



Phytochemical, Antioxidants and Antimicrobial Activities of Leaf Extract of *Sida rhombifolia* on Selected Pathogenic Organism Causing Skin Infections

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Abstract

Sida rhombifolia belongs to the family *Malvaceae*. It is commonly known as Bala or Atibala. *Sida rhombifolia* is claimed by folklore for treating various ailments. The aim of this study is to determine selected Phytochemical screening, Antioxidant and Antimicrobial activities of leaf extract of *S. rhombifolia* on selected bacteria agents causing skin infections. Phytochemicals screening was determined using Spectrophotometric method to determine Alkaloid 4.681 %, Flavonoid 1.892 %, Terpenoid 0.516, Saponin 1.628 % and Tannin 0.116%. Antioxidant of the leaf extract was determined with Diphenyl Picryl Hydrazyl having 65.81% while Ferric Reducing Antioxidant Properties had 8.112 % scavenging capacity. The antimicrobial activity of *S. rhombifolia* leaf extract was also determined on selected pathogenic organisms causing skin infections using borehole method. The bioactive compounds of the leaves was extracted using 95% ethanol and extract was diluted into different concentration using distilled water, which was used to investigate antibacterial (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Micrococcus luteus* and *Micrococcus varians*) and antifungi (*Aspergillus flavus*, *Trichophyton rubrum*, *Aspergillus niger* and *Fusarium spp*) activity at different concentration of 20, 40, 60 and 80 mg/ml. Results showed that the extracts of *S. rhombifolia* is effective in inhibiting all the test organisms with highest inhibitory zone of 22.0 mm at 80 mg/ml for *S. aureus* and least at 20 mg/ml with 11.5 mm for *M. luteus*. *A. niger* had the highest inhibitory zone of 28.0 mm at 80 mg/ml, while *T. rubrum* had the lowest inhibitory effect at 10.0 mm. Minimum Inhibitory Concentration was also carried out which reveals highest and the minimum inhibitory zone for the same organism. *S. rhombifolia* extract demonstrated a good Antibacterial activity against selected organism causing skin infection, this leaf could be used in the treatment of skin infections caused by any of the selected microorganism.

Keywords: *Sida rhombifolia*, Phytochemical screening, *Malvaceae*, Antioxidant

Introduction

Sida rhombifolia commonly called Bala or Atibala is claimed by folklore for various ailments like rheumatism, seminal weakness, and diarrhea. *Sida rhombifolia* belonging to family *Malvaceae* possess insecticidal and antimicrobial properties. *Malvaceae* is a cosmopolitan family of herbs, shrubs and trees. Modern research carried out on the *Malvaceae* plants revealed that most of the plants belonging to this family are medicinally important as they contain biologically active compounds (Masih et al., 2014). *S. rhombifolia* is a small erect woody, very variable annual or perennial under shrub about 1.5 meters high with rough branches and stellate hairs. Leaves are very variable in shape up to 5mm by 18mm, short petioled, rhomboid-lanceolate to lanceolate, serrated towards the top, entire towards the base. The flowering and fruiting in

the plant start from September to December. Flowers are yellow or white, axillary, solitary or in pairs. The leaves are reduced on the flowering branches. The fruits are depressed, globose, schizocarpic, enclosed within the calyx, separating into one-seeded indehiscent unit. Seeds are black and smooth (Ajeet et al., 2018). *Sida rhombifolia* is classified thus, Kingdom- Plantae, Division- Tracheophyta, Class- Mangnaliopsida, Order- Malvales, Family- *Malvaceae*, Genus- *Sida*, Species- *Sida rhombifolia* Linn.

