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CHALCONES FROM *NYCTANTHUS ARBORTRISTIS* AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

The present study deals with isolation as well as characterization of two new biologically active chalcones which has been isolated from extracts of the leaves of *Nyctanthus arbortristis*. Compound I was isolated from ethanol fraction and characterized as 4'-hydroxy, (5, 5' diprenyl) 4-methoxy chalcone, and compound II was isolated from water fraction and characterized as 3, 4, 4' trihydroxy [5-5' bis-O-(7-methyl 5-oxooctanoate) β- D Glucosidic] Chalcone. Their structures were determined by several color reaction, spectral analysis and chemical degradations. The study revealed their antimicrobial and antifungal potential.

KEYWORDS: Antimicrobial activity; Chalcone; *Nyctanthus arbortristis.*

INTRODUCTION

The use of plants for treating diseases is as old as the human species. This medicinal plant has significantly used for primary health care in South American countries specially.[1] Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action.[2] This plants is well known by their pharmacological properties around the world.

Nyctanthus arbortristis is a well documented tropical plant in India commonly known as *Harsingar, Parijat* and *night jasmine*. *Nyctanthus arbortristis* belonging to oleaceae family has been selected. The use of plants for treating disease is old as the human species. It is used in the ayurvedic system of medicine for the treatment of various diseases, such as, fever, rheumatism; diaphoretic, diuretic and intestinal warm infections. The distilled extract of the leaves is used as a specific treatment for obstinate sciatica,[3] seeds are used for the treatment of piles. The young leaves of *parijat* are used as gynecological troubles with pipper,[4] and it also possess anti-leishmanial, anti-viral activities.[5] The bark of this plant mixed with *Terminalia arjuna* is rubbed on the body to cure internal injuries and to repair broken bones,[3] Antimicrobial properties of medicinal plants are being increasingly reported day by day from different parts of the world,[6,7] As part of our research program on ployphenolics in plants for their efficient utilization, we have isolated here compound [I] from ethanolic extract and compound [II] from water extract from the leaves of *Nyctanthus arbortristis*. The present paper deals with the isolation, spectral characterization and antimicrobial activity of the chalcone from the leaves of *N. arbortristis*.

MATERIAL AND METHODS

Plant materials the leaves of *Nyctanthus arbortristis* is collected from garden in Jhansi (U. P.) in the month of February 2009. Voucher specimen has been kept in the herbarium of the faculty of science (B. B. Colllege, Jhansi) with identification no. 3281.

Characterization techniques All the Melting point was determined by Hofler Bock monoscope and was uncorrected. IR spectrums were carried out on Perkin-Elmer infra-cord model 157. The Mass spectrum recorded on DART-MS (Direct analysis in real time) on JEOL- ACCU TOF JMS- T100 CC, mass spectrum having a dart source. ^{1}H and ^{13}C NMR was recorded at Bruker 300 MHz and 75 MHz respectively. The phase sensitive ROESY spectrum was recorded using standard pulse sequence.

Extraction and Isolation Chromatography techniques were used to isolate the compounds. The column chromatography technique most commonly used to separate the compounds into several fractions according to the affinity of the solvent used. The structure of the compounds were tried to establish by spectroscopic methods.

Shade dried powered leaves (900 gm) of the plant were extracted with different solvent sequentially in the increasing order of polarity: Hexane, Carbon tetra chloride, Benzene, Ethyl acetate, Ethanol, Methanol and water through the soxhlet apparatus. The ethanol and water fraction of the extract after successive extraction by different solvents gave brownish substance as compound-[I] and dark brown crystalline compound-[II]. Further they were separated by column chromatography over silica gel and eluted with CH_2Cl_2: MeOH for compound [I] and ethyl acetate: ethanol for compound [II], and purified by rechromatographed on silica gel eluted with $CHCl_3$: MeOH gave compound [I] For the purification of another compound paper chromatography is used with solvents EtOAC: MeOH: H_2O (12:1:1) ratio. The yellow bands appear, cut this band and dissolved in MeOH, concentrated under reduced pressure to give compound [II]. These were studied separately.

