UMYU Scientifica, Vol. 1 NO. 2, December 2022, Pp 147 – 156
https://doi.org/10.56919/usci.1222.018
ISSN: 2955 – 1145 (print); 2955 – 1153 (online)
A periodical of the Faculty of Natural and Applied Sciences, UMYU, Katsina

ORIGINAL RESEARCH ARTICLE

Qualitative and Quantitative Analysis of the Phytochemicals of *Azadirachta indica*, *Nauclea latifolia*, *Vernonia ambigua*, and *Artemisia annua* Distillates.

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ARTICLE HISTORY

Received September 19, 2022
Accepted December 17, 2022
Published December 30, 2022

KEYWORDS

Medicinal plants, Phytochemical, Quantitative Analysis, Steam distillation, Volatile constituents.

ABSTRACT

In order to treat a variety of human health issues, plants are a beneficial source for getting numerous pharmacologically active chemicals. This study aimed to qualitatively and quantitatively determine the phytochemical constituents of *Artemisia annua*, *Azadirachta indica*, *Nauclea latifolia*, and *Vernonia ambigua*. The volatile constituents of these plants were extracted using steam distillation. The phytochemical constituents were qualitatively and quantitatively determined using the standard methods. The results of the phytochemical screening show the presence of glycosides, terpenoids, alkaloids, terpenoids, saponins, and tannins. The quantitative Analysis shows that *Azadirachta indica* has the highest concentration of alkaloid (0.18 mg/mL), flavonoid (0.06 mg/mL), and total phenolic compounds (0.11 mg/mL). It was concluded that the distillates of the plants studied possess significant phytoconstituents. Further study needs to identify relevant compounds from the distillates for a better understanding of their mechanism of actions and activities. Furthermore, toxicology and in vivo studies should performed to deduce the safety of ingesting the distillates.

INTRODUCTION

To treat various human health issues, plants are a very valuable source for getting numerous pharmacologically active compounds (Verpoorte, 2009). Plants have been the foundation and core component of numerous traditional medicine systems for thousands of years (Schippmann, et al., 2006). According to estimates, between 40,000 and 70,000 plant species, or around 25% of all known plant species, are utilized as medicines by people in different parts of the world (Ramawat and Goyal, 2008).

According to Hansel et al. (2007), the phytomedicines used in phytotherapy are typically used as an extract (alcohol or water, essential oil, or distillate), which contains many secondary metabolites, frequently with different structural groups. Severally, it was nearly impossible to identify a single secondary metabolite that could account for the extract's bioactivity or its use in conventional medicine (Wink, 2012). The activity of an extract may result from synergistic interactions between several secondary metabolites that are present but are undetectable when single components are assessed in isolation (Hamoud, et al., 2015). Furthermore, these extracts are frequently used to treat various health conditions rather than just one illness.

It is now possible to track the medicinal worth of plants and the bioactive components that have a particular physiologic impact on people using phytochemical screening (Uche, et al., 2017). Several important plant bioactive compounds include glycosides, alkaloids, flavonoids, saponins, cardenolides, proanthocyanidins, polyphenolic chemicals and tannins (Ahmed, et al., 2013). Due to their biological activity and potential for application in the treatment and prevention of disease, these chemicals are sought after (Akinwumi, et al., 2020).

For over a century, the use of steam in phytotherapy has been adopted in both rural and urban communities in the treatment of various diseases, especially in the treatment of malaria and typhoid fever. But this has been done without knowledge of the phytochemicals present in the steam, or their concentrations, which could be a factor in the suppressive and curative processes. Hence, the need to study some of the plants used in steam phytotherapy, to establish the phytochemicals present in them, as well

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How to cite: Muhammad Z., Masanawa A. A. and Abdulazeez R. (2022). Qualitative and Quantitative Analysis of the Phytochemicals of *Azadirachta indica*, *Nauclea latifolia*, *Vernonia ambigua*, and *Artemisia annua* Distillates. UMYU Scientifica, 1(2), 146 – 155. https://doi.org/10.56919/usci.1222.018

as the quantity of these phytochemicals responsible for the suppressive/curative processes. This would provide an insight on the possible phytochemicals responsible for the healing process when the steam of these plants are employed in phytotherapy.

The present study aims to investigate *Azadirachta indica*, *Nauclea latifolia*, *Veronica ambigua*, and *Artemisia annua* for the distillates' qualitative and quantitative phytochemical constituents.

MATERIALS AND METHODS

Reagents used

Unless stated otherwise, all chemicals and reagents used were Analar grade (BDH Chemicals Limited, Poole England): Chloroform, Acetic anhydride, Ammonia, Sulphuric acid, Acetic acid glacial, Methanol, Ethanol, Hydrochloric acid, Dragendorff's reagent, Meyer's reagent, Lead sub-acetate, Sodium hydroxide, Ferric chloride, Folin-Ciocalteu reagent (EMSURE Merck Darmstadt, Germany), Gallic acid, Sodium carbonate, Quercetin (EMSURE Merck Darmstadt, Germany), Aluminum chloride, Potassium acetate, Atropine (AR-grade Sigma-Aldrich, St Louis MO), Phosphate buffer, BCG solution, Catechin, Vanillin reagent, Liebermann-Burchard reagent.

Plant material collection

The leaves of *Azadirachta indica, Nauclea latifolia, Veronica ambigua, and Artemisia annua* were collected from Afaka Village, Igabi Local Government Area of Kaduna state Nigeria, in February 2021. The plants were characterized and identified at the Department of Biological Science, Nigerian Defence Academy.

Preparation of Plant Sample

The leaves of *Azadirachta indica, Nauclea latifolia, Veronica ambigua, and Artemisia annua* were air-dried at room temperature and ground into a powdered form using a mortar and pestle.

