Bioefficacy of Turmeric Rhizome Extracts with Alum on Microbes: An in Vitro Approach

Lawrence O. Amadi*, Joy S. Ekechi, Seth M. Akporutu

Department of Microbiology, Faculty of Science, Rivers State University, P.M.B 5080, Nkpolu-Oroworukwo, Port Harcourt, Nigeria

*Corresponding author: lawrenceamadi@gmail.com

Received January 11, 2019; Revised March 10, 2019; Accepted April 07, 2019

Abstract Bioefficacy of crude Turmeric rhizome extracts and in combination with alum against four (4) bacterial and five (5) fungal species were determined using disc diffusion (DD) and agar well diffusion (AWD) methods respectively. The extracts with or without Alum were active against all the test microbes in dose-dependent manner by inhibiting their growth. The highest diameter of inhibition zone (DIZ) was observed with ethanolic turmeric extract (ETE) and alum (ETE+Alum) at 0.3g concentration on Gram negative bacteria; Escherichia coli (16.6±0.8mm) and Pseudomonas fluorescens (15.3±1.1mm) and Gram positive bacterium; Staphylococcus aureus (15.0±0.0mm) whereas strong antimycotic activity occurred with Alum in the order Aspergillus terreus (17.5±1.0mm) > A. flavus (17±1.0mm) > S. cerevisiae (14mm) > C. albicans (12±1.0mm) by DD respectively. Using AWD bioassay, alum exhibited the best activity against Bacillus cereus (17.8±1.0mm), S. aureus (16.0 ± 0.7mm) and 14.0mm on P. fluorescens and E. coli whereas ETE+Alum demonstrated highest antimycotic activity on A. terreus (35±1.0mm) > Penicillium crystallium and A. flavus (33.0mm) > S. cerevisiae (24.0mm). Furthermore, the demonstration of apparent antimicrobial activity on both Gram positive and Gram negative bacteria as well as against moulds and yeasts by extracts of Turmeric rhizome’ and Alum is suggestive of broad spectrum activity. In contrast, however, the high activity of Ofloxacin (OFL) and Ketoconazole (KTA) against test microbes highlights their superiority to the extracts with or without alum. However, enhancement of bioefficacy of Turmeric rhizome extracts was achieved by incorporation of Alum and such novel approaches to research with safe, natural products would provide an alternative to antibiotic and antifungal treatment of diseases of plants, animals and humans in future.

Keywords: alum, bioefficacy, microbes, antimycotic activity, turmeric extract


1. Introduction

Curcuma longa, a rhizomatous herb belongs to the family Zingiberacaea which includes more than 80 species is a native of Southeast Asia but extensively cultivated in tropical and subtropical countries of the world [1-5]. Turmeric obtained from C. longa comprise of three (3) curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), sugars, proteins, volatile oils (natlantone, turmerone and zingiberone), a-phellandrene, sabinen, cineol, borneol, sesquiterpenes, minerals and resins [6,7,8,9].

Turmeric and its derivatives have been reported to possess evidence-based broad spectrum activity as an important nutraceutical as well as in human health. It has been effectively used as antimicrobial, anticancer, anti-diabetic, anti-inflammatory, anti-ulcerogenic, antimalarial, antioxidant, insect repellent, antiallergic, wound healing, plays anti-aging role, modulates several crucial pathways against life-threatening diseases as well as in foods as colorant, flavourant, curry spice, preservative and additive, together with its low toxicity (doses of 12 g/day, safe to humans) [9-21].

Curcumin suppresses the activity of many bacteria and fungi such as Staphylococcus aureus, Salmonella paratyphi, Bacillus cereus, B. subtilis, B. macerans, B. licheniformis, Azotobacter, Helicobacter pylori [19,22,23,24,25] and phytopathogenic fungi; Phytophthora infestans, Botrytis cinerea, Rhizoctonia solani, Puccinia recondita, phytophagous fungi; Fusarium solani, Helminthosporum oryzae, dermatophytes; Trichophyton rubrum, T. mentagrophytes, Epidermophyton floccosum and Microsporum gypseum, others such as Cryptococcus neoformans, Sporothrix schenckii, Fonsecaea pedrosoi, Exophiala jeaneselmei, and against Candida including some Fluconazole resistant strains and clinical isolates of C. albicans, C. glabrata, C. krusei, C. tropicalis, etc., respectively [3,9,18,26,27,28,29].

