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GOSSYPIUM HIRSUTUM L. AND *GOSSYPIUM BARBADENSE* L.: DIFFERENCES IN PHYTOCHEMICAL CONTENTS, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES.

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ABSTRACT

Gossypium L. generally called cotton plants are used indiscriminately in traditional medicine without cognisance to the fact that there are different cotton species and the likelihood of differences in phytochemical content and ultimately, their medicinal capabilities. This study reports the differences in the phytochemical contents, antioxidant and antimicrobial properties of extracts of leaves of two cotton species, *Gossypium hirsutum* L. and *Gossypium barbadense* L. Leaf samples from mature *G. hirsutum and G. barbadense* plants were collected, shade-dried and powdered. Phytochemical contents were quantified while antioxidant activity was tested through 1,1-diphenyl-2-picrylhydrazyl **(**DPPH) scavenging activity and reducing power. Aqueous, decocted aqueous and ethanol leaf extracts of *G. hirsutum* and *G. barbadense* were tested against *Escherichia coli* (known to cause gastrointestinal infections and urinary tract infections), *Staphylococcus aureus* (known to cause skin infection), *Klebsiella pneumoniea* and *Pseudomonas aeruginosa* (known to cause bronchial infections and pneumonia). The results showed that tannins and phenols were present in leaves of *G. hirsutum* but absent in *G. barbadense.* Leaves of *G. hirsutum* contained significantly higher (at $p<0.05$) amount of total glycosides (156.44 ± 2.05 mg /100g) and flavonoid (120.85 ± 0.32 mg /100g, twice as much) than *G. barbadense. Gossypium hirsutum* showed higher DPPH free radical scavenging activity and reducing power than *G. barbadense. Gossypium hirsutum* showed higher zones of inhibition (mm) in all organisms than *G. barbadense.* It can therefore be concluded that *G. hirsutum* leaves have higher phytochemical contents as well as antioxidant and antimicrobial activities than *G. barbadense. Gossypium hirsutum* is therefore recommended for use as against *G. barbadense* where the choice is available.

Keywords: Antimicrobial, Antioxidant, 1,1-diphenyl-2-picrylhydrazyl **(**DPPH), *Gossypium hirsutum, Gossypium barbadense,* Phytochemical

INTRODUCTION

The cotton genus *Gossypium* (Family Malvaceae) comprises of about 50 species and few new species continue to be discovered (Wendel *et al*., 2009). The species of this genus are generally shrubs or shrub-like plants which are extraordinarily diverse in morphology and adaptation (Wendel *et al*., 2009). The name "cotton" (English) originated from the Arabic term "al qutn" and it describes species that produce spinnable fibres (lint) on their seed coat (Lee, 1984). The name 'cotton plant' is actually used for four species in the genus *Gossypium*: *G. hirsutum* L., *G. barbadense* L., *G. arboretum* L. and *G. herbaceum* L. that were domesticated independently as a source of textile fiber (Brubaker *et al*., 1999). Today *G. hirsutum* and *G. barbadense* are the major cultivated species with *G. hirsutum* accounting for 90% of world cotton production (Jerkins, 2003) while *G. barbadense* represents approximately 5% (Wu *et al*., 2005). In addition to their economic importance, the leaf, root, bark and seeds of *Gossypium* species have been widely explored for their medicinal values. *Gossypium barbadense* extracts are sold for use in alternative medicine for treatment of hypertension, fungal infections and as an abortifacient or emmenagogue or menstruation stimulant (Hasrat *et al.,* 2004; Mans *et al*., 2004). *Gossypium hirsutum* has been shown to have bioinsecticidal and antitrypanocidal properties (Atawodi *et al*., 2003; Abe *et al*., 2004; Abdullahi 2004), antiviral (Fasola *et al*., 2011) as well as antimicrobial properties (Omojasola and Awe, 2004). Other *Gossypium* species such as *G. herbaceum* (Singh *et al*., 2011; Dhamija *et al*., 2011; Kumar *et al*., 2011) and *G. arboretum* (Saidu and Abdulahhi, 2011) have also been shown to have medicinal properties.

Gossypium barbadense and G. hirsutum are the most commonly found *Gossypium* species in Nigeria (mostly in the south), with *G. barbadense* occurring

the most. In nature, *G. hirsutum* is a perennial shrub that grows to approximately 1.5 – 2 m in height, while *G. barbadense* grows to approximately 3 m in height. *Gossypium hirsutum* is heliotropic, its leaves are generally flat and track the sun to maximize light adsorption throughout the day to reduce photobleaching and transpiration (Wise *et al*., 2002). *Gossypium hirsutum* is widely branching, the stem more or less stellate – pubescent, having gland dots throughout while *G. barbadense* is arborescent with the stem sparsely stellate to pubescent to glabrous and prominently gland dotted. *Gossypium hirsutum* has long petiolate, cordate, weakly 3-5 lobes, lobes broadly triangle to ovate, acute to acuminate leaves whereas *G. barbadense* has petiolate, cordate, 3-7 lobes, palmately 7-9 nerved, glabrate, lobes ovate, entire, acuminate with 1-5 foliar nectarines beneath. The stipules of *G. hirsutum* are subulate to falcate and 5-15 mm long whereas those of *G. barbadense* are subulate to falcate and 10-50 mm long (Fryxell, 1984). *Gossypium hirsutum* flowers are cream in colour while *G. barbadense* has yellow flowers. The nectar of *G. hirsutum* contains higher concentration of sugar than that of *G. barbadense* (Moffet, 1983). *Gossypium hirsutum* has been shown to have larger anatomical and physiological features (Lu *et al*., 1997; Wise *et al*., 2002) compared to *G. barbadense* which according to Wise *et al.* (2002) may limit the yield potential of G. *barbadense* in certain growing environments.

