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CLERODANE DITERPENES FROM *POLYALTHIA LONGIFOLIA VAR. PENDULA* AS NOVEL ANTI-TUBERCULAR AGENTS

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ABSTRACT

Over the ages people have relied upon nature to meet their fundamental needs, including medicines for the treatment of wide variety of diseases. The second most significant cause of death in the world is tuberculosis (TB) from bacillus *Mycobacterium tuberculosis.* Although the adverse reaction to first line therapy is less, many patients experience untoward effects while on multi-drug therapy leading to non-compliance. With these in perspective, our research group is in pursuit of novel anti TB drugs from natural products.

We isolated four compounds belonging to clerodane class of diterpenes from the plant *Polyalthia longifolia* var *Pendula*. Among these 16α-hydroxycleroda3,13 (14)Z-dien15,16olide (**1**), 16Oxo-cleroda3,13(14)E-diene15 oic acid (**2**), methyl (Z)-3-formyl-5-((8aR)-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)pent-2-enoate (**3**), 5-methoxy-4-(2-((8aR)-1,2,4a,5-tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)ethyl)furan-2(5H)-one (**4**). Compounds 1, 2 & 4 showed potent anti TB activity (MIC 3.125µg/ml) *Mycobacterium tuberculosis* H37Rv. They also have shown significant antibacterial activity against gram +ve (*Staphylococcus aureus (*NCIM *2122)*) and gram -ve

(*Escherichia coli (*NCIM 2137*)*) bacteria at 50µg/mL indicating at good selectivity against *Mycobacterium tuberculosis*.

Keywords: *Polyalthia longifolia var Pendula* seeds, Anti tubercular activity, MABA assay, Anti-bacterial activity, Antifungal activity

1. INTRODUCTION:

Across history, mankind has relied on Nature to satisfy human needs, including medicines for the treatment of a variety of ailments. Plant systems continue to play a significant function in health care and are well chronicled for their utilization in many civilizations **[1, 2]**. The World Health Organization (WHO) estimated in 1985 that approximately 65 % of the world's population relied predominantly on plant-derived traditional medicines for primary health care, while plant products also play an important, albeit more indirect, role in the health care systems of the remaining population, pretty much exclusively domiciled in developed countries **[3]**.

The second most significant cause of death in the world is tuberculosis (TB) from bacillus *Mycobacterium tuberculosis.* About nine million new cases of TB have been identified by the World Health Organisation (WHO, Geneva), of which 1.3 million died **[4]**. Microbial derived natural products have a long history in the treatment of TB. Rifampicin, Isoniazid, Pyrazinamide, and Ethambutol are the most effective oral anti-TB drugs.

Although the adverse response is minimal, many victims experience terrible side effects when taking many drugs simultaneously, which is the primary justification of noncompliance **[5]**. Certain major causes of resistance include incoherent and prolonged treatment, prescription of inappropriate combinations and substandard drugs **[6]**. Multi-drug-resistant strains (MDR) are not only difficult to treat but are more lethal. All such complications can be prevented by having synergistic medications included in the usual treatment. In the treatment of different diseases, some combinations have been tried to improve efficacy, narrower treatment duration, improve conformity and reduce resistance level of risk.

Research into natural products has provided various drugs, and a new compound, isolated from plants has recently been found active against MDR TB. Natural flora serves millions of lives with use of drugs or supplements **[7, 8]**. The main objective of this study is study anti-TB activity of secondary metabolites obtained from the plant *Polyalthia longifolia var Pendula*.

Polyalthia longifolia var Pendula (Annonaceae) is a routinely planted tree that may significantly suppress noise emissions. The bark is used in traditional medicine for treating fever, skin diseases, hypertension, diabetes, and helminthiasis **[9]**. Prior studies on this plant revealed that the acetone extract of the leaves contains two clerodane diterpenoids with antifeedant effects **[10]**. Diterpenoids isolated from hexane extract of seeds of *P. longifolia* have shown remarkable antibacterial and antifungal action **[11]**. The cytotoxic activity of *P. longifolia* leaf extract was further investigated across several cancer cells, and the mechanism of apoptosis induction was also reported **[12]**. Thereby, our investigation focuses on the *in vitro* antitubercular and anti-microbial activities from secondary metabolites isolated from the methanolic extract *of Polyalthia longifolia var Pendula* seeds.

