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## Analysis of phytochemical profile and antibiofilm activity of Stem Bark extract of *Terminalia Arjuna Wt & Arn* against the human pathogen *Candida albicans*

**Bhagyashree Shridhar Bansode, Ashwini Khanderao Jadhav, Namdev Vitthal Ghule, Datta Phule, R Srinivas, G Shankar and Sankunny Mohan Karuppaiyl**

### Abstract

The bark of *Terminalia arjuna* (Roxb.) *Wt.* and *Arn.* is used in Ayurveda. Biofilm-related infections caused by *Candida albicans* is a serious clinical challenge for immune compromised populations. The preliminary phytochemical analysis of water extract of arjuna stem bark showed presence flavonoids, phenolic compounds, carbohydrates, tannins and glycosides. FTIR analysis confirmed the presence of alcohol, alkanes, Carboxylic acids and aliphatic compounds. Extract was analysed for activity against growth and virulence attributes of *Candida*. It showed minimum inhibitory concentration (MIC) at 0.5 mg/ml for planktonic growth and 4 mg/ml for Biofilm of *C. albicans* ATCC 3017 and 4 mg/ml for planktonic growth of clinical strain of *Candida albicans* GMC 03. Extract also inhibited yeast to hyphal dimorphism at (0.5–4 mg/ml), while adhesion to a solid surface at 0.5–2 mg/ml. For the first time, Arjuna stem bark extract revealed the antibiofilm potential and ten phytochemicals are identified through GCMS analysis.

**Keywords:** *Terminalia arjuna*, phytochemicals, biofilm, *Candida albicans*, drug resistance

### 1. Introduction

*Terminalia arjuna Wt & Arn*, commonly known as Arjuna is a tree which belongs to the family Combretaceae. It is found in abundance throughout Uttar Pradesh, South Bihar, Madhya Pradesh, Delhi and Deccan regions of India and also seen in forests of Sri Lanka, Burma, mountains. In the Indian system of medicine, ayurveda the bark has been used as a coolant, astringent, aphrodisiac, cardiogenic, infarctions, ulcers, spermatorrhoea, leucorrhoea, diabetes, cough, tumour, excessive perspiration, asthma, inflammation and skin disorders etc. The bark powder has been found to possess cardioprotective, anti-ischemic, antioxidant action [1], hypercholesterolemia properties [2], fungicidal, antibacterial [3], Anti-inflammatory, immunomodulatory and antinociceptive properties [4]; it is also useful for the treatment of obesity, hypertension and hyperglycemia [5].

*Candida albicans* is an opportunistic fungal pathogen of the humans, it colonize the gastrointestinal (GI) tract, reproductive tract, oral cavity, and skin of most humans [6, 7]. *Candida* can form biofilms on the surface of various solid materials, which are used for medical prostheses. The National Institutes of Health estimate that biofilms are responsible for 80% of all microbial infections in the United States [8]. In individuals with healthy immune systems, *C. albicans* is often harmless, kept in balance with other members of the local microbiota. However, alterations in the host microbiota (e.g. due to antibiotics), changes in the host immune response (e.g., during stress, infection by another microbe, or immunosuppressant therapy), or variations in the local environment (e.g. shifts in pH or nutritional content) can enable *Candida albicans* to overgrow and cause infection. These infections range from superficial mucosal and dermal infections, such as thrush, vaginal yeast infections, and diaper rash, to hematogenously disseminated infection with sizable mortality rates (approaching 40% in some cases) [9]. *Candida* biofilms show resistance to anti-fungal agents including the widely prescribed anti-fungal drug, Fluconazole [10]. Emergence of resistance to conventional antibiotics and side effects of the synthetic chemical drugs have increased the problems in antimicrobial therapy [11]. In such circumstances use of products of natural origin for anti-infective and therapeutic purposes is attractive.

In this study for the first time the antibiofilm and antivirulence properties of the extract is reported against the human pathogen, *Candida albicans*. We are reporting the phytochemistry of the water extract of the Bark of *Terminalia arjuna Wt & Arn* which revealed the presence of Bio-active constituents which are known to exhibit medicinal as well as physiological activity.

## 2. Materials and Methods

### 2.1. Collection, authentication and preparation of extracts of tree bark

Tree barks were collected from Kinwat, Maharashtra State of India in the month of September 2015. It was authenticated by a Botanist Dr. R. M. Mulani. Barks were sliced into small pieces, shade dried and then ground into a powder using an electrical blender. The powder was subjected to solvent extraction so that 25 gm of powder was extracted in 250 ml of distilled water for 5 h. The extract was filtered and concentrated and the obtained powder was weighed and kept in sterile bottles under refrigerated conditions until use. For activity testing the solutions of water extract of *Terminalia arjuna* Wt & Arn tree bark was prepared by dissolving known quantity of the extract in DMSO.

### 2.2 Phytochemicals analysis

Phytochemical analysis of the test sample was carried out according to standard methods [12-15].

#### 2.2.1. Ferric chloride test for phenolic compounds and tannins

Take 2 ml of extract in a test tube and add ferric chloride solution drop by drop. Appearance of bluish black precipitate indicated presence of phenolic compounds and tannins.

#### 2.2.2 Molisch's test for carbohydrates

The extract was mixed with Molisch reagent, and then added conc. H<sub>2</sub>SO<sub>4</sub> along the sides of the test tube to form layers. Appearance of reddish violet ring the interference indicated the presence of carbohydrates.

#### 2.2.3 Ninhydrin test for proteins

Few drops of ninhydrin added to the extract. Appearance of blue colour indicated presence of amino acid whereas proteins may rarely give positive result.

#### 2.2.4 Lead acetate test for flavonoids

To the alcoholic solution of the extract add few drops of 10% lead acetate solution. Appearance of yellow precipitate indicated presence of flavonoids.

#### 2.2.5 Keller-Killiani test for glycosides

A total of 1mL of glacial acetic acid, few drops of ferric chloride solution and conc. H<sub>2</sub>SO<sub>4</sub> (Slowly through the sides of the test tube) were added to the extract. Appearance of reddish brown ring at the junction of the liquids indicated the presence of de-oxy sugars.

### 2.3. Culture, media, chemicals and culture conditions

*Candida albicans*, ATCC 3017 was obtained from the Institute of Microbial Technology, Chandigarh, India. The strain was maintained on Yeast-Peptone-Dextrose (YPD) agar slants at 4° C. A single colony from the yeast extract-peptone-dextrose (YPD) agar plates was inoculated in 50 ml of YPD broth (pH 6.5), in a 250 ml Erlenmeyer flask. The flasks were incubated at 30 °C on an orbital shaker at 120 rpm for 24 h. Cells from the activated culture were harvested by centrifugation for 5 min at 2000 g speed. Collected cells were washed three times and resuspended with phosphate buffer saline (PBS) (10mM phosphate buffer, 2.7 mM potassium chloride and 137 mM sodium chloride, pH 7.4). For susceptibility testing, RPMI-1640 medium with L-glutamine, without sodium bicarbonate and buffered with 165 mM

MOPS (3-[N-morpholine] propane sulphonic acid) pH 7, was prepared and filter-sterilized using 0.2 um, bacteriological filters. Various concentrations of extracts were prepared in RPMI-1640 medium by double dilution.

