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Abstract

The antibacterial and antifungal activity of *Garcinia kola* of small and large seeds varieties were extracted in ethanol and water (cold and hot) and tested against some selected clinical bacterial and fungal isolates; *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis* and *Pseudomonas aeruginosa* at concentrations of 10, 20, and 30 mg/ml. The same concentrations were used against the fungi *Candida albicans* and *Aspergillus niger.* Agar well diffusion method was employed to determine the antimicrobial activity of the extracts against test microorganisms. The results showed that the small seed ethanol and aqueous (hot water) extracts exhibited more antimicrobial activity at a concentration of 30 mg/ml, with zones of inhibition ranging from 17 to 23mm for ethanol. The aqueous (hot water) extracts showed zones of inhibition ranging from 20 to 27mm. The extracts also showed antifungal activity against *Aspergillus niger*. The minimum inhibitory concentration (MIC) showed antimicrobial activity at lowest concentration of 0.008mg/ml and maximum concentration of 5.0mg/ml against *Staphylococcus aureus.* There were presence of phytochemical compounds such as flavonoids, tannins, saponins, steroids, cardiac glycosides, and reducing sugars. The results imply that the ethanol and aqueous extracts of *Garcinia kola* seed posses strong antibacterial and antifungal properties when compared with standard antibiotics amoxicillin; ciproxin; tetracycline and streptomycin used during the investigation, and hence its potential as a useful chemotherapeutic agent in the treatment of bacterial and fungal infections in humans.

Key Words: Phytochemical, *Garcinia kola* ,antibiotic, bacteriostatic,chemotherapeutic, resistant.

Introduction

They are physical or chemical agents that either kill or inhibit the growth of microorganisms. They could occur in form of physical or chemical agents such as temperature, radiations, sound waves, disinfectants, antiseptics, synthetic chemotherapeutic agents, antibiotics, and phytotherapeutic agents. They are widely employed to reduce microbial load on animate and inanimate surfaces or in the cure of diseases associated with microorganisms mostly bacteria and fungi. The action of these agents could either irreversibly inhibit growth of bacteria and

hence are said to be "bactericidal" or reversibly inhibit the growth of a microorganism due to continuous contact with the agent it is referred to as being " bacteriostatic" **(**Rajesh and Rattan, 2008**).** The emergence of resistance of bacteria to antibacterial drugs (antibiotics) today has become a common phenomenon Consequently antibiotic resistance has imposed both a biological cost as well as an economic cost (Chabot *et al.,* 1992; Chen *et al*.,1992; Chessin *et al*.,1995). Drug reaction and side effects, increased risk of malignancy, fake and adulterated drugs have added to the problem of antibiotic resistance (Green, 2007). In addition to the problem of treatment failure, most MRSA infections have often been associated with increased hospitalization periods and mortality (Engemann *et al*., 2003).

.A number of studies have validated the use of plants in the treatment of disease conditions. A typical example is *Garcinia kola*, a tropical plant of the African continent which has been the subject of investigation as a potential source of numerous antimicrobial compounds (Sibanda *et al*., 2010).

Garcinia kola, generally known in Nigeria as bitter kola or Guttiferae (Nosiri *et al.,*2010) is found in moist forest and grows as a medium size tree, up to 12 m high. It is cultivated and distributed throughout west and central Africa. It has been referred to as a 'wonder plant' because almost every part of it has been found to be of medicinal importance. It is commonly called "Agbilu" in Igbo land and "Namijin goro" in Hausa and "Orogbo" in Yoruba land of Nigeria (Adegboye *et al*., 2008). It produces a characteristic orange-like pod with seeds covered with a skin or husk. The edible seed is valued in Nigerian houses as a substitute for the true kola nuts (*Cola nitidais*). Generally, the mechanical cleansing effect and antimicrobial substances in the seed are seen as major beneficial effects of chewing this nut (Han *et al*., 2005, Nwaokorie *et al.,* 2010).

Phytochemical analysis of extracts from both root, stem and seed of Garcinia kola and other members of the genus show that they contain reasonable amounts of phenolic compounds including biflavonoids (GB-1,GB-2), xanthones and benzophenones (Onunkwo *et al*., 2004; Okunji *et al*., 2007).

