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Research Article

# Comparative Qualitative Analysis of the Phytochemical Load of Water, Methanol, Ethyl Acetate and Hexane Extracts of Six Selected Medicinal Plants

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**ABSTRACT**

The effectiveness of herbal medicine based on the paradigm of synergicity may depend on the phytochemical load of the plant. However, the effectiveness of the extraction method used by herbal practitioners who commonly macerate plants in water or dry gins or boil in water to prepare their treatment still remains unclear. To ascertain the effectiveness of these local practices, the phytochemical load of water, methanol, ethyl acetate and hexane solvent extraction methods were evaluated and compared for six plants locally used for the folk medicine. The plants were screened for tannins, flavonoid, cardiac glycosides, phenolics, steroids, terpenes, saponin, carbohydrates, phlobatannins and alkaloids. All 10 phytochemicals screened were presents for various solvent extractions methods. The most effective extraction methods were water and methanol with the highest phytochemical load for all six selected phytochemicals investigated. All 10 phytochemical were identified for water and methanol extracts of *Psidium guajava*, *Vitex doniana a*nd *Kola acuminata*. Hexane extraction showed the lowest phytochemical load. These results suggest that the local practices of herbal practitioners using water and dry gin (an alcohol) for the preparation of their treatments are effective in extracting necessary phytochemicals and are in conformity with basic scientific methods of extraction. However, treatments prepared with dry gin, an alcohol which is known to have adverse effect in the body may not be advisable for patients to consume and therefore water extraction will be ideal for such practices.

**Keywords:** effectiveness, solvent extraction, herbal medicine, herbal practitioners, phytochemicals

## INTRODUCTION

Herbal medicine which basically relies on plants for the treatment of various diseases and illness and began as far back as time immemorial since the existence of mankind, is fast growing and given so much attention in the world today[1]. The safety of this medicine gives it an extra advantage over orthodox medicine, serving as alternative to a wide population of the world particularly in Asia and Africa[2].

Unlike orthodox medicine whose principle is based on specificity, isolating and using only a specific compound for the treatment of a disease[3], alternative medicine believes on synergicity; the combine effect of more than one compound in treating an illness[4]. With this thought, herbal medicine believes it is the assembly of various phytochemicals within a plant that combines together in treating a disease[5].

Herbals medicine is highly advanced in Asia particularly China[6]. The herbal practitioners are well trained and have laid scientific basis in the production and administration of their drugs. In Africa, it is not the case though herbal medicine is highly appreciated. The herbal practitioners use their common initiative in the production and administration of their treatments without any scientific knowledge which can ensure the efficacy and safety. This has been the greatest challenge of herbal medicine in this part of the globe since the World Health Organization supports the use of traditional medicine provided they are proven to be efficacious and safe[7]. It becomes necessary to relate the basic practices of herbal medicine to scientific practices and establish the fundamental guidelines for drug production, and administration of treatment.

Most herbal practitioners usually prepare their treatments either by macerating herbs in water or dry gins, or boil in water without necessarily understanding the role of these extraction processes and their implication in the treatment of diseases. These practices are very common with herbal practitioners in Africa and have shown to be effective in the treatment and cure of many diseases. The effectiveness is thought to be based on the synergic effect of the phytochemicals extracted during this extraction process[8]. To ascertain the effectiveness of these local practices, the phytochemical load of various solvent extraction methods were evaluated and compared for six plants locally used for the folk medicine.

## MATERIALS AND METHODS

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Table 1: Phytochemical screening of water, methanol, ethyl acetate and hexane from six indigenous medicinal plant extracts

