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## RESEARCH ARTICLE

# Comparative study of the *in vitro* phytochemicals and antimicrobial potential of six medicinal plants [version 1; peer review: 1 approved with reservations, 2 not approved]

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## Abstract

**Background:** This study sought to investigate the antimicrobial activity of six plants used in traditional medicine in Africa.

**Methods:** The antimicrobial activity of the six medicinal plant extracts (aqueous and ethanol) were evaluated against *Proteus mirabilis* (ATCC 21784), *Pseudomonas aeruginosa* (ATCC27856) were *Aspergillus fumigatus* using the agar-well diffusion protocol. The activities of these extracts were compared with the positive controls chloramphenicol and griseofulvin. Similarly, the phytochemicals from the extracts were qualitatively assayed and their percentage yield calculated by standard methods.

**Results:** The bacterial organisms used, *P. mirabilis* and *P. aeruginosa*, were slightly-to-highly susceptible to aqueous and ethanolic extracts from the various test plants, while *A. fumigatus* was insensitive to the treatments. The ethanolic extracts of the sampled plants showed superior inhibitory performance on the target bacteria to the aqueous extracts. Aqueous and ethanolic extracts of *Aframomum melegueta*, *Moringa oleifera* and *Cola nitida* showed inhibitory consistency against the target bacteria. Superior inhibitory activity was observed for ethanol extracts of *A. melegueta* seed and *M. oleifera* pod against *P. mirabilis* and *P. aeruginosa*. Variations in phytochemicals were noticed across solvents and plant parts for all plants. Phenols were detected in the aqueous and ethanolic extracts of *C. nitida* and *Cola acuminata*, but relatively appeared denser in extracts of *A. melegueta* seed and *Chrysophyllum albidum* fruits. The extracts of *C. nitida*, *C. acuminata* and *A. melegueta* tested positive for the presence of flavonoids, which were undetected in *C. albidum* and *M. oleifera* seed and pod extracts. None of the extracts showed the presence of every phytochemical assayed during the study.

**Conclusions:** Extracts of the medicinal plants assessed in this study showed antibacterial potential. Developing new methodologies that

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preserve the bioactive potency of phyto-extracts for optimal microbicidal activity is promising for development of safe, non-reactive pharmaceuticals.

### Keywords

Antimicrobial activities, Medicinal plants, Phytochemicals, Bacteriostatic, Bacteriocidal

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## Introduction

The ethnobotanical use of medicinal plants and/or their derivatives (essential oil, resins and soluble extracts) in Africa dates back to early civilization (Sofowora, 1993). This practice is globally perceived as comparatively cheaper and more widely accessible to most rural or less-privileged economies of the world than modern synthetic drugs (Lawal *et al.*, 2012). Statistics as presented by Fabricant & Farnsworth (2001) in the bulletin of the World Health Organization showed that close to 65% of the world population relied on medicinal plants for their primary healthcare drugs (Eddouks & Ghanimi, 2013). Consequently, it is estimated that about 39% of drugs developed since 1980 have being from natural plants, their derivatives or analogues (Newman & Cragg, 2007; Verpoorte *et al.* (2010). In addition, Ramawa *et al.* (2009) and Verpoorte *et al.*, (2010) noted that approximately 25% of the currently used modern drugs are derived from plants, a number largely composed of analgesics (e.g. morphine), cardiotonics, chemotherapeutics and antimalarials (e.g. quinine and artemisinin). In recent decades, there have been growing global concerns on the rising cost of buying synthetic drugs, assessing their toxicological profile, and redressing their periodic side-effects and unstable efficacy (Gupta *et al.*, 2016). These undermine their continuous use in modern healthcare delivery and cause a renaissance of herbal screening as well as the chronopharmacological process (Westh *et al.*, 2004). The clinical and pharmacokinetics sustainability of the efficacy of many synthetic antibiotics, prophylactics and curative drugs is threatened by the growing emergence of multi-drug resistant pathogenic strains and cost of production (Bandow *et al.*, 2003). These concerns caused researchers to shift focus and exploit more intensely natural plants and their allies (ferns, fungi and algae) for safer generic bioequivalents to synthetic drugs or substitutions with stable therapeutic values (Rojas *et al.*, 2003; Savoia, 2012). Synthetic drugs use in animal farming also have implications for the global development of organic foods.

Plant-derived medicine accounts for more than a quarter of today's pharmacopoeia and over US\$3.5 billion in annual export value of pharmaceutical (Eddouks & Ghanimi, 2013). While the global inventory of ethnobotanicals is growing, the catalogue of their bioactive compounds that can improve human health is constantly being updated. Over 12,000 bioactive metabolites (primary and secondary) and pigments of plant origin with a wide range of biological activities as well as therapeutic values were documented. Osemwegie *et al.* (2014) noted the inadequacy of current data in capturing the global totality of medicinal plants due to either neglect of plants in remote ecozones or bias against other related plant biota. While the prehistoric and historic knowledge of numerous health-beneficial plants in Africa seems threatened in recent times, their use in primary healthcare delivery predates modern medicine (Pasewu *et al.*, 2008). This has facilitated the hybridization of both traditional and modern primary healthcare systems in some continents of the world (Eddouks & Ghanimi, 2013).