According to Estrada-Reyes *et al.* (2004), genetic diversity even as narrow as variety level affects phytochemical composition and medicinal value. Though, literature on *Gossypium* species is confusing and authors disagree on the identity of species, subspecies, sections, varieties, forms, races and cultivars that have been distinguished (Brink and Achigan-Dako, 2012), it does not however, silence the fact that different *Gossypium* species such as *G. hirsutum* and *G. barbadense*, are likely to have differences in phytochemical composition and medicinal value.

Levels of phytochemicals such as alkaloids, glycosides, phenols, saponins and tannins were compared. Phenolics, alkaloids and glycosides are compounds that have been documented to possess medicinal properties (Rosbard *et al*., 1999). The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strain have been reported by Cragg and Newman (2001), Edeoga *et al.* (2005) and Kubmarawa *et al.* (2007). 1, 1 – diphenyl- 2-picrylhydrazyl **(**DPPH) is free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. It is therefore used as a substrate to evaluate the antioxidative activity of antioxidants. DPPH assay has been widely applied in a number of studies to evaluate the radical scavenging ability of antioxidants (Brad-Williams *et al*., 1995). DPPH scavenging activity is compared based on the IC_{50} value. IC_{50} value is the concentration of the extract required to scavenge 50% of DPPH radical. According to Oktay *et al.* (2003), reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Jayanthi and Lalitha, 2011).

However, in Nigeria, little attention is paid to species differences in *Gossypium* and as such traditional medicine practitioners believe that any plant producing cotton lint would suffice for medicinal use irrespective of the species; with the belief that they are varieties of the same species (personal communication with practitioners). Generally there is no scientific report on the possibility of the two species having the potential to substitute each other in medicinal use. This study therefore investigated the differences in the phytochemical contents, antimicrobial and antioxidant properties of *G. hirsutum* and *G. barbadense*, in order to establish the species with possible higher medicinal value.

MATERIALS AND METHODS

Collection of plant material

Leaves of *G. hirsutum* L. and *G. barbadense* L. were collected in the morning from mature trees at flowering stage and taken to the Herbarium of Department of Botany, University of Lagos, where they were identified and authenticated as *G. hirsutum* and *G. barbadense*. Collected leaves of both *Gossypium* species were cleaned and air-dried for a period of 14 days in the laboratory. Dried

leaves were pulverized using electric blender and sieved to obtain fine powder of the samples.

Quantitative Phytochemical Screening

Phytochemical analysis of the powdered *G. hirsutum* and *G. barbadense* leaves were carried out using the method of Harborne (1973), Van Buren and Robinson(1981), Obadoni and Ochuko (2007) and Bohm and Kocipai-Abyazan (1994) to quantify the amount of alkaloids, flavonoids, tannins, phenols, saponins, and glycosides.

Antioxidant activity tests

1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity assay

The free radical scavenging activity of methanol extracts against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was evaluated by the method of Ursini *et al.* (1994). One millitre of various concentrations (25- 100 μg/ml) of leaf extracts were diluted in 3 ml ethanol and mixed with 3 ml DPPH solution. The reaction mixture was shaken vigorously and incubated in the dark for 30 minutes. The absorbance of the solution was measured against a blank at 517 nm afterwards. Percentage inhibition of DPPH was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_o - A_1) / A_o] * 100$$

Where A_o is the absorbance of the blank sample and A_1 is the absorbance of the tested sample.

The IC_{50} values of the extracts were determined from the graph of DPPH activity over concentration of extract. IC_{50} value is the concentration of the extract required to scavenge 50% of free radicals (Gawron-Gzella *et al.*, 2012).

Reducing power assay

The reducing power of the extracts was determined by the method described by Oyaizu (1986). One millilitre of various concentrations (25-100 μg/ml) of the leaf extracts were diluted in 1 ml distilled water. They were mixed with sodium phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). They were incubated at 50 °C for 20 min, 2.5 ml trichloroacetic acid (10%) was added to the mixtures and centrifuged at 3000 rpm for 10 min. The supernatant was dissolved in equal volume of distilled water, and 0.5 ml ferric chloride (0.1 %) added. Absorbance was read at 700 nm using a UV/visible spectrophotometer and was compared with that of ascorbic acid as standard.

Antimicrobial test

Preparation of plant extracts

Decocted aqueous and ethanolic extracts of the plant materials were prepared using the method described by Oyagade *et al.* (1999). Decocted aqueous and ethanolic extraction of the plant materials were carried out by suspending 20 g of pulverized leaf samples in 200 ml of water and 200 ml in 95% ethanol respectively. A preliminary test had shown that the aqueous extract at 25 °C did not show any activity against the test organisms hence the decocted aqueous extract was prepared in a water bath at 75 °C for one and half hours. The ethanolic extraction was done at room temperature and the extracts were decanted and filtered through Whatman filter paper No 10. The filtered extracts were sterilized using membrane filter and evaporated to dryness at 40 °C. The residues obtained were reconstituted in distilled water and 95% ethanol at stock concentration of 0.5 g/ml and stored in the refrigerator at 4 °C before use.

Test organisms

Isolates of *Escherichia coli, Staphylococcus aureus, E. coli* ATCC 25922*, Pseudomonas aeruginosa, Klebsiella pneumoniea* and *Shigella sonnei* were collected from Microbiology Department of the University of Lagos Teaching Hospital, Idi-Araba, Lagos.