Concentration of the solvent used i.e. Dimethyl Sulfoxide (DMSO) was never >1 %. Whereas Fluconazole was used as a standard antifungal drug. Fluconazole (Forcan, Cipla Pvt. Ltd., Mumbai, India) was purchased from local market. 2, 3-bis (2-methoxy-4-nitro-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) and menadione were purchased from Sigma-Aldrich Chem. Ltd. Mumbai, India.

### 2.4. Minimum inhibitory concentration (MIC) for planktonic growth

Effect of extract on the growth of planktonic cells of *Candida albicans* was studied by using the standard broth micro dilution methodology, as per Clinical and Laboratory Standards Institute (CLSI) guidelines [16]. Briefly, various concentrations of the extract (ranging from 0.25 to 4 mg/ml) were prepared in RPMI-1640 medium, in 96 well plates. Wells without test extract served as a control. Inoculum from washed *Candida albicans* cells were added to each well so that to get 1×10<sup>3</sup> cells/ml. The plates were incubated at 35° C for 48 h. To analyze the growth, absorbance was read using a microplate reader (at 620 nm) (Multiskan EX, Thermo Electron Corp., USA). The lowest concentrations of extract which caused 50% reduction in the absorbance compared to that of control were considered as minimum inhibitory concentrations (MICs) for growth of *Candida albicans* [17].

### 2.5. Adhesion assay

Effect of extract on the adherence of *Candida albicans* to a solid surface (i.e. polystyrene) was studied using a microplate based assay. Various concentrations (0.25 – 4 mg/ml) of extract were prepared in PBS. Wells without extract was kept as controls, while Fluconazole was used as a standard antifungal drug. Fifty micro liters of cell suspension was added to each well to obtain 1 × 10<sup>7</sup> cells/ml. The final volume of the assay system in each was kept at 100 µl. The plates were incubated at 37 °C for 90 min at 100 rpm in an orbital shaking incubator to allow attachment of cells to the surface. After the incubation, wells were washed with PBS to remove non-attached cells. The density of the adherence in each was analyzed as relative metabolic activity (RMA) using the XTT-assay. More than a 50% reduction in RMA compared to the control was considered significant [18, 19].

### 2.6. Morphogenesis

Serum induced yeast to hyphal morphogenesis in *Candida albicans* was studied in a microplate-based assay. Various concentrations of test molecules were prepared in 20% serum in deionized water. Cells were inoculated to get 1 × 10<sup>6</sup> cells/ml, in test and control wells. Final volume of assay system in each well was kept at 200µl. The plate was incubated at 37 °C at 200 rpm; on an orbital shaker for 2 h. Later cells were observed microscopically for formation of germ tube forms. Every time 100 cells were counted and the number of yeast and hyphae were noted. Concentration which inhibited ≥ 50% of hyphae compared with that of control was considered the MIC for morphogenesis [19, 20].

### 2.7. Biofilm formation

*Candida albicans* biofilms were developed on polystyrene surface of 96-well plates as per standardized in vitro biofilm

model. Cell suspension of  $1 \times 10^7$  cells /ml was prepared in PBS and 100  $\mu$ l was inoculated in each well. In the adhesion phase, plate was incubated at 37 °C for 90 minutes at 100 rpm to allow attachment of cells on the surface. Non-adhered cells were removed by washing the wells by sterile PBS for two to three times. Two hundred  $\mu$ l of the RPMI-1640 medium was added to each well and the plates were incubated at 37 °C for 48 h to allow biofilm formation. To observe its effect on development of biofilms, RPMI-1640 medium along with various concentrations (i.e. 0.25 to 4 mg/ml) of the extracts were added to each well immediately after adhesion phase and incubated for 48 h at 37 °C. To analyze activity against mature *Candida albicans* biofilms, the extracts were added to 24 h mature biofilms and further incubated for 48 h at 37 °C. After incubation, wells were washed to remove any nonattached planktonic cells. The presences or absence of biofilms structure in the wells were confirmed by inverted light microscope (Metzer, India) and Photographs were captured by Labomed microphotography system (Labomed, India) at x 200 magnification. Finally, biofilm growth was analyzed and confirmed with XTT metabolic assay [17].

### 2.8. Biofilm Quantitation by XTT assay

Biofilm growth was quantitated using XTT i.e. [2, 3-bis (2-methoxy-4-nitro-sulphophenyl)-2H-tetrazolium-5-carboxanilide] metabolic assay. The XTT solution was prepared by mixing 1 mg ml<sup>-1</sup> XTT salt in PBS and stored at 20 °C. Prior to use, menadione solution prepared in acetone was added to XTT obtain final concentration of 4  $\mu$ M. The wells containing biofilms were washed with PBS to remove non adhered cells. Later 100  $\mu$ l of XTT-menadione solution was added to the wells and incubated for 5 h in dark, at 37° C. The color formation by water soluble formazan product was measured at 450 nm using a microplate reader (Multiskan EX, Thermo Electron Corp., USA). Wells without extract were considered as control, while those without biofilms were the blank. The concentration of extract which caused  $\geq 50\%$  lowering in RMA was considered MIC for biofilm [19, 21].

### 2.9. Microscopic analysis

Germ tube formation and biofilms were observed under an inverted light microscope (Metzer, India). Photographs were taken by a Labomed microphotography system (Labomed Korntal, Germany) at x 200 magnification. Further for scanning electron microscopy (SEM), samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 hour at 4 °C. Samples were post-fixed in 2% aqueous solution of osmium tetroxide for 4 hour, then dehydrated in a series of graded alcohols and finally dried to a critical drying point with a Critical point dryer unit. The samples were mounted over stubs, and gold coating will be performed using an automated gold coater (model JOEL JFC-1600) for 3 min. Photographs was taken under a scanning electron microscope (Model JOEL-JSM 5600) [19, 22].

### 2.10. Statistical analysis

Values mentioned are the mean with standard deviations obtained from three different observations. Values in the

control and treatment groups for various molecules as well as results obtained in XTT were compared using Student's t - test. A value of P < 0.05 was considered statistically significant [23].

### 2.11. GC- MS analysis

The GC-MS analysis were performed using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a 5973N mass selective detector (a single quadrupole instrument) and a HP-5MS capillary column of 30 m length, 250  $\mu$ m internal diameter (i.d.) and 0.25  $\mu$ m film thickness was used. The column oven was programmed initially from 50 °C with 2 min holdup time to the final temperature of 280 °C with 10 °C/min ramp and the final temperature holdup time was 5 min (Total run time 30 min). Helium gas was used as the carrier gas in constant flow mode with flow rate of 1.0 ml/min. The inlet and GC/MS interface temperatures were kept at 200 °C and 280 °C, respectively. The samples were introduced in splitless injection mode. The temperatures of the EI source and quadrupole analyzer were kept at 230 °C and 150 °C, respectively. The mass spectrometer was scanned from m/z 29 to 600.

