International Journal of Traditional and Natural Medicines, 2021, 11(1): 1-15

Modern Scientific Press

International Journal of Traditional and Natural Medicines **ISSN: 2167-1141**

Journal homepage: www.ModernScientificPress.com/Journals/IJTNM.aspx *Florida, USA*

Article

Comparative Analysis of Phytochemical Screening of Some Selected Plant Leaves from Western Nigeria

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Article history: Received 5 August 2021, Revised 1 October 2021, Accepted 10 November 2021, Published 17 December 2021.

Abstract: The importance and efficacy of natural and traditional plants cannot be over-emphasized. The concentration of these phytochemicals varied from plants to plants hence the combination of one or more of the plants in treatment of different ailments in traditional medicine. Qualitative and quantitative analysis of phytochemical constituents of some five Nigerian plants leaves were carried out using aqueous extract of the plant leaves. The following constituents were analysed using standard methods: alkaloids, glycosides, flavonoids, phenol, saponin and anthraquinone. Their level of presence and concentrations were determined using standard methods. The concentrations of alkaloids ranged between Alkaloid: 0.366±0.00-2.163±0.01 (mg/g), Glycosides: 0.223±0.02 - 1.543±0.01 (mg/g), Phenol: 0.367±0.02 - 1.707±0.01 (mg/g), Saponin: 0.747±0.01 - 1.891±0.00 (mg/g), Flavonoid: 0.918±0.01 - 4.237±0.00 (mg/g) and Anthraquinone: 1.647±0.01 - 1.707±0.01 (mg/g) for the five samples. There is significant difference in the concentrations of alkaloids from the five different plants ($p<0.05$). There is no significant difference in the saponin content of *Gossypium hirsutum* and *Zingiber officinate* but there is significant difference in that of *Calotropis procera, Alcalypha wikesina* and *Justicia calycina*. *Zingiber officinate* is rich is flavonoids, *Calotropis procera* is rich in alkaloids and saponin. It could be concluded that all the plant leaves possess the bioactive compounds tested for which can be used in treatment of differs ailments when compounded as their concentrations varies from one plant to the other.

Keywords: Phytochemistry, Phytochemicals, Traditional Medicine, Therapeutic, Nigerian plants.

1. Introduction

Phytochemicals are non-nutritive compounds from plants which have preventive and protective properties against diseases. They have anti-bacterial, antioxidants, enzymes stimulant, anti-cancer and so many other characteristics (Sridhar, *et al.,* 2014; Arawande *et al.,* 2018; Pereira *et al.,* 2020). In general, plants containing these phytochemicals are usually called medicinal plants. Medicinal plants have been known to contain some organic compounds with specific physiological action on the human body system (Yadav and Agarwala, 2011; Arawande 2013; Alamgir, 2017; Das *et al.,* 2020). Phytochemicals are found in the various parts of the plants: leaves, stems, bark or roots (Arawande *et al*. 2012; Sathya *et al*., 2013; Das *et al.,* 2020). For some times now, researchers have focus on maximizing the uses of various parts of plants and vegetables for traditional medicines and plant drugs against numerous diseases in the health sector because they are safer (little or no adverse effects) (Sathya *et al*., 2013; Arawande *et al.,* 2015; Alamgir, 2017; Pereira *et al.,* 2020).

As a term, *phytochemical* is generally used to describe plant compounds that are under research with several effects on health and are not scientifically defined as essential nutrients. Regulatory agencies governing food labeling in Europe and the United States have provided guidance for industry limiting or preventing health claims about phytochemicals on food product or nutrition labels (Usunobun *et al.,* 2015; Evya *et al*., 2017). They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators (Jamuna *et al.,* 2013; Senguttuvan, *et al.,* 2014; Arawande *et al.,* 2018). Some phytochemicals have been used as poisons and others as traditional medicine because of their versatility (Ketron, and Osheroff, 2014; Raikwar *et al.,* 2018; Mtewa *et al.,* 2021).

