World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Comparative phytochemical analysis of four medicinal plants traditionally used for malaria therapy in Nigeria

Adeleke Martina TV * and Ndah Sharon

Department of Plant Science and Biotechnology, Rivers State University, Nkpolu, Port Harcourt, Nigeria.

World Journal of Biology Pharmacy and Health Sciences, 2022, 10(01), 080–085

Publication history: Received on 09 March 2022; revised on 20 April 2022; accepted on 22 April 2022

Article DOI: https://doi.org/10.30574/wjbphs.2022.10.1.0069

Abstract

Nature provides us not just with food in abundance, but also with medicines, such that we could eat our food as medicine. Herbs are becoming more prominent as medicine because synthetic drugs with all their side effects are not completely safe. Herbs are however safer, as they are taken first as food, and in addition have qualities that are medicinal. The medicinal value of a plant lies in the bioactive phytochemical constituents of the plant. Among the most important of these bioactive phytochemicals are alkaloids, tannins, flavonoids, phenolics, saponins and cyanogenic glycosides. Qualitative and quantitative phytochemical analysis of four known anti-malarial plants was carried out on some phytochemical parameters of their leaves, namely- *Cymbopogon citratus, Carica papaya, Azadirachta indica and Ocimum gratissimum. C. papaya* gave the highest values for alkaloids (64.8 mg/g) and flavonoids (104.6 mg/g), followed by *A. indica*, which also gave the highest value for saponins (82 mg/g) and cyanogenic glycosides (14 mg/g). All the plant samples analyzed contained all five of the secondary metabolites in varying quantities, hence they are used in combination as herbal therapy for malaria. Alkaloid which was present in all four plants is known for its anti-malarial ability to suppress Plasmodium, and same with the other phytochemicals analyzed. The presence of these phytochemicals prove that these plants have great medicinal values and have commercial potential in the pharmaceutical industry. The comparative analysis of the phytochemicals in these plants helps one to combine them in the right way for best therapeutic results based on knowledge of their constituents.

Keywords: Phytochemicals; Medicinal plants; Quantitative anaylsis; Flavonoids; Qualitative analysis; Alkaloids

1. Introduction

Nature provides us not just with food in abundance, but also with medicines, such that we could eat our food as medicine. Herbal sources of active ingredients in conventional medicine help in managing intractable diseases; for this reason, trade of plant materials have been increased [1]. Herbs are becoming more prominent as medicine because synthetic drugs with all their side effects are not completely safe. Herbs are however safer, because they are taken first as food, and in addition have qualities that are medicinal. For various reasons, including resistance of strains of Plasmodium to known anti-malarial drugs [2], local African populations usually fall back on plant –based treatments. Hence, herbal renaissance is happening all over the globe [3]. Plant parts have medicinal values, but the concentration of the active components varies from structure to structure [4], and from plant to plant. These medicinal properties lie in some chemical substances in plants which affect the metabolic and physiological functions in the human body.

Natural compounds isolated from various parts of plants such as leaves, fruits, stem, roots and seeds have been shown to possess excellent medicinal values. Scientists therefore have embarked on a mission to survey the flora extensively in order to discover more potential plants.

* Corresponding author: Adeleke Martina TV
Department of Plant Science and Biotechnology, Rivers State University, Nkpolu, Port Harcourt, Nigeria.

The need to search for, and develop more effective anti-malarial, anti-fungal, and anti-bacterial drugs that are inexpensive, with less side effects and readily available to people in developing countries like Nigeria, has necessitated this study. Plants produce vast and diverse organic compounds, the great majority of which do not appear to participate directly in their growth and development. These substances, traditionally referred to as secondary metabolites, are often differentially distributed among limited taxonomic groups within the plant kingdom [5].

Plants have the ability to synthesize almost an unlimited number of chemical substances. In many cases, some of these chemicals serve in plant defense mechanism against pathogens, insects, and herbivores. Generally, any part of the plant may contain the various active ingredients.

