# Evaluation of Antimicrobial Activities of the Ethanolic Extracts of Leaf of *Senna alata* and Bark of *Piliostigma thonningii* and the effect of their combination against Skin Infections.

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**Abstract**

The skin is the largest, multi-layered organ with a protective function. However, a breach as a result of damage to the epidermis causes micro-organisms to penetrate and cause infections. This study evaluated the antimicrobial activities of the extracts of leaf of *Senna alata* and bark of *Piliostigma thonningii* as well as possible effect of the extracts combination in varying ratios. Ethanolic extracts of leaf of *S. alata* and bark of *P. thonningii* were evaluated for antimicrobial activities against selected Gram-positive - *Staphylococcus aureus,* Gram-negative - *Escherichia coli*, *Pseudomonas aeruginosa Citrobacter freundii*, *Yersinia enterocolitica*; and fungal strain - *Candida albican* using agar well diffusion method at 100, 200 and 300 mg/mL and in combination at 75:25, 25:75 and 50:50 ratios against selected microorganisms. Gentamicin and Nystatin were used as positive controls. Triplicate zones of inhibition were measured after 24 and 72 hours for bacterial and fungal isolates respectively. The extracts of *S. alata* and *P. thonningii* had means of zones of inhibition ranging from 24.00±1.06 to 13.00±0.00 and 30.00±0.43 to 15.00±1.00 against *S. aureus* and 21.50±0.25 to 18.00±0.00 and 19.00±0.43 to 17.00±0.81 against *E. coli* respectively. Only the extract of *S. alata* showed antifungal activity with mean of zones of inhibition ranging from 30.00±0.53 to 12.00±0.82 against *C. albicans*. The synergistic activity *S. alata* and *P. thonningii* at ratio 50:50 produced the highest activity against *S. aureus* and *E. coli*. Considering these antimicrobial activities observed, the two extracts have shown interesting potentials in the treatment of skin infections.

**Keywords:** *S. alata*, *P. thonningii*, Antimicrobial activities, Skin infections

## Introduction

Skin is the outer layer of the body. It is the largest organ of the integument in humans, made up of multiple layers of mesodermal tissues and guards the underlying muscles, bones, ligaments as well as internal organs[1]. Among other functions, the skin plays a crucial role in protecting the body against invading pathogens but when there is a breach in its lines of defense, harmful microorganisms such as bacteria, fungi and viruses adhere or gain entry and may cause skin infections that may threaten or eliminate life[2]. The prevalence of skin infections is recently of public health concern affecting the quality of life, morbidity and is directly responsible for nearly half of all deaths in many developing countries[3].

Skin infections accounts as the fourth most frequent cause of all human disease, affecting about 1.9 billion people among all age group at a particular time especially in places of poor level of hygiene and the leading reason for seeking medical intervention globally[4]. Antibiotics are generally employed in the treatment of skin infections either administered topically or orally but due to the development of resistance as a result of selective pressure other alternative treatment sources were sought. World Health Organization in 2017 suggested the use of medicinal plants as alternative sources for potential therapeutic agents due to the presence of phytochemicals such as tannins, alkaloids, phenolic compounds, and flavonoids which exhibits antimicrobial activities. Traditionally, leaf of *Senna alata* and bark of *Piliostigma thonningii* are employed in the treatment of several infections such as typhoid, diabetes, malaria, asthma, ringworms, tinea infections, scabies, eczema and Leprosy[5, 6]. Therefore, this study seek to evaluate the antimicrobial activities of the extracts of leaf of *Senna alata* and bark of *Piliostigma thonningii* as well as possible effect of the extracts combination in varying ratios against different microorganisms.

## Materials and Methods

### Collection and Treatment of Plant Materials

Leaf of *S. alata* and bark of *P. thonningii* were collected from a location within Ilorin, Kwara State. The leaf and the bark were authenticated at the herbarium of the Department of Plant Biology, University of Ilorin where voucher numbers of UILH/001/947 and UILH/03/1288 were issued for *S. alata* and *P. thonningii* respectively. Plants materials were oven-dried at 40 $^{o}$C milled into powder and kept in a dry glass jars for further analysis.

### Extraction of Plants

As described by Mishra *et al.*, in 2009, 250 g each of the powdered leaf of *S. alata* and bark of *P. thonningii* was macerated separately in 70 % ethanol and allowed to stand for 48 hours. Each mixture was decanted and more solvent added. This process was repeated two times and the final and the suspensions were filtered, concentrated in *vacuo*, resultant extracts were kept in a dry glass jars and refrigerated at 4˚C until needed for analysis.