2.MATERIALS AND METHODS

2.1 Collection of plant material:

The seeds of the plant were collected in the grounds of Andhra University, Visakhapatnam, in the month of July 2018. The identity of the plant was confirmed by Dr. S.B. Padal and a voucher specimen (No. 22293) has been deposited in the Herbarium of the department of Botany, Andhra University, Visakhapatnam, India.

2.2 Extraction and Isolation:

Polyalthia longifolia var Pendula fresh seeds (25kg, **Figure 1**) were dried under shade at room temperature. The dried seeds were powdered (14kg) and macerated at room temperature with 10% ethyl acetate: methanol for 7 days and filtered. The filtrates were collected, concentrated under reduced pressure to a syrupy mass (yield: 1.2 kg). Six fifty grams (650 g) of the extract were fractionated using a silica gel column chromatography of 60-120 mesh by sequential elution of 2.5L of n-hexane, ethyl acetate and methanol each **(Figure 2)** (mother column). From mother column we have collected 10% ethyl acetate: n-hexane fraction (50 g), which has the highest clerodane diterpenes level, was subjected to column chromatography using n-hexane, graded with ethyl acetate (n-hexane: ethyl acetate (100:0, 99:1, 98:2, 96:4 up to 0:100) (Fraction 1) to obtain the compounds 1 (5g) & 2 (5g) **(Figure 3)**. From fraction 1 column we have collected 5% ethyl acetate: n-hexane fraction (5 g), and again subjected to column chromatography using silica gel (100-200) mesh to obtain compounds 3 (1g) and 4 (1g) (Fraction 2) **(Figure 3)**. The isolated compounds were analysed spectroscopically by FTIR, ^{1}H NMR, ^{13}C NMR, and MASS spectrometry.

2.3 Anti-bacterial activity:

Agar well-diffusion method was followed to determine the antimicrobial activity on both gram positive (*Staphylococcus aureus*

*(*NCIM 2122)) and gram negative bacteria (*Escherichia coli (*NCIM 2137)). All the isolated compounds were weighed accordingly and 1mg/ml stock solutions were prepared using DMSO as a vehicle and Rifampicin was used as a standard drug. Into the sterilized agar medium, 100 µl of bacterial suspension (*E. coli & S. aureus)* was added, whose OD was checked at 535 nm before the preparation of bacterial suspension (OD = 0.6-0.8). The inoculated agar medium was poured into sterilized petri plate and after 15 minutes, wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. All isolated compounds, control and Rifampicin; each of 100 µl & 50 µl quantity were added into the bores using micropipette. The petri plate was incubated at 37ºC up to 24 hrs to determine the zone of inhibition **[13]**.

2.4 Anti-fungal activity:

Antifungal activity of all the compounds was evaluated by the agar well diffusion method. All the compounds were tested for their *in vitr*o growth inhibition activity against *Candida albicans* (NCIM 3102) **[14]**. For the evaluation of the antifungal activities of the samples, agar well diffusion method was used. All the compounds were weighed accordingly and 1mg/ml stock solutions were prepared using DMSO as a vehicle. In the same way fluconazole, which is used as a standard drug was prepared. Into the sterilized Sabouraud's medium, 100 µl of bacterial suspension (*Candida albicans*) were added. The Sabouraud's agar medium was poured into sterilized petri plate, after 15 minutes bores were made on solidified agar medium **[15]**. In each of these plates, sterile cork borers were used to make wells (10 mm diameter and roughly 2 cm apart). All isolated compounds, control and fluconazole of 100 µl & 50 µl concentration was added in the petri plate. The petri plate was incubated at 26ºC up to 48 hrs to determine the zone of inhibition**.**

2.5 Anti tubercular activity:

The isolated compounds were tested for anti TB activity (according to NCCL standards 1985) against *Mycobacterium tuberculosis* H37Rv by Micro-plate Alamar Blue Assay (MABA) **[16]**.