Antimicrobial activity assay

Antimicrobial activities of decocted aqueous and ethanol extracts was determined by well method on nutrient agar (Oyagade *et al.*, 1999). Sterile culture media (Mueller Hinton agar) were poured into sterile Petri dishes and allowed to solidify. Test organisms were aseptically introduced and evenly spread using sterile swap sticks on the surface of the gelled Mueller Hinton Agar plates. Three wells of 5.0 mm were aseptically punched on each agar plate using a sterile cork borer, allowing at least 30 mm between adjacent wells and between peripheral wells and the edge of the Petri dish. Fixed volumes (0.1 ml) of the extracts

were then introduced into the wells in the plates. A control well was in the center with 0.01 ml of the extracting solvent. The plates were kept at room temperature for 40 minutes for pre-diffusion of the extracts to occur and then incubated at 37 °C for 24 hours. The resulting zones of inhibition were measured using a ruler calibrated in millimeters.

Statistical Analysis

All analyses were carried out based on three replicates. The data obtained with extracts of *G. hirsutum* were compared with those of extracts of *G. barbadense*. Statistical test for significance was done with Student's t-test ($p < 0.05$) using GraghPad Prism 6 software and data plotted graphically using Microsoft Excel 2007. Data are presented as means with standard errors.

RESULTS AND DISCUSSION

Quantitative Phytochemical Screening

The results of the quantitative phytochemical analyses of both *Gossypium* species are shown in figure 1. Tannins and phenols were present in *G. hirsutum* but absent in *G. barbadense*. *Gossypium hirsutum* plants had significantly higher (at $p<0.05$) amount of total glycosides (156.44 ± 2.05 mg /100 g) and flavonoid (120.85 ± 0.32 mg /100 g, twice as much) than *G. barbadense* (95.8 ± 1.52 mg /100 g and 67.62 ± 1.39 mg /100 g respectively) plants. Omojasola and Awe (2004) have also reported the presence of saponins, tannins and phenolics in *G. hirsutum* but their result showed absence of flavonoid, alkaloids and cardiac glycoside in *G. hirsutum*. The medicinal value of plant lies in the phytochemical (bioactive) constituents of the plant which shows various physiological effects on human body (Edeoga *et al*, 2005). Therefore, through phytochemical screening, one could detect the various important compounds which may be used as the basis of modern drugs for curing various diseases (Sheikh *et al.*, 2013). Most phytochemicals are antioxidant agents which essentially reduce the damages caused in tissue during physiological processes (Ezeonu and Ejikeme, 2016). Phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders (Badam *et al.*, 2002; Gupta and Tandon, 2004; Kamalakkannan *et al.,* 2005; Chew *et al.*, 2009). With the higher variant and quantity of phytochemicals that was obtained in *G. hirsutum* compared with *G. barbadense*, it suffice to say that *G. hirsutum* appear to possess higher medicinal potential than *G. barbadense*.

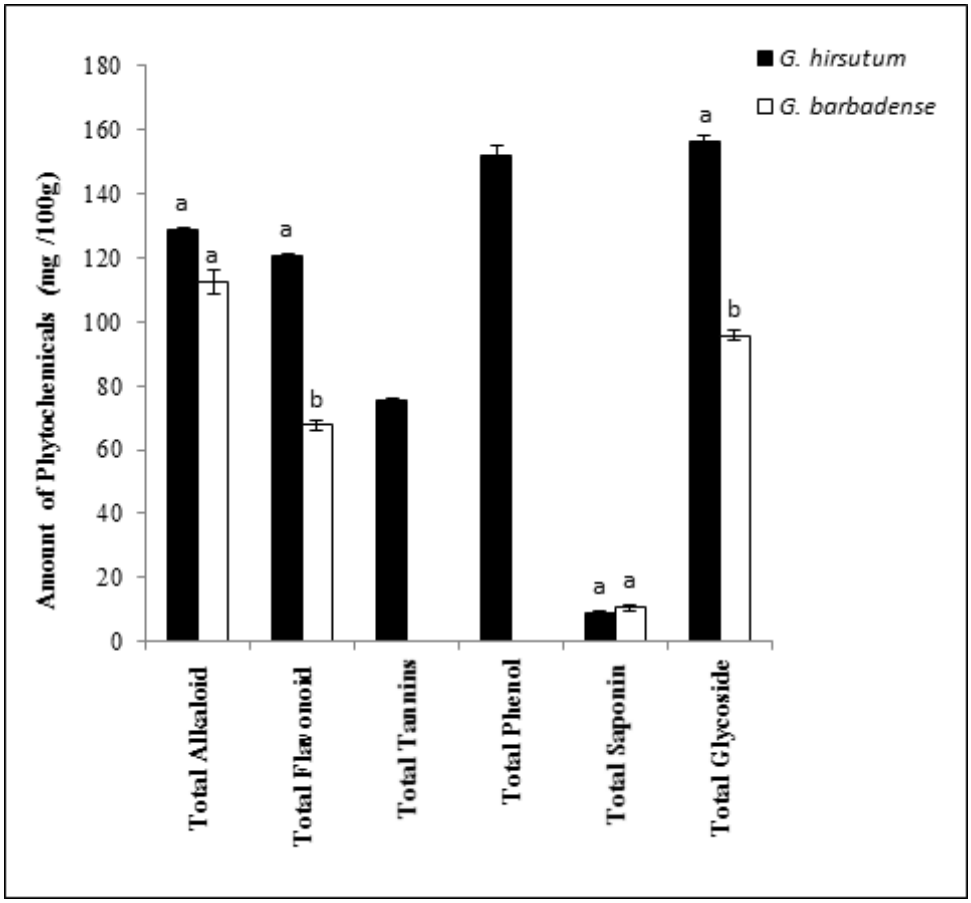

Figure 1: Means of some phytochemicals present in leaves of *G. hirsutum* and *G. barbadense* (mg/100g) with error bars. Bars with the same letters in each of the compound are not significantly different ($p>0.05$).