Antimicrobial Activity of Compounds Most plants contains several compounds with antimicrobial properties for protection against aggressor agents, especially microorganism. The chemical structure of some antimicrobial compounds, obtained from plants. It has been reported that the antimicrobial activity had done on the bacteria as well as fungi with agar disc diffusion method.[8]

Phytochemical screening- Ethanolic and aqueous extracts were subjected to qualitative phytochemical tests followed by methods

Antibacterial Assay

Agar disc diffusion method

The chalcone to be tested at a concentration of 0.002g/ml in solvent DMSO were placed with the help of whatman filter paper disc (6mm) in the Petri dish containing nutrient agar. Inoculation of the plates was done at 37^0C for 48hrs. Ruler is used for measuring radius of inhibition zone. The inhibition zone size is proportional to how sensitive the organism to the particular chalcone on the dish. The results obtained were compared with the standard β-naphthol. The obtained results presented in the table no.1. The glycosidic chalcone [II] exhibited the larger inhibition zone as compared to chalcone [I] The microbial activity of an agent might be due to protein binding capacity of phenolics.[9]

Antifungal assay

The antifungal activity for *Aspergillus niger* was carried out on potato dextrose agar medium[10] was used for the preparation of plates. The sterile filter paper disc (6mm) soaked with antifungal agent and incubated for 7 days at 25^OC. The zone of inhibition is reported in the table 1.

Microorganism Tested

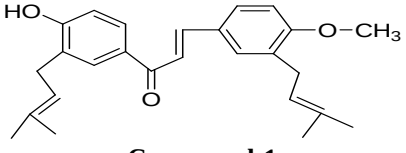
Bacterial strains used in this study were purchased from medical college Jhansi (UP). There are Bacteria Gram – ive (*E.coli*) and Gram + ive (*S. aureus*). They were cultured overnight at 37^0C in Muller and Hilton Broth in nutrient agar and fungal culture *A. Niger* was maintained on potato dextrose agar and incubated at 25^0C for 7 days.

The isolated chalcone and chalcone glycoside were seemed for their antimicrobial activity by using zone inhibition techniques against gram negative bacteria E. coli and gram positive bacteria namely *S. aureus.*

The action mechanisms of natural compounds are related to disintegration of cytoplasmic membrane, destabilization of the proton motive force (PMF), electron flow, active transport and coagulation of the cell content. The structure of the bacterial cell that is considered targets for action by the component of natural products.[11]

RESULT AND DISCUSSION

Compound [I] was obtained as a brownish substance, molecular weight 390.47 compatible with the molecular formula $C_{26}O_3H_{30}$ on the basis of DART, m.p. 200^0 C. The characteristic band in the IR spectrum showed the presence of hydroxyl group (3430 cm^{-1}), non-chelated C=O (1630-1632 cm^{-1}) and C-H stretching (2980 cm^{-1}) functions in the molecule. In addition to UV spectrum maxima at 247, 300sh, 360[12] indicating for chalcone. The ^{I}H NMR spectrum (DMSO at 300 MHz) displayed singlet at δ 7.36 and δ 7.94 unambiguously assigned to C-α and C-β suggesting the chalcone molecule.[13] The occurrence of signals at δ 3.15, δ 2.94 and δ 5.4 for the presence of prenyl group. The presence of doublet at δ 1.99 and δ 2.04 for methyl group assign to prenyl group. The appearance of signal at δ 7.10 and δ 6.69 suggesting the H-2' and H-3' proton.[14] An Additional singlet at δ 3.75 confirms the Methoxy group in the molecule. The position of prenyl group confirmed by the ^{13}C NMR and DART spectrum. The ^{13}C NMR (DMSO at 75 MHz) showed resonances at δ 130.0(C-2, C-6), δ 113.22(C-5), δ 115.6(C-2', C-6'), and δ 114.0(C-5').[15] The signal for C-5, C-5' indicating the carbon- carbon linkage.[16] The signal at δ 27.01 (C-7, C-7'), δ 126.0 (C-8, C-8'), δ 139.9 (C-9, C-9') for prenyl carbon. The presence of signal at δ 17.9 and δ 25.8 for methyl carbon and δ 118.0 and δ 134.2 showed the C-α and C-β carbon,[17] The above deliberations were corroborated by DART-MS spectrum (scheme no.1) which exhibited typical features expected from the fragmentation pattern of chalcone. With peaks at 359.446 (-OCH_3), 189.206(-$C_{12}O_2H_{13}$), 304.35 (-C_4H_7 Prenyl group), 335.374 (-Prenyl group), 279.27 (removal of both prenyl group), 200.25 and 131.136. It is clear from all the spectral determination the compound 1 has a prenyl group at C-5 and C-5' position so the compound[1] is characterized as "4' Hydroxyl (5, 5' diprenyl) 4 methoxy Chalcone".