## 3. Results

### 3.1 Phytochemical analysis

The preliminary phytochemical analysis of *T. arjuna* Wt. & Arn. stem bark water extract showed the active compounds presence in high concentration, such as flavonoids, phenolic compounds, carbohydrates, tannins and glycosides (Table 1).

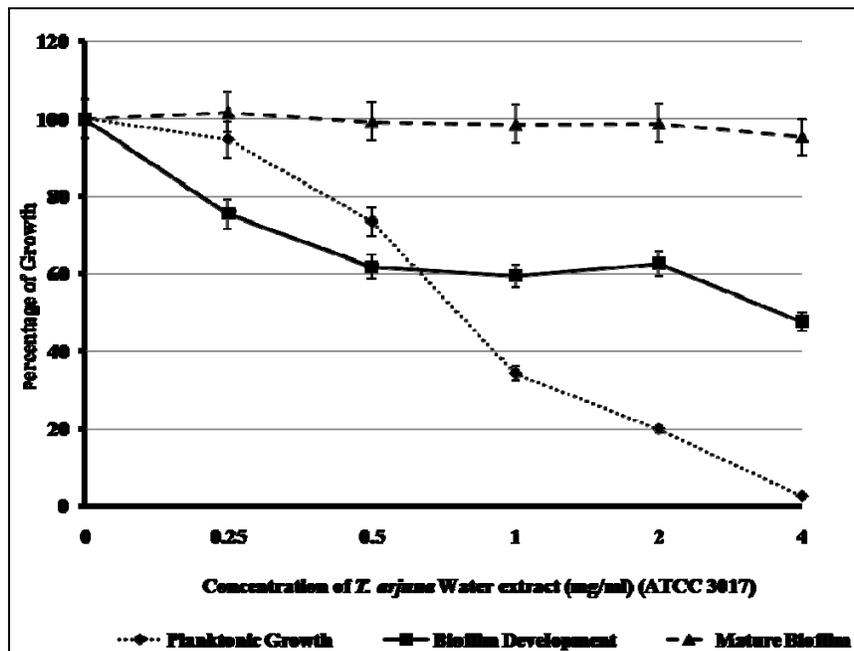
**Table 1:** Preliminary phytochemical analysis of *T. arjuna* Wt & Arn bark extract

| Phytoconstituents  | Test Name              | Conclusion |
|--------------------|------------------------|------------|
| Phenol Test        | FeCl <sub>3</sub> Test | +++        |
| Carbohydrates Test | Molisch's test         | ++         |
| Amino acid test    | Ninhydrin Test         | -          |
| Flavonoids test    | Alkaline test          | +++        |
| Glycosides test    | Keller Kelliani's test | ++         |

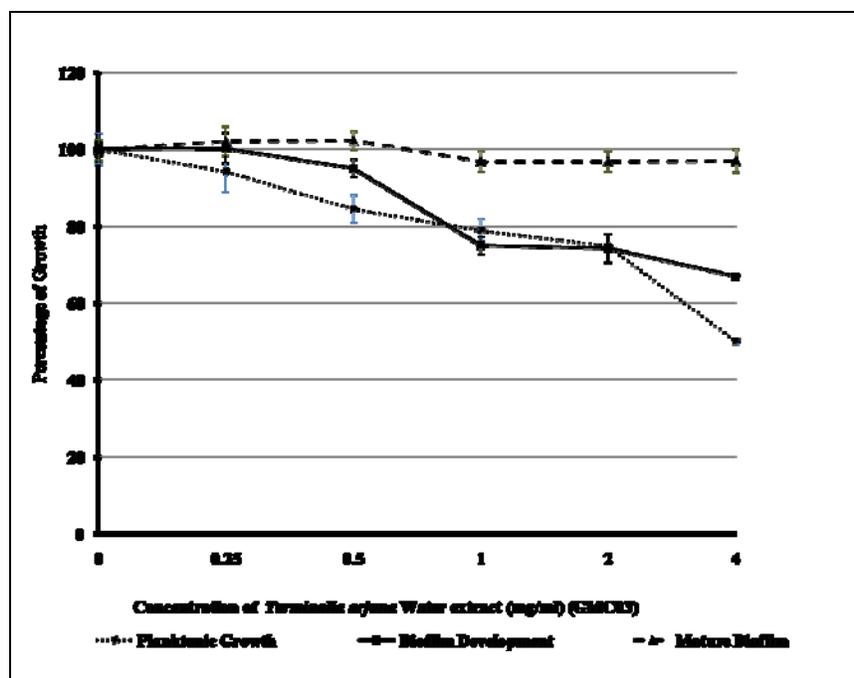
+ Present in low concentration; ++ Present in high concentration; +++ Present in very high concentration; - Absent.

### 3.2 Activity of *T. arjuna* stem bark water extract against planktonic and Biofilm growth of *C. albicans*

Water extract of *Terminalia arjuna* Wt & Arn showed MIC at a concentration of 0.5 mg/ml for planktonic growth and 4 mg/ml for Biofilm of *C. albicans* ATCC 3017 (Figure 1) and 4 mg/ml for planktonic growth of clinical strain of *Candida albicans* GMC 03 (Figure 2). Mature biofilms of *Candida albicans* consisted of multiple layers of a dense network of filamentous and yeast form cells. Extracellular polymeric substances secreted by biofilm cells are deposited along the entire cellular network which gives a thick biofilm structure. In mature stages, biofilms are more resistant to environmental factors as well as antifungal drugs. In the present study water extract of *Terminalia arjuna* Wt & Arn was not showed any inhibitory effect on mature biofilm of *Candida albicans* (Figure 1 and Figure 2).



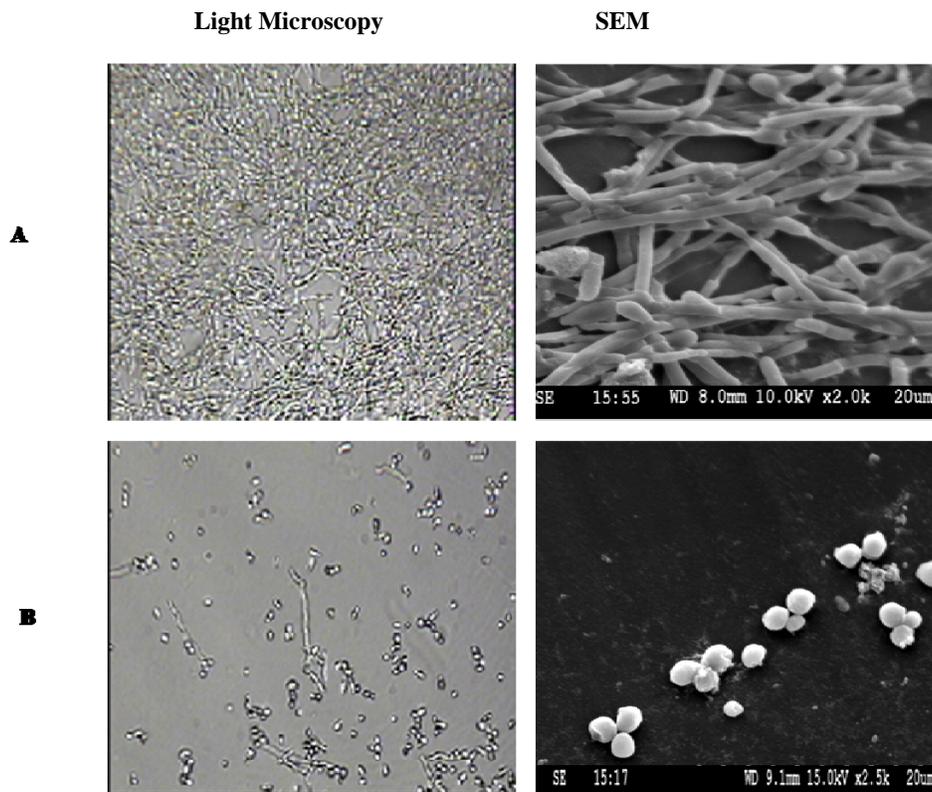
**Fig 1:** Effect of water extract of *Terminalia arjuna* Wt & Arn on Planktonic growth, Developing Biofilm and Mature Biofilm of *Candida albicans* ATCC 3017. The percentage biofilm formation by *C. albicans* ATCC 3017 in the presence of the water extract of *Terminalia arjuna* Wt & Arn was analysed as a function of the Relative Metabolic Activity in an XTT-assay.