| PLANTS | SOLVENT | PHYTOCHEMICALS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TAN | FLA | CAG | PHE | STE | TER | SAP | CAR | PHL | ALK |
| *Vitex doniana* | Water | + | + | + | + | + | + | + | + | + | + |
| | Methanol | + | + | + | - | + | + | + | + | + | + |
| | Ethyl acetate | + | + | + | + | + | + | + | - | - | - |
| | Hexane | - | - | + | + | + | + | + | + | - | - |
| *Psidium guajava* | Water | + | + | + | + | + | + | + | + | + | - |
| | Methanol | + | + | + | + | + | + | + | + | + | + |
| | Ethyl acetate | - | + | + | + | + | + | + | + | + | - |
| | Hexane | - | + | + | - | + | + | - | + | + | - |
| *Kola acuminata* | Water | + | + | + | + | + | + | + | + | + | + |
| | Methanol | + | + | + | + | + | + | + | + | + | + |
| | Ethyl acetate | - | + | + | + | + | + | + | + | + | - |
| | Hexane | - | + | - | - | + | + | + | + | + | - |
| *Carica papaya* | Water | + | - | + | - | + | + | + | + | + | + |
| | Methanol | + | - | + | - | + | + | + | + | + | + |
| | Ethyl acetate | - | - | + | - | + | + | - | + | - | - |
| | Hexane | + | - | + | - | + | + | - | + | - | - |
| *Vigna radiata* | Water | - | - | + | - | + | + | + | + | + | + |
| | Methanol | - | - | + | - | + | + | - | + | - | + |
| | Ethyl acetate | - | - | + | - | + | + | - | + | - | - |
| | Hexane | - | - | + | - | + | + | + | + | - | - |
| *Azadirachta indica* | Water | - | - | + | - | + | + | - | + | + | + |
| | Methanol | - | - | + | - | + | + | + | + | + | + |
| | Ethyl acetate | - | - | + | + | + | + | - | + | - | + |
| | Hexane | - | - | + | - | + | + | - | + | - | - |

*Legend: TAN : Tannins FLA : Flavanoids CAG : Cardiac glycosidesPHE : Phenolics STE : Steroids TER : Terpenoids SAP : Saponin CAR : Carbohydrates PHL : Phlobatannins ALK : Alkaloids*
*+ Present, - absent*

Table 2: Number of phytochemicals identified from plant extract for water, methanol, ethyl acetate and hexane

| | *Vitex doniana* | *Psidium guajava* | *Kola acuminata* | *Carica papaya* | *Vigna radiata* | *Azadirachta indica* |
|---|---|---|---|---|---|---|
| Water | 10 | 9 | 10 | 8 | 7 | 6 |
| Methanol | 9 | 10 | 10 | 8 | 5 | 7 |
| Ethyl acetate | 7 | 8 | 8 | 4 | 4 | 6 |
| Hexane | 6 | 6 | 6 | 5 | 5 | 4 |

Collection of plant materials: The leaves, stems or roots of six medicinal plants (*Vitex doniana, Psidium guajava, Kola acuminata, Carica papaya, Vigna radiata, and Azadirachta indica*) were collected from uncultivated farmlands located at South Eastern parts of Nigeria.

Solvent extraction: The plant samples were air-dried at room temperature and ground into uniform powder using a Thomas- Willey milling machine. 80 grams of each plant was divided into 4 portions of 20g for water, methanol, ethyl acetate and hexane extractions. 20g of the powdered sample was soaked into 80ml of each solvent in a beaker and stirred continuously until the mixture is homogenous. The beaker was heated at $42^0$C for 5min in a water bath and extract were filtered using Whatman filter paper No 42 (125 mm). The filtrate was collected and kept at room temperature for further analysis.

Phytochemical analysis: Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by[9,10,11.]

Test for tannins: 1ml of filtrate was added to 1m of distilled water in a test tube. A few drops of 10% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponin: 1ml of filtrate was mixed with 4 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoid: 1ml of 10% NaOH was mixed with 1ml of filtrates, shake vigorously and observe for the development of a yellow colouration.

Test for cardiac glycosides: 1ml of concentrated sulphuric acid gently poured on the walls of an incline test tube containing 1ml of plant filtrate. Dropwise was added 10% Ferric Chloride solution and observe for a brown, violet, or greenish ring.