### Characterization of Extracts

The extracts of *S. alata* and *P. thonningii* were examined for physical characteristics, which include: colour, odour and texture. Also, the pHs of the extracts were determined.

### Collection of Isolates

Cultures of *Staphylococcus aureus* (five different strains), *Pseudomonas aeroginosa*, *Escherichia coli*, *Citrobacter freundii*, *Yersinia enterocolitica* and *Candida albicans* were collected from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Ilorin. Bacterial and fungal Isolates were sub-cultured on Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) (Oxoid, Basingstoke –UK) and incubated aerobically at 37 $^{o}$C and 25 $^{o}$C for 24 and 72 hours respectively. The stock cultures were maintained on NA at 4°C until required.

### Medium Preparation

For the bacteria and fungi culture, NA and SDA were prepared by dissolving 14 g and 27.5 g of the dry agar in 500 mL of distilled water and slightly heated while mixing for

complete dissolution on a burner. The prepared agar solutions were sterilized by autoclaving at 121 $^{o}$C for 15 minutes. On cooling, the sterile media was then poured into sterile petri dishes and then allowed to solidify in the plates.

**Standardization of Innocula**

The stored organisms were stabilized under room temperature. Aseptically, using a sterile inoculating loop, each of the organisms was transferred into a tube containing sterile broth solution and adequately mixed afterwards. All tubes were incubated for 24 hours at 37 $^{o}$C after which they were standardized to 0.5 MacFarland turbidity standards to yield a final organism density of $10^8$ and $10^6$ cfu/ mL for bacteria and fungi culture respectively.

**Antimicrobial Activity of Extracts**

The antimicrobial activity of ethanolic plant extracts was screened against selected microorganisms by using the agar well diffusion method[7]. Standardized innocula were seeded into prepared Mueller Hinton agar (MHA) (Oxoid, Basingstoke - UK) for bacteria and Sabouraud dextrose agar (SDA) for the fungus. Using sterile cork borer No 6 (6 mm in diameter), wells were made in the seeded agar plates. A 100 µl of prepared plant crude extract of 100, 200 and 300 mg/mL were added into each well and allowed to pre- diffused for 45 minutes and incubated at 37 °C for 24 hours for bacteria and at 25 °C for 24 hours for the fungus *C. albicans.* The degree antimicrobial activity were evaluated by measuring the zones of inhibition diameter in millimeters (mm) around the wells. Gentamicin (10 µg/ disc) and Nystatin (335 mg/mL) were used as positive controls. All assays were performed in triplicate and results presented as mean ± standard deviation.

Furthermore, *S. aureus* and *E. coli* showing zones of inhibition ≥ 15mm were considered for the extracts combination antimicrobial assay using the ager well diffusion method. Both extracts were combined in three different ratios of 75:25, 25:75 and 50:50 respectively using a concentration with widest antibacterial activity

**Results**

The ethanolic extract of *S. alata* was dark green in colour, sticky to touch and had an astringent odour. The ethanolic extract of *P. thonningii* was deep brown in colour, rough to touch, and had a bark-like smell. The pHs of the two extracts were 5.0 and 5.3 for the *S. alata* and *P. thonningii* respectively, indicating that they were less acidic.

The two extracts exhibited varying degrees of activities against the selected bacterial and fungus pathogens that are primarily known to cause dermal infections as well as other human infections. The antimicrobial activities of ethanolic extracts of both *S. alata* and *P. thonningii* compared favorably with positive controls used (Gentamicin and Nystatin) and inhibitions were concentration dependent among the Gram positive bacteria but differ among the Gram negative bacteria which exhibited slightly lower inhibitions as the concentration increases. *Ps. aeruginosa* was not inhibited by extract of *P. thonningii.*