The medicinal properties of these plants have not been sufficiently harnessed. The difficulty encountered with alternative medicine has been that of reliable documentation of known traditional herbal medicine since uses vary from tribe to tribe. Indigenous medical practices have been the subject of much attention in the literature of various disciplines to date but the specificity, mode of action and clinical efficacy of most traditional plants have not been established in a manner consistent with standards of modern medicine (Adeshina *et al*., 2000; Yadav and Agarwala 2011; Senguttuvan, *et al.*, 2014; Olasupo *et al.,* 2018).

However, alternative medicine cannot be discredited since a large number of modern day medicines have their origin from plants, hence the importance and efficacy of natural and traditional plants cannot be overemphasized (Arawande *et al.*, 2015). The concentration of these phytochemicals varied from plants to plants hence the combination of one or more of the plants in treatment of different ailments in traditional medicine are usually adopted. For instance, the drugs used for treatment of malaria, a prevailing ailment in tropical Africa: chloroquine and more recently artemisinin have their origin from plant sources - from *Cinchona* bark and *Artemisia annua,* respectively (Senguttuvan *et al.,* 2014; Adeshina *et al*., 2013).

The Plants

(i) *Alcalypha wilkesiana:* The plant *Acalypha torta* belongs to the family Euphorbiaceae which is a family of dicotyledonous plants that includes shrubs and trees. They are primarily found in the tropical region of Africa. Euphorbiaceae plants have been intensively investigated and contain alkaloids, saponins, flavonoids, tannins, resins and carbohydrate amongst others. Various pharmacological activities have been reported for some *Acalypha* species. *A. wilkesiana* have been reported to have *in vitro* antimicrobial activities. Antioxidant activities have also been reported in *A. guatemalensis* and *A. hispida* (Seebaluck *et al.,* 2015; Atawodi *et al.,* 2017; Abdulmumin *et al.,* 2020).

(ii) *Zingiber officinale*: *This* is a tropical shrub widely grown in Nigeria. It is commonly known in English as ginger, 'atale' or 'aje' by Yorubas, jinja by Efiks/Ibibios of Cross River and Akwa Ibom States (Osabor *et al.,* 2015). It is a semi-woody perennial herb and it's 3 to 4 feet high from the root stock. It grows rapidly; the leaves and flowers are used medicinally. The plant is used in traditional medicine for the treatment of several ailments such as stomach disorder, diabetes, wounds, rheumatism, snake bite etc. in different parts of the world (Weldner 2000; Ali *et al.,* 2008; Osabor *et al.,* 2015; Funck *et al.,* 2016; Beristain-Bauza *et al.,* 2019).

(iii) *Calotropis procera*: *C. procera* belong to family *Asclepiadaceae* and it is a large broadleaf evergreen plant with a strong odour, common in the tropical regions: Africa and Asia which is commonly known as Milkweed. *C. procera* is used as a folk medicine for the treatment of various diseases. It has been reported that the plant possess potential anthelmintic, antimicrobial, anticancer, anticoagulant, analgesic, anti-inflammatory, purgative and antipyretic properties and is also used in the treatment of leprosy, leucoderma, liver and abdomen (Shivkar and Kumar, 2003; Kareem *et al.,* 2008; Rani *et al.,* 2017; Nadeem *et al.,* 2019).

(iv) ***Gossypium hirsutum:*** Cotton has been used by humans for approximately 5,000 years. Archaeologists have found evidence that cotton has been used to produce garments in Africa, Pakistan and the Americas since 2500 BC (Wang and Memon, 2020). Cultivated cotton produced today belongs to the genus *Gossypium* which consists of over 50 species. The majority of cotton produced worldwide today is *Gossypium hirsutum*, commonly known as upland cotton. There are 3 other species commercially produced including *G. barbadense* or Pima cotton and two Asian species, *G. herbaceum* and *G. arboretum* (Lewis *et al.*, 2000).

