

Portion of final extract (2.0 mL) was added to 0.1mM 2, 2-diphenyl, 1-picryl hydrazyl (DPPH) solution (2.0 mL). The mixture was kept in a dark room for 30 minutes. At end of 30 minutes, the absorbance of the mixture was determined spectrophotometrically at wavelength, λ_{max} 517 nm against an equal amount of DPPH and methanol as a blank. The experiment was repeated thrice.

The percentage of DPPH• scavenging (RSA %) was estimated using the equation:

$$\% \text{ scavenging of DPPH}^{\bullet} = \left[\frac{Ao - At}{Ao}\right] \times 100 \quad \ldots\ldots\ldots\ldots\ldots 1$$

Where A_0 = absorbance of the control and At = absorbance of the test extracts.

Hydrogen peroxide scavenging experiment

The radical scavenging activity of individual extracts was determined using the hydrogen peroxide method [72].

Portion of final extract (2.0 mL) was added to 2mM hydrogen peroxide solution (4.0 mL), solution of phosphate buffer of pH 7.4 (1.0 mL) was added and the mixture was allowed to stand for 10 minutes. At end of 10 minute interval absorbance of mixture was determined spectrophotometrically at maximum wavelength of 230 nm against the phosphate buffer solution as blank.

The percentage scavenging of H_2O_2 was calculated using the equation:

$$\% \text{ scavenging of } H_2O_2 = \left[\frac{Ao-A1}{Ao}\right] \times 100 \quad \ldots\ldots\ldots\ldots 2$$

Where A_0 = absorbance of the control (phosphate buffer with H_2O_2 and A_1 = absorbance of the test extracts.

Ferric reducing antioxidant power (FRAP) experiments

The reducing powers of the individual extracts that reflected their antioxidant activity were determined using the modified Fe^{3+} to Fe^{2+} reduction assay (Hu *et al*., 2016).

Portion of the final extract (1.0 mL) was added to 0.2M sodium phosphate buffer solution (2.5 mL), followed by 1% (w/v) potassium hexacyanoferrate (III) solution (2.5 mL). The mixture was placed in a vortex machine for agitation while being incubated at 50 ˚C for 20 minutes. At end of 20 minutes, 10% (w/v) trichloroacetic acid (2.5 mL) was added and the mixture was placed in a centrifuge running at 3000 rpm for 10 minutes. The supernatant solution (2.5 ml) was mixed with deionized water (2.5 mL) and 0.1 % (w/v) iron (III) chloride solution (0.5 mL). Perl's Prussian blue color was measured at maximum wavelength λmax 700 nm against a blank.

Preparing linoleic acid emulsion

Linoleic acid (0.5608 g), Tween 20 emulsifier (0.5608g) and 0.2M phosphate buffer of pH 7.0 (100 mL) was mixed and homogenized.

Preparing iron (III) thiocyanate

10L ethanol (75%), 0.2 mL ammonium thiocyanate (30% w/v), and 0.2 mL iron (II) chloride (2 mM $FeCl_2$ in 3.5 % HCl)

Ferric thiocyanate (FTC) in a linoleic acid system

The antioxidant activity of selected plant extracts was determined by a linoleic acid system as described as follows.

Portion of the final extract solution (1.0 mL) was dissolved in ethanol (5.0 mL) was mixed with linoleic acid emulsion (5.0 mL). The reaction mixture was incubated in the dark room at 37 ˚C. for 20 minutes. At end of 20 minute period, to the mixture is added 75% ethanol (10 mL), iron (III) thiocyanate solution (0.4 mL). The resulting mixture is used to determine absorbance at wavelength of 500 nm using a spectrophotometer as compared to blank made of linoleic acid emulsion (5 mL) and phosphate buffer (5 mL).

Phytochemical screening

Samples were sun-dried, pulverized and passed through a sieve (about 0.5 mm pore size) to obtain a fine dry powder. Aqueous extract of the sample was prepared by soaking 100 g of the powdered samples in 200 ml of distilled water for 24 hours. The extracts were filtered using Whatman filter paper No 42 (125 mm). Chemical tests were carried out on the aqueous extract and on the powdered samples to identify the constituents using standard procedures. Color intensity was used to categorize the presence of each phytochemical substance.

Test for Alkaloids

Crude powder (0.5 g) was defatted with 5% ethyl ether for 15 minutes. The defatted sample was extracted for 20 min with hydrochloric acid (5 mL) on a boiling water bath. The resulting mixture was centrifuged for 10 minutes at 3000 rpm. The filtrate (1 mL) was treated with few drops of Mayer's reagent and another portion (1 mL) was reacted with Dragendroff's reagent and turbidity was observed [73, 74].

Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% aqueous iron (III) chloride solution was added and observed for brownish green or a blue-black coloration.

Test for terpenoids: (Salkowski test):

The extract (5 mL) was mixed with chloroform (2 mL) and concentrated sulfuric acid (3 mL) was added cautiously to form a layer. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids.

Test for cardiac glycosides (Keller-Killani test)

The extract (5 mL) was treated with glacial acetic acid (2 mL) then ferric chloride solution (1 drop) was added. This was followed by concentrated sulfuric acid (1 mL). A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for steroids

Acetic anhydride (2 mL) was added to ethanolic extract of the sample (0.5 mL) followed by sulfuric acid (2 mL). The color changed from violet to blue or green in some samples indicating the presence of steroids.

Test for saponins

Powdered sample (2 g) was boiled in distilled water (20 mL) in a water bath and filtered. The filtrate (10 mL) was mixed with distilled water (5 mL) and shaken vigorously for a stable persistent froth. The frothing was mixed with olive oil (3 drops) and shaken vigorously, then observed for the formation of emulsion.

Test for phytosterol

The aqueous extract (5 mL) was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and extracted with ether. The ether layer was evaporated and the residues were tested for the presence of phytosterol. The residue was dissolved in diluted acetic acid (5 drops); then added acetic anhydride (2 mL) followed by concentrated sulfuric acid (.4 drops). A bluish green color indicates the presence of phytosterol.

Test for Ascorbic acid:

Iodine (0.5 g) was dissolved in 1% freshly prepared potassium iodide solution (100 mL). Iodine solution (1 drop) was added to 0.1% starch solution (1 mL) and left to stand. Aqueous extract of the sample was added drop by drop until the blue-black color of the starch iodine complex disappears leaving a colorless solution. The colorless solution indicates the presence of ascorbic acid.

Statistical analysis: In this study excel version 2016 to design the standards curves and charts.

RESULTS AND DISCUSSION

Wild and home-grown plants have remarkable roles and contributions to animal and human health as they provide food and dietary supplements thereby enhancing food security and health. The knowledge about the nutritional and medicinal values of home-grown and wild vegetables is passed on to generations through folklore and it needs scientific documentation if it is to be of great help to the human race.

Plants contain bioactive chemical substances that produce remarkable physiological and biochemical actions in the human body. These bioactive constituents include alkaloids, tannin, flavonoids, and phenolic compounds. Plant-derived natural products have received considerable attention in recent years due to diverse pharmacological properties including antioxidant and antitumor activity. Natural products/dietary phytochemical have aroused considerable interest in recent years as potential therapeutic agents to counteract free radical mediated diseases [13]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds. Phytochemicals include compounds with various biological properties which allow plants to cope up with environmental challenges including exposure to radiation and toxins [75]. They are bioactive compounds (secondary metabolites) found in plants that work with nutrients and dietary fibers to protect against diseases. Certain phytochemicals are almost structurally identical to compounds isolated from human beings. Most plants with antidiabetic properties have been found to contain secondary metabolites such as glycosides, alkaloids, and flavonoids [1]. It has been shown that many plants exhibit efficient antioxidant properties owing to their phenolic content. Earlier report indicated that phytochemical screening of this species revealed the presence of alkaloids, flavonals, flavones, flavanols, saponins, flavonoids, and steroids [76]. Alkaloids such as soladunalinidine, solasonine, and solamargine have been isolated from leaf of *Solanum* species [77].

Literature on the content of phenols and flavonoids as well as antioxidant activity of several solanum genera are available [10, 16, 18, 78-81] but a comparison with those growing in Uganda is deficient. This study intended to quantify and compare the content of phenols and flavonoids as well as the antioxidant efficiency of aqueous extracts of three solanum genera selected from Kabale in Southwest Uganda.

Experimental results on tests performed to determine the qualitative presence of key natural products and metabolites in aqueous leafy extracts of *S. angiuvi*, *S. macrocarpon* and *S. nigrum* are presented in Table 1 below.

Table 1: Qualitative tests on leaf extracts on chemical groups present in the leaves of *Solanum anguivi; S. macrocarpon* and *S. nigrum* .sampled from Kabale

Test	*S.nigrum*	*S. macrocarpon*	*S. anguivi*
Mayer's test (alkaloids)	+	+	+
Born-Trager reaction (quinones)	-	-	+
Ferric chloride test (tannins)	+	+	+
Ferric chloride test in saturated sodium acetate (Gallic acid)	+	+	+
Shinoda test and magnesium powder(Flavonoids)	+	-	+
Test index foam (Saponins)	+	+	+
Raymond reaction Cardenolides)		-	-

Kedde reaction (Steroids)		-	+
Ethanoic acid test + ethanoic anhydride and sulfuric acid (Terpenoids)	+	-	+
Picric acid test(Cyanogenic derivatives)	-	-	-

Results on qualitative analysis of aqueous extract from leaves of *S. anguivi*, *S. macrocarpon* and *S. nigrum* are shown in Table 1 above. The results indicate all leaves sampled contained flavonoids, polyphenols, alkaloids, saponins and tannins so revealing that phytochemicals normally sought for from food supplements are present. So these leaves have medicinal values as well as providing food contents in form of carbohydrates, mineral salts, proteins and vitamins.

