Original Article

Isolation and Characterization of Two New Compounds from *Phaseolus trilobus* Ait.

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ABSTRACT

This paper describes isolation and characterization of two new bioactive compounds A and B which were isolated from the methanolic extract from the stems of *Phaseolus trilobus* Ait. Structures of these Compounds were elucidated on the basis of spectral data including Mass NMR, IR, UV spectroscopy and chemical analysis. Compound A and B were also screened against various gram positive and gram negative bacteria and fungi and showed good results.

Keywords: *Phaseolus trilobus* Ait.; Leguminosae; Flavone glycoside; Zone of inhibition.

INTRODUCTION

Phaseolus trilobus Ait. belongs to family Leguminosae, commonly known as Mugani and mainly found in India, Burma, Afghanistan, Abyssinia and Nubia. It is widely used by tribal people in the treatment of hepatic disorders, and their leaves are also considered as a sedative. Effectiveness of this plant have also been reported for treatment of inflammations, fever, burning sensation, thirst, piles, dysentery, cough, gout and biliousness[1,2]. Literature survey revealed that various constituents of plant have also been reported [3-6]. This study describe the isolation and structure elucidation of two new compounds **A** and **B** from methanolic extract of the stem. The structures of these compounds have been characterized as 3, 5, 7, 3′-tetrahydroxy-8-methoxyflavone-3-O-β-D-xylopyranosyl-(1→2)-O-β-D-galactopyranosyl-7-O-α-L-rhamnopyranoside (A) and 5, 7, 2′-trihydroxy -3-methoxy flavone-7-O-β-D-galactopyanosyl-(1→4)-O-β-D-arabinopyranosyl-2′-O-α-L- rhamno-pyranoside (B) by various spectroscopic techniques.

MATERIALS AND METHODS

General experimental procedures

All the melting points were determined on a melting point apparatus and are uncorrected. The IR spectra were recorded on FT-IR spectrophotometer, Shimadzu 8400S. UV Spectra were determined on Shimadzu-120 double beam

spectrophotometer. The NMR spectral data were obtained on Bruker DRX- 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) in $CDCl_3$ with tetramethylsilane as internal standard. Chemical shifts values are reported in ppm (δ) units and coupling constant (*J*) in Hz. The FABMS were recorded on a Jeol-SX (102) mass spectrometer. Paper Chromatography was performed using Whatman filter paper No. 1. Column and thin layer chromatography were carried out on silica gel (60-120 mesh) and silica gel-G (60 mesh) respectively.

Plant material

The stems of *Phaseolus trilobus* Ait. were collected from Sagar region and were taxonomically authenticated by the Department of Botany, Dr.H.S.Gour University sagar. A voucher specimen (No. Bot/Her/2822) has been deposited in the Natural Products Laboratory, Department of Chemistry, Dr.H.S.Gour University.

Extraction and Isolation

The shade dried and powdered stems (6 kg) of the plant were extracted with petroleum ether (at 40-60°C) in Soxhlet apparatus for five days. The stems were successively extracted with methanol for four days. The MeOH soluble fraction of the plant was concentrated under reduced pressure, to yield a light brown viscous mass (3.6g) which was further subjected to TLC examination over silica gel-G using n BAW (4:1:5) as solvent and I_2 vapors as visualizing agent. It showed two spots, indicating it to be a mixture of two compounds **A** and **B**. These compounds were separated and purified by column chromatography over silica gel using $CHCl_3$: MeOH in various proportions. After removal of the solvent and crystallization from ether, above eluates yielded compound **A** (0.72g) and compound **B** (0.61g) respectively.

RESULTS AND DISCUSSION

Study of Compound A

Compound A was isolated as yellow amorphous powder. Flavonoidal glycosidic nature of compound was confirmed by Molisch and Shinoda tests [7-8]. UV spectrum of compound exhibits absorption bands at 266, 310 and 350 nm, indicating C-3-O-substituted flavonol skeleton. Presence of hydroxyl (3400 cm^{-1}), α-β unsaturated carbonyl (1651 cm^{-1}) and aromatic ring (1600, 1560, 1506 cm^{-1}) were confirmed by IR spectroscopy.

