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Isolation of Phytosterols from the Methanolic Extract of Leaves of *Ficus Dalhousiae* Miq.

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ABSTRACT

The aim of the present study was to isolate the compounds from the leaves of *Ficus dalhousiae* Miq and subsequently evaluate their antibacterial and antifungal activity. The crude methanolic extract was obtained by using continuous soxhlation technique using soxhlet apparatus. The antibacterial activity of isolated compounds AUCP-1, AUCP-2, AUCP-3 and plant extract were carried using cup plate method against six bacterial species i.e., *Bacillus subtilis*, *Staphylococcus epidermitis*, *Klebsiella pneumonia*, *Staphylococcus Aureus*, *Escherichia Coli* and *Pseudomonas aeruginosa*. Antifungal activity was done on three species of fungi namely *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* using agar diffusion method. It is note-worthy that three compounds AUCP-1, AUCP-2 & AUCP-3 were isolated first time from the crude methanolic extract of *Ficus dalhousiae Miq*. The compounds were identified as β-sitosterol, β-stigmasterol and β -stigmasterol-D-glucoside respectively, based on physical properties and spectroscopic (IR and NMR) data as well as literature reports. The in vitro test results shows that the antibacterial as well as the antifungal activities of the isolated compounds were found to be lower than the plant extract which in-turn was lower than the reference drugs.

Keywords: *Ficus dalhousiae Miq,* Extraction, Isolation, Antibacterial, Antifungal, β -Sitosterol, β -Stigmasterol and - β Stigmasterol-D-glucoside.

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INTRODUCTION

Compounds which have biological activities and are derived from natural sources e.g., plants, animal and microorganisms, are defined as natural products. Natural products have been used by human societies for thousands of years. Natural products have provided considerable value to the pharmaceutical industry over the past half century. In particular, the therapeutic areas of infectious diseases and oncology have benefitted from numerous drug classes derived from natural product sources[1].

Ficus dalhousiae Miq which belongs to the family Moraceae is commonly known as kal-aal or soma-valika or pei-aal. The plant grows in Kerala[2-6].According to the Ayurvedic literature of India, Ficus has been explored for its various medicinal properties viz. haemostatic, anti-inflammatory, antiseptic, diarrhea, dysentery, skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia and deficient lactation[7].

Phytosterols are a large group of compounds that are exclusively found in plants. They are structurally related to cholesterol[8]. These are important nutrients present in vegetable oils and products made from them, nuts ,cereal products, vegetables, fruits and berries have been classified as richest or significantly rich source[9]. Among various phytosterols, β-sitosterol, β-stigmasterol and its glucosidic derivatives occupy a unique position as they are considered as good biomarkers due to their biological activity[10]. Broadly β-sitosterol is found to be possessing antioxidant, antidiabetic effect [11], anti-inflammatory and anti-pyretic effect[12], antifertility[13,14]. Whereas β-stigmasterol is found to be possessing antibacterial and anticholinesterase activity,antifungal activity, antioxidant, hypoglycaemic and thyroid inducing properties[15].

In continuation of our ongoing studies we earlier reported that the ethanolic extract of *Ficus dalhousiae* Miq is possessing antihyperglycaemic activity[16], anti-inflammatory activity[17]and gastroprotective activity[18]. In the present study the leaf methanolic extract of *Ficus dalhousiae* Miq has been tested for antimicrobial, antifungal activity and isolation of β-sitosterol, β-stigmasterol and β-stigmasterol glucoside is carried out.

MATERIAL AND METHODS

Plant Material

The leaves of *Ficus dalhousiae* Miq plant belonging to the family *Moraceae* were collected from Kerala and Tamil Nadu states (Coimbatore, Dundigal, Namkkal, Niligiri, Salem, Theni, Tirunaveli and Vellore districts) and was identified and authenticated by Head Department of Botany at Osmania University.