*Aframomum melegueta* (Roscoe) K. Schumann (Alligator pepper), *Chrysophyllum albidum* G. Don (Cherry), *Cola nitida* (Vent.) Schott and Endl., *Cola acuminata* (P. Beauv.) Schott and

Endl. (Kolanuts), and *Moringa oleifera* Lam. (Moringa) were listed among over 5,000 species of documented medicinal plants reported by Mahomoodally (2013). These plants, from ethnobotanical surveys, were found to be common in many Nigerian culture and traditional festivities, featuring as masticatory and spiritual materials at traditional marriage, coronation and invocation ceremonies (Anwar *et al.*, 2007; Idu *et al.*, 2007; Pasewu *et al.*, 2008). They are also ethnopharmacologically valuable in maintaining, preventing and improving health, and in treating different forms of illness in many African nations (Duraipandiyar *et al.*, 2006; Idu *et al.*, 2007; Mahomoodally, 2013). While the data on West African medicinal plants is hardly current, reports are inconsistent on the use of these plants as insecticides, antimicrobial, molluscicides and nematocides across cultures (Odugbemi, 2006). Similarly, the therapeutic scope and potency of biological active constituents of plants are to a large extent improved by ecosystem factors, methods of extraction of biometabolites, polarity of extraction solvent(s) used and part of plant assayed (Saini *et al.*, 2016; Shitan, 2016). The emerging human allergic response or side effects from synthetic drugs usage, explosion of multi-drug resistant microorganisms, and global paradigm for more efficient stable biopharmacopoeia have increased toxicological, mechanism of action, ethnobotanical and phytometabolomic investigations of medicinal plants. This present study therefore aims to compare the antimicrobial potential, phytochemical profile and solubility of the phytometabolome of selected medicinal plants in aqueous and ethanol media.

## Methods

### Materials

Sterile distilled water, ethanol, dilute hydrochloric acid, 0.1% ferric chloride solution, microbiological media, antibiotics, sodium chloride, barium chloride, chloroform, acetic anhydride and H<sub>2</sub>SO<sub>4</sub>, NaOH were purchased from Ayo-Sigma (ZSA) Chemicals Ltd in Jos, Plateau State, Nigeria.

*Pseudomonas aeruginosa* (ATCC27856), *Proteus mirabilis* (ATCC21784) and *Aspergillus fumigatus* strains were used as target organisms for the study. The cultures of bacterial strains used were obtained from immune-compromised patients of the University Health Centre by personnel of the institution's laboratory. These were preliminarily observed to develop antimicrobial resistance. These strains were later sub-cultured and stored in the Microbial Bank of the Microbiology Laboratory, Landmark University, OmuAran. *Aspergillus fumigatus* was obtained from composited plant materials and kept in similar banks as the bacterial strains. Each microbe was cultured in bijou bottles containing agar slants of nutrient agar (LAB-008) and Sabourad Dextrose Agar (LAB-009).

### Preparation of plant materials for extraction

*Cola acuminata* [Pal. De Beauv.] Schott and Endl., *Cola nitida* (Vent.) Schott and Endl., *Aframomum melegueta* (Roscoe) K. Schum, *Moringa oleifera* Lam, and *Chrysophyllum albidum* G. Don were randomly collected from rainforests in the southern belt of Nigeria. These medicinal plants that were selected based on existing ethnobotanical data were authenticated using picture books of tropical medicinal plants (Fayaz & Ramachandran, 2015;

Mueller & Mechler, 2005). Identification was corroborated by the Curator, University of Ilorin Herbarium in Nigeria.

The seeds of plants used for this study were rid of their coat, air-dried for 14 days and then pulverized with a stainless-steel electric blender. This was then sieved with a 325-mesh sieve before storage in labeled, air-tight, sterile universal bottles. The *Moringa*, seeds and pods were also air dried separately for 5 days before milling to a powder while the two species of kola nuts (*Cola nitida* and *Cola acuminata*) were also sorted, air-dried for 14 days, pounded with a mortar and pestle, air-dried again for another 4 days and stored in air-tight jars on the laboratory bench. In the same vein, the fruit pulp and the fruit apicarp of *Chrysophyllum albidum* were manually separated from the seeds, air dried together for 12 days and also stored in air-tight jars.

### Preparation of aqueous extracts

A total of 80 g of each of the pulverized plant materials was weighed, poured into a labeled 500-ml conical flask and then soaked with 400 ml distilled water prior to each being vortexed for effective extraction using a multipurpose vibrator for 18 h. The preparations were then filtered with a steam-sterilized white handkerchief fixed in a glass filter funnel that drained to labeled 250 ml conical flasks. The residue from each plant extraction was stored in labeled cylindrical covered jar and refrigerated while each filtrate was further processed using a rotary evaporator (Model R-205V) at 55°C until a thick concentrate was obtained. This was later transferred into 250 ml beakers and further concentrated on a Water bath at 50°C until a paste was formed (dry crude extract). The paste was later spatulated into freshly labeled sterile universal bottles and weighed (weight of the dry extracts = weight of the universal bottle containing extract – weight of empty universal bottle).