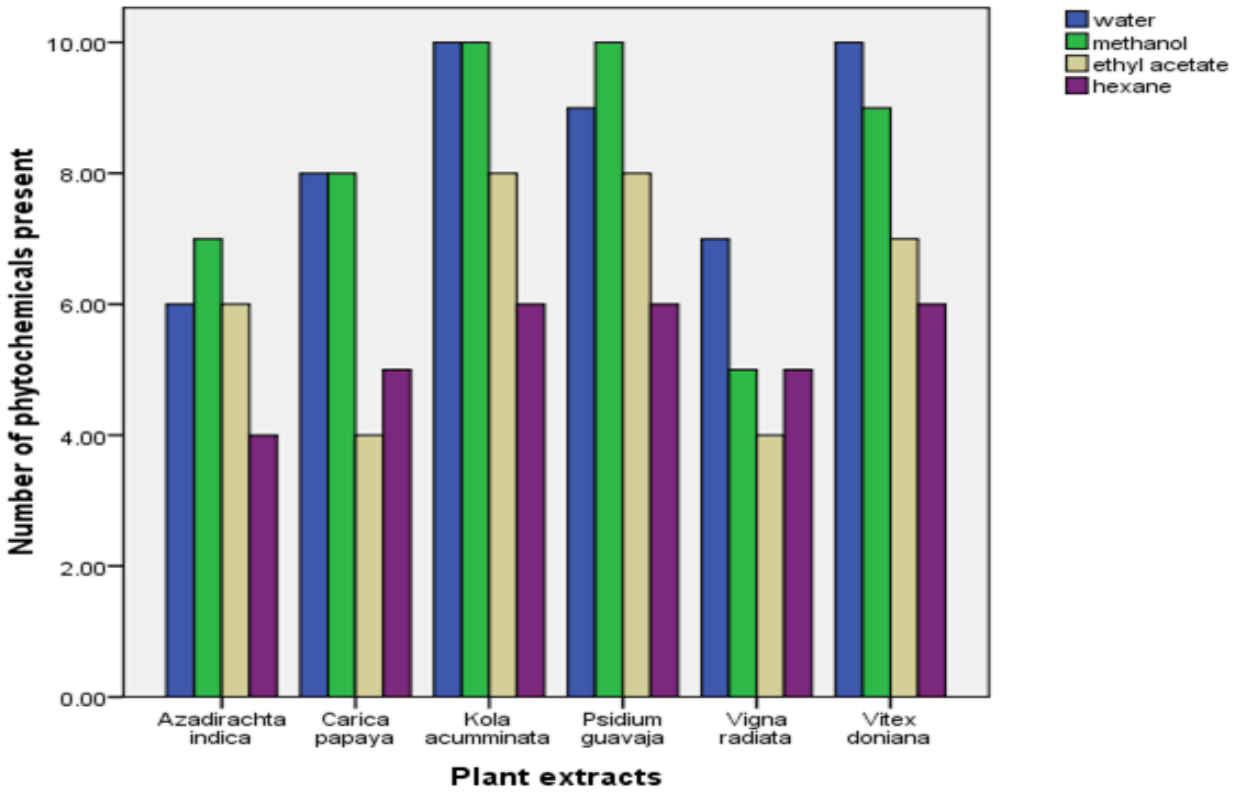


*Figure 1: Numerical presentation of identified phytochemicals in water, methanol, ethyl acetate and hexane extracts of selected medicinal plants*

Test for Phenolics: 2ml of distilled water was added in a test tube containing 1ml of extract. This was followed by 0.5ml of Ammonium hydroxide and 1ml of Amyl alcohol. The test was allowed at room temperature for 30mins and observed for the formation of precipitate and colour change.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Test for steroids: a dark brown colour was indicative of phlobatannins when 2ml of Acetic anhydride followed by 2ml of concentrated sulphuric acid was added into a test tube containing 1ml of filtrate.

Test for Terpenoids: 1ml of extract was placed into a test tube and added 0.4ml of chloroform, 0.6ml of concentrated sulphuric acid was poured gently into the tube at an inclined portion. A reddish brown colouration was indicative of the presence of terpenoids.