Their antibacterial activities are due to flavonoids especially biflavonoid type GB1 which are well known for their antioxidant activities and this has been demonstrated using methicillin-resistant Staphylococcus aureus (MRSA), vancomycin- resistant enterococci (VRE) (Han et al., 2005; Nwaokorie *et al*., 2010).

The aims of this study were (i) to determine the antimicrobial and antifungal potency of *G.kola* seeds in different solvents, against a spectrum of pathogenic microorganisms (ii) it also investigated the bioactive and phytochemical potentials of the extracts from the plant seeds on selected pathogenic microorganisms.

Materials and Methods

Colection of plant materials

Fresh large and small seeds of *Garcinia kola* were purchased from OJa- oba market in Ilorin, Kwara State, Nigeria. The seeds were authenticated in the herbarium of the Plant Biology Department, University of Ilorin, Nigeria

Preparation of seed extract

Aqueous extraction (cold water)

The method described by Okigbo and Omodamiro (2006) and Okigbo and Mmeka (2008) was used with slight modifications.

Aqueous extraction (hot water

Twelve grams (12g) of the plant extract was soaked in 100ml of hot sterile distilled water boiled for 30 mins and added into a conical flask and agitated on a rotary shaker for 48hrs. The extract was filtered five times

using muslin cloth then stored at 4^{o}C in a refrigerator.

Organic solvent using ethanol

Twelve grams (12g) of the powdered plant material was soaked in 100ml of 95% ethanol for 24hrs at room temperature with occasional stirring. The content was filtered using sterile Whatman ® No.6 filter paper inserted in a funnel and the extract collected was stored in the refrigerator at 4^{o}C until required for use.

Collection and maintainace of test microorganisms

Test organisms were selected based on those available, which subsequently covered a broad range/spectrum of microorganisms (Gram positive and negative). Thus five bacteria isolates and two fungi were used as test organisms.

The organisms *Klebsiella pnuemoniae, Staphylococcus aureus , Escherichia coli and Candida albicans* were collected from University of Ilorin Teaching Hospital Ilorin, Kwara State, Nigeria, while B*acillus subtilis , Pseudomonas aeruginosa* and *Aspergillus niger* were collected from stock samples of the Microbiology Department University of Ilorin.

The bacteria isolates were maintained on Nutrient agar slant and stored at 4^{o}C in a refrigerator. *Candida albicans and Aspergilus niger* were maintained on Potato Dextrose agar (PDA) slants. Both bacteria and fungi were sub-cultured onto fresh media at regular intervals of three months to avoid contamination and loss of viability.

Sterility test of seed extract

Each of the six large and small seed extracts (ethanolic, aqueous hot and cold water) was tested for growth of contaminants.

One milliliter (1ml) of standard seed extract was inoculated aseptically unto Nutrient Agar and incubated at 37^{o} C for 24hrs. The plates were observed for any sign of visible growth. No growth on the plates indicated/signified that the extracts were sterile. The extracts were then assessed for antimicrobial and antifungal activity.

Sterilization of materials

Fawole and Oso (2007) methods were used to sterilize all glass wares

The test organisms

Distinct colonies from stored slants were inoculated using a sterile inoculating loop and needle (for bacteria and fungi respectively) onto sterile Nutrient agar and Potato dextrose agar plates and incubated at 37^{o}C for 24hrs for bacteria while fungi were incubated at 30^{o}C for 72hrs

Antimicrobial susceptible testing

Determination of antibacterial activities of extracts

The antibacterial activity of the plant extracts on the test organisms was determined using the agar well diffusion method described by Irobi *et al.,* (1994) with slight modifications.

Determination of antifungal activities of extracts

The agar diffusion method was used to determine the antifungal properties of the six extracts on. Potato Dextrose Agar on Petri dishes was seeded with inoculum of *C. albicans,* using cotton swabs. Wells of 5mm diameter were cut on the seeded plates using sterile cork borer. The agar plugs were carefully removed by use of sterile forceps. Each well was filled with 0.1ml of different concentrations of the seed extract.