(v) ***Justicia calycina:*** *Justicia* is the largest genus of Acanthaceae, with approximately 600 species (Corrêa and Alcântara 2012; Jahan. e*t al.*, 2020). Plants of the genus *Justicia* are known for the production and accumulation of phytochemicals: saponin, alkaloids, lignans, terpinoids, and flavonoids. It is an important source of therapeutic drugs used in blood boosting. They also contain essential oils used to treat inflammations. Lignans are the major components of the active extracts of the species of Justicia, exhibiting important pharmacological properties (Wasshausen and Wood 2003; Correa *et al.*, 2014; Ugboko *et al.*, 2020).

Phytochemical screening refers to the extraction, screening and identification of the medicinally active substances found in plants which include: flavonoids, alkaloids, carotenoids, tannin, antioxidants, phenolic compounds and so on. The difficulty encountered with alternative medicine has been that of reliable documentation of known traditional herbal medicine since uses vary from tribe to tribe. The aim of the study is to carry out comparative study on the qualitative and quantitative analysis of the phytochemical constituents of some selected plant leaves from the South Western Nigeria so as to be able to use them as a mixture (‘compounded drug) in taking care of various diseases or ailments.

Figure 1: *Alcalypha wilkesiana*

Figure 2: *Zingiber officinale*

Figure 3: *Calotropis procera*

Figure 4: *Gossypium hirsutum*

Figure 5: *Justicia calycina*

2. Materials and Methods

2.1. Collection and Preparation of Samples

Fresh plant leaves of: *Gossypium hirsutum*, *Zingiber officinale*, *Alcalypha torta*, *Calotropis procera* and *Justicia calycina* were obtained from local gardens within Owo town and authenticated at the Environmental Biology Laboratory of the Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo, Nigeria. Fresh and matured leaves were harvested from the plants, properly washed with distilled water to remove foreign matters, air-dried, ground and sieved using a 5mm sieve mesh. The powdered samples were properly labeled using the following codes: S1: *Gossypium* *hirsutum*, S2: *Zingiber officinale*, S3: *Calotropis procera*, S4: *Alcalypha wilkesiana* and S5: *Justicia calycina*.

2.2. Preparation of Aqueous Extracts of the Plant Leaves

The powdered samples were soaked in sterilized container using distilled water as the solvent and labeled properly using the method of Amir *et al.*, 2005. The mixture was allowed to stay for 72 hours for complete extraction of the phyto-constituents (bioactive compounds) of the samples. The content was then filtered using a sintered funnel. The extract was then concentrated using rotary evaporator. This was then weighed and used for the analysis.

2.3. Phytochemical Screening of the Extracts

Phytochemical screening of the plant extracts was determined using the methods of Harbone (1973), Odebiyi and Sofowora (1978), Sofowora (1993).

2.3.1. Determination of saponin

0.2g of the extract/sample was shaken with 5mL distilled water and then heated to boiling. Frothing showed the presence of saponin. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (soluble).

2.3.2. Determination of flavonoids

0.2g of the extract was dissolved in dilute NaOH and HCl was added. A yellow solution which turned colourless was observed (Trease and Evans, 1989).

2.3.3 Cardiac glycosides

100mg of extract was dissolved in 1mL of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under-layered with 1ml of concentrated H_2SO_4. A brown ring obtained at the interface indicated the presence of deoxysugars, characteristic of cardenolides (Odebiyi and Sofowora, 1978).

2.3.4. Determination of alkaloids

3g of extract was stirred with ethanol containing tartaric acid. The filtrate was poured into a beaker and Mayer’s reagent was added. Precipitation in the beaker indicated the presence of alkaloids (Banso and Ngbede, 2006).

2.3.5. Determination of phenols

Total polyphenols were determined according to the Folin–Ciocalteau reagent method (Singleton *et al.,* 1999). The absorption was determined after 60 minutes at a wavelength of 720 nm with a Jenway 6305 UV-VIS Spectro 230V spectrophotometer. The results were expressed as gallic acid equivalents (mg GAE/100 g).

