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Original Research

Evaluation of Antibacterial and Antifungal Activity of Bioactive Chemical Compounds Isolated from *Candida albicans*

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Abstract:

Aims and Objective: The aims of this research were analysis of the bioactive chemical products of *C. albicans* and evaluation of antibacterial and in *vitro* antimicrobial activity of plant extracts on *Candida albicans.*

Method: Bioactives (chemical compounds often referred to as secondary metabolites) were analyzed using gas chromatography-mass spectroscopy (GC-MS) techniques, then the in *vitro* antibacterial and antifungal activity of the *Candida albicans* methanolic extract was evaluated.

Results: GC-MS analysis of *Candida albicans* revealed the existence of the: methyl ester-2-methyl-7,9-methyl-bor ,dicarbonyl-(n-4-pinocarvone)[1,2-bis(dimethylphosphi, Lycoxanthin , 12-Butanol , 3-chloro-,(R*,R*)- , 8,11-Octadecadiynoic acid , methyl ester , 6-Acetyl-β-d-mannose , Thieno[2,3-c]furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-tetra , 17-Octadecynoic acid , N,N'-bis(2-hydroxyethyl)- , 5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione , 1-Propyl-3,6-diazahomoadamantan-9-ol , Stearic acid , α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β- , Lactose , Cyclopropanebutanoic acid , Estra-1,3,5(10)-trien-17β-ol , Benzenemethanol,4-[(ethylpropyl)amino]-2-methyl-3,5-dinitro- and [1,1'-Bicyclopropyl]-2-octanoic acid , 2'-hexyl-,methyl ester, D-Glucose , 6-O-α-D-galactopyranosyl- , α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- , 6-Acetyl –β-d-mannose , 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- and Pyrimidin-2-ol,4-(3,4-dimethoxyphenyl)-6-phenyl, Orcinol, Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide, 2H-Oxecin-2-one.3.4.7.8.9.10-hexahydro-4-hydroxy-10-methyl-.[4, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), Maltol, 2-Tridecyl-5-(acetylamino)tetrahydro-γ-pyrone, Cycloundecanone , oxime, D-Glucose,6-O-α-D-galactopyranosyl, 6-Acetyl-ß-d-mannose, 5-Hydroxymethylfurfural, 1-Gala-l-ido-octonic lactone. *Candida albicans* metabolites was very highly active against *Staphylococcus epidermidis* (5.97±0.04).

Keywords: Candida albicans, Secondary metabolites, Antibacterial and Antifungal activity, GC/MS.

Introduction:

Candida albicans is a common commensal fungus that lives in the oropharynx, gut, and genitourinary tracts, as well as on the skin of healthy people. Diseases caused by Candida species can range from mild, skin-only mucocutaneous conditions to severe, systemic infections that pose a serious risk of death [12]. Candida's normal equilibrium can be disrupted by a number of variables, both systemic and local, genetic and environmental, leading to a shift from commensal to pathogenic and opportunistic infections. The virulence features of Candida that result in candidiasis also affect the pathophysiology of the infection's initiation and development [3]. Due to dysbiosis of the resident microbiota, immunological dysfunction, and disruption to the muco-intestinal barrier, *C. albicans* is able to thrive and spread throughout the body .Invasive Candida infections characterized by a high number of *C. albicans* in the blood are called candidemias. Using its virulence factors, Candida actively contributes to the pathophysiology of infection development and spread. Colonization, or the start of an infection, is caused by one set of virulence factors, whereas infection dissemination is aided by another. Angular cheilitis, median rhomboid glossitis, and chronic mucocutaneous candidiasis are just a few of the secondary and additional kinds of disease caused by Candida [4]. This study aimed to determine the antibacterial and in *vitro* antimicrobial activities of plant extracts against *Candida albicans*, as well as to analyze the bioactive chemical products of *C. albicans* .

Materials and Methods:

Growth conditions and determination of metabolites:

In potato dextrose agar slants, *Candida albicans* was isolated and kept alive [5]. Spores were raised in a liquid culture of potato dextrose broth (PDB) and cultured for 16 days at 25°C and 130 rpm. After the culture had been infused, the extraction was carried out by adding 25 ml of methanol to 100 ml of liquid culture in an Erlenmeyer flask. The mixture was shaken for 10 minutes at 130 rpm after being incubated at 4^{o}C for 10 minutes. By separating the metabolites from the liquid culture and drying them with a rotary evaporator at 45 °C. Before being used for GC-MS, the residue was dissolved in 1 ml of methanol, filtered through a 0.2µm syringe filter, and kept at 4 ^{o}C for 24 hours.