This is in agreement with what was published [82]. Phytochemicals have beneficial effects in a ways like, polyphenols reduce blood pressure while saponins may prevent cancer [83, 84] yet presence of tannins has been associated with causing cancer of the gullet[34, 85] excessive daily consumption of the leaves sampled in this study should be avoided because tannins, solasonine and solamargine are anti-nutritive [86, 87]. The presence of glycosides in the leaves of *S. anguivi*, *S. macrocarpon* and *S. nigrum* indicated the leaves have anti-inflammatory properties so consumption of the selected leaves can increase the ptotential to resist diseases and oxidative stress [79, 88]. The presence of glycosides in leafy extracts of these plants may treat cholesterol disorders and diabetes I and II [13] when consumed in appropriate quantities. The leaves contain saponins which are useful healthy and nutritional products.

Saponins are glycosides which are essential healing diseases that affect the trachea and lowering cholesterol levels. Presence glycosides, monosaccharides and polysaccharides in leaves imparts antioxidative properties to the leaf extracts of the selected plants. It is necessary to feed on a varied diet of vegetables to harness these benefits. Terpenes are present in leafy extract of *S. anguivi* and *S. nigrum* but were not detected in S. macrocarpon indicated that only when vegetables are eaten or used in combination that synergy can be achieved if treatment of an ailment has to effected. It is not surprising that in traditional medicine several plants are bundled up for therapeutic measures.

Flavonoid content of the aqueous extracts of leaves of *S. anguivi*, *S. macrocarpon* and *S. nigrum* was determined spectrophotometrically using reaction of flavonoids with chromogenic system $NaNO_2$-Al (III)-NaOH. The mechanism involves the reaction of any aromatic ring bearing a quercetin group. When Al (III) is added followed with NaOH, mixture turned from yellow to red and the absorbance was measured at wavelength of 510 nm [89-91]. The total flavonoid content of the dry leaves was expressed as milligrams of Quercetin equivalents per gram dry mass (mg QE/g) in Table 2. Each leaf extract was analyzed in triplicate [92].

The variation of absorbance of aqueous quercetin of different concentrations was plotted to give Figure 2 shown below.

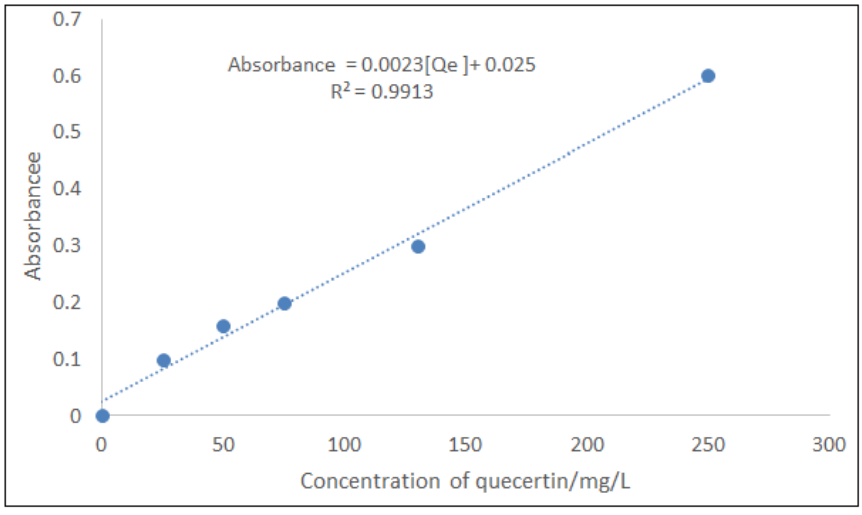

Figure 2: Plot of absorbance against concentration quercetin

As seen in Figure 2 above, there was a linear relation between absorbance and concentration of quercetin.

The line graph obtained is: Absorbance = 0.0023[Qe] + 0.025 with R^2 = 0.9913. This was used as standard curve for defeminizing concentration of flavonoids in quercetin equivalents Concentration mg/L

of total flavonoids obtained from standard curve of quercetin from line equation, absorbance = 0.0023 [Qe] + 0.025, in Figure 2 by using the absorbance of the analyte under test and reading off the corresponding concentration from the line or calculating using the equation. The concentrations were averaged for each sample and recorded in Table 2

Table 2: Total flavonoid and polyphenol contents of aqueous extracts of leaves of *S. anguivi; S. macrocarpon* and *S. nigrum* from Kabale (*n* = 3)

Plant	Polyphenols GAE/g/of dry weight	Flavonoids/mg QE/g	DPPH scavenging at IC_{50} / µg/mL	H_2O_2 Scavenging at IC_{50} / µg/mL
Ascorbic acid			3.30 ± 0.15	16.25 ± 0.25
S. anguivi	17.50 ± 0.70	7.40 ± 0.30	7.80 ± 0.25	6.90 ± 0.15
S. macrocarpon	104 ± 0.45	35.90 ± 0.60	45.60 ± 0.30	27.00 ± 0.35
S. nigrum	97.85 ± 0.15	16.40 ± 0.40	42.90 ± 0.20	17.90 ± 0.20

The total polyphenols in the aqueous leafy extracts was determined using the Folin-Ciocalteu reagent (Singleton *et al*., 1999) whereby the crude extract (100 µL) was mixed with Folin-Ciocalteu reagent (0.2 mL); and deionized distilled water (2 mL) and of 15% Na_2CO_3 (1mL) and allowed to stand for two hours. Aliquot of mixture formed was placed in cuvette of spectrophotometer. The absorbance of the mixture was measured at wavelength of 765 nm after standing for two hours at room temperature. Absorbance of solutions of gallic acid of different concentrations were used to plot standard curve shown in Figure 2.

The total concentrations of polyphenols were read from Figure 2 or calculated using the linearized equation using the absorbance of the mixture being analyzed and expressed as mg of gallic acid equivalent (mg GAE/100g) sample because the assay yields concentration of all polyphenols. The experiment were triplicated. Standard curve of absorbance of a different concentrations of standard gallic acid was plotted as shown in Figure 3 / Figure 3: Plot absorbance against concentration of gallic acid standard Figure 3 shows the linear variation of absorbance as concentration of gallic acid increased.

The line graph followed the equation Absorbance = 0.003[GAE] + 0.02 and R^2 = 0.996 of gallic acid (0–300 g/mL) and expressed in gallic acid equivalents (GAE) per gram dry extract weight in Table 2. In terms of concentration of polyphenols in the aqueous extracts of leaves sampled from Kabale showed *S. macrocarpon* was best source with 104.00 ± 0.45 mg GAE /g of dry leaves followed by *S. nigrum* with 97.85± 0.15 mg GAE /g and the least concentration was in *S. anguivi* having 17.50 ± 0.70 mg GAE /g.

These results indicated that feeding on leaves of *S. macrocarpon* would give higher polyphenol benefit than the other studied plants. However most Ugandans use *S. nigrum* because of the traditional knowledge passed from generations before. The data showed that the leaves of *S. anguivi*, *S. macrocarpon* and *S. nigrum* when consumed in sufficiently large quantity would contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against diseases caused by presence of free radicals in bodies [93]. Polyphenols are important antioxidants in plant materials. They contribute as terminators of free radicals. Polyphenols donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties.

Flavonoid content of aqueous extracts of selected leaves of sampled solanum species from Kabale revealed a similar concentrations variation to polyphenols above with *S. macrocarpon* yielding 35.90 ± 0.60 mg QE/g; followed by S. nigrum with 16.40 ± 0.40 mg QE/g and then S. anguivi with 7.40 ± 0.30 mg QE/g of dry leaves. In terms of dietary contribution, feeding on S. macrocarpon would contributing higher supplements of flavonoids than traditional *S. nigrum* yet consumption of leaves of *S. anguivi* would provide a below average quantity of these nutrients as evidenced by results of this study. Flavonoids include flavanals, flavanols, and flavones and have various chemical and biological properties including radical quenching [94].

The presence of flavonoids in the leaf extracts of all samples studied indicated that consumption of these leaves whether as food or medicine would provide formidable ingredients to combat free radicals once inside the body. It may be necessary to feed on the leaves following a routine to avoid accumulation of anti-nutrients in them [95].

The presence of compounds such as polyphenols and flavonoids in leaf extracts of *S. anguivi*, *S. macrocarpon* and *S. nigrum* may account for the antioxidant potential and the use of these plants in food and herbal medicines. As phytochemical compositions of the sampled leaves used in this study varied can be used to assert that it would be highly beneficial to use the leaves in combination for synergistic effects, as well as harnessing different nutrients provided by each species.

Abundance, distribution and activities of pharmacologically useful compounds in herbs is affected by geographical location, genetic variations,

environment [96-99]. Flavonoids, saponins and alkaloids exert a wide range of biological effects [100]. Antioxidant, antiproliferative and DNA repair are biological properties of phytochemicals present in plants which allow plants to cope up with environmental challenges including exposure to radiation and toxins [75]. These plant metabolites work with nutrients and dietary fibers to protect against diseases.

The determination of antioxidant capacity uses the diphenylpicrylhydrazyl (DPPH) radical. In this test, the scavenging of DPPH is followed by monitoring the decrease in color intensity at wavelength of 517 nm resulting from reduction by the antioxidant, electrons or coupling with a radical species.

DPPH results test the ability of compounds donating hydrogen or quench free radicals. Thus it can relied on to assess how ingredients in extract can increase safety and value of foods. Solutions of DPPH of different concentrations in methanol were prepared and separate mixed with alliqouts of leafy extracts and allowed to stand. Absorbance, A then was read at wavelength of 517 nm, against the methanol blank to remove the influence of the color of the samples.

The radical-scavenging activity expressed as percentage of inhibition according to the following formula [101].

Inhibition% (mg/mL) = $\frac{Acontrol - Asample}{Acontrol}$ x 100

The scavenging of DPPH at IC_{50} was observed highest for *S. macrocarpon* at 45.60 ± 0.30 µg/mL and least for *S. anguivi* at 7.80 ± 0.25 µg/mL yet that for *S. nigrum* at 42.90 ± 0.20 ug/mL was also high. These average results indicate that leaves of S. macrocarpon are the best at providing protection against oxidaitive stress followed by *S. nigrum* and leaf extracts of *S. anguivi* are poor. Dietary ingredients like vegetables are consumed with the interest of providing resources for free radical scavenging. Since *S. macrocarpon* and S. *nigrum* provide nearly equal capacity to reduce free radicals, it would be better to recommend their use in foods and herbal medicine where the aim is to help mediate effects of oxidative stress and degeneration caused by it [93, 94].

