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Research Article

ANTIMICROBIAL ACTIVITY OF THE EXTRACTS AND ISOLATED COMPOUNDS OF *CLERODENDRUM PHLOMIDIS*

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ABSTRACT

Objective: In the present work we made an attempt to assess the *in vitro* antimicrobial activity of the extracts and isolated compounds from roots of *Clerodendrum phlomidis* of family Verbenaceae against *Staphylococcus aureus*, *Streptococus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus niger*.

Method: Root extracts (Pet ether, Chloroform, Ethyl acetate and Ethanol) of *Clerodendrum phlomidis* was prepared by using Soxhlet apparatus. 30gms of ethanol extract was subjected to column chromatography. Three compounds such as Phenyl acetic acid, Ethyl- 2- hydroxy -4- methyl benzoate, 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one, was isolated and characterised by IR, NMR and Mass Spectroscopy. Different dilutions of extracts (106.66µg/ml, 200 µg/ml, 320 µg/ml and 400µg/ml) and isolated compounds (20, 40, 60 µg/ml) were prepared and their antimicrobial screening was carried out by disc diffusion method. The MIC of the extracts and isolated compounds was determined by broth dilution method of NCCLS. The selected microorganisms were also tested against standard drugs like (Amikacin 30µg/disc and Ketoconazole 20µg/disc).

Results: The present experiment showed that ethanol extract at 106.66µg/ml showed a significant result against *Escherichia coli* was observed as the most sensitive (15.33mm). Chloroform extract also shows good antimicrobial activity against *Staphylococcus aureus* with a zone of inhibition of (14.67mm). Among the isolated compounds, Ethyl- 2- hydroxy -4- methyl benzoate shows good antimicrobial activity than other two compounds against *Staphylococcus pyogenes*, and *Candida albicans* with a zone of inhibition of 9mm respectively at 60µg/ml the zone of inhibition observed was also supported by the results obtained from MIC.

Conclusion: In the present work, three compounds were isolated by column chromatography such as Phenyl acetic acid, Ethyl- 2- hydroxy -4- methyl benzoate, 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one. The above compounds and their antimicrobial activity were not reported earlier. Ethyl- 2- hydroxy -4- methyl benzoate shows good antimicrobial activity than other two compounds.

Keywords: *Clerodendrum phlomidis*, Extracts, Column chromatography, Verbenaceae.

INTRODUCTION

Clerodendrum phlomidis is a common shrub of arid plains, low hills and tropical deserts. They are distributed throughout the drier parts of India (Andhrapradesh, Uttarpradesh, Delhi, Gujarat, and Tamilnadu). *Clerodendrum phlomids* Linn, (Syn. *Clerodendrum multiflorum* (Burn.f) O.Kuntze, *Volkameria multiflorum* (Burn.f). (Verbenaceae) is an important and well known medicinal plant extensively used in Ayurveda and Siddha system of medicine for treatment of various ailments. The popular therapies include on inflammation, diabetic, nervous disorders, asthma, rheumatism, digestive disorders, and urinary disorders. It has been reported that pectolinaringenin, scutellarein, 7 hydroxy flavone has been isolated from this plant.[1] Decoction of its root which is slightly aromatic and astringent is used as a demulcent in gonorrhea. It is also given to children during convalescence from measles.[2] Roots contain clerodin, clerodendrin A, clerosterol, ceryl alcohol. The ethanol extract of leaves exhibited hepatoprotective activity. The aqueous extract of leaves exhibited in vitro anthelmintic activity. The plant also exhibited anti diabetic activity.[3,4] The methanolic extract *Clerodendrum phlomidis* Linn shows minor tranquilizing property.[5] It is used in amrit nectar tablets (Amrit nectar tablets containing 38 herbs.) The effect of aqueous and alcoholic extract of Amrit nectar tablet on rat liver microsomal lipid peroxidation is good.[6]Methanolic extract of leaves of Clerodendrum phlomidis shows significant inhibitory against castor oil induced diarrhoea.The extract also showed a significant reduction in charcoal meal test in rats.[7] Ethyl acetate and hexane extracts of leaves and stems of Clerodendrum phlomidis showed antifungal activity against plant and human pathogens.[8]