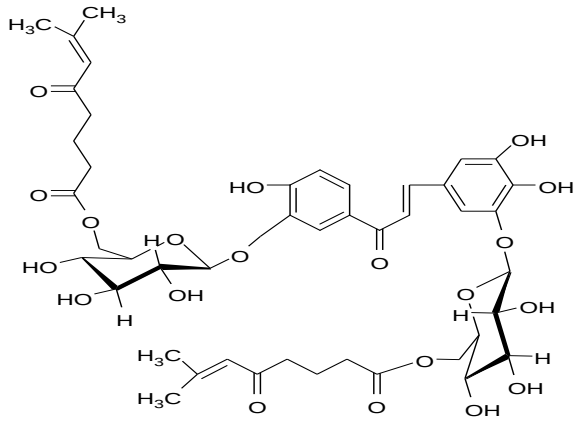


Compound-1

Compound [II] molecular weight 916.698 compatible with the molecular formula $C_{45}O_{20}H_{56}$, m. p. 150^0C was found as dark brown substance. On the basis of its spectroscopic data IR 3400cm^{-1} for hydroxyl group, 1630 cm^{-1} non chelated C=O, 2980 cm^{-1} (C-H) stretching and 1180-1130 cm^{-1} O-glycosidic nature of the chalcone,[18,19] UV spectrum (Me OH) 239, 260, 371 were characteristic of chalcone. The bathochromic shift of 62 nm suggest 4' hydroxyl group with Na OH and 30 nm bathochromic shift with Na OAc/ H_3BO_3 revealed orthodihydroxyl group,[12] In ^{I}H NMR spectrum (DMSO-d_6 300 MHz) 3 signals at δ 8.06 (H-2',H-6') (20), δ 7.10 (H-3') and δ 6.98 (H-2,H-6)[7] assigned in aromatic region . Two signals at δ 7.36(IH, s, J 15) and δ 7.45 (IH, s, J 15) were assigned to α- H and β-H respectively confirm the chalcone molecule.[20]

The anomeric proton signal at δ 4.13 (2H, S) were assigned to H-7', H-7" of D-glucose, while one signal at δ 3.93 (H-12', H-12") indicate the acylation of the glucose.[21,15] Out Of the remaining proton signals in aliphatic region at δ 2.53-δ 1.89 were assigned to nCH_2. The presence of doublet at δ 1.14 correlated well with methyl carbons at C-20', C-20", C-21', C-21". The presence of β-D glucosidic was confirmed by ^{13}C NMR and ^{1}H-^{1}H ROESY spectrum. In ^{13}C NMR (DMSO at 75 MHz) spectrum of this compound showed 29 signals for 45 carbons. 15 signals were assigned to the Chalcone skeleton. Among the 6 carbon signals belong to aromatic carbon bearing an oxygen atom δc 151.1 (C-4'), δc 150.9 (C-5'), δc 188.2 (C=O), δc 158.0 (C-3), δc 160.0 (C-4), and δc 151.0 (C-5). The signal at C-5, C-5' shows hydroxylation substitution on this site.[15] Two carbon signals δc 128.1 and δc 136.3 were attributed to C-α and C-β carbons. Beside the 15 carbon signal of the chalcone nucleus the ^{13}C NMR exhibited another 14 carbon signals in which 6 carbon signals for glucose, the anomeric signal at δc 104.6 was assigned to C-7',C-7" of D-glucose, remaining aliphatic carbons were attributed to oxo-octanoate moiety (nCH_2 C=O) attached to glucose moiety. The up field signal at δc 45.4 and δc 45.2 were ascribable to C-14', C-14", C-16', C-16".[22] A number of carbons in up field region together showed the nCH_2 chain attributed to C-8',C-8"(75.1), C-9'.C-9"(77.8), C-10',C-10"(71.3),C-11',C-11"(77.7),C-12',C-12"(62.5).[23] A signal at δc 25.9 is indicative for 4 methyl carbons (C-20', C-20", C-21', C-21"). In the ^{I}H-^{I}H- ROESY spectrum the compound showed correlation between proton signal of δ 2.53 (H-12',H-12") and δ 3.93 (H-14',H-14"), which suggest the attachment of nCH_2 chain to glucose and signal at δ 1.14 (Methyl proton) and δ 2.06 (H-18',H-18") showed the presence of methyl group at C-20',C-20" position. ^{I}H NMR and ^{13}C NMR spectra showed characteristic shift values and multiplicities of β-D glycoside.[24]