**Fig 2:** Effect of water extract of *Terminalia arjuna* Wt & Arn on Planktonic growth, Developing Biofilm and Mature Biofilm of *Candida albicans* Clinical strain GMC 03. The percentage biofilm formation by Clinical strain of *C. albicans* GMC 03 in the presence of the Water extract of stem bark of *Terminalia arjuna* Wt & Arn was analyzed as a function of the RMA in an XTT-assay.

Prevention of biofilm was also confirmed with microscopic observations which showed the absence of characteristic biofilm structure at the MICs of the water extract of stem bark of *Terminalia arjuna* Wt & Arn (Figure 3). Treatments with effective concentrations of water extract of *Terminalia arjuna* Wt & Arn caused removal of *C. albicans* biofilm cells. Only a

few hyphae and yeast forms were seen to remain on the solid surface (Figure 3 (B)) compared to the dense network of cells in the control (Figure 3 (A)). Formation of a dense network of filamentous forms and yeast cells was completely prevented. The antibiofilm activity was confirmed by Scanning Electron Microscopy (SEM) (Figure 3 Second Column).

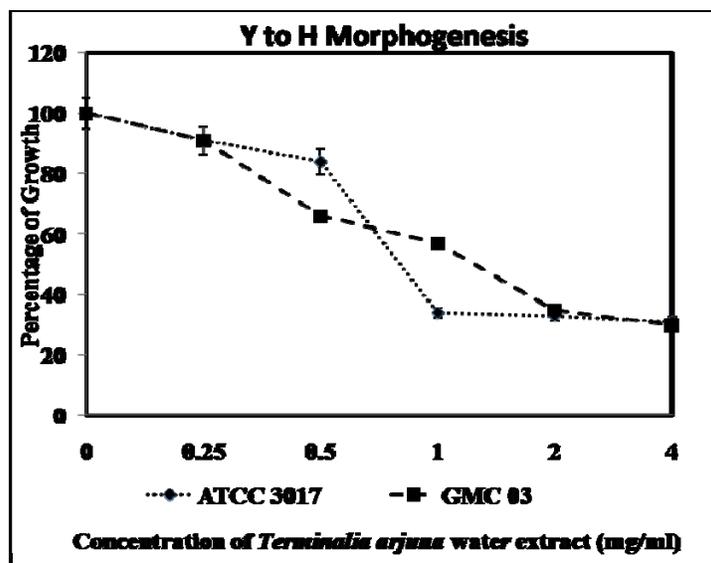


**Fig 3:** Effect of water extracts of stem bark of *Terminalia arjuna* Wt & Arn. on biofilm formation by *Candida albicans*. Panels in first column show light photomicrographs (magnification x 200) while, the second column represent scanning electron micrographs under different treatment conditions. (A) Control (*C. albicans* ATCC 3017) (B) represent biofilm preventive activity of Water extracts of *Terminalia arjuna* Wt & Arn at 4 mg per ml Concentration.

**3.3 Activity of *T. arjuna* stem bark water extract on Yeast to hyphal morphogenesis and Adhesion property of *Candida***

Yeast to hyphae switching was exhibited with an MIC at

concentration of 1 mg/ ml of ATCC 3017 and 2 mg/ml of *C. albicans* GMC 03 ( Figure 4) by *T. arjuna* stem bark water extract.



**Fig 4:** Effect of water extract of stem bark of *Terminalia arjuna* Wt & Arn. against yeast (Y) to hyphae (H) morphogenesis of *Candida albicans* 3017 and GMC 03.

Water extract of stem bark of *Terminalia arjuna* Wt & Arn was found to prevent adhesion of *Candida albicans* to polystyrene surfaces. Analysis of metabolic activity by the XTT assay revealed that water extracts of *Terminalia arjuna*

Wt & Arn was the most active inhibitors of adhesion. It exhibited antiadhesion activities with an MIC at concentration of 1 mg/ ml of ATCC 3017 (Figure 5(A)) and 2 mg/ml of *C. albicans* GMC 03 (Figure 5(B)).

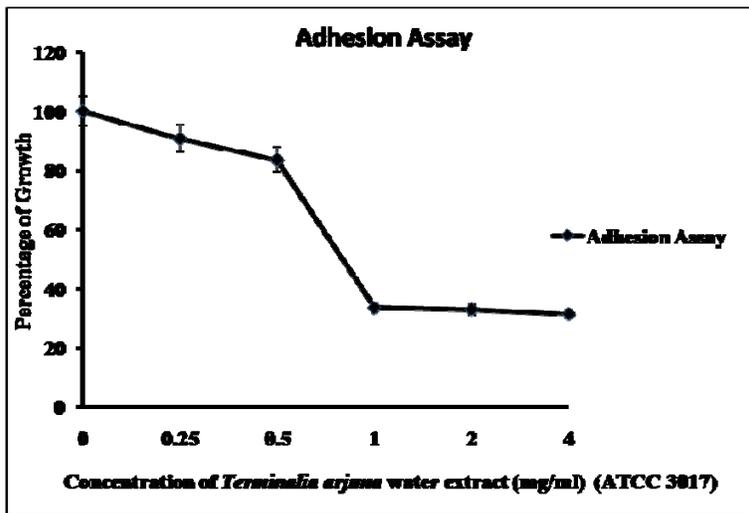


Fig 5 (A): Antiadhesion activity of water extract of *Terminalia arjuna* Wt & Arn. against *Candida albicans* ATCC 3017.