Spectral analysis of bioactive chemical compounds using gas chromatography-mass spectrometry (GC/MS)

Analysis was conducted using GC-MS (Agilent 789 A) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250oC). Ionization voltage was 70 eV and ion source temperature was 230oC. Scan range was 41- 450 amu. The components were identified by comparing their retention times to those of authentic samples of WILEY MASS SPECTRAL DATA BASE Library.

Determination of antibacterial and antifungal activity:

The test pathogens (*Escherichia coli*, *Staphylococcus aureus*, *Proteus mirabilis* and *Staphylococcus epidermidis*) were swabbed in Muller Hinton agar plates. 90µl of fungal extracts was loaded on the bored wells. The wells were drilled with a 0.5 cm diameter hole. The plates were inspected 24 hours after being incubated at 37C [6]. The diameter of the inhibition zones surrounding the discs was measured following the incubation. Candida albicans isolate was diluted to around 105 colony forming units (CFU) per milliliter before being suspended in potato dextrose broth. Onto the surface of potato dextrose

agar, they were "flood infected, and then dried. The conventional agar well diffusion method was used [7]. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Gramineae poaceae*, *Nerium olender*, *Ricinus communis*, *Datura stramonium*, *Linum usitatissimum*, *Anastatica hierochuntica*, *Cassia angustifolia*, *Euphorbia lathyrus*, *Rosmarinus oficinalis*, *Mentha viridis*, *Artemisia annua*, *Quercus infectoria*, *Citrullus colocynthis*, *Althaea rosea*, *Coriandrum sativum*, *Origanum vulgare*, *Urtica dioica*, *Equisetum arvense*, *Foeniculum vulgare*, *Nigella sativa*, and *Ocimum basilicum*,) were delivered into the wells. The plates were incubated for 48 h at room temperature. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent [67]. The tests were carried out in triplicate.

Statistical analysis:

On an SPSS (Version 11.6) database, the acquired data were examined using statistical tests including mean value and analysis of variance (ANOVA).

Results and Discussion:

The GC-MS chromatogram of the forty five peaks of the compounds detected were methyl ester-2-methyl-7,9-methyl-bor ,dicarbonyl-(n-4-pinocarvone)[1,2-bis(dimethylphosphi, Lycoxanthin , 12-Butanol , 3-chloro-,(R*,R*)- , 8,11-Octadecadiynoic acid , methyl ester , 6-Acetyl-β-d-mannose , Thieno[2,3-c]furan-3-carbonitrile,2-amino-4,6-dihydro-4,4,6,6-tetra , 17-Octadecynoic acid , N,N'-bis(2-hydroxyethyl)- ,5-Methyl-6-phenyltetrahydro-1,3-oxazine-2-thione , 1-Propyl-3,6-diazahomoadamantan-9-ol , Stearic acid , α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-,Lactose, Cyclopropanebutanoic acid , Estra-1,3,5(10)-trien-17β-ol , Benzenemethanol,4-[(ethylpropyl)amino]-2-methyl-3,5-dinitro- and [1,1'-Bicyclopropyl]-2-octanoic acid , 2'-hexyl-,methyl ester, D-Glucose , 6-O-α-D-galactopyranosyl- , α-D-Glucopyranoside , O-α-D-glucopyranosyl-(1.fwdarw.3)-β- , 6-Acetyl –β-d-mannose , 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- Pyrimidin-2-ol,4-(3,4-dimethoxyphenyl)-6-phenyl, Orcinol, Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide, 2H-Oxecin-2-one.3.4.7.8.9.10-hexahydro-4-hydroxy-10-methyl-.[4, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), Maltol, 2-Tridecyl-5-(acetylamino)tetrahydro-γ-pyrone, Cycloundecanone , oxime, D-Glucose,6-O-α-D-galactopyranosyl, 6-Acetyl-ß-d-mannose, 5-Hydroxymethylfurfural, 1-Gala-l-ido-octonic lactone.

