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Quantification of Total Phenolic and Flavonoid Contents in *Cassia Tora* and *Laptedenia Hastata* Leaves from Seven Irrigation Areas of Kano State Nigeria

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ABSTRACT
The aim of this work was to quantify the total phenolic (TPC) and flavonoid contents (TFC) of Cassia tora and laptadeniahastata vegetables consumed in Kano state, Nigeria. Presence of TPC in the leaves of cassia tora and Laptadiniahastata from different sampling areas are as follows: Bebeji sample had 4.41±0.02 mg/g gallic acid equivalent (GAE) and 15.69 ±0.01mg/g GAE, Chalawa sample had 3.41±0.09 mg/g GAE and 6.479±0.036 mg/g GAE, Minjibir sample had 4.72±0.07 mg/g GAE and 7.33±0.03 mg/g GAE, Tudun Wada sample had 5.16±0.09 mg/g GAE and 2.583±0.036mg/g GAE, Garun Malam sample had 5.39±0.03 mg/g GAE and 5.33±0.07 mg/g GAE,Kura sample had 4.5±.01 mg/g GAE and 7.542±0.072 mg/g GAE),Tiga sample had 5.62±0.01mg/g GAE and 14.00±0.132 mg/g GAE respectively whereas, TFC are :Bebeji sample had 17.68±0.01mg/g rutin equivalent (RE) and 6.725±0.06 mg/g RE, Chalawa sample had 10.35±0.04 mg/g RE and 6.392±0.034 mg/g RE ,Minjibir sample had 11.23±0.09 mg/g RE and 5.42±0.03 mg/g RE, Tudun Wada sample had 9.96±0.07 mg/g RE and 6.35±0.02 mg/g RE, Garun Malam sample 16.82±0.04 mg/g RE and 6.52.0.04 mg/g RE, Kura sample had 14.37±0.07 mg/g RE and 6.56±0.03 mg/g RE), Tiga sample had 22.64±0.09 mg/g RE and 5.88±0.42 mg/g RE respectively. Based on the results of this investigation, it can be concluded that Cassia tora and Laptadeniahastata are rich sources of phenolic compounds which are natural antioxidant of high value.

Keywords: Cassia tora, laptedenia hastata, leaves, phenolic,flavonoid

INTRODUCTION

It is well known fact that drugs obtained from plants don't show any side effects. So that herbal drugs are used in large proportion in the world (Jeffrey and Herbert,1993). These plants Casssia tora and Laptadenia hastate belongs to family Caesalpinioldeae and Asclepiadaceae. These plants are very easily available in northern part of Nigeria.They contain very important chemical constituents like alkaloids, glycosides, phenol,and flavonoid (Mohd*et al*,2013).Phenolics are the naturally occurring compounds, which occurs in different part of plants both in free state.They are found to have many biological activities including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic,antiangiogenic, anticancer, protein kinase inhibition etc. A variety of dietary plant phenolics inhibits tumors development in experimental animal models. Phenolics compounds have the pharmacological effects like their ability to inhibit the release of histamines, the adhesion of blood platelets and the action of lensaldosereductase, to block the inflammatory effects of hepatotoxins, and to act as a heart stimulant(Rai and Nath,2005) . Based on the strong evidence of biological activities of phenolic compounds, the study was focused on quantification of total phenolic and flavonoid content in these selected plant species.

MATERIALS AND METHODS

Sample collection

The plant materials used in this study were collected from seven different irrigation areas of Kano-state Nigeria (Viz:BBJ=Bebeji, TWD=Tudun Wada, GNM=Garun Malam, TIG=Tiga, KUR=Kura, CHL=Chalawa, MJB=Minjibir).

Sample Preparation

The plant materials were washed properly with distilled water and air dried at room temperature until dried and ground into fine powder.

Extraction of Total Phenolic Content

To 400g each of the finely powdered vegetable samples. 10 ml of aqueous acetone (70%) was added and suspended in water bath for

20 min at room temperature. The suspension was centrifuged for 10 min at 3000 rpm. The supernatant was diluted with methanol to achieve a volume of 10 ml and kept on ice (supernatant A). Each plant was extracted in triplicate.

