



Phytochemicals and biological testing of *Syzygium guineense* seeds extract against *Ascaris suum* and five pathogenic microbes

Sheila M. Maregesi*, Rogers Mwakalukwa, Muhaddisa H. Kassamali

Pharmacognosy Department - School of Pharmacy. Muhimbili University of Health and Allied Sciences.
P.O. Box 65013, Dar Es Salaam, Tanzania

Received: 14-06-2016 / Revised: 25-07-2016 / Accepted: 27-07-2016 / Published: 31-07-2016

ABSTRACT

This work is a continuation of our previous study where the crude ethanolic extract of *Syzygium guineense* seeds demonstrated anthelmintic activity against a non pathogenic worm *Pherithema posthuma*. The current work aimed at confirmation of anthelmintic activity using the pathogenic worm *Ascaris suum*, determination of antimicrobial activity and qualitative phytochemical analysis. Anthelmintic activity was confirmed but lower compared to the standard drug Albendazole (API). The extract showed weak to moderate antibacterial and anticandidal activity (MIC values ranging from 0.156 - 1.25 mg/ml) against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella typhi* (ATCC 6539), and *Candida albicans* (ATCC 90025). Detected phytochemical compounds were; steroids, terpenes, flavonoids and tannins. Studies to elucidate the phytoconstituents responsible for the exhibited activities and establishment of safety status by using animal models are ongoing.

Keywords: *Syzygium guineense*, *Ascaris suum*, Ethanolic extract, Paralysis, Death, Anthelmintic activity, Antimicrobial activity, Phytochemicals



INTRODUCTION

Medicinal plants as a natural source for the treatment of various diseases is known since ancient times. Referring to World Health Organization report of more than a decade ago, about 20,000 plant species were used for medicinal purposes [1]. Infections due to microbes and intestinal worm infestation are common in developing countries of the tropical and subtropical climate ideal for the survival of most pathogenic organisms, especially worms which may even be found in the soil.

Soil transmitted helminth is the most important group of intestinal worms affecting two billion people worldwide. The main species for infestation are *Ascaris lumbricoides* (roundworms), *Trichuris trichiura* (whipworms) and *Necator americanus/Ancylostoma duodenale* (hookworms) [2]. Despite causing considerable morbidity and mortality, intestinal infestation is among the Neglected Tropical Diseases (NTDs). Globally, the burden of disease due to hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* amounts to 39 million

disability-adjusted life-years (DALYs) lost as compared to malaria (35.7 million) [3].

Various studies have revealed anthelmintic and antimicrobial potentials of plants and a number of bioactive compounds identified [4,5]. Hence, traditional medicine plants provide useful antimicrobial and anthelmintic herbal preparations and their biomolecules could be developed into new drugs to solve resistance of the currently used conventional anthelmintic and antimicrobial drugs. *Syzygium guineense* is among medicinal plants used for treatment of various diseases such as, dysentery, naso-pharyngeal affections, pulmonary troubles diarrhea among others. [6] These claims could be associated with the reported antibacterial activity of *S. guineense* including; antimycobacterial activity of the root bark [7], activity against *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* due to arjunolic and asiatic acids from the leaves [8] and activity against gram positive gram and negative bacteria by alkaloids and anthraquinones as the bioactive compounds [9].

*Corresponding Author Address: Dr. Sheila M. Maregesi, Pharmacognosy Department - School of Pharmacy. Muhimbili University of Health and Allied Sciences. P.O. Box 65013, Dar Es Salaam, Tanzania; E-mail: smaregesi@hotmail.com

Based on our previous work on crude ethanolic extract of *S. guineense* seeds that demonstrated interesting anthelmintic against a non pathogenic worm *Pherithema posthuma* [10], We tested the extract against pathogenic worms *Ascaris suum* to justify further scientific research. In addition, we worked on antimicrobial testing and phytochemical aspects. We are reporting results obtained from *in vitro* testing against *Ascaris suum*, four pathogenic bacteria, *Candida albicans* and detected phytochemical groups.