Antibacterial activity of secondary metabolites of *Candida albicans* on five pathogenic bacteria

In the current study, Bioactivity of the methanolic extract of *Candida albicans* and standard antibiotics against the five tested pathogens *Escherichia coli* , *Staphylococcus aureus* , *Proteus mirabilis* , *Staphylococcus epidermidis*. Antibacterial activity recorded 3.00±0.02 , 5.00±0.04 , 4.12±0.03 and 5.97±0.04 for methanolic extract of *Candida albicans* metabolites. While recorded 1.03±0.01 , 2.17±0.01 , 3.59±0.02 , and 1.70±0.01 respectively for Streptomycin and 1.99±0.02 ,

1.89±0.01 , 0.93±0.01 , 2.04±0.01 respectively for Kanamycin. *Candida albicans* metabolites was very highly active against *Staphylococcus epidermidis* (5.97±0.04).

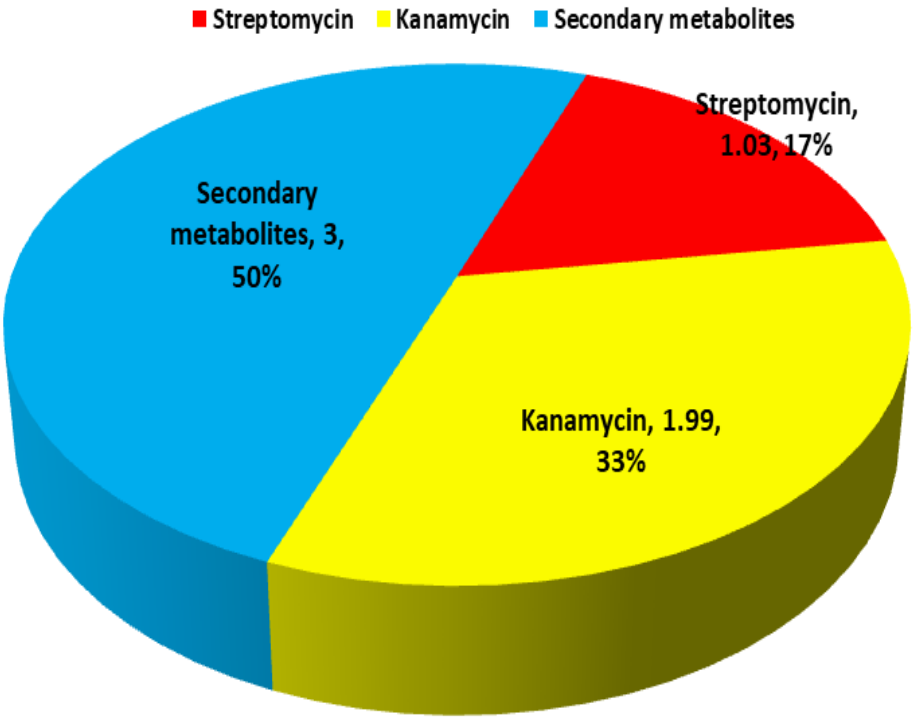

Figure 1. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Escherichia coli*

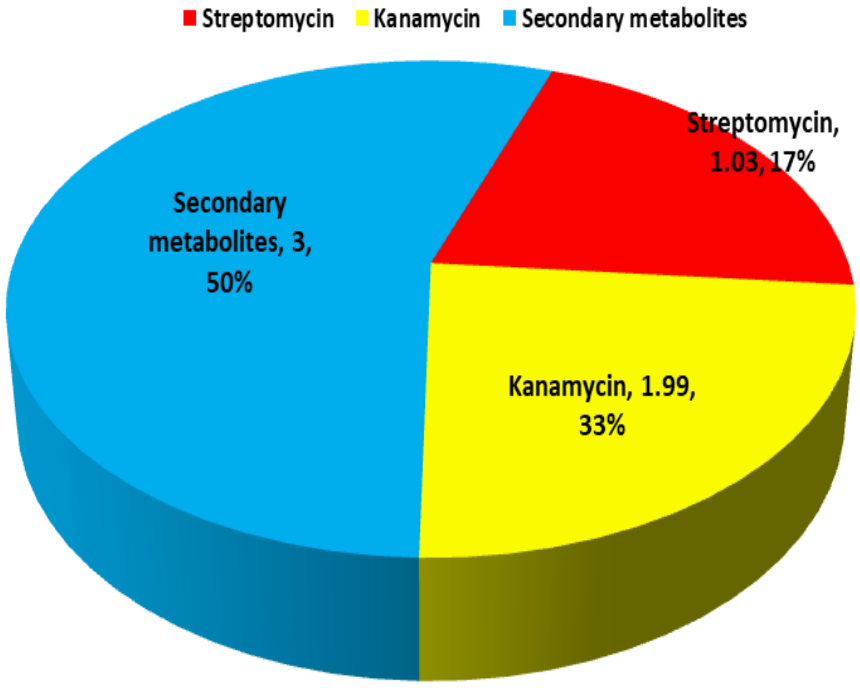

Figure 2. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Staphylococcus aureus*