Table 1: Means of zones of inhibition of different concentrations of *S. alata* extract and gentamycin against five strains of *S. aureus*

| | Zones of Inhibition (mm) | | | | |
|---|---|---|---|---|---|
| **Samples** | **Strain 1** | **Strain 2** | **Strain 3** | **Strain 4** | **Strain 5** |
| S100mg/mL | 13.33 ± 0.53 | 15.67 ± 1.11 | 20.33 ± 0.74 | 13.00 ± 0.00 | 18.00 ± 0.53 |
| S200mg/mL | 14.00 ± 1.70 | 18.67 ± 1.06 | 23.33 ± 0.42 | 17.00 ± 1.14 | 19.43 ± 0.47 |
| S300mg/mL | 15.33 ± 1.12 | 20.00 ± 0.82 | 24.00 ± 1.06 | 18.50 ± 0.25 | 22.33 ± 0.82 |
| Gentamycin | 25.00± 1.06 | 22.00± 0.74 | 22.00± 0.53 | 25.00± 0.42 | 21.00± 0.53 |

**Mean ±SD, n = 3;**

S100 = 100 mg/mL concentration of *S. alata* extract; S200 = 200 mg/mL concentration of *S. alata* extract; S300 = 300 mg/mL concentration of *S. alata* extract

Table 1 and 2 shows the means of zones of inhibition of different concentrations of *S. alata* and *P. thonningii* extracts against gentamycin using five strains of *S. aureus* (Gram positive). Both extracts were generally active against the five different strains of *S. aureus* with 300mg/mL exhibiting the highest mean zones of inhibition of 24.00 ± 1.06 mm with strain 3 and 30.00± 0.43 mm with strain 4 while the least were at 100mg/mL for strain 4 (13.00 ± 0.00 mm) and strain 3 (15.00 ± 1.00 mm) for *S. alata* and *P. thonningii* extracts respectively.

Table 2: Means of zones of inhibition of different concentrations of *P. thonningii* extract and gentamycin against different strains of *Staphylococcus aureus*

| | Zones of Inhibition (mm) | | | | |
|---|---|---|---|---|---|
| **Samples** | **Strain 1** | **Strain 2** | **Strain 3** | **Strain 4** | **Strain 5** |
| P100mg/mL | 18.00 ± 1.24 | 19.67 ± 0.68 | 15.00 ± 1.00 | 22.00 ± 0.81 | 19.00 ± 0.26 |
| P200mg/mL | 20.50 ± 0.01 | 23.00 ± 0.43 | 18.67 ± 0.72 | 25.00 ± 0.25 | 23.00 ± 0.00 |
| P300mg/mL | 22.00± 0.81 | 25.00± 0.25 | 20.00± 0.68 | 30.00± 0.43 | 25.00± 0.72 |
| Gentamycin | 19.00± 0.68 | 22.00± 0.72 | 20.00± 0.25 | 22.00± 0.43 | 20.00± 0.00 |

**Mean ±SD, n = 3;**

P100 = 100 mg/mL concentration of *P. thonningii* extract; P200 = 200 mg/mL concentration of *P. thonningii* extract; P300 = 300 mg/mL concentration of *P. thonningii* extract.

Table 3: Means of zones of inhibition of different concentrations of *S. alata* extract and against selected Gram negative bacteria

| Samples | Zones of Inhibition (mm) | | | |
|---|---|---|---|---|
| | ***C. freundii*** | ***Ps. Aeruginosa*** | ***E. coli*** | ***Y. enterocolitica*** |
| S100mg/mL | 25.00 ± 0.82 | 25.00 ± 1.06 | 21.50 ± 0.25 | 35.33 ± 0.82 |
| S200mg/mL | 23.67 ± 1.06 | 23.33 ± 0.42 | 20.00 ± 1.14 | 32.43 ± 0.47 |
| S300mg/mL | 20.67 ± 1.11 | 22.33 ± 0.74 | 18.00 ± 0.00 | 30.00 ± 0.53 |
| Gentamycin | 20.00 ± 0.00 | 18.00 ± 0.00 | 22.00 ± 0.00 | 22.00 ± 0.00 |

**Mean ±SD, n = 3;**

S100 = 100 mg/mL concentration of *S. alata* extract; S200 = 200 mg/mL concentration of *S. alata* extract; S300 = 300 mg/mL concentration of *S. alata* extract

Table 4: Means of zones of inhibition of different concentrations of *P. thonningii* extract against Gram negative bacteria

| Samples | Zones of Inhibition (mm) | | | |
|---|---|---|---|---|
| | ***C. freundii*** | ***Ps. Aeruginosa*** | ***E. coli*** | ***Y. enterocolitica*** |
| P100mg/mL | 20.67 ± 0.68 | 0.00 | 17.00 ± 0.81 | 22.00 ± 0.26 |
| P200mg/mL | 20.00 ± 0.43 | 0.00 | 18.00 ± 0.25 | 22.00 ± 0.00 |
| P300mg/mL | 22.00± 0.81 | 0.00 | 19.00± 0.43 | 30.00± 0.81 |
| Gentamycin | 20.00± 0.87 | 0.00 | 22.00± 0.81 | 20.00± 0.43 |