Control experiments were set up with crude extracts and sterile distilled water used as positive and negative controls respectively. Plates were incubated at 30^{o}C and zones of inhibition were measured after 24hrs. Ketoconazole and streptomycin were used as standard antifungal agents to compare with the activity of the crude extract.

The radial growth method was used to assay for the extracts effect against *Aspergillus niger*, 1ml of various concentrations of the plant extract was separately plated into PDA. The agar-extract mixture was poured into sterile Petri dishes and allowed to gel (Smith, 1977; Oloke *et* al., 1988). Mycelia plugs of test fungus measuring 5mm in diameter were cut with a sterile cork borer from the advancing margin of fungal colonies. The plugs were placed at the center of each agar medium containing different concentrations of the seed extracts .

Control experiments were set up containing the mycelia plugs of test fungus with crude extracts as positive control while distilled water was used as negative control. All plates were incubated at 30^{o}C for 72hr.

2.7.3. Determination of minimum inhibitory concentration (MIC)

The MIC of the extract was determined using the method of Akinpelu and Kolawole (2004); Adegboyega *et al.,* (2008).

MBC of plant extract on bacterial Isolates

The MBC of the extract was determined using Adegboye *et al., (2008)*; method.

2.8 Antibiotics and antifungal used in this study

The following antibiotics which were available as powder, capsule and caplet were used in this study: Amoxycillin; Erythromycin; Sreptomycin Ciprofloxacin, and Ketoconazole.

2.8.1 Preparation of antibiotic dilution

The antibiotics used were reconstituted by dissolving 500 mg of powder/granules in a 500 ml of distilled water to get a concentration of 1.0 mg/ml; while 500 mg of powder in 250 ml of sterile distilled water gave a concentration of 2 mg/ml. The prepared dilutions of antibiotics were used to compare with the antimicrobial effect of the extract at concentration of 30 mg/ml. Reconstitution of antifungal was carried out in the same manner as antibiotics.

Phytochemical screening test for the extract

A small portion of the dry extract was subjected to the phytochemical test using Trease and Evans (1983) methods as described by Adegboye *et al., (2008)* to test for alkaloids, tannins, flavonoids, steroids, saponins, reducing sugars and cardiac glycoside.

Test for alkaloids

Exactly 0.5 g of the plant extract was dissolved in 5 ml of 1% HCl on steam bath.1ml of the filtrate was treated with two drops of Dragendorff's reagent. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

Test for tannins

One gram (1g) of the extract was dissolved in 20 ml of distilled water and filtered. Two to three drops of 10% of Ferric chloride ($FeCl_{3)}$ was added to 2 ml of the filtrate. The production of a blackish-blue or blackish-green colouration was indicative of tannins. To another 2 ml of the filtrate was added 1 ml of bromine water. A precipitate was taken as positive for tannins.

Test for flavonoids

A 0.2 g of the extract was dissolved in 2 ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated Hydrochloric acid (HCl). The occurrence of a red or orange colouration was indicated presence of flavonoids.

Test for saponins

Freshly prepared 7% blood agar medium was used and wells were made in it. The extract in methanol was applied with distilled water and methanol used as negative control, while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35^{0}C for 6 hrs, complete haemolysis of the blood around the extract was indicative of saponins.

2.9.5. Test for steroids

Half a gram (0.5 g) of the extract was dissolved in 3 ml of Methyltrichloride ($CHCl_3$) and filtered. To the filtrate was added concentrated Tetraoxosulphate vi acid (H_2SO_4) to form a lower layer. A reddish brown color was taken as positive indication for steroids.

Test for cardiac glycoside

Half gram (0.5 g) of the extract was dissolved in 2 ml of glacial acetic acid containing 1 drop of 1% Ferric chloride ($FeCl_3$). This was under laid with concentrated Tetraoxosulphate vi (H_2SO_4). A brown ring obtained at the interface indicated the presence of a deoxy sugar, characteristic of cardiac glycosides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring might form just above the ring and gradually spread throughout this layer.