### Preparation of ethanol extracts

A similar weight (80 g) of each pulverized plant material was weighed and soaked in 400 ml of 95% ethanol in labeled 500 ml conical flasks prior to being vortexed with a bench-top reciprocal shaker (E5850) for a period of 18 h. The mixture was then filtered and processed using the similar extraction protocol for aqueous extraction.

The percentage extraction yield is expressed as:

Extraction yield (%) =  $\frac{\text{Weight of dry extract (g)}}{\text{Weight of sample used for extraction (g)}} \times 100$

Where: weight of dry extract is the actual weight of the extracts and the weight of the sample used for the extraction (g) is the initial weight of the samples measured (80 g).

### Phytochemical analysis

Phytochemical screening of both the aqueous and ethanol extracts of the different plant materials was done according to Tiwari *et al* (2011). The concentrates were solubilized with either distilled water or ethanol to assay saponins, phenolics, flavonoids and terpenoids of each plant specimen respectively.

A total of 0.10g of each of the plant extracts was weighed in a labeled sterile universal bottle, appropriately solubilized with 2 ml of either distilled water or ethanol and transferred into clean labeled test-tubes. The solutions were heated over a Bunsen flame for 3 min, agitated, filtered with Whatmann filter paper (32 mm), cooled, agitated again continuously for 2 min, left to stand for 10 min and observed for froth. In another protocol, phenols, flavonoids and terpenoids were qualitatively investigated using previously described methods (Harborne, 1998). The intensity of color change was used as indicator, observed and rated mildly intense (+), strongly intense (++), extremely intense (+++). Color intensity was visually judged using an RGB color chart.

### Preparation of culture media

A total of 28 g of nutrient agar (NA) and 65 g of Sabouraud dextrose agar (SDA) were each weighed into a sterile conical flask containing 1000 ml of distilled water and mixed vigorously. Each flask was then corked with an absorbent cotton wool stopper after which it was autoclaved for 15 mins at 121°C and later allowed to cool. This was then dispensed to labeled Petri dishes under a laminar flow chamber where they were allowed to solidify. The bottom of each of the plates was marked into 4 quadrants while they were still inverted.

### Standardization of test organisms

In this study, the McFarland turbidity standard method, as described by Forbes *et al.* (2007) was used. The 0.5 McFarland standard, which is equivalent to  $1.5 \times 10^8$  bacteria/ml was prepared according to standard methods (Zapata & Ramirez-Arcos, 2015). Furthermore, normal saline suspensions of the pure culture of each target bacteria were prepared. Turbidity was comparable to the 0.5 McFarland standards by visual determination. Similarly, a serial dilution of *Aspergillus fumigatus* suspension was done and the  $10^{-3}$  diluent was then used.

### Preparation of positive and negative controls

A concentration of 5 mg/ml of chloramphenicol and griseofulvin was prepared, serving as positive controls for antibacterial and antifungal activities, respectively. A solution of 0.85% NaCl was prepared and used as negative control.

### Assessment of antimicrobial activity

The agar well diffusion method was used for the antimicrobial assay (Murray *et al.*, 2016; Ncube *et al.*, 2008). Previously prepared nutrient agar plates were flooded evenly with 1 ml  $1.5 \times 10^8$  bacteria/ml of each bacterial strain in triplicate. These were left for 15 min after which four 6 mm wells were bored aseptically with a sterile cork-borer into each inoculated agar plate. Next, 400 mg from each of the prepared crude extract was dissolved in 1 ml of sterile distilled water or ethanol, as appropriate. A total of 100 µl of each plant extracts was then used to fill 4 equidistant wells. For the controls, 100 µl each of the commercial antimicrobials (positive control) and normal saline (negative control) solutions was used to fill agar wells. The plates were all allowed to incubate at 36°C for 24 h for bacteria and room temperature for 48 h for the fungus. These were then observed for zones of inhibition around the wells. Diameters of

zones of inhibition were measured in millimeters with a meter rule.

## Results

### Yields

The ethanol and water used in this study elicited varying degrees of solubility of plant phytochemicals. The water extract had the highest yield (yield range, 2.13–22.88%) of the soluble phytochemicals compared to 95% ethanol (yield range, 1.76–22.74%) (Table 1). A marked contrast was observed between the aqueous and ethanol extract yields of *Chrysophyllum albidum* and *Cola nitida* respectively. Ethanol had the least phytochemical yield value of 1.76% for *Moringa oleifera* pod. This was apparently lower than the lowest yield value for the aqueous (2.13%) extract of the same plant material (Table 2).

