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Estimation of Rutin, Quercetin and Antioxidant Assay of Some Food Plant Samples (Fruits, Seeds and Leaves) Grown in Adamawa and Gombe States, Nigeria.

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ABSTRACT

Antioxidants are known to play a key role in the protective influence exerted by plant food. Regular consumption of vegetables and fruits has been recognized as reducing the risk of chronic diseases. Antioxidants are responsible for prevention of the damaging effects of free radicals and toxic products of their metabolism. In this research, commonly grown and consumed plant food samples in the north east of Nigeria, were selected under classification as fruits, leaf and seed samples. Rutin and quercetin contents of crude the samples (Fruits, Leafs and Seeds) were determined using high performance liquid chromatography (HPLC) and the antioxidant activities assay was measured using UV-Spectroscopy by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method. The results shown that the rutin content in the fruit, leaf and seed samples analyzed range from 27.53 to 77.30 µg/g, 31.99 to 91.68 µg/g and 4.01 to 17.64 µg/g respectively while the concentration of quercetin in the studied fruit, leaf and seed samples range between 3.87 – 47.96 µg/g, 0.44 – 2953.22 µg/g and 1.71 – 41.7 µg/g respectively. The DPPH IC50 values of the crude extracts of samples FC LH, LG, SO, LI and SM, showed that they have very strong, strong, moderate, weak, very weak and antioxidant activity respectively. The extracts of crude samples FA, FB, FD, FE, LF, LJ, SK, SL and SO did not show any antioxidant reaction. The significant amounts of these antioxidants confirm the nutritional and medicinal value of the food samples.

Keywords: Antioxidant Assay, Rutin, Quercetin, HPLC, UV Spectroscopy and Fruits samples

INTRODUCTION

Free radicals are continuously produced within living cells as a result of multiple biochemical and physiological processes [1,2,3]. In addition, numerous exogenous sources, including xenobiotics and radiation, can induce free radicals [4,5,6,7]. Because of their high reactivity, free radicals can damage diverse cellular macromolecules, and when the accumulation of these radicals exceeds the limits of what the natural cellular antioxidant effects can neutralize, numerous pathological effects may manifest in the cells [8,9,10]. The identification of certain alkaloids and phenolic compounds in food plant some how provide an alternative method for medicine and remedies as many studies have proved to alleviate certain ailments in treating liver cancer, gall bladder problems, kidney stones, menstrual pains and etc [11,12,13,14,15]. All these problems have lead to the development of new drugs derived from plants, which is believed to be safer and more effective [16,17,18,19,20]. The objective of this study was determined the health promoting properties of the food plant samples using high performance liquid chromatographic (HPLC) and UV – spectrophotometric methods. The structures of quercetine and Rutin are presented in figures 1and 2 below.

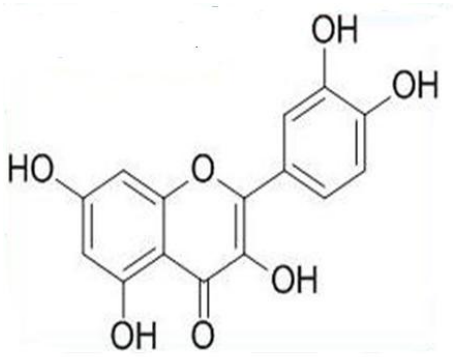

Fig1: Structure of Quercetin [21].

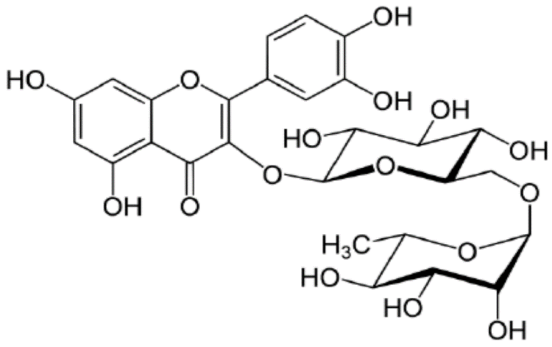

Fig2: Structure of Rutin [22].

Materials and Methods

All the samples (selected fruits, herbs and seeds) were collected from Madagali, Numan and Yola agricultural farms in Adamawa state except *Azanza garckeana* that was collected from Baule, Yiri and Wange in Gombe State of Nigeria.The samples were identified by renowned taxonomist. They were washed thoroughly with distilled water to remove foreign materials.The samples were oven dried at 60^0C, then cut and ground in a mortar.Then, the samples were sieved with laboratory sieves (30mm size) to obtain homogenous particle size using the BS410/1986 laboratory test sieve.