Sida rhombifolia is one of the 200 species in *Sida*. It grows in tropical and warm regions, and distributed throughout the tropics. *Sida rhombifolia* Linn is known for its wide range of medicinal uses (Opie, 2016). It is used for treating stings and bites of scorpion, snake and wasp (its flowers), skin diseases and sores (its

stem), treat stomach disorders, stomach pain, digestion problem (its roots), malaria, flatulence, diarrhea (its root decocted), dysentery (roots), irritable bowel syndrome, gastritis, enteritis, hemorrhoids (its roots and leaves), diabetes (its leaves), chicken pox, blood cleaning, fatigue, headache and migraine headache (its fruits), eye problems, conjunctivitis, toothaches (roots), fever, gum infection, swelling, tonic, wounds (root and leaves), ophthalmia and swelling (its leaves), cuts and wounds (its leaves) are some of the example to mention. In Ethiopia, *Sida rhombifolia* is also widely used by herbalists to treat different human diseases. These include use of its leaves to treat disease, wounds and inflammations, rabies and skin bleeding. In Jimma area, it is locally known as karaba, and its stems are used as tooth brush and the leaves and stem barks are used for the treatment of wound (Lopez et al., 2002 and Ajeet et al., 2018).

The plant growths that leads to the production of various chemical compounds are called phytochemical and also refer to as "secondary metabolites" which consist of, flavonoids, alkaloids, Coumadin's, tannins, phenols, phlobatannins, anthraquinones, terpenoids, cardiac glycosides, steroid and phytosteroid. It is known that the phytochemical compounds identified have medicinal significance (Aruoma, 2003 and Anooj et al., 2019).

Sida rhombifolia have been used as good sources of many modern drugs for treatment of several human diseases such as cardiovascular, cancer, malaria, mental diseases, etc. Most of these modern drugs have been obtained or discovered from medicinal plants. Hence, there is need to test for the phytochemical, antioxidant and antimicrobial activities of *Sida rhombifolia* leaf extract on the selected microorganisms which can cause skin infections in both human and animal.

Materials and Methods

Materials

Sida rhombifolia was collected from Igbole area, Igboora, Ibarapa Central Local Government, Oyo State, Mueller Hinton Agar and 99 % Ethanol were purchased.

Sample preparation

The leaf of *Sida rhombifolia* was collected, rinsed with distilled water and air dried. The air-

dried leaf was grinded into powdery form using electric blender and stored in a sterile air tight container for further work. 80g of plant powder was extracted with 500mls of ethanol for 72hrs using cold extraction method. The extract was concentrated in a water bath, transferred into pre-weighed sample container and air dried at 40°C until about the time of experiments.

Phytochemical Screening Determination Test for Alkaloids

One ml of 2NHCl was added to the concentrated extract and filtered. Five (5ml) ml each of Boromocresol green and phosphate buffer was added to the filtrate and shake together. Chloroform was added in successive volumes (1–4 ml) to the mixture in a separating funnel and was mixed vigorously. The chloroform layer was separated and the volume was made up to 10 ml. Absorbance was measured at 470 nm using UV Spectrophotometer (Woldeyes et al., 2012).

Test for Flavonoids

The extract was evaporated in 200µl of the solvent and 5 ml of aluminum chloride i.e., 0.1 M was added to the residue and incubated for 40 min, the absorbance was measured at 480nm using UV Spectrophotometer (Ekramul et al., 2002 and Assam et al., 2010).

Test for Terpenoids

Two (2 ml) ml of chloroform and 3 ml of concentrated sulphuric acid was added to 5 ml of the extract to check for the presence of Terpenoids which was indicated by a reddish-brown interface at wavelength of 520 nm in form of polyphenolic using UV Spectrophotometer (Ekramul et al., 2002 and Assam et al., 2010).



Figure 1: Image of *Sida rhombifolia*



Test for Tannins

The mixture of extract (100 µl), water (8.4 ml), Folin-Ciocalteu reagent (500 µl) and sodium bicarbonate (1 ml) was incubated for 30 min and read at 580nm absorbance using Spectrophotometric method (Tambe and Bhambar, 2014).

Test for Saponins

Equal volume of distilled water (2 ml) and leaf extract (2 ml) was added together and shake for 15 minutes in a graduated flask. Appearance of 1cm layer of a lather shows the conformation of saponins with absorbance measured at wavelength of 203 nm using UV Spectrophotometer (Tambe and Bhambar, 2014).

Antioxidant activity

The antioxidant potential of the samples was measured by evaluating their ability to reduce compounds by donating electrons using the FRAP assay.