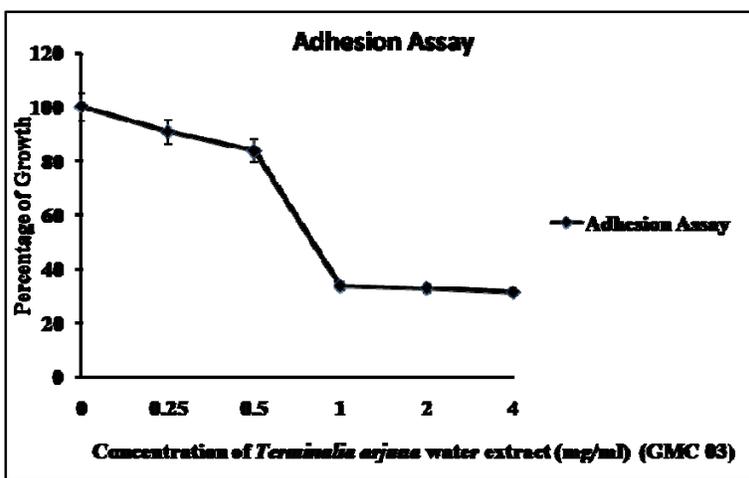


Fig 5 (B): Antiadhesion activity of water extracts of stem bark of *Terminalia arjuna* Wt & Arn. against *Candida albicans* ATCC 3017.

3.4 GC-MS analysis

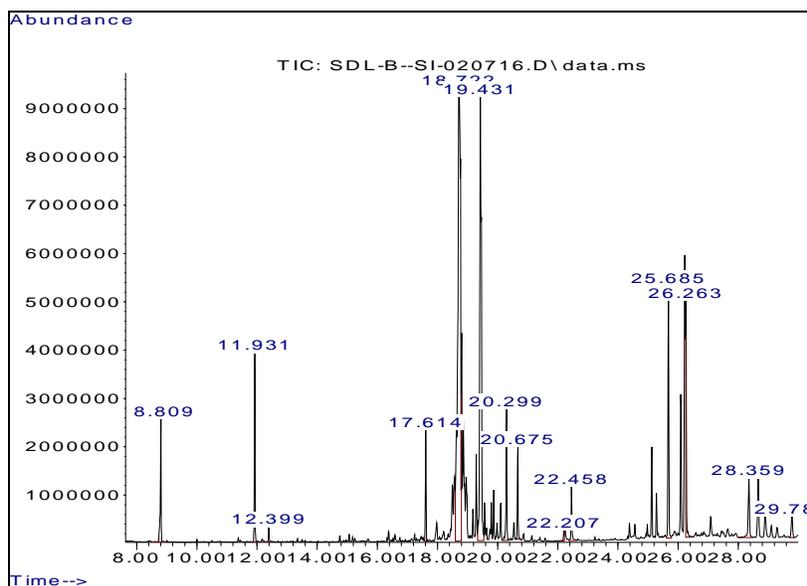
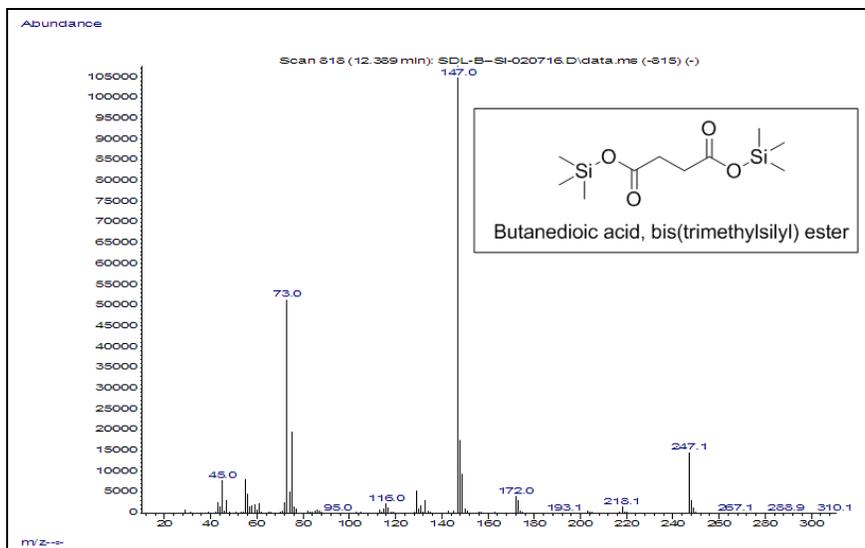
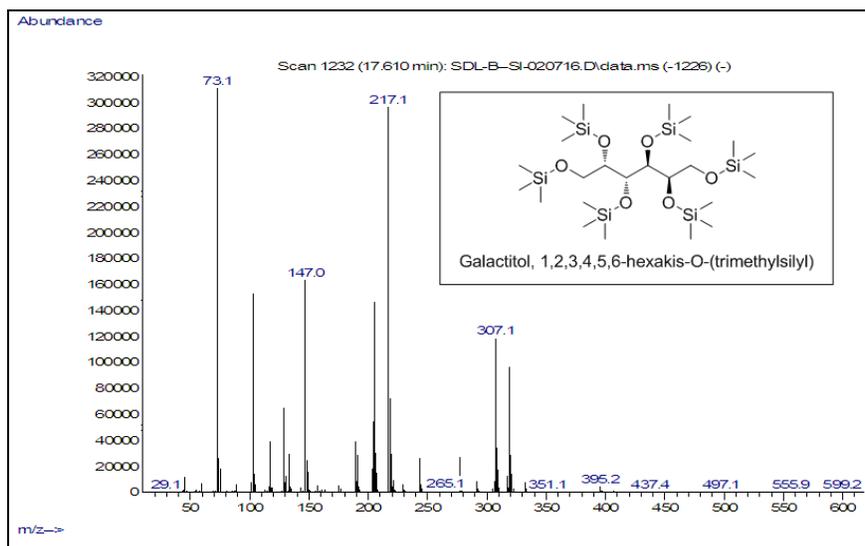


Fig 6: Gas chromatogram spectrum of water extract of the stem bark of *Terminalia arjuna* Wt & Arn stem bark

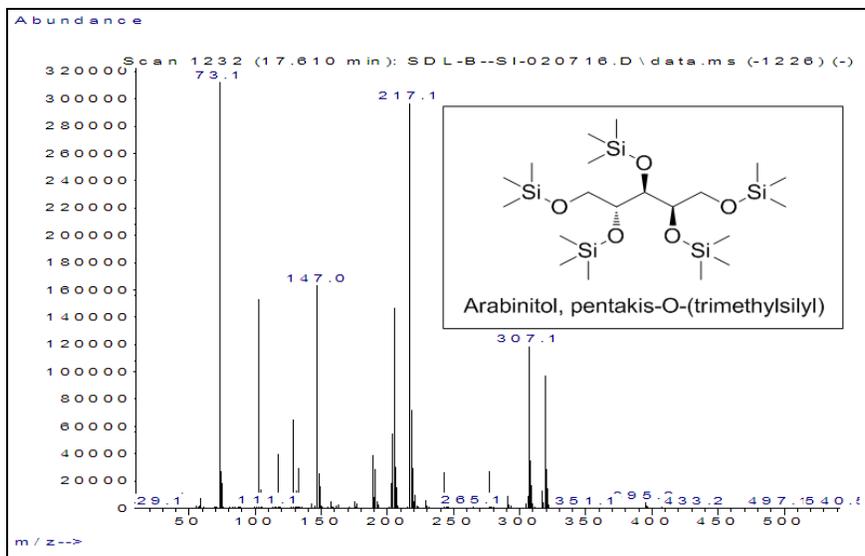
**1. Butanedioic acid, bis(trimethylsilyl) ester**