**Table 1.** Weight and percentage yield of aqueous extract from the plant samples.

| Plant                        | Weight of extract | % Yield |
|------------------------------|-------------------|---------|
| <i>Cola nitida</i>           | 8.46              | 10.58   |
| <i>Cola acuminata</i>        | 6.31              | 7.89    |
| <i>Aframomum melegueta</i>   | 3.17              | 3.96    |
| <i>Chrysophyllum albidum</i> | 18.30             | 22.88   |
| <i>Moringa oleifera</i> seed | 13.44             | 16.80   |
| <i>Moringa oleifera</i> pod  | 1.70              | 2.13    |

### Phytochemicals

Assessment of the phytochemical profile of each plant extract showed a marked variation in the phytochemical content of each plant material used in this study. Phenol was detected in all the plant materials evaluated, with the exception of *Moringa oleifera* seeds and pods. Saponin, flavonoids and terpenoids showed inconsistent distributions across the various plant extracts studied, with *Moringa oleifera* seeds having the highest terpenoid concentration in aqueous extracts compared to the visual color density of its pod extracts (Table 3). Conversely, *Aframomum melegueta* was negative for terpenoids in the two extractants used for this study. Levels of saponin were observed to be low but present in *Cola nitida* and moringa seeds of both aqueous and ethanol extractants respectively (Table 4).

**Table 2.** Weight and percentage yield of ethanolic extract from the plant samples.

| Plant Extract                | Weight of extract (g) | % Yield |
|------------------------------|-----------------------|---------|
| <i>Cola nitida</i>           | 18.19                 | 22.74   |
| <i>Cola acuminata</i>        | 5.11                  | 6.39    |
| <i>Aframomum melegueta</i>   | 3.17                  | 4.55    |
| <i>Chrysophyllum albidum</i> | 4.74                  | 5.93    |
| <i>Moringa oleifera</i> seed | 3.37                  | 4.21    |
| <i>Moringa oleifera</i> pod  | 1.70                  | 1.76    |

**Table 3.** Phytoconstituents of the aqueous plant extracts.

| Phytochemical/Extract | <i>C. nitida</i> | <i>C. acuminata</i> | <i>A. melegueta</i> seed | <i>C. albidum</i> | <i>M. oleifera</i> seed | <i>M. oleifera</i> pod |
|-----------------------|------------------|---------------------|--------------------------|-------------------|-------------------------|------------------------|
| Phenolics             | ++               | +                   | +                        | ++                | -                       | -                      |
| Saponin               | ++               | -                   | -                        | -                 | ++                      | -                      |
| Flavonoids            | +                | +                   | +                        | -                 | -                       | -                      |
| Terpenoids            | +                | -                   | -                        | +                 | +++                     | +                      |

-, absent; +, present in low amounts; ++, present in medium amounts; +++, present in high amounts.

**Table 4.** Phytoconstituents of the ethanolic plant extracts.

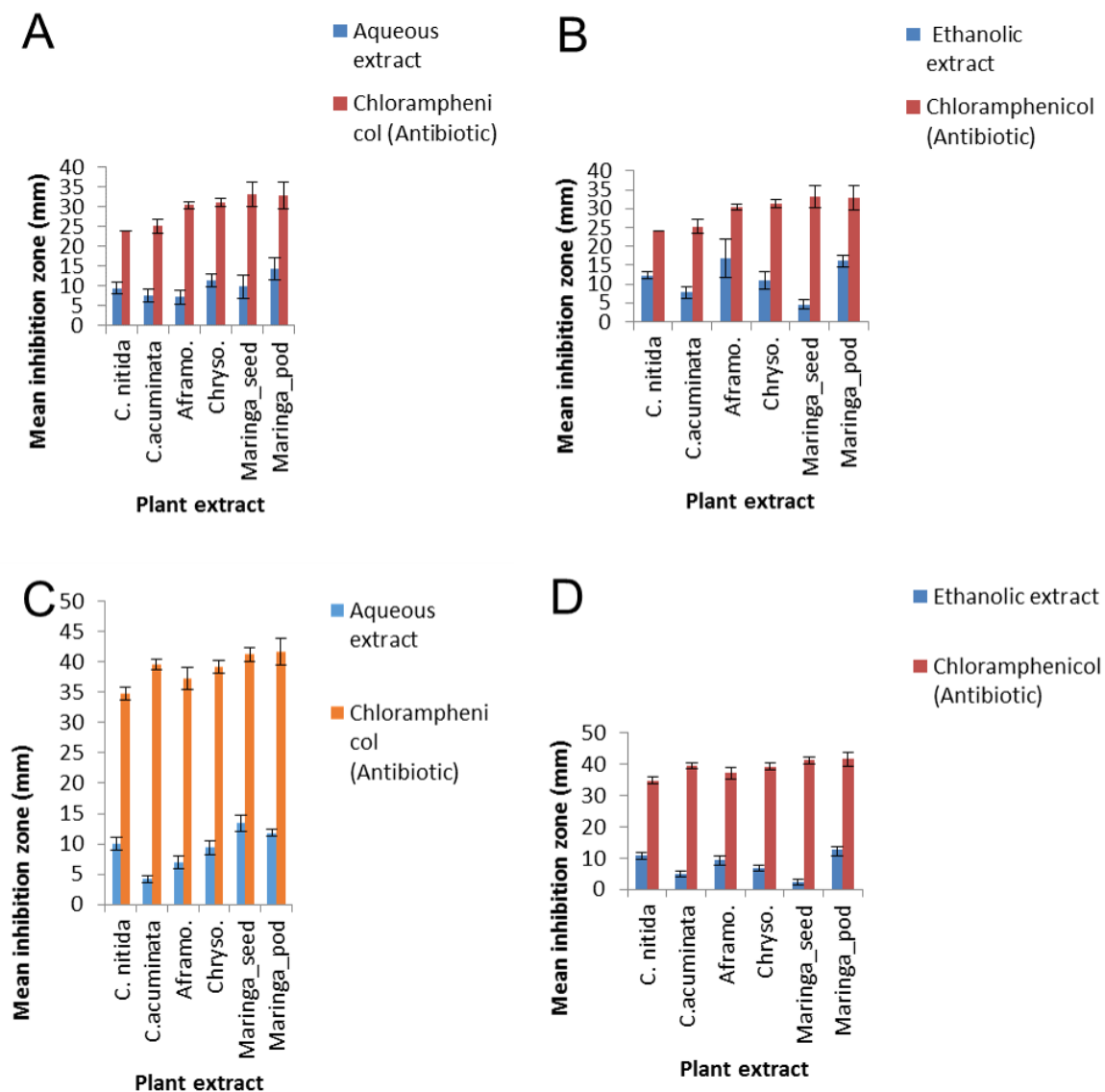
| Phytochemical/Extract | <i>C. nitida</i> | <i>C. acuminata</i> | <i>A. melegueta</i> seed | <i>C. albidum</i> | <i>M. oleifera</i> seed | <i>M. oleifera</i> pod |
|-----------------------|------------------|---------------------|--------------------------|-------------------|-------------------------|------------------------|
| Phenolics             | ++               | +++                 | +++                      | +++               | -                       | -                      |
| Saponin               | ++               | -                   | -                        | -                 | -                       | -                      |
| Flavonoids            | ++               | ++                  | +++                      | -                 | -                       | -                      |
| Terpenoids            | +                | +                   | -                        | -                 | -                       | +                      |