Plants are endowed with various phytochemical molecules such as, terpenoids, phenols, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites which are a rich source for free radical scavengers [6, 7]. Most active compounds from collected data belong to the same compound classes as the malarial drugs of natural origin, e.g. the alkaloid class for quinine, and the terpenoid class for artemisinin [8]. Thousands of plant varieties used in folklore medicine have been studied for treatment of cancer, diabetes, arthritis, malaria, infectious diseases, etc. However, unveiling the medicinal value of several plant species still remains an area of research interest to be studied thoroughly.

In this study, four plant species used by traditional health practitioners especially for malaria therapy, *Cymbopogon citratus, Azadirachta indica, Ocimum gratissimum* and *Carica papaya*, were examined. *C. citratus*, commonly known as lemon grass, is a tropical perennial herb belonging to the family Poaceae (true grasses) [9]. It is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root, *C. papaya* is commonly called paw-paw. *C. papaya* belongs to the family *Caricacae* and it is cultivated in most tropical countries. *A. indica* is commonly known as Neem plant. It is one of the fast growing trees and is resistant to drought. Lastly, *O. gratisimum is* an aromatic herb, commonly known as clove basil, African basil or as scent leaf in Nigeria. The aim of this study was to screen, analyze for, and compare some secondary metabolites which constitute the medicinal properties of these plants.

2. Material and methods

This experiment was conducted in the Department of Plant Science and Biotechnology, Rivers State University, Port Harcourt, Nigeria. Fresh, healthy and mature leaves of *C. citratus, C. papaya, O. gratissimum and A. indica* were sourced and authenticated from the Departmental garden and the Taxonomy Unit of the Department, respectively. The leaves were rinsed free of dirt and air dried until crispy, then they were blended and sieved through a 0.5 mm mesh. The powders were stored separately in sealed containers until use.

2.1. Preparation of stock solution

Two different solvents were used for extraction. 30 grams of the plant materials were soaked in 200 ml of ethanol and distilled water in a conical flask and left for 48 hours. After 48 hours, the mixture was filtered through a Whatman filter paper No. 42, boiled for 5 mins and cooled in a desiccator. The filtrate was stored as the stock solution in the refrigerator till when used.

2.2. Qualitative Phytochemical Analysis

2.2.1. Test for Flavonoids

The stock solution (5 ml) was added to 1ml of conc. Sulphuric acid and 0.5 g of Mg. A reddish coloration is formed, that disappears after sometime indicates the presence of flavonoids.

2.2.2. Test for Alkaloids

To 5 ml of the stock solution, 2 ml of HCl was added, then 1ml of Dragendroff's reagent was added to this same mix. An orange red precipitate with turbidity that forms indicate the presence of alkaloids.

2.2.3. Test for Saponins

To 1 ml of stock extract solution, some distilled water was added in a test tube and shaken vigorously. A persistent froathing of the mix indicates the presence of saponins.

2.2.4. Test for Tannins

To 2 ml of stock extract solution in a test tube, 2 ml of water was added, and 1 to 2 drops of diluted ferric chloride solution was added. A dark green coloration indicates the presence of tannins.

2.2.5. Test for Glycosides

To 2 ml of the extract, 2 ml of glacial acetic acid with 1-2 drops of 2 % $FeCl_3$ was added and mixed. This mixture was poured into another test tube with 2 ml of conc. H_2SO_4. Presence of a brown ring at the interface indicates glycosides.

2.3. Quantitative Phytochemical Analysis

2.3.1. Determination of Alkaloids

The method used here was Harbone's method [10]. 5 g of the crude powder of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The solution was allowed to settle and the phosphate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.3.2. Determination of Flavonoids

Flavonoid Determination was done using the method of Boham and Kocipaiabyazan [11], 5 g of the plant sample was extracted with 100ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

2.3.3. Determination of Tannins

Precisely 0.1 g of the sample was weighed into a 50 ml conical flask and shaken for 1 hour in a mechanical shaker. This was then filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1 M $FeCl_3$ in 0.1 M HCL and 0.008 m potassium fenocyanide. The absorbance was measured at 120 nm within 10 minutes.