The DART MS spectrum exhibited typical features expected from the fragmentation pattern (scheme no.-2) with peak 861.602(-C_4H_7), 601.434, 833.602(-C_5OH_7), 846.568, 886.625, 902.672 (-CH_3), 311.180(-$C_9O_2H_{13}$), 464.354(-$C_{23}O_{10}H_{28}$) and 885.617. The cleavage of the ethereal of compound [II] by acid hydrolysis furnished aglycon with mp. 210^0C and the sugar fraction which was identified as β- D Glucose by paper chromatography These evidences were adequate compound[II] to characterized as 3, 4, 4' trihydroxy [5-5'bis-O-(7-methyl-5-oxooctanoate) β- D Glucosidic] Chalcone.



Compound-2

It is found that compound-1 and compound-2 of *Nyctanthus arbortristis* leaves contained flavonoids, and chalcones. In vitro antibacterial and antifungal activities were examined from compound-1 and compound-2 against three pathogenic bacteria (two gram positive and negative) and one pathogenic fungus was investigated by agar disk diffusion methods.[25]

In present work the compounds, obtained from *Nyctanthus arbortristis* show strong activity against most of the tested bacterial and fungal strains. The results were comparing with standard antibiotic drugs. In this screening work, extracts of leaves were found to be active against organism. Results show that the plant rich in chalcone as well as phenolic compounds have been shown to possesss antimicrobial activities against a number of microorganisms.