The scavenging of hydrogen peroxide at IC_{50} shown in Table 2 ranged from an average as high as 27.00± 0.30 for leaf extracts of *S.macrocarpon* through 17.90 ± 0.20 for *S. nigrum* to as low as 6.90 ± 0.15 for *S. anguivi.* The data suggests that consumption of leaves of *S. anguivi* may not be as instrumental as consuming leaves of *S. macrocarpon* but all leaves can serve the purpose of providing defence against oxidants if consumed in relative proportions to the quantity of capacity to reduce oxidizing agents. So one using *S. macrocarpon* may need less mass of leaves to get a similar performance to one consuming *S. anguivi* leaves. The set of results in agreement with what was published by some authors [102] probably because they also used water at room temperature to extract leaves. However, there are discrepancies in some values probably caused by geographical and environmental conditions where the plants were collected from as compared to where the authors in literature collected their samples.

Phytochemicals modulate metabolism preventing oxidative stress and cancers. They are present fruits, leaves and roots of certain herbs, shrubs and trees [33]. Thus the therapeutic values of plants stems from providing curative substances, minerals and vitamins that have specific physiological effects in the human body.

Flavonoids, saponins, alkaloids, phenols, phytates, tannins, cyanides, terpenoids and steroids were important phytochemicals reported to be present in fruits of *S. marcrocarpon* [35] and the aqueous leafy extracts, revealed presence of alkaloids, flavonoids and tannins in the present study. The differences in phytochemical composition in this study from those in literature have been attributed to factors like solvent used, method of extraction, geographical and environmental uniqueness [82, 103, 104] and this has been observed in many studies in different parts of the world [96-99]. Plant nutrients show different health enhancing effects to life processes [100, 105] reported *Solanum melongena* fruits lowered lipid and cholesterol in rats because they contained flavonoids. Similarly, flavonoids and saponins extracted from fruits and leaves have been shown to exhibit antioxidant, anti-inflammatory, hypocholesterolaemic and antimicrobial effects [106-111]. Alkaloids impart bitter taste to plant extracts like that from *S. macrocarpon* and other eggplants [110]. The curative effects of alkaloids to high blood pressure, malaria and cancer appear in many studies [109, 112]. *S. melongena* leafy extracts showed analgesic and central nervous system depression effects due to presence of alkaloids [113]

Total Polyphenol Content

Polyphenols obtained from plant extracts readily undergo reduction-oxidation reactions which enable them to perform antioxidant activities [19]. The hydroxyl groups present on them facilitate free radical scavenging by donating hydrogen. Thus why they are very vital in life processes, especially in relieving free radicals.

Since polyphenols form complexes with the Folin–Ciocalteu reagent whose intensity depends on concentration of polyphenols in each extract, the reaction was adapted and followed colorimetrically by reading absorbance of mixtures of leafy extracts with the reagent. The results were derived from a calibration curve y = 9.53x - 0.13, with R^2 = 0.996 of gallic acid in a range of concentrations 0–250 µg/mL and expressed

in gallic acid equivalents (GAE) per gram dry extract weight in Table 2. The content of polyphenols extracted to water ranged from 104 to 17.50 mgGAE/g representing an approximate five-fold variation. *S. macrocarpon*, *S. nigrum* and *S. anguivi* had average polyphenol content of 104 ± 0.45, 97.73 ± 0.15 and 17.50 ± 0.70 mg GAE/g of dry weight, respectively. Presence of hydroxyl groups on polyphenols readily dissolve in water, hence water is frequently used to extract them. Whereas it was reported that TPC was 0.704 mg GAE/g fresh weight of *S. nigrum* in a water extract [16] we report a value nearly ten times high for each gram of dry leaves. This could have been a result many factor like environmental, seasonal or genetic variations. The values of content of polyphenols obtained in this study for S. macrocarpon are similar to those published [114] and yet polyphenol content of *S. anguivi* adduced in this study are less compared to those in the literature [65]. Variations in TPC in plants are caused by many factors like the duration of extraction, geographical variation and methods of extraction just to mention. So divergence of results from those in literature was expected. *S. macrocarpon* was proved to be the richest source of polyphenols yet *S. anguivi* leaves would supply least.

The need for extraction of polyphenols and flavonoids from plants is important because they possess unique functions, structures and interactions with food components in human bodies. S Many factors may retard solid-liquid extraction such as nature of solvent used, extraction time duration, temperature and particle sizes [115, 116] thus why in this study the powder was extracted for 24 hours to increase amount of active ingredients percolating in the aqueous phase [117].

Total Flavonoid Content

The flavonoid concentration of aqueous extracts from leaves was determined by observing color intensity formed from interaction of leafy extracts with complex of aluminium chloride in NaOH method. The results were derived from the calibration curve Absorbance = 0.0023[Qe] + 0.025 with R^2 = 0.9913 of quercetin in range of concentrations from 0–100 µg/mL) and expressed in quercetin equivalents (QE) per gram dry extract weight (Table 2). The flavonoid content extracted to water ranged from 6.6 to 16.42 mg QE/g of dry leaves, representing an approximate three-fold variation. While the smallest amounts of flavonoids were found in *S. nigrum* (6.61 ± 0.40, *S. anguivi*. and *S. macrocarpon* contained 6.97 ± 0.60 and 16.42 ± 0.30 mg QE/g respectively) and the results agree with those in literature [114].

Flavonoids are plant metabolites whose antioxidant activity depends on the number and position of free OH groups [14]. It was reported that a TFC of 21.53 mg QE/g dry weight in the methanol extract of *C. tora [15]*, yet that for fresh leaves of *S, nigrum* extracted in water was 0.64 mg catechin equivalents/g [16] and as reported in the literature, genetic diversity; as well as biological, environmental, seasonal and year-to-year variations significantly affect the flavonoid content of vegetables [15].

DPPH Radical Scavenging Activity

The data on DPPH radical scavenging activities of aqueous extracts of solanum genera selected from Kabale are presented in Figure 4. All the extracts had their DPPH radical scavenging efficiency increasing as concentration of extract was increased so radical scavenging capacity was proportion to concentration.

The greatest DPPH radical scavenging activity of with a minimum IC_{50} value was recorded for *S. macrocarpon* 45.60 ± 0.30 µg/mL followed by *S. nigrum* 42.90 ± 0.20 µg/mL and *S. anguivi* 7.80 ± 0.20 µg/mL. All data were compared with the IC_{50} value of standard ascorbic acid as presented in Table 2.

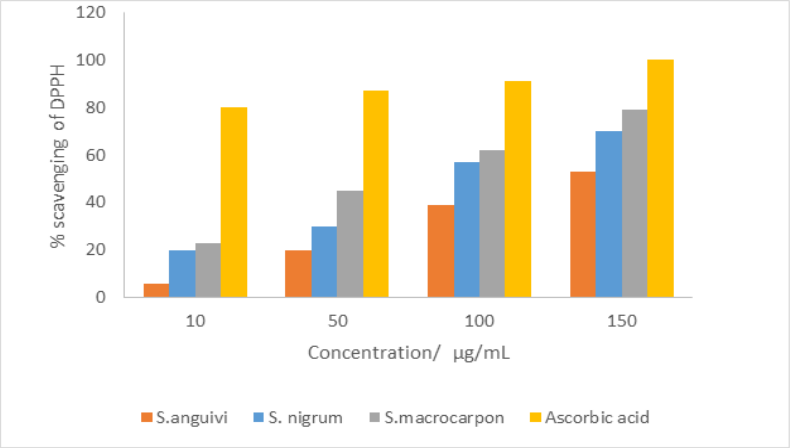

Figure 4: Comparison of DPPH radical scavenging activity of standard ascorbic acid and three selected solanum genera from Kabale at different concentrations

From Figure 4 above, it is observed that ascorbic acid removed radicals at highest rate as a standard. Like the standard, all tested leafy extracts showed progressive increase in capability to counteract free radicals as concentration increased and this has been reported as concentration dependence of the reactions [102]. DPPH radical is stable and readily takes up free radicals or electrons [42]. The DPPH radical being colored easily quantifies quenching of radicals by plant extracts [43]. When DPPH takes up electron or free radical it changes color from blue to yellow, this causes the absorption peak at wavelength of 517nm disappears [44]. In this study extracts from leaves of *S. macrocarpon* was superior to *S. nigrum* at removing DPPH radicals and least performance was provided by *S. anguivi* probably due to least total content of polyphenols and flavonoids in the extract of *S. anguivi*. In terms of food supplement or herbal medicine with best radical scavenging properties leaves of *S. macrocarpon* is listed yet in its absence even leaves of *S. anguivi* would facilitate the diet or medicine being administered but the quantity used must be larger than would be used for *S. macrocarpon* alone.

Hydrogen Peroxide Scavenging Activity

The capacity of the aqueous leafy extracts of selected solanum genera from Kabale in scavenging hydrogen peroxide was carried out and the data obtained was plotted in Figure 5. They showed lower performance than the reference standard ascorbic acid. The reference standard and the aqueous plant extracts had their capacity to scavenge for radicals from hydrogen peroxide increasing with increasing concentration of the test reagent. The greatest radical scavenging activity with minimum IC_{50} value was recorded for *S. macrocarpon* at 27.00 ± 0.35µg/mL, followed by *S. nigrum* providing 17.90 ± 0.20 µg/mL and *S.anguivi* showed 6.90 ± 0.15µg/mL. Thus leaves of *S. macrocarpon* would serve as best antioxidants in herbal medicine, preservative or food.