Multi-drug bacterial resistance has become a major health concern and such phenomena have been partly attributed to abuse such as overdose and even imbalance of strains in gut microbiota, etc. [30,31,32]. This
development necessitates the studies for synergistic effects of antibiotics or nanoformulations in combination with Alum or plant’s derivatives to produce antimicrobial cocktail with a broader spectrum of activity and reduction of adverse side effects of antimicrobials [16,33,34].

Alum has been used for years for a variety of purposes such as drug (in Ayurveda and unani medicinal systems), food processing and preservation, domestic and industrial water treatments, antibacterial agent, cosmetics and pharmaceutical industries [35-39].

Synergistic enhancement of antibacterial activity of curcumin with various antimicrobial drugs have been reported with promising outcomes [5,40,41,42] but assays using Alum, a potent antimicrobial has never been performed. Therefore, this study investigates the bioefficacy of crude Turmeric rhizome (Curcuma longa) extracts with Alum on some bacterial and fungal species.

2. Materials and methods

2.1. Preparation of Turmeric Rhizome Sample and Extraction Procedure

Fresh Turmeric rhizomes, Curcuma longa were purchased from Ultra Modern, Nkpolu-Oworukwo, Market, Mile 3, Port Harcourt, Rivers State. The samples were washed, sliced and dried in hot air oven at 70°C for 72h, then pulverized with a mechanical grinder [43]. Twenty (20.0) grammes of the powder was weighed and extracted with 100ml sterile distilled water and 100ml 98% ethanol respectively. Then soaked in respective solvents for 24h and filtered with Whatman No. 1 filter paper. The filtrates were concentrated by evaporation in water bath at 60°C for 12-15h and 24h for ethanolic and aqueous extracts respectively. The resultant filtrates were reconstituted in 70% ethanol and sterile distilled water to obtain final concentrations of ethanolic Turmeric extract (ETE) and aqueous Turmeric extract (ATE) as 100, 200 and 300mg/ml (w/v) and stored at 4°C for further experiment.

2.2. Preparation of Combination of Turmeric Rhizome Extracts with Alum

Potassium aluminium sulphate (Vickers Laboratories, Ltd, England) was prepared by reconstituting appropriate quantity in sterile distilled water (w/v). Different concentrations of extracts (ETE and ATE) were used in combination with alum at a ratio of 1:1 to make final concentrations of 100, 200 and 300mg/ml respectively.

2.3. Test Bacteria

The test microorganisms were obtained from the stock cultures of the Department of Microbiology, Rivers State University, Nkpolu-Oworukwo, Port Harcourt. They include four bacteria and five fungi. Staphylococcus aureus, Escherichia coli and Bacillus cereus were clinical isolates and Pseudomonas fluorescens obtained from the soil. Cultures of each bacterium was subcultured on nutrient agar (NA; Titan Biotech Ltd, India) plates, confirmed for viability and subsequently held on nutrient agar slants and kept at 4±1°C prior to susceptibility testing.

2.4. Fungal Species

Five fungal species were used for antymycotic activity. They are; Saccharomyces cerevisiae and Candida albicans (yeasts) obtained from palmwine and vaginal swab respectively; Aspergillus flavus, A. terreus and Penicillium crystallium (moulds) obtained from bread and soil respectively. These fungi were held in the stock cultures of the Department of Microbiology. Cultures of each fungus was further grown for viability, confirmed and maintained on Sabouraud’s dextrose agar (SDA; Titan Biotech Ltd, India) and slants at 5°C until further use.

2.5. Antimicrobial Activity Assay

In vitro susceptibility test was determined by paper discs and agar well diffusion methods [44]. [42] Antibacterial activity was performed on the ethanolic and aqueous extracts of the rhizome of Curcuma longa with and without alum. Activity was performed against four (4) bacterial species; Bacillus cereus, Staphylococcus aureus, Escherichia coli and Pseudomonas fluorescens. Sterile discs (6mm diameter) were made of Whatman filter paper as described by Ochei and Kolhatkar, [45] and impregnated with about 5-10µl of different concentration of test extracts and Alum (100mg/ml, 200mg/ml and 300mg/ml [46] and air dried to eliminate residual solvents and placed on surface-dried inoculated medium (Mueller Hinton agar (MHA) Titan Biotech., Ltd, Bhiwadi-301019, Rajasthan, India) and with 10µg Ofloxacin (OFL; Abtek Biologicals Ltd., UK) commercially prepared antibiotic discs as positive control. In contrast, AWD method was determined using MHA plates with wells equidistant from one another and previously spread-plated with 24h old culture of test bacteria (after adjustment to 0.5 McFarland turbidity standard) and with 20mg/ml OFL as control. All the plates were incubated at 37°C for 24-48h. The zone of inhibition was measured and their mean diameters were recorded [47,48].