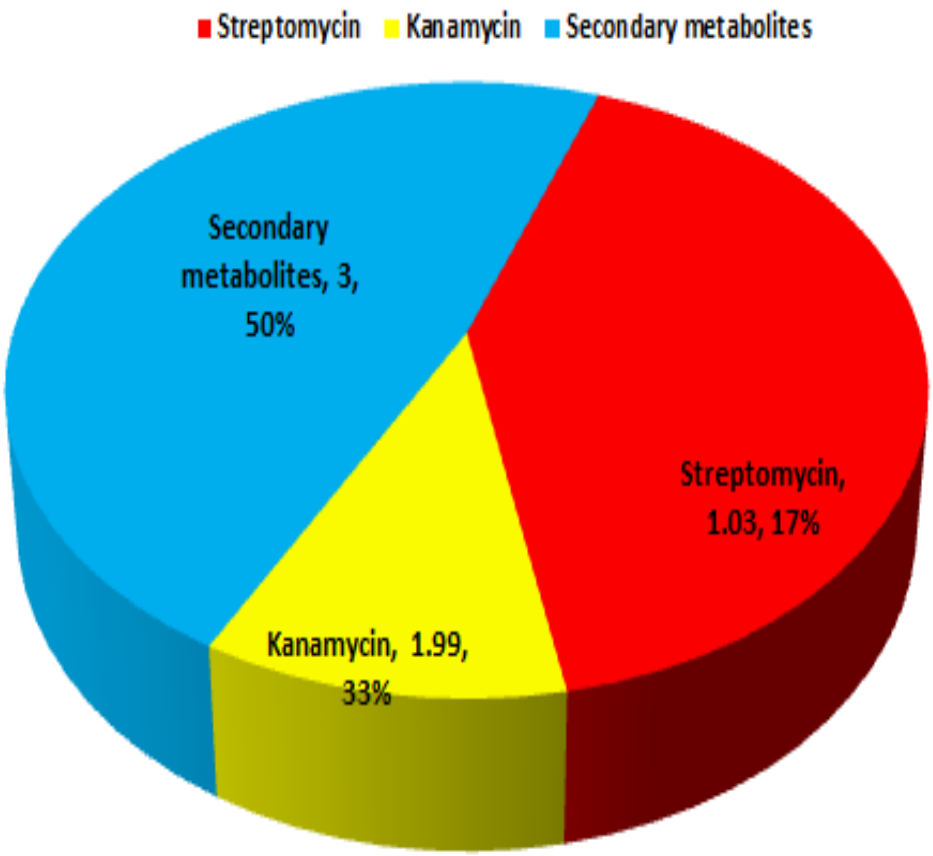

Figure 3. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Proteus mirabilis*

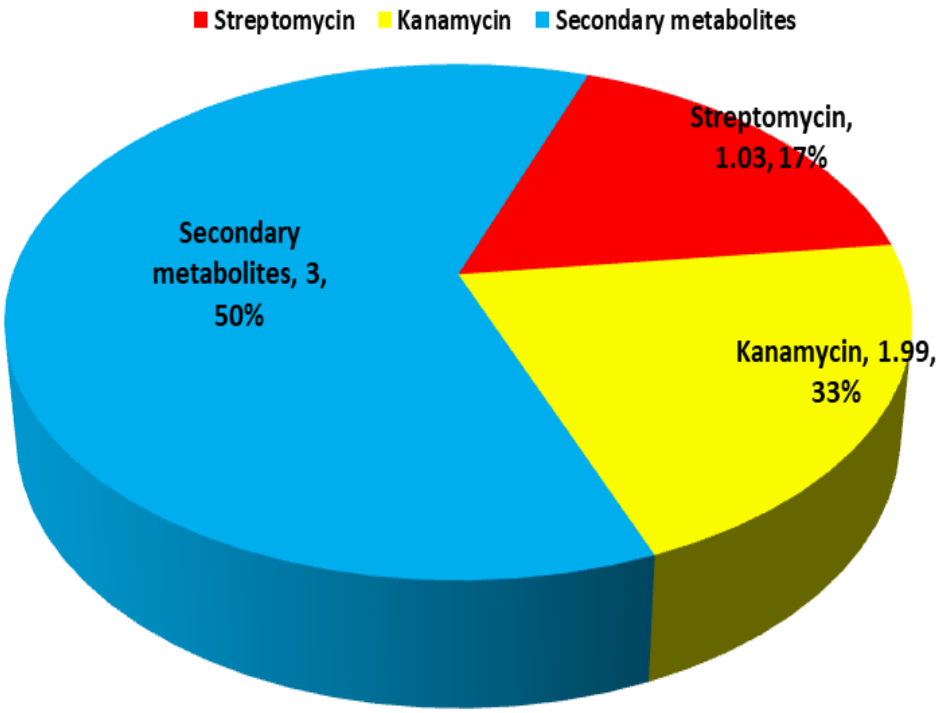

Figure 4. Metabolite products, Streptomycin and Kanamycin as anti-Bacterial activity against *Staphylococcus epidermidis*

Table 1. Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of plants to *Candida albicans*.

S. No.	Plant extract	Diameter of zones of inhibition (mm) After 48 hr.		Mean Standard Deviation
		Replicate 1	Replicate 2	
1.	*Gramineae poaceae* (Crude)	7	7.3	7.97±0.14
2.	*Nerium olender* (Alkaloids)	4.2	4.5	4.21±0.21
3.	*Ricinus communis* (Alkaloids)	2	2.7	2.94±0.20
4.	*Linum usitatissimum* (Crude)	4.7	5	4.97±0.15
5.	*Anastatica hierochuntica* (Crude)	5	5	5.11±0.19
7.	*Cassia angustifolia* (Crude)	7.0	6.5	6.35±0.26
8.	*Mentha viridis* (Crude)	6.0	4.7	5.59±0.15
9.	*Artemisia annua* (Crude)	5.3	4.9	5.73±0.29
10.	*Quercus infectoria* (Crude)	7.2	7.5	6.79±0.11
11.	*Citrullus colocynthis* (Crude)	4.5	5.0	4.21±0.21
12.	*Althaea rosea* (Crude)	6.0	5.4	5.99±0.16
13.	*Coriandrum sativum* (Crude)	5.7	5.0	5.82±0.19
14.	*Melia azedarach* (Crude)	4.1	3.6	3.98±0.26
15.	*Origanum vulgare* (Crude)	6.2	6.0	6.09±0.29
16.	*Urtica dioica* (Crude)	3.7	3.0	3.78±0.15