Method of Steam Distillation

Water (200 mL) was added to a flask containing 50 g of the powdered leaves. On a heating mantle, the resultant suspensions were heated. The volatile compounds were condensed and collected as distillates in conical flasks. For two hours, the distillation process was conducted. The distillates obtained were stored in a volumetric flask and maintained in the refrigerator (Victor and Chidi, 2009).

Phytochemical Analysis

The distillates underwent phytochemical examination using the general approach proposed by (Ankita and Sapan, 2018). Essential phytochemical screening was done using straightforward chemical techniques to identify the presence of secondary plant metabolites in the distillates.

Test for Saponins

To 20 mL of deionized water, 5.0 mL aliquot of the distillate was added which was then rapidly agitated. Foaming that persist indicates the presence of saponins.

Test for Alkaloids

To 1 g of each distillate, 6 mL of diluted hydrochloric acid was added, mixed, boiled, cooled, and filtered. The filtrate was split into two parts and put through the subsequent tests.

Two drops of Dragendorff's reagent were applied to the initial portion. Alkaloids were present as evidenced by the precipitate turning red.

Two drops of Meyer's reagent were applied to the second portion. Alkaloids were detected as a creamy white precipitate.

Test for Tannins

To 1 mL aliquot distillate, 10 mL deionized water was added and then treated with 4 drops of lead sub-acetate solution. A cream precipitate denoted the presence of tannins.

Test for Flavonoids

NaOH Tests: A few drops of sodium hydroxide solution were added in a test tube containing 2 ml of distillate. The presence of flavonoids was revealed by the production of a bright yellow colour that turned colourless upon adding a few drops of diluted HCl.

Test for Glycosides

Keller-kilani test: The distillates and concentrated H_2SO_4 were combined with two drops of a 2% $FeCl_3$ solution and 2 mL of glacial acetic acid. The presence of cardiac steroidal glycosides was revealed by a brown ring that formed between the layers.

Test for Anthraquinones

Chloroform (5 mL) was added to 0.5 g of the distillate, which was then agitated and filtered. 1 mL of diluted ammonia was added after the chloroform layer was pipette into a another test tube. A resulting red-pink color in the lower layer of ammonia indicates a positive result.

Test for Steroids/Terpenoids

Salkowski's Test: Chloroform was used to extract 1 gm of the material, which was then filtered. The filtrate was carefully combined with 2 ml of concentrated H_2SO_4 to generate a lower layer of sulfuric acid. The presence of a steroidal ring at the interphase was indicated by a reddish-brown color.

Libermann- Burchard's Test: the sample (2mL), 2 mL of acetic acid anhydride with 2 mL of concentrated H_2SO_4 was mixed. A green color in the upper layer with a reddish

to pink color at the interphase of the two layers indicated a positive result for triterpene.

Quantitative Phytochemical Evaluation

Estimation of total phenols content

Using the Folin-Ciocalteu reagent (FCR) as an oxidizing agent and gallic acid as a standard, the total phenolic content of the distillates was calculated (Singleton and Rossi, 1965).

Preparation of reagents:

Standard gallic acid solution: A concentration of 1 mg/mL was prepared by dissolving 10 mg of gallic acid in 10 mL of methanol. This served as the stock standard solution. The stock solution was then serially diluted to different concentrations using a micropipette to measure, 20 µL, 40 µL, 60 µL, 80 µL, and 100 µL and diluting each volume to 10 mL with distilled water to yield the following concentrations 2, 4, 6, 8 and 10 µg/mL respectively.

Preparation of blank solution: 1.5 mL Folin Ciocalteau reagent, 1 mL distilled water, and 4 mL 20% sodium carbonate were mixed in a 20 mL volumetric flask.

Experimental procedure for the phenolic content evaluation

Separate test tubes were filled with 1 mL of the test sample solution and varying concentration of the standard gallic acid. To each test tubes, distilled water (1 mL) was added, followed by 1.5 mL of Folin Ciocalteu's reagent. The mixes were wrapped in foil and left at room temperature for five minutes. Afterward, 4 mL of 20% (w/w) Na_2CO_3 was added to each. The mixes were stirred and submerged for 30 minutes in a water bath that was heated to 40^{O}C. To stop the reaction, the test tubes were submerged in an ice water bath. Using a UV-VIS spectrophotometer at 765 nm, the test samples' and standards' absorbance was measured. The total phenol content of the samples were calculated as mg/g using the formula:

$$Total\ Phenol\ content\ (\%\ w/w) = \frac{\text{GAE x V x 100}}{\text{W}}$$

Where,

GAE = Gallic acid equivalents (mg/mL),

V = Volume of test sample (mL),

W = weight of sample extract (distillate)(g)

Estimation of total flavonoids content

The aluminum chloride colorimetric method used to determine the total flavonoid concentration. The flavonoid concentration of the extracts was expressed in mg of quercetin equivalent/gm of dry extract using quercetin as the standard (Kumar *et al.,* 2014).

Preparation of reagents

Quercetin standard solution: A stock solution with a concentration of 1 mg/mL was prepared by dissolving 10 milligrams of quercetin in 10 mL of distilled water. To obtain the varied concentrations of 20, 40, 60, 80, and 100 µg/mL, the stock solution was serially diluted.

Blank Solution: 1.5 mL of 95% methanol, 0.2 mL of 1 M potassium acetate, 0.2 mL of 10% aluminum chloride, and 2.8 mL of distilled water were measured and placed in a 20 mL volumetric flask and mixed.