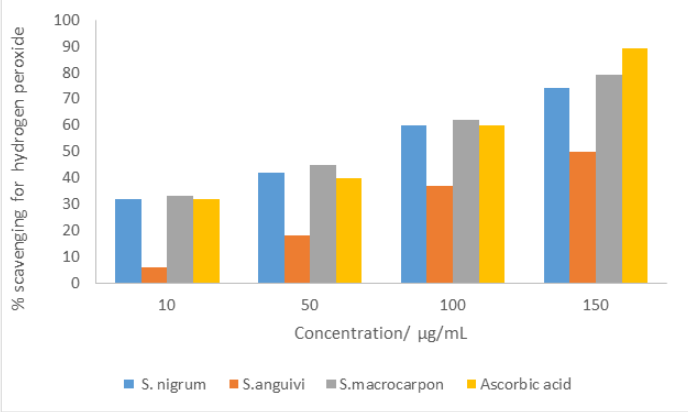

Figure 5: Comparison of hydrogen peroxide radical scavenging activity of ascorbic acid and aqueous extracts of dry leaves of solanum genera selected from Kabale at different concentrations

It can be observed from Figure 5 that hydrogen peroxide scavenging increased as concentration of extracts increased, so consumption of these leaves must be continuous if man has to harness good protection to health from vegetables. Hydrogen peroxide is a powerful oxidizing agent, stimulates cellular proliferation [36], or differentiation [37]. It was reported that hydrogen peroxide is produced in a biological system by oxidizing enzymes such as superoxide dismutase [38]. Its accumulation is known to cause oxidative stress and inflammation reactions, which were related to pathological conditions like cancer, diabetes, and cardiovascular diseases [39, 40]. This happens because hydrogen peroxide readily decomposes generating hydroxyl radicals that initiates lipid peroxidation and damage of cellular organelles [41]. Regulation of hydrogen peroxide generation using aqueous extracts from the selected solanum genera from Kabale in Figure 5 predicts chance for use of the leaves *S. anguivi*, *S. macrocarpon* and *S. nigrum* as medicine or food compliments that can alleviate radicals generated in living systems.

Ferric Reducing Antioxidant Power *(FRAP)*

The capacity of leafy extracts of selected plants is shown in Figure 6. Like the radical scavenging activity, all the aqueous extracts from the selected solanum genera from Kabale reduced iron (III) ions in a manner that increased with increase in concentration of the extracts form the dried leaves. The most reducing extract was *S, macrocarpon* followed by that from, *S. nigrum a*nd the least reducing was from *S. anguivi.* However all extracts tested in this study showed lower degree of reducing power than ascorbic acid that was used as standard. The ability of compounds present in the leafy extracts of solanum genera selected from Kabale and even from other plants in reducing iron (III) ions to iron (II) ions in hexacyanoferrate (III) complex was reported to act as a potential indicator for antioxidant activity [45]. It is on record that in the experiments in which the reducing power of antioxidants on iron (III) ions is determined, the yellow color test solution changes to green and blue depending on the reducing power of extracts or compounds [46, 47]. The presence of reducing agents in the aqueous

leafy extracts being studied transformed Fe^{3+} to Fe^{2+}, which was monitored by measuring absorbance at wavelength of 700 nm [48].

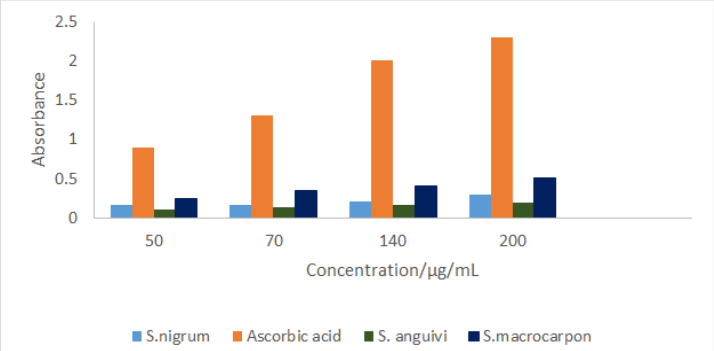

Figure 6: Variation of capacity to reduce Iron (III) ions of selected Solanum genera from Kabale at concentrations ranging from 50 to 200 µg/mL

From Figure 6 it can be asserted that extracts from leaves of *S. macrocarpon* showed highest reducing action to iron (III) ions in hexacyanoferrate (III) yet leaves of *S. anguivi* exhibited least reducing power. The higher tendency for extracts from leaves of S.macrocarpon in reducing iron (III) ions showed that the extracts contained larger total concentration of polyphenol and flavonoid than the others studied because capacity of natural antioxidants to reduce iron (III) ions is proportional to total concentrations of flavonoids and polyphenols [102]. Thus feeding on leaves of or/and using leaves of *S. macrocarpon* in medicine would serve better to retard transition metals from generating free radicals at pace higher than either *S. nigrum* or *anguivi*

The Thiocyanate- Iron (III) Complex in a Linoleic Acid System

Oxidation of lipids in living systems may cause oxidative damage which leads to stress because it produces lipid hydro-peroxides which can decompose to free radicals. Failure to overcome free radicals can cause damage to cells and induce degenerating diseases [50].

Lipid hydro-peroxides are stable at room temperature, but are decomposed by heat, UV light or by transition metals [51]. The capacity of leafy extracts to act as antioxidants was carried out using the linoleic acid- thiocyanate system at 37 ^{o}C mixing it with 100 µg/mL of extracted leafy samples. The products oxidize Fe^{2+} to Fe^{3+} ions.

On the addition of thiocyanate (SCN-), it gives a thiocyanate- iron (III) complex which shows maximum absorbance at wavelength of 500 nm. The effect of leafy extracts of selected genera from Kabale in retarding oxidation of linoleic acid is presented in Figure 7. The absorbance of the control increased to 2.00 ± 0.02 at 70 h, then decreased. This was due to the formation of products, which retarded peroxide formation. This result can be interpreted as revealing that when antioxidants are present in living systems, the oxidation of lipids is slow, and so the color development from thiocyanate- iron (III) complex was retarded. Of the solanum species studied, minimum absorbance was observed for mixtures containing extracts of leaves of *S. macrocarpon* because there was minimum peroxide formation in observed time intervals yet extracts of *S. anguivi* exhibited maximum absorbance due to its low antioxidant activity. The species with higher polyphenol or flavonoid contents showed lower absorbance due to minimum peroxidation and the vice versa is true *S. anguivi* which contained less total quantity of flavonoids and polyphenols than the rest.

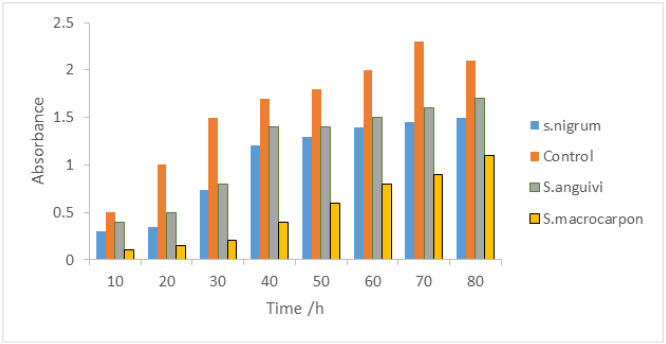

Figure 7: Capacity of aqueous extracts from leaves of solanum genera selected from Kabale to resist oxidation in the ferric thiocyanate–linoleic acid system at different time intervals at a concentration of 100 µg/mL

From Figure 7 above, it can be observed that the increase in absorbance was slowest for extracts from leaves of *S. macrocarpon* because it contained the highest combined concentrations of polyphenols and flavonoids so it possessed he highest antioxidant concentration and quenched the lipid oxidation best. The increase in absorbance was highest for extracts from leaves of *S. anguivi* indicating that they contained less concentration of antioxidant. Linoleic acid more readily underwent oxidation in presence of extracts from leaves of *S. anguivi* than in presence of leafy extracts of *S. macrocarpon* because leafy extracts from *S. anguivi* offered less protection against oxidation of linoleic acid in the thiocyanate-iron (III) system because it contained less total concentration of flavonoid and polyphenols as compared to *S. macrocarpon*. The results in Figure 7 above reveal that consuming leaves of *S. macrocarpon* provides higher chances of retarding oxidation of lipids than *S.anguivi* and *S. nigrum*. So the greatest reducing antioxidant power was recorded for *S.macrocarpon*, followed by *S. nigrum, and S. anguivi* compared to standard ascorbic acid.

CONCLUSIONS

In this study, phenol and flavonoid content of *S. anguivi, S. macrocarpon* and *S. nigrum* growing in Kabale were determined. The presence of higher concentrations of polyphenols and flavonoids in S. macrocarpon revealed that its leaves would serve as a very rich source of antioxidants needed to increase nutritive values of food or could be used as medicinal supplement. Similarly the powder of dry leaves of *S. anguivi, S. nmacrocarpon* or *S. nigrum* can serve as preservatives food since they are rich in antioxidants. The polyphenol content of dry leaves of: *S. anguivi* was 17.50± 0.70; that for *S. macrocarpon* was 104 ± 0.45 and *S. nigrum* was 97.80 ± 0.15 GAE/g yet the flavonoid content was 7.40 ± 0.30, 35.00 ± 0.60 and 16.40 ± 0.40 mg/QE/g for *S. anguivi, S. macrocarpon* and *S. ngrum* respectively. The DPPH scavenging at IC_{50} were 7.80 ± 0.25 ; 45.60 ± 0.30 and 42.90 ± 0.20 respectively yet hydrogen peroxide scavenging stood at IC_{50} were 6.89± 0.15; 27.00 ± 0.35 and 17 .90 ± 0.20 ug/mL in the respective order for *S. anguivi, S. macrocarpon* and *S. nigrum.* The available data suggests the plants are very good food supplements.

From the adduced results, aqueous extract of leaves of *S. anguivi, S. macrocarpon* and *S, nigrum* possess a powerful antioxidant activity so it may offer good protection against oxidative damage to body cells. It can be suggested that the selected solanum genera could be used as readily available sources of natural antioxidants, which can be used as dietary supplements for reducing the oxidative burden in degenerative diseases such as cancer, inflammation, diabetes, which develop due to oxidative stress in the body. The traditional herbalists and local population may continue using these plants as reliable sources of antioxidants in sauce, soup or/and herbal medicines.