Ferric reducing antioxidant power (FRAP)

Benzie and Strain (1996) method was used to determined FRAP assay. The FRAP reagent consisted of 10 mM TPTZ in 40 mM HCl, 250 mM sodium acetate buffer (pH 3.6) and 20 mM FeCl₃ was freshly prepared by mixing TPTZ solution, FeCl₃ solution and acetate buffer in a ratio of 1:1:10. An extract solution (100 µl) was mixed with 900 µl of FRAP reagent. The mixture was incubated at 37°C for 4 minutes and the absorbance was estimated at 593 nm. BHT was used as a standard. The results were expressed as µg BHT equivalent/mg sample.

Antibacterial Activity

The antibacterial activity of the extract was tested against selected bacteria (*Staphylococcus aureus*, *Staphylococcus epidermis*, *Micrococcus luteus* and *Micrococcus varians*) by using 0.5 McFarland standard of 24-

hour culture growth of bacterial which was spread on the surface of freshly prepared Mueller Hinton Agar with a sterile swap stick. Distilled water was used to prepare plant extract with concentration ranging from (20mg/ml, 40mg/ml, 60mg/ml and 80mg/ml) for the antibacterial analyses. The antibacterial activity of the extract was tested using the plate bore well method and leaf extract was impregnated into the wells by using sterile pipettes. The plates were incubated for 24 hours and the zones of inhibition around the wells was measured and recorded.

Antifungal Activity

Antifungal effect of *Sida rhombifolia* leaf extract was determined on selected fungi (*Trichophyton rubrum*, *Aspergillus niger*, *Aspergillus flavus* and *Fusarium spp*) using bore well method. 0.5 McFarland standard was prepared and spread on the surface of freshly prepared Potatoes Dextrose Agar using sterile swap stick and the leaf extract prepared at different concentration was pipette into the well. The plates were incubated at 27°C for 72 hours. The zone of inhibition on the plates were observed and recorded.

Minimum Inhibitory Concentration of Extract

The Minimum Inhibitory concentration is known to be lowest concentration of the compound that inhibit the growth of Microorganism (Kumar et al., 2007). The MIC value was determined by varying concentration of ethanol leaf extracts of 80mg/ml which has the highest inhibitory zone in all the isolates (20, 40, 60 and 80mg/ml) and impregnated into the prepared Mueller Hinton Agar that contain the selected organism. The plates were incubated at 37°C for 18hrs for bacteria and room temperature for fungi. The lowest concentration preventing all visible growth was taken as the minimal inhibitory concentration.

Result

Table 1: Phytochemical Analysis of *Sida rhombifolia*

Selected phytochemical parameters	Percentage composition (%)
Alkaloid	4.681
Flavonoid	1.892
Saponin	1.628
Terpenoid	0.516
Tannin	0.116

The photochemical analysis of *Sida rhombifolia* is shown in table 1 with the presence of alkaloids, flavonoids, Saponins, Terpenoids,

Tannins in ethanol leaf extract of *Sida rhombifolia*. The ethanol extract of *Sida rhombifolia* demonstrated highest percentage



in Alkaloids with 4.681 %, followed by
Flavonoids at 1.892 %, Saponin 1.628 %,

followed by Terpenoids of 0.516 % and Tannins
had the lowest percentage of 0.116 %.

Table 2: Antioxidant Properties of *Sida rhombifolia*

Antioxidant parameters	Percentage composition (%)
DPPH	65.81%
FRAP	8.112%

Key words: DPPH (Diphenyl, Picryl Hydrazyl), FRAP (Ferric Reducing Antioxidant Properties)

The result in table 2 showed the anti-oxidative
properties of *Sida rhombifolia* leaf extracts
under study with Diphenyl, Picryl Hydrazyl
(DPPH) having the highest percentage

(68.81%) while Ferric Reducing Antioxidant
Properties (FRAP) had the lowest percentage of
scavenging capacity (8.112%).

Table 3: Antibacterial Activities of Leave Extract of *Sida rhombifolia* on the Selected Organisms.