-, absent; +, present in low amount; ++, present in medium amounts; +++ present in high amounts.

### Antibacterial activity

The plant extracts investigated showed various degrees of antimicrobial activity against the target bacterial organisms. The observed zone of inhibition from the edge of each well differed from one target organism to the other. Raw data for zones of inhibition are available on OSF (Nwonuma, 2019). All the plant extracts assayed showed mild inhibition activity compared to the positive control (Chloramphenicol), to which the target bacteria were radically susceptible to (Figure 1). The negative control wells, containing normal saline solutions, showed no inhibitory activity. Plant extracts derived using ethanol solvents showed better inhibitory capacity than the aqueous extracts against *Pseudomonas aeruginosa*. In addition, *Pseudomonas aeruginosa* was

the most susceptible target bacterium to the plant extracts used for the study. A susceptibility contrast was, however, observed for *Proteus mirabilis* treated with the aqueous extracts of *Cola nitida*. Similarly, the aqueous extracts of *Chrysophyllum albidum* at 400 mg in 1 ml of distilled water had the best inhibition activity against *Pseudomonas aeruginosa* and *Proteus mirabilis* (11.3 mm and 9.4 mm) respectively. The highest inhibitory activities were observed for the ethanol extracts of *Cola nitida* (11.8 mm), *Aframomum melegueta* (15.8 mm) and moringa pod (13.4 mm) against *Pseudomonas aeruginosa*. The susceptibility results obtained for both aqueous and ethanol assays of investigated plants on *Proteus mirabilis* vary and were consistently low.



**Figure 1.** Comparative inhibitory performance (mm  $\pm$  SD) of plant extracts on *Pseudomonas aeruginosa* and *Proteus mirabilis*. Plants aqueous extracts inhibition performance on *Pseudomonas aeruginosa* (A) and *Proteus mirabilis* (C). Ethanol extract of plants inhibitory performance against *Pseudomonas aeruginosa* (B) and *Proteus mirabilis* (D).

## Antifungal activity

*Aspergillus fumigatus* showed no visible susceptibility to the various plant extracts investigated with no clear inhibition zone.

## Discussion

Herbal practice is now assuming a global dimension, attracting interest primarily in the standardization of the production chain, quality control and indigenous knowledge inventorying (Venkatasubramanian *et al.*, 2018). The use of herbal knowledge in the development of pharmaceutical industries and primary healthcare in many nations of the world is rapidly growing amidst rising clinical health and unstable therapeutic concerns associated with synthetic drugs. Pharmacopeias derived from natural herbs is now rife in the global pharmaceutical market and has become a huge investment (Mahomoodally, 2013). Furthermore, all the plants selected for this study were indigenous, with ethnomedicinal implications at lower minimum inhibitory concentrations (MICs); they elicit pharmacological, biological or toxicological effects on humans (Ramawa *et al.*, 2009). Conversely, their administrations in healthcare practice in this part of the world remained poorly standardized and without clear therapy specificity. Although every one of the plant materials used for this study are misconceived as having multifunctional bioactive compounds for curing numerous ailments (cancer, fever, infections, inflammations, hypertension, diabetics, obesity, dementia, etc.) and zero side effect in humans, they showed varying level of antimicrobial potential against two prevalent clinical Gram-negative target bacteria.