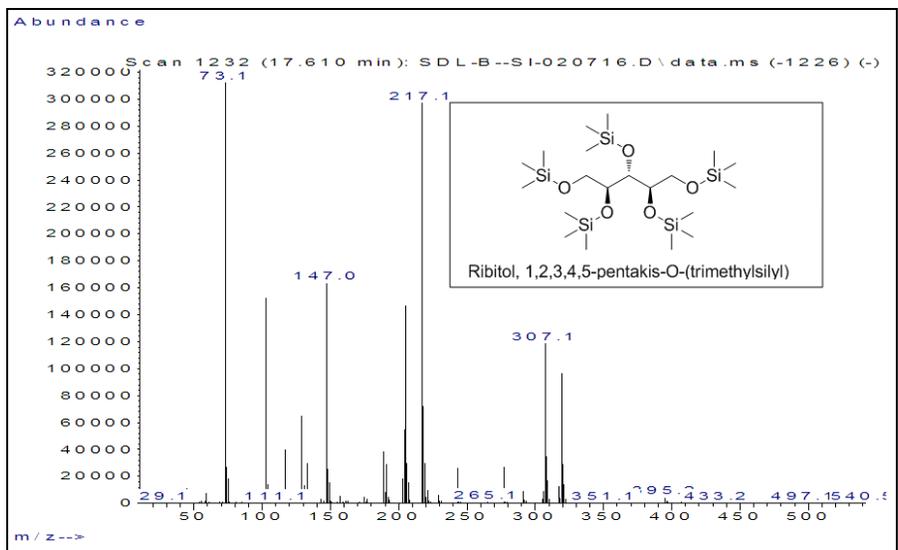
**2. Galactitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)**



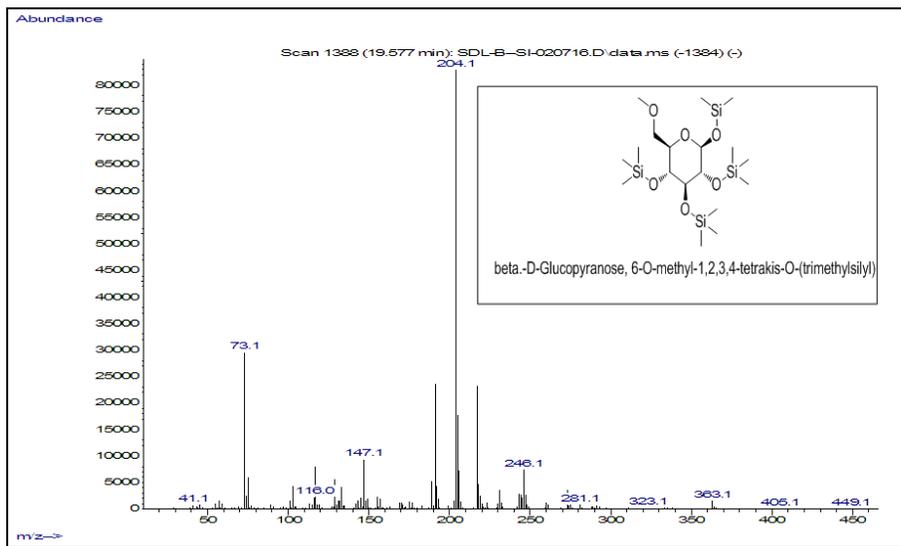
**3. Arabinitol, pentakis-O-(trimethylsilyl)**



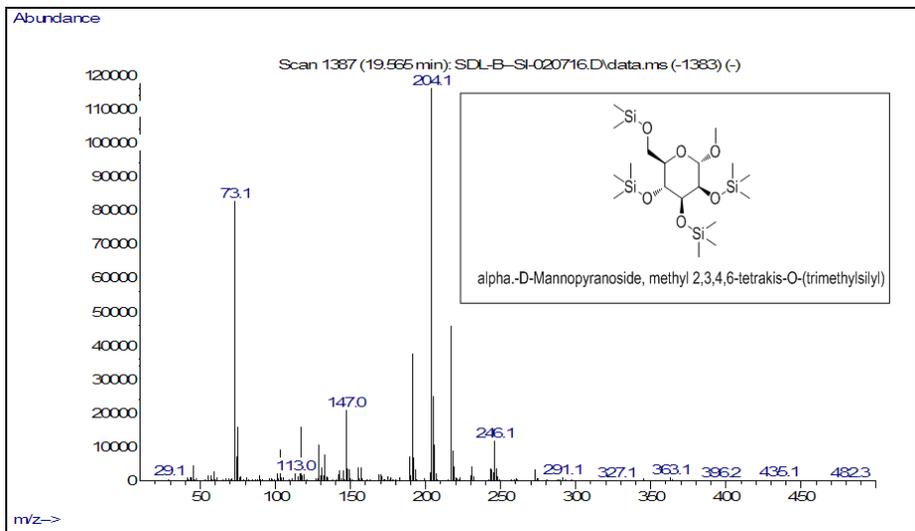
**4. Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)**



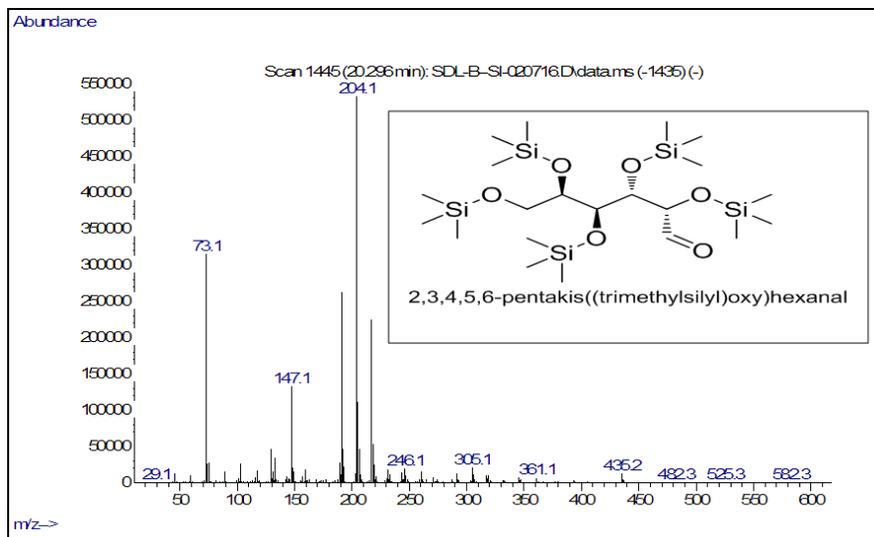
**5. beta.-D-Glucopyranose, 6-O-methyl-1, 2, 3, 4-tetrakis-O-(trimethylsilyl)**