2.3.6. Test for anthraquinones

0.2 g of plant extract and 4 mL of chloroform added with proper shaken together for 5 minutes. The mixture was then filtered. 2mL of 10% ammonium hydroxide was added to the filtrate. A bright pink, red or violet colour at the upper layer indicates the presences of anthraquinones (Sofowora, 1993).

2.4. Quality control and assurance

All chemicals used were of analytical grade (99% purity). Equipment were standardized and calibrated as described by manufacturers' procedure before use. Distilled water was used for the preparation of reagents. Laboratory apparatuses were washed with detergent, rinsed with distilled water and oven-dry before use. Samples were analysed in triplicates.

2.5. Statistical Analysis

Data generated through triplicate analyses were compared by one-way analysis of variance (one-way ANOVA) to test for significant difference using statistical package for the social sciences (SPSS) version 16.0 while means of the group were compared using Duncan Multiple Range Test (DMRT) (Daniel, 2003).

3. Results and Discussion

Tables 1 and 2 show both results of the qualitative and the quantitative phytochemical constituents of the five samples *Gossypium spp*. (S1), *Zingiber officinale* (S2), *Calotropis gigantae* (S3), *Alcalipha wilkesiana* (S4) and *Justicia calycina* (S5) leaves. The table showed that six phytochemicals were tested for: Alkaloids, glycosides, phenol, saponin, flavonoids and anthraquinone. The results showed that all parameters tested for were present in all the five samples in varying concentrations (low, medium and high concentrations). For *Gossypium spp.* (S1), the result showed that alkaloid, glycosides, phenol and saponin were present in low concentration but only flavonoids and anthraquinone were present in high concentration, this result correlate with the work of Gupta and Prakash, (2020) and Odebiyi and Sofowora (1979). The table also showed that *Zingiber officinale* (S2) has all parameters tested, but has more phenol, flavonoids and anthraqiuonone in medium and high concentrations respectively. *Zingiber officinale* has been useful in the treatment of ailments such as stomach disorder, diabetes, wounds, rheumatism, snake bite, baldness, toothache, respiratory disorders and a lot of others disorders (Osabor *et al.,* 2015; Funck *et al.,* 2016; Beristain-Bauza *et al.,* 2019).

Table 1: Qualitative Analysis of the Phytochemical Constituents of the samples

Sample	Alkaloids	Glycosides	phenol	Saponin	Flavonoids	Anthraquinone
S1	+	+	+	+	++	++
S2	+	+	++	+	+++	++
S3	+	+	+	+++	+++	++
S4	+	+	+	+	++	++
S5	+++	++	++	++	+	++

Legend: + = Present in low concentration, ++ = Present in medium concentration, +++ = present in high concentration. S1: *Gossypium hirsutum*, S2: *Zingiber officinale*, S3: *Calotropis procera*, S4: *Alcalypha wilkesiana* and S5: *Justicia calycina*.

Table 2: Quantitative Analysis of the Phytochemical Constituents of the samples

Sample	Alkaloid (mg/g)	Glycosides (mg/g)	Phenol (mg/g)	Saponin (mg/g)	Flavonoid (mg/g)	Anthraquinone (mg/g)
S1	0.366±0.00[d]	0.953±0.01[c]	0.367±0.02[e]	0.752±0.00[d]	1.020±0.02[d]	1.647±0.01[c]
S2	0.354±0.00[e]	0.223±0.02[e]	1.707±0.01[a]	0.747±0.01[d]	4.237±0.00[a]	1.693±0.01[b]
S3	0.461±0.01[b]	0.763±0.91[d]	0.545±0.00[c]	2.454±0.00[a]	2.285±0.00[b]	1.683±0.01[b]
S4	0.433±0.00[c]	1.420±0.02[b]	0.440±0.02[d]	0.975±0.01[c]	1.059±0.00[d]	1.707±0.01[a]
S5	2.163±0.01[a]	1.543±0.01[a]	1.523±0.01[b]	1.891±0.00[b]	0.918±0.01[c]	1.657±0.01[c]

Results are expressed as means ± standard deviation (n=3). Values with different superscripts in each row are significantly different (P< 0.05). S1: *Gossypium hirsutum*, S2: *Zingiber officinale*, S3: *Calotropis procera*, S4: *Alcalypha wilkesiana* and S5: *Justicia calycina*.