Antioxidant activity tests

1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity assay

The IC_{50} values of the DPPH scavenging activities of leaf extracts of *G. hirsutum* and *G. barbadense* are presented in figure 2. IC_{50} value is the concentration of the extract required to scavenge 50% of free radicals (Gawron-Gzella *et al*., 2012). The IC_{50} value of *G. hirsutum* (40.05 ± 3.25 µg/ml) was significantly lower ($p = 0.05$) than that of *G. barbadense* (55.15 ± 5.09 µg/ml). The IC_{50} value of the standard antioxidant drug, ascorbic acid (35.23 ± 0.21 µg/ml) was significantly lower ($p = 0.05$) than that of *G. hirsutum* and *G. barbadense* plants as expected. α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method offers the first approach for evaluating the antioxidant potential of a compound, an extract or other biological sources (Kedare and Singh, 2011). This method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis (Rahman *et al.,* 2015). The IC_{50} values obtained in this report, shows that less amount of leaf extracts of *G. hirsutum* (40.05 ± 3.25 µg/ml) is required to scavenge 50% of DPPH compared to *G. barbadense* (55.15 ± 5.09 µg/ml). This is an indication that *G. hirsutum* has stronger antioxidant capability than *G. barbadense.*

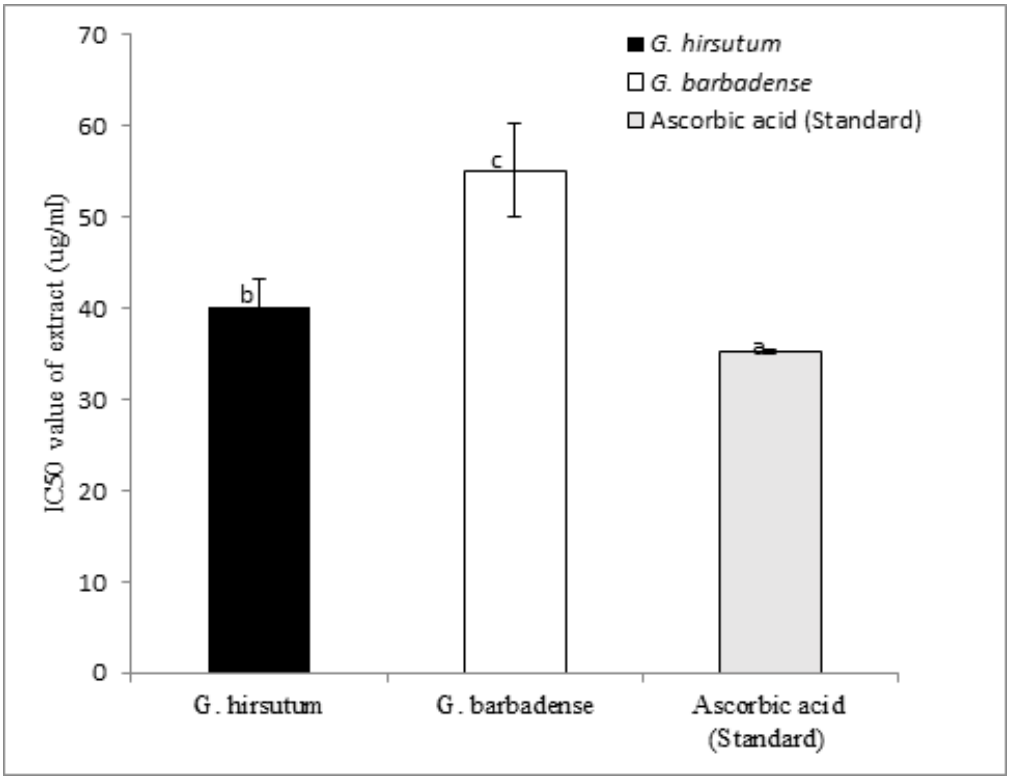

Figure 2: IC_{50} values of DPPH scavenging activities of leaf extracts of *G. hirsutum* and *G. barbadense* and the standard drug, ascorbic acid. IC_{50} value is the concentration of the extracts required to scavenge 50% of DPPH. Bars with different letters are significantly different at $p<0.05$.

Reducing power assay

The results of the reducing power assay, of the leaf extracts of *G. hirsutum* and *G. barbadense* at different concentrations (25 μg/ml to 100 μg/ml) are shown in figure 3. The absorbance of the reaction mixture of the leaf extracts of *G. hirsutum* and *G. barbadense* increased with an increase in the concentration of leaf extracts. *Gossypium hirsutum* and *G. barbadense* showed significantly lower absorbance when compared to ascorbic acid (standard). *Gossypium hirsutum* shows a significantly higher ($p<0.05$) absorbance than *G. barbadense* at all concentrations except with the use of 25 μg/ml extract. Increased absorbance of the reaction mixture indicated increased reducing power of the extracts (Soni and Sosa, 2013), thus it suffice to say that from this result, *G. hirsutum* has a significantly higher ($p<0.05$) reducing power than *G. barbadense*. The reducing property is generally associated with the presence of reductants (Loganayaki *et al.*, 2013). It has been reported that the reducing properties are generally associated with the presence of reductones (reducing agents), which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Kumar *et al.*, 2012). The reducing capacity of a compound may reflect its antioxidant potential (Rao *et al.*, 2010; Soni and Sosa, 2013). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Soni and Sosa, 2013). *Gossypium hirsutum* exhibited a higher antioxidant activity than *G. barbadense* via reducing power assay in a similar manner that it did with DPPH-scavenging assay.