Experimental procedure for the flavonoid content evaluation

Separate test tubes were filled with 1 mL of the test sample and the various concentrations of the standard quercetin solutions. To each of the test tubes 1.5 mL of methanol was added. Followed by 100 µL of aluminum chloride reagent, then 100 µL of a 1 M potassium acetate solution. Each test tube was then filled with 2.8 mL of distilled water. Aluminum foil was used to cover the test tubes, which were then left to sit at room temperature for 30 minutes. A UV-VIS spectrophotometer was used to measure the absorbance of the test sample and standards at 420 nm in comparison to a blank. Each distillate's total flavonoid concentration was determined. The total flavonoid content in each of the distillates was calculated as mg/g plant diatillate using the formula:

$$Total\ flavonoid\ content\ (\%\ w/w) = \frac{\text{QE x V x 100}}{\text{W}}$$

Where,

QE = the concentration of quercetin established from the calibration curve, mg/ mL

V = the volume of extract, mL

M = weight of plant extract gm

Determination of total alkaloid content

Preparation of reagents

Three reagents are prepared for use in this estimation adopting John *et al.*, (2014) method.

Preparation of reagents

Atropine standard solution: 10 mg Atropine was dissolved in 10 mL methanol, producing 1000 µg/mL concentration.

Blank solution: 5 mL of BCG solution and 5 mL of pH 4.7 phosphate buffer were mixed, then 5 mL of chloroform was used to extract the mixture. The extract was collected in volumetric flasks measuring 10 mL, and the volume was then adjusted with chloroform.

Test samples solutions:

25 mL of 2N HCl was used to dissolved 25 mg of the test sample. 10 mL of chloroform was used to wash 1 mL of the solution in a separatory funnel (3 times). The pH of the washed aqueous parts was adjusted to neutral with 0.1 N NaOH.

Experimental procedure for the Alkaloid content evaluation

The washed test sample solutions and a suitable aliquot (20, 40, 60, 80, and 100 µL) of the 1000 µg/ mL standard stock solution were transferred to separate separating funnels. Then, 3 mL of BCG solution and 3 mL of pH 4.7 phosphate buffer were added. Each solution was vigorously agitated and extracted with 1, 2, 3, and 4 mL of chloroform. Each extract was collected in a volumetric flask measuring 10 mL, and the corresponding volumes were filled with chloroform to the appropriate levels. In a UV-VIS Spectrophotometer, the absorbance of each test sample and the standards complexed in chloroform was measured at a wavelength of 470 nm in comparison to the blank (John, *et al.*, 2014). A standard curve was used to determine the Atropine equivalence (AE) of the test samples. The total Atropine content in the test samples were computed in mg/g using the formula:

$$Total\ Alkaloid\ content\ (\%\ w/w) = \frac{\text{AE x V x 100}}{\text{W}}$$

Where,

AE = the concentration of quercetin established from the calibration curve, mg/mL

V = the volume of extract, mL

M = weight of plant extract, gm

Determination of total tannin content (TTC)

For the purpose of estimating the amount of tannin in a test sample, the Acidified Vanillin method of evaluating tannin content was used, and the results were expressed as mg catechin equivalent per gram of extract, as specified by Broadhurst and Jones (1978); and Ferreira *et al.,* (1999).

Preparation of reagents

Standard catechin solution: To prepare a stock solution with a concentration of 1 mg/mL, 10 mg of catechin was dissolved in 10 mL of methanol. Then, serial dilution was performed to prepare various concentrations of 20, 40, 60, 80, and 100 µg/mL.

Blank solution: A clean test tube was wrapped in aluminum foil and 500 µL of methanol was measured in it, along with 3.0 mL of vanillin reagent and 1.5 mL of conc. HCl and horoughly mixed.

Test sample solution: the sample was dissolved in an appropriate quantity of methanol.

Experimental procedure for the Tannin content evaluation

Cleaned test tubes were wrapped in aluminum foils. The various standard catechin solution concentrations and 0.5 mL of test sample solutions were pipetted into various test tubes. 3.0 mL of vanillin reagent, then 1.5 mL of conc. HCl and the solutions were properly mixed. The reaction mixtures were let to stand at 25 ^{O}C for 15 minutes. A UV-VIS spectrophotometer was used to measure the absorbance of the test samples and standards at 500 nm in comparison to a blank. A standard curve for the catechin standards was prepared and used to determine Catechin equivalence of the test samples. The Total Tannin Content of the samples were calculated using the formula.

$$Total\ Tannin\ content\ (\%\ w/w) = \frac{\text{CE x V x 100}}{\text{W}}$$

Where,

CE = Catechin equivalents (mg/mL),

V = Volume of test sample (mL),

W = Sample weight (g)

Determination of total Phytosterols (plant steroid) content

Test Sample preparation and Analysis:

20 mg of distillates were suspended in 20 mL of chloroform covered properly and heated for 30mins at 60 ^{0}C in a water bath with shaking. A further 20 mL of chloroform were used to re-extract the solutions. The same solvent (SM) was used to adjust the volume to 50 mL.

2 mL of Liebermann-Burchard (LB) reagent and 5 mL of extracted solutions were added to 10 mL volumetric flasks. Chloroform was used to adjust the volume. A UV-Vis spectrophotometer was used to detect the absorbance five minutes after the addition of the reagent LB at a 625 nm wavelength.

Preparation of blank:

5 mL of chloroform was measured into a volumetric flask (10 mL), 2 mL of LB reagent was added and filled to the 10 mL mark with chloroform. It was kept for 5 min, then the absorbance was measured at 625 nm.

RESULTS

Phytochemical composition

Phytochemical screening of various distillates was carried out following the methods reported in literature and the results are presented in Table 1. Saponins, tannins, terpenoids/steroids, alkaloids and cardiac glycoside were detected in all four distillates. Anthraquinone was present in

only *azadirachta indica and vernonia ambigua*, while flavonoids was absent in all.