The ^{1}H-NMR spectrum of compound showed a doublet at δ_H 6.49 (1H, d, *J* = 2.2 Hz) assigned for H-6 in Ring A. Doublets at δ_H 6.96 (1H, d, *J* = 8.6 Hz) and δ_H 6.95 (1H, d, *J* = 8.5 Hz) assigned for H-2′ and H-4′ in Ring B. Another double doublet at δ_H 7.63 (1H, dd, *J* = 8.5-2.2 Hz) and a triplet at δ_H 7.37 (1H, t, *J*= 8.1) assigned for H-5′ and H-6′ respectively. A singlet at δ_H 12.93 and δ_H 10.42 assigned for hydroxyl groups at C-5 and C-3′ positions. The three –proton singlet observed at δ_H 3.86 confirmed the presence of methoxy group at C-8 position. The anomeric proton signals at δ_H 5.41 (1H, d, *J* = 7.8 Hz, H-1″), δ_H 4.52 (1H, d, *J* = 7.5 Hz, H-1‴) and δ_H 5.54 (1H, d, *J* = 1.9 Hz, H-1⁗) were assigned to H-1″ of D-galactose, H-1‴ of D-xylose and H-1⁗ of L-rhamnose respectively. Two coupling constants at (*J* = 7.8 Hz) of H-1″ and (*J* = 7.5 Hz) of H-1‴ confirmed the β – anomeric configuration for the D-galactose and D-xylose. A coupling constant at (*J* = 1.9 Hz) of H-1⁗ confirmed the α-anomeric configuration for the L-rhamnose respectively[9,10].

The FAB-MS (Positive Ion Mode) experiment revealed a molecular ion peak at m/z 756, corresponding to the molecular formula of $C_{33}H_{40}O_{20}$. Mass spectrum showed characteristic fragments at *m/z* 610, 478 and 316 were found by subsequent losses from the

molecular ion of each molecule of L-rhamnose, D-xylose and D-galactose .

Acid hydrolysis of compound **A** with 10% ethanolic H_2SO_4 gave aglycone **A-1**, melting point 225-226 °C, molecular formula $C_{16}H_{12}H_7$, *m/z* 316 (FABMS) which was identified as 3, 5, 7, 3′- tetrahydroxy-8-methoxy flavone. The obtained aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to Paper chromatography, examination on Whatman filter paper No. 1 was carried out and it showed the presence of D-galactose (R_f 0.16), D-xylose (R_f 0.28) and L-rhamnose (R_f 0.37). Quantitative estimation of sugars revealed that all the three sugars were present in equimolar ratio 1:1:1[11]. Periodate oxidation of compound **A** confirmed that all sugars were present in the pyranose form[12]. The position of the sugars moieties in compound **A** were determined by permethylation followed by acid hydrolysis, it yielded methylated aglycone and methylated sugars. The methylated aglycone was identified as 3, 7-dihydroxy-5, 8, 3′ trimethoxy flavone which confirmed that glycosidation was involved at C-3-OH and C-7-OH positions of aglycone. The methylated sugars were identified as 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.01), 3, 4, 6-tri-O-methyl-D-galactose (R_G 0.42) and 2, 3, 4-tri-O-methyl-D-xylose (R_G 0.95) by paper chromatography with authentic samples. Therefore, it was concluded that C-1′′-OH of D-galactose was attached with OH group at C-3 position of aglycone, C-2′′-OH of D-galactose was linked with C-1′′′-OH of D-xylose and C-1′′′′ of L-rhamnose was attached with OH group at C-7 position of aglycone. The interglycosidic linkage (1→2) was found between D-galactose and D-xylose. Enzymatic hydrolysis of compound A with takadiastase enzyme liberated L-rhamnose indicated the presence of α linkage between L-rhamnose and as proaglycone 3, 5, 7, 3′-tetrahydroxy-8-methoxyflavone-3-O-β-D-xylopyranosyl - (1→2) - O -β-D-galactopyranoside. Proaglycone on further hydrolysis with almond emulsin enzyme liberated D-xylose first followed by D-galactose and aglycone. Thus, compound **A** was identified as 3, 5, 7, 3′- tetrahydroxy-8-methoxy flavone-3-O-β-D-xylopyranosyl-(1→2)-O-β-D-galactopyranosyl-7-O-α-L-rhamnopyranoside (Figure 1).