Antioxidant Analysis

Chromatographic conditions

HPLC System Agilent 1200 Series with auto-sample and Diode Array detector with variable wavelengths was used at Wave Length of 254 nm. Flow rate used was 1.2 ml/minute. The Column Temperature was 30 ºC and run length for analysis was 25 min. Column specification is Agilent (Eclipse) XDB-C18, 5µm, 4.6x250 mm, PN: 990967-902 BN: USNHO 25774 and LN: B11169. A security guard of C18, 4.0 X 3.0 mm was attached to the column.

Mobile phase preparation

Mobile phase was prepared by mixing a portion of acetonitrile 20 % and 0.3 % formic acid 80 % (V/V) and the pH was adjusted to 3.0 with perchloric acid. The resultant solvent was filtered using 0.45 µm x 47 mm nylon membrane filter and sonicated for 30 min before use.

Preparation of diluent

Diluent was prepared by mixing 50 mL of the HPLC grade methanol and 50 mL of water in a volumetric flask and the pH was adjusted with 1 mL glacial acetic acid and the solution was sonicated for 30 min before use.

Rutin standard preparation

A portion (10.0 mg) of rutin reference standard was weighed and dissolved with 100 % methanol sonicate for 10 min and make up to mark in a 10 mL volumetric flask with methanol to get a 1 mg/mL stock solution. The resulting stock solution was filtered using 0.2 µm x 13 mm syringe filter. Different concentrations (1.5625 – 100 µg/mL) were prepared from the stock solution by serial dilution using the diluent to make up the volumes.

Quercetin standard preparation

A 10.0 mg portion of the Quercetin reference standard was weighed and dissolved with 100 % methanol sonicate for 10 min and make up to mark in 10 mL volumetric flask with methanol to get a 1 mg/mL stock solution. The resulting stock solution was filtered using 0.2 µm x13 mm syringe filter. Different concentrations (1.5625 - 100 µg/mL) from the stock solution was prepared by serial dilution using the diluent to make up the volumes.

Sample preparation

The method described by [23] was adopted with slight modification. A portion of 1.0 g of the sample was weighed and 10 mL of methanol/water (50/50 v/v) was added and extracted by shaking on a mechanical shaker for 1 h and decanted. The resultant solution was centrifuged at 4500 rpm for 10 min and the supernatant filtered using 0.2 µm x 13 mm nylon syringe filter and 10 µL of the filtrate was introduced into the column for HPLC analysis.

Antioxidant Activity

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the method previously reported by [24] and modified by [25]. A solution of 0.1 mM of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (50,100,200,400 and 800 µg/mL). The mixtures were vigorously s62haken and allowed to stand at room temperature for 30 min. Then, the absorbance was measured at 517 nm using a UV-VIS spectrophotometer. Gallic acid and Trolox were used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The percent DPPH scavenging effect was calculated by using following equation:

DPPH Scavenging Effect (%) or % Inhibition= A_ (o-A_1)/A_0 ×100

Where Ao is the absorbance of control reaction and A1 is the absorbance in the presence of the extract samples and reference standard. All the tests were performed in triplicates and the results were averaged. The inhibition concentration (IC50) value of the sample was calculated using Log dose inhibition curve.

Antioxidant Activity

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the method previously reported by Blois, M. (1958) and modified by [25].

RESULTS AND DISCUSSION

Rutin, quercetin contents and the IC_{50} values of the samples (Fruits, Leafs and Seeds) were determined and presented in Table 1 below:

Table 1: Rutin Content, Quercetin Content and the IC_{50} values in the Fruit, Leaf and Seed Samples

Sample Code	Rutin Content (µg/g)	Quercetin Content (µg/g)	IC_{50} (µg/mL)
FA	43.99±0.11	29.57±0.21	279.60
FB	27.53±0.11	24.78±0.17	380.33
FC	60.99±0.06	47.96±0.27	36.02
FD	28.47±0.06	3.87±0.04	306.66
FE	77.30±0.42	25.59±0.03	316.57
LF	37.24±0.07	40.09±0.04	409.44
LG	278.56±0.00	2953.22±2.8	101.49
LH	91.68±0.21	185.96±0.5	62.05
LI	31.99±0.48	0.44±0.00	205.99
LJ	33.83±0.23	39.79±0.01	395.81
SK	7.94±0.78	1.79±0.19	653.87
SL	14.36±0.29	2.96±0.36	315.18
SM	4.01±0.01	1.71±0.54	228.94
SN	14.21±0.13	5.90±0.00	200.49
SO	17.64±0.13	41.70±1.28	654.76
Gallic Acid (Positive control)	NA	NA	36.49
Trolox (Positive control)	NA	NA	11.84