Table 1: Results of the Qualitative Analysis of the phytochemical screening of the distillates

Test/ Sample	*Azadirachta indica*	*Vernonia ambigua*	*Nauclea latifolia*	*Artemisia annua*
Saponins	+	+	+	+
Flavonoid	-	-	-	-
Tannins	+	+	+	+
Terpenoids/steroids	+	+	+	+
Alkaloids	+	+	+	+
Anthraquinone	+	+	-	-
Cardiac glycoside	+	+	+	+

Positive = + negative = -

Quantitative Analysis of the phytochemical screening of the distillates

Total Alkaloid content of Plant Distillates

The quantitatively analyzed total alkaloid content of the distillates were estimated to be 0.18 mg /mL, 0.12 mg/mL, 0.01 mg/mL, and 0.01 mg/mL for *azadirachta indica, vernonia ambigua, artemisia annua and nauclea latifolia* respectively as shown in figure 1. From the result, *azadirachta indica* has the highest content of alkaloid while *artemisia annua* and *nauclea latifolia* has the lowest content of alkaloid.

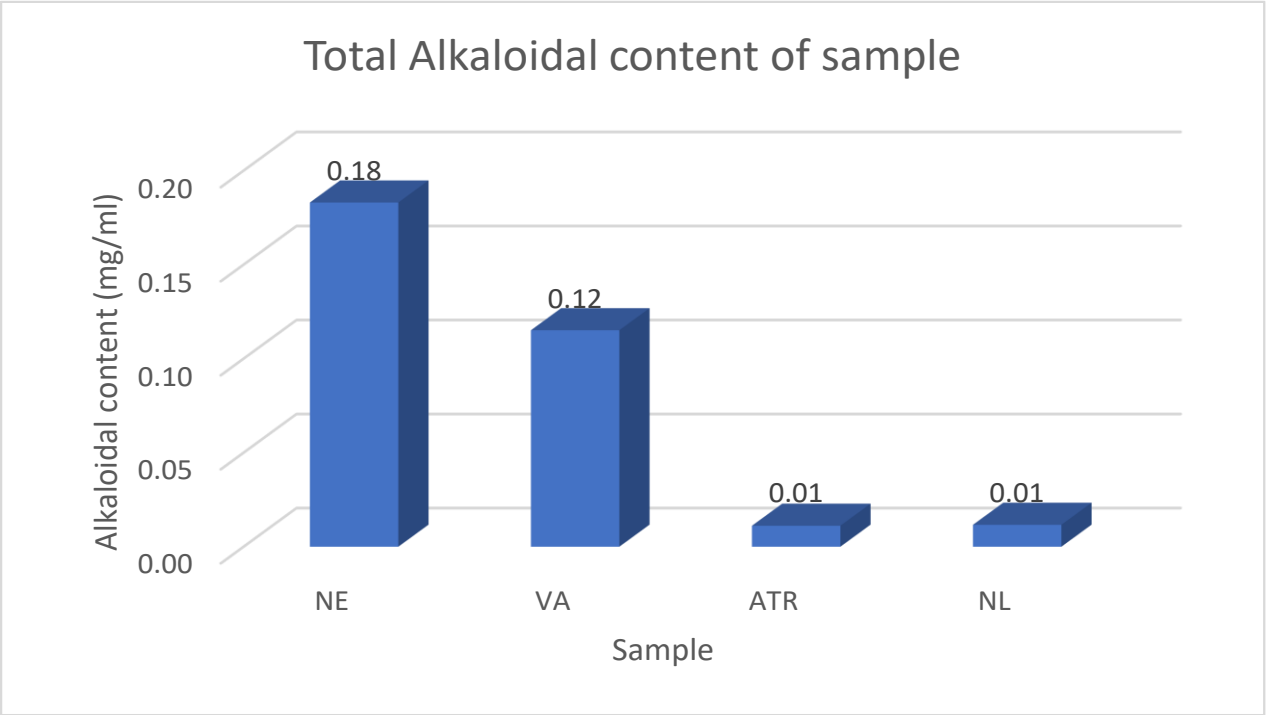

Figure 1: Concentration of total alkaloid content of plant distillates

Total Flavonoid content of Plant Distillates

The result of the quantitatively analysis total flavonoid content of the distillates was estimated to be 0.06 mg /mL, 0.00 mg/mL, 0.00 mg/mL, and 0.03 mg/mL for *azadirachta indica, vernonia ambigua, artemisia annua and nauclea latifolia* respectively as shown in figure 2. The result revealed *azadirachta indica* to contain the highest content of flavonoids when compared to other distillates.