Löwenstein Jensen (LJ) medium growth was suspended in sterile Middlebrook 7H9 broth supplemented with 0.2 % glycerol and 10% OADC (oleate-albumin dextrose-catalase) enrichment, with a 1:20 dilution employed as the MABA inoculum. All procedures were carried out while maintaining appropriate safety hoods. To prevent evaporation of medium in the test wells during incubation, 200 µl of sterile deionized water was injected to the outside perimeter wells of a sterile 96 well plate. The 96-well plate was loaded with 100 µl of Middlebrook 7H9 broth, and the

compounds were serially diluted on the plate. The final drug concentrations tested were 25, 12.5, 6.25, 3.125, 1.6 and 0.8 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink.

3.RESULTS AND DISCUSSION:

3.1 CHEMISTRY

From Fraction 1 column chromatography 16α-hydroxycleroda3,13(14)Zdien15,16 olide **(1),** obatained as white powder (5gm), 16Oxo-cleroda3,13(14)E-diene15 oic acid **(2)** obtained as yellow gum (5gm), and from Fraction 2, Methyl (Z)-3-formyl-5-((8aR)-1,2,4a,5-tetra methyl-1,2,3,4,4a,7,8,8a- octa hydro naphthalen-1-yl) pent-2-enoate (methyl ester) **(3),** obtained as plae yellow oil (1gm) (Figure.3) and 5-methoxy-4-(2-((8aR)-1,2,4a,5 tetra methyl-1,2,3,4,4a,7,8,8a-octa hydro naphthalen-1-yl) ethyl) furan-2 (5H)-one (methyl ether) **(4)**, obtained as pale-yellow oil (1gm). We have got 3 known compounds which are reported earlier **[17]**. The compound 4 obtained from fraction 2 shows IR absorptions at 2932, 1762, 1648 cm^{-1} indicated the presence of carboxylic acid and a double bond respectively and ^{1}H NMR have shown four methly signatures at 17 (δ 0.81),18 (δ 1.59),19 (δ 1.06), and 20 (δ 0.77) positions, as well as two -diene bonds at the 3 (δ 5.19) and 14 (δ 5.64) positions, clearly shown the characteristic feature for clerodane diterpene skeleton. ^{1}H NMR and ^{13}C NMR upon thorough scrutiny compound 4 is closely relating to the compound containing acetoxy group which reported earlier **[17]**. Both are differing by acetoxy and methoxy groups have shown in **(Figure 4)**. Spectral investigations, including FTIR, 1D and 2D NMR measurements, MASS spectroscopy was used to determine the structures of all lactones produced. Complete spectral data are mentioned in **Table 1 and Table 2**.

3.1.1 Compound 1:

16α-hydroxycleroda3,13(14)Zdien15,16 olide obtained as white powder, m.p. 100-101^{o}C:1H NMR (CDCl3, 400 MHz): δ 1.45 (2H, m, H1), 2.04 (2H, m, H2), 5.19 (1H, m, H3), 1.15 (1H, m, H6), 1.467 (2H, m, H7), 1.452 (1H, m, H8), 1.45 (2H, m, H10), 1.50 (1H, m, H11), 2.28-2.36 (2H, m, H12), 5.83 (1H, s, H14), 6.02 (1H, s, H16), 0.82 (3H, d, J=5.1Hz, H17), 1.60 (3H, s, H18), 1.01 (3H, s, H19), 0.78 (3H, s, H20); 13C NMR (CDCl3, 100MHz): δ 18.2 (C1), 26.8 (C2), 120.4 (C3), 144.2 (C4), 38.1 (C5), 36.7 (C6), 27.3 (C7), 36.3 (C8), 38.6 (C9), 46.4 (C10), 34.7 (C11), 21.3 (C12), 171.5

(C13), 116.8 (C14), 172.0 (C15), 99.4 (C16), 16.0 (C17), 18.1 (C18), 19.9 (C19), 18.2 (C20). IR (KBr) Vmax cm^{-1}: 3435, 2922, 1751(C=O), 1650(C=C), ESI *m/z* $[M]^+$ 319.2 (calcd for $C_{20}H_{31}O_3$, 319.22).