Study of Compound B

Compound B was isolated as yellow amorphous powder. Flavonoidal glycosidic nature of compound was confirmed by Molisch and shinoda tests. UV spectrum of compound exhibit absorption bands at 265, 312 and 352 nm, indicating C-3-O substituted flavonol skeleton. Presence of hydroxyl (3460 cm^{-1}), α-β unsaturated carbonyl (1650 cm^{-1}) and aromatic ring (1600, 1562, 1508 cm^{-1}) were confirmed by IR spectroscopy.

The ^{1H}NMR spectrum of compound showed two doublets at δ_H 6.16 (1H, d, *J* = 1.5 Hz) and δ_H 6.85 (1H, d, *J* = 2.0 Hz) were assigned for H-6 and H-8 in Ring A. Another double doublet at δ_H 7.91 (1H, dd, *J* = 2.0-8.0 Hz) was assigned for H-6′ position of Ring B. Two singlets at δ_H 12.91 and δ_H 10.85 were assigned for hydroxyl group at C-5 and C-2′ positions and a singlet at δ_H 3.81 confirmed the presence of methoxy group at C-3 position. The anomeric proton signals at δ_H 4.38 (1H, d, *J* = 6.4 Hz, H-1′′), 5.13 (1H, d, *J* = 7.5 Hz, H-1′′′) and 5.53 (1H, d, *J* = 1.9 Hz, H-1′′′′) were assigned to H-1′′ of D - arabinose, H-1′′′ of D-galactose and H-1′′′′ of L- rhamnose respectively. Two coupling constants at (*J* 6.4 Hz) and (*J* 7.5 Hz) confirmed the β anomeric configuration for the D-arabinose and D-galactose at H-1′′ and H-1′′′. A coupling constant at (*J* 1.9 Hz) of H-1′′′′ confirmed the α configuration for L-rhamnose.

The FAB-MS (Positive Ion Mode) experiment revealed a molecular ion peak at

m/z 740, corresponding to the molecular formula of $C_{33}H_{40}O_{19}$. Mass spectrum showed characteristic fragments at *m/z* 594, 432 and 300 were found by subsequent losses from the molecular ion of each molecule of L-rhamnose, D-galactose and D-arabinose.

Acid hydrolysis of compound B with 10% ethanolic H_2SO_4 gave aglycone B-1, melting point 274-275 °C, molecular formula $C_{16}H_{12}O_6$, 300 m/z (FABMS) identified as 5, 7, 2′-trihydroxy-3-methoxy flavone. The obtained aqueous hydrolysate was neutralized with $BaCO_3$ and the $BaSO_4$ was filtered off. The filtrate was concentrated and subjected to Paper chromatography, examination on Whatman filter paper No.1 was carried out and it showed the presence of D-galactose (R_f 0.16), D-arabinose (R_f 0.21) and L-rhamnose (R_f 0.37). Periodate oxidation of compound **B** confirmed that all sugars were present in the pyranose form. The position of the sugars moieties in compound B were determined by permethylation followed by acid hydrolysis, it yielded methylated aglycone and methylated sugars. The methylated aglycone was identified as 5, 2′- dihydroxy- 3, 7-dimethoxy flavone which confirmed that glycosidation was involved at C-7-OH and C-2′-OH positions of aglycone. The methylated sugars were identified as 2, 3, 4, 6- tetra-O-methyl-D-galactose (R_G 0.88), 2, 3-di-O-methyl-D-arabinose (R_G 0.64) and 2, 3, 4-tri-O-methyl-L-rhamnose (R_G 1.01) by paper chromatography with authentic samples. Therefore it was concluded that C-1″ of D-arabinose was attached with OH group at C-7 position of aglycone, C-4″ of D-arabinose was linked with C-1‴ of D-galactose and C-1‴ of L-rhamnose was attached with OH group at C-2′ position of aglycone. The interglycosidic linkage (1→4) was found between D-galactose and D-arabinose. Enzymatic hydrolysis of compound B with takadiastase enzyme liberated L-rhamnose indicating the presence of the α-linkage between L-rhamnose and as proaglycone 5,7,2′- trihydroxy-3-methoxyflavone-7-O-β-D-galactopyranosyl-(1→4)-O-β-D-arabinopyranoside[13]. Proaglycone on further hydrolysis with almond emulsion enzyme liberated D-galactose first followed by D - arabinose and aglycone. Thus compound **B** was identified as 5, 7, 2′-trihydroxy-3-methoxy flavone-7-O-β-D-galactopyranosyl-(1→4)-O-β-D-arabinopyranosyl-2′-O-α-L-rhamnopyranoside (Figure 2).

