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Phytochemical Analysis and Antimicrobial Activity of Chloroform Extract of *Abutilon indicum*

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Abstract: Powdered *Abutilon indicum* leaves were extracted from chloroform. Thin layer chromatography technique in combination with column chromatography was used to separate the chemically active constituents. The constituents were subjected to IR, NMR, EIMS analysis to identify the compounds contained in the extract. The antimicrobial activity of the plant extract was examined on Bacillus subtils, Klebsiella pneumonia, Pseudomonas aeruginosa, E.coli and the effect was compared with that of chloramphenicol. The comparative results showed that chloroform extract of *Abution indicum* can serve well as an antimicrobial agent.

Keywords: *Abutilon indicum,* Column Chromatography, Antimicrobial Activity, Spectroscopy, Bacteria

1. Introduction

Abutilon indicum is an erect, woody, small shrub in the Malvaceae family. It is commonly known as Indian Mallow, Atibala, Kanghi. It is widely distributed in the tropical and subtropical countries of America, Asia and Australia. The stem of the plant is tinged with purple color and covered with minute hairs. The leaves are alternate, cordate and acute. It bears solitary flowers which are yellowish with five petals. Its fruit is like a capsule, densely pubescent with conspicuous and horizontally spreading beak. The seed are blackish or brownish in color. Traditionally, the entire plant has medicinal value. The leaves are used as demulcent, aphrodisiac, laxative, sedative and diuretic. It is also used in ulcers and in bleeding disorders. A decoction made from leaves is useful to cure gout and tuberculosis. Leaves are also used as eyewash, mouthwash. The leaves extract in combination with other herbs is used to treat jaundice [1, 2], in reducing fever and in treatment of snake bite [3]. The leaf powder in combination with wheat flour is reported to cure uterus displacement [4]. The seeds are demulcent, laxative, expectorant and used in piles, gonorrhea, fever, bronchitis and in urinary disorders. The root is used as a pulmonary sedative and diuretic. It is also used in treatment of leprosy [5].

A survey of literature shows that the plant material is extracted in different solvent mediums and the extracts used for investigation of chemically active constituents. The author has already analyzed the plant root, seeds and leaves for their chemical composition [6]. The present studies are extension of the earlier work in which we are interested in investigating chemically active constituents in chloroform extract and finding its application in antimicrobial activity.

2. Materials and Methods

2.1. Collection of Plant Material

The leaves of plant *Abutilon indicum* were collected from Kotdwar region of District Pauri Garhwal (India). The plant was authenticated by Dr. Arun Agarwal, Associate Professor, Govt. P. G. College Uttarkashi, Uttarakhand, India.

2.2. Extraction & Isolation

The air dried powdered plant leaves (9 kg) were extracted with methanol using soxhlet extractor for 24 hours. The solvent was evaporated by rotatory evaporator to get the residue. The residue was dissolved in water and then partitioned with n-hexane, chloroform and ethyl acetate respectively. Only chloroform extract (54gm) was taken for

analysis. A Small quantity of chloroform fraction was spotted on TLC plates. Different solvent systems were developed for spotting the TLC plates and the more efficient were used for separation through column chromatography. The solvent system of hexane/chloroform (8:2, 7:3) and hexane/acetone (9:1), hexane/ethylacetate (8:2) gave better separation of the compounds and were used in the TLC monitoring and in column chromatography.

The chloroform extract was subjected to column chromatography over a silica gel column. The column was eluted with solvent increasing polarity (hexane, chloroform to ethylacetate). Total 10 fractions were obtained. After evaporating the solvents on water bath, all the collected fractions were subjected to TLC analysis, using I_2 vapour as locating reagent. On the basis of R_f values, same fractions were pooled together. The fraction 2 was loaded on silica gel & eluted with hexane: $CHCl_3$ (8:2), one spot was obtained which was further chromatographed on silica gel with eluant hexane: acetone (9:1), to get a pure compound 1. The fraction 4 was loaded on silica gel & eluted with n hexane: $CHCl_3$ (7:3) to obtain compound 2. The fraction 6 was loaded on silica gel and eluted with hexane: ethyl acetate in (8:2), to get compound 3.

3. Analysis of the Compounds

3.1. Compound 1

Molecular formula: $C_{30}H_{50}O$,

IR: 3319, 2923, 2850, 1660, 1466, 1440, 1198, 1042, 822, 750 & 680 cm^{-1}

^{1}H NMR: ($CDCl_3$, & 400 MHz), δ 4.67(1H, brs, H-29a), 4.57(1H, brs, H-29b), 3.18(1H, dd, J=11.6, 5.2, H-3), 2.39(1H, d, J= 9.6 & 4.0Hz, H-19), 1.72(3H, d, J=1.3 CH_3-30), 1.06(3H, s, CH_3-26), 0.83(3H, s, CH_3-25), 0.91(3H, s, CH_3-27), 0.74(3H, s, CH_3-24), 0.78(3H, s, CH_3-28), 0.94(3H, s, CH_3-23).