MATERIALS AND METHODS

Collection ad identification of *Clerodendrum phlomidis*

The roots of *Clerodendrum phlomidis*, were collected from Chennai, Tamil Nadu, India. The plant material was identified by Dr. Sasikala Ethirajulu, Research officer, CCRAS, Govt.of India, Chennai. The roots of *Clerodendrum phlomidis* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The dried powder of the roots was extracted sequentially by hot continuous percolation method using Soxhlet apparatus, using different polarities of solvents like petroleum ether, chloroform, ethyl acetate and ethanol. The dried root powder was packed in Soxhlet apparatus and successively extracted with petroleum ether by for 24 hrs. Then the marc was subjected to chloroform for 24 hrs, and the marc was subjected to ethyl acetate for 24 hrs and then marc was subjected to ethanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Phytochemical Analysis

The extracts were used for preliminary phytochemical screening of Phytochemicals such as alkaloids (Wagner and Dragendroff's tests), flavonoids (Shinodas and lead acetate tests), Phenols (ellagic acid and ferric chloride test), tannins (gelatin test), saponins (foam test), sterols (Liberman – Burchard and Salkowski tests) and glycosides (Molishs test, Benedicts) following Harbone method.[9]

Antimicrobial activity

Four bacterial cultures viz. two Gram positive (*Staphylococcus aureus* 33592, *Streptococus pyogenes* 49399), and Gram negative (*Escherichia coli* 010536, *Pseudomonas aeruginosa* 35032), Two fungal strains viz. (*Aspergillus niger* 16404), (*Candida albicans* 10321) were obtained from Bose Clinical laboratory Madurai, Tamilnadu. Mueller Hinton Agar (MHA) and Sabouraud dextrose Agar (SDA) obtained from Himedia laboratories (Mumbai). Amikacin was from Cipla Pharmaceuticals, Mumbai. Ketoconazole obtained from Triveni Interchem Pvt, Ltd. Vapi, Gujarat.

Table 1: Phytochemical screening of root extracts of *Clerodendrum phlomidis*

S. No.	Name of the test	Pet ether	Chloroform	Ethyl acetate	Ethanol
1.	Liberman Burchard (for terpenes & steroid)	+	+	-	-
2.	Salkowski (for steroid & terpenes)	+	+	-	-
3.	Mayer's (for alkaloids)	-	-	-	-
4.	Legal,s test for glycosides	-	+	-	+
5.	Test for Phenolics ($Fecl_3$)	-	-	+	+
6.	Shinoda (for flavonoids)	-	-	+	+

Preparation of inoculums

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton Agar (MHA) for bacteria and Sabouraud dextrose Agar (SDA) for fungi that were incubated without agitation for 24 hrs at 37°C and 25°C respectively. The cultures were diluted with fresh Mueller-Hinton and Sabouraud dextrose Agar to achieve optical densities corresponding to $2.0 \cdot 10^6$colony forming units (CFU/ml) for bacteria and $2.0 \cdot 10^5$spore/ml for fungal strains.

Assay of antimicrobial activity

The disc diffusion method[10] was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 20ml of molten media into sterile petriplates. The plates were allowed to solidify for 30 minutes and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. Four different dilutions of extracts (106.66µg/ml, 200µg/ml, 320µg/ml and 400µg/ml) and (Amikacin 30µg/disc and Ketoconazole 20µg/disc) standards were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, antibacterial activity was recorded by measuring the diameter of zone of inhibition. Amikacin was used as positive reference standard. The entire test was performed in triplicate. The antifungal activity was assayed by inoculating the fungal spores on Sabouraud dextrose agar (SDA) medium pre-impregnated with disc containing plant extracts. Ketoconazole was used as positive reference standard against fungal strains.

The minimum inhibitory concentration (MIC) of extracts of roots was determined by broth dilution method by NCCLS. The lowest concentration of the plant extracts inhibiting the visible growth of organism was considered as MIC.[11]

The isolated compounds from ethanol extract (compound A, B, & C) were also screened for antimicrobial activity by using the same above method in different concentrations like (20µg/ml, 40µg/ml and 60µg/ml). Zone of inhibition and minimum inhibitory concentration was determined as above. Amikacin and Ketoconazole were used as the positive reference standard for the bacteria and fungi.