3.1.2 Compound 2:

16Oxo-cleroda3,13(14)E-diene15 oic acid obtained as yellow gum, m.p. 168-170 ^oC:

1H NMR (CDCl3, 400 MHz): δ 1.38-1.46 (2H, m, H1), 2.05-2.10 (2H, m, H2), 5.20 (1H, m, H3), 1.71 (1H, m, H6), 1.38-1.46 (2H, m, H7), 1.68-1.71 (1H, m, H8), 1.41 (2H, m, H10), 1.25-1.26 (1H, m, H11), 2.53-2.60 (2H, m, H12), 6.45 (1H, s, H14), 9.49 (1H, s, H16), 0.84 (3H, d, J=5.1Hz, H17), 1.60 (3H, s, H18), 0.98 (3H, s, H19), 0.68 (3H, s, H20); 13C NMR (CDCl3, 100MHz): δ 18.2 (C1), 26.8 (C2), 120.9 (C3), 143.8 (C4), 38.0 (C5), 36.7 (C6), 27.5 (C7), 36.2 (C8), 39.1 (C9), 46.4 (C10), 37.1 (C11), 19.6 (C12), 153.5 (C13), 137.6 (C14), 170.8 (C15), 196.5 (C16), 14.5 (C17), 17.9 (C18), 20.02 (C19), 18.2 (C20). IR (KBr) Vmax cm^{-1}: 2924,1 695(C=0), 1640(C=C), 1450, 1379, 1257, 1224, 1178, 1110, 1099, 1043, 1006, 925, 879, 816, 797. ESI [M - H2O] + *m/z* 318, (calcd for $C_{20}H_{30}O_3$, 318.5).

3.1.3 Compound 3:

Methyl (Z)-3-formyl-5-((8aR)-1,2,4a,5-tetra methyl-1,2,3,4,4a,7,8,8a- octa hydro naphthalen-1-yl) pent-2-enoate (methyl ester) as pale yellow oil, m.p. 171-172 ^oC : 1H NMR (CDCl3, 400 MHz): δ 1.43 (2H, m, H1), 2.13 (2H, m, H2), 5.23 (1H, m, H3), 1.26 (1H, m, H6), 1.44 (2H, m, H7), 1.46 (1H, m, H8), 1.43 (2H, m, H10), 1.70 (1H, m, H11), 2.53 (2H, m, H12), 6.44 (1H, s, H14), 9.51 (1H, s, H16), 0.86 (3H, d, J=5.1Hz, H17), 1.60 (3H, s, H18), 1.01 (3H, s, H19), 0.69 (3H, s, H20) OMe (1H, s, 3.82); 13C NMR (CDCl3, 100MHz): δ 19.4 (C1), 20.8 (C2), 120.5 (C3), 144.0 (C4), 38.1 (C5), 26.9 (C6), 36.5 (C7), 37.1 (C8), 39.2 (C9), 46.6 (C10), 27.5 (C11), 36.9 (C12), 153.2 (C13), 138.9 (C14), 174.5 (C15), 194.8 (C16), 16.19 (C17), 18.18 (C18), 18.05 (C19), 19.98(C20). IR (KBr)Vmax cm^{-1}: 2952, 2725(CHO), 1725(C=O), 1697(C=O), 1642(C=C).

3.1.4 Compound 4:

5-methoxy-4-(2-((8aR)-1,2,4a,5tetra methyl-1,2,3,4,4a,7,8,8a-octahydro naphthalen-1-yl)ethyl) furan-2 (5H)-one (methyl ether) as pale yellow oil, m.p.110-113^oC:

1H NMR (CDCl3, 400 MHz): δ 1.39 (2H, m, H1), 1.69(2H, m, H2), 5.19(1H, m, H3), 1.26 (1H, m, H6), 1.44 (2H, m, H7), 1.46 (1H, m, H8), 1.35 (2H, m, H10), 1.50 (1H, m, H11), 5.64 (1H, s, H14), 5.86 (1H, s, H16), 0.81 (3H, d, J=5.1Hz, H17), 1.59 (3H, s, H18), 1.06 (3H, s, H19), 0.77 (3H, s, H20) OMe (1H, s, 3.5); 13C NMR (CDCl3, 100MHz): δ 18.2(C1), 26.7 (C2), 120.4 (C3), 144.4 (C4), 38.1 (C5), 36.6 (C6), 27.3 (C7), 36.3 (C8), 38.6 (C9), 46.4 (C10), 34.7 (C11), 21.3(C12), 168.7 (C13),

117.6 (C14), 170.7 (C15), 104.4 (C16), 15.9(C17), 17.9 (C18), 19.8 (C19), 18.1(C20), OMe (56.89). IR (KBr) Vmax cm^{-1}: 2932, 1762(C=O), 1648(C=C).