Quantitative Determination of Total Phenolic Content

200µL of supernatant A was transferred to a test tube (4tube/sample) and dried. 600µL of sulfuric acid (0.4M) was added to the test tubes. To three tubes, 900µL of Rhodanine solution (0.667%) and to the fourth tube 900 µL of methanol was added. The fourth tube was set as a blank. After 10 min 600µL of potassium hydroxide solution (0.5M) was added to all of the test tube. 6 min later 13 mL of distilled water was also added. After 25 min the absorbance of red –purple solution was measured at 520 nm against the blank using Varian UV-Vis spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian).The concentration of total phenolic content in the test sample was obtained from the calibration plot of the standard by interpolation and expressed as mg gallic acid equivalent (GAE)/g of dried plant material. All the determinations were carried out in triplicate as per the method described by Mahdi *et al*, (2011).

Extraction of Total Flavonoid

1g of each dried plant material was used for the preparation of the extract. Samples were extracted with 7.5 mL (95%) ethanol at 40°C for 10 min; the extraction process was repeated thrice. The solvent was evaporated at 40°C.The dried extract was used for further analysis.

Sample preparation for flavonoid content

50 mg of the extract was dissolved in 5 ml methanol and sonicated for 45 minutes at 40°C followed by centrifugation at 1,000 rpm for 10 min. The clear supernatant was collected and used for analysis.

Determination of total flavonoid

0.6 ml of the extract was separately mixed with 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixture was measured against blank at 420 nm wavelength with a Jenway UV-Vis spectrophotometer. The concentration of total flavonoid content in the sample was obtained from the calibration plot by interpolation and expressed as mg rutin equivalent (RE)/g of dried plant material. All the determinations were carried out in triplicate(Marinova*et al.,* 2005).

RESULTS AND DISCUSSION

In this study the total phenolic content of Cassia tora from the sampling areas varied between 22.647±0.096 to 9.961±0.073mg/g GAE with Tiga sample having the highest value and Tudun wada sample had the least as shown in Figure 1. The values obtained in this work for total phenolic content in Cassia tora was similar to the results obtained by Jia *et. al.,* (1998), who reported Mulberry to have 9.84mg/g GAE of total phenolic content. Sumitra *et al*. (2012), also reported Cassia tora to have 34.76±0.02mg/g GAE total phenolic content. Kumar *et. al.,* (2013), reported that, Cassia tora had 4.25 mg/g GAE total phenolic content, while Ouedraogo *et. al.,*(2011), reported Amaranthus to contain 12.73±1.61 mg/g GAE total phenolic content.

The highest total phenolic content of 7.542±0.072 mg/g GAE in Laptedenia hastate was found in Kura sample. Figure 2, shows Tudun wada sample has the least phenolic acid content of 2.583±0.036mg/g GAE, while Minjibir, Chalawa, Kura, Tiga and Garunmalam samples have total phenolic content of 7.333±0.036, 6.479±0.036,7.542±0.072, 5.333±0.072 mg/g GAE respectively.Another research reported by Aliero *et. al.,* (2013) shows that, Laptedenia hastate leaves contained 37.77 mg/g GAE of total phenolic content. Available literature reports shows that Laptedenia hastate, Cassia tora, Amaranthus and Hibiscus sabdariffa Calyx leaves,by Bello *et. al.,* (2013), Vedpriya*et. al.*,(2010), Chitindingu et al,(2007) and Chinedu*et. al.*,(2011) contained 3.77± 1.12mg/g GAE, 13.15±0.78 mg/g GAE,1.127 ± 0.133 mg/g GAE and 29.2 mg.GAE/g total phenolic content respectively .The values obtained in this work are less than those reported by the above workers. This might be due to differences in varieties and soil formation (Kumar *et. al.,* 2013).

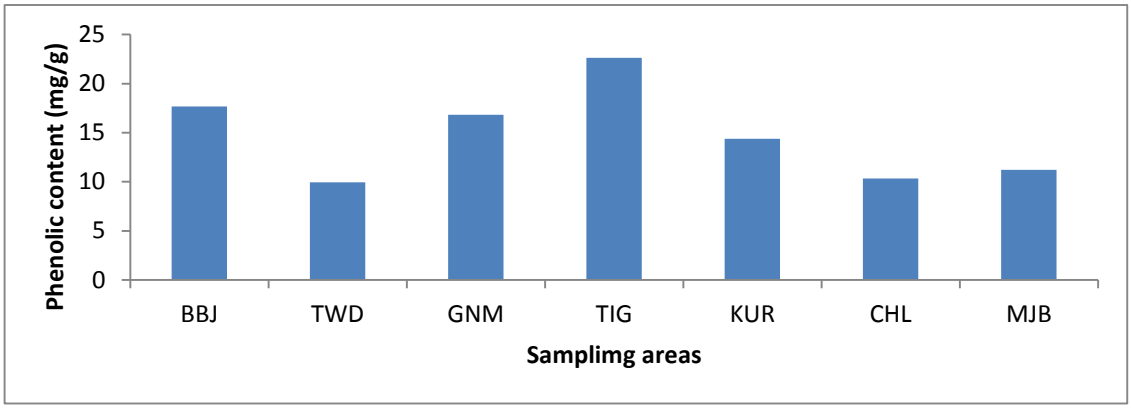

Figure 1: Mean concentration of total phenoilc content in cassia tora from the sampling areas
Key: BBJ=Bebeji, TWD=Tudun Wada, GNM=Garun Malam, TIG=Tiga, KUR=Kura,CHL=Chalawa, MJB=Minjibir