2.3.4. Determination of Saponins

The method used was that of Obadoni and Ochuko [12]. The sample (2 g) was weighed and put into a conical flask and 100 cm^3 of 20 % aqueous ethanol was added. The sample was then heated over a hot water bath for 4 hours with continuous stirring at about 55 ˚C. The mixture was filtered and the residue re-extracted with another 200 ml of 20 % ethanol. The combined extract was reduced to 40 ml over water bath at about 90˚C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 55 % aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to constant weight, the saponnin content was then calculated.

2.3.5. Determination of Cyanogenic Glycosides

Alkaline Titrimetric Method was used. About 5 g of the sample was weighed into a clean distillation flask, 20 ml distilled water was added and the sample was allowed to stand overnight for proper hydrolysis to be attended. The sample was distilled into 20 ml Sodium hydroxide containing 0.5 g crystal. The distillate was titrated with 0.02 N silver Nitrate in the presence of 0.2 ml of 5 % Potassium Iodide and 1 ml 6 N Ammonia hydroxide solution to a permanent turbidity.

3. Results

3.1. Qualitative Phytochemical screening

The phytochemical screening of the leaves of *C. citratus, C. papaya, A. indica and O. gratissimum* showed by their precipitate colorations that they all contained alkaloids, flavonoids, tannins, saponins and cyanogenic glycosides in varying concentrations. The observed quality of the test samples of the plant species, showing the presence of these secondary metabolites, is shown in Table 1.

Table 1 Qualitative Phytochemical screening of different medicinal plants

Test	*C. citratus*	*C. papaya*	*A.indica*	*O. gratissimum*
Alkaloid	+ +	+ +	+ +	+ +
Flavonoid	+ +	+ + +	+ +	+ +
Saponins	+	+	+ + +	+
Tannins	+	+	+	+
Cyanogenic Glycoside	+	+	+	+

Note: (+) = presence

3.2. Quantitative Phytochemical Screening

The quantity of the secondary metabolites present in the plant species were measured in mg/g. The result of the quantitative phytochemical screening of the leaves of the medicinal plants is presented in Fig. 1. Flavonoids were found to be the most abundant constituent in all the plants screened, followed by alkaloids; while tannins had the least values, followed by cyanogenic glycosides.

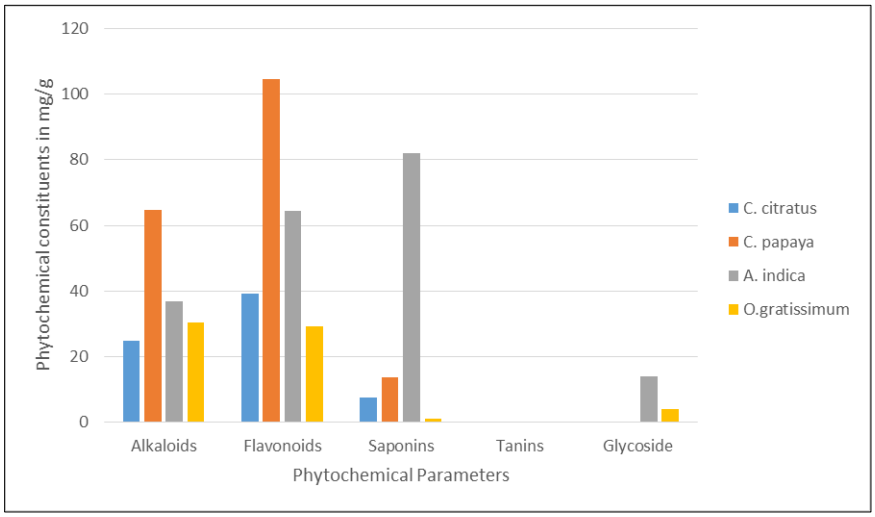

Figure 1 Comparison of some phytochemical constituents of four medicinal plants

4. Discussion

The use of medicinal plants to treat and manage various forms of diseases and dysfunctions is becoming increasingly popular and has received wide acceptance. Nigeria, an important nation of biodiversity, is enriched with herbal resources. Reports on the effects of these medicinal plants on animal and human health are diverse. The chemical evaluation of medicinal plants and their isolates have transformed traditional medicine from an almost invisible trade into a modern industrial enterprise, capable of making significant contribution to both health care delivery and economic growth of most developing countries [13].