3.2 Antibacterial Activity:

The isolated compounds (Compound 1, 2, 3, & 4), were tested for antibacterial activity against *Staphylococcus aureus* bacteria (gram +ve) and *Escherichia coli* (gram -ve) bacteria. The standard drug Rifampicin showed MIC of 18.5 µg/ml against *Staphylococcus aureus* and 19.5 µg/ml against gram *E.Coli* bacteria. The control (DMSO) produced no zone of inhibition. The results were presented in the **Table 3 and figure 5**.

3.3 Anti-fungal activity

The isolated compounds (Compound 1, 2, 3, & 4) were tested for antifungal activity against *Candida albicans.* The standard drug Fluconazole produces and MIC of 12.5 µg/ml against *Candida albicans*. The results were represented in **Table 4 and Figure 6**.

3.4 *In Vitro* antitubercular activity screening

For all the isolated compounds (Compound 1, 2, 3, & 4), anti-tubercular activity was done by MABA assay against *Mycobacterium tuberculosis* H37Rv. The results are shown in **Table 5** represented in **Figure 7**.

Antibacterial and cytotoxic activities was reported earlier for the compounds 1, 2 & 3 **[17]**. Anti-tubercular activity of all the isolated compounds 1, 2, 3 & 4 were isolated from *Polyalthia longifolia* seeds exhibited excellent anti-tubercular efficacy at a minimum inhibitory concentration of 3.125 µg/ml by Microplate Alamar Blue Assay (MABA) against the *Mycobacterium tuberculosis* MTB H37Rv bacilli strain which are same as standard drugs. They also have shown significant antibacterial activity against gram +ve (*Staphylococcus aureus (*NCIM 2122*)*) and gram -ve (*Escherichia coli* (NCIM 2137)) bacteria at 50µg/mL indicating at good selectivity against *Mycobacterium tuberculosis*. The comparatively extended therapeutic period of the Tuberculosis will deeply affect the gut and other areas of our body's microbial ecology. As a result, it is always sought after to find specific anti-mycobacterial medicines such as INH, which has almost minimal action against other microorganisms. The anti-tubercular results clearly confirm that potency is increased with the groups containing the lactone moiety along with the ester containing groups.

Figure 1: Seeds of *Polyalthia logifolia* var Pendula linn

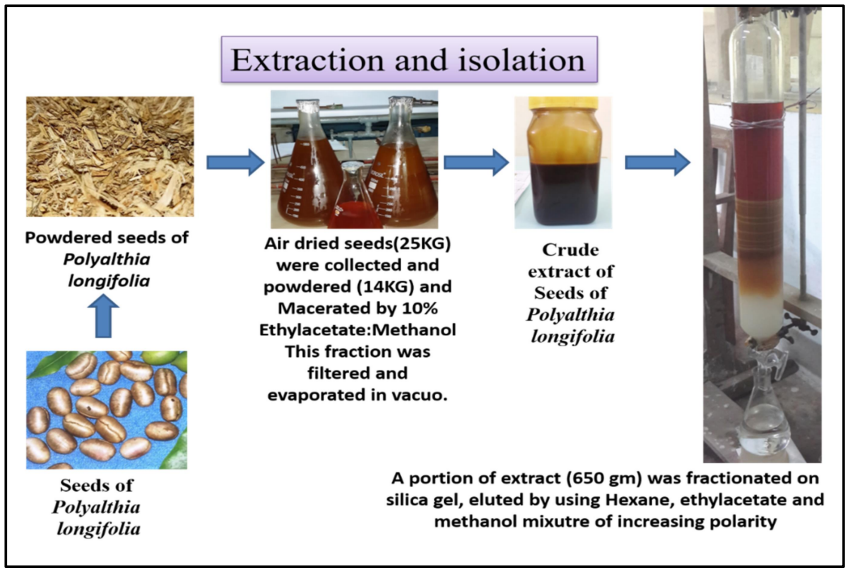


Figure 2: Extraction and isolation of seeds of *Polyalthia logifolia* var Pendula linn