MATERIALS AND METHODS

Standard drugs, chemicals, reagents: Reference drugs include; Abendazole (API) USP, manufactured by Supharma chem (Gujarat, India). Gentamicin sulphate 40mg/ml injection bought from INTAS Pharmaceuticals Ltd. (Ahmedabad, India) and Fluconazole was purchased from CADILA Pharmaceutical Ltd. (Dholka, India). Absolute ethanol (Scharlau) purchased from KAS MEDICS Dar es Salaam, Tanzania, Sabraud's Dextrose Broth (SDB) bought from Biotech Laboratory Ltd. Ipswich, United Kingdom. Iodonitrotetrazolium chloride was bought from SIGMA (Sigma-Aldrich) St. Louis, USA.

Microtitre plates: Polystyrene, Nonpyrogenic Tissue Culture Plate, 96 well, U- bottomed with Low evaporation Lid polystyrene plates were purchased from Becton Dickinson Labware Europe. (38800 Le Pont De Claix, France.

Collection of plant materials and Extraction: *Syzygium guineense* fruits were bought from the local market Kisutu, washed with clean water to remove dirt and then washed with distilled water. The seeds were manually separated from the fruit pulp. The seed were then dried at room temperature. The dried *S. guineense* seeds were ground to a course-fine powder by using a mortar and pestle followed by an electric grinder. 660 grams of powder was macerated using 1.3L of absolute ethanol and was left for 72 hours. The extraction process was repeated three times to ensure complete extraction. The extract was double filtered by using cotton wool and filter paper then dried on the rotary evaporator at 40°C.

Test organisms:

Pathogenic worm: Adult roundworms - *Ascaris suum* were obtained from Mbezi slaughterhouse of Dar es Salaam, Tanzania .

Microorganisms: The five microorganisms used in this study namely; *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Salmonella typhi* (ATCC 6539), and *Candida albicans* (ATCC

90025) were obtained from the Department of Microbiology and Immunology, School of Medicine, Muhimbili University of Health and Allied Sciences.

Evaluation of the anthelmintic activity: The anthelmintic activity was carried out on adult roundworms (*Ascaris suum*) using the protocol described by Nilani's team [11] with minor modification. From the stock solutions (1g/ml) of the ethanolic extract and albendazole (API) and used to prepare testing concentrations of 100, 80, 50 and 30mg/ml in normal saline as a vehicle. Experiments were done in triplicate for each test concentration and all procedures were done at room temperature, as follows; a group of six *Ascaris suums* were released into 50ml of the various testing concentrations. Group one was treated as negative control in normal saline and group two served as a positive control and received the standard drug Albendazole (API). Observations were made for the time taken to cause paralysis and death of the individual worms in each group. For each concentration, the mean time for paralysis (hours) was noted when no movement of any sort could be observed except when the worm was shaken vigorously, Similarly. the mean time of death of worm (hours) was concluded when the worms lost their motility completely even after applying an extenal stimuli.

Evaluation of the antimicrobial activity: The broth micro-dilution method described by Masola's team [12] was used to determine the minimum inhibitory concentrations (MICs) of tested microbes. The 96-well microtitre plates were first preloaded with 50 µL of the Saboraulds dextrose broth in each well followed by an addition of 50 µL of the extract (5mg/mL) into the first wells of each row tested to make a total volume of 100 µl in the first wells. After thorough mixing 50 µl were drawn from each of the first row wells and put into the next row wells. The process was repeated down the columns to the last wells at the bottom where 50 µL was discarded. Thereafter, 50 µL of the bacterial and fungal suspensions (0.5 Mac Farhland standard turbidity) were added in each well to make the final volume of 100 µL in each well.