Spectroscopic Investigation

Analytical thin layer chromatography was performed on pre-coated sIlica gel plates. Visualization of the spots on TLC plates was achieved either by exposure to iodines vapours or UV light or dipping in anisaldehyde followed by heating the plate under a stream of hot air. Column chromatography was performed using silica gel and the column was eluted with ethyl acetate – n-hexane as solvent system. ^{1}H-NMR spectra were recorded on bruker avance 500 Mhz instrument using $CDCl_3$ as reference and the chemical shift was reported in ppm with respect to TMS as internal standard. Mass spectra were recorded on ESI mass spectrometer. Infra red spectra were recorded on perkin elmer infrared spectrophotometer .Melting point were recorded on stuart SMP3 melting point apparatus.

Extraction and Isolation

The leaves of *Ficus dalhousiae Miq* were washed and dried .Weighed leaves were grinded into a fine powder. The petroleum ether and methanolic extracts of the leaves were prepared by soxhlation. In this extraction process 150 gms of dried powder was extracted with 1200ml of petroleum ether and methanol separately at 30-50^{0}C. Phytochemical screening of extract showed the presence of different chemical constituents.

Thin layer chromatography of the crude extract was carried out using pre coated TLC plates. N-hexane and ethyl acetate (7:3) was used as the solvent. The results of TLC showed the presence of three compounds viz. β-sitosterol, β-stigmasterol, β-stigmasterol glucoside, which were further isolated using column chromatography.

For their isolation plant extract was taken and dissolved in a minimum quantity of chloroform, ethyl acetate and adsorbed on silica gel .The slurry formed was allowed to dry . A neat and dried column was taken. A cotton plug was put at the base of column and silica gel was poured into the column and the dried extract was added gradually. The column was eluted with n-hexane and ethyl acetate (7:3). Equal sized fractions were collected sequentially and carefully labeled for later analysis. These fractions were further subjected to IR, Proton NMR (500MHz), Carbon-13 NMR (500 MHz) and LC-MS to ascertain the chemical structure.

Experimental Procedure for Biological Assays

The plates were inoculated by dipping a sterile swab into inoculums. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid. The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60^0C after each application. Finally the swab was passed round the edge of the agar surface. The inoculation was dried for few minutes, at room temperature, with the lid closed. A bore was ditched in the plate and the compounds solution was added in the bore. The plates were placed in an incubator at 37^0C within 30 minutes of preparation for bacteria and 22^0C for fungal. After 48 hrs incubation for bacteria and 7-days for fungal, the diameter of zone (including the diameter disc) was measured and recorded in mm. The measurements were taken with a ruler, from the bottom of the plate, without opening the lid.

Identification Tests

Test for tannins and phenolic compounds

i. $FeCl_3$ solution test: 5% $FeCl_3$ solution was added to 2-3ml of the test solution. Appearance of deep blue black colour indicated the presence of tannins.
ii. Dilute HNO_3 test: To 2-3ml of the test solution, dilute HNO_3 solution was added. Appearance of reddish to yellow colour indicated the presence of tannins.
iii. Gelatin test: To 5ml of test solution, 1 % gelatin solution containing 10% NaCl was added. Presence of tannins was confirmed by formation of white precipitate.
iv. Chlorogenic acid: To 2-3ml of the test solution, aqueous ammonia was added. Gradual formation of green colour when exposed to air indicated the presence of tannins.

Test for sterols and triterpenoids

i. Libermann-buchard test: 2-3ml of test solution was treated with few drops of acetic anhydride, boiled and cooled. Then con. H_2SO_4 was added from the sides of the test tube. Brown ring at the junction of two layers and the upper layer turns green which confirmed the presence of steroids and formation of deep red colour indicated the presence of triterpenoids.
ii. Salkowski test: 2-3ml of test solution was treated with chloroform and few drops of concentrated sulphuric acid were added, shaken well and allowed to stand for some time. Appearance of red colour at the lower layer indicated the presence of steroids and formation of yellow coloured lower layer indicated the presence of triterpenoids**[19].**