Similarly, antymycotic activity of extracts of Turmeric, Alum and their combinations were evaluated on SDA. Fungal cultures of 1-4d were aseptically plated and equidistantly spaced from one another and incubated for varying times depending on growth rate of each organism. This was as briefly as 1d (24h) for fast growing species, e.g., yeasts, followed by moulds. An antymycotic drug, Ketoconazole (20mg/ml; Janseen, Pharmaceuticals, New Jersey, USA) was used as control both for DD and AWD methods respectively. Colony diameters were measured and the mean and standard deviation determined.

2.6. Statistical Analysis

Means of duplicate measurements and standard deviations (SD) were determined for each sample using Microsoft Excel® 2016.
3. Results

Inhibitory activity of the extract and Alum on test bacteria occurred in a dose related fashion respectively (Table 1). Comparatively, better results were achieved with ethanolic extract but Alum depicted highest activity on *E. coli*. However, the control, Ofloxacin was the most effective agent (Table 1).

Table 2. Depicts ethanolic extract with Alum (Et + Alum) as the most potent antibacterial agent on *E coli*, *P. fluorescens* and *S. aureus* respectively. In contrast, aqueous extract and its combination with alum exhibited weak activity independent of dosage.

Better inhibitory effect was observed with Alum alone, with activity being more prominent against *B. cereus* and *S. aureus* and dose dependent whereas Ofloxacin (control) exhibited the best and very strong activity on all the test bacterial species (Table 3).

Ethanolic extract with alum (Et + Alum) depicted better activity on test bacteria than aqueous extract and its combination (Table 4). Comparatively, the results indicate that ethanolic extract with Alum was much more beneficial with respect to bioefficacy (Table 2 and Table 4).

Table 1. Inhibitory activity of extract and Alum at different concentrations by disc diffusion method

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Diameter of inhibition zone (DIZ) in mm ± SD</th>
<th>Aqueous (mg/ml)</th>
<th>Aq + Alum (mg/ml)</th>
<th>Et + Alum (mg/ml)</th>
<th>Ofloxacin (OFL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>6.0</td>
<td>6.0</td>
<td>6.5±0.7</td>
<td>7.3±0.3</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td></td>
<td>8.0±0.7</td>
<td>10.4</td>
<td>12.4±0.1</td>
<td>6.9±0.1</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Legend: Aq = Aqueous; Et = Ethanolic. Each of the number represents the SD after two experiments.

Table 2. Inhibitory activity of extracts and Alum at different concentrations by disc diffusion method

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Diameter of inhibition zone (DIZ) in mm ± SD</th>
<th>Aqueous (mg/ml)</th>
<th>Aq + Alum (mg/ml)</th>
<th>Et + Alum (mg/ml)</th>
<th>Ofloxacin (OFL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>6.0</td>
<td>6.0</td>
<td>6.5±0.7</td>
<td>6.0</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td></td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>6.0</td>
<td>7.2</td>
<td>7.3±0.3</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Legend: Aq = Aqueous; Et = Ethanolic. Each of the number represents the SD after two experiments.

Table 3. Inhibitory activity of extract and Alum at different concentrations by agar well method

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Diameter of inhibition zone (DIZ) in mm ± SD</th>
<th>Aqueous (mg/ml)</th>
<th>Aq + Alum (mg/ml)</th>
<th>Et + Alum (mg/ml)</th>
<th>Ofloxacin (OFL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>10.3±0.3</td>
<td>11.5±0.3</td>
<td>14.0±0.7</td>
<td>6.0±0.0</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td></td>
<td>9.8±0.3</td>
<td>12.0</td>
<td>14.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>10.0</td>
<td>11.8±0.2</td>
<td>17.8±1.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Each of the number represents the SD after two experiments.

Table 4. Inhibitory activity of extracts and Alum at different concentrations by agar well method

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Diameter of inhibition zone (DIZ) in mm ± SD</th>
<th>Aqueous (mg/ml)</th>
<th>Aq + Alum (mg/ml)</th>
<th>Et + Alum (mg/ml)</th>
<th>Ofloxacin (OFL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td></td>
<td>100</td>
<td>200</td>
<td>300</td>
<td>100</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>B. cereus</em></td>
<td></td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Legend: Aq = Aqueous; Et = Ethanolic. Each of the number represents the SD after two experiments.