Gentamicin sulphate (50 - 0.024 µg/mL) and Fluconazole were used as standard positive drug for bacteria and fungal respectively. 10% DMSO was used as a negative control. The plates were then incubated at 37°C for 24 h. To determine the MICs, 50 µL of 0.02% *p*-iodonitrotetrazolium (INT) chloride dye was added in each well followed by incubation for 1 h at 37°C. Bacterial or fungal growth was indicated by a change to pink colour. The lowest concentration which showed no

bacterial or fungal growth was considered as the MIC.

Chemical qualitative analysis: Test methods were employed following the standard procedures using relevant reagents/chemicals [13] as outlined below;

- i. *Steroids*: 2 ml of acetic anhydride was added to 0.5 g of the ethanol extract with 2 ml of Sulphuric acid. A colour change from violet to blue to green indicated the presence steroids.
- ii. *Terpenoids*: 5ml of the extract was mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer (Salkowski test). A reddish brown coloration at the interface indicated the presence of terpenoids.
- iii. *Saponins*: 0.5 to 1 g of the extract was dissolved in 5ml of water and the tubes were shaken vigorously, formation of 1 cm layer of foam indicated the presence of saponins.
- iv. *Flavonoids*: 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of the plant extract followed by the addition of concentrated sulphuric acid. A yellow coloration indicated presence of flavonoids.
- v. *Tannins*: About 0.5 g of the ethanolic extract was boiled in 20 ml of water in a test tube. It was then filtered and a few drops of 0.1% Iron III chloride added. The appearance of green/bluish black colour indicated the presence of tannins.

RESULTS AND DISCUSSION

Our literature search could not find reports of *Syzygium guineense* seeds indicated for treatment of diseases caused by microbes or worms. Interestingly, the present study has demonstrated antimicrobial and anthelmintic activities against pathogenic organisms.

Anthelmintic activity: The assessment was based on the time required to cause 100 % paralysis and 100% death giving results presented in Figure 1. At all tested concentrations, the crude ethanolic extract of *S. guineense* required longer time to cause paralysis and death than albendazole (API). At lower concentrations of 50 mg/ml and 30 mg/ml of crude ethanolic extract of *S. guineense* the time required to effect 100% death was slightly higher by 0.025% compared to the negative control. Also, at the concentration of 100 mg/ml, the time required to effect 100% death for *G. guineense* ethanolic extract was 6% higher than that of albendazole (API). It implies that at higher concentration, the bioactive contents in the crude extract are reasonably high to exhibit anthelmintic activity comparable to albendazole (API) despite the

presence of non active compounds in the matrix. It can thus be expected that, active fractions or compounds could yield products exhibiting same anthelmintic effect as albendazole (API).

Regarding worms sensitivity; *A. suum* obtained from the pigs are less sensitive than *Pherithema posthuma* used in our previous work. At the concentration of 30 mg/ml of ethanolic extract 5400 and 7720 minutes was required to effect 100% paralysis and 100% death of *A. suum* versus 20 and 60 minutes for *P. posthuma* [10]. The lower sensitivity of *Ascaris suum* is undoubted because even at the highest tested concentrations i.e. 100 mg/ml of albendazole (API), time required to effect 100% paralysis and 100% death were 1,240 and 2,838 minutes respectively compared to the 194 and 487 minutes obtained from the the tablets whose Albendazole content is low [10]. Resistance is most likely since pigs are normally de-wormed several times before slaughtering, but we could not identify which drug(s) had been used to treat the pigs and for how long since the worms were collected the from the slaughterhouse.