17.	*Equisetum arvense* (Crude)	5.6	5.9	5.69±0.14
18.	*Foeniculum vulgare* (Crude)	3.0	4.2	3.72±0.19
19.	*Nigella sativa* (Crude)	4.8	4.5	4.55±0.35
20.	*Ocimum basilicum* (Crude)	4.0	4.0	4.67±0.19
21.	Amphotericin B	7.2	7.5	7.55±0.24
22.	Fluconazol	6.9	7.0	7.98±0.16
23.	Control	0.0	0.0	0.0

In *vitro* antimicrobial activity of plant extracts on *Candida albicans*

Diameter of zones of inhibition (mm) After 48 hr. for two repeated were (*Gramineae poaceae* (Crude) (7.0 and 7.3 mm), *Nerium olender* (Alkaloids) (4.2 and 4.5 mm), *Ricinus communis* (Alkaloids) (2 and 2.7 mm), *Linum usitatissimum* (Crude) (4.7 and 5.0 mm), *Anastatica hierochuntica* (5 and 5 mm), *Cassia angustifolia* (Crude) (7.0 and 6.5 mm), *Mentha viridis* (6.0 and 4.5 mm), *Artemisia annua* (Crude) (5.3 and 4.9 mm), *Quercus infectoria* (Crude) (7.2 and 7.5 mm), *Citrullus colocynthis* (Crude) (4.5 and 5.0 mm), *Althaea rosea* (Crude) (6.0 and 5.4 mm), *Coriandrum sativum* (Crude) (5.7 and 5.0), *Melia azedarach* (Crude) (4.1 and 3.6 mm), *Origanum vulgare* (Crude) (6.2 and 6.0), *Urtica dioica* (Crude) (3.7 and 3.0 mm), *Equisetum arvense* (Crude) (5.6 and 5.9 mm), *Foeniculum vulgare* (Crude) (3.0 and 4.2 mm), *Nigella sativa* (Crude) (4.8 and 4.2), and *Ocimum basilicum* (Crude) (4.0