In the present study, the analytical results for the four plant samples investigated show that they all contain some phytochemical compounds (Table 1, Fig. 1) which possess good medicinal properties. The secondary metabolites are always the major point of interest as they have been extensively investigated as a source of medicinal ingredients [7]. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [14]. Of all the ones screened for in this study, the flavonoids were the most abundant, followed by the alkaloids. [15] Tijani et al [15] has shown that plant-derived alkaloids from African medicinal plants have a great potential for anti-malarial drug development. C. papaya had the highest values for flavonoids (104.6 mg/g) and alkaloids (64.8 mg/g), followed by *A. indica* which appeared to have all five secondary metabolites except tannins which it had here in trace amount. *A. indica* gave the highest value for Saponins (82 mg/g) and cyanogenic glycosides (14 mg/g) of the four plants (Fig 1). This agrees with the findings of Pandey et al [16] that says that *A. indica* leaves showed presence of saponins, flavonoids, phenol, tannins, alkaloids, glycosides, and alkaloids. The parts of the Neem tree *(A. indica*) have undergone

extensive chemical investigation, and more than 150 bioactive chemical compounds have been isolated from it [17]. The benefit of plants containing bioactive anti-malarial compounds, particularly the bitter principles (alkaloids and terpenoids), include their use in the preparation of traditional remedies against malaria, fever, infection and inflammation, amongst others. *C. ctratus* and *O. gratissimum* on the other hand had much lower values than *C. papaya* and *A. indica*, in the quantitative analysis for these secondary metabolites; especially for alkaloids, flavonoids and saponins. Nevertheless, when used in combination with other similarly therapeutic plants, their effects are cumulative.

Alkaloids as one of the most efficient, therapeutically significant plant substance, its presence in the leaves of all the plant samples analyzed shows that these plants can be effective anti-malaria agents since quinine, an alkaloidal drug, is anti-malaria. No wonder one or more of these medicinal plants studied are included in the herbal cocktail for treating malaria traditionally [18, 19]. However, since the breakdown of these herbs in treating malaria have to do with the liver especially and kidney [20], they could become toxic if combined and used indiscriminately.

5. Conclusion

In conclusion, getting to know comparatively the quantity of alkaloids, flavonoids, saponins, tannins and cyanogenic glycosides in these four plants –*A. indica, C. papaya, C. citratus* and *O. gratissimum*, it would guide anyone in knowing what to include in a herbal cocktail with not too much of anyone of these secondary metabolites, lest it becomes toxic. For instance, a combination of *C. papaya* and *A. indica* in a traditional herbal cocktail for malaria treatment may not be a good idea, for they are both high in alkaloids and flavonoids. Inspite of the usefulness of traditional herbal cocktails therefore, in treating malaria, their toxicity levels would however need to be ascertained.

Compliance with ethical standards

Acknowledgments

The authors are grateful for the assistance of Clement, Awajikere Jonathan and the laboratory staff of the Plant Anatomy and Physiology Research laboratory of the University of Port Harcourt, Choba, Rivers State, Nigeria.

Disclosure of conflict of interest

The authors have not declared any conflict of interest.

References

[1] Suresh K, Deepa P, Harisaranraj R,. and Achudhan VV. (2008). Antimicrobial and Phytochemical Investigation of the Leaves of Carica papaya, Cynodond actylon (L), Euphorbia hirta (L), Melia azedarach (L), and Psidium guajava (L), Ethnobotanical Leaflets, (1): 1-8.