Isolation by Column chromatography

It was reported in the literature survey that ethanol extract of Clerodendrum phlomidis has the highest content of phenolic and flavonoid components compared to other extracts like (pet ether, chloroform ethyl acetate and ethanol).[12] Henceforth ethanol extract was selected for column chromatographic isolation and wet packing method was followed. Initially 3/4th of the column is filled with pet ether and then silica gel (100 – 200 mesh size) is added slowly to ensure uniform packing. 30gms of ethanol extract was chromatographed over a column of 600gms silica gel by gradient elution.

Fractions 15 – 18 eluted with Pet ether: Chloroform (50:50) led to the isolation of compound A, which on concentration and filtration yielded a residue which was recrystallised in chloroform. Fraction 26 – 30 eluted with Chloroform: Ethyl acetate (90:10) elution led to the isolation of compound B, which on concentration and filtration yielded a residue which was recrystallised in chloroform. Fractions 50- 54 eluted with Ethyl acetate: Methanol (90:10) led to the isolation of compound C, which on concentration and filtration yielded a residue which was recrystallised in chloroform. The crystals of compound A, B & C were dissolved in minimum amount of chloroform and are then subjected for thin layer chromatography. It was found that all the compounds shows only single spot in the solvent system used. The isolated compounds are characterized by IR (KBr), ^{1}H NMR and [13]CNMR and ESI - MS spectra.

Table 2: TLC analysis *Clerodendrum phlomidis*

S. No.	Compounds	Solvent system	No of spot	R_f value
1.	Compound A	Pet ether: Chloroform (50: 50)	1	0.56
2.	Compound B	Chloroform: Ethyl acetate (90:10)	1	0.64
3.	Compound C	Ethyl acetate : Methanol (90: 10)	1	0.72

Identification test

Test for phenols

Few crystals of compound B gave green colour with 2 to 3 drops of 10% ferric chloride solution indicating the presence of phenolic hydroxyl group.

Test for flavonoids

Few crystals of compound C dissolved in chloroform treated with 0.5g of magnesium turnings and few drops of conc. HCl from the sides of the test tube. Appearance of pink colour shows the presence of flavonoids.

Spectroscopical investigations

Melting point was determined by determined in open–glass capillaries on Stuart SMP10 melting point apparatus and were uncorrected. The IR (KBr) spectrum was recorded on a Shimadzu UV 168A and Perkin Elmer 1600 FTIR spectrometer, respectively. The 1H-NMR and 13C-NMR spectra were recorded on a Bruker R32 (400 MHz) in DMSO –d with TMS as an internal standard (chemical shifts in δ, ppm). TLC was performed with silica gel 60 G F254 and spots were visualized by iodine vapors or ultraviolet light The electron spray ionization mass spectrum (ESI - MS) was acquired on a Bruker Daltronics Esquire 3000 plus ion trap mass spectrometer. All solvents were analytical reagent grade.

Characterization for isolated compounds[13]

Compound A

IR (KBr) 1686 cm^{-1} (the carbonyl group of the aromatic acids), 3071-2917cm^{-1}(stretching frequencies of aromatic C-H vibration). ^{1}H NMR spectrum (400 MHz, $CDCL_3$), (a) Aromatic proton; doublet; δ8.20 – 8.10ppm, 2H. (b) Aromatic proton; multiplet; δ7.58- 7.45ppm, 3H. (c) – CH_2 – proton; singlet; δ1.3, 2H. (d) – OH – proton of COOH group; singlet; δ11.00ppm. The [13]C NMR spectra in $CDCL_3$, (a)

Carbon of phenyl ring resonates at δ128 – 130ppm, (b) Tertiary carbon resonates at δ133ppm), (c) Methylene carbon resonates at δ76ppm, (d) Carbonyl carbon resonates at δ172ppm. The mass of the molecular ion peak of compound A is found to be m/z 136, corresponding to the molecular formula ($C_8H_8O_2$). Ion peaks were also observed at m/z 59, 78.