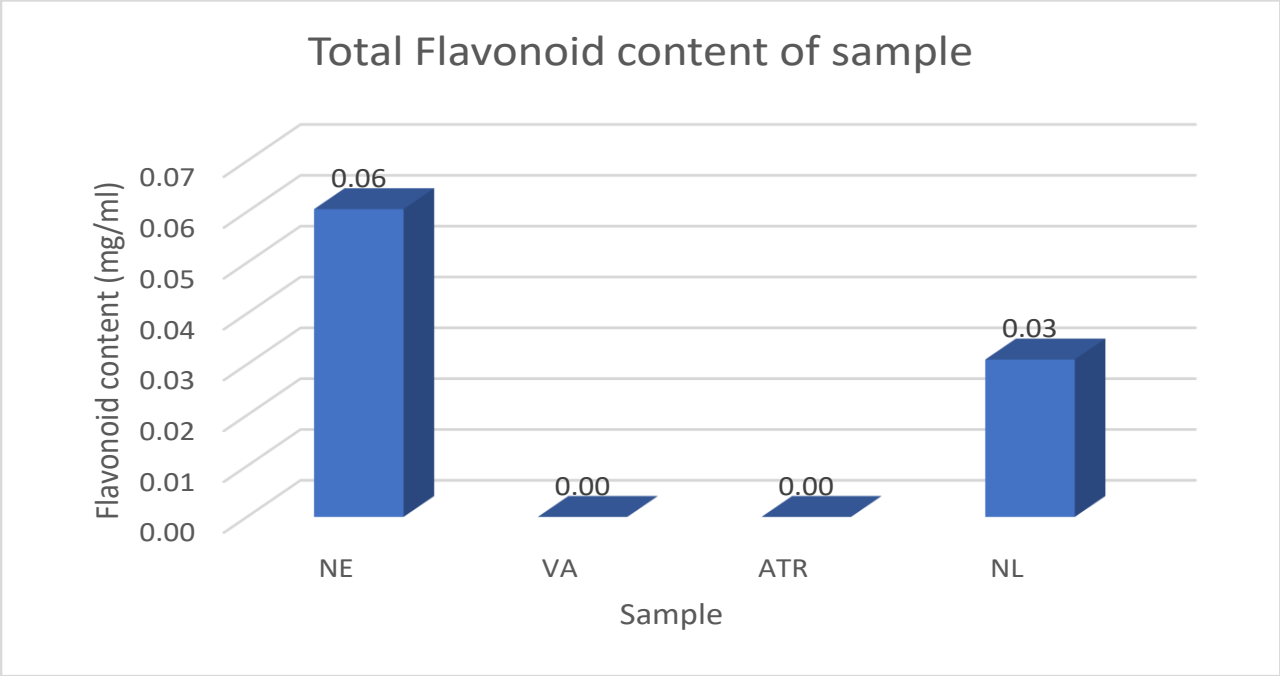

Figure 2: Concentration of total flavonoid content of plant distillates

Total Phenolic content of Plant Distillates

The result of the quantitatively analysis total phenolic content of the distillates was estimated to be 0.11 mg /mL, 0.02 mg/mL, 0.00 mg/mL, and 0.00 mg/mL for *azadirachta indica, vernonia ambigua, artemisia annua and nauclea latifolia* respectively as shown in figure 3. The result revealed *azadirachta indica* to contain the highest content of phenols when compared to other distillates.

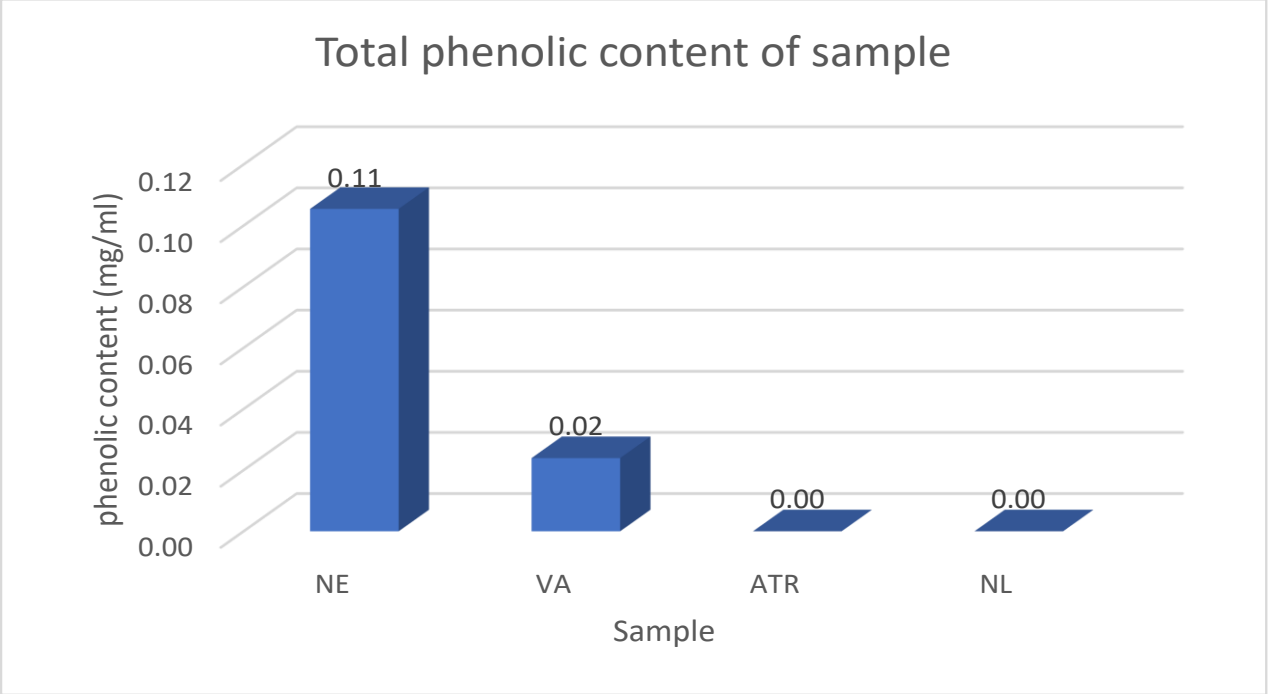

Figure 3: Concentration of total phenolic content of plant distillates

Total Steriodal content of Plant Distillates

The result of the quantitatively analysis total steroidal content of the distillates was estimated to be 0.00 mg /mL, 0.00 mg/mL, 0.00 mg/mL, and 0.00 mg/mL for *azadirachta indica, vernonia ambigua, artemisia annua and nauclea latifolia* respectively as shown in figure 4. The result revealed that none of the distillate contain a significant content of steroids.

