

Evaluation of the Antimicrobial Activity of *Erythrina abyssinica* Leaf Extract

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ABSTRACT

Natural plant products have been important for the development of new active molecules for drug development since the ancient times. This is particularly due to the presence of secondary metabolites in plants, which are known for their antimicrobial activity. Thus, this study focused on investigating the antimicrobial activity of *Erythrina abyssinica* against *Candida albicans* and *Staphylococcus aureus*. *Erythrina abyssinica* is a medicinal plant which has been used traditionally for the treatment of various infections, snakebites and some sexually transmitted diseases. However, not much scientific studies have been done to validate the use of *Erythrina abyssinica* as a medicinal plant. The bark was extracted using solvent-solvent extraction method. The extracts were tested for their antimicrobial activity using the agar disc diffusion assay. Antimicrobial activity was observed in most extracts with the ethyl acetate extract showing the highest zone of inhibition of 25 mm and dichloromethane showing the least zone of inhibition against *C. albicans*. The minimum inhibitory concentrations (MIC) for all the extracts were determined using the broth dilution assay. The dichloromethane and hexane extracts were the most potent with MICs of 62.5 µg/ml. However, the hexane extract showed the highest zone of inhibition of 23 mm against *S. aureus* whilst dichloromethane was found to be the most potent with an MIC of 15.6 µg/ml against *C. albicans* by broth dilution assay. Minimum fungicidal concentrations for all the extracts were 500 µg/ml except for ethyl acetate which was 250 µg/ml. The minimum bactericidal concentration for all the extracts was greater than 500 µg/ml except for hexane showing that extracts inhibited growth of *S. aureus* but did not kill the cells. Toxicity studies showed that all extracts may not be toxic to human cells. Therefore, these results scientifically validate the use of the *Erythrina abyssinica* bark for the treatment of various ailments.

Keywords: Antimicrobial; *Erythrina abyssinica*; *Candida albicans*; *Staphylococcus aureus*

INTRODUCTION

About 80% of the African population uses herbal medicine for the treatment of known and unknown illnesses, and this has increased the world market for herbal medicine to approximately US 60 billion [1]. Different plant parts can be used as source of medicine and can be consumed raw, cooked, boiled or administered as decoction, syrup, poultice, infusion, or pounded paste which is applied topically, used in herbal baths or taken orally. *Erythrina abyssinica* is a plant commonly found in Zimbabwe, Sudan and Ethiopia south to Angola and Mozambique and is well known as an herbal plant used by traditional healers [2]. The bark is commonly used in traditional medicine to treat snakebites, malaria, sexually transmitted diseases like syphilis and gonorrhoea, amoebiasis, cough, liver inflammation, stomach ache, colic and measles.

Roasted or powdered bark is applied to burns, ulcers and swellings. The liquid from crushed bark of green bark is used to treat conjunctivitis caused by chlamydia trachomatis (trachoma). The sap is drunk as an anthelmintic and against vomiting. The pounded flowers are used to treat dysentery and a maceration of flowers is drunk as an abortifacient, also applied externally to treat earache [3]. The roots are taken to treat peptic ulcers, epilepsy, malaria, blennorrhagia and schistosomiasis. The leaves are also used to treat peptic ulcers diarrhoea and applied to wounds, painful joints and skin diseases. The fruit of the *Erythrina abyssinica* are also taken to treat asthma and meningitis. Thus, this study focused on validating the use of *Erythrina abyssinica* as a medicinal plant by investigating its antimicrobial activity against *C. albicans* and *S. aureus*.

Candida albicans is an example of dimorphic fungi and is the most cause of systemic mycoses. *Candida albicans* is a member of the

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human gut flora found at about 40% to 60% in healthy humans. However under favourable conditions, *C. albicans* can become pathogenic and can infect the skin, genitals, mouth and blood [4]. *Staphylococcus aureus* is another microorganism which frequently causes infections such as skin, wound and deep tissue infections and is responsible for some life threatening conditions like pneumonia, endocarditis and septicemia. The bacterium is one of the most common causes of nosocomial infections worldwide and has been reported to be multidrug resistant [5].