Characterization and spectroscopic data

Compound 1 (AUCP-1) is a white powder with m.p. 136-138 ^{0}C. Molecular formula $C_{29}H_{50}O$. **IR(KBr) υ_{max} cm^{-1}** 3425 (OH stretch), 2953, 2921, 2851, 2373, 2338 (C-H stretch), 2272 (C=C stretch), 1607,1441 (C-C stretch) , 1103(C-O stretch), 87(=C-H band). **^{1}H-NMR (500 MHz, $CDCl_3$):** δ 7.71(1H, m, H-3), 7.52(1H, m, H-6), 7.35(1H, m, H-23), 7.12(H,m,H-22), 6.47(1H, m, H-3), 5.78 (1H, m, H-20), 5.35 (5H, m) , 5.0-4.96 (m, 9H), 4.30-4.08 (m, 5H), 3.63-2.81 (m, 4H), 2.32 (m, 3H), 2.01(m, 5H), 1.42(m, 3H), 0.97 (m, 9H) ppm. **^{13}C-NMR (500MHz, $CDCl_3$):** δ 151.38, 146.44, 141.75, 123.65, 122.90, 117.94, 65.35, 64.46, 60.85, 52.11, 49.89, 47.88, 47.64, 46.50,

45.18, 33.29, 31.04, 30.42, 29.97, 29.91, 29.71, 29.17, 29.02, 28.35, 26.39, 25.67, 24.12, 23.95, 21.89. **LC-MS** m/z: 414M^+.

Compound 2 (AUCP-2) is a white powder with m.p. 168-169^{0}C.; Molecular formula $C_{29}H_{48}O$; **IR (KBr) υ_{max} cm^{-1}** 3427 (O-H), 2962, 2923, 2853, 2357, 2347 (C-H stretch), 2268 (-C≡C-stretch), 1736 (C=O), 1625 (C=C stretch), 1447 (C-H bend), 1292,1100 (C-O stretch), 967, 873 (C-H bend). **^{1}H-NMR (500 MHz, CDCl$_3$):** δ 7.72(1H, m, H-3), 7.53(1H, m, H-6), 7.35(1H, m, H-23), 7.13(H,m,H-22), 5.53(1H, m, H-3), 4.29 (1H, m, H-20), 4.15 (5H, m), 2.76(m, 9H), 2.32 (m, 5H), 2.02 (m, 4H), 1.57 (m, 3H), 1.42 (m, 5H), 0.97(m, 3H), 0.94 (m, 9H) ppm. **^{13}C-NMR (500MHz, CDCl$_3$):** δ 173.88, 170.18, 151.19, 147.25, 135.91, 133.74, 133.30, 129.88, 129.81, 124.38, 124.04, 120.82, 119.12, 101.67, 62.51, 53.10, 51.32, 34.87, 34.52, 34.08, 33.17, 31.92, 31.43, 30.88, 30.53, 30.21, 30.03, 29.44, 29.34. **LC-MS** m/z: 412M^+.