and 4.0 mm) were effective against *Candida albicans*, Table 1. *Quercus infectoria* (Crude) (7.2 and 7.5 mm) was very highly active against *Candida albicans*. There are a number of potent antifungal medications available for the treatment of candidiasis. However, following treatment, isolates may display intrinsic or secondary drug resistance [8]. Therefore, using natural compounds as substitute agents to control fungi is thought to be an intriguing alternative to synthetic fungicides [9]. Although phytochemicals (metabolites derived from plants) have antibacterial properties, they also induce immunity in the oral cavity, which indirectly lowers the incidence of oral illnesses [10]. According to [11], mouthwash containing 20 ml of a tea catechin solution has an antiplaque effect for up to 90 minutes. Menthol, menthone, methyl esters, and terpenoids, or the derivatives of monoterpenes, make up the majority of the compounds in mint leaves. Terpenoids primarily damage membranes of microorganisms to exert their antimicrobial action. Aloe vera gel's chemical makeup is a complicated blend. It includes a latex ingredient that has been proven to have bacteriostatic properties. Plant extracts produce an unfavorable oral environment for microbe. This alters the oral environment and inadvertently inhibits bacteria growth. Although phytochemicals (metabolites derived from plants) have antifungal properties, they also induce immunity in the oral cavity, which indirectly lowers the incidence of oral illnesses [12].

Conclusion:

The current study identifies a few easily accessible household medicinals that have the potential to be employed as alternative medicines and as adjuncts to conventional therapy. This information will be of great use to developing countries with inadequate oral health care facilities for populations and limited financial resources. The metabolites of *Candida albicans* were extremely effective in inhibiting the growth of Staphylococcus epidermidis.

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References:

1. Deorukhkar S.C. Virulence Traits Contributing to Pathogenicity of Candida Species. J. Microbiol. Exp. 2017;5.
2. Gale C., Gerami-Nejad M., McClellan M., Vandoninck S., Longtine M.S., Berman J. Candida albicans Int1p Interacts with the Septin Ring in Yeast and Hyphal Cells. Mol. Biol. Cell. 2001;12:3538–3549.
3. Maza P.K., Bonfim-Melo A., Padovan A. Candida albicans: The ability to invade epithelial cells and survive under oxidative stress is unlinked to hyphal length. Front. Microbiol. 2017;8
4. Moyes D.L., Wilson D., Richardson J.P., Mogavero S., Tang S.X., Wernecke J., Höfs S., Gratacap R.L., Robbins J., Runglall M., et al. Candidalysin is a fungal peptide toxin critical for mucosal infection. Nature. 2016;532:64–68.
5. Hussein JH, Ubaid JM, Hameed IH. Gas chromatography – mass spectrum analysis of volatile components of methanolic leaves extract of Cordia myxa. International Journal of Current Pharmaceutical Review and Research. 2017; 7(6).
6. Kadhim MJ, Kaizal AF, Hameed IH. Medicinal plants used for treatment of rheumatoid arthritis: A review. International Journal of Pharmaceutical and Clinical Research. 2017; 8(11).
7. Hameed, I.H., Al-Rubaye A.F. and Kadhim, M.J. Antimicrobial Activity of Medicinal Plants and Urinary Tract Infections. International Journal of

Pharmaceutical and Clinical Research. 2017; 8(11).

8. Bianchi A, Zambonelli A, D′Aulerio AZ. Ultra structural studies of effects of allium sativum on phytopathogenic fungi in vitro. Am Phytopathol Soc 1997;81:1241-6.
9. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564-82.
10. Haslam E. Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. J Nat Prod 1996;59:205-15.
11. Hamilton-Miller JM. Anti-cariogenic properties of tea (Camellia sinensis). J Med Microbiol 2001;50:299-302
12. Osato JA, Santiago LA, Remo GM, Cuadra MS, Mori A. Antimicrobial and antioxidant activities of unripe papaya. Life Sci 1993;53:1383-9