MATERIALS AND METHODS

Chemicals and reagents

Chemicals and reagents used in this study were purchased from Sigma-Aldrich, USA. Reagents used include, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), nutrient agar, nutrient broth, absolute methanol, hexane, ethyl acetate, tetrazolium, ethanol, glycerol and sodium chloride. Distilled water was obtained from the department of biochemistry.

Plant collection and identification

Erythrina abyssinica bark was collected from Glen Norah suburb in Harare Province (Coordinates 17.9113°S, 30.9790°E) and was identified by a botanist at Harare Gardens. The bark was allowed to sun dry, then was pulverised by use of a mortar pestle, weighed and then stored at room temperature.

Extract preparation

The dried powdered bark of *E. abyssinica* was soaked overnight in absolute methanol in a ratio of 1:4, for the extraction of its constituents. The extract was filtered and allowed to evaporate at room temperature. About 75% of the dried methanol extract was dissolved in 30% methanol and solvent-solvent extraction followed. Different solvents were added to the 30% methanol extract in their increase in polarity and constituents were extracted according to their polarity. The solvents used for the extraction were hexane, ethyl acetate and dichloromethane. The extracts were dried, weighed and stored at room temperature in air tight bottles.

Test organisms

Glycerol stocks of the test organisms, *S. aureus* and *C. albicans* were provided by Prof. S. Mukanganyama from the Department of Biochemistry, University of Zimbabwe. The microorganisms were resuscitated by culturing them overnight in nutrient broth at 37°C and 150 rpm. *S. aureus* and *C. albicans* were subculture on nutrient agar in a 37°C non shaking incubator overnight. Some glycerol stocks were prepared and stored in a refrigerator at 4°C.

Agar disc diffusion assay

S. aureus and *C. albicans* cells from glycerol stocks were grown overnight at 37°C, 150 rpms in 20 ml of nutrient broth. Cells were then inoculated in nutrient agar to a final concentration of 1x10⁶ CFU/ml. Each dried plant extract was dissolved in absolute ethanol and a certain volume (equivalent to 500 µg) was impregnated on whatman paper discs (6 mm in diameter). The extracts on paper discs were allowed to dry and then placed on inoculated agar in petri dishes. The petri dishes were refrigerated at 4°C for 2 hours to allow the diffusion of the extracts into the agar. The plates were then incubated overnight at 37°C. Growth or inhibition of growth was observed the following day.

Broth dilution assay

A volume of 100 µl of fresh broth cultures of 1x10⁶ CFU/ml of each microorganism were placed in 96 well plates. Plant extracts (100 µl) prepared by two fold dilution from 500 µg/ml to 0 µg/ml were added to cells in 96 well micro titre plates. The plates were labelled respective of the plant extracts. Distilled water was added to give a final volume of 300 µl in each well. The negative controls contained nutrient broth only and the positive controls contained cells only. Miconazole, a known antifungal and erythromycin, an antibiotic were also tested under the same conditions. The plates were covered with a parafilm and incubated at 37°C overnight. Viability of cells was then determined by adding 25 µl of a tetrazolium salt, MTT at 2 mg/ml. Plates were left to stand for three hours and colour change was observed. MTT assesses the viability of live cells through a reduction reaction of the yellow tetrazole to give purple formazan with the intensity depending on the concentration of the cells present. This reaction is catalysed by oxidoreductase enzymes in viable cells. Thus, the intensity of the colour was measured using a spectrophotometer at 540 nm. The lowest concentration of the extract that inhibited growth was recorded as the MIC. Minimum bactericidal concentration and minimum fungicidal concentration were determined by re-culturing cells from wells that had no viable cells present from the MTT assay. Cells were streaked onto nutrient agar and incubated at 37°C overnight. Growth of the cells on agar shows presence of viable cells in the well. The lowest extract concentration which showed no growth of bacteria or fungi on the media was the MBC or MFC value.