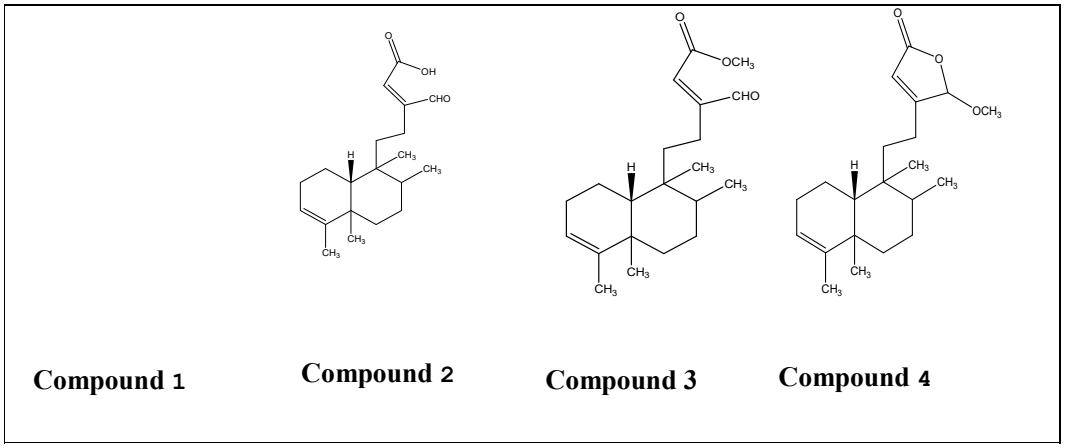
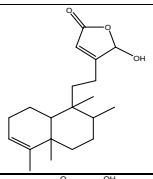
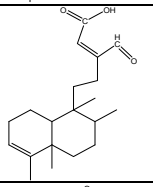
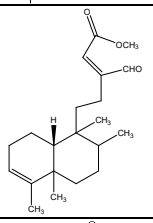
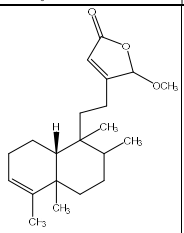


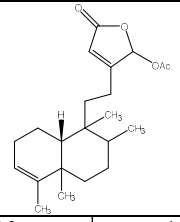
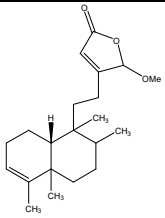
Figure 3: Structures of isolated compounds from seeds of *Polyalthia longifolia*

Table 1: Comparision of NMR spectral data of Compound 1 and 2 with the compounds reported earlier [17], and Spectral DATA of isolated compounds (Compound 3 and 4)