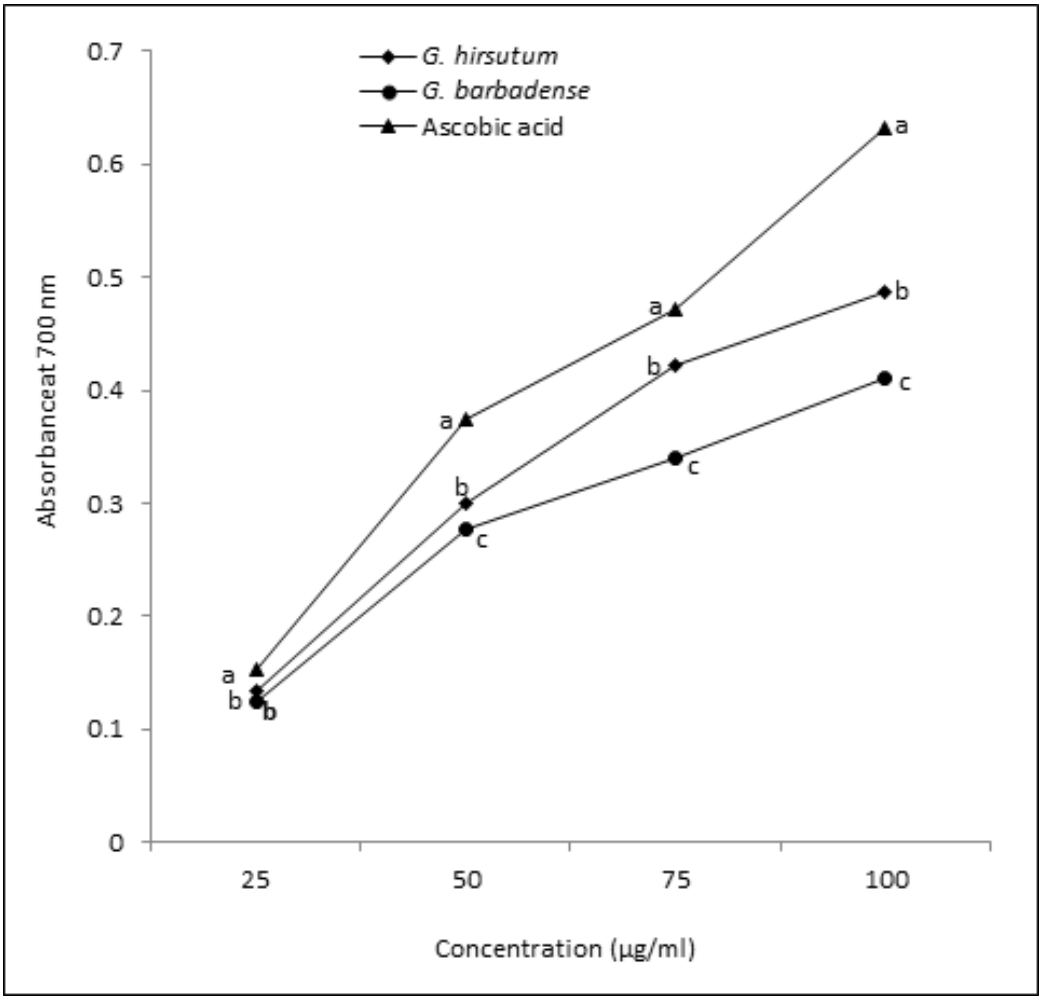

Figure 3: Reducing power activity of leaf extracts of *G. hirsutum* and *G. barbadense*. (Bars with same letters at each stage of growth are not significantly different at $p < 0.05$ using student's t-test).

Antioxidant activities have been associated with the biologically active compounds present in plants (Srivastava *et al.,* 2012). Tannins, saponins, terpenoids, flavonoids, phenols, ascorbic acid, and many other phytochemicals have been reported to have antioxidant capabilities (Krishnaiah *et al*., 2007, Ansari *et al*., 2013). Antioxidant properties are been reported to be positively correlated with the amount of flavonoids and phenolics contained in the plant extract (Sharma *et al.,* 2009, Manach *et al*., 2005, Zhang *et al.,* 2014; Rahman *et al.,* 2015). All the tested phytochemicals were found to be present in *G. hirsutum*, and *G. hirsutum* even had a higher quantity of phytochemicals common to both species. These probably accounts for the higher antioxidant activities via DPPH scavenging ability and reducing power demonstrated by *G. hirsutum* compared to *G. barbadense*.

Antimicrobial test

The pharmacological action of a plant cannot be ascertained by the result of phytochemical studies only hence a need for antimicrobial tests (Mujeeb *et al*., 2014). Antimicrobial test was performed by agar disc diffusion method by using six bacterial strains. The zone diameter of inhibition (mm) of some pathogenic organisms by decocted, ethanol and aqueous leaf extracts of *G. hirsutum* and *G. barbadense* are shown in table 2. There was no reaction observed with non-decocted aqueous extracts. More reactions of organisms to extract were observed with decocted aqueous extracts than ethanolic leaf extracts of both *Gossypium* species. It is not surprising therefore that traditional medicine practitioners use aqueous decoctions of *Gossypium* plants for treatments. Decocted aqueous leaf extracts of *G. hirsutum* recorded higher zones of inhibition (mm) of all the pathogens tested than leaf extracts of *G.*

barbadense but only the differences in the inhibition zones of *Escherichia coli, Staphylococcus aureus* and *Shigella soonei* were significant at $p < 0.05$. Ethanolic leaf extracts of *G. hirsutum* caused higher zones of inhibition (mm) of all the pathogens tested than leaf extract of *G. barbadense* but the differences were not significant at $p<0.05$.