Spectral Data of compound A

It has yellow amorphous, m.p. 246-247°C, m.f. $C_{33}H_{40}O_{20}$ $[M]^+$ 756 found (%) C 51.90, H 5.24, O 42.54, Calcd for m.f. $C_{33}H_{40}O_{20}$ found (%) C 52.3 , H 5.29, O 42.3, UV (MeOH) λ_{max} nm 266, 310 and 350. IR (kBr) υ_{max} (cm^{-1}); 3400, 1651, 1600, 1560, 1506. ^{1H}NMR (300 MHz, $CDCl_3$); 12.93 (1H, s, 5-OH), 10.42 (1H, s, 3′-OH), 3.86 (3H, s, OCH_3), 6.49 (1H, d, *J* 2.2 Hz, H-6), 6.96 (1H, d, *J* 8.6 Hz, H-2′), 6.95 (1H, d, *J* 8.5 Hz, H-4′), 7.37 (1H, t, *J* 8.1 Hz, H-5′), 7.63 (1H, dd, *J* 8.5, 2.2 Hz, H-6′), 5.41 (1H, d, *J* 7.8 Hz, H-1″), 3.78 (m, H-2″), 3.56 (1H, dd, *J* 9.6, 3.3 Hz, H-3″), 3.76 (1H, d, br, *J* 2.5 Hz, H-4″), 3.68 (1H, br, t, *J* 6.0 Hz, H-5″), 3.76 (1H, dd, *J* 10.2, 3.6 Hz, H-6″), 4.52 (1H, d, J 7.5 Hz, H-1‴), 3.00 (1H, dd, J 7.5, 8.9 Hz, H-2‴), 3.14 (1H, dd, J 8.9, 8.4 Hz, H-3‴), 3.24 (m, H-4‴), 3.58 (1H, dd, J 5.2, 11.3 Hz, H-5‴), 5.54 (1H, d, *J* 1.9 Hz, H- 1⁗), 4.03 (1H, dd, *J* 3.4, 1.9 Hz, H-2⁗), 3.84 (1H, dd, *J* 9.5, 3.5 Hz, H-3⁗), 5.49 (1H, t, *J* 9.5 Hz, H-4⁗), 3.62 (1H, dd, *J* 9.6, 6.2 Hz, H-5⁗), 1.27 (1H, d, *J* 6.3 Hz, H-6⁗). ^{13}C NMR (75 MHz, $CDCl_3$); δ 159.30 (C-2), 135.20 (C-3), 180.0 (C-4), 164.10 (C-5), 100.50 (C-6), 158.0 (C-7), 128.10 (C-8), 159.10 (C-9), 108.0 (C-10), 123.10 (C-1′), 116.60 (C-2′), 162.30 (C-3′), 117.70 (C-4′), 119.50 (C-5′), 128.70 (C-6′), 100.60 (C-1″), 78.60 (C-2″), 73.90 (C-3″), 69.50 (C-4″), 74.20 (C-5″), 61.30 (C-6″), 104.0 (C-1‴), 76.10 (C-2‴),

76.30 (C-3‴), 70.20 (C-4‴), 65.70 (C-5‴), 100.20 (C-1⁗), 71.90 (C-2⁗), 72.30 (C-3⁗), 73.90 (C-4⁗), 71.50 (C-5⁗), 18.30 (C-6⁗).