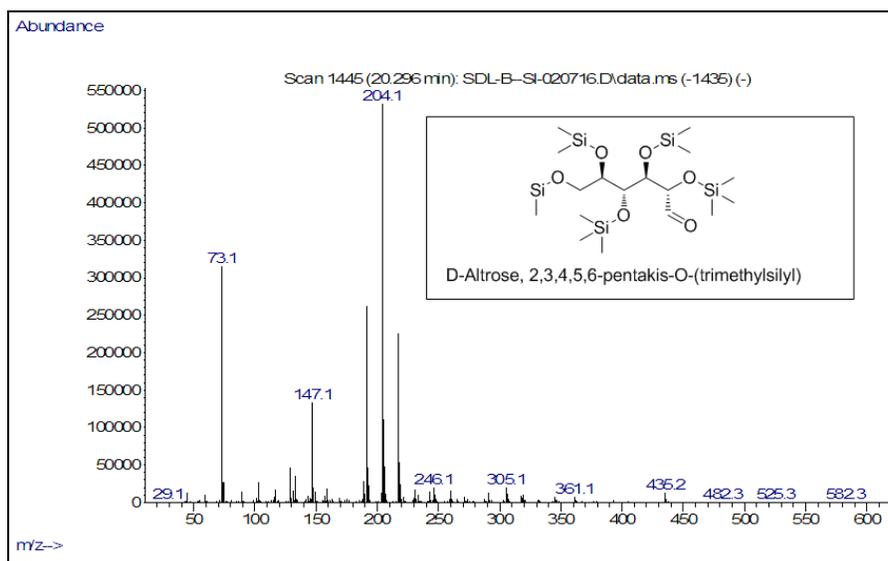
**6. alpha.-D-Mannopyranoside, methyl 2, 3, 4, 6-tetrakis-O-(trimethylsilyl)**



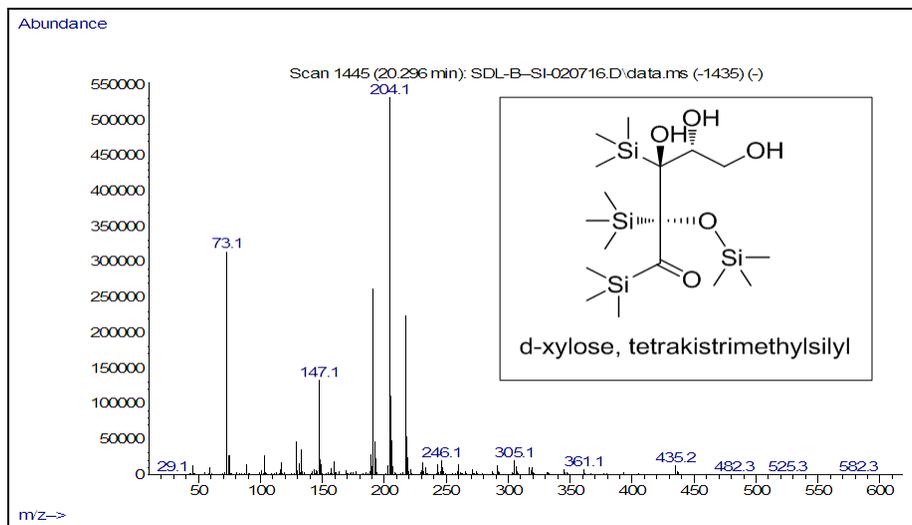
## 7. Talose, 2, 3, 4, 5, 6-pentakis-O-(trimethylsilyl)-



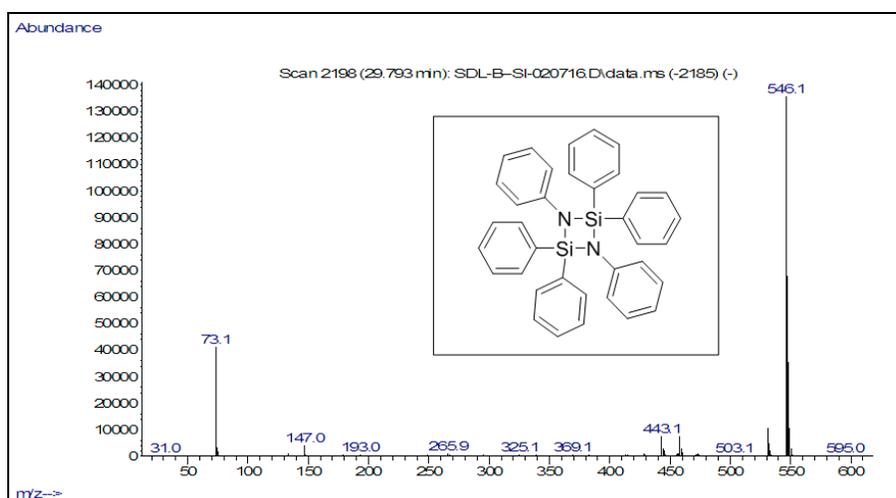
## 8. D-Altrose, 2, 3, 4, 5, 6-pentakis-O-(trimethylsilyl)



## 9. D-Xylose, tetrakis (trimethylsilyl)



## 10. Cyclodisilazane, hexaphenyl



**Fig 7:** The individual mass fragments of some major compounds from water extract of *T. arjuna* Wt & Arn stem bark detected by GC-MS analysis.

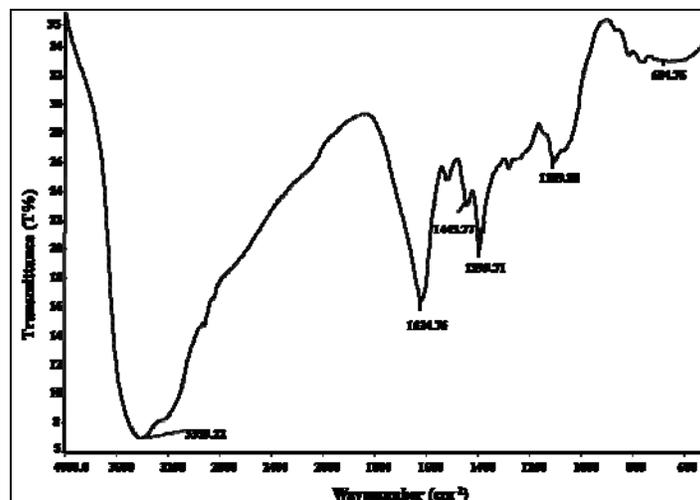
**Table 2:** Phyto components detected in the water extract of *Terminalia arjuna* Wt & Arn stem bark by GC-MS analysis