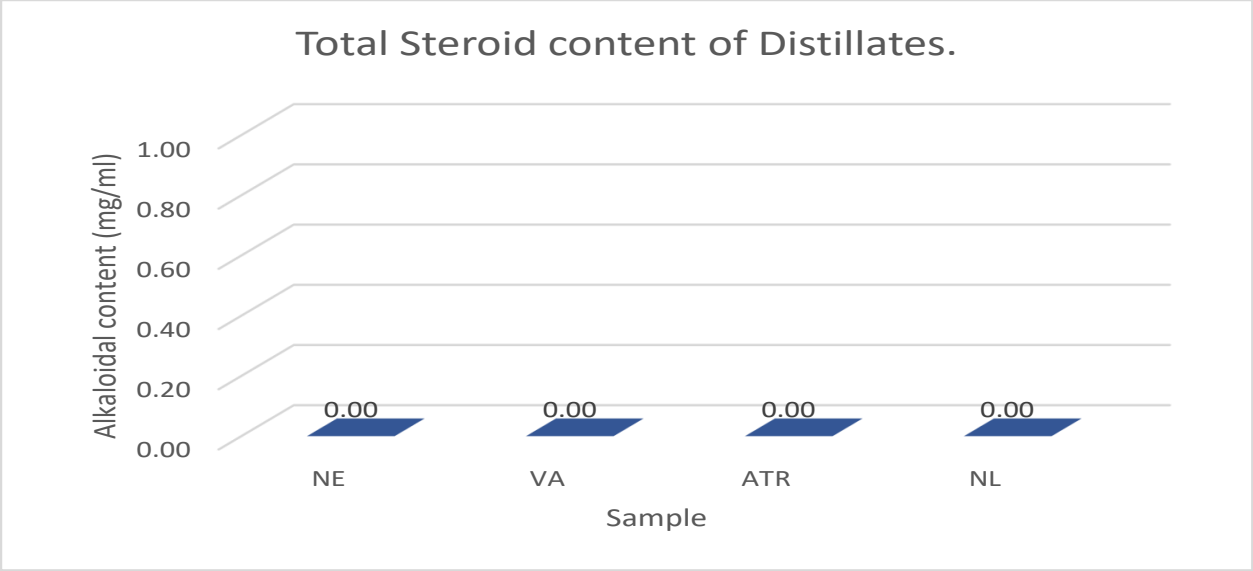

Figure 4: Concentration of total steroidal content of plant distillates

The total Tannin content of plants distillates

The result of the quantitatively analysis total steroidal content of the distillates was estimated to be 0.00 mg /mL, 0.00 mg/mL, 0.00 mg/mL, and 0.00 mg/mL for *azadirachta indica, vernonia ambigua, artemisia annua and nauclea latifolia* respectively as shown in figure 5. The result revealed that none of the distillates contain a significant content of tannins.

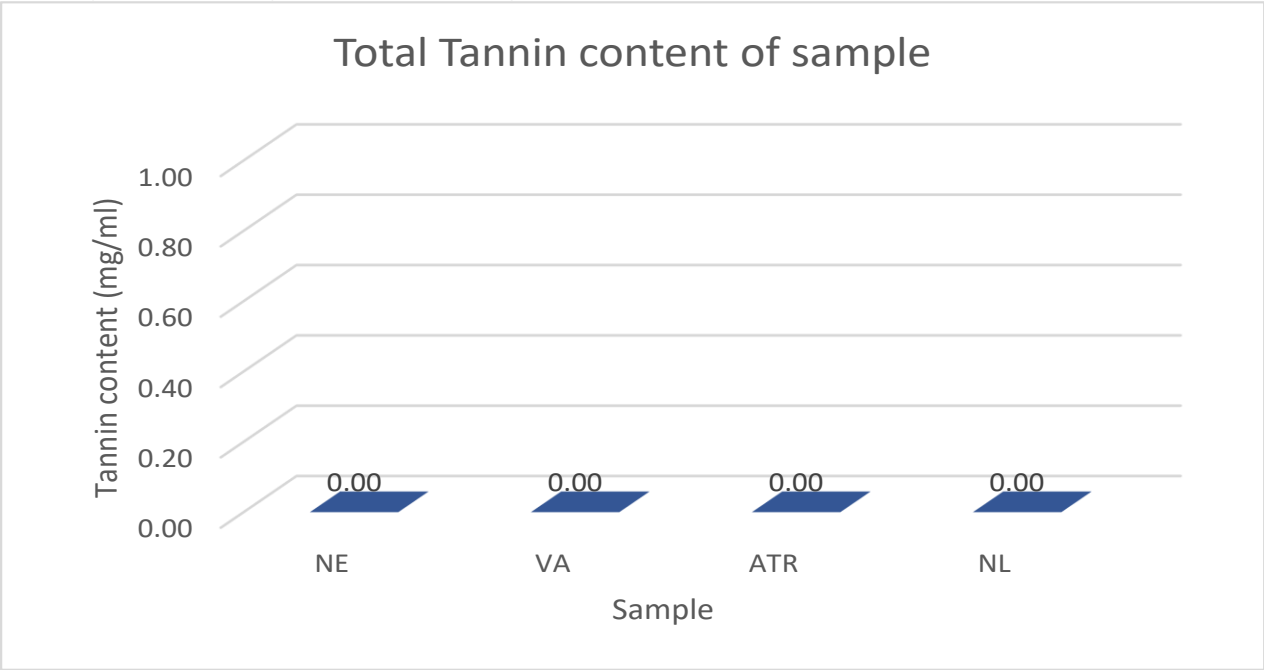

Figure 5: Concentration of total tannin content of plant distillates.

Note :

NE = *Azadirachta indica*

VA = *Veronica ambigua*

ATR = *Artemisia annua*

NL = *Nauclea latifolia*

DISCUSSION

Studies on plant extracts have shown the significance of plant chemicals as possible sources of non-toxic, easily biodegradable substances and they may serve as precursors for the synthesis of useful drugs (Sofowora, 1993). The results of the qualitative Analysis of the secondary metabolites in the distillates were shown in table 1. The results of the quantitative Analysis of the

phytochemical screening revealed the concentration of each phytochemical in the distillates.