Haemolytic assay

A volume of 5 ml of sheep blood in EDTA was centrifuged at 1000 rpm and 4°C for 10 minutes. The supernatant and buffy coat layer was carefully discarded. The pellet collected was washed 3 times with phosphate buffered saline (PBS). The erythrocyte pellet (100 µl) was diluted using 900 µl of PBS and 50 µl of the diluted sample was mixed with 100 µl of plant extract. This was done for plants that showed antimicrobial activity against both *S. aureus* and *C. albicans*, at their MIC values. For the positive control, 50 µl of diluted erythrocytes were mixed with 10% Sodium Dodecyl Sulphate (SDS). The negative control had 100 µl of PBS. The contents were then incubated at 37°C for 60 minutes. After incubation 850 µl of PBS were added to each reaction vessel and thoroughly mixed. The tubes were centrifuged at 300 rpm for 3 minutes and the supernatant was put into respective wells of a 96 well ELISA plate. The absorbance was read at 545 nm. Percentage lysis was determined using the positive control as 100% lysis. Percentage haemolysis of the extracts was calculated by the formula:

$$\left(\frac{\text{Absorbance of red blood cells with plant extracts}}{\text{Absorbance of red blood cells with SDS}} \right) \times 100\%$$

RESULTS

Plant extractions

The total mass of the pounded *Erythrina abyssinica* bark powder was 37 g. The methanol extract obtained was 5.6 g and 75% of it was further partitioned by solvent-solvent extraction. The solvents used in solvent-solvent extraction were hexane, dichloromethane and ethyl acetate. The yields of plant extracts obtained are shown in Table 1.

Inhibition of growth of *S. aureus* and *C. albicans* by *E. abyssinica* extracts

The effects *E. abyssinica* extracts on the growth of *S. aureus* and *C. albicans* is shown by zones of inhibition in Table 2. All the extracts inhibited both species with *C. albicans* being inhibited the most. Although all the extracts inhibited both species, the effects were less than that of positive controls, erythromycin and miconazole. The ethyl acetate extract showed the highest zone of inhibition of 25 mm and dichloromethane showed the least with 12 mm against *C. albicans*. On the other hand, the hexane extract showed the highest inhibition zone of 23 mm and dichloromethane showed the least with 11 mm against *S. aureus*.

MIC and MBC determination

The dichloromethane and hexane extracts of *E. abyssinica* were found to have MICs of 62.5 µg/ml whilst methanol and ethyl acetate had 125 µg/ml against *C. albicans* (Table 3). The MBC values were found to be 500 µg/ml for all the extracts except for ethyl acetate which had 250 µg/ml (Table 4). Dichloromethane and hexane extracts were also found to have the MIC values of 15.6 and 31.25 µg/ml respectively against *S. aureus* (Table 3). The MBCs values were more than the highest concentration tested, >500 µg/ml for all the extracts except for hexane which had 31.25 µg/ml (Table 4).

Toxicity effects

Haemolysis assay was carried out to assess the effect of the active extracts on mammalian (sheep) red blood cells. The haemoglobin released by red blood cells when they lyse was measured at 545 nm against a positive control of 10% SDS. The MICs of plant extracts obtained against both species were used to determine haemolytic activity. Table 5 shows that all tested extracts had haemolytic activity less than 50%.

Solvent	Mass of extract in grams	Percentage yield (%)
Methanol	5.6	15
Hexane	0.75	0.18
Dichloromethane (DCM)	0.28	0.07
Ethyl acetate	0.03	0.01

Table 1: Yields of different extracts of *Erythrina abyssinica*.

Plant extract	Inhibition zone (mm)	
	<i>S. aureus</i>	<i>C. albicans</i>
Dichloromethane	11 ± 0.78	12
Ethyl Acetate	21	25 ± 1.4
Hexane	23.5 ± 2	21 ± 1
Methanol	22	23 ± 1
Erythromycin	30 ± 2	-
Miconazole	-	31 ± 2

Antimicrobial activity of *Erythrina abyssinica* extracts. The commercial drugs erythromycin and miconazole were used as the positive controls against *S. aureus* and *C. albicans* respectively. Values are means ± SD for N=2 incubations.