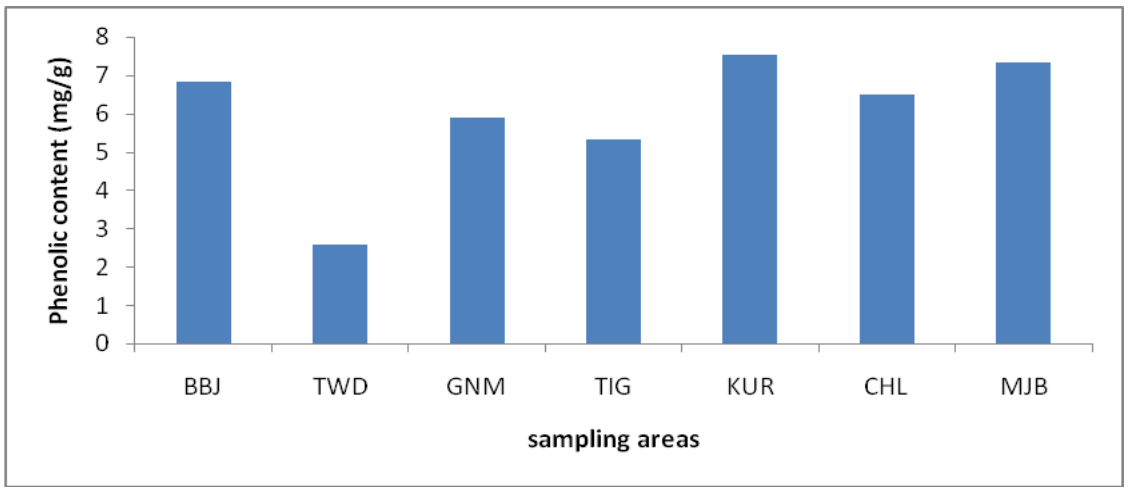

Figure 2:Mean concentration of total phenolic content in Laptadeniahastata
Key: BBJ=Bebeji, TWD=Tudun Wada, GNM=Garun Malam, TIG=Tiga, KUR=Kura,CHL=Chalawa, MJB=Minjibir

Figure 3 showed that, In this study the total flavonoid content in Cassia tora from the sampling areas varied between 5.625±0.062mg/g RE and 3.417 ± 0.095mg/g RE. Tiga having the highest value and Chalawa the least. The value obtained in this work (4.146 ± 0.029 mg/g RE) of flavonoid content of cassia tora was observed to be in agreement with other result published by Kumar et al., (2013), who reported Cassia alata to have 4.25 mg/g QE of flavonoid.content. Sumitra *et al*., (2012), reported cassia tora leaves to have 34.76±0.02mg/g QE of flavonoid content. Ouedraogo *et al*., (2011) also reported A. viridis have value of 1.51±0.30 mg/g QE/ of total flavonoid.

Figure 4 showed that the highest total flavonoid content of 6.725±0.068mg/g RE in Laptedenia hastata was found in Bebeji. Tiga has the least flavonoid content of 5.42±0.034 mg/g RE, while Tudun wada, Garun malam, Minjibir, Kura ,Chalawa have flavonoid content of 6.353, 6.524, 5.882±0.424, 6.569±0.034 and 6.392±0.034 mg/g RE respectively. Available literature shows that, laptedenia hastate leaves (Bello *et al*., 2013), contained 10.50±0.30mg/g RE total flavonoid. Other works showed that cassia tora, M. dioica leaves and selected Uganda traditional medicinal food were reported by Andabati *et al*. (2014); Kumar *et al*., (2013) and Sumitra *et al*. (2012), to have contained 0.3±0.1 to 162.2± 3.5 mg/g CE,2.89 mg/g QE and 46.67±1.04mg/g QE of flavonoid content.