The tested microorganisms were observed to be most susceptible to the fruit, seed and pod extracts (ethanol and aqueous) of *Cola nitida*, *Aframomum melegueta* and *Moringa oleifera* respectively. This comparative variation maybe attributed to a number of factors involving the chronopharmacology, storage concentration and separation of electric charges (polarity) of the solvent used and composition of bioactive metabolites in the extracts (Wikaningtyas & Sukandar, 2015). The structural and induced resistance of the target microbes coupled with the diversity and richness of relevant solvated metabolites could have equally accounted for the observed variations of inhibitory capacity in the medicinal plants investigated. The choice of ethanol and water for this study is consistent with several other antimicrobial studies involving medicinal plants that used distilled water or ethanol as preliminary extractants of bioactive metabolites of (Ezeifeke *et al.*, 2004). The aqueous extractant was observed to have solvated more constituents as supported by the percentage yield results across the investigated plant materials than ethanol. This finding is inconsistent with the mean zones of inhibition results obtained for the ethanol extract, which suggests better antimicrobial activity by ethanol derived plant extracts (Ahmad *et al.*, 1998; Abu-Shanab *et al.*, 2004; Bacon *et al.*, 2017; Cowan, 1999; Mothana, *et al.*, 2010).

While the mechanism underlying the solubility preference of secondary metabolites in aqueous or ethanol is not fully understood, it is logical to assume that the aqueous extract selectively solvated more of the non-cytotoxic or biologically weak secondary metabolites contrary to the ethanol (95%) that extracted lower but optimized consortium of potent biologically active

phytochemicals (Nascimento *et al.*, 2000). It is also philosophical to correlate the superior inhibition action of the ethanol extracts of the plants investigated to the capacity of the extractant to solvate phytochemicals that structurally modulate through reactive functional groups for optimal antimicrobial activity (Vinoth *et al.*, 2012; Wink, 2015). This may therefore suggest that the full representation of phytochemicals assayed in the various plant extracts studied was selective or limited and accounted for the huge variations in the mean zones of inhibition obtained for the experimental extracts and positive control (chloramphenicol) tested. Phenols was detected in all the plant extracts except *Moringa oleifera*. This may have accounted for the observed antimicrobial capacity of tested extracts and phytotherapeutic popularity the medicinal plants screened (Bukar *et al.*, 2010; Manisha & Vibsha, 2004). The cellular proteins disruption potential and interspecific interactions of phenols or other phytochemicals (flavonoids, tannins and polyketides) may be assumed to be responsible for multi-therapeutic uses of the investigated plants. This finding is, however, inconsistent with the report of Saini *et al.* (2016) and Fahal *et al.* (2018), who both observed higher deposits of flavonoids than phenols in the plant seeds and pods. Difference in the extraction and susceptibility test protocols may have accounted for the contradiction in experimental observations and the detection of more of the assayed phytochemicals in the fruit extracts of *Colanitida*.

Although this study showed that *Pseudomonas aeruginosa* is relatively more sensitive to both crude aqueous and ethanol-based plant extracts than *Proteus mirabilis*, the mechanism underlying the sensitivity reaction remains unclear. In theory, the plant extracts are made up of biochemicals with specific and non-specific modes of actions that compromise the resistance of the target bacteria. The potency and efficacy of the extracts are measured by collective modes of action of the phytochemicals and the corresponding reaction(s) of the target organisms (structural and inductive resistance). Mild resistance posed by *Proteus mirabilis* could have induced by chemical signaling mechanism (Venkatasubramanian *et al.*, 2018). Further study on the isolation and identification of the phytochemical agent(s) responsible for the observed antimicrobial activity using modern techniques involving High Performance Liquid Chromatography and gas chromatography may be required for better understanding of the mode of action. The inhibition of growth of target bacteria is also attributed to the destruction of cell protein machinery such as ion channels and pumps, enzyme actions, cytoskeleton function and membrane biochemical modulation (Ekpendu, 1995; Idowu *et al.*, 2006; Wink, 2015). The structural complexity of the test fungus *Aspergillus fumigatus* may have been responsible for its non-susceptibility to all the investigated extracts compared to the positive control of commercial griseofulvin.