Table 2: Effects of *Erythrina abyssinica* bark extracts by disc diffusion assay on *S. aureus* and *C. albicans*.

Plant extract	MIC (µg/ml)	
	<i>S. aureus</i>	<i>C. albicans</i>
Dichloromethane	15.6	62.5
Ethyl acetate	62.5	125
Methanol	62.5	125
Hexane	31.25	62.5
Erythromycin	62.5	-
Miconazole	-	125

Concentrations of the plant extracts were diluted from 500 µg/ml to 0 µg/ml. Values are means ± SD for N=2 incubations.

Table 3: Minimum inhibitory concentrations for *Erythrina abyssinica* bark extracts against *S. aureus* and *C. albicans*.

Plant extract	MBC (µg/ml)	MFC (µg/ml)
	<i>S. aureus</i>	<i>C. albicans</i>
Dichloromethane	>500	500
Ethanol	>500	500
Hexane	31.25	500
Ethyl acetate	>500	250
Miconazole	-	250
Erythromycin	125	-

MBC and MFC were determined by reculturing cells from wells that did not show viability by MTT assay. The lowest concentration that showed growth was regarded as the MBC or MFC. Values are means ± SD for N=2 incubations.

Table 4: Minimum fungicidal concentrations and Minimum bactericidal concentrations for *Erythrina abyssinica* bark extracts against *S. aureus* and *C. albicans*.

Extract	Percentage haemolysis at the MIC
Hexane	15.5%
Methanol	39.7%
Ethyl acetate	15.4%
Dichloromethane	9.1%

The MIC of plant extracts obtained against both extracts was used to determine hemolysis activity. Values are means ± SD for N=2 incubations.

Table 5: Effect of *Erythrina abyssinica* on sheep erythrocytes.

DISCUSSION

A wide range of active compounds were extracted from the bark of *E. abyssinica* using solvents of increasing polarity. All the plant extracts showed antimicrobial activity against both *S. aureus* and *C. albicans* by the agar disc diffusion assay. The highest inhibition zone was 25 mm for ethyl acetate extract and the lowest inhibition zone was 12 mm for dichloromethane extract against *C. albicans*. Hexane showed the highest zone of inhibition of 23 mm and dichloromethane the lowest of 11 against *S. aureus*. Minimum inhibition concentrations and the minimum fungal concentrations of the extracts were determined using the broth dilution assay. The technique is quick, sensitive, and quantitative with reproducible results. The MICs for all the plant extracts ranged from 62.5 µg/µl to 125 µg/µl and the MFCs for most extracts were 500 µg/µl against *C. albicans*. However, results showed that most of *S. aureus* grew when re-cultured suggesting that *Erythrina abyssinica* extracts can only inhibit the *Staphylococcus aureus* bacteria, but does not kill. Only the hexane extract showed an MBC of 31.25 µg/ml. Variation in the degree of activity were because of the different constituents and structures of natural products present [6].

Haemolysis assay was carried out to investigate the toxicity of the *E. abyssinica* extracts. The assay is based on the destruction of the membrane lipid bilayer of red blood cells exposing haemoglobin. The haemoglobin absorbs light at 545 nm and is detected by a spectrophotometer [7]. Haemolysis is related to the concentration and potency of the extract, the more the active biomolecules absorbed the more the lysis [8]. Coloured extracts absorb light at 545 nm and may lead to false positives [9]. Methanol extract showed the greatest haemolysis of 39.7% at the MIC of 125 µg/ml. The toxicity of the methanol suggests the extract may not be suitable for drug development. The other three extracts showed tolerable haemolysis at 62.5 µg/ml as the MIC and may be suitable for drug development. Haemolysis for other extracts was hexane 15.5%, ethyl acetate 15.4% and DCM with the lowest at 9.1%.

CONCLUSION

The results of the study showed that *E. abyssinica* may have antimicrobial activity against *C. albicans* and *S. aureus*. This therefore verifies the use in the treatment of the bark by traditional healers. The extracts may not be toxic at minimum inhibitory concentrations.

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