Test organisms	Inhibitory zone (mm)			
	20mg/ml	40mg/ml	60mg/ml	80mg/ml
<i>Micrococcus luteus</i>	11.5	12.0	13.5	16.0
<i>Micrococcus varians</i>	12.5	13.5	16.5	20.5
<i>Staphylococcus epidermis</i>	14.5	15.5	18.0	18.5
<i>Staphylococcus aureus</i>	13.5	19.5	21.0	22.0

Table 3 showed the antibacterial activity of *Sida rhombifolia* with different zone of inhibition, at 20 mg/ml *Staphylococcus epidermis*, *Staphylococcus aureus*, *Micrococcus varians*, *Micrococcus luteus* had inhibition value of 14.5, 13.5, 12.5 and 11.5 respectively. At 40mg/ml *Staphylococcus aureus*, *Staphylococcus epidermis*, *Micrococcus varians* and *Micrococcus luteus* with an inhibition value of 19.5, 15.5, 13.5 and 12.0. At 60mg/ml

Staphylococcus aureus, *Staphylococcus epidermis*, *Micrococcus varians* and *Micrococcus luteus* with an inhibition value of 21.0, 18.0, 16.5 and 13.5 respectively. The 80mg/ml demonstrated the highest inhibitory zone for all the organism used, *Staphylococcus aureus*, *Micrococcus varians*, *Staphylococcus epidermis* and *Micrococcus luteus* with an inhibition value of 22.0, 20.5, 18.5 and 16.0 respectively.

Table 4: Antifungal Activities of Leave Extracts of *Sida rhombifolia* on the Selected Organisms

Isolates	Inhibitory zone (mm)			
	20mg/ml	40mg/ml	60mg/ml	80mg/ml
<i>Trichophyton rubrum</i>	10.0	12.0	15.0	20.0
<i>Fusarium spp</i>	11.0	13.0	16.0	21.0
<i>Aspergillus flavus</i>	11.0	14.0	18.0	23.0
<i>Aspergillus niger</i>	13.0	18.0	22.0	28.0

The leaf extract of *Sida rhombifolia* demonstrated a good antifungal effect on all the organisms used. At 20g/ml *Trichophyton rubrum*, *Fusarium spp*, *Aspergillus flavus* and *Aspergillus niger* had an inhibition value of 10.0, 11.0, 11.0 and 13.0mm. At 40mg/ml *Trichophyton rubrum*, *Fusarium*, *Aspergillus flavus* and *Aspergillus niger* had an inhibition

value of 12.0, 13.0, 14.0, 18.0mm respectively. At 60mg/ml *Trichophyton rubrum*, *Fusarium*, *Aspergillus flavus* and *Aspergillus niger* with an inhibition value of 15.0, 16.0, 18.0 and 22.0mm and at 80mg/ml *Trichophyton rubrum*, *Fusarium*, *Aspergillus flavus* and *Aspergillus niger* had an inhibition value of 20.0, 21.0, 23.0 and 28.0 mm respectively.



Table 5: The Minimum Inhibition Concentration (MIC) of Leave Extract of *Sida rhombifolia* on the Selected Organisms
Inhibitory zone (mm)

Test organisms	20mg/ml	40mg/ml	60mg/ml	80mg/ml
<i>Micrococcus luteus</i>	14.5	15.0	16.5	17.0
<i>Staphylococcus epidermis</i>	14.5	15.0	16.0	17.5
<i>Staphylococcus aureus</i>	12.5	15.5	17.0	18.5
<i>Micrococcus varians</i>	14.0	15.5	20.5	21.0

Table 6: Minimum Inhibition Concentration (MIC) of Leave Extract of *Sida rhombifolia* on the Selected Fungi
Inhibitory zone (mm)

Isolates	20mg/ml	40mg/ml	60mg/ml	80mg/ml
<i>Trichophyton rubrum</i>	10.0	12.0	15.0	18.0
<i>Aspergillus flavus</i>	12.0	12.0	15.0	20.0
<i>Fusarium spp</i>	12.0	14.0	17.0	22.0
<i>Aspergillus niger</i>	13.0	17.0	20.0	25.0