Acid hydrolysis of compound A

70 mg of Compound A was dissolved in 15 mL of methanol and refluxed with 10% H_2SO_4 on water bath for 6 h. The contents were concentrated and allowed to cool and residue was extracted with diethyl ether. The ether layer was washed with water and the residue was chromatographed over silica gel using a mixture of $CHCl_3$ and MeOH as solvent to give aglycone **A-1**. This was identified as 3, 5, 7, 3′-tetrahydroxy-8-methoxyflavone.

Spectral data of Compound B

It has yellow amorphous powder, m.p. 294-295°C, m.f. $C_{33}H_{40}O_{19}$ $[M]^+$ 740 found (%) C 53.38, H 4.22, O 40.85, Calcd for m.f. $C_{33}H_{40}O_{19}$ found (%) C 53.44, H 4.18, O 41.02 UV(MeOH) λmax nm 274, 321 and 375. IR (kBr) υ_{max} (cm^{-1}); 3460, 1650, 1600, 1562, 1508. 1HNMR (300 MHz, $CDCl_3$); δ 12.91 (1H, s, 5-OH), 10.85 (1H, s, 2′-OH), 3.81 (3H, s, OCH_3), 6.16 (1H, d, *J* = 1.5 Hz, H-6), 6.85 (1H, d, *J* = 2.0 Hz, H-8), 7.41 (1H, dt, *J* = 2.0 - 8.0 Hz, H-4′), 6.97-7.04 (2H, m, H-3′, 5′), 7.91 (1H, dd, *J* = 2.0 - 8.0 Hz H-6′), 4.38 (1H, d, *J* = 6.4 Hz, H- 1″), 3.66 (1H, dd, *J* = 6.4 - 8.5 Hz, H-2″), 3.62 (1H, dd, *J* = 3.4 - 8.3 Hz, H-3″), 3.35 (m, H-5″), 5.13 (1H, d, *J* = 7.5 Hz, H-1‴), 3.44-3.76 (4H, m, 2‴, H-3‴, H-4‴, H-5‴), 3.83 (1H, dd, *J* = 11.0 - 7.0 Hz, H-6_a‴), 4.21 (1H, dd, *J* = 11.0 - 4.0 Hz, H-6_b‴), 5.53 (1H, d, *J* = 1.9 Hz, H-1⁗), 4.02 (1H, dd, *J* = 3.4 - 1.9 Hz, H-2⁗), 3.83 (1H, dd, *J* = 9.5 - 3.5 Hz, H-3⁗), 5.48 (1H, t, *J* = 9.5 Hz, H-4⁗), 3.63 (1H, dd, *J* = 9.6 - 6.2 Hz, H-5⁗), 1.28 (1H, d, *J* = 6.3 Hz, H-6⁗). ^{13}C NMR (75 MHz, $CDCl_3$); δ 158.60 (C-2), 142.20 (C-3), 180.10 (C-4), 163.50 (C-5), 100.20 (C-6), 166.50 (C-7), 94.40 (C-8), 159.40 (C-9), 105.80 (C-10), 117.30 (C-1′), 156.80 (C-2′), 117.0 (C-3′), 132.90 (C-4′), 119.50 (C-5′), 128.50 (C-6′), 60.10 (C-3-OMe), 105.80 (C-1″), 71.10 (C-2″), 73.80 (C-3″), 69.70 (C-4″), 66.50 (C-5″), 105.30 (C-1‴), 73.10 (C-2‴), 75.10 (C-3‴), 70.20 (C-4‴), 71.30 (C-5‴), 62.10 (C-6‴), 100.10 (C-1⁗), 71.90 (C-2⁗), 72.30 (C-3⁗), 73.90 (C-4⁗), 71.50 (C-5⁗), 18.30 (C-6⁗).