Compound 3 (AUCP-3) is a white colour with m.p. 288-290 ^{0}C; Molecular formula $C_{35}H_{58}O_6$; **IR (KBr) υ_{max} cm^{-1}** 3422 (O-H stretching), 3405, 2920, 2851, 2370 (C-H stretching), 1630 (C=CH stretching), 1442 (C-C stretching), 1103 (C-O stretching), 1011, 875 (C=C-H bending). **^{1}H-NMR (500 MHz, CDCl$_3$):** *δ* 5.35 (1H, m, H-6), 5.20 (1H, dd, H-22), 5.06 (2H, m, H-23, H-1'), 4.59 (1H, d, H-6'b), 4.44 (1H, dd, H-6'a), 4.33 (2H, m, H-3', H-4'), 4.10 (1H, m, H-2'), 4.01 (1H, m, H-5'), 3.98 (1H, m, H-3), 2.75 (1H, d, H-4b), 2.50 (1H, m, H-4a), 2.13 (1H, m, H-2b), 2.03 (1H, m, H-20), 1.97 (1H, m, H-12b),1.87 (1H, m, H-7b), 1.83 (1H, m, H-16b), 1.73 (1H, m, H-2a), 1.71 (1H, m, H-1b), 1.57 (1H, m, H-24), 1.54 (2H, m, H-7a, H-25), 1.53 (1H, m, H-15b), 1.42 (2H, m, H-11a, H-11b), 1.37 (1H, m, H-8),1.26 (1H, m, H-16a), 1.10 (1H, m, H-12a), 1.08 (1H, m, H-17), 1.03 (1H, m, H-1a), 1.01 (1H, m, H-15a), 0.97 (3H, s, H-19), 0.95 (2H, m, H-28), 0.92 (3H, br,s, H-21), 0.91 (3H, m, H-14), 0.89 (1H, br s, H-9), 0.87 (3H, d, H-26), 0.85 (3H, br,s, H-29), 0.83 (3H, br s, H-27), 0.66 (3H, s, H-18). **^{13}C-NMR (500MHz, CDCl$_3$):** δ 141.14, 138.52, 129.64, 122.08, 102.83, 78.80, 78.71, 78.33, 75.53, 71.91, 63.03, 57.12, 56.49, 51.69, 50.56, 42.56, 41.12, 40.15, 39.65, 37.63, 37.69, 37.26, 32.38, 32.13, 30.48, 29.52,25.89, 24.60, 21.59, 21.50, 19.60,19.42, 12.56, 12.25. **LC-MS** m/z: 575 M^{+1}.

RESULT AND DISCUSSION

Characterization of the isolated compounds from the leaf of *Ficus dalhousiae Miq* (FDM)

The three compounds (AUCP-1, AUCP-2 & AUCP-3) are isolated from the methanolic extract of the leaves of FDM. Three compounds were characterized to be β -Sitosterol (AUCP-1), β -Stigmasterol (AUCP-2) & β-Stigmasterol-D-glucoside (AUCP-3) respectively. (Fig 1)

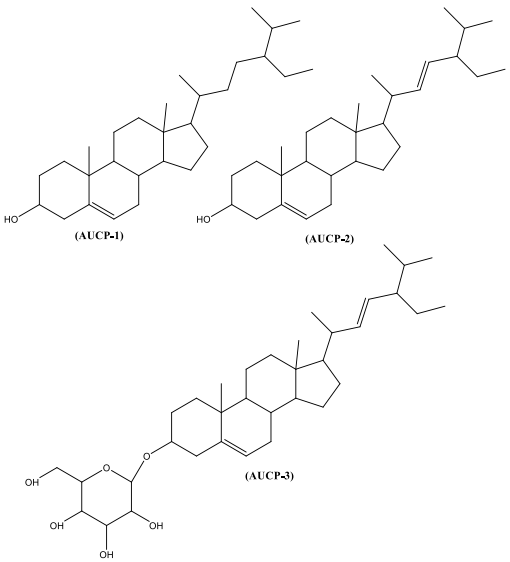

Fig 1: The structure of the compounds AUCP-1, AUCP-2 & AUCP-3 isolated from the roots of *Ficus dalhousiea Miq*.

The compounds were characterized by using spectroscopic techniques such as NMR, MASS & IR. The structural elucidation was done by comparing the observed spectral and melting points data with the reported data of these compounds in the literature.