The minimum inhibitory concentration as shown in table 5 and 6 revealed the highest zone of inhibition at 80 mg/ml for both bacteria (*Micrococcus luteus*, *Staphylococcus epidermis*, *Staphylococcus aureus* and *Micrococcus varians* with 17.9, 17.5, 18.5 and 21.0) and fungi (*Trichophyton rubrum*, *Aspergillus flavus*, *Fusarium* and *Aspergillus niger* with an inhibition value of 18.0, 20.0, 22.0 and 25.0

mm and the lowest at 20 mg/ml with *Micrococcus luteus* having 14.5mm, *Staphylococcus epidermis* 14.5 mm, *Staphylococcus aureus* 12.5mm, and *Micrococcus varians* at 14.0mm, *Trichophyton rubrum* 10.0 mm, *Aspergillus flavus* 12.0 mm, *Fusarium spp* 12.0 mm and *Aspergillus niger* with an inhibition value of 13.0 mm.

Table 7: Susceptibility of Selected Organism to Commercial Antibiotic Disc (Gram Positive)
Inhibition zone (mm)

Isolate	CAZ	CRX	GEN	CPR	OFL	AUG	NIT	AMP
<i>Staphylococcus aureus</i>	-	-	-	16.0	13.5	-	24.5	-
<i>Micrococcus luteus</i>	-	-	-	19.5	19.5	-	16.5	-
<i>Staphylococcus epidermis</i>	-	-	14.5	23.5	25.5	-	18.0	-
<i>Micrococcus varians</i>	-	-	16.5	31.5	32.0	-	28.0	-

Key words: CAZ-Ceftazidime, CRX- Cefuroxime, GEN- Gentamicin, CPR- Ciprofloxacin, OFL- Ofloxacin, AUG- Amoxicillin/Clavulanate, NIT- Nitrofurantoin, AMP- Ampicillin

The result in Table 7 shows that in Gentamicin, *Micrococcus varians* had the highest susceptibility zone of 16.5mm in table 7 and *Staphylococcus epidermis* has the least antimicrobial susceptibility zone of 14.5 mm. In Ciprofloxacin, *Micrococcus varians* had the highest susceptibility zone of 31.0 mm, followed by *Staphylococcus epidermis* with 23.0

mm, *Micrococcus luteus* 19.5 mm while *Staphylococcus aureus* has the least susceptibility zone of 16.0 mm. In Ofloxacin, *Micrococcus varians* had the highest susceptibility zone of 32.0 mm, next to it is *Staphylococcus epidermis* with 25.5 mm, *Micrococcus luteus* 19.5 mm and *Staphylococcus aureus* had the least



susceptibility zone of 13.5 mm. From Nitrofurantoin *Micrococcus varians* had the highest zone of inhibition of 28.0 mm, *Staphylococcus aureus* 24.5 mm,

Staphylococcus epidermis 18.0 mm and *Micrococcus luteus* had the least zone of inhibition of 16.5mm respectfully.

Table 8: Susceptibility of Selected Fungi to Commercial Antibiotics

	<i>Aspergillus flavus</i>	<i>Trichophyton rubrum</i>	<i>Aspergillus niger</i>	<i>Fusarium spp</i>
Nystatin	10.0	12.0	12.0	23.0
Fluconazole	15.0	16.0	14.0	25.0
Cotrimoxazole	16.0	14.0	12.0	26.0
Griseofulvin	-	16.0	14.0	27.0

From Table 8, there is zone of susceptibility for all the commercial antibiotic used against the selected fungi causing skin infection except *Aspergillus flavus* where there is no susceptibility zone in Griseofulvin. *Fusarium spp* had the highest susceptibility value in Griseofulvin with 27.0 mm while *Aspergillus flavus* had the lowest susceptibility zone in Nystatin with 10.0 mm.

Discussion

The results obtained in table one (1) showed that the phytochemical evaluation of the leaf extracts of *Sida rhombifolia* indicate the presence of Alkaloids, Flavonoids, Saponins and Tannins. Yang Hong et al., (2016) reported that the alkaloid fraction of aerial parts of *Sida rhombifolia* possesses antibacterial activities. The abundance of these phytochemicals are indicators that revealed the antibacterial activities of these *Sida rhombifolia* leaf extracts. This study is a reflection of work reported by Yang Hong et al., 2016.