^{13}CNMR: ($CDCl_3$)(100MHz): δ 38.7(C-1), 27.4(C-2), 79.0(C-3), 40.1(C-4), 55.5(C-5), 18.6(C-6), 34.2(C-7), 40.7(C-8), 50.8(C-9), 37.2(C-10), 21.1(C-11), 25.2(C-12), 38.3(C-13), 42.9(C-14), 27.2(C-15), 35.7(C-16), 43.1(C-17), 48.3(C-18), 48.1(C-19), 151.0(C-20), 29.9(C-21), 40.0(C-22), 28.1(C-23), 14.3(C-24), 16.1(C-25), 16.6(C-26), 14.8(C-27), 18.3(C-28), 109(C-29)19.45(C-30)

.EI/MS m/z: 426[M^+](20), 411 [M-CH_3]$^+$(25), 408[M-H_2O] (30), 393[M-CH_3-H_2O]$^+$(35), 220 [M-C_5H_{28}}$^+$(80), 218[M-$C_{14}H_{24}O$]$^+$(55), 207[M-$C_{16}H_{23}$]$^+$(25), 133(14).

3.2. Compound 2

M. F.: $C_{17}H_{14}O_7$

IR: ($CHCl_3$MHz): 3314, 1642, 1603, 1350 & 870cm^{-1}.

^{1}HNMR($CDCl_3$): δ11.93(1H, s, OH-5), 10.87(1H, s, OH-7), 9.32(1H, s, OH-3), 7.85(2H, d, J=8.5Hz, H-ii & H-vi), 6.95(2H, d, J=8.9Hz, H-iii & H-v), 6.69(1H, J=2.1Hz, H-8), 3.92(3H, s, OMe-6 and 3.75(3H, s, OMe-iv).

^{13}CNMR($CDCl_3$)(75MHz): δ175.89(C-4), 158.90(C-2)157.52(C-4'), 152.20(C-10), 151.50(C-6), 149.22(C-8), 136.50(C-3), 131.52(C-5), 130.10(C-ii &C-vi), 121.82(C-i), 115.40(C-iii), 115.42(C-v), 105.10(C-9)91.80(C-7), 56.80(OMe-7) & 55.41(OMe-iv).

EI/MS, m/z [M]+330(100), 315(19), 318(6), 183(26) and 135(33).

3.3. Compound 3

M. F.: $C_{29}H_{50}O$

IR: (KBr): 3421, 2935, 2866, 1653, 145875, 1062, 883, 800.46cm^{-1}.

^{1}HNMR: ($CDCl_3$)(400MHz): δ 5.34(1H, m, d, j=5.2Hz, H-6), 3.53(1H, m, H-3), 1.44(1H, t, H-8), 1.14(1H, d, H-17), 1.12(1H, d, H-14), 1.01(3H, s, CH_3-19), 0.93(1H, m, H-24), 0.92(3H, d, J=6.2Hz, CH_3-21), 0.84(3H, d, J=8.0Hz, CH_3-29), 0.83(3H, d, j= 7.2Hz, CH_3-26), 0.79(3H, d, J=7.2Hz, CH_3-27), 0.67(3H, s, CH_3-18). ^{13}CNMR($CDCl_3$)(75MHz):

37.30(, C-1), 31.82(C-2), 71.90(C-3), 42.43(C-4), 140.90(C-5)121.89(C-6), 31.12(C-7), 32.02(C-8), 50.83(C-9), 36.60(C-10), 21.12(C-11), 40.31(C-12), 42.6(C-13), 56.80(C-14), 24.31(C-15), 30.24(C-16), 56.20(C-17), 12.05(C-18), 19.5(C-19), 36.30(C-20), 18.7(C-21), 34.01(C-22), 26.0(C-23), 15(C-24), 29.8(C-25), 18.81(C-26), 19.84(C-27), 23.11(C-28), 12.01(C-29).

EI/MS, m/z:414(22), 399(11), 396(14), 381(68), 329(28), 303(24), 275(12), 273(18), 255(37).