Antimicrobial activity: The crude ethanolic extract of *Syzygium guineense* showed antimicrobial activity of different levels on the tested microorganisms as presented in Table 1. Although observed activities against all tested microbes were lower compared to standard drugs, our findings remain interesting based on the fact that, they have shown some activity against gram negative bacteria that are difficult to treat due to the chemical nature of their cell walls [14]. Furthermore, sensitivity of *Escherichia coli* and *Klebsiella pneumonia* with the MIC value of 156µg/ml is not very different from suggested MIC value of 100µg/ml for the crude extract to be considered active [15]. Our findings are in agreement with previous reports of a related species *Syzygium cumini* where, the ethanolic leaf extract and aqueous seed extract showed a very high antimicrobial property for wide range of gram positive and gram negative bacterial strains [16] and crude hydroalcoholic extract demonstrated good antibacterial activity against multi-resistant strains of *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Staphylococcus aureus* as well as antifungal activity against *Candida krusei* [17].

Phytochemicals: Out of five tests, four phytochemical groups were detected in the crude ethanolic extract of *Syzygium guineense* seed as presented in Table 2. The detected phytochemical groups could be responsible for the observed activities such as tannins previously reported to produce anthelmintic activities due to their ability to bind to free proteins in the gastrointestinal tract

of host animal [18] or glycoprotein on the cuticle of the parasite and thereby cause deaths [19].

CONCLUSION

The confirmation of anthelmintic activity against the pathogenic *Ascaris suum* and antimicrobial activities shown by *Syzygium guineense* seed

extract are encouraging results. Thus, our team is working towards safety verification using animal experiments and isolation/identification of bioactive compounds(s) or fraction(s). More important is that, the relevant local ministry will be informed about our findings for proper measures of conservation of *Syzygium guineense* plant and related species.

Table 1: Antibacterial and Antifungal activities of the *Syzygium guineense* seeds

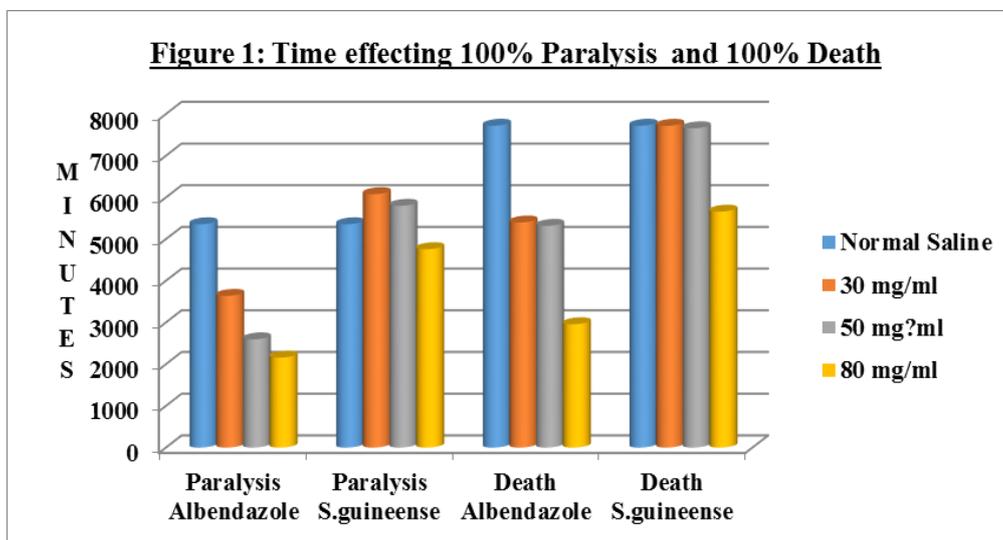
PATHOGENIC MICROORGANISM	MINIMUM INHIBITION CONCENTRATION (MIC mg/ml)	
	ETHANOL EXTRACT	STANDARD DRUG
		GENTAMYCIN
<i>Escherichia coli</i>	0.156	0.00063
<i>Klebsiella pneumonia</i>	0.156	0.00033
<i>Salmonella typhi</i>	1.25	0.00125
<i>Staphylococcus aureus</i>	0.625	0.00033
		FLUCONAZOLE
<i>Candida albicans</i>	0.625	0.00625

Table 2: Phytochemicals of ethanolic seed extract

COMPOUNDS	RELATIVE AMOUNTS
Steroids	(+++)
Terpenoids	(+++)
Saponins	(-)
Flavonoids	(++)
Tannins	(+++)