Acid hydrolysis of compound B

75 mg of Compound **B** was dissolved in 15 ml ethanol and refluxed with 10% H_2SO_4 on water bath for 6 h. The contents were concentrated and allowed to cool and residue was extracted with diethyl ether. The ether layer was washed with water and the residue was chromatographed over silica gel using $CHCl_3$: MeOH as solvent to give aglycone B-1.

ANTIMICROBIAL ACTIVITY OF COMPOUND A AND B

The antimicrobial activities of compounds **A** and **B** were determined by Kirby Bauer method [14-15]. The various bacterial and fungal species were first incubated at 37°C for 24 hrs for the proper growth of microbes then prepared agar plate (Muller Hinton Agar Media for the bacteria and Potato Dextrose Agar media for the fungus). Antibacterial and Antifungal activities of compound A and B were determined with Ciprofloxacin and Fluconazole as standard. Anti microbial activities and antifungal activities of Compound A and B are represented in figure 3 and 4. Figure 3 and 4

ACKNOWLEDGEMENT

Authors are grateful to Head, SAIF, CDRI, Lucknow (U.P.) for recording various spectral and elemental analysis and SCAN

Research laboratory, Bhopal (M.P.) for providing microbial facilities. I also thankful to Head, Department of Chemistry, Dr. H.S.Gour Central University, Sagar (M.P.) for providing necessary laboratory facilities. One of the author Prakrati Yadav is thankful to UGC- New Delhi for financial support.

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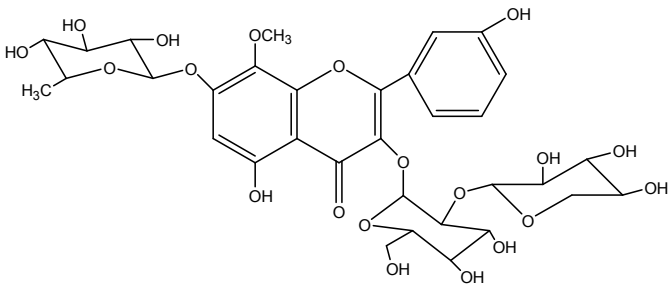
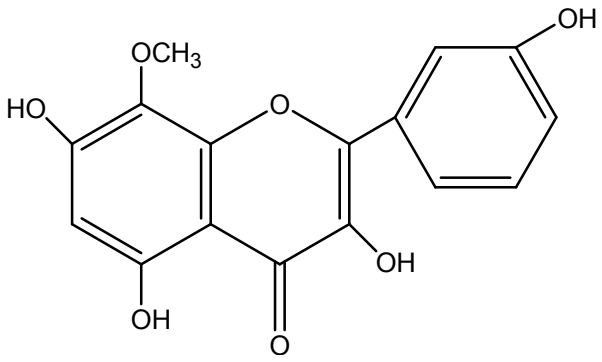
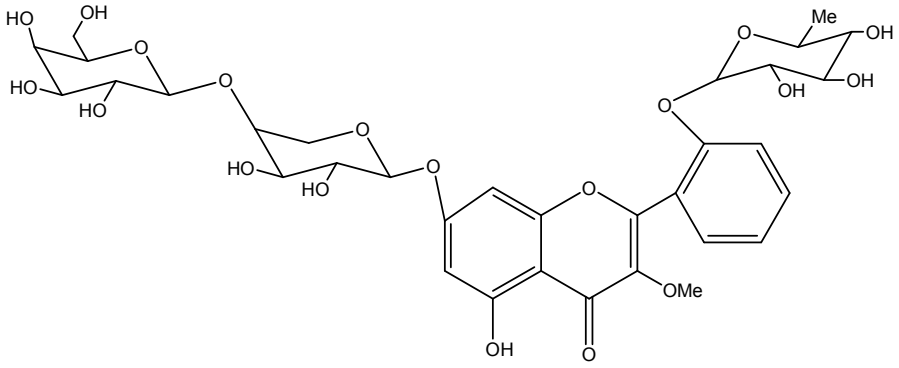


(A)



(A-1)