Test for reducing sugars

The method of Tiwari *et al.*,(2010) was used for reducing sugars.1ml each of Fehling's solutions I and II was added to 2 ml of the aqueous solution of the extract. The mixture was heated in a boiling water bath for about 2 – 5 min. The production of a brick red precipitate indicated the presence of reducing sugars

Preparation of culture media

Three culture media were used during the course of the research were Nutrient agar (NA), Potato dextrose agar (PDA), and Nutrient broth. All media were prepared according to manufacturer's specifications.

Results and Disscussion

Table 1 compares the physical properties of pH values and the colour expressions of the various extracts. The pH values of all the aqueous extracts either hot or cold for both large and small seeds showed they were in the neutral range (7.0-7.3) while the ethanolic extracts showed lower pH ranges indicating they were acidic. The colour expression of the extracts ranges from a deep coffee brown to light cream, the darker shades of brown were more associated with the ethanolic solvent used which is believed to have released a large quantity of the phytochemical principles found in the extract.

The investigations done on *Garcinia kola* extract revealed that the plant possesses antimicrobial activities against the tested bacterial isolates which supports the conclusions of Mboto *et al*., (2009) and Nosiri and Abba (2010) they reported *Garcinia kola* exhibited antimicrobial effects on a microorganisms. *Garcinia kola* extract was found to exhibit its antimicrobial activity at a final concentration which ranged from 10 to 30 mg/ml (Tables 2 and 3) with maximum effect at 30mg/ml this is in agreement with Adegboye *et al*., (2008) they reported 20mg/ml as an effective final concentration for antimicrobial effect. The extracts exhibited activities against five test organisms out of seven except (*K.pneumoniae and C.albicans*) ,the bacterial isolates comprising of both Gram-positive and Gram- negative organisms. The results show that the extract possess broad spectrum activities. *S. aureus* showed the least zone of inhibition of 12 mm while *E. coli* had the highest zone of inhibition of 25 mm. This concurs with Ghamba *et al*., (2011), they also identified bitter kola to have strong antibiotic activities and found the plant to be very effective against disease causing microorganisms such as *E. coli, Staph. aureus, P. aeruginosa, Salmonella spp. Streptococcus spp, Candida albicans, Vibrio and Neisseria gonorrhea.*

The observation that all extracts of the small seed were effective against *S. aureus* indicates that the small seed extracts will be effective in the treatment of some staphylococcal infections (Table 3). This result agrees with findings by Sibanda *et al.*,(2010) they suggested the use of *G. kola* crude extracts at low concentration for treatment of staphylococcal infections .

On the other hand zones of inhibitions exhibited by standard antibiotics used

ranged from 12 to30 mm at final concentration of 1mg/ml while at concentration of 2mg/ml it ranged from16 to 30mm as presented in Table 4. Comparing the zones of inhibition by the four antibiotics used, Amoxycylin showed a broad spectrum of activity being more effective over *S. aureus* and it also displayed a tendency to maintain a saturation point since it tended to maintain similar diameter for zone of inhibition even at higher concentration (2mg/ml).

The phytochemical analysis of the extract of G. kola showed the presence of flavonoids, tannins, cardiac glycoside, steroids, saponins and trace reducing sugars (Table 5) this is in agreement with Adegboye *et al*.,(2008) and Eminedoki *et al*.,(2010).they also found similar products in *G kola* The small seed crude extract possess higher concentration of phytochemicals than the large seed crude extrtract.This might be attributed to its effectiveness on *Staphphylococcus aureus.*

These phytochemical compounds are known to play important roles in bioactivity of medicinal plants. The medicinal value of these plants lays in these phytochemical compounds and as such produce definite physiological actions in the human body.

Hodek *et al*., (2002) confirms that flavonoids which are part of the phytochemical constituents of *G. kola* exhibit a wide range of biological activities, one of which is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals, and thus promotes good health. Flavonoids also exhibit anti-inflammatory, antiangionic, anti-allergic effects, and analgesic and antioxidant properties. These observations support the usefulness of *G. kola* in folklore remedies for the treatment of various infections.

The Minimum Inhibitory Concentration of the plant extract against the tested bacterial isolates was also determined. The Minimum Inhibitory Concentration ranged from 0.008 to 5.00 mg/ml. The standard antibiotic, streptomycin had Minimum Inhibitory Concentration values ranging from 0.0157 to 0.5 mg/ml (Table 6).