Compound B

IR (KBr) 3422 cm^{-1} (phenolic –OH) group, 1654 cm^{-1} (carbonyl group of the ester), ^{1}H NMR spectrum (400 MHz, $CDCL_3$), (a), Aromatic proton; doublet; δ6.85 – 6.82ppm, 1H. (b), Aromatic proton; doublet; δ7.55 – 7.52ppm. (c) Aromatic proton; doublet; δ6.39 – 6.30ppm, 1H. (d), -CH_2- proton; quartet; δ3.71 – 3.68ppm, 2H. (e), - CH_3 – proton; triplet; δ3.29 – 3.23ppm, 3H. (f), - CH_3 – proton associated with phenyl ring system; singlet; δ2.50ppm, 3H. (g), OH proton; singlet; δ212.60ppm. The [13]CNMR spectra in $CDCL_3$, (a), Carbon (secondary) of aromatic ring resonates at δ144 – 103ppm. (b), Tertiary carbon of aromatic ring resonates at δ148ppm. (c), carbon of methyl groups resonates at δ76 – 70ppm, (e), carbon carbonyl group resonates at δ160ppm. (d), carbon of methylene group resonates at δ98ppm. The mass of the molecular ion peak of compound B is found to be m/z 180, corresponding to the molecular formula ($C_{10}H_{12}O_3$). Ion peaks were also observed at m/z 15, 73, 94.

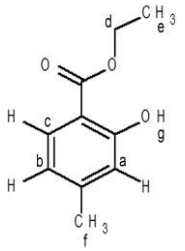
Compound C

IR (KBr), 3330-3612 cm-1 (OH) groups, 1655cm^{-1}(carbonyl group), 3089cm^{-1} (-C-H aromatic). 2849cm^{-1} (C-H aliphatic). ^{1}H NMR spectrum (400 MHz, $CDCL_3$), (a), aromatic proton, singlet; δ8.33, 2H, (b), aromatic proton, doublet; δ6.50ppm, 2H. (c), aromatic proton, doublet; δ6.85 – 6.81, 1H. (d), aromatic proton, triplet, δ7.36 – 7.33ppm, 1H. (e), Hydroxyl protons showed a sharp singlet at δ13.05 and two broad peak at 10.72 and 9.60ppm respectively. (f), methoxy proton showed a singlet at δ3.75ppm. The [13]CNMR spectra in $CDCL_3$, (a), Secondary carbon resonates at δ115 – 157ppm, aromatic C and C=C. (b), Tertiary carbon resonates at δ93 – 104ppm, aromatic tertiary carbon. (d), Carbonyl carbon resonates at δ180ppm and (e), methoxy carbon resonates at δ59ppm. The mass of the molecular ion peak of compound C is found to be m/z 300.1, corresponding to the molecular formula ($C_{16}H_{12}O_6$). Ion peaks were also observed at m/z 75, 149.2, 207, 237, 281, 327, 347, 345, 355, 400, 425, 500.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the extracts revealed the presence of steroids, terpenoids, glycosides, phenolic compounds and flavonoids. From the positive test for phenolic compounds given by B, it is assumed to be compound containing phenol nucleus. The B is white crystalline needles like substance with melting point 210° - 212° C. On subjection to IR spectroscopic analysis, The IR spectrum of compound ET 2 shows that broad band at 3422 cm^{-1} is due to the phenolic –OH group. The carbonyl group of the ester occurs at 1654 cm^{-1}. ^{1}H NMR spectrum (fig.1) of compound B shows (a), singlet signal at δ6.39ppm, 1H**,** (b), doublet signal at δ6.85 – 6.82ppm, 1H, (c) doublet signal at δ7.55 – 7.52ppm, 1H corresponds to aromatic protons. (d), Quartet signal at δ3.7 – 4.1ppm, 2H corresponds to methylene group, (e), strong triplet signal at **δ** 1.22 – 0.976ppm, 3H, correspond to methyl groups. (f), Singlet at δ 2.50 assigned to methyl group associated with phenyl ring system. (g), Singlet at δ12.60 assigned to phenyl – hydroxy proton. The [13]CNMR spectra (fig.2) of compound B in $CDCL_3$ suggested that, (a), secondary carbon of aromatic ring resonates at δ144 – 103ppm, (b), tertiary carbon of aromatic resonates at δ148ppm, (c) carbon of methyl groups resonates at δ76 – 70ppm, (e), carbon of carbonyl group resonates at δ160ppm, and (d), carbon of methylene group resonates at δ98. The mass of the molecular ion peak of compound B is found to be m/z 180, corresponding to the molecular formula ($C_{10}H_{12}O_3$). Ion peaks were also observed at m/z 15, 73, 94. Based on the above spectral data's the compound B is found to be Ethyl- 2- hydroxy -4- methyl benzoate.