	COMPOUNDS											
Position	Compound 1				Compound 2				Compound 3		Compound 4	
	Reported NMR Values		Values of our NMR		Reported NMR Values		Values of our NMR					
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	18.2	1.45-1.48	18.2	1.45	18.1	1.38-1.46	18.2	1.38-1.46	19.4	1.43	18.2	1.39
2	26.7	1.95-2.05	26.8	2.04	26.8	2.05-2.14	26.8	2.05-2.10	20.8	2.13	26.7	1.69
3	120.4	5.18	120.4	5.19	120.8	5.21	120.9	5.20	120.5	5.23	120.4	5.19
4	144.2	-	144.2	-	144.3	-	143.8	-	144.0	-	144.4	-
5	38.1	-	38.1	-	38.2	-	38.0	-	38.1	-	38.1	-
6	36.6	1.15-1.20	36.7	1.15	36.8	1.73-1.79	36.7	1.71	26.9	1.26	36.6	1.26
7	27.3	1.43-1.47	27.3	1.467	27.7	1.38-1.46	27.5	1.38-1.46	36.5	1.44	27.3	1.44
8	36.2	1.40-1.45	36.3	1.452	36.3	1.68-1.45	36.2	1.68-1.71	37.1	1.46	36.3	1.46
9	38.6	-	38.6	-	39.3	-	39.1	-	39.2	-	38.6	-
10	46.4	1.35	46.4	1.45	46.6	1.38-1.40	46.4	1.41	46.6	1.43	46.4	1.35
11	34.7	1.50-1.53	34.7	1.50	37.1	1.25-1.29	37.1	1.25-1.26	27.5	1.70	34.7	1.50
12	21.3	2.28-2.35	21.3	2.28-2.36	19.2	2.53-2.61	19.6	2.53-2.60	36.8	2.53	21.3	-
13	171.2	-	171.5	-	157.5	-	153.5	-	153.2	-	168.7	-
14	116.7	5.82	116.8	5.83	134.1	6.47	137.6	6.45	138.9	6.44	117.6	5.64
15	172.3	-	172.0	-	170.6	-	170.8	-	174.5	-	170.7	-
16	99.4	6.03	99.4	6.02	194.3	9.53	196.5	9.49	194.8	9.51	104.4	5.86
17	16.0	0.80	16.0	0.82	15.9	0.84	14.5	0.84	16.19	0.86	15.9	0.81
18	18.0	1.58	18.1	1.60	18.1	1.59	17.9	1.60	18.18	1.60	17.9	1.59
19	19.8	1.00	19.9	1.01	19.9	0.99	20.02	0.98	18.05	1.01	19.8	1.06
20	18.2	0.76	18.2	0.78	18.1	0.68	18.2	0.68	19.9	0.69	18.1	0.77
OCH_3	-	-	-	-	-	-	-	-	-	3.82	-	-
OMe	-	-	-	-	-	-	-	-	-	-	56.89	3.5

Table 2: Properties of all isolated compounds of *Polyalthia longifolia*

Compound Codes	Structure	Properties
Compound 1		$C_{20}H_{30}O_3$ Exact Mass: 318.2 Mol. Wt.: 318.5 m/e: 318.2 (100.0%), 319.2 (22.1%), 320.2 (2.9%) C, 75.43; H, 9.50; O, 15.07
Compound 2		$C_{20}H_{30}O_3$ Exact Mass: 318.2 Mol. Wt.: 318.5 m/e: 318.2 (100.0%), 319.2 (22.1%), 320.2 (2.9%) C, 75.43; H, 9.50; O, 15.07
Compound 3		$C_{21}H_{32}O_3$ Exact Mass: 332.2 Mol. Wt.: 332.5 m/e: 332.2 (100.0%), 333.2 (23.2%), 334.2 (3.2%) C, 75.86; H, 9.70; O, 14.44
Compound 4		$C_{21}H_{32}O_3$ Exact Mass: 332.2 Mol. Wt.: 332.5 m/e: 332.2 (100.0%), 333.2 (23.2%), 334.2 (3.2%) C, 75.86; H, 9.70; O, 14.44

S. No.	Compound with Acetoxy group [17]	Compound with methoxy group (compound 4)
1.		

H-2	**1.92**
H-3	**5.17**
H-14	**5.95**
H-17	**0.80**
H-18	**1.57**
H-19	**0.98**
H-20	**0.75**
OAc	**2.16**

H-2	**1.62**
H-3	**5.19**
H-14	**5.64**
H-17	**0.81**
H-18	**1.59**
H-19	**1.06**
H-20	**0.77**
OMe	**3.5**

Figure 4: Comparisions of ^{1}H NMR spectra with reported compound

Table 3: MIC(µg/ml) for Isolated compounds

Sample	Gram +Ve	Gram -Ve
codes	50µg/ml	50µg/ml
STD	18.5±0.34	19.5±0.4
1	12.3±0.2	12.9±0.5
2	15.95±0.5	14.95±0.6
3	5.3±0.3	5.4±0.3
4	5.1±0.4	5.5±0.4

Standard (Rifampicin)-18.5mm; (against *Staphylococcus aureus* and 19.5 against *E.coli*); Control (DMSO)-No zone; NA = not active