Calotropis procera (S3) has all the phytochemicals tested for but saponin and flavonoids were present in high concentrations while anthraquinone was present in medium concentrations. *C. procera* flowers has been reported to cause temporary paralysis of red stomach worm in sheep and notably reduces egg count percent of gastrointestinal nematodes in naturally infected sheep (Kareem *et al.*, 2008; Rani *et al.*, 2017; Nadeem *et al.*, 2019). *Alcalipha wikesiana* (S4) has all the phytochemicals in low concentrations for alkaloids, glycosides, phenol and saponin, while flavonoids and anthraquinone were in high concentrations. *Acalypha spp.* is used medicinally for the treatment of some fungal skin diseases. The plant is also useful traditionally in the treatment of neonatal jaundice traditionally and antioxidant activity (Onocha and Oloyede 2011; Odigie *et al.*, 2015; Odiegwu *et al.*, 2021).

Alkaloid is present in high concentration in *Justicia calysina* (S5), while glycosides, phenol, saponin and anthraquinone were present in moderate concentration; and flavonoids in low concentrations. This results were in line with the work of Madziga *et al.*, (2010); Arawande *et al.*, (2018); Dada *et al.*, (2019). *Justicia calysina* has been reported to exhibit pharmacological properties such as antiviral, antitumoral, anti-inflamatory, and antiplatelet aggregations activities and so on (Wasshausen and Wood 2003; Correa *et al.*, 2014; Ugboko *et al.*, 2020).