| No. | Name of compound  | Molecular formula  | Molecular Weight | Retention Time | Nature of the Compound |
|-----|---|--|------------------|----------------|------------------------|
| 1   | Butanedioic acid, bis(trimethylsilyl) ester                           | C <sub>10</sub> H <sub>22</sub> O <sub>4</sub> Si <sub>2</sub> | 262.45           | 12.389         | Ester                  |
| 2   | Galactitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)                    | C <sub>24</sub> H <sub>62</sub> O <sub>6</sub> Si <sub>6</sub> | 615.26           | 17.610         | Sugar alcohol          |
| 3   | Arabinitol, pentakis-O-(trimethylsilyl)                               | C <sub>20</sub> H <sub>52</sub> O <sub>5</sub> Si <sub>5</sub> | 513.05           | 17.610         | Sugar alcohol          |
| 4   | Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl)                        | C <sub>20</sub> H <sub>52</sub> O <sub>5</sub> Si <sub>5</sub> | 513.05           | 17.610         | Sugar alcohol          |
| 5   | beta.-D-Glucopyranose, 6-O-methyl-1,2,3,4-tetrakis-O-(trimethylsilyl) | C <sub>19</sub> H <sub>46</sub> O <sub>6</sub> Si <sub>4</sub> | 482.91           | 19.577         | Sugar ester            |
| 6   | alpha.-D-Mannopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl)  | C <sub>19</sub> H <sub>46</sub> O <sub>6</sub> Si <sub>4</sub> | 482.91           | 19.565         | Sugar alcohol          |
| 7   | Talose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)                         | C <sub>21</sub> H <sub>52</sub> O <sub>6</sub> Si <sub>5</sub> | 541.06           | 20.296         | Sugar alcohol          |
| 8   | D-Altrose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)                      | C <sub>21</sub> H <sub>52</sub> O <sub>6</sub> Si <sub>5</sub> | 541.06           | 20.296         | Sugar alcohol          |
| 9   | D-Xylose, tetrakis(trimethylsilyl)                                    | C <sub>17</sub> H <sub>42</sub> O <sub>5</sub> Si <sub>4</sub> | 438.85           | 20.296         | Sugar alcohol          |
| 10  | Cyclodisilazane, hexaphenyl   | C <sub>36</sub> H <sub>30</sub> N <sub>2</sub> Si <sub>2</sub> | 546.81           | 29.793         | Aromatic hydrocarbon   |

### 3.5 FTIR Spectroscopic analysis

The FTIR spectrum of water extract of *Terminalia arjuna* Wt & Arn stem bark showed the presence of various functional groups of the active components based on the peaks values in the region of IR radiation (Figure 8). The vibrational frequency of FTIR observed at 3359 cm<sup>-1</sup> represents the presence of alcoholic -OH and stretching observed at 1624 cm<sup>-1</sup> assign the presences of carbonyl group (-C=O)

analogous to carboxylic acid and ketonic group respectively. While stretching at lower wave number 1399 cm<sup>-1</sup> corresponding to (-C-H, -C-N) assigns the presence of aliphatic chains. Furthermore, the stretching at 1109 cm<sup>-1</sup> corresponding to (-C-O-) assigns the presence of alcoholic group. The results of FTIR analysis confirmed the presence of alcohol, alkanes, Carboxylic acids and aliphatic compound.



**Fig 8:** FTIR spectrum of water extract of *Terminalia arjuna* Wt & Arn stem bark

#### 4. Discussion

The bark of Arjuna has been used for centuries in Ayurveda primarily as a cardiotonics for the treatment of various cardiovascular disorders. In addition, TA bark powder and extract become recently available as over-the-counter supplements marketed for maintaining a healthy heart. Studies in animal models have shown that organic extracts of TA bark can decrease blood pressure [24] and heart beat rate, and counteract actions of norepinephrine and isoproterenol (ISO) - and ischemia/reperfusion-induced myocardial injury [25]. The beneficial effect of TA bark powder has been shown in animal studies such as role of *Terminalia arjuna* as an antioxidant agent on ischemic perfused rat heart [26] and in Clinical studies such as in a double blind, cross over design, placebo controlled study. Many hydrophobic components of TA bark powder have been shown to exert a variety of biological activities, including hypotension [24], cardiotoxic effect [1], negative inotropy and chronotropy [27], antioxidants [28], growth suppression and apoptosis [29]. In contrast to *Terminalia arjuna* organic extracts, aqueous extract shows promising and relatively safe cardiotoxic that can be beneficial to the healthy heart and the inotropy therapy for chronic heart failure [1]. Keeping this usefulness of Arjuna, it can be therefore used as a therapeutic strategy for the infections caused by *Candida* to the humans.

In this present study, the preliminary phytochemical analysis in *T. arjuna* Wt & Arn bark water extract showed the active compounds presence in high concentration, such as flavonoids, phenolic compounds, carbohydrates and tannins and glycosides which are known to give the medicinal property to Arjuna. Anti-candida properties of bark extract of Arjuna are not reported by any research group. In this study we found that water extract of arjuna is a good inhibitor of the growth and yeast to hyphal form of the human fungal pathogen, *Candida albicans*. It inhibited the biofilm formation of *Candida albicans* which is normally resistant to antifungal agents like Fluconazole. For the first time MIC values of the bark extract of *Terminalia arjuna* is established against the virulence factors of *Candida albicans*. This is a novel property of the extract of *Terminalia arjuna*. Toxicity reports are not available on the extract of *Terminalia arjuna*. Our chemical analysis of the water extract of the bark of *Terminalia arjuna* by GC-MS analysis identified Ten compounds namely (1) Butanedioic acid, bis(trimethylsilyl) ester, (2) Galactitol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl), (3) Arabinitol, pentakis-O-(trimethylsilyl), (4) Ribitol, 1,2,3,4,5-pentakis-O-(trimethylsilyl), (5) beta.-D-Glucopyranose, 6-O-methyl-1,2,3,4-tetrakis-O-(trimethylsilyl) (6) alpha.-D-Mannopyranoside, methyl 2,3,4,6-tetrakis-O-(trimethylsilyl) (7) Talose, 2,3,4,5,6-pentakis-O-(trimethylsilyl) (8) D-Altrose, 2,3,4,5,6-pentakis-O-(trimethylsilyl) (9) D-Xylose, tetrakis (trimethylsilyl) and (10) Cyclodisilazane, hexaphenyl. These molecules are reported for the first time from the water extract of the bark of *Terminalia arjuna*. Bioactive properties of the molecules are not known. The results of FTIR analysis confirmed the presence of alcohol, alkanes, Carboxylic acids and aliphatic compound. This study opens up the possibility of exploring the bioactive potential of these molecules and the use of water extract of the bark of *Terminalia arjuna* Wt & Arn for the treatment of candidiasis caused by *Candida albicans*.

#### 5. Conclusions

This study first time demonstrates the antibiofilm potential of

water extract of the bark of *Terminalia arjuna* Wt & Arn which need to be further explored as a therapeutic strategy against biofilm associated infections of *C. albicans*. The information obtained in this study indicates that water extract of the bark of *Terminalia arjuna* Wt & Arn exhibit promising activity against growth and the virulence factors of *C. albicans*. This is novel property of the extract of *Terminalia arjuna*. Toxicity reports are not available on the extract of *Terminalia arjuna* as such Arjunarishta has a long history of use in humans. These Arjuna properties could be effective drugs against *Candida albicans*. Hence, water extract of stem bark of arjuna can be developed into novel therapeutic strategies for prevention and eradication of *Candida* biofilms. In the present investigation, GC-MS analysis of water extract of the bark of *Terminalia arjuna* Wt & Arn. bark showed 10 peaks of different phytoconstituents. The *arjuna* bark extract revealed the presence of Bio-active constituents which are known to exhibit medicinal as well as physiological activity.

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