Structure Elucidation of AUCP-1

The compound AUCP-1 was obtained as white powder having M.P 136-138 ^{0}C with R_f value of 0.55 (Hexane and ethyl acetate 80:20 %). IR (KBr) spectrum of AUCP-1 shows a stretching band at 3425 indicates the presence of hydroxyl group. The strong band at 2953 cm-1 represents C-H stretch of alkenes, whereas the bands at 2921 & 2853 cm-1 indicates the C-H stretching of methylene and methyl groups. The IR data suggests that the compound AUCP-1 could be an alcohol possessing a C=C double bond in its chain. In the ^{1}H-NMR spectrum of AUCP-1, the peaks at δ 0.94, 0.97, 1.42 & at 1.53 indicates the presence of protons of six methyl (-CH_3) groups. The peaks at δ 4.29&5.53 indicate the presence of olefinic protons, the ^{13}C-NMR showed signals at δ 146.44 and 123.65 which can be assigned to C-5 and C-6 indicating the presence of C=C olefin[20]. The value at δ 64.46 can be attributed to C-3 β hydroxyl group attached carbon. The ^{13}C-NMR data showed 29 signals respectively for six methyl, nine methylene, eleven methane and three quaternary carbon atoms. The observed IR and NMR data were found to be consistent with the reported data of β -Sitosterol[21-23]. The observed melting point was also found to be in good agreement with reported melting point of β -Sitosterol i.e., 133^{0}C. Thus based on similarities of spectral data and melting point data, the chemical structure of AUCP-1 was proposed to be identical with that of β -Sitosterol (AUCP-1) (Fig 1)[24]

Structure Elucidation of AUCP-2

The compound was obtained as white powder with R_f values of 0.38 (Hexane and ethyl acetate 80:20 %). IR (KBr) spectrum of AUCP-2 has bands at 3427 cm^{-1} is due to presence of OH group, the strong band at 2962 cm^{-1} represents CH- stretching of alkene, whereas the band at 2923 2853 cm^{-1} indicates the C-H stretching of methylene and methyl groups. The IR data suggests that the compound AUCP-2 could be an alcohol possessing a C=C double bond in its chain. In the ^{1}H-NMR spectrum of AUCP-2, the peaks at δ 0.94, 0.97, 1.42 & 1.57 indicates the presence of protons of six methyl (-CH_3) groups whereas, the peaks at the peak at δ 4.29 indicated presence of protons of carbon attached to oxygen (hydroxyl group). The peaks at δ 4.15 & 5.53, indicates the presence of olefinic protons in AUCP-2. The ^{13}C-NMR showed signals at δ 147.25, 124.38, 135.91 and at 129.88 ppm, which are assigned to C-5, C-6 and C-22 , C-23 double bonds respectively. The δ value at 62.51 ppm is due to C-3 - β hydroxyl group. Totally in ^{13}C-NMR spectra showed 29 signals respectively, that can be assigned to six methyl, nine methylene, eleven methane and three quaternary carbon atoms. The observed IR and ^{1}H-NMR data were found to be consistent with the reported melting point of β -stigmasterol (i.e., 170 0C)[25]. Thus, based on these observations, the chemicals structure of AUCP-2 was proposed to be identical with that of β -stigmasterol (AUCP-2) (Fig1)

Structure Elucidation of AUCP-3

The compound AUCP-3 is obtained as white powder with m.p 288-290^{0}C. Its IR spectrum showed the absorption band for hydroxyl group at 3422, aliphatic groups at 2920 and 2851, C=C at 1630, CH_2 at 1442, C-O at 1103 and peaks at 3405 & 1011 were characterized as glycoside compound. The ^{1}H-NMR spectrum of AUCP-3 shows two tertiary methyl group at δ 0.66 (CH_3-18) and 0.99 (CH_3-19), three secondary methyl group at 0.92 (CH_3-21), 0.87 (CH_3-26) and 0.83 (CH_3-27), one of the primary methyl group at 0.85 (CH_3-29), one proton with olefinic substitution at δ 4.59 (H-6), two protons with substituted olefinic at 5.20 (H-22) and 5.04 (H-23) and one anomaric proton at δ 4.10. The ^{13}C-NMR spectrum of AUCP-3 showed there are 35 carbon atoms in the molecules. An anomaric carbon at δ 102.83 indicated the presence of single monosaccharide moiety. The four methane resonances at 78.80, 78.71,75.53 and 71.91 as well as the methylene resonance at 63.03 were due to C-2', C-3',C-4' C-5' and C-6' respectively of β -D- glucopyranoside. The olefinic resonance at δ 122.08, 138.52 & 129.64 corresponds to C-6, C-22 and C-23 methine carbons. The signal at δ 141.43 is corresponds to C-5 quaternary carbon of the sterol moiety. Based on the data described above and comparison with physical and spectroscopic data of the reported compound, the AUCP-3 is confirmed as β -stigmasterol-D-glucopyranoside[26].