RECOMMENDATIONS

There is need to perform in vivo and vitro experiments to deduce the efficacy of selected solanum genera on mammals. It will be necessary isolate and identify antioxidant components and study the individual antioxidant potential, which may lead to the inclusion of these compounds in different antioxidant formulations.

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Interest

We hereby declare there is no conflict of interest in this work. So work can be published on open access basis.

REFERENCES

1. Craig W, and Beck L.(1999) Phytochemicals: Health protective effects. Can J Diet Pract Res; 60 (2):78-84.
2. Uruquiaga, I. & Leighton, F. (2000). "Plant polyphenol antioxidants and oxidative stress", *Biological research*, 33, 159-165.
3. Wargovich, M.I. (2000). Anticancer properties of fruits and vegetables. *Hortscience*; 35, 573- 575.
4. Lind, E.M. & Tallantire. A.C. 1975. Some common flowering plants of Uganda. Oxford University Press, Nairobi.
5. Sengendo. B.K.A. 1982. Studies in the taxonomy « / Solanum L sect. Oliganthes. B.Sc. Dissertation, Makerere University. Kampala, Uganda
6. Bukenya-Ziraba, R. and Bonsu, K.O., (2004) *Solanum macrocarpon* L. In: Grubben, G.J.H. & Denton, O.A. (Editors). PROTA 2: Vegetables/Légumes, [CD-Rom]. PROTA, Wageningen, Netherlands.
7. Bukenya, Z.R. and J.F. Carasco (1995) Solanum (Solanaceae) in Uganda Bothalia 25,1: 43-59
8. Oboh,G.; M. M. Ekperigin, and M. I. Kazeem, (2005)"Nutritional and haemolytic properties of eggplants (Solanum macrocarpon) leaves," *Journal of Food Composition and Analysis*, vol. 18, no.2-3, pp. 153–160.
9. Messiaen, C.M.(1992)*The Tropical Vegetable Garden*, Macmillan Press Limited, London and Basingstoke.
10. Komlaga, G.; G. H. Sam, R. A. Dickson, M. L. K. Mensah, and T. C. Fleischer, (2014) "Pharmacognostic studies and antioxidant properties of the leaves of Solanum macrocarpon," *Journal of Pharmaceutical Sciences and Research*, vol. 6, no. 1, pp. 1–4,

11. Odetola, A.A; Y. O. Iranloye, and O. Akinloye,(2004) "Hypolipidaemic potentials of solanum melongena and solanum gilo on hypercholesterolemic rabbits," *Pakistan Journal Nutrition*, vol. 3, no. 3, pp. 180–187.
12. Sanchez-Mata, M.C; W. E. Yokoyama, Y.-J. Hong, and J. (2010) Prohens, "a-Solasonine and a-solamargine contents of gboma (*Solanum macrocarpon* L.) and scarlet (*Solanum aethiopicum* L.) eggplants," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 9, pp. 5502–5508..
13. Devi S, Singh R. (2016) Antidiabetic activity of methanolic extract of *Nepeta hindostana* herb in streptozotocin induced diabetes in rats. Int J Pharm Pharm Sci; 8(7):330-5.
14. Panche, A.N.; Diwan, A.D.; Chandra, S.R.(2016) Flavonoids: An overview. *J. Nutr. Sci. 5*, e47.
15. Kumar, V.; Roy, B.K. (2018) Population authentication of the traditional medicinal plant Cassia tora L. based on ISSR markers and FTIR analysis. *Sci. Rep.* , *8*, 10714. [CrossRef]
16. Adebooye, O.C.; Vijayalakshmi, R.; Singh, V. (2008) Peroxidase activity, chlorophylls and antioxidant profile of two leaf vegetables (Solanum nigrum L. and Amaranthus cruentus L.) under six pretreatment methods before cooking. *Int. J. Food Sci. Technol. 43*, 173–178.
17. Shrestha, P.M.; Dhillion, S.S.(2006) Diversity and traditional knowledge concerning wild food species in a locally managed forest in Nepal. *Agrofor. Syst*, *66*, 55–63.
18. Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I. and Bahorun, T. (2005) Phenolics as Potential Antioxidant Therapeutic Agents: Mechanism and Actions. Mutation Research, 579, 200-213. https://doi.org/10.1016/j.mrfmmm.2005.03.023
19. Lee, Y.H.; Choo, C.; Watawana, M.I.; Jayawardena, N.; Waisundara, V.Y.(2015) An appraisal of eighteen commonly consumed edible plants as functional food based on their antioxidant and starch hydrolase inhibitory activities. *J. Sci. Food Agric*, *95*, 2956–2964. [CrossRef]
20. Bors, W.; Michel, C. (2002) Chemistry of the antioxidant effect of polyphenols. *Ann. N.Y. Acad. Sci.*, *957*, 57–69
21. Burri, S.C.M.; Ekholm, A.; Håkansson, Å.; Tornberg, E.; Rumpunen, K.(2017) Antioxidant capacity and major phenol compounds of horticultural plant materials not usually used. *J. Funct. Foods*, *38*, 119–127.
22. Magder, S. (2006) Reactive Oxygen Species: Toxic Molecules or Spark of Life? Critical Care, 10, 208-216. https://doi.org/10.1186/cc3992
23. Mitjavila, M.T. and Moreno, J.J. (2012) The effects of polyphenols on oxidative stress and the arachidonic acid cascade. Implications for the prevention/treatment of high prevalence diseases. Biochemical Pharmacology, 84, 1113-1122. https://doi.org/10.1016/j.bcp.2012.07.017
24. Gupta, V.K. and Sharma, S.K. (2006) Plants as Natural Antioxidants. Natural Product Radiance, 5, 326-334.
25. Rahman K. (2007). Studies on Free Radicals, Antioxidants, and Co-Factors Clinical Interventions in Aging 2, 219-236.
26. Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010) Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. Pharmacognosy Reviews, 4, 118-126. https://doi.org/10.4103/0973-7847.70902
27. Halliwell, B. (1995) How to Characterize an Antioxidant: An Update. Biochemical Society Symposia, 61, 73-101. https://doi.org/10.1042/bss0610073
28. Brewer, M.S. (2011) Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. Comprehensive Reviews in Food Science and Food Safety, 10, 221-247. https://doi.org/10.1111/j.1541-4337.2011.00156.x
29. Liu, R.H. (2003) Health Benefits of Fruit and Vegetables Are from Additive and Synergistic Combinations of Phytochemicals. American Journal of Clinical Nutrition, 78, 517S-20S. https://doi.org/10.1093/ajcn/78.3.517S
30. Fridovich, I. (1999) Fundamental Aspects of Reactive Oxygen Species, or What's the Matter with Oxygen? Annals of the New York Academy of Sciences, 893, 13-18. https://doi.org/10.1111/j.1749-6632.1999.tb07814.x
31. Hinneburg, I., Damien Dorman, H.J. and Hiltunen, R. (2006) Antioxidant Activities of Extract from Selected Culinary Herbs and Spices. Food Chemistry, 97, 122-129. https://doi.org/10.1016/j.foodchem.2005.03.028
32. Simic, M.G. (1988) Mechanisms of Inhibition of Free-Radical Processes in Mutagenesis and Carcinogenesis. Mutation Research, 202, 377-386. https://doi.org/10.1016/0027-5107(88)90199-6
33. Tripoli E, Guardia ML, Giammanco S, Majo DD, Giammanco M (2007). Citrus flavonoids: molecular structure, biological activity and nutritional properties - a review. Food Chemistry 104:466-479.
34. Dougnon TV, Bankolé HS, Johnson RC, Klotoé JR, Dougnon G, Gbaguidi F, Assogba F, Gbénou J, Sahidou S, Atègbo JM, Rihn BH, Loko F, Boko M, Edorh AP (2012). Phytochemical screening, nutritional and toxicological analyses of leaves and fruits of *Solanum macrocarpon* Linn (Solanaceae) in Cotonou (Benin). Food and Nutrition Sciences 3:1595-1603.
35. Sodipo, O.A.; F. I. Abdulrahman, J. C. Akan and J. A. Akinniyi, (2008) "Phytochemical Screening and Elemental Constituents of the Fruit of *Solanum macrocarpum* Linn," *Continental Journal of Applied Sciences*, Vol. 3, pp. 85-94.