Results are presented in Mean±SD
NA = Not Applicable

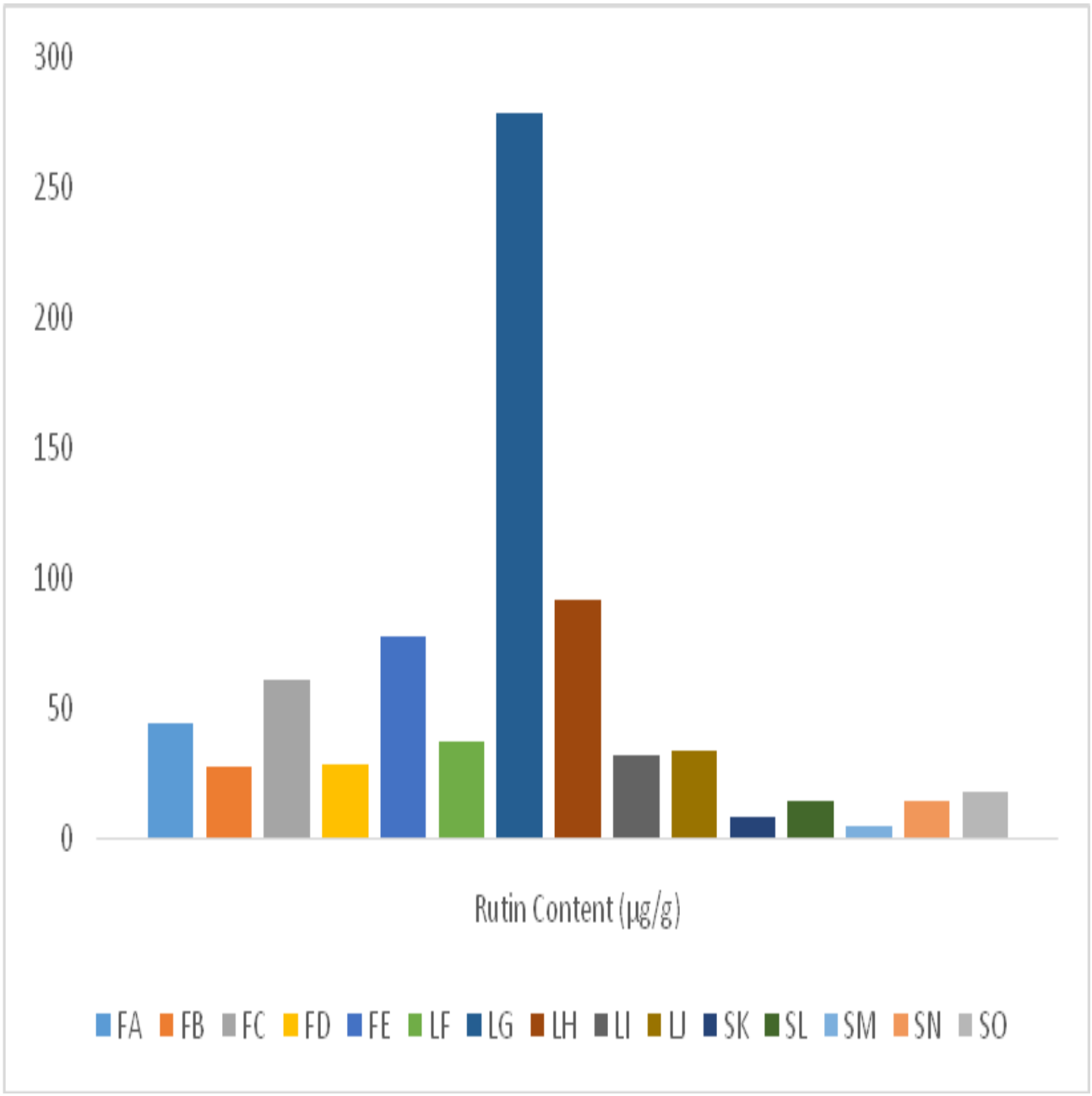

Fig 3: Rutin contents of the sample (µg/g)