Antibacterial activity results

The antibacterial tests were carried out to evaluate the antibacterial activities of isolated compounds AUCP-1, AUCP-2, AUCP-3 and plant extract using cup plate method against six bacterial species

i.e., *Bacillus subtilis*, *Staphylococcus epidermitis*, *Klebsiella pneumonia*, *Staphylococcus Aureus*, *Escherichia. Coli* and *Pseudomonas. aeruginosa*. The activities of the compounds were expressed in terms of growth inhibition zones (given in mm). The results of the antimicrobial activity are given in Table-1.

Table 1:- Antibacterial activity of AUCP-1, AUCP-2 & AUCP-3 isolated compounds and Plant extract of FDME (Zone of inhibition in mm)

S. No	Bacterial Strain	Standard Drug (Gentamycin) 50 µg/ ml	Concentration of the test compounds	AUCP-1	AUCP-2	AUCP-3	Plant Extract
1	*Bacillus subtilis*	18 mm	50 µg/ ml	7 mm	-	-	10 mm
			100 µg/ ml	14 mm	-	-	17 mm
2	*Staphylococcus epidermitis*	18 mm	50 µg/ ml	8 mm	-	-	12 mm
			100 µg/ ml	13 mm	-	-	16 mm
3	*Klebsiella pneumonia*	17 mm	50 µg/ ml	7.5 mm	-	-	13 mm
			100 µg/ ml	10 mm	-	-	14 mm
4	*Staphylococcus aureus*	18 mm	50 µg/ ml	-	7 mm	-	10 mm
			100 µg/ ml	-	10mm	-	13 mm
5	*Escherichia Coli*	18 mm	50 µg/ ml	-	10 mm	-	13 mm
			100 µg/ ml	-	13 mm	-	16 mm
6	*Pseudomonas aeruginosa*	16 mm	50 µg/ ml	-	9 mm	-	12 mm
			100 µg/ ml	-	11 mm	-	14 mm

Table 2:- The anti-fungal activity AUCP-1, AUCP-2 & AUCP-3 isolated compounds and Plant extract of FDME (Zone of inhibition in mm)

S. No	Fungal Species	Standard Drug (Ampothericin-B) 50 µg/ ml	Concentration of the test compounds	AUCP-1	AUCP-2	AUCP-3	Plant Extract
1	*Candida albicans*	18 mm	50 µg/ ml	-	-	-	-
			100 µg/ ml	-	-	10 mm	16 mm
2	*Aspergillus niger*	17 mm	50 µg/ ml	-	-	6 mm	8 mm
			100 µg/ ml	-	-	7 mm	15 mm
3	*Aspergillus flavus*	16 mm	50 µg/ ml	-	-	-	-
			100 µg/ ml	-	-	8 mm	14 mm