The strongest inhibitory efficacy as shown by high DIZ values occurred with ketoconazole (KTA, control), followed by Alum at 0.3g concentration. The entire result can be represented in this order KTA > Alum > ETE + Alum > ETE whereas ATE and ATE + Alum are ineffective (Figure 1). Inhibitory of Alum positively influenced all the species but much more on *A. flavus*, *A. terreus*, *S. cerevisiae* and *C. albicans*. Similar trend was observed with ETE+Alum and ETE on these species at lower concentration but incomparable to Alum at 0.3g. However, the potency of these antymycotics on test fungal species was dose dependent and phenomenal.

The high DIZ values of the antymycotics on test fungi generally exhibited apparent enhancement of activity by agar well diffusion (AWD) method with KTA being the most effective (Figure 2). Similar trend of inhibitory activity on the various species occurred with ETE+Alum, ETE and Alum being more effective. ATE+Alum and ATE showed relatively weak effect. *A. terreus*, *A. flavus* and *P. crystallium* were more susceptible to ETE+Alum and ETE whereas Alum and ATE+Alum depicted stronger activity on *A. flavus* and *C. albicans*. 

The high DIZ values of the antymycotics on test fungal species can be represented in this order KTA > Alum > ETE + Alum > ETE whereas ATE and ATE + Alum are ineffective (Figure 1). Inhibitory of Alum positively influenced all the species but much more on *A. flavus*, *A. terreus*, *S. cerevisiae* and *C. albicans*. Similar trend was observed with ETE+Alum and ETE on these species at lower concentration but incomparable to Alum at 0.3g. However, the potency of these antymycotics on test fungal species was dose dependent and phenomenal.
4. Discussion

Phytochemicals extracted from different natural sources have been tested successfully in vitro and in vivo for their antimicrobial activity and safety. Curcumin and Turmeric oil which are chemical components of Turmeric rhizome and their derivatives have been reported to have both antibacterial and antifungal inhibitory effects [3,9,18,29,49]. This study revealed that crude Turmeric rhizome extracts, Alum and their combinations/cocktails were inhibitory to the test microorganisms (bacteria and fungi) respectively. Susceptibility of these microbial species occurred in a dose dependent manner and correlates with previous reports [3,13,14,50]. The inhibitory effect on Gram negative and Gram positive bacterial species as well as on yeasts and moulds is suggestive of broad spectrum antimicrobial activity. This corroborates several reports of different solvent extracts of Turmeric on different bacteria including B. cereus, E. coli, S. aureus, Pseudomonas species, etc. [4,19,29] of which the first three species are test bacteria but reports on P. fluorescens is scarce.

Ethanolic extract of Turmeric with Alum was more efficacious and presents captivating targets for future research and development. Several reports revealed that Turmeric activity on bacterial isolates may be associated with polyphenols, curcumin or Turmeric oil [18,19,51,52,53,54]. However, the precise mechanism or target of most plant bioactive components against bacteria, in general, is not yet elucidated but several mechanisms have been suggested to be involved, viz; the disruption of pathogen membranes, interruption of DNA/RNA synthesis and function, interference with intermediary metabolism, induction of coagulation of cytoplasmic constituents, and the interruption of normal cell communication (quorum sensing, QS) [55,56,57,58].

In addition, It has been also proposed that, in the case of phenolic compounds, which are commonly present in aqueous plant extracts, antimicrobial activity may be also related with their capacity to chelate iron [59] which is necessary for almost all bacteria survival, for which an increased growth and virulence with iron availability has been reported [60]. Despite the differences in cellular
system between bacteria and fungi, one or more of these phyto-components have also been linked to antifungal activity against C. albicans due to their adsorption potential or other mechanisms not properly understood, thus preventing their growth [9,42,61,62] which may account for the present results. In contrast, Alum’s activity has been attributed to low acidity or deleterious effects on bacterial/fungal cell wall which may also be responsible for the present results. The combination (of Turmeric extracts and Alum) impacted more on Gram negative bacteria than on Gram positive probably due to variation in activities among bacteria which may reflect differences in cell wall structures and composition. This may have positively influenced fungi as well (Figure 1 and Figure 2). Synergistic/additive effect of Turmeric rhizome extracts with Alum resulted in enhanced activity against

5. Conclusion

Crude Turmeric rhizome extracts, Alum and their combinations were inhibitory against test bacterial and fungal species and depicted broad spectrum activity. The addition of Alum enhanced the bioefficacy of Turmeric extracts and such novel approaches to research with safe, natural product would provide an alternative to antibiotic and antifungal therapy.

Conflict of Interests

The authors declare they have no conflict of interests.

References