[2] Trape JF. (2002). Combating malaria in Africa. Trends Parasitol, 18:224–230

[3] Roshan A, Verma, NK, Gupta AA. (2014). Brief Study on Carica papaya- A Review. International Journal of Current Trends in Pharmaceutical Researc;h. 2(4):541-550.

[4] Lohidas, J, Manjusha, S. and Jothi, GGG. (2015). Animicrobial activity of Carica papaya. Plant Archives, 15(2): 1179-1186.

[5] Croteau, R. Kutchan, TM & Lewis, NG. (2000). Natural products (secondary metabolites). Biochemistry and Molecular Biology of Plants, pp. 1250–1318.

[6] Zheng W, Wang SY. (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170.

[7] Gracelin, DH, Britto, AJ. and Kumar, BJR. (2012). Qualitative and quantitative analysis of Phytochemicals in five Pteris species. International Journal of Pharmacy and Pharmaceutical Sciences, 5: 105-107.

[8] Chiyaka C, Garira W. and Dube S. (2009). Effects of treatment and drug resistance on the transmission dynamics of malaria in endemic areas. Theor Popul Biol, 75:14–29

[9] Uraku, AJ, Okaka, ANC, Ogbanshi, ME. And Onuoha, SC. (2015). Nutritive and anti-nutritive potentials of Cymbopogon citratus leaves. American Journal of Food and Nutrition, 6(1): 14-22.

[10] Harborne, JB. (1973)Phytochemcial Methods. Chapman and Hall Ltd, London, pp. 378.

[11] Boham BA, Kocipai-Abyazan R. (1974). Flavonoids and condensed tannins from leaves of Hawallan vaccinium vaticultum and V. calycinium. J Pac Sci., 48: 458-463

[12] Obadoni BO and Ochuko PO. (2001). Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci., 8: 203-208.

[13] Bekono, BD, Ntie-Kang, F, Onguéné, PA, Lifongo, LL, Sippl, W, Fester, K. and Owono, LC. (2020). The potential of anti-malarial compounds derived from African medicinal plants: a review of pharmacological evaluations from 2013 to 2019. Malaria Journal, 19(1):1-35.

[14] Bankole, AE, Adekunle, AA. Sowemimo, AA. et al. (2016). Phytochemical screening and in vivo antimalarial activity of extracts from three medicinal plants used in malaria treatment in Nigeria. Parasitol Res., 115:299–305 https://doi.org/10.1007/s00436-015-4747-x

[15] Tijani VPK, Zofou D. and Ngemenya MN. (2008). The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. Afr J Trad Cam., 5:302–21.

[16] Pandey, G, Verma, KK. and Singh, M. (2014). Evaluation of phytochemical, antibacterial and free radical scavenging properties of Azadirachta indica (neem) leaves. Int J Pharm Pharm Sci, 6(2): 444-447.

[17] Brahmachari G. (2004). Neem- an omnipotent plant: a retrospection. Chembiochem. 5(4): 408-421. DOI: 10.1002/cbic.200300749

[18] Akinpelu, M, Gerald, EI, Ayodele, BR, Akpu, AO. and Chuks, IM. (2018). Evaluation of herbal cocktail used in the treatment of malaria on liver tissue of adult Wistar rats. Journal of Medicinal Plants Research, 12(28):508-521.

[19] Moronkeji, A, Eze, GI, Igunbor, MC, Ogbonna, AA. and Moronkeji, AI. (2019). Histomorphological and biochemical evaluation of herbal cocktail used in treating malaria on kidneys of adult wistar rats. IJARP, 3(7):57-66.

[20] Wojcikowski K, Johnson DW, and Gobé G. (2004). Medicinal herbal extracts -- renal friend or foe? Part one: the toxicities of medicinal herbs. Nephrology (Carlton), 9(5):313-8. doi: 10.1111/j.1440-1797.2004.00310.x. PMID: 15504145.