The quantitative Analysis of *azadirachta indica* revealed a significant quantity of some phytochemicals. From the result obtained by Ankita and Sapan (2018) on *azadirachta indica,* the total phenol was 241.642 mg/mL and 178.218 mg/mL in the leaves and stem, while the total flavonoids were found to be 85.386 mg/mL and 82.781 mg/mL in the leaves and stem respectively. Khanal (2021) also reported the total alkaloids and flavonoids of the plant to be 10.67% and 13.8% respectively. These results were found to be significantly higher than what was obtained in the present study; total alkaloid 0.18 mg/mL, flavonoids 0.06 mg/mL and phenol 0.11 mg/mL. The significant difference could be attributed to the extraction method, solvent used, geographical area and the weather at the time of collection.

The result of the qualitative phytochemical screening of *vernonia ambigua* is similar to the result reported by Abubakar *et al.,* (2011) with the exception of flavonoids. Unfortunately, there was no report found on the quantitative phytochemical analysis of the plant, in spite of exhaustive literature search.

Quantitative phytochemical screening of *artemisia annua* was carried out by Masoumeh *et al.,* (2012), from their analysis, the total phenol of different parts of *artemisia annua* differs significantly between 11.22 mg/g to 16.94 mg/g, while the total flavonoid ranges from 11.62 mg/g to 63.74 mg/g. Pushpa *et al.,* (2015) reported total phenol content between 22.7 mg/g to 255.5 mg/g, while total flavonoids were between 11.15 mg/g to 161.20 mg/g when different solvents were employed in extraction. Despite the significant difference between the present study and literature, it was established that the part of plant and solvent used for extraction have a significant effect on the quantity of phytochemicals.

According to Omotugba *et al.,* (2019) *nauclea latifolia* does not contain saponin, flavonoids and phenols, this is in contrast with the present study. The quantitative phytochemical screening of *nauclea latifolia* revealed the quantity of some phytochemicals presents in the plants. The result revealed alkaloids to be 0.01 mg/mL, and flavonoids 0.03 mg/mL, the result of steroid and tannin are found not be significant. This was lower than alkaloid 0.32%, flavonoids 10.75 mg/g, and phenolics 58.08 mg/g reported (Frankly *et al.,* 2018).

Based on the present study, despite all the distillates confirming the presence of alkaloid, the distillate of *azadirachta indica* (NE) was found to have the highest concentration of alkaloid (0.18 mg/mL), this can be attributed to the high level of antimalarial activity reported for it. Biological and therapeutic properties have been associated with alkaloids which are widely used in body physiological effects (Nwalo, *et al.,* 2017) linked with anti-helminths, tumor, and cancer cells (Wink, *et al.,* 1993). The distillate of the same plant was also found to contain the highest level of total phenolic compounds (0.11 mg/mL) and total flavonoids (0.06 mg/mL) quantitatively. The presence of flavonoids confers potency in scavenging free radicals (Nwalo, *et al.,* 2017), reducing blood-lipids content, glucose (Atoui, *et al.,* 2005), some activities of the flavonoids are in synergies with other related chemicals (Fleischer, 2003). The concentrations of steroidal compounds and tannins were found to be low in the distillates despite testing positive in the qualitative Analysis. Tannins have been reported to be an important source of protein that protect cell membrane lipids, protein, nucleic acid and against cardiovascular and brain dysfunction when taken accurately (Han, *et al.,* 2007). The least concentration of steroids in the distillates satisfied the known activity to reduce the production of pro-inflammatory cytokines (Fleischer, 2003).

CONCLUSION

The present study showed the phytochemical constituents (qualitatively and quantitatively) present in *Azadirachta indica, Nauclea latifolia, Vernonia ambigua, and Artemisia annua* leaves distillates. To our best knowledge, this study is the first to report the quantitative Analysis of distillates of *Azadirachta indica, Nauclea latifolia, Vernonia ambigua, and Artemisia annua*. It is recommended that further studies should be perform to identify individual relevant compounds from the distillates for a better understanding of their mechanism of actions and activities. In addition, toxicology and *in vivo* studies should also be performed to deduce the safety for inhalation of the distillates.

ACKNOWLEDGEMENT

We would like to acknowledge the entire staff of Applied Science, Kaduna Polytechnic Kaduna Nigeria.

REFERENCES

Abubakar, B. A., Aliyu M.M., Mikhail, S.A., Hamisu, I. and Adebayo, O.O. (2011). Phytochemical screening and antibacterial activities of vernonia ambigua, vernonia blumeoides and vernonia oocephala (asteraceae). Acta Poloniae Pharmaceutica n Drug Research. 68(1): 67-73, 067-073.qxd (ptfarm.pl)

Ahmed, M., Khan, M.I., Khan, M.R., Muhammad, N., Khan, A.U., and Khan, A.R. (2013). Role of Medicinal Plants in Oxidative Stress and Cancer. OMICS Publishing Group. 2: 641. **[Crossref]**

Akinwunmi, O.A., Adekeye, D.K., and Olagboye, S.A. (2020). Phytochemical quantification, In vitro antioxidant and antidiabetic potentials methanol and dichloromethane extracts of Triclisia subcordata (Oliv) leaves. Trends in Phytochemical Research Journal. 4 (1): 17-24. https://dorl.net/dor/20.1001.1.25883623.2020.4.1.4.6

Ankita, S., and Sapan P. (2018). Preliminary phytochemical screening and quantitative analysis of secondary metabolites of Mentha arvensis and Azadirachta indica. International Journal of Advanced Research and Development. 3(1):114-118.