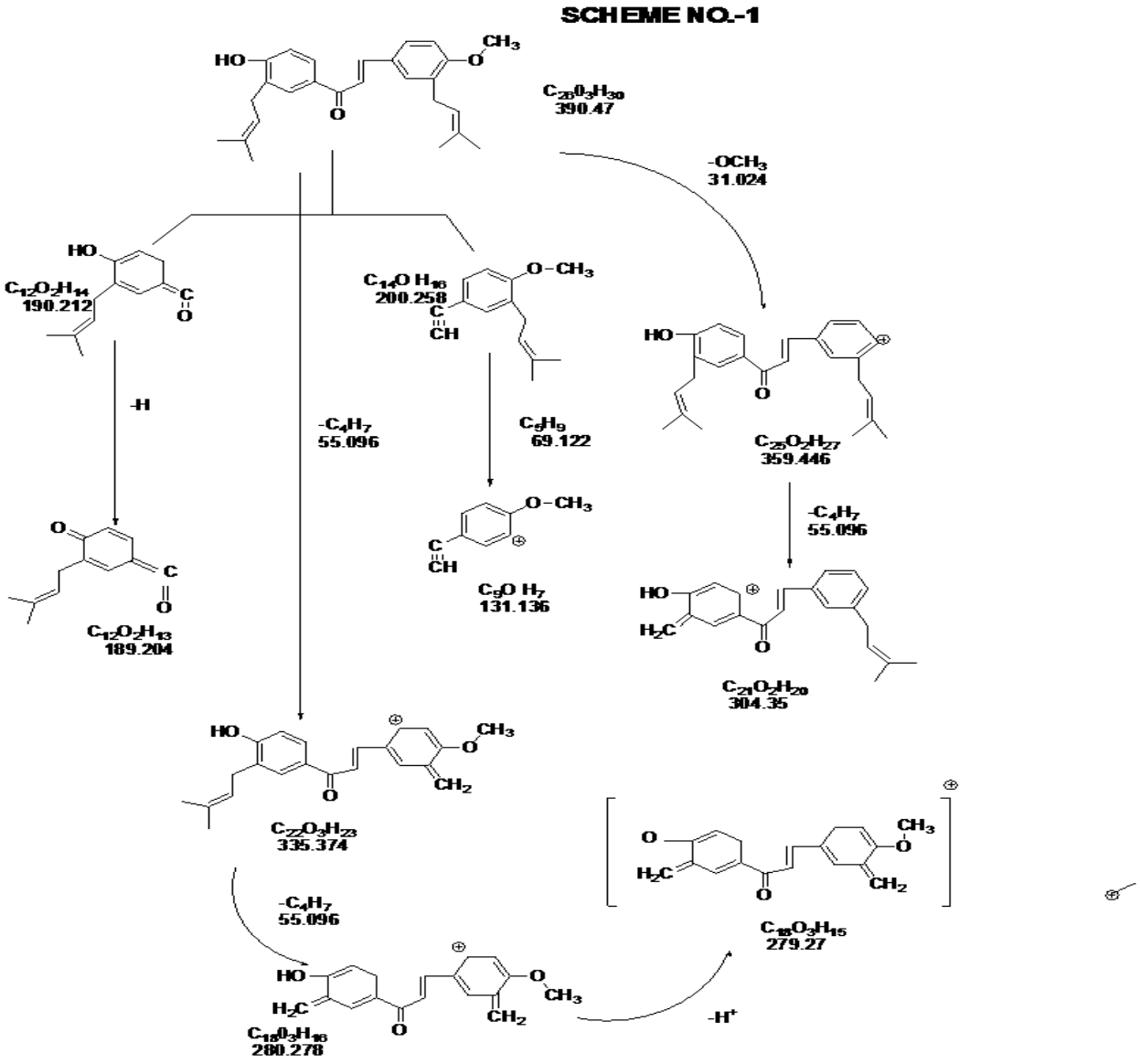
Compound I: mp. 200^oC, UV (MeOH, λmax, nm): 247, 300, 360; IR (KBr, ν, cm^{-1}): 3439(OH), 1630(C=O non chelated), 2980 (C-H Stretching), DART-MS: 359.446, 189.206, 304.35, 335.374, 279.27, 131.136. δ^{1}H(DMSO at 300 MHz) (ppm) 6.98(2H, H-2,H-6), 8.06(1H, H-3), 3.15(1H, H-7a), 2.94(1H, H-7'b), 5.4(2H, H-8,H-8"), 1.99(6H, d,H-10,H-10'), 2.04(6H,d,H-11,H11'), 7.10(2H,d,H-2',H6'), 6.69(1H,s H-3'), 7.36(1H,s, H-α), 7.94(1H,s, H-β), 3.75(3H,s, 0CH3). ^{13}C NMR(DMSO at 75 MHz) δ (ppm): 127.0(C-1), 130.0(C-2, C-6), 118.8(C-3), 159.2(C-4), 113.2(C-5), 120.0(C-1'), 115.6(C-2',C-6'), 108.7(C-3'), 158.1(C-4'), 114.0(C-5'), 27.01(C-7,C-7'), 126.0(C-8,C-8'), 139.9(C-9,C-9'), 17.9(C-10,C-10'), 25.8(C-11,C-11'), 163.1(C=O), 118.0(C-α), 134.2(C-β).

Compound II: mp. 150^0C, UV (MeOH, λmax, nm): 239, 266, 371; 251,281,433(+NaOH); 274, 339, 384