4. Antimicrobial Screening

The antimicrobial activity of the chloroform extract was followed according to the standard Disc Diffusion method [7]. The method involved preparation of Muller Hinton agar which was poured into sterilized petri dishes. Sterilized cotton swabs were impregnated with the respective bacteria culture in nutrient broth and then swabbed on the prepared agar plates. The sterilized what man filter paper discs of 5mm diameter were prepared and the leaf extract (25µl) was loaded on each of the discs with the help of micro pipette. These sterile impregnated discs with plant extract were dried and placed on the agar surface carefully with help of forcep. All the Agar plates were incubated at 37°C for 24 hours. The standard drug used was chloramphenicol (25µgm/ml) in dimethyl sulphoxide (DMSO). The antimicrobial activity of the plant extract was noted by presence or absence of inhibition zone and diameter of the zones were measured in millimeter and averaged for each plate at the end of the incubation period. The results are shown in Table 1.

5. Result and Discussion

Three compounds were isolated from the chloroform extract of the plant leaves by repeated chromatographic separation and purification over silica gel. The structure of the isolated compounds were confirmed by spectroscopic techniques like IR, ^{1}HNMR, ^{13}CNMR, EIMS and identified as Lupeol (Compound 1), 3, 5, 7 trihydroxy 4', 6methoxy flavone (Compound 2) and beta sitosterol (Compound 3) as shown in Figures 1, 2, 3.

5.1. Compound 1

Compound 1 was isolated as white powder, m. p. 213-215°C. The IR spectrum of the compound 1 showed a very intense broad absorption at 3319cm^{-1} and moderately intense band at 1198 and 680cm^{-1} were observed for the O-H bond vibration of hydroxyl group. The out of plane C-H vibration of the unsaturated part was observed at 822cm^{-1}. The corresponding C=C vibration was shown at 1660cm^{-1}. The stretching and bending vibration of methyl group were noticed by the intense peak at 2923cm^{-1} & medium intensity band at 1466cm^{-1}. The strong band at 2850cm^{-1} & medium band at 1440cm^{-1} were noticed due to vibration of methylenic part & moderately intense band at 750cm^{-1} was attributed to the rocking vibration of methylenic part. The C-C vibration was noticed as a weak band at 1042cm^{-1}.

The ^{1}HNMR spectrum of compound 1 showed a doublet of doublet (j=11.6Hz, 5.2) of one proton intensity at δ3.18 due to the proton attached to the carbon having the hydroxyl group or typical of oxymethine proton at C-3. These chemical shift and coupling constant values lead to the conclusion of β orientation of the hydroxyl group at C-3. The spectrum showed seven singlets at δ 0.74, 0.78, 0.84, 0.91, 0.97, 1.03 & 1.70 (3H each) assignable to the protons of methyl groups at C-4(H-23, H-24), at C-10(H-25), C-8(H-26), C-14(H-27), C-17(H-28) and C-20(H-30) respectively. A pair of broad singlets at δ4.57 and δ 4.70 (1H each) which indicated the presence of terminal isopropenyl moiety or due to the vinylic protons at (H-29b, H-29a). This indicated that compound 1 belongs to the lupane class of terpenoids. A sextet of one proton at δ 2.38 assignable to 19 β-H, is characteristics of lupeol. The ^{13}CNMR spectrum showed seven methyl groups at δ 28.1(C-23), 18.3(C-28), 16.1(C-25), 15.9(C-26), 14.3(C-24), 14.8(C-27), 19.45(C-30). The signals due to an exo methylene group at δ 109.0(C-29), and at δ 151.0(C-20). The signal at δ 79.0 was appeared due to deshielding effect of OH group attached at C-3. The DEPT-135 experiments showed that presence of 30 carbons in the compound, out of which seven methyls, eleven methylenes, six quaternary carbons and six methine carbons. The EI-MS of the compound showed molecular ion peak at m/z 426.38 consistent with the molecular formula $C_{30}H_{50}O$.

Other peaks at m/z 385(M+-41), 220(M+-$C_{15}H_{26}$), 218(M+-$C_{14}H_{20}O$) and 207(M+-$C_{16}H_{27}$) were characteristics for lupane skeleton. Based upon this ^{1}HNMR &^{13}CNMR data of compound 1 and in

Comparison of its physical and spectral data with published values [8-11] confirmed the identity of compound 1 as Lupeol.