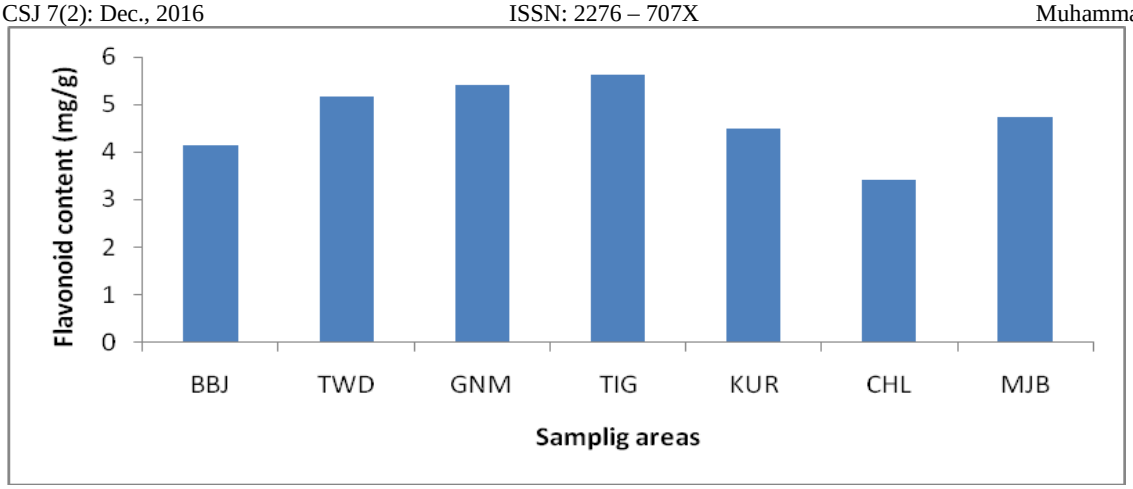

Figure 3: Mean concentration of flavonoid content in cassia tora from the sampling areas
Key: BBJ=Bebeji, TWD=Tudun Wada, GNM=Garun Malam, TIG=Tiga, KUR=Kura,CHL=Chalawa, MJB=Minjibir.

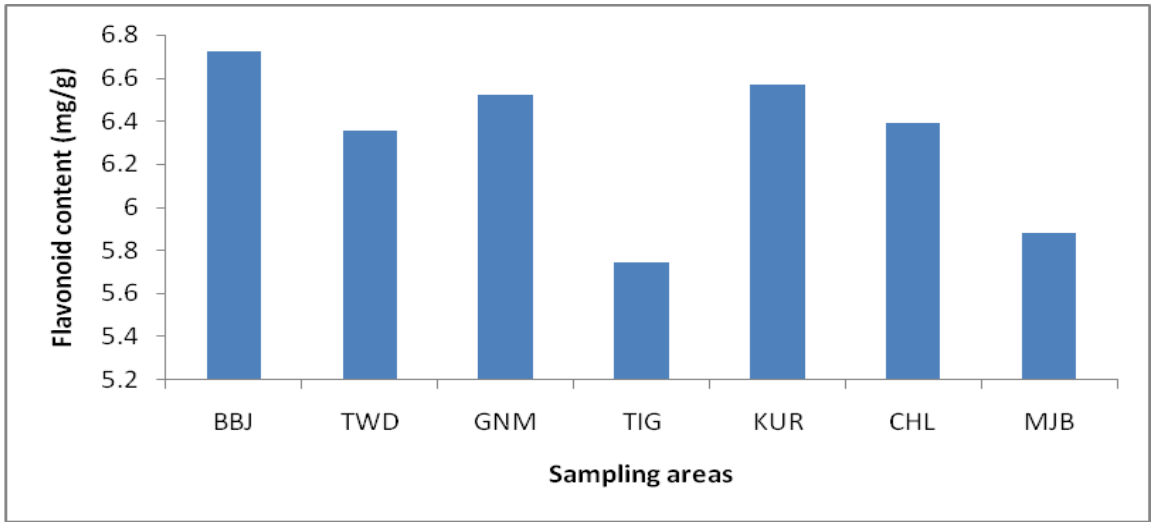

Figure 4; Mean concentration of flavonoid content in Laptadeniahastata from the sampling areas.

Key: BBJ=Bebeji TWD=Tudun Wada, GNM=Garun Malam, TIG=Tiga, KUR=Kura, CHL=Chalawa,MJB=Minjibir.

CONCLUSION

Based on the results obtained in this investigation, it could be concluded that Cassia tora and Laptadenia hastate are rich sources of phenolic and flavonoid compounds which are natural antioxidants of high value.

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