Figure 1. Structure of Compound A and A-1



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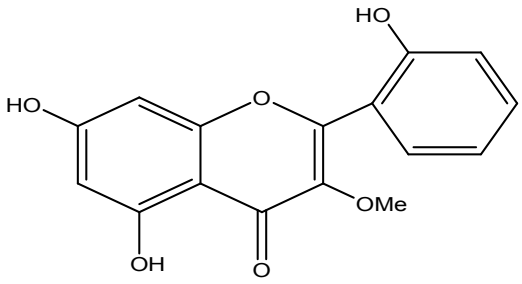

(B-1)

Figure 2. Structure of Compound B and B-1

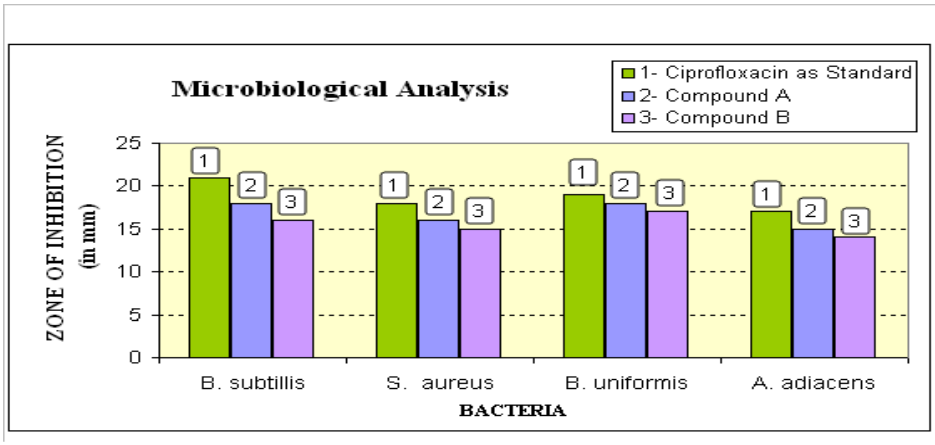

Figure 3. Impact assessment of Compound A, B and Ciprofloxacin on Zone of Inhibition (in mm) against bacteria *B.subtilis, S.aureus,B.uniformis* and *A.adiacens.*

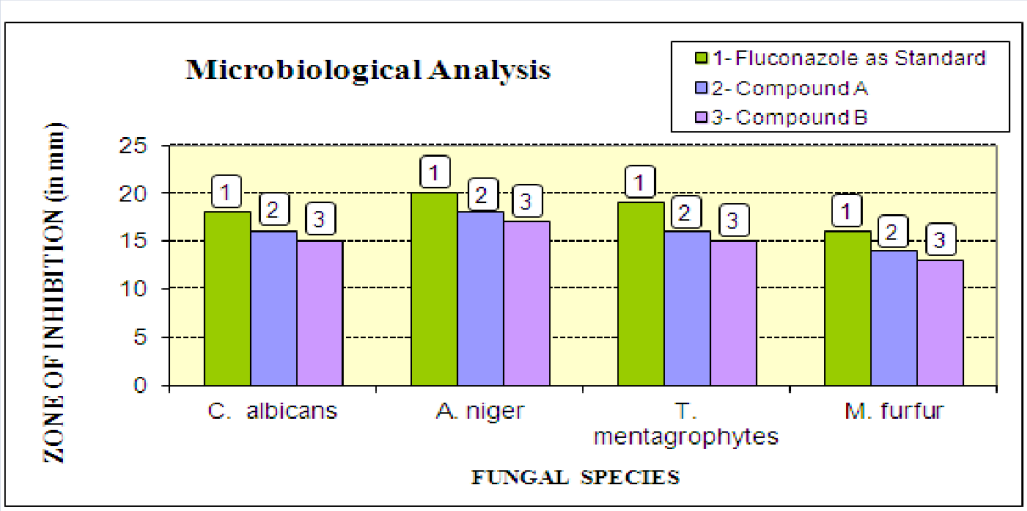

Figure 4. Impact assessment of Compound A, B and Fluconazole on Zone of Inhibition (in mm) against fungi *C.albicans, A.niger, T.mentagrophytes* and *M.furfur*.