Compound A was isolated as White crystals, melting point 75° C - 76° C. Its IR spectrum shows that The IR spectrum of compound A, shows that the stretching frequencies of aromatic C-H vibration occur in the range of 3071 – 2917 cm^{-1}. There is an intense band at 1686 cm^{-1} which correspond to the carbonyl group of the aromatic acids. ^{1}H NMR spectrum (fig 3) of compound A shows, (a), doublet signal at δ8.113 – 8.109 ppm 2H, (b), triplet signal at δ7.510 – 7.459ppm, 2H, (c), triplet signal at δ7.647 – 7.598ppm 1H, corresponds to aromatic protons.(d), The singlet at δ 11.0ppm is due the carboxylic hydroxyl group. (e), Strong singlet at δ 1.3ppm is assigned to the methylene hydrogen. The [13]CNMR spectra (fig.4) in $CDCL_3$ of shows, (a), Carbon (Secondary) of phenyl ring resonates at δ128 – 130ppm, (b), Tertiary carbon of phenyl ring resonates at δ133ppm. (c), Methylene carbon resonates at δ77 - 76 ppm, (d), Carbonyl carbon resonates at δ172ppm. The mass of the molecular ion peak of A is found to be m/z 136. Corresponding to the molecular formula ($C_8H_8O_2$). Ion peaks were also observed at m/z 15, 73, 94. Based on the above spectral data's the compound A is found to be phenyl acetic acid.



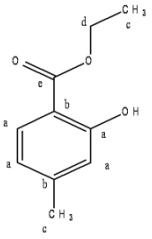
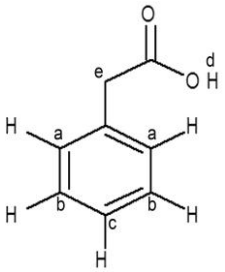


Fig. 1: ^{1}H NMR of compound B. Fig. 2: [13]C NMR of compound B



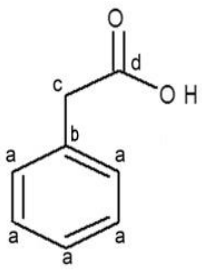


Fig. 3: ^{1}H NMR of compound A. Fig. 4: [13]C NMR of compound A

Compound C was isolated as yellow coloured crystals melting point 222° C – 225° C. Its IR spectrum shows that the IR spectrum of compound ET 3 suggested that absorption at the range from 3330 to 3612 $cm^{-1}$is due to the -OH groups. The -C-H (aromatic) absorption falls at 3089cm^{-1}. Absorption at 2849cm^{-1} is for C-H (aliphatic). The sharp absorption at 1655cm^{-1}shows the presence of carbonyl group at ring. ^{1}H NMR spectrum (fig.5) of compound C shows that (a), singlet signal at δ8.33ppm, 2H, (b), doublet signal at δ6.50ppm, 2H, (c), doublet signal at δ6.83 – 6.81ppm, 1H, (d) triplet signal at δ7.38 - 7.36ppm, 1H, corresponds to aromatic protons. (e), Singlet at δ13.07ppm, 1H corresponds to hydroxyl proton. (f), Broad peak at δ10.72 and (g), δ9.60ppm corresponds to hydroxyl proton. (h), Singlet signal at δ3.75ppm, 3H assigned to methoxy proton. The [13]CNMR spectra (fig. 6) in $CDCL_3$ of compound C shows that, (a), Secondary carbon of aromatic ring resonates at δ115 – 157ppm, (b), tertiary carbon of aromatic resonates at δ93 - 104ppm. (c), Carbonyl carbon of aromatic ring resonates at δ180ppm. (d), Methoxy carbon resonates at δ59ppm. The mass for parent ion (M^+ ions) of is m/z 300. Based on the Spectral Data the tentative structure of compound C was proposed to be 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4*H*-chromen-4-one. The molecular formula is deduced as $C_{16}H_{12}O_6$.