- The results of the minimum bactericidal concentration of the seed extract against the test organisms showed that the extracts were effective when compared with the zones of inhibition of the standard antibiotics (Table 7). This agrees with Adegboye *et al*., (2008) they reported *G kola* 2008) seed extracts exhibited bactericidal effects very well when compared to standard antibiotics.

- The results of the minimum bactericidal concentration of the seed extract against the test organisms showed that the extracts were effective when compared with the zones of inhibition of the standard antibiotics (Table 7). This agrees with Adegboye *et al*., (2008) they reported *G kola* 2008) seed extracts exhibited bactericidal effects very well when compared to standard antibiotics.

- On the other hand, antifungal test of *G.kola* crude extracts on *C.albicans* showed no visible effect (Tables 8 and 9) indicating the extracts lack antifungal effect on *C. albicans* this is similar to the work reported by Okigbo and Mmeka (2008) they found *G kola* to have small effects on fungi. The result obtained from this study might be due to the concentration used, which were relatively low, consequently could not exert any antifungal effect on this fungus. This is however contrary to findings of Madubunyi (1995) who reported antifungal activity of *G. kola* extracts on *C. albicans.*. *Aspergilus niger* shows susceptibility to the extract with 30mg/ml being the most effective (Tables 8 and9).
- Table 10 shows the sensitivity of the antifungal Ketocnazole and Streptomycin. Both antifungal showed antifungal activity at higher concentration of 2mg/ml with zone of inhibition of 11mm for *A. niger* while *C. candida* exhibited zone of inhibition

of 15mm. this agrees with Banso (2005) whose findings indicate that at higher concentrations most antifungal exhibit higher antifungal activity.

Conclusion

G. kola seeds extract exhibited strong antibacterial activity against the tested clinical bacterial isolates at the different treatment regimens i.e. 10mg/ml, 20mg/ml and 30mg/ml concentration of the extracts. This may be attributed to the presence of these phytochemical compounds identified in this study. The low minimum inhibitory concentration values observed for the ethanol extracts are good starting point for further research that can lead to the isolation, purification and characterization of active compounds for the development of a new antimicrobial drug.

Table-1 Physical properties of *G. kola* crude extracts

S/No.	Type of seed	Medium of extraction	pH	Colour
1.	Large seeds	Alcohol	3.5	Coffee brown
2.	Large seeds	Cold water	7.3	Creamy brown
3.	Large seeds	Hot water	7.0	Cream
4.	Small seeds	Alcohol	4.0	Coffee brown
5.	Small seeds	Cold water	7.2	Brown
	Small seeds	Hot water	7.3	Brown

Table-2 The sensitivity patterns of some bacterial isolates to large seed crude extracts as measured by zone of inhibition.

Microorganism	Medium of extraction								
	Alcohol			Cold water			Hot water		
	Concentration								
	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)
	Zone of inhibition (mm)								
B. subtilis	14	18	20	11	13	16	20	20	24
E. coli	18	21	24	22	25	25	18	20	20
P. aeruginosa	14	19	21	11	15	18	13	17	17
K. pneumoniae	-	18	19	-	-	-	16	17	20
S.aureus	12	14	16	-	-	-	19	20	22

– =Resistant

*mm= Mean of three replicate

Table-3 The sensitivity patterns of some bacterial isolates to small seed crude extracts as measured by zone of inhibition

Microorganism	M**e**dium of extraction								
	Alcohol			Cold water			Hot water		
	Concentration								
	10 (mg/ ml)	20 (mg/ ml)	30 (mg/ ml)	10 (mg/m l)	20 (mg/m l)	30 (mg/m l)	10 (mg/m l)	20 (mg/ ml)	30 (mg/ ml)
	Zone of inhibition (mm)*								
B. subtilis	15	16	17	15	16	16	21	25	25
E. coli	16	21	21	19	21	23	18	20	22
P. aeruginosa	19	20	20	10	12	15	17	18	20
K. pneumoniae	-	-	-	-	-	-	-	-	-
S.aureus	12	14	23	21	25	29	22	25	27