Table 2: Zones of inhibition (mm) of some pathogenic organisms by leaf extracts of *G. hirsutum* and *G. barbadense*

	PATHOGENIC ORGANISMS					
	Escherichia coli	*Staphylococcus aureus*	*Shigella sonnei*	*Pseudomonas aeruginosa*	*E. coli* ATCC 25922	*Klebsiella pneumoniea*
Decocted aqueous extract						
G. hirsutum	11.67±2.65a	15.00±1.73a	12.67±2.65a	12.67±5.57a	11.67±4.36a	12.67±4.36a
G. barbadense	10.33±1.00b	11.00±1.73b	8.33±2.16b	10.54±7.00a	10.00±1.73a	9.33±2.00a
Ethanol extract						
G. hirsutum	11.33±5.29a	11.33±2a	8.67±2.00a	11.00±1.73a	9.67±5.57a	10.00±1.73a
G. barbadense	10.67±7.20a	9.67±2.65a	7.00±1.73a	9.67±2.65b	9.00±3.00a	9.00±1.73a

Means of zones of inhibition of a particular microbe, in same column, by same extract type, followed by the same letters are not significantly different at $p=0.05$.

Antimicrobial activities of leaf extracts of *G. hirsutum* against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* have been reported by Omojasola and Awe (2004) and Essien *et al.* (2011) reported antimicrobial activities of the essential oil from the leaf extracts of *G. barbadense* against *Staphylococcus aureus* and *Escherichia coli.* Decocted aqueous leaf extract showed more antimicrobial activities than ethanolic leaf extracts. Results of the antimicrobial test can therefore, be related to the higher quantity of phytochemicals found in leaves of *G. hirsutum* than in leaves of *G. barbadense.* Tannins and phenols were absent in *G. barbadense*; also *G. hirsutum* had a significantly higher ($p<0.05$) quantity of flavonoids than *G. barbadense.* Phytochemical compounds such as alkaloids, saponins, tannins, phenols and flavonoids have been known to be biologically active and thus partially responsible for the antimicrobial activities of plants, hence their use in traditional medicine (Rao *et al.*, 2010; Ajibesin *et al.*, 2011; Nethathe and Ndip, 2011; Aderiye *et al.*, 2014).

CONCLUSION AND RECOMMENDATION

This is the first report on the biochemical differences between two *Gossypium* species; other biochemical studies have been on the comparison of a *Gossypium* species with another plant species. There are phytochemical differences between *G. hirsutum* and *G. barbadense* as well as differences in antioxidant and antimicrobial activities of the studied species and this could be related to species differences

REFERENCES

Abdullahi, Y.M. and Muhammed, S. 2004. Assessment of the Toxic Potentials of some Plants Powders on Survival and Development of *Callosobuchus maculatus. African Journal of Biotechnology* 3(1):60 – 62.

Abe, F., Nagafuji, S., Okabe, H., Akahane, H., Estrada-Muniz, E., Huerta-Reyes, M. and Chilpa, R. 2004. Trypanocidal Constituents in Plants 3. Leaves of *Garcinia intermedia* and Heartwood of *Calophyllum brasiliense. Biological and Pharmaceutical Bulletin* 27:141-143.

Aderiye, B.I., David, O.M. and Atere, V.A. 2014. Administration of Cashew Extracts in the Treatment of Some Infections and

Diseases. *Advancement in Medicinal Plant Research*, 3(3): 75-86.

Ajibesin, K.K., Bala, D.N. and Umoh, U.F. 2011. The Use of Medicinal Plants to Treat Sexually Transmitted Disease in Nigeria: Ethnomedical Survey of Niger Delta region. *International Journal of Green Pharmacy* 3(5): 181-191.

Ansari, A.Q., Ahmed, S.A., Waheed, M. A. and Sayyed, J.A. 2013. Extraction and Determination of Antioxidant Activity of *Withania somnifera* Dunal. *European Journal of Experimental Biology* 3(5): 502-507

Atawodi, S.E., Bulus, T., Ibrahim, S., Ameh, D.A., Nok, A.J., Mamman, M. and Galadima, M. 2003. *In vitro* Trypanocidal Effect of Methanolic Extract of Some Nigerian Savannah Plants. *African Journal of Biotechnology* 2(9):317 – 321.

Badam, L., Bedekar, S.S., Sonawane, K.B. and Joshi, S.P. 2002. *In vitro* Antiviral Activity of Bael (*Aegle marmelos* Corr.) Upon Human Coxsackieviruses B1-B6. *Journal of Communicable Diseases* 34(2): 88–99.

Bohm, B.A. and Kocipai-Abyazan, R. 1994. Flavonoids and Condensed Tannins from Leaves of *Hawaiian vaccinium vaticulatum* and *V. calycinium*. *Pacific Science* 48: 458-463.

Brad-Williams W., Cuvelier M.E. and Berset C. 1995. Use of a Radical Method to Evaluate Antioxidant Activity. *Lebensmittel-Wissenschaft und-Technologie* 28(1): 25-30.

Brink, M. and Achigan-Dako, E.G. 2012. *Fibres*. Plant Resources of Tropical Africa (PROTA), Wageningen Netherlands. pp. 196

Brubaker, C.L., Bourland, F.M. and Wendel, J.E. 1999. The Origin and Domestication of Cotton. *In*: C.W. Smith and J.T. Cothrea (eds.) *Cotton: Origin History Technology and Production*. John Wiley and Sons Inc. New York. pp 3-31.

Chew, Y.L., Goh, J.K. and Lim, Y.Y. 2009. Assessment of *in vitro* Antioxidant Capacity and Polyphenolic Composition of Selected Medicinal Herbs from Leguminosae Family in Peninsular Malaysia. *Food Chemistry*. 116(1):13–18.

Cragg, G.M. and Newman, D.J. 2001. Natural Product Drug Discovery in the Next Millennium. *Pharmaceutical Biology* 39 (Suppl. 1): 8 -17.

Dhamija, H.K., Parashar, B. and Singh, J. 2011. Anti-depression Potential of Herbal Drugs: An Overview. *Journal of Chemical and Pharmaceutical Research* 3(5): 725-735.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. 2005. Phytochemical Constituent of Some Nigerian Medicinal Plants. *African Journal Biotechnology* 4(7): 685-688.