Ethanol extract exerts good antibacterial activity against *Escherichia coli* with MIC value of 106µg/ml and a zone of inhibition of 15.33mm. Ethanol extract also exerts good antibacterial activity against *Streptococcus pyogenes* with a zone of inhibition of 12.93mm at 400µg/ml (Table 3& 4). Pet ether extract exerts good antibacterial activity against *Streptococcus pyogenes*, with a MIC value of 120µg/ml. Pet ether extract produce higher zone of inhibition against *Streptococcus pyogenes* with 14.50mm at 320µg/ml. (Table 3& 4). Chloroform extract exerts good antibacterial activity against *Staphylococcus aureus* with a MIC value of 200µg/ml and a higher zone of inhibition against *Staphylococcus aureus* with 14.67 and 14.33mm at 200µg/ml and 320µg/ml. Ethyl acetate extract exerts a

good antifungal activity against *Aspergillus niger* with a MIC value of 200µg/ml and higher zone of inhibition of 13.87mm at 400µg/ml.

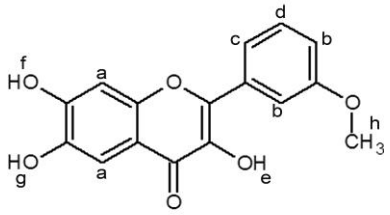


Fig. 5: ^{1}H NMR of compound C

(Table 3& 4). All the extracts do not produce inhibition against *Pseudomonas aeruginosa* at different concentration used.

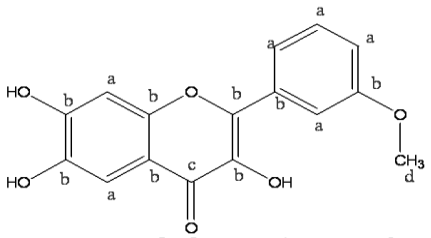


Fig. 6: [13]C NMR of compound C

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents, the first step towards this goal is *in vitro* antibacterial activity. The extracts of higher plant can be very good source of antibiotics against various bacterial pathogen.[14]The present study carried out on the plant samples revealed the presence of medicinally active constituents. The presence of phenols and steroids in the extracts could be responsible for the observed antimicrobial property. The antimicrobial activities of phenolic compounds may involve multiple modes of action for eg, oils degrade the cell wall, interact with the composition and disrupt cytoplasmic membrane[15] damage membrane protein, interfere with membrane integrated enzymes,[16] cause leakage of cellular components, coagulate cytoplasm, deplete the proton motive force, change fatty acid and phospholipid constituents, impair enzymatic mechanism for energy production and metabolism, alter nutrient uptake and electron transport.

The isolated compounds from ethanol extract such as Phenyl acetic acid (Compound A), ethyl- 2- hydroxy -4- methyl benzoate (Compound B) and 3,6,7-trihydroxy-2-(3-methoxyphenyl)-4H-chromen-4-one (Compound C) were screened for *in vitro* antimicrobial activity. It was found that Compound B (Ethyl -2-hydroxy -4-methyl benzoate) shows higher antimicrobial activity than other two compounds against all microorganism used in the study. All three compounds do not produce inhibition against *Pseudomonas aeruginosa* at different concentration used. The higher zone of inhibition was observed against *Streptococcus pyogenes* with 9mm and also against *Candida albicans* with 9mm, with MIC value of 60µg/ml. (Table 5& 6).

In the isolated compounds, ethyl- 2- hydroxy -4- methyl benzoate (Compound B) shows good antimicrobial activity than other two compounds, this may be due to the presence of ester group and phenolic hydroxyl group. The organic acid esters widely used as food preservatives are the alkyl esters of p – hydroxyl benzoic acid, commonly referred to as parabens. Ethyl- 2- hydroxy -4- methyl benzoate, possess the chemical structure similar to parabens, may exert the similar antimicrobial activity like that of parabens. The antimicrobial activity increases with hydrophobicity and therefore with the length of ester side chain.[17]

Table 3: Minimum inhibitory concentrations of various extracts of *Clerodendrum phlomidis*

Name of the Organism	Pet ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract
E.coli	400	320	-	106
Staphylococus aureus	320	200	320	320
Streptococus pyogenes	120	200	200	200
Pseudomonas aeruginosa	-	-	-	-
Candida albicans	200	-	320	200
Aspergillus niger	200	200	200	320