─ ,=Resistant

*****mm= Mean of three replicates in mm

Table-4 Sensitivity patterns to standard antibiotics as measured by zone of inhibition

Microorganism	Zone of inhibition (mm)*****							
	Antibiotics							
	1 (mg/ml)				2 (mg/ml**)**			
	Amoxy	Cipro	TCN	Strep	Amox	Cipro	TCN	Strep
B. subtilis	-	-	-	-	-	-	-	-
E. coli	12	28	-	-	22	30	-	25
P. aeruginosa	13	-	18	12	16	-	21	13
K. pneumonia	29	20	-	30	23	20	-	-
S.aureus	23	28	-	22	23	-	-	-

─ =Resistant, *****mm= Mean of three replicates in mm
Amoxy = Amoxycilin
Cipro = Ciproxin
TCN = Tetracyclin
Strep = Streptomycin

Table-5 Some phytochemicals found in *Garcinia kola* crude extract.

	Phytochemicals	Type of seed	
S /No.		Large seeds	Small seeds
1.	Alkaloids	-	-
2.	Steroids	+	+
.3	Cardiac glycosides	+	+
4.	Flavonoids	+	++
5.	Tannins	+	++
6.	Saponins	+	+
7.	Reducing sugars	+	++

─ = Negative
+ = Positive
++ = High concentration

Table-6 The minimum inhibitory concentrations (MIC) exhibited by *G. kola* extracts against some selected bacterial isolates.

S /No.	Bacterial isolates	Concentration *G.kola* extract (mg/ml)					
	Medium of extraction						
		Ls OH	Ls cold	Ls Hot	Ss OH	Ss cold	Ss Hot
1.	*B. subtilis*	1.00	0.25	0.25	0.5	0.25	0.1
2.	*E. coli*	0.25	0.125	0.5	0.1	0.5	0.1
3.	*P. aeruginosa*	0.25	2.5	0.1	0.25	2.5	0.125
4.	*K. pneumoniae*	0.125	2.5	0.1	0.004	2.5	1.0
5.	*S.aureus*	0.008	5.0	5.0	0.008	5.0	5.0

Ls = Large seeds
Ss = Small seeds
OH = Ethanol

Table-7 The minimum bactericidal concentrations (MBC) exhibited by *G. kola* extracts against some selected bacterial isolates.

S /No.	Bacterial isolates	Concentration *G.kola* extract (mg/ml)					
	Medium of extraction						
		Ls OH	Ls **cold**	Ls Hot	Ss OH	Ss cold	Ss Hot
1.	*B. subtilis*	1.00	0.25	0.50	1.00	0.25	0.10
2.	*E. coli*	0.25	0.10	0.50	0.10	0.50	1.00
3.	*P. aeruginosa*	0.25	2.5	0.1	0.004	2.5	1.0
4.	*K. pneumoniae*	0.125	2.5	0.1	0.004	2.5	1.0
5.	*S.aureus*	0.25	5.0	5.0	0.008	5.0	5.0

Ls = Large seeds
Ss = Small seeds
OH = Ethanol

Table-8 The sensitivity patterns of some fungal isolates to Small seed crude extracts as measured by percentage inhibition.

Microorganism	Medium of extraction								
	Ethanol			Cold water			Hot water		
	Concentration								
	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)
	Zone of inhibition **(%)***								
A. niger	65.6	84.3	100	10.2	10.2	12.6	41.6	52.5	56.0
C.albicans	-	-	-	-	-	-	-	-	-

* %= Mean of three replicates in percentage
ー , =Resistant

Table-9 The sensitivity patterns of fungal isolate to large seed crude extracts as measured by percentage inhibition.

Microorganism	Medium of extraction								
	Ethanol			Cold water			Hot water		
	Concentration								
	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)	10 (mg/ml)	20 (mg/ml)	30 (mg/ml)
	Zone of inhibition **(%)***								
A. niger	33.4	38.2	53.4	10.1	20.5	26.1	30.8	42.6	48.3
C.albicans	-	-	-	-	-	-	-	-	-

* %= Mean of three replicates in percentage

—, =Resistant

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