Atoui, K., Mansouri, A., Bosku, G., and Kefalas, P. (2005), Tea and herbal infusions: their antioxidant activity and phenolic profile, Food Chemistry. 89: 27-36. https://doi.org/10.1016/j.foodchem.2004.01.075

Broadhurst, R. B., and Jones, W. T. (1978). Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture. 29(9): 788–794. **[Crossref]**

Ferreira, D., Nel, R. J. J., and Bekker, R. (1999). Condensed Tannins. In Comprehensive Natural Products Chemistry. pp. 747–797. **[Crossref]**

Fleischer, T. (2003), Xylopia aethiopica A Rich: A chemical and biological Perspective. Journal of University of Science and Technology. 23:24-31. Xylopia Aethiopica (Dunal) A. Rich.: A Chemical Biological Perspective | Semantic Scholar

Franklyn, N.I., Emmanuel, I.N., Kazeem, O.K., Opeyemi, C.E., Olubanke, O.O., and Shalom, N.C. (2018). Bioactive Screening and In Vitro Antioxidant Assessment of Nauclea latifolia Leaf Decoction. AIP Conference Proceedings 1954, 030015. **[Crossref]**

Hamoud, R., Reichling, J., and Wink, M. (2015). Synergistic antibacterial activity of the alkaloid sanguinarine with EDTA and the antibiotic streptomycin against multidrug-resistant bacteria. Journal of Pharmaceutical and Pharmacology. 67: 264–273. **[Crossref]**

Han, X., Shen, T., and Lou H. (2007), Dietary Polyphenols and their biological significance, International Journal of Molecular Science. 8: 950-988. **[Crossref]**

Hänsel, R., Sticher, O., and Steinegger, E. (2007). Pharmakognosie—Phytopharmazie, 8th edition, Springer Verlag, Heidelberg, Germany, Pp. 153. **[Crossref]**

John, B., Sulaiman, C. T., George, S., and Reddy, V. R. K. (2014). Spectrophotometric estimation of total alkaloids in selected justicia species. International Journal of Pharmacy and Pharmaceutical Sciences. 6(5): 647–648. https://innovareacademics.in/journal/ijpps/Vol6Issue5/9556.pdf

Khanal, S. (2021). Qualitative and Quantitative Phytochemical Screening of Azadirachta indica Juss. Plant Parts. International Journal of Applied Science and Biotechnology. 9(2): 122-127. **[Crossref]**

Kumar, D., Jamwal, A., Madaam, R., and Kumar, S. (2014). Estimation of Total Phenols and Flavonoids in Selected Indian Traditional Plants. Journal of Pharmaceutical Technology, Research, and Management. 2(1) : 77–86. https://doi.org/10.15415/jptrm.2014.21006

Masoumeh, M., Zahra, M., Parastoo, Z.M., Mehdi, A., Helen, H. and Fatemeh, F. (2012). Essential Oil Composition, Total Phenol, Flavonoid, Anthocyanin and Antioxidant Activities in Different Parts of Artemisia annua L. in Two Localities (North of Iran). Journal of Medicinal Plants and By-products. 1: 13-21. **[Crossref]**

Nwalo, F.N., Echeta, J.O., Ude, G. D. and Itumoh, M.O. (2017). Determination of phytochemical composition and antioxidative properties of selected medicinal plant in Ikwo, Ebonyi state, FUNAI Journal of Science and Technology. 3(2): 41-55. https://fjst.org/wp-content/uploads/2015/04/Overview_Preliminary-pages-1.pdf

Omotugba, S. K., Kehinde, A. S., Ampitan, T. A. and Amusa, T. O. (2019). Phytochemical screening of selected browse species in federal college wildlife management new bussa, Niger state. Journal of Research in Forestry, Wildlife & Environment. 11(4):23-27. https://www.ajol.info/index.php/jrfwe/article/download/193018/182143

Pushpa, R., Tanuj, K.A., Pankaj, G. and Ashish T. (2015). Qualitative and quantitative phytochemical analysis of Artemisia indica Willd. Journal of Chemical and Pharmaceutical Research. 7(4):942-949. https://www.jocpr.com/articles/qualitative-and-quantitative-phytochemical-analysis-of-artemisia-indica-willd-3254.html

Ramawat, K.G., and Goyal, S. (2008). The Indian herbal drugs scenario in global perspectives. In: Ramawat, K.G., and Merillon, J.M (eds) Bioactive molecules and medicinal plants. Springer Berlin, Heidelberg. Pp. 82. **[Crossref]**

Schippmann, U., Leaman, D.J., and Cunningham, A.B. (2006) Plants as source of medicines: New perspectives. In: Bogers RJ, Craker LE, Lange D, eds. Medicinal and aromatic plants – Agricultural, commercial, ecological, legal, pharmacological, and social aspects. Dordrecht: Springer. Pp. 71.

Singleton, V. L., and Rossi, J. A. J. (1965). Colorimetry of Total Phenolics with Phosphomolybdic acid Reagents. American Journal of Enology and Viniculture. 16(3):144–158. https://www.ajevonline.org/content/16/3/144

Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.

Uche, F.I., Abed, M.N., Abdullah, M.I., Drijfhout, F.P., McCullagh, J., Claridge, T.W., Richardson, A., and Li, W.W. (2017) Isochondodendrine and 2'-norcocsuline: additional alkaloids from Triclisia subcordata induce cytotoxicity and apoptosis in ovarian cancer cell lines. RSC Advances. 7(70): 44154-44161. **[Crossref]**

Verpoorte R. (2009). Medicinal plants: A renewable resource for novel leads and drugs. In: Ramawat KG, ed. Herbal drugs: Ethnomedicine to modern medicine. Berlin Heidelberg: Springer-Verlag. Pp. 54

Victor, N. O. and Chidi O. (2009). Phytochemical constituents of some selected medicinal plants. African Journal of Pure and Applied Chemistry. 3 (11): 228-233. **[Crossref]**

Wink, M. (2012). Medicinal plants: Source of anti-parasitic secondary metabolites. Molecules. 17: 12771–12791. **[Crossref]**

Wink, M., Schmeller, T., and Latz-Brüning, B. (1998). Mode of action of Allelochemical alkaloids: Interaction with neuroreceptors, DNA and other molecular targets. Journal of Chemical Ecology. 24: 1881 – 1937. **[Crossref]**