Molisch test of Carbohydrates: a few drops of Molisch reagent was added in a test tube containing 1ml of filtrate. 1ml of concentrated sulphuric acid was gently added and observed for a purple ring colour change.

Test for alkaloids: 4ml of 1% hydrochloric acid was added to 1ml of filtrate into test tube, mixed and warm solution in a warm bath. The presence of turbidity or precipitate after the addition of 1ml of Mayer's reagent followed by 1ml of Dragendoff's reagent was indicative for alkaloids.

**DATA ANALYSIS**

Data was presented in graphs and tables using Statistical Programme for Social Sciences (SPSS) version 16.0.

**RESULTS**

Phytochemical screening of six medicinal plant extract show the presence of tannins, flavonoid, cardiac glycosides, phenolics, steroids, terpenes, saponin, carbohydrates, phlobatannins and alkaloids (Table 1).

*Vitex doniana* showed the presence of all 10 phytochemicals screened in the water extract. Only phenolics were absent for the methanol extract with the other nine present. Both ethyl acetate and hexane showed the presence of seven phytochemicals. All 10 ten phytochemical for *Psidium guajava,* were present in methanol extract, followed by water extract with 9 phytochemicals present. Hexane extract was the least with just 6 phytochemicals identified present. For *Kola acuminata*, all ten phytochemicals were present for water and methanol extrcts ethyl acetate extract identified 8 while hexane identified 6 of the phytochemicals. Water and methanol extract identified 8 extracts of *Carica papaya* while only 4 were identified for ethyl acetate and hexane extracts. *Vigna radiata* water extract identified 7 phytochemicals, 5 for methanol and ethyl acetate extracts and 4 for hexane extract. Methanol extract for *Azadirachta indica* identified 7 phytochemicals. 6 were

identified for water extract and 4 each for ethyl acetate and hexane (table 2).

Water and methanol extract showed the presence of most phytochemicals in all the plants while ethyl acetate and hexane identified some of the phytochemicals. Most of the phytochemicals not identified were tannins, phenolics, cardiac glycosides and alkaloids.

## DISCUSSION

Phytochemicals particularly secondary metabolites found in minute quantities are very important in plant due to their protective role in preventing attacked from bacteria, fungi, insects and other plant predators[12]. These phytochemicals in actual sense resembles the immune system in animals in preventing or fighting against attacks from foreign bodies[13]. With these notions, herbs have been used to support the immune system of man to treat certain diseases[14.] Presently, this herbal medicine is widely accepted across the globe as an alternate to orthodox medicine. The effectiveness of this medicine based on the paradigm of synergicity may depend on the phytochemical load of the plant. Though herbal medicine has shown to be effective to treat diseases, the effectiveness of the media for extraction used by herbal practitioners who commonly use water or dry gins in cold or hot to prepare their treatment still remains unclear.

In this study, water, methanol, ethyl acetate and hexane solvents were used as solvents for extraction to compare the phytochemical load of some selected medicinal plant. Phytochemical analysis showed the presence of all 10 phytochemicals screened; tannins, flavonoid, cardiac glycosides, phenolics, steroids, terpenes, saponin, carbohydrates, phlobatannins and alkaloids for various extraction methods. The most effective extraction methods were water and methanol. These extractions showed the highest phytochemical load for the six selected phytochemicals investigated. All 10 ten phytochemical were identified for *Psidium guajava*, *Vitex doniana a*nd *Kola acuminata* for water and methanol extractions. For water and methanol extraction of *Carica papaya*, *Vigna radiata* and *Azadirachta indica*, the identified phytochemicals varied between 6 and 8. Hexane extraction showed the lowest phytochemical load. These results suggest that the local practices of herbal practitioners using water and dry gin (an alcohol) for the preparation of their treatments are effective in extracting necessary phytochemicals and are in conformity with basic scientific methods of extraction.

This study encourages the use of water and dry gin by herbal practitioners for preparation of their treatments. However, treatments prepared with dry gin, an alcohol which is known to have adverse effect in the body may not be advisable for patients to consume and therefore water extraction will be ideal for such practices.

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