This preliminary study confirmed all the plants investigated as having antibacterial property and corroborating their prevalent use in Nigeria herbal healthcare services. Similarly, ethanol as an extraction solvent optimized more effectively the antimicrobial agents released in the plant extracts compared to the aqueous solvent, supporting the common practice as well as offering the scientific basis to employing tincture for most local herbal preparations. The therapeutic usage of *Moringa*

*oleifera* leaf however predominates over other plant parts (e.g. roots, barks, pods and fruits) in this part of the world in herbal healthcare predisposing it to possible exploitation for pharmacopeia. It was observed from this study that its seeds had phytochemical efflux with low antimicrobial activity relative to the other plants screened. While *Chrysophyllum albidum* was the least ethnobotanically popular of the plant studied (Adewoye *et al.*, 2011; Idu *et al.*, 2007; Okoli & Okere, 2010), its fruit extracts proved to have antimicrobial activity that is comparable with the fruits of kola nuts and the alligator pepper. Qualitative assays for flavonoids, terpenoids, phenolics and saponin in all the extracts showed the prevalence of phenolics and inconsistent distribution of the other phytochemicals. Consequently, this by no means accounted for the total phytochemical representations of the plant extracts studied due to non-detection of some unsolvated ones but

proved that they have the potential for use as safe, cheap and alternative sources of antimicrobials, and other pharmaceuticals (Abdul *et al.*, 2010; Abu-Shanab *et al.*, 2004).

### Data availability

Complete raw data containing the zones of inhibition for each extract are available on OSF. DOI: <https://doi.org/10.17605/OSF.IO/5APVE> (Nwonuma, 2019).

Data are available under the terms of the [Creative Commons Zero “No rights reserved” data waiver](#) (CC0 1.0 Public domain dedication).

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### Patchima Sithisarn

Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand

The concept of the work is very interesting about the antimicrobial activities of medicinal plants in Africa. Though there are some crucial points needed to be clarified scientifically.

1. In the title, declaration of 6 plants, though there are 5 plants. It is needed to clarify that they are different preparations of extracts not different species.
2. It is crucial scientifically to describe the methods e.g. quantification of *A. fumigatus* and its experimental standardization as it is fungi. How the fungi grows and be inhibited by extracts in agar which should be different from other organisms which are bacteria that should not be measure in exact same way.
3. Crucial statistical analysis and validity of the data is required to compare antimicrobial activities of the plants to antibiotic of choices.
4. Phytochemical analysis in the research is qualitative. It may not be used as a referable or validate results in a quality scientific paper.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Partly

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

I cannot comment. A qualified statistician is required.

**Are all the source data underlying the results available to ensure full reproducibility?**

No

**Are the conclusions drawn adequately supported by the results?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Antimicrobial, antiviral targeting in pathogenic zoonotic organisms from phytochemicals.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.**

Reviewer Report 19 March 2019

<https://doi.org/10.5256/f1000research.18691.r43814>

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**Bhim Pratap Singh** 

Department of Biotechnology, Mizoram University, Aizawl, Mizoram, India

The work done by Nwonuma et al. is a interesting work but lacking proper execution. I felt that authors should have done the assays at-least till quantitative analysis.

1. Major issue is the use of strain ATCC 21784, which shows as *Rhodococcus* sp. (21784) at ATCC site ([https://www.google.com/search?q=ATCC+21784&rlz=1C1RUCY\\_enIN705IN705&oq=ATCC+217](https://www.google.com/search?q=ATCC+21784&rlz=1C1RUCY_enIN705IN705&oq=ATCC+217))
2. Were *Aspergillus fumigatus* using, bad language, language of the manuscript needs revision.
3. *Aspergillus fumigatus*, accession number?

I would suggest a serious revision of the manuscript before accepting for indexing.

**Is the work clearly and accurately presented and does it cite the current literature?**

No

**Is the study design appropriate and is the work technically sound?**

No

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Partly

**Are the conclusions drawn adequately supported by the results?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Microbial secondary metabolites, traditional medicinal plants, DNA fingerprinting

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Reviewer Report 04 February 2019

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**Armen Trchounian** 

Research Institute of Biology, Faculty of Biology, Department of Biochemistry, Microbiology and Biotechnology, Yerevan State University, Yerevan, Armenia

The manuscript is of partial interest, the study is too preliminary and poor and should not be considered for indexing in this journal.

Comments:

1. Abstract: Results are not actually summarized. Conclusions can be altered for the reflection of all content of the article. The presented conclusions are incomprehensible.
2. A voucher specimen must be deposited in a recognized herbarium (collection) in case of plants, or otherwise an appropriate chemical fingerprint is required for future reference.
3. There are no proper negative controls. Particularly sterile distilled water and ethanol should be used as negative controls at the concentrations they present in the final test solution. Taking into account that ethanol has antiseptic properties how the authors can distinguish detected antimicrobial activity as result of ethanol or tested phytochemicals action.
4. In figure 1 there are six columns regarding the inhibition zones of positive control chloramphenicol differing each other against the same bacterial strains. This is not clear: why?
5. Most good journals do not accept agar diffusion studies to determine the antimicrobial activity of plants. Many factors influence the agar diffusion assay for plant extracts and results between different laboratories cannot be compared. Agar diffusion assays may work well for single chemical compounds but not for plant extracts containing compounds with different polarities. Non-polar compounds do not diffuse well into the aqueous agar matrix and this underestimates activity. MIC using serial dilution delivers reproducible results to compare results in different laboratories and only extracts with MICs less than 0.1 mg/ml are considered, as interesting ones. Using crude extracts of plant materials with concentrations above 1000 µg/ml in antimicrobial screening protocols should be avoided, because using high concentrations of plant crude extracts can bring to false positive results (Rios and Recio, 2005). During the current study authors used 400 mg/ml concentration of the extracts in antimicrobial tests, which is too high. The concentrations of positive controls are also too high (5 mg/ml)