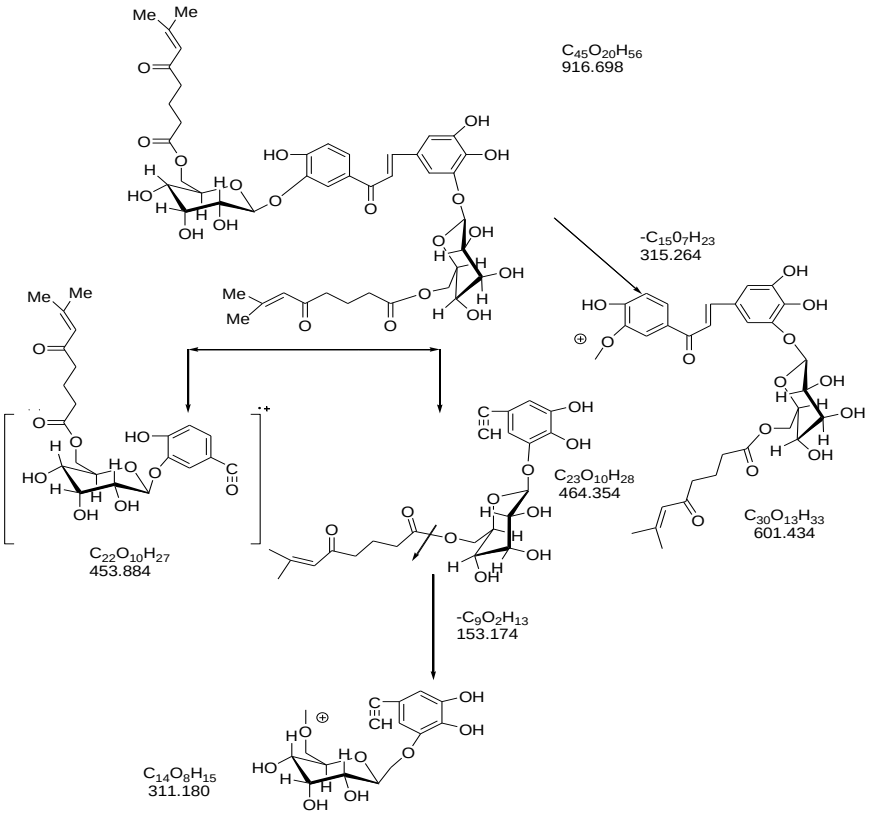
(dec)(+NaOAc), IR (KBr, ν, cm^{-1}): 3400 (OH), 1630 (C=O non chelated), 2980 (C-H Stretching), 1180-1130 (O-glycosodic nature). DART-MS: 861.602, 601.434, 833.602, 846.568, 886.625, 902.672, 885.617, 464.354, 311.180. δ ^{1}H (DMSO at 300 MHz) (ppm): 8.06(2H,d,H-2'H-6'), 7.1(1H,s, H-3'), 7.36(1H,s, J15,H-α), 7.45(1H, s, J15, H-β), 6.98(2H,s, H-2, H-6), 4.13(2H, s , H-7',H-7"), 3.04(2H,s,H-8',H-8"), 3.13(2H, s, H-9',H-9"), 3,20(2H, s,H-10',H-10"), 2.97(2H, s, H-11',H-11"), 3.93(4H,s, H-12',H-12"), 2.53(8H, s, H-14;,H-14", H-16',H-16"), 1.89(4H,s, H-15',H-15"), 2.06(2H, s, H-18',H-18"), 1.12(12H, d,H-20',H-20",H-21;,H-21"). ^{13}C NMR(DMSO at 75 MHz)δ (ppm): 127.1(C-1),131.0(C-2), 158.0(C-3), 160.0(C-4), 151.0(C-5), 131.7(C-6), 128.1(C-α), 136.3(C-β), 188.2(C=O), 118.0(C-1'), 123.6(C-2'), 137.1(C-3')151.1(C-4'), 150.9(C-5'), 121.0(C-6'), 104.6(C-7',C-7"), 75.1(C-8',C-8"), 77.8(C-9',C-9"), 71.3(C-10',C-10"), 77.7(C-11',C-11"), 62.5(C-12',C-12"), 170.6(C-13',C-13"), 45.4(C-14',C-14"), 42.2(C-15',C-15"), 45.2(C-16',C-16"), 173.8(C-17',C-17"), 122.4(C-18',C-18"),130.5(C-19',C-19"), 25.7(methyl carbon). ROESY linkage: 1.14(CH_3)—2.06(H-18',H-18"), 2.53(H-12',H-12")—3.93(H-14',H-14"), 4.13(H-7',H-7")—3.93(H-12',H-12").

Table 1: Antimicrobial Activity (Diameter of inhibition zone in mm).

MO'S	Compound-I	Compound-II (Control , β- Naphthol)
E. Coli	9mm	15mm
S. Aureus	7mm	12mm
A. Niger (fungi)	6.1mm	7.6mm