Table 4: Zone of inhibition produced by the extracts of *Clerodendrum phlomidis*

Zone of inhibition in mm (*Mean diameter of zone of inhibition)						
Extract conc (µg/ml)	***Escherichia coli***	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Pseudomonas aeruginosa***	***Candida albicans***	***Aspergillus niger***
Control	-	-	-	-	-	-
Pet ether						
106.66	8.33 ± 0.33	7.83 ± 0.16	13.33 ± 0.33	-	11.33 ± 0.33	8.33 ±0.33
200	9.33 ± 0.33	7.93 ± 0.06	12.67± 0.33	-	12.33±0.33	10.33±0.33
320	8.66±0.33	10.33±0.33	14.50 ±.0.10	-	12.87 ± 0.13	12.33±0.33
400	9.66±0.33	9.66±0.33	14.17±0.13	-	13.93 ± 0.06	12.33±0.33
Chloroform						
106.66	-	13.33±0.33	9.83±0.16	-	-	10.33±0.33
200	-	14.67±0.66	9.90 ± 0.10	-	-	11.83 ±0.16
320	9.93 ± 0.06	14.33±0.33	11.67±0.66	-	9.66±0.33	12.83±0.16
400	10.33±0.33	13.90 ±0.17	10.87±0.13	-	11.67±0.33	13.33±0.33
Ethyl acetate						
106.66	-	-	11.93±0.06	-	9.90 ± 0.10	10.93± 0.06
200	-	-	11.90 ± 0.10	-	9.93±0.06	11.90±0.10
320	-	9.83 ± 0.16	11.93±0.06	-	10.90 ±0.10	12.93±0.06
400	-	9.90 ± 0.10	11.87±0.13	-	12.87 ± 0.13	13.87±0.13
Ethanol						
106.66	15.33 ± 0.16	7.83 ± 0.16	11.87±0.13	-	10.93 ± 0.06	8.90 ± 0.10
200	-	7.87± 0.13	11.67±0.66	-	11.90± 0.10	9.93 ± 0.06
320	9.93 ± 0.06	9.90± 0.10	12.87± 0.13	-	11.93 ± 0.06	9.87 ± 0.13
400	9.90 ± 0.10	9.93±0.06	12.93±0.06	-	11.87± 0.13	9.90 ± 0.10
Amikacin/Ketoconazole	17.83± 0.16	19.90±0.10	22.27±0.13	20.33±0.33	16.67±0.33	17.93±0.06

* = Mean of 3 determinations; - = No inhibition; Zones measuring > 12 mm were acceptable as sensitive

Table 5: Minimum inhibitory concentrations of isolated compounds from *Clerodendrum phlomidis*

S. No.	Name of the organism	Minimum inhibitory concentration (µg/ml)		
		Compound A	Compound B	Compound C
1	*Escherichia coli*	-	40	-
2	*Staphylococcus aureus*	60	40	-
3	*Streptococcus pyogenes*	-	40	-
4	*Pseudomonas aeruginosa*	-	-	-
5	*Candida albicans*	60	40	60
6	*Aspergillus niger*	-	40	60

Table 6: Zone of inhibition of isolated compounds from *Clerodendrum phlomidis*

Zone of inhibition in mm (*Mean diameter of zone of inhibition)						
Compound concentration (µg/ml)	***Escherichia coli***	***Staphylococcus aureus***	***Streptococcus pyogenes***	***Pseudomonas aeruginosa***	***Candida albicans***	***Aspergillus niger***
Compound A **20**						-
40	-	-	-	-	4	-
60	-	-	-	-		-
	-	4	-	-		
Compound B						
20	3	-	2	-	-	-
40	5	5	5	-	4	3
60	8	8	9	-	9	5
Compound C **20**		-	-	-		
40	-	-	-	-	-	-
60	-	-	-	-	-	-
	-				7	3

* = Mean of 3 determinations; - = No inhibition

CONCLUSION

Our experiment proposes *Clerodendrum phlomidis* as important medicinal plant as its root extracts showed good antimicrobial activity against all organisms except *Pseudomonas aeruginosa.* In the isolated compounds, ethyl- 2- hydroxy -4- methyl benzoate shows good antimicrobial activity than other two compounds, this may be due to this may be due to the presence of ester group and phenolic hydroxyl group. All the isolated compounds showed no inhibition against *Pseudomonas aeruginosa.* Ethyl- 2- hydroxy -4- methyl benzoate, possess the chemical structure similar to parabens, may exert the similar antimicrobial activity like that of parabens. The antimicrobial activity increases with hydrophobicity and therefore with the length of ester side chain

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