Essien, E.E., Aboaba, S.O. and Ogunwande, I.A. 2011. Constituents and Antimicrobial Properties of Leaf Essential Oil of *Gossipium barbadense*. *Journal of Medicinal Plants Research* 5(5):702-705.

Estrada-Reyes, R., Aguirre Hernándezc, E., García-Argáez, A., Hernández, M.S., Linares, E., Bye, R., Heinze, G. and Martínez-Vázquez, M. 2004. Comparative Chemical Composition of *Agastache mexicana* subsp. *mexicana* and *A. mexicana* subsp. *xolocotziana*. *Biochemical Systematics and Ecology* 32: 685–694.

Ezeonu, C.S. and Ejikeme, C.M. (2016). Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. *New Journal of Science 2016*. Article ID 5601327, doi:10.1155/2016/5601327

Fasola, T.R., Adeyemo, F.A., Adeniji, J.A. and Okonko, I.O. 2011. Antiviral Potentials of *Gossypium hirsutum* Extracts on Yellow Fever Virus. *New York Science Journal* 4(10):30-35.

Fryxell, P.A. 1984. Taxonomy and Germplasm Resources. *In*: R.J. Kohel and C.F. Lewis (eds.) *Cotton*. American Society of Agronomy Crop Science and Soil Science Madison Wisconsin. pp 27-58.

Gawron-Gzella, A., Dudek-Makuch, M. and Matławska, I. 2012. DPPH Radical Scavenging Activity and Phenolic Compound Content in Different Leaf Extracts From Selected Blackberry Species. *Acta Biologica Cracoviensia Series Botanica* 54(2): 32–38.

Gupta, A.K. and Tandon, N. 2004. *Reviews on Indian Medicinal Plants*. Vol. 1. Indian Council of Medicinal Research, New

Delhi, India, 543pp.

Harborne, J.B. 1973. Phytochemical Methods. . Chapman and Hall Ltd, London. pp. 49-188.

Hasrat, J.A., Pieters, L. and Vlietinck, A.J. 2004. Medicinal Plants in Suriname: Hypotensive Effect of *Gossypium barbadense*. *Journal of Pharmacy and Pharmacology* 56: 381–387.

Jayanthi, P. and Lalitha, P. 2011. Reducing Power of the Solvent Extracts of *Eichhornia crassipes* (Mart.) Solms. *International Journal of Pharmacy and Pharmaceutical Sciences* 3(3):126–128.

Jerkins, J.N. 2003. Cotton. *In*: Organization for Economic Co-operation and Development (ed.) *Traditional crop breeding practices: an historical review to serve as a baseline for assessing the role of modern biotechnology*, Paris. pp 61-70.

Kamalakkannan, N., Stanely, M. and Prince, P. 2005. Antihyperlipidaemic Effect of *Aegle marmelos* fruit Extract in Streptozotocin-Induced Diabetes in Rats. *Journal of the Science of Food and Agriculture* 85: 569–573.

Kedare, S.B. and Singh, R.P. 2011. Genesis and Development of DPPH Method of Antioxidant Assay. *Journal of Food Science and Technology* 48(4): 412 – 422.

Krishnaiah, D., Sarbatly, R. and Bono, A. (2007). Phytochemical Antioxidants for Health and Medicine - A Move towards Nature. *Biotechnology and Molecular Biology* 1(4): 97 – 104.

Kubmarawa, D., Ajoku, G.O., Enworem, N.M. and Okorie, D.A. 2007. Preliminary Phytochemical and Antimicrobial Screening of 50 Medicinal Plants from Nigeria. *African Journal of Biotechnology* 6: 1690-1696.

Kumar, K.H.N., Rakshitha, H.R., Navyashree, S.N. and Chauhan, J.B. 2012. *In-vitro* Antioxidant Studies of *Pritchardia arecina*. *International Journal of Pharmaceutical Sciences and Drug Research*. 4(2):140–142.

Kumar, S.P., Singh, S.S., Singh, N.P. and Mayur, P. 2011. In-vitro *Antioxidant* Activity of *Gossypium herbaceum* Linn. *International Research Journal of Pharmacy* 2(7): 166 -170.

Lee, J.A. 1984. Cotton as a World Crop. *In*: R.J. Kohel and C.F. Lewis (eds.) *Cotton*. American Society of Agronomy, Madison, Wisconsin. pp. 1-25.

Loganayaki, N., Siddhuraju, P. and Manian, S. 2013. Antioxidant Activity and Free Radical Scavenging Capacity of Phenolic Extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *Journal of Food Science and Technology* 50(4): 687–695.

Lu, Z., Chen, J., Percy, R.G. and Zeiger, E. 1997. Photosynthetic Rate Stomatal Conductance and Leaf Area in Two Cotton Species (*Gossypium barbadense* and *Gossypium hirsutum*) and Their Relation to Heat Resistance and Yield. *Australian Journal of Plant Physiology* 24: 693 – 770.

Manach, C., Williamson, G., Morand, C., Scalbert, A. and Remesy, C. 2005. Bioavailability and Bioefficacy of Polypheols in Humans. I. Review of 97 Bioavailability Studies. *The American Journal of Clinical Nutrition* 81: 230-242.

Mans, D.R.A., Toelsie, J.R., Jagernath, Z., Ramjiawan, K. and van Brussel, A. 2004. Assessment of Eight Popularly-Used Plant Derived Preparations for their Spasmolytic Potential using the Isolated Guinea-Pig Ileum. *Pharmaceutical Biology* 42: 422-429.

Moffet, J.O. 1983. Pollination of Entomophilous Hybrid Seed Parents Hybrid Cotton. *In*: C.E. Jones and R.J. Little (eds.) *Handbook of Experimental Pollination of Biology*. Van Nostrand Reinbold, New York. Pp 508-514.