36. Geiszt, M.; Leto, T.L.(2004) The Nox family of NAD (P) H oxidases: Host defense and beyond. *J. Biol. Chem*, *279*, 51715–51718. [CrossRef]
37. Li, M.O.; Sanjabi, S.; Flavell, R.A. (2006) Transforming growth factor-β controls development, homeostasis, and tolerance of T cells by regulatory T cell-dependent and-independent mechanisms. *Immunity*, *25*, 455–471. [CrossRef]
38. Buettner, G.R. (2011) Superoxide dismutase in redox biology: The roles of superoxide and hydrogen peroxide. *Anti-Cancer Agents Med. Chem.*, *11*, 341–346.
39. Mahmoud, E.A.; Sankaranarayanan, J.; Morachis, J.M.; Kim, G.; Almutairi, A.(2011) Inflammation responsive logic gate nanoparticles for the delivery of proteins. *Bioconjug. Chem*, *22*, 1416–1421. [CrossRef]
40. De Gracia Lux, C.; Joshi-Barr, S.; Nguyen, T.; Mahmoud, E.; Schopf, E.; Fomina, N.; Almutairi, A.. (2012) Biocompatible polymeric nanoparticles degrade and release cargo in response to biologically relevant levels of hydrogen peroxide. *J. Am. Chem. Soc. 134*, 15758–15764. [CrossRef]
41. Saed-Moucheshi, A.; Pakniyat, H.; Pirasteh-Anosheh, H.; Azooz, M.M. (2014) Chapter 20—Role of ROS as Signaling Molecules in Plants. In *Oxidative Damage to Plants*; Ahmad, P., Ed.; Academic Press: San Diego, CA, USA; pp. 585–620.
42. Chithiraikumar, S.; Gandhimathi, S.; Neelakantan, M. (2017) Structural characterization, surface characteristics and non-covalent interactions of a heterocyclic Schiff base: Evaluation of antioxidant potential by UV–visible spectroscopy and DFT. *J. Mol. Struct.*, *1137*, 569–580. [CrossRef]
43. Dudonné, S.; Vitrac, X.; Coutiere, P.; Woillez, M.; Mérillon, J.-M. (2009) Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *J. Agric. Food Chem. 57*, 1768–1774. [CrossRef]
44. Chang, L.-W.; Yen, W.-J.; Huang, S.C.; Duh, P.-D. (2002) Antioxidant activity of sesame coat. *Food Chem.78*, 347–354. [CrossRef]
45. Meir, S.; Kanner, J.; Akiri, B.; Philosoph-Hadas, S. (1995) Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J. Agric. Food Chem*, *43*, 1813–1819. [CrossRef]
46. Ferreira, I.C.; Baptista, P.; Vilas-Boas, M.; Barros, L. (2007) Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chem. 100*, 1511–1516. [CrossRef]
47. Zou, Y.; Lu, Y.; Wei, D.(2004) Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. in Vitro. *J. Agric. Food Chem. 52*, 5032–5039. [CrossRef]
48. Amarowicz, R.; Pegg, R.; Rahimi-Moghaddam, P.; Barl, B.; Weil, J.(2004) Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem. 84*, 551–562.
49. Benzie, I.F.; Strain, J.J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.*, *239*, 70–76.
50. Sy, C.; Caris-Veyrat, C.; Dufour, C.; Boutaleb, M.; Borel, P.; Dangles, O. (2013) Inhibition of iron-induced lipid peroxidation by newly identified bacterial carotenoids in model gastric conditions: Comparison with common carotenoids. *Food Funct*, *4*, 698–712. [CrossRef]
51. Ayala, A.; Muñoz, M.F.; Argüelles, S.(2014) Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid. Med. Cell Longev*, *2014*, 360438.
52. Atanu ,F. O.; U. G. Ebiloma and E. I. Ajayi (2011) A review of the pharmacological aspects of *Solanum nigrum* Linn Biotechnology and Molecular Biology Review Vol. 6(1), pp. 001-007, Available online at http://www.academicjournals.org/BMBR ISSN 1538-2273
53. Cooper M.R. and Johnson, A.W (1984). Poisonous Plants in Britain and other effects on Animals and Man. Ministry of Agriculture, Fisheries Food, 161: 219-220.
54. Dan C.G; Kouassi, K.N ; Ban, Kl.; Nemlin GJ. Kouame, PL.(2014) Influence of maturity stage on nutritional and therapeutic potentialities of *Solanum anguivi* L berries (Gnagnan) cultivated in Cote d'Ivoire. International journal of nutrition and food science 3(2): 1-5 DOI:10.11648/j.ijnfs.s.20140302.11
55. Dike, M.C. (2010). "Proximate, Phytochemical and Nutrient composition of some fruits, seeds and leaves of some plant species at Umadike, Ngeria", ARPN. *J. Agric. Biol. Sci.* Vol. 5, 7-16.
56. Adeleke, R.O. & Abiodun, A.O. (2010). "Chemical composition of Three Traditional Vegetables in Nigeria", *Pakistan Journal of Nutrition*., (9), 858-860.
57. Asaolu, S.S., Adefemi, O.S., Oyakilome, I.G., Ajibola, K.E. & Asaolu, M.F. (2012). "Proximate and Mineral Composition of Nigerian Leafy Vegetables", *Journal of Food Research*.,1(3), 214-218.
58. Oyeyemi S.D.; Ayeni, M.J.; Adebiyi A.O.; Ademiluyi, B.O.; Tedela, P.O. and Osuji I.B. (2015) Nutritional Quality and Phytochemical Studies of *Solanum anguivi* (Lam.) Fruits Journal of Natural Sciences Research, 5(4). www.iiste.org ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online).
59. Oyeyemi, S.D. & Tedela, P.O (2014). "Nutritional quality and phytochemical properties of *Anchomones difformis* leaves (Blume) Engl", *Ind. J. Sci. and Tech.* 2(4), 66-77.

60. Elekofehinti, O.O., Adanlawo, I.G., Saliu, J. A. & Sodehinde, S.A. (2012). "Saponins from *Solanum anguivi* fruits exhibit hypolipidermic potential in *Rattus novergicus* Der", Pharmacia Lett; 4(3), 811-814.
61. Johnson, M.; Wesely, E.G.; Selvan, N.; Chalini, K.; (2010): Comparative phytochemical and isoperoxidase studies on leaf and leaves derived callus of Solanum anguivi L . J. Chem. Pharm. Res. 2(4) 899-906.Joshi V., Verma T., Shetty P. R.(2009) Antioxidant potential of *Bauhinia purpurea* Linn. Leaves. *International Journal of Pharmaceutical Research*;1:51–55. [Google Scholar]
62. Ravi, V., Saleem, S.M., Maiti, P.P., Gauthaman, K. & Kamamurthy, J. (2009). "Phytochemical and Pharmacological evaluation of *Solanum nigrum* L", *African Journal of Pharmacy and Pharmacology*, 3(9), 455-457.
63. Santhi, K. & Nadanakunjidam, S. (2011). "Pharmacognostical studies on *Solanum nigrum* L., *AD*". *Plant Sci*., 24(1), 339-341.
64. Manach, C.; Scalbert, A., Morand, C.; Remesy, C,; and Jimenez L. (2004) Polyphenols: Food sources and bioavailability. American journal of clinical nutrition: 79, 727-747
65. Adanlawo, I.G. & Akanji, M.A. (2008). "Effect of saponin extract from *Solanum anguivi* Lam. fruits on serum cholesterol concentration of albino rats", *Rece Pig. Med. Pla*., 9(19), 1-7.
66. Taha M., Banat F., Rababah A., Ereifej K. and Wade Y. (2010). Optimization of Extraction Conditions of Total Phenolics, Antioxidant Activities,and Anthocyanin of Oregano, Thyme,
67. Yen, G.-C.; Chuang, D.-Y. (2000) Antioxidant properties of water extracts from Cassia tora L. in relation to the degree of roasting. *J. Agric. Food Chem. 48*, 2760–2765. [CrossRef] [PubMed]
68. Losada-Barreiro, S.; Bravo-Díaz, C. (2017) Free radicals and polyphenols: The redox chemistry of neurodegenerative diseases. *Eur. J. Med. Chem. 133*, 379–402.
69. Harborne J.B.(1973), *Phytochemical methods*, (London: Chapman and Hall, Ltd) 49-188
70. Trease G.E. and Evans W.C.,(1989) *Pharmacognsy* (Brailliar Tiridel Canada: Macmillian publishers) 11th edn., 290
71. Huffman MA. (2003) Animal self-medication and ethno-medicine: Exploration and exploitation of the medicinal properties of plants. Proc Nutr Soc ;62(2):371-81
72. Amir M and Kumar S. (2004) Possible industrial application of genus solanum in twenty first century - A review. J Sci Ind Res.63:116-24.
73. Juneja D, Shrivastava PN, Guha MK, Saxena RC.(2007) Preliminary phytochemical screening of some folklore medicinal plants for their anti-inflammatory activity. Pharmacogn Mag 2007;3:11.
74. Kadhim MJ, Kaizal AF, Hameed IH. (2017) Medicinal plants used for treatment of rheumatoid arthritis: A review. *International Journal of Pharmaceutical and Clinical Research*. 8(11).
75. Hameed I.H,; Cotos, R.M.C, and Hadi M.Y. (2017) A Review: *Solanum nigrum* L. Antimicrobial, Antioxidant properties, Hepato-protective effects and Analysis of Bioactive Natural Compounds *Research J. Pharm. and Tech 2017; 10(11): 4063-4068.* DOI: 10.5958/0974-360X.2017.00737.5
76. Jasim H, Hussein AO, Hameed IH, Kareem MA.(2015) Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 7(4): 56-72.
77. Kadhim WA, Kadhim, M.J., Hameed, I.H. (2017) Antibacterial Activity of Several Plant Extracts Against *Proteus Species*. *International Journal of Pharmaceutical and Clinical Research*. 8(11).
78. Prasath G. S, Prakash, Srinivasan P.T., Subramanian S (2016): Studies on the phytochemical screening and free radical scavenging potentials of *Solanum nigrum* leaves extract *Asian J Pharm Clin Res, Vol 9, Issue 6, 316-321* DOI: http://dx.doi.org/10.22159/ajpcr.2016.v9i6.14506
79. Richelle, M., I. Tavazz and E. Offord, (2001). Comparison of the anti-oxidant activity of commonly consumed polyhenolic beverages (coftee, cocoa and tea) prepared per cup serving. J. Agri. Food Chem., 49: 3438-3442.
80. Sharp, G.J., (1997). The final word on Nutrition: phytochemicals, RDA and other Nutrients. http:/ defaut / HSF/Miscellianeus//HSF Head. Htm.
81. Mbegbu E.C, Ezechukwu C.S, Ejere V.C., Nnamonu E, I. and Ugwu G. C. (2019) Phytochemistry, acute toxicity and blood profile of albino rats treated with fruit extract of *Solanum macrocarpon* J. Pharmacognosy Phytother. Vol. 11(2), pp. 43-51, July-September DOI: 10.5897/JPP2019.0543 Article Number: E3F451161796
82. Assogba-Komlan F. and P. Anihouvi, "Pratiques Cul- turales et Teneur en Eléments (2007) Anti Nutritionnels (Nitrates et Pesticides) du *Solanum macrocarpum* au Sud du Bénin," *African Journal of Food, Agriculture, Nutrition and Development*, Vol. 7, No. 4, pp. 1-21.
83. Sanchez-Mata, M.C; W. E. Yokoyama, Y.-J. Hong, and J. (2010) Prohens, "a-Solasonine and a-solamargine contents of gboma (*Solanum macrocarpon* L.) and scarlet (*Solanum aethiopicum* L.) eggplants," *Journal of Agricultural and Food Chemistry*, vol. 58, no. 9, pp. 5502–5508..
84. Kariuki AC, Njoroge GN (2011). Ethnobotanical and antimicrobial studies of some plants used in Kibwezi (Kenya) for management of lower respiratory tract infections. African Journal of