Table 4: MIC (µg/ml) of anti-fungal activity for isolated compounds

Sample codes	MIC (µg/ml)	
	50µg/ml	100µg/ml
STD	12.5±0.34	14±0.1
1	10.5±0.1	11.4±0.2
2	9.6±0.4	10.6±0.1
3	8.6±0.2	9.5±0.1
4	7.9±0.1	9±0.2

Standard (Rifampicin)-18.5mm; (against *Staphylococcus aureus* and 19.5 against *E.coli*); Control (DMSO)-No zone; NA = not active

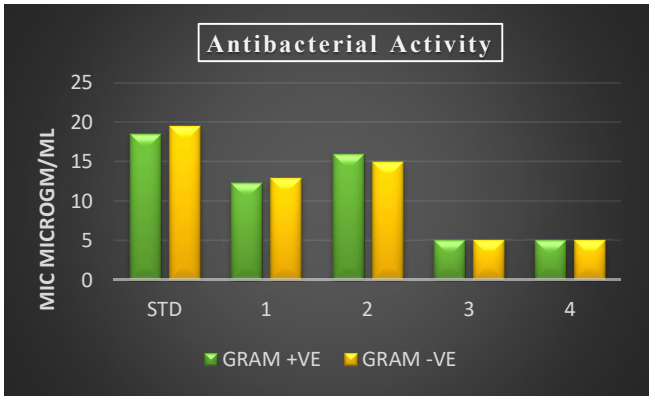


Figure 5: MIC **for anti-bacterial activity of isolated compounds**

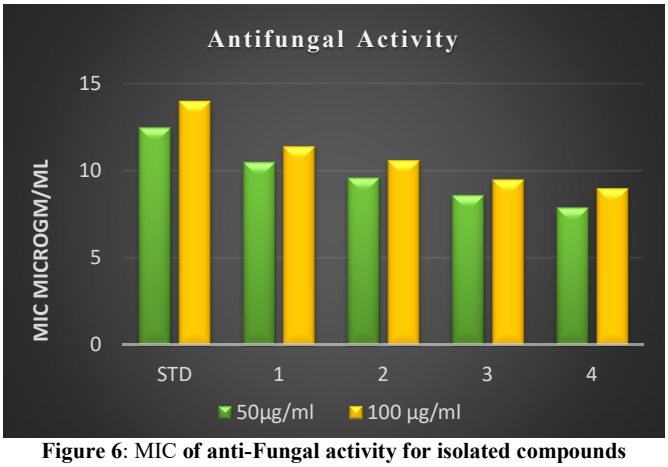


Figure 6: MIC **of anti-Fungal activity for isolated compounds**

Table 5: MIC of anti-tubercular activity for isolated compounds

S.No	Sample code	MIC(µg/ml)
1	Compound 1	3.125
2	Compound 2	3.125
3	Compound 3	6.25
4	Compound 4	3.125
5	PAS	12.5
6	RIFAMPICIN	6.25

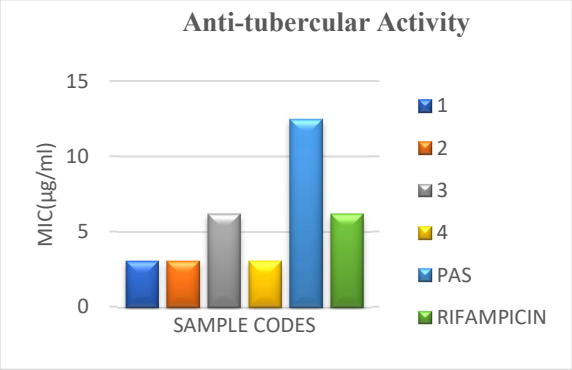


Figure 7: MIC of anti-tubercular for isolated compounds

4. CONCLUSION:

Finally, the conclusion is nature is an evergreen resource for finding the novel lead molecules. The isolated compounds which we found from seeds of *Polyalthia longifolia* shown a potent antibacterial, antifungal and antitubercular activity. *Polyalthia longifolia* seeds has been shown to have antitubercular activity. As a result, we may certainly declare that nature is capable of curing all ailments.

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