**Mean ±SD, n = 3;**

P100 = 100 mg/mL concentration of *P. thonningii* extract; P200 = 200 mg/mL concentration of *P. thonningii* extract; P300 = 300 mg/mL concentration of *P. thonningii* extract

Table 3 and 4 shows the means of zones of inhibition of different concentrations of *S. alata* and *P. thonningii* extracts against selected Gram negative bacteria. These extracts generally exhibited significant level inhibition against all selected Gram negative bacteria with *Y. enterocolitica* showing the highest level of mean zones of inhibition 35.33 ± 0.82 mm and 22.00 ± 0.26 mm at 100mg /mL for *S. alata* and *P. thonningii* respectively. *Ps. aeruginosa* was not inhibited by *P. thonningii* at all concentrations.

Table 5 shows the means of zones of inhibition of different concentrations of *S. alata* extract and nystatin against five (5) different strains of *Candida albicans.* Strain 4 and 5 exhibited the highest mean zones of inhibition of 30.00 ± 0.53 mm and 30.00 ± 0.00 mm at 300 mg/mL respectively while *P. thonningii* extracts had no activity against *C. albicans* at all concentrations. Each extract at concentration of 300 mg/mL produced widest antibacterial activity and

hence, 300 mg/mL concentration selected for combination.

Table 5: Means of zones of Inhibition of different concentrations of *S. alata* extract and nystatin against different strains of *Candida albicans*

| **Samples** | **Zones of Inhibition (mm)** | | | | |
|---|---|---|---|---|---|
| | **Strain 1** | **Strain 2** | **Strain 3** | **Strain 4** | **Strain 5** |
| S100mg/mL | 12.00 ± 1.12 | 12.00 ± 0.82 | 15.00 ± 1.06 | 25.50 ± 0.25 | 20.33 ± 0.82 |
| S200mg/mL | 18.00 ± 1.70 | 15.67 ± 1.06 | 17.33 ± 0.42 | 28.00 ± 1.14 | 25.43 ± 0.47 |
| S300mg/mL | 20.33 ± 0.53 | 16.67 ± 1.11 | 18.33 ± 0.74 | 30.00 ± 0.00 | 30.00 ± 0.53 |
| Nystatin | 33.00± 0.82 | 35.00± 0.25 | 35.00± 1.12 | 35.00± 0.82 | 55.00± 0.42 |

**Mean ±SD, n = 3;**

S100 = 100 mg/mL concentration of *S. alata* extract; S200 = 200 mg/mL concentration of *S. alata* extract; S300 = 300 mg/mL concentration of *S. alata* extract.

Table 6: Means of zones of inhibition of different combination ratios of *S. alata and P. thonningii* against *S. aureus* and *E. coli.*

| **Organisms** | **Zones of inhibition of different samples (mm)** | | | | | |
|---|---|---|---|---|---|---|
| | **SP100:0** | **SP 0:100** | **SP 50:50** | **SP:25:75** | **SP 75:25** | **Gentamycin** |
| *S. aureus* | 24.00±1.12 | 25.00±0.86 | 25.00±1.06 | 22.00±0.25 | 20.00±0.82 | 20.00±0.25 |
| *E. coli* | 21.00±1.24 | 19.00±0.26 | 28.00±1.00 | 26.00±0.81 | 25.00±0.68 | 20.00±0.43 |