Traditional, Complementary and Alternative Medicines 8(2):144-149.
85. Zhu, H., Wang, Y., Liu, Y., Xia, Y., Tang, T., (2010). Analysis of flavonoids in Portulaca oleracea L. by UV –Vis spectrophotometry with comparative study on different extraction technologies. Food Analytical Methods 3, 90 –97.
86. Magalhães, L.M., Almeida, M.I.G.S., Barreiros, L., Reis, S., Segundo, M.A.,(2012). Automatic aluminum chloride method for routine estimation of total flavonoids in red wines and teas. Food Analytical Methods 5, 530 –539.
87. Pękal, A., Pyrzynska, K., (2014). Evaluation of aluminium complexation reaction for flavonoid content assay. Food Analytical Methods 7, 1776 – 1782.
88. Atanassova, M., Georgieva, S. and Ivancheva, K., (2011). Total phenolic and total flavonoid contents, antioxidant capacity and biological contaminants in medicinal herbs. *Journal of the University of Chemical Technology & Metallurgy*, *46*(1).
89. Kale MA, Bindu SM, Khadkikar P. (2015) Role of antioxidants and nutrition in oxidative stress: A review. Int J Appl Pharm;7(1):1-4.
90. Ruba AA, Mohan VR. (2016) *In vitro* antioxidant potential of whole plant of *Andrographis echioides* (L.) nees (Acanthaceae). Int J Pharm Pharm Sci ;8(6):70-5.
91. Akwaowo, E.U., B.A. Ndon and E.U. Etuk, (2000). Minerals and antinutrients in fluted pupkin (*Telfairia occidentialis Hook. F.*). J. food Chem., 70: 235-240.
92. Borokini TI, Ayodele AE (2012). Phytochemical screening of Tacca leontopetaloides (L.) Kuntze collected from four geographical locations in Nigeria. International Journal of Modern Botany 2(4):97- 102
93. Aberoumand, A and; Deokule, S.S. (2009): Studies of nutritional values of some wild edible plants from Iran and India Pak. J. Nutri. 8: 26-31.
94. Ubani CS, Oje OA, Ihekogwo FNP, Eze EA, Okafor CL (2012). Effect of varying soil minerals and phytochemical parameters on antibacterial susceptibility of Mitracarpus villosus ethanol extracts using samples from south east and south-southern regions of Nigeria. Global Advanced Research Journal of Microbiology 1(7):120-125.
95. Ullah N, Khurram M, Amin MU, Khan TA, Khayyam SU, Khan FA, Najeeb U, Ullah S (2012). Impact of geographical locations on Menthaspicata antibacterial activities. Journal of Medicinal Plants Research 6(7):1201-1206
96. Sandhar HK, Kumar B, Prasher S, Tiwari P, Salhan M, Sharma P (2011). A review of phytochemistry and pharmacology of flavonoids. InternationalePharmaceuticaSciencia 1(1):25-41.
97. Jawad Kadhim, N.; Al-Rekaby L.S.; Redha, A.A. and Chappell, J. (2019): Chemical composition and antioxidant capacity of eggplant parts during vegetative and flowering stage Journal of Physics: Conf. Series 1294 (2019) 092013IOP Publishing doi:10.1088/1742-6596/1294/9/092013
98. Aryal , S, Kumar BM , Danekhu K, Kunwar P., Gurung R. and Koirala N (2019)Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal *Plants* 2019, *8*, 96; doi:10.3390/plants8040096 www.mdpi.com/journal/plants
99. Tatiya AU, Tapadiya GG, Kotecha S, Surana SJ (2011). Effect of solvents on total phenolics, antioxidant and antimicrobial properties of Bridelia retusa stem bark. Indian Journal of Natural Products and Resources 2(4):442-447.
100. Dent M, Dragovic-Uzelać V, Penić M, Brnčić M, Bosiljkov T, Levaj B (2013). The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in Dalmatian wild sage (Salvia officinalis L.) extracts. Food Technology and Biotechnology 51(1):84-91.
101. Sudheesh S, Presannakumar G, Vijayakumar S, Vijayalashmi NR (1997). Hypolipidemic effect of flavonoids from Solanum melongena, Plant foods for Human Nutrition 51:321-330.
102. Vinson JA, Hao Y, Su X, Zubik L (1998). Phenol antioxidant quantity and quality in foods: vegetable. Journal of Agricultural and Food Chemistry 48:3630-3634.
103. Francis G, Kerem Z, Makkar HPS, Becker K (2002). The biological action of saponins in animal systems: a review. British Journal of Nutrition 88:587-605
104. Cushnie TPT, Lamb AJ (2005). Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 26(5):343-356
105. Soetan KO, Oyekunle MA, Aiyelaagbe OO, Fafunso MA (2006). Evaluation of the antimicrobial activity of saponins extract of Sorghum bicolor L. Moench. African Journal of Biotechnology 5(23):2405-2407
106. Chinedu SN, Olasumbo AC, Eboji OK, Emiloju OC, Arinola OK, Dania DI (2011). Proximate and phytochemical analyses of Solanum aethiopicum L. and Solanum macrocarpon L. fruits.Research Journal of Chemical Sciences 1(3):63-71.
107. Mir MA, Sawhney SS, Jassal MMS (2013).Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. Wudpecker Journal of Pharmacy and Pharmacology 2(1):1-5.
108. Roberts MF, Wink M (1998). Alkaloids: biochemistry, ecology and medicinal applications. Plenum Press, New York.
109. Vohora SB, Kumar I, Khan MS (1984). Effect of alkaloids of Solanummelongena on the central nervous system.Journal of Ethnopharmacology 11(3):331-336.
110. Olusola B. Adewale, A. Onasanya, Adewale O. Fadaka, H. Iwere, Scholastica O. Anadozie, Olukemi A. Osukoya, I. I. Olayide (2014) *In vitro* antioxidant effect of aqueous extract of *Solanum*

macrocarpon leaves in rat liver and brain Oxid Antioxid Med Sci *; 3(3):225-229 ISSN: 2146-838*

111. Wang, H., Provan, G.J. and Helliwell, K. (2004) Determination of Rosmarinic Acid and Caffeic Acid in Aromatic Herbs by HPLC. Food Chemistry, 87, 307-311. https://doi.org/10.1016/j.foodchem.2003.12.029
112. Zuraida Ab Rahman, Mohd Waznul Adly Mohd Zaidan, Ayu Nazreena Othman, Muhamad Aizuddin Ahmad, Sanimah Simoh, Muhammad Aiman Haiqal Ismail (2019) Optimizing Extraction of Phenolics and Flavonoids from *Solanum ferox* Fruit Natural Science, Vol. 11, (No. 4), pp: 99-105
113. AOAC,(1990) "Official Methods of Analysis (Section 923.03 and 962.09)," 15th Edition, Washington DC, Association of Official Analytical Chemists.
114. Aruoma, O.I. (1999) Free Radicals, Antioxidants and International Nutrition. Asia Pacific Journal of Clinical Nutrition, 8, 53-63. https://doi.org/10.1046/j.1440-6047.1999.00036.x
115. Audigié, . D;. G. Dupont and T. Zonszain, (1978)"Manipulation d'Analyse Biochimique, " Doin., Paris, pp. 27-74.
116. Ayeleso A. O., Oguntibeju O. O., Brooks N. L.(2014) *In vitro*study on the antioxidant potentials of the leaves and fruits of *Nauclea latifolia* . *The Scientific World Journal.* 2014;2014:8. doi: 10.1155/2014/437081.437081 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
117. Bello,S.O.; B. Y. Muhammad, K. S. Gammaniel (1994)., "Preliminary evaluation of the toxicity and some pharmacological properties of the aqueous crude extract of solanum melongena," *Research Journal of Agriculture, Biology and Science*, vol. 1, no. 1, pp. 1–9, 2005.
118. Bors, W.; Michel, C. (2002) Chemistry of the antioxidant effect of polyphenols. *Ann. N.Y. Acad. Sci.*, *957*, 57–69
119. Bruneton , J.(1999) "Parmacognosie-Phytochimie-Plantes Médi- cinales, 3rd Édition, Éditions Tec & Doc et Médicales Internationales, Cachan, Paris, p. 1120.
120. Bursal E., Gülçin I. (2011) Polyphenol contents and *in vitro* antioxidant activities of lyophilised aqueous extract of kiwifruit (Actinidia deliciosa) *Food Research International*;44(5):1482–1489. doi: 10.1016/j.foodres.2011.03.031. [CrossRef] [Google Scholar]
121. Cheek, P.R. (1971) "Nutritional and Physiological Implications of Saponins: A Review," *Canadian Journal of Animal Science*, Vol. 51, No. 3, pp. 621-632. doi:10.4141/cjas71-082
122. Chumark P., Khunawat P., Sanvarinda Y., (2008) The in vitro and ex vivo antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa oleifera* Lam. leaves. *Journal of Ethnopharmacology.*116 (3):439–446. doi: 10.1016/j.jep.2007.12.010. [PubMed] [CrossRef] [Google Scholar]
123. Crozier A., Jaganath I. B., Clifford M. N.(2009) Dietary phenolics: chemistry, bioavailability and effects on health. *Natural Product Reports.* 26(8):1001–1043. doi: 10.1039/b802662a. [PubMed] [CrossRef] [Google Scholar]
124. Dougnon, V.T.; H. S. Bankolé, P. A. Edorh, J. T. Dougnon, M. Gouissi, A. Hounkpatin, S. Montcho, H. Azonhè, J.-R. Klotoé and M. Boko, (2012)"Evaluation of the Microbiological Quality of the Leaves of *Solanum macrocarpum* L. Cultivated with the Chicken's Droppings and Water of Marsh in Cotonou (Republic of Benin)," *International Journal of Biosciences*, Vol. 2, No. 2, pp. 45-52.
125. Eddine, L.S., Djamila, B. and Redha, O.M. (2016) Solvent pH Extraction Effect on Phytochemical Composition and Antioxidant Properties of Algerian Matricaria Pubescens. Journal of Pharmacy Research, 10, 106-112.
126. Elekofehinti O. O., Adanlawo I. G. and Fakoya A. (2012). *Solanum anguivi* saponins inhibit basal erythropoiesis in Rattus norvegicus. Indian Journal of Pharmaceutical and Health Sciences. Volume 2, Issue 3: 416-419.
127. Emebu, P.K. and J. U. Anyika, (2011)"Vitamin and Antinutrient Composition of Kale (*Brassica oleracea*) Grown in Delta State, Nigeria," *Pakistan Journal of Nutrition*, Vol. 10, No. 1, pp 76-79.
128. Freeman B. L., Eggett D. L., Parker T. L. (2010) Synergistic and antagonistic interactions of phenolic compounds found in navel oranges. *Journal of Food Science.*;75(6):C570–C576. doi: 10.1111/j.1750-3841.2010.01717.x. [PubMed] [CrossRef] [Google Scholar]
129. Fu R., Zhang Y.-T., Guo Y.-R., (2013). Antioxidant and anti-inflammatory activities of the phenolic extracts of *Sapium sebiferum* (L.) Roxb. Leaves. *Journal of Ethnopharmacology*; 147(2):517–524. doi: 10.1016/j.jep.2013.03.058. [PubMed] [CrossRef] [Google Scholar]
130. Guimarães-Beelen, P.M; T. T. Berchielli, R. Beelen, J. A. Filho and S. G. de Oliveira,(2006) "Characterization of Condensed Tannins from Native Legumes of the Brazilian- northeastern Semi-Arid," *Scientia Agricola*, Vol. 63, No. 6, pp. 522-528.
131. Gupta,S.S. () "Prospects and Perspective of Natural Plant Products," *Indian Journal of Pharmacology*, Vol. 26, No. 1, pp. 1-12.
132. Hach, (1999)"Digesdahl Digestion Apparatus: Instruction Manual for Models 23130-20-21," Hach USA, p. 95.
133. Halliwell B., Gutteridge J. M. C. (1984) Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochemical Journal,* 219(1), 1–14. doi:

10.1042/bj2190001. [PMC free article] [PubMed] [CrossRef] [Google Scholar]
134. Hameed, I.H., Al-Rubaye A.F. and Kadhim, M.J. (2017) Antimicrobial Activity of Medicinal Plants and Urinary Tract Infections. *International Journal of Pharmaceutical and Clinical Research*. 8(11).
135. Harborne J.B.(1973), *Phytochemical methods*, (London: Chapman and Hall, Ltd) 49-188
136. Houghton, P.J. and A. Raman,(1998) "Laboratory Handbook for the Fractionation of Natural Extracts," Chapman and Hall, New York, pp. 130-207. doi:10.1007/978-1-4615-5809-5
137. Jaitak V., Sharma K., Kalia K., (2010) Antioxidant activity of *Potentilla fulgens*: an alpine plant of western Himalaya. *Journal of Food Composition and Analysis*;23(2):142–147. doi: 10.1016/j.jfca.2009.02.013. [CrossRef] [Google Scholar]
138. Karakaya S. (2004) Bioavailability of phenolic compounds. Critical review of food science and nutrition. 44, 453-464.
139. Katalinic, V.; Milos, M.; Kulisic, T.; Jukic, M. (2006) Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chem*, *94*, 550–557
140. Kosar, M., Dorman, H.J.D. and Hiltunen, R. (2005) Effect of an Acid Treatment on the Phytochemical and Antioxidant Characteristics of Extracts from Selected Lamiaceae Species. Food Chemistry, 91, 525-533. https://doi.org/10.1016/j.foodchem.2004.06.029
141. Kumar T., Jain V. (2014) Antinociceptive and anti-inflammatory activities of bridelia retusa methanolic fruit extract in experimental animals. *The Scientific World Journal.* 2014:12. doi: 10.1155/2014/890151.890151 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
142. Lin, S.Y.; H.-Y. Liu, Y.-L. Lu and W.-C. Hou, (2005)"Antioxidant Activities of Mucilages from Different Taiwanese Yam Cultivars," *Botanical Bulletin of Academia Sinica*, Vol. 46, pp. 183-188.
143. Losada-Barreiro, S.; Bravo-Díaz, C. (2017) Free radicals and polyphenols: The redox chemistry of neurodegenerative diseases. *Eur. J. Med. Chem. 133*, 379–402.
144. Luqman S., Srivastava S., Kumar R., Maurya A. K., Chanda D. (2012) Experimental assessment of *Moringa oleifera* leaf and fruit for its antistress, antioxidant, and scavenging potential using *in vitro* and *in vivo* assays. *Evidence-Based Complementary and Alternative Medicine*;2012:12. doi: 10.1155/2012/519084.519084 [PMC free article] [PubMed] [CrossRef] [Google Scholar]
145. Magalhães L. M., Barreiros L., Reis S., Segundo M. A. (2014) Kinetic matching approach applied to ABTS assay for high-throughput determination of total antioxidant capacity of food products. *Journal of Food Composition and Analysis.* 33(2):187–194. doi: 10.1016/j.jfca.2014.01.003. [CrossRef] [Google Scholar]
146. Mc-Graw Hill (1987). Encyclopedia of Food Agricultural Nutrition Vol. 1. (5th ed). MC-Graw Hill Book . Company, New York. 11-120
147. Menezes-Benavente L., Teixeira F. K., Kamei C. L. A., Margis-Pinheiro M. (2004) Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a Brazilian *indica* rice (*Oryza sativa* L.) *Plant Science.*;166(2):323–331. doi: 10.1016/j.plantsci.2003.10.001. [CrossRef] [Google Scholar]
148. Meot-Duros L., Magné C. (2009) Antioxidant activity and phenol content of *Crithmum maritimum* L. leaves. *Plant Physiology and Biochemistry*. 47(1):37–41. doi: 10.1016/j.plaphy.2008.09.006. [PubMed] [CrossRef] [Google Scholar]
149. Miliauskas G., Venskutonis P. R., Van Beek T. A.(2004) Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chemistry*, 85(2):231–237. doi:10.1016/j.foodchem.2003.05.007. [CrossRef] [Google Scholar]
150. Naczk, M. and Shahidi, F. (2004): Extraction and analysis of phenolics in foods. Journal of chromatography A. 1054: 95- 111.
151. Olaniyi,A.A; J. S. K. Ayim, A. O. Ogundaini and T. A. Olugbade, (1998)"Essential Inorganic and Organic Chemistry," Omoade Printing Press, Ibadan, p. 582.
152. Osei,M.K.; B. Banful, C. K. Osei, and M. O. Oluoch, (2010)"Characterization of african eggplant for morphological characteristics," *Journal of Agricultural and Science Technology*, vol. 4, no. 3, pp.33–37.
153. Otshudi,A.L.; A. Vercruysse and A. Foriers, (2000)"Contribution to the Ethnobotanical, Phytochemical and Pharmacological Studies of Traditionally Used Medicinal Plants in the Treatment of Dysentery and Diarrhoea in Lomola Area, Democratic Republic of Congo (RDC)," *Journal of Ethnopharmacology*, 71(3), 411-423. doi:10.1016/S0378-8741(00)00167-7
154. Oyeyemi, S.D., Arowosegbe, S. & Adebiyi, A.O. (2014). "Phytochemical and Proximate Evaluation of *Myrianthus arboreus* (P. Beau.) and *Spargonophorus sporgonophora* (Linn.) Leaves", *IOSR Journal of Agriculture and Veterinary Science*, 7(9), 01-05.
155. Rajani C., Ruby, K.M; Aastha, S. and Jaya D. (2012) Int. J. Pharm. Sci. Rev. Res., 15(1), 14, 65-71.
156. Rice-Evans, C. A.; Miller, N. J.; Paganga, G.(1996) Structure-antioxidant activity relationships of flavonoids and phenolic acid. *Free Radical Biol. Med. 20*, 933–956.
157. Roginsky V. (2003) Chain-breaking antioxidant activity of natural polyphenols as determined

during the chain oxidation of methyl linoleate in Triton X-100 micelles. Arch. Biochem. Biophys. 2003; 414: 261-270.

158. Shahidi F.(1997) : Natural antioxidant: Chemistry, health effects and amplifications, ed. F. Shahidi, Champaign, IL: AOCS PMMress. 1-7
159. Shils,E.M.; M. Shike, A. C. Ross, B. Caballero and R. J. Cousings, (2006)"Modern Nutrition in Health and Disease," 10th Edition, Lippincott Williams and Wilkins, A Wolters Klumer Company, UK, pp. 280-281
160. Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999) cited in Milan S. Stanković,2010, Total Phenolic Content, Flavonoid Concentration and Antioxidant Activity of *Marrubium peregrinum* L. Extracts Terebinth, and Pomegranate, Journal of Food Science, Institute of Food Technologists. 75(7):1750-3841.
161. Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L., Hawkins Byrne D. (2006) Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis.* 19(6-7):669–675. doi: 10.1016/j.jfca.2006.01.003. [CrossRef] [Google Scholar]
162. Tourtoglou C., Nenadis N., Paraskevopoulou A.(2014) Phenolic composition and radical scavenging activity of commercial Greek white wines from *Vitis vinifera* L. cv. Malagousia. *Journal of Food Composition and Analysis.* 33(2):166–174. doi: 10.1016/j.jfca.2013.12.009. [CrossRef] [Google Scholar]
163. Wong, C. C.; Li, H. B.; Cheng, K. W.; Chen, F. (2006) A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chem. 97*, 705–711.