The antioxidant properties of *Sida rhombifolia* as shown in table 2 indicate that DPPH is one of the compounds that has a proton free radical with a characteristic absorption at 65.81%, which decreases significantly on exposure to proton radical scavengers. It is well accepted that the DPPH radical scavenging by antioxidants is attributed to their hydrogen donating ability. The FRAP scavenging assay is based on the principle that Sodium Nitroprusside in aqueous solution at physiological spontaneously generates 8.112%, which interact with oxygen to produce Nitrate and Nitrite ions that can be estimated by the use of Griess reagent (Assam et al., 2010).

The ethanol extracts of *Sida rhombifolia* in table 3 exhibited a significant inhibitory effect on all the test bacteria. The extracts were administered to four different organisms

(*Micrococcus luteus*, *Micrococcus varians*, *Staphylococcus aureus* and *Staphylococcus epidermis*) at different concentrations (20mg/ml, 40mg/ml, 60mg/ml and 80mg/ml), *Staphylococcus aureus* had the highest zone of 22.0 mm at 80.0 mg/ml while *Micrococcus luteus* had the lowest zone of inhibition of 11.5mm at 20 mg/ml. The results obtained showed that 80mg/ml has the highest inhibitory zone while 20mg/ml has the lowest inhibitory zone which is also applicable to the Minimum inhibitory concentration result in table 5 and 6. It also shows that the inhibitory zone increased as the concentration increases this was in line with the work done by Masih et al (2014) in his work titled Antibacterial properties of selected medicinal plants against pathogenic bacteria. The antifungal activities of the extracts of *Sida rhombifolia* leave was tested against *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton rubrum* and *Fusarium spp* and there were significant zone of inhibition (Table 4) for all the fungi isolates used, even at the lower concentration (20, 40, 60 and 80mm), plant extract were active against all the selected organisms at all concentrations with highest inhibitory concentration recorded in *Aspergillus niger* with 28.0mm at 80mg/ml while lowest was recorded in *Trichophyton rubrum* at 20.0 mg/ml with an inhibitory zone of 10.0 mm.

The Antibiotic Susceptibility Test against selected Organisms in table 7and 8 shows that CAZ-Ceftazidime, CRX- Cefuroxime, AUG-Amoxicillin/Clavulanate and AMP- Ampicillin had no effect on all the bacterial used in this research, GEN- Gentamicin had no effect on *Staphylococcus aureus* and *Micrococcus luteus* but was able to inhibit *Staphylococcus epidermidis* and *Micrococcus varians* but Ciprofloxacin, Ofloxacin and Nitrofurantoin were able to inhibit the growth of all the selected bacteria used. Also, the leaf extracts showed appreciable level of potency against the



selected bacteria which have ability to cause skin infection. For fungi, all the antibiotic used (Nystatin, Fluconazole, Cotrimoxazole and Griseovulvin) were able to inhibit the growth of *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton rubrum* and *Fusarium spp* except Griseovulvin which had no effect on *Aspergillus niger* with highest susceptibility zone recorded in *Fusarium spp* with 27.0 mm zone of inhibition in Griseovulvin. This study is in line with the work reported by Kumar et al (2007).

This finding justifies the traditional uses of *Sida rhombifolia* leaf for medicinal purposes as reported by Konate et al., 2010 and Ramachandran et al., 2014 where the plant part has records of being used as rheumatoid, diarrhea, ulcers, inflammation, swelling, infectious disease, chicken pox, blood purification, tiredness headache, fever, infection with gum, tonsillitis, cuts, injuries, ophthalmia, diabetes, chest and diarrhea on oral administration.

Conclusion

The results of this study support the traditional application of the plant which reveal that the leave extract possess compounds that have antimicrobial properties and can be used as antimicrobial agents in novel drugs for the treatment of bacteria and fungi that can cause skin infections.

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