Scheme 2 Part 1



SCHEME NO.2

Part- 2

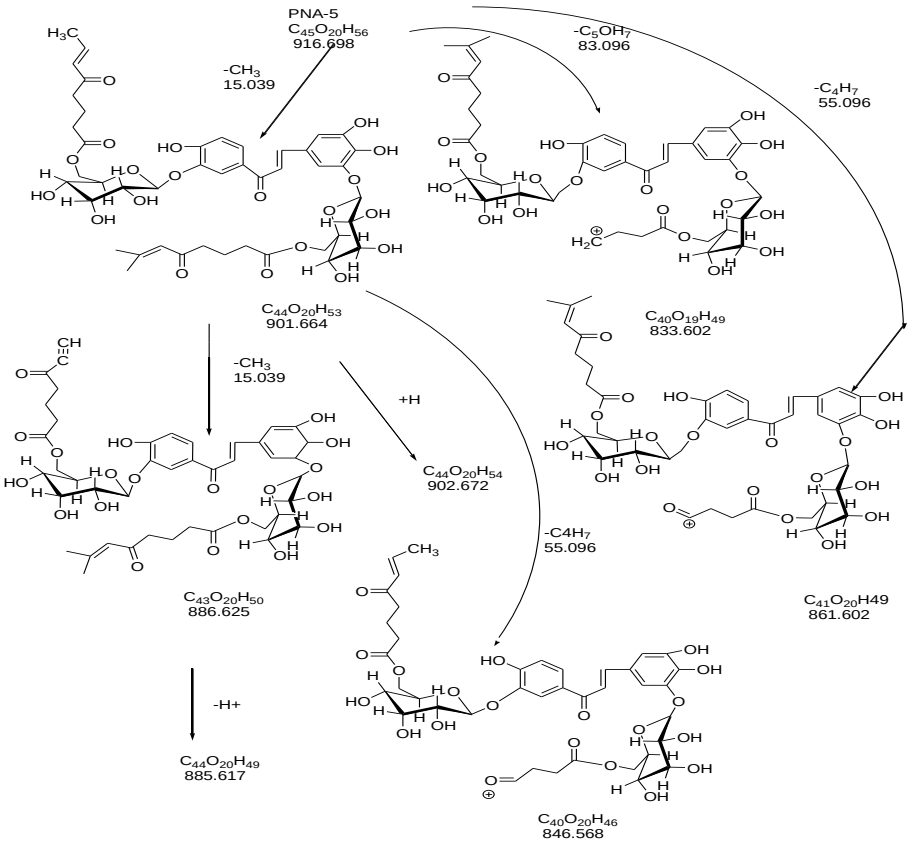


Table 2: ^{1}H and ^{13}C NMR data for compound I (recorded in DMSO δ in ppm).

Position	δ H	δC
2	6.98(IH)	130.0
3	8.06(1H)	118.8
4	------	159.2
5	--------	113.2
6	6.98(1H)	130.2
1’	------	120.0
2’,6’	7.10(2H)	115.6
3’	6.69(1H)	108.7
4’	-------	158.1
5’	---------	114.0
PRENYL		
7,7’	3.15(a)2.94(b)(4H)	27.01
8,8’	5.4(2H)	126.0
9,9’	--------	139.9
10,10’	1.99(6H)	17.9
11,11’	2.04(6H)	25.8
α	7.36(1H)	118.0
β	7.94(1H)	134.2
OCH3	3.75(3H)----	--
C=O	------	163.

Table 3: ^{1}H and ^{13}C NMR data for compound II (recorded in DMSO δ in ppm).

Position	δH	Roesy (linkage)	δ C
1	----		127.1
2,6	6.98(2H,S)		131.0
3	----		158.0
4	----		160.0
5	----		151.0
2’,6’	8.06 (2H)		123.6,121.0
α	7.36 (1H,S,J=15)		128.1
β	7.45 (1H,S,J=15)		136.3
1’	----		118.0
3’	----		137.1
4’	----		151.1
5’	-----		150.9
Glu			
7’,7”	4.13 (2H,S,)	3.93(H12;,H12”)	104.6
8’.8”	3.04 (2H,S)		75.1
9’,9”	3.13 (2H,S)		77.8
10’,10”	3.20 (2H,S)		71.3
11’,11”	2.97 (2H,S)		77.7
12’,12”	3.93 (2H,S)		62.5
13’,13”	----		170.6
14’,14”	2.53 (4H,S)	3.93(H12;,H12”)	45.4
15’,15	1.89 (4H,S)		42.2
16’,16”	2.53 (4H,S)		45.2
17’,17	----		173.8
18’,18”	2.06 (2H,S)		22.4
19’,19”	----		30.5
Methyl carbons	1.14 (12H,d)	2.06(H18’,H18”)	25.7

CONCLUSION

The present study exhibited the isolation, purification and characterization of two new chalcones and their antimicrobial activity. The research revealed the antimicrobial effect of Chalcone from ethanol and water fraction of *Nyctanthus arbortristis* against some of the pathogenic organism. As a result is assured that these compounds inhibit the growth of these microorganisms.

However, Water fraction exihibited significant inhibitory activity then ethanolic fraction against tested microorganisms.

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