Key: (-) = Not detected; (++) = detected in high amount and; (+++) = detected in higher amount

Figure 1: Time effecting 100% Paralysis and 100% Death



REFERENCES

1. Gullece M *et al.* Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria mylanderian*. *Phytomedicine* 2006; 13: 515- 521.
2. World Health Organization. Soil-transmitted helminth infections. 2016; <http://www.who.int/mediacentre/factsheets/fs366/en/>, (Accessed on 05/07/2016)
3. Stephenson LS. *et al.* Malnutrition and parasitic helminth Infections. *Parasitology*. 2000; 121 suppl 1: S 23- S 38.
4. Mali RG, Mehta AA. A review on Anthelmintic Plants. *Natural Product Radiance*. 2008; 7(5): 466-475.
5. Cowan MJ. Plant Products as Antimicrobial Agents *Clinical Microbiology Reviews*. 1999; 12: 564-582.
6. Cimanga KR *et al.* Antibacterial and antifungal screening of extracts from six medicinal plants collected in Kinshasa-Democratic Republic of Congo against clinical isolate pathogens. *Journal of Pharmacognosy and Phytotherapy*. 2014; 6(3): 24-32.
7. Nvau JB *et al.* Antimycobacterial evaluation of some medicinal plants used in plateau State of Nigeria for the treatment of tuberculosis. *Agriculture and biology Journal of North America*. 2011; 2(9): 1270-1272.
8. Djoukeng JD *et al.* Antibacterial triterpenes from *Syzygium guineense* (Myrtaceae). *Journal of Ethnopharmacology*. 2005; 101(1-3): 283-286.
9. Bankefa E. *et al.* Phytochemical and antibacterial properties of *Syzygium guineense* root extract. *Journal of Scientific Research*. 2014; 2 (3): 21-25.
10. Maregesi S *et al.* Determination of Mineral contents, Cytotoxicity and Anthelmintic activity of *Syzygium guineense* fruit. *Saudi Journal of Medical and Pharmaceutical Sciences*. 2016; 2(54): 95-99.
11. Nilani P *et al.* Anthelmintic Activity of *Moringa Oleifera* Seed Oil- Validation of Traditional Use. *Journal of Advanced Scientific Research*. 2012; 3(2): 65-66.
12. Masola SN *et al.* Assessment of antimicrobial activities of crude extracts if stem and root barks from *Adansonia digitata* (Bombacaceae) (African baobab). *African Journal of Biotechnology*. 2009; 8: 5076-5083.
13. Ahumuza T, Kirimuhyuza C. Qualitative (Phytochemical) Analysis and Antifungal Activity of *Pentas decora* (De Wild), a Plant Used Traditionally to Treat Skin Fungal Infections in Western Uganda. *Research in Pharmaceutical Biotechnology*. 2011; 3: 75-84.
14. Maregesi SM *et al.* Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. *Journal of Ethnopharmacology*. 2008; 119: 58-66.
15. Cos P *et al.* Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of concept'. *Journal of Ethnopharmacology*. 2006; 106: 290-302.
16. Shylaja P *et al.* Phytochemical and antimicrobial properties of *Syzygium cumini* an ethnomedicinal plant of Javadhu hills. *Research in Pharmacy*. 2011; 1(1): 22-32.
17. Oliveira GF *et al.* Antimicrobial activity of *Syzygium cumin* (Myrtaceae) leaves extract. *Journal of Microbiology* 2007; 38: 381-384.
18. Athanasiadou S *et al.* Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. *Veterinary Parasitology*. 2001; 99: 205-19.
19. Thompson DP, Geary TG. The structure and function of helminth surfaces. In: *Biochemistry and Molecular Biology of Parasites*. 1st ed.; Marr JJ, ed. New York: Academic Press. 1995; pp. 203-32.