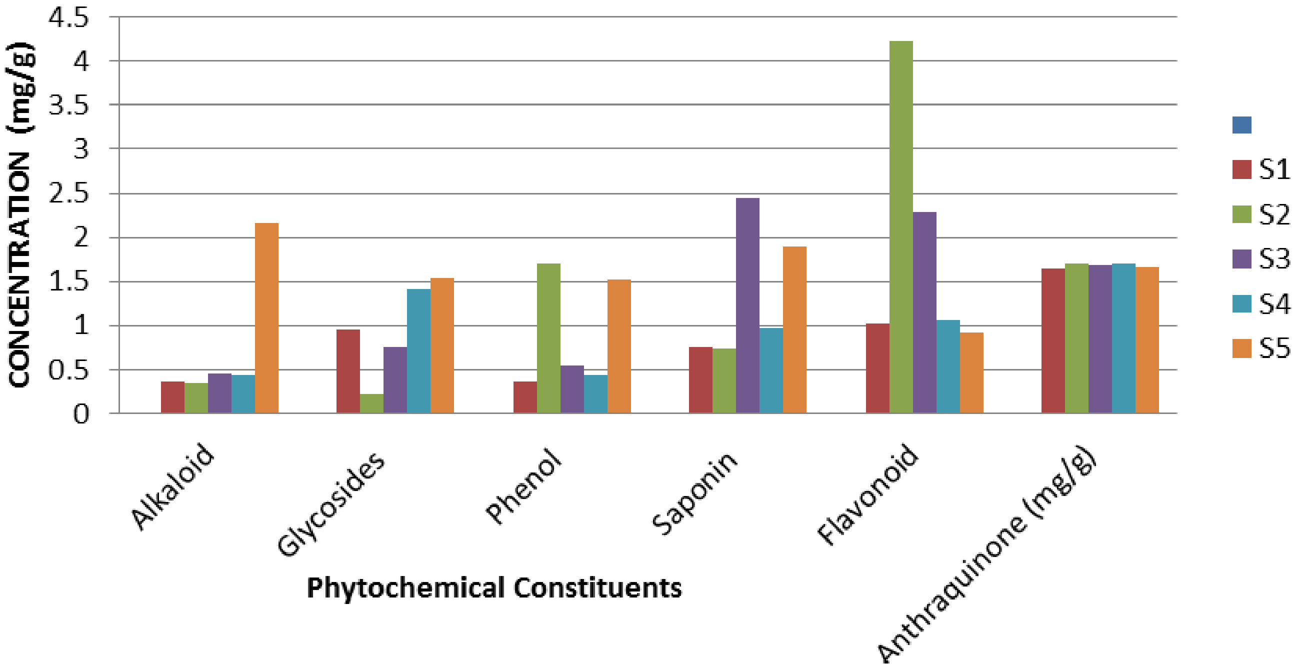

Fig. 6: Quantitative Results of the Phytochemical Constitutes of the five leaves samples

Table 2 showed the statistical values for the comparative analysis of the phytochemical constituents of the plant samples while figure 6 provides the chart.

The concentrations of alkaloids ranged between 0.366±0.00 to 2.163±0.01 mg/g for the five samples. The highest value is from *Justicia calycina* (S5) while the lowest is from *Zingiber officinale* (S2). There is significant difference in the concentrations of alkaloids from the five different plants. The concentrations of glycoside were between 0.223 ±0.02 mg/g and 1.543±0.01 mg/g for the samples. The highest is from *Justicia calycina* (S5) while the lowest is from *Zingiber officinale* (S2). Phenol content ranged between 0.367±0.02 to 1.707±0.01 mg/g. *Zingiber officinale* has the highest value while Gossypium hirsutum has the lowest value. The concentration of saponin ranged between 0.975±0.01 to 2.454±0.00 mg/g, the highest was from *Calotropis procera* while the lowest was from *Zingiber officinale* (sample 2). The concentration of flavonoid ranged between 0.918±0.01 to 4.237±0.00 mg/g. The highest value was from *Zingiber officinale* while the lowest was from *Justicia calycina*. Phenolic compounds have been found useful as an antioxidant, , antiviral, antitumor, anti-inflammatory, regulatory of the immune system and analgesic according to Tenase *et al.*, (2019); Shovo *et al.*, (2021). The concentration of Anthraquinone ranged between 1.647±0.01 and 1.707±0.01 mg/g. The five samples were rich in flavonoid and anthraquinone compared to other parameters determined. *Zingiber officinate* is rich in flavonoids.

Comparing the result of the five samples, it can be seen that *Zingiber officinate* contains higher flavonoid concentration than the remaining samples. The presence of Alkaloid, glycosides, phenol, saponin and glycosides makes the plants a good source of phytochemicals. This agrees with the work of Madziga *et al.*, (2010); Arawande *et al.*, (2018); Dada *et al.*, (2019). Saponin from plants are found to have various biological effects such as antimicrobial, anti-oxidant, anti-infllammatory, antitumor and immune boosting agent according to Wei *et al.*, (2020) and Cheng *et al.*, (2020).

4. Conclusion

The phytochemical screening of the five samples showed the presence of glycosides, alkaloids, saponins, flavonoids, phenols and anthraquinone which are sources of medicinal herbs. There is significant difference in the alkaloid, glycosides and phenol contents of the five samples. There is no significant difference in the saponin content of *Gossypium hirsutum* and *Zingiber officinate* but there is significant difference in that of *Calotropis procera*, *Alcalypha wikesina* and *Justicia calycina*. *Zingiber officinate* is rich is flavonoids, *Calotropis procera* is rich in alkaloids while *Calotropis procera* is rich in saponin. There is no significant difference in the concentration of anthraquinone in *Zingiber officinate* and *Calotropis procera* same with *Gossypium hirsutum* and *Justicia calycina.* It could be concluded that all the plant leaves possess the bioactive compounds tested for which can be used in treatment of differs ailments when compounded as their concentrations varies from one plant to the other. Further research can be tailored towards comparing the concentration of these active compounds in the root, stem and bark of the plants.

Potential Conflicts of Interest

The authors declare no conflict of interest.

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