**Mean ±SD, n = 3;**

SP = Ratio of *S. alata* to *P. thonningii*

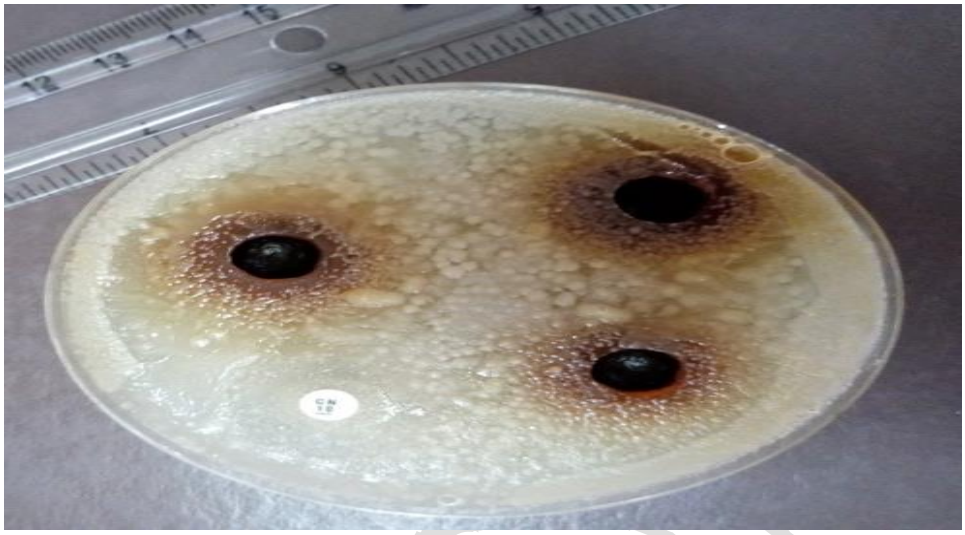

**Plate 1: Showing the combined inhibition of *S. alata and P. thonningii* against *S. aureus* at SP 50:50**

**Plate 2: Showing the combined inhibition of *S. alata and P. thonningii* against *E. coli* at SP 75:25**

Table 6 shows the means of zones of inhibition of different combination ratios of *S. alata and P. thonningii* against *S. aureus* and *E. coli.* At 50: 50 ratio of *S. alata and P. thonningii* extracts, the highest means zones of inhibition of 25.00±1.06 and 28.00±1.00 mm were observed against *S. aureus* and *E. coli* respectively.

**Discussion**

The increasing prevalence of skin infection globally due to inappropriate use of antibiotics, availability of immunosuppressed, compromised host as well as poor level of personal hygiene and environmental sanitation contributing significantly to the spread of resistant pathogens especially in developing countries[8]. Therefore, suggesting the need to identify novel sources of antimicrobial agents especially of plants origins which are capable of producing secondary metabolites with therapeutic values would be a great idea[9, 10].

This study was designed to evaluate the antimicrobial activity of extract of *S. alata* and *P. thonningii* against selected Gram positive (*S. aureus*), Gram negative (*E. coli. C. freundii, Y. enterocolitis and Ps. aeruginosa*) and different strains of *C. albicans* causing skin infection as well as other human infections. At an overall, extract of leaf of *S. alata* had significant level of inhibition against both bacteria and fungus while the extract of bark of *P. thonningii* exhibited a better antibacterial activity and had no activity against *C. albicans*. This can be attributed to the presence of secondary metabolites such as tannins, alkaloids, flavonoids, terpenes, anthraquinone, saponins, phenolics, quinones, reducing sugars, steroids, and volatile oils[6] and the presence of newly identified active phytochemical; 2β - methoxyclovan-9α-ol (1) and methyl-ent-3β-hydroxylabd-8(17)-en-15-oate (2) phytochemicals in *P. thonningii* as reported by Orishadipe *et al.,* 2018. This suggests its therapeutic value and supports their traditional claims in the treatment of skin infections[9].

In this study, leaf of *S. alata and* bark of *P. thonningii* exhibited activity against gram positive, negative and fungus which compares favorably with the standard antibiotics (Gentamicin and Nystatin). This indicates that the plants extracts have good prospect for production of drugs with wide spectrum of activity. Although, the Gram negative bacteria were more sensitive than the Gram positive bacteria and the *C. albicans*. This could be attributed to the variation in the make-up of their cell wall. This is similar to the findings of Akinjogunla *et al.,* (2009) and Obeidat, 2011.

The synergistic activity of *S. alata* and *P. thonningii* at ratio SP 50:50 had highest activity against *S. aureus* and *E. coli*. This suggests that the combination of the two extracts enhances a broader spectrum of activity with a different mode of action capable of combating resistance. This is similar to the report of Masoumian a nd Mohammad in 2017.

## Conclusion

Based on these findings, further study is required to investigate the potential to isolate antimicrobial phytocompounds from the ethanolic extracts of leaf of *S. alata and* bark of *P. thonningii* appears promising as it will foster the development of pharmaceutical, skin care products and combat multi-drug resistant microbes.

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