6. In the article, it is stated that bacterial test strains (*Pseudomonas aeruginosa* (ATCC27856) and *Proteus mirabilis* (ATCC21784)) were isolated from immune-compromised patients of the University Health Centre and available in Microbial Bank of the Microbiology Laboratory, Landmark University. But the ATCC reference numbers were given to them. Are they available in ATCC's microorganism collection?
7. The discussion must be completely rearranged.
8. Language is poor.

**Is the work clearly and accurately presented and does it cite the current literature?**

No

**Is the study design appropriate and is the work technically sound?**

No

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Partly

**Are the conclusions drawn adequately supported by the results?**

No

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Microbiology, Microbial Biotechnology, Plant Biotechnology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.**

Author Response 04 Feb 2019

**Osarenkhoe Osemwegie**, Landmark University, Omuara, Nigeria

We sincerely thank you for the thorough analytics and comments raised. We have already initiated efforts at addressing the issues of comment and wish to respond to some of the issues we disagreed in your reviewer's comment.

1. *There are no proper negative controls. Particularly sterile distilled water and ethanol should be used as negative controls at the concentrations they present in the final test solution. Taking into account that ethanol has antiseptic properties how the authors can distinguish detected antimicrobial activity as result of ethanol or tested phytochemicals action.*

The method adopted only used ethanol as an extractant of bioactive phytochemicals of the test medicinal plants and not meant for solubilizing the crude for antimicrobial assay. We regret such error and appreciate your pointing it out. Similarly, the extracts were only suspended as crude in distilled water. Therefore, the antiseptic reference of ethanol is not appropriate.

1. In figure 1 there are six columns regarding the inhibition zones of positive control chloramphenicol differing each other against the same bacterial strains. This is not clear: why?

The method only affords **one positive and one negative control per treatment**. No inhibition was observed and recorded (suppressed column) for the negative control treatments hence the number of columns presented. Differing reaction by the same strain of bacterium to in vitro antibiotic treatment is a possibility possible linked to undetectable slow differing changes in the local micro-environmental conditions.

1. *Most good journals do not accept agar diffusion studies to determine the antimicrobial activity of plants. Many factors influence the agar diffusion assay for plant extracts and results between different laboratories cannot be compared. Agar diffusion assays may work well for single chemical compounds but not for plant extracts containing compounds with different polarities. Non-polar compounds do not diffuse well into the aqueous agar matrix and this underestimates activity. MIC using serial dilution delivers reproducible results to compare results in different laboratories and only extracts with MICs less than 0.1 mg/ml are considered, as interesting ones. Using crude extracts of plant materials with concentrations above 1000 µg/ml in antimicrobial screening protocols should be avoided, because using high concentrations of plant crude extracts can bring to false positive results (Rios and Recio, 2005). During the current study authors used 400 mg/ml concentration of the extracts in antimicrobial tests, which is too high. The concentrations of positive controls are also too high (5 mg/ml). One 2019 (Arunkumar et al., 2019) reference has been used to reflect currency and spread of literature review. On the technical dept. of the methods, the preference for disc technique in susceptibility test expressed by the version 1 report is scientifically debatable and appreciated. Well diffusion method is also listed among the approved routine antimicrobial susceptibility testing methods in clinical laboratory practices. Selection of method is influenced by a range of factors that may include the nature of culture media, test material and target organism. Literature as recent as 2019 also exists that have employed this method (Mounyr et al., 2016 – Journal of Pharmaceutical Analysis; Arunkumar et al., 2019 – Molecules). Ethanol used on crude was 0.1% while the concentration of crude extract is 200mg/ml instead of 400mg/ml. The three (3) reviewers called fungal spore suspension concentration ( $10^5$  spores/ml) which is provided in the Assessment of antimicrobial activity).*

We partially concur with you that well diffusion method is becoming antiquated but its ineffectiveness in investigating the antimicrobial property of plant extracts remains debatable and it is still receiving acceptance in reputable journal outlets (Balouri et al., 2016 – <http://dx.doi.org/10.1016/j.jpha.2015.11.005>). We wish for you to know that the first intent of the study was to prove that all the test plants have antimicrobial activity. We refer you to the last paragraph of the discussion which clearly mentioned that the work is preliminary. The concentration used was set based on the outcome of ethnobotanical survey of the location of study and it still generated the expected result despite disagreeing with Rios and Recio, 2005. All other comments on the voucher number for the test plants, reconstruction of the abstract and the discussion, expressive narration of the results will be addressed. While we do not understand what you mean by the language is poor, we wish to appeal for your guidance in the use of the right language hence we would be glad you return to use the edited version of the article. Thank you.

**Competing Interests:** No competing interests were disclosed.

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