From the antimicrobial results it is inferred that crude extract exhibited good activity when compared to isolated compounds with reference to standard drug *Gentamycin* against all bacterial strains. The antibacterial activity were tested at two different concentrations i.e., 50 µg/ ml and 100 µg/ ml. The results showed that, in the case of *Bacillus subtilis,* AUCP-1 showed 14 mm zone of inhibition, where as the extract showed zone of inhibition 17 mm at 100 µg/ ml when compared to standard drug *Gentamycin* which was 18 mm zone of inhibition at 50 µg/ ml concentration.A similar trend was observed in the case of *Staphylococcus epidermitis* (i.e) AUCP-1 showed 10 mm zone of inhibition, where as the plant extract exhibited 16 mm zone of inhibition at 100 µg/ ml ,compared to *Gentamycin* which showed 18 mm zone of inhibition at 50 µg/ ml.AUCP-1 showed 10 mm zone of inhibition, where as crude extract showed 14 mm zone of inhibition at 100 µg/ ml again compared to standard drug which showed 17 mm zone of inhibition at 50 µg/ ml against *Klebsiella pneumonia*.AUCP-2 and AUCP-3 were inactive against *Bacillus subtilis, Staphylococcus epidermitis and Klebsiella pneumonia*. In the case of *Staphylococcus Aureus* AUCP-2 showed 10 mm zone of inhibition & crude extract showed 13mm zone of inhibition at 100 µg/ ml compared to standard drug which showed 18 mm zone of inhibition at 50 µg/ ml concentration.AUCP-2 showed 13 mm zone of inhibition and plant extract showed 16 mm zone of inhibition at 100 µg/ ml concentration ,compared to standard drug which showed 18 mm zone of inhibition at 50 µg/ ml concentration against *Escherichia Coli*. In the case of *Pseudomonas aeruginosa*, AUCP-2 showed 11 mm zone of inhibition where as crude

extract showed 14 mm zone of inhibition at 100 µg/ ml of concentration compared to standard drug which showed 18 mm zone of inhibition at 50 µg/ ml concentration.AUCP-1 and AUCP-3 were inactive against *Staphylococcus aureus*, *Escherichia Coli* and *Pseudomonas aeruginosa*.

Antifungal Activity

Antifungal activity was done on three species of fungi namely *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus* using agar diffusion method. The activities of the compounds were shown in terms of growth inhibitions zones (given in mm). The result of the antifungal activities is given in Table-2.The antifungal activities were tested by taking *Amphotericin-B* as standard anti-fungal drug.

The antifungal activity results were found to be similar to that of antibacterial activity results. In general the plant extract showed good antifungal activity when compared to their isolated compounds i.e., AUCP-1, AUCP-2 & AUCP-3. The antifungal activities were tested at two different concentrations i.e., 50g/ml and 100g/ml. The results showed that compounds AUCP-1 and AUCP-2 were inactive against all three fungal strains. In the case of *Candida albicans* AUCP-3 showed 10 mm zone of inhibition whereas the plant extract exhibited 16 mm zone of inhibition at 100/ml concentration compared to standard drug which showed 18 mm zone of inhibition at 50µg/ml concentration.AUCP-1 showed 7 mm zone of inhibition where as plant extract showed 15 mm zone of inhibition at 100 µg /ml concentration. The standard drug showed 17 mm zone of inhibition at 50 µg /ml against *Aspergillus niger*. In the case of *Aspergillus flavus* AUCP-3 showed 8mm and plant extract showed 14 mm zone of inhibition at 100 µg /ml concentration , compared to standard drug which exhibited 16 mm zone of inhibition at 50 µg /ml concentration.

CONCLUSION

In conclusion, three compounds AUCP-1, AUCP-2 & AUCP-3 were isolated from the crude methanolic extract. The compounds were identified as β-sitosterol, β-stigmasterol and β-stigmasterol-D-glucoside respectively, based on physical properties and spectroscopic (IR and NMR) data as well as literature reports. It is interestingly to note that, these three compounds were first time isolated from *Ficus dalhousiae Miq*. The in vitro test results shows that the antibacterial as well as the antifungal activities of the isolated compounds were found to be lower than the plant extract which in-turn is lower than to the reference drugs. The overall result was also found to be consistent with that of the crude extract. The observed antibacterial and antifungal activities of the crude extract and isolated compounds is in agreement and justify the traditional use of the plant for the treatment of different bacterial and fungal infections.

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