Mujeeb, F., Bajpai, P., and Pathak, N. 2014. Phytochemical Evaluation, Antimicrobial Activity, and Determination of Bioactive Components from Leaves of *Aegle marmelos* . *BioMed Research International*, 2014, 497606. http://doi.org/10.1155/2014/497606

Nethathe, B.B. and Ndip, R.N. 2011. Bioactivity of *Hydnora africana* on Selected Bacterial Pathogens: Preliminary Phytochemical Screening. *African Journal of Microbiological Research* 5(18): 2820–2826.

Obadoni, B.O. and Ochuko, P.O. 2001. Phytochemical Studies and Comparative Efficacy of the Crude Extracts of some Homostatic Plants in Edo and Delta

States of Nigeria. Global *Journal of Pure and Applied Sciences* 8(2): 203-208.

Oktay, M., Gülçin, İ. and Küfrevioğlu, Ö.İ. 2003. Determination of *in vitro* Antioxidant Activity of fennel (*Foeniculum vulgare*) seed extracts. *LWT-Food Science Technology* 36(2): 263-271.

Omojasola, P.E. and Awe, S. 2004. The Antibacterial Activity of the Leaf Extracts of *Anarcadium occidentale* and *Gossypium hirsutum* Against Selected Microorganisms. *Bioscience Resource Communications* 16(1): 25-28.

Oyagade, J.O., Awotoge, O.O., Adewumi, J.T. and Thorpe, H.T. 1999. Antimicrobial Activity of some Nigerian Medicinal Plants: Screening for Antibacterial Activity. *Journal of Bioscience Research Communication* 11:193 – 197.

Oyaizu, M. 1986. Studies on Product of Browning Reaction Prepared from Glucose Amine. *Japanese Journal of Nutrition* 44: 307-315.

Rahman, M.M., Islam, M.B., Biswas, M., Khurshid, and Alam, A.H.M. 2015. In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Research Notes* 8:621.

Rao, A.S.V.C., Reddy, S.G., Babu, P.P. and Reddy, A.R. 2010. The Antioxidant and Antiproliferative Activities of Methanolic Extracts from Njavara Rice Bran. BMC *Complementary and Alternative Medicine* 10: 4.

Rosbard, K., Prernlzler, P.D., Tucker, D., Swatzitang, G., and Glover, W. 1999. Phenolic Compounds and Their Role in Oxidative Processes in Fruits. *Food Chemistry* 66: 401-436.

Saidu, T.B. and Abdullahi, M. 2011. Phytochemical Determinations and Antibacterial Activities of the Leaf Extracts of *Combretum molle* and *Gossypium arboretum*. *Bayero Journal of Pure and Applied Science* 4: 132–136.

Sharma, R.K., Chatterji, S., Rai, D.K., Mehta, S., Rai, P.K., Singh, R.K., Watal, G. and Sharma, B. 2009. Antioxidant Activities and Phenolic Contents of the Aqueous Extracts of Some Indian Medicinal Plants. *Journal of Medicinal Plant Research* 3: 944-948. http://www.academicjournals.org/article/article1380521885_Sharma%20et%20al.pdf.

Sheikh, N., Kumar, Y., Misra, A. K. and Pfoze, L. 2013. Phytochemical screening to validate the ethnobotanical importance of root tubers of *Dioscorea* species of Meghalaya, North East India. *Journal of Medicinal Plants Studies* 1: 62–69.

Singh, P.K., Singh, S., Kumar, V. and Krishna, A. 2011. Ethnoveterinary healthcare practices in Marihan sub-division of District Mirzapur Uttar Pradesh India. *Life Sciences leaflets* 16:561 –569.

Soni, A. and Sosa, S. 2013. Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts. *Journal of Pharmacognosy and Phytochemistry* 2 (4): 22-29.

Srivastava, J., Kumar, S. and Vankar, P.S., 2012. Correlation of Antioxidant Activity and Phytochemical Profile in Native Plants. *Nutrition and Food Science* 42 (2): 71-79. https://doi.org/10.1108/00346651211212024

Ursini, F., Maiorino, M., Morazzoni, P., Roveri, A. and Pifferi, G. 1994. A Novel Antioxidant Flavonoid (IdB) Affecting Molecular Mechanisms of Cellular Activation. *Free Radical Biology and Medicine* 16: 547–553.

Van Buren, T.P. and Robinson, W.C. 1981. Formation of Complexes between Protein and Tannin Acid. *Journal of Agricultural and Food Chemistry* 1: 77.

Wendel, J.E., Brubaker, C., Alvarez, I., Cronn, R. and Stewart, J.M. 2009. Evolution and Natural History of the Cotton Genus. *In*: A.H. Paterson (ed.) *Genomics of Cotton Plant Genetics and Genomics Crops and Models* 3. Springer New York. pp 3-22.

Wise, R.R., Sassenrath-Cole, G.F. and Percy, R.G. 2002. A comparison of leaf anatomy in field grown *Gossypium hirsutum* and *Gossypium barbadense*. *Annals of Botany* 86:731-738. http://naldc.nal.usda.gov/naldc/download.xhtml?id=316&content=PDF.

Wu, Z., Soliman, K.M., Zipf, A., Saha, S., Sharma, G.C. and Jenkins, J.N. 2005. Isolation and Characterization of Genes Differentially Expressed in Fiber of *Gossypiumbarbadense* L. *The Journal of Cotton Science* 9: 116-174.

Zhang, Y., Luo Z., Wang, D., He, F. and Li, D. 2014. Phytochemical profiles and antioxidant and antimicrobial activities of the leaves of *Zanthoxylum bungeanum*. *Scientific World Journal*. 2014: 181072. PMC4134800