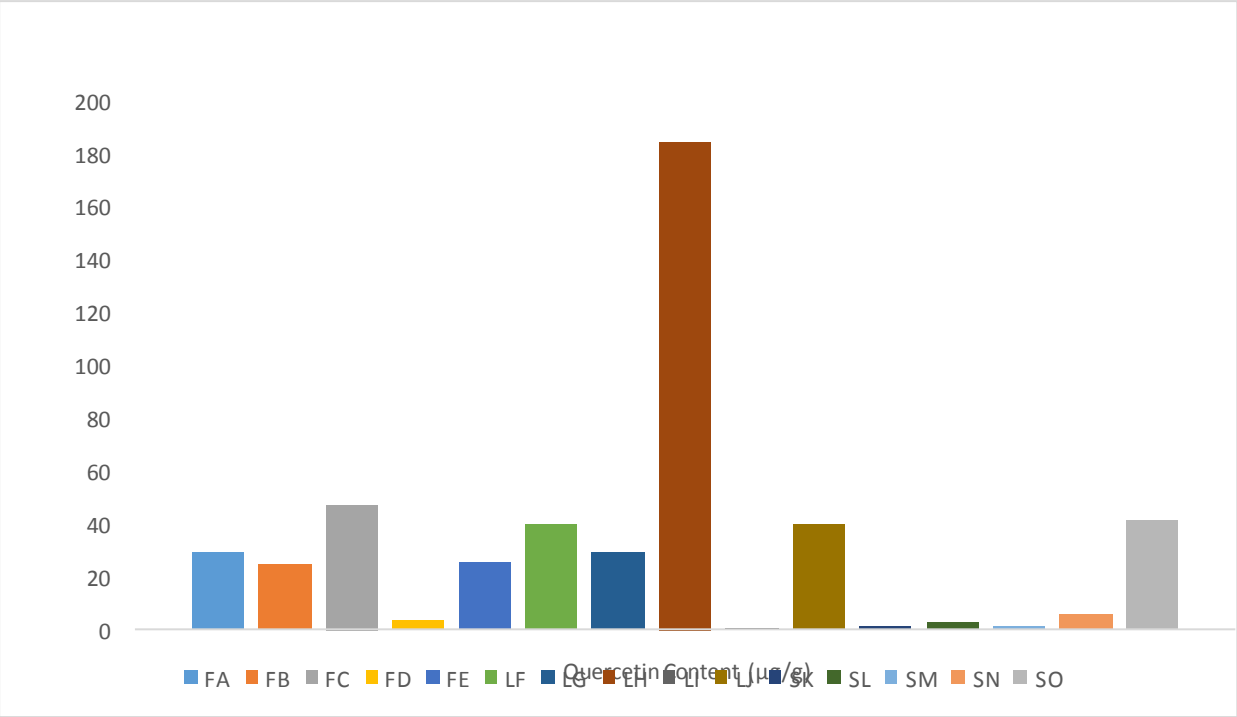

Fig 4:Quercetine contents of the plant samples (µg/g)

Rutin and Quercetin Content

The rutin content in the studied fruit, leaf and seed samples range from 27.53 to 77.30 µg/g, 31.99 to 91.68µg/g and 4.01 to 17.64 µg/g respectively as presented in table 1 and figures 2and 3 above. This shows that the studied leaf samples are richer in rutin content followed by the fruit and the seed samples. Except for sample LI, most of the studied leaf samples show significantly, higher quercetin content while the least quercetin content was observed in the studied seed samples except for sample SO. Quercetin content for LG was found to be higher than the value (2340 µg/g) reported by [26] for caper p. The findings in this study is supported by reports [27], which says that quercetin is commonly found in vegetables and fruits.

DPPH radical- scavenging activity

Based on the classification of level of antioxidant activity at IC50 (µg/mL), samples FC LH, LG, SO, LI and SM has very strong, strong, moderate, weak, very weak and very weak antioxidant activity respectively. Figure 4 shows the IC50 vales of the samples as compared with the standard. However, this does not imply that they are not good antioxidants because studies by [23] showed that antioxidant activity is sensitive to free radical source. IC_{50} values 0.43 mg/mL in red grapes, 176.2 mg/mL in cucumber, 0.0002 mg/mL in pomegranate and 15.97 mg/mL in Wheat have been reported by [27]. The findings in this study shows that majority of the studied fruit samples does not have good antioxidant activity using DPPH method and most of the studied seed samples have very weak antioxidant activity.

CONCLUSION

The crude samples analysed are good source of Rutin and Quercetin. Samples FA, FB, FD, FE, LF, LJ, SK, SL and SO did not show antioxidants activity using DDPH method while samples FC > LH > LG > SO > LI have good antioxidant activity. Most of the fruit samples analysed did not show good antioxidant activity using DPPH method and majority of the studied seed samples have very weak antioxidant activity. The significant amounts of these antioxidants confirm the nutritional and

medicinal value of the food samples anased which helps in boosting human immune system.

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