5.2. Compound 2

The compound 2 was isolated as light yellow amorphous powder having melting point 270°-272°C. It gave positive test for flavonoids. The UV spectrum showed absorption maxima at 270 & 340nm. These values indicated that compound 2 was 3 hydroxy flavones. The IR(KBr) spectrum showed absorption bands at 3314cm^{-1} which appeared due to presence of OH group. One absorption peak was observed at 1645 cm^{-1} due to presence of unsaturated ketone, and a band at 1604 was observed due to aromatic region. The ^{1}HNMR spectrum showed the two singlets were observed at δ 3.74 and 3.92 due to presence of two methoxyl group that were present on C- iv & C-6 respectively. Three singlets that were observed at δ9.31, 10.84 & 11.91 due to three OH groups present at C-3, C-7 & C-5. A singlet peak was observed at δ 6.68, due to H-8. The doublets at δ 7.84 was observed due to ortho coupling(1H, J=8.5, 8.9Hz) between H-ii & H- vi and at 6.96 was observed due to ortho coupling between H-iii & H- iv. The ^{13}CNMR spectrum showed the signal at δ 158.9(C-2), 136(C-3) & 175(C-4) confirmed that compound 2 was a 3 substituted flavone. The ^{13} CNMR spectrum showed 17 carbon signal indicating presence of seventeen carbons in the compound2. Out of these 10 are quaternary 158.78(C-2), 136(C-3), 175(C-4), 131(C-5), 151(C-6), 91.8(C-7), 105(C-9), 152(C-10), 120.9(C-i) & 157(C-iv), rest five were methine carbons 149(C-8), 130.1(C- ii), 115(C-iii), 115.42(C-v) & 130(C- vi). The EIMS showed the molecular ion peak of the compound at m/z 330 which corresponds to the molecular formula $C_{17}H_{14}O$. Comparison of its physical and spectral data with published values [12, 13] confirmed the identity of compound 2 as 3, 5, 7, trihydroxy IV, 6 dimethoxy flavone.

5.3. Compound 3

The Compound 3 was isolated as white crystal with melting point 135°-137°C which gave positive Lieberman-Burchard test for steroids. The IR spectrum in KBr of compound 2 showed a characteristic absorption band at 3421cm^{-1} due to vibration of O-H bond of hydroxyl group. Absorption bands were noticed at 2935 and 2866cm^{-1} due to aliphatic C-H stretching of (-CH_2 &-CH) respectively. The C=C stretching appeared as a weak band at 1653cm^{-1}. The absorption band at 1062cm^{-1} was noticed due to C-O stretching. The ^{1}HNMR spectrum of compound 3 showed a one proton multiplet at δ 3.52 and one proton multiplets at δ 5.36 for H-3 & H-6 respectively. The two peaks (3H each) as a singlets at δ0.68 &1.02 appeared due to presence of two methyl group H-18 & H-19, present at tertiary carbons C-10 and C-13 respectively. In addition, two doublets at δ 0.94 & 0.88 assigned to the two methyl group at H-21& H-29 attached to C-20 and C-28 respectively. Similarly one three proton doublet at 0.83 for H-26 and other three proton doublet at 0.85 assigned to H-27, both attached to C-25. In the ^{13}CNMR spectrum total 29 signals for different carbons were observed. Out of these, six signals were observed for methyl groups present at δ 11.86(C-18), 19.40(C-19), 18.79(C-21), 18.81(C-26), 19.84(C-27)& 11.95(C-29), eleven signals were observed for methylene at δ 37.28(C-1), 31.69(C-2), 42.83(C-4), 31.69(C-7), 21.10(C-11), 39.80(C-12), 24.37(C-15), 28.25(C-16), 33.98(C-22), 26.14(C-23) & 11.95(C-29), nine signals were observed for methane (-CH) group at δ 71.82(C-3), 121.72(C-6), 31.69(C-8), 50.17(C-9), 56.79(C-14), 56.09(C-17), 36.52(C-20), 45.88(C-24) & 28.91(C-25) and three signals were observed for quaternary carbons(C) for δ 140.70(C-5), 36.52(C-10) and 42.33(C-13). The EIMS spectrum showed a molecular ion peak at 414 &

other fragment ion peak appeared at m/z 399, 396, 381, 329, & 303. The last two fragment ions 329 & 303 were diagnostic for sterols having Δ^5 unsaturation. Other important fragments were observed at m/z 273 (loss of side chain, M^+-side chain), & at 255(loss of M^+-side chain-H_2O) respectively.

Comparison of its physical and spectral data with published values [14, 15] confirmed the identity of the compond 3 as Betasitosterol.

Table 1. *Antimicrobial activity of leaves of A. indicum in Chloroform extract using Disc Diffusion method.*

Test organism	zone of inhibition in(mm)	Chloramphenicol(positive Control)	DMSO
Bacillus subtills	13.2	25	nil
Klebsiella pneumonia	8.3	28	nil
Pseudomonas aeruginosa	15.0	23	nil
E. coli	15.2	22	nil

Table 1 summarizes the results obtained for antimicrobial activity of the *Abutilon indicum* plant leaves extract. The values shown in the table are average values of the inhibition zone around the discs. It is indicated from the result that though the extract is working as antibacterial agent for all the four types of bacteria, yet the highest values of inhibition zone are observed on E. coli. it is this effect that makes the leave powder a good candidate for consumption in stomach problems. It also shows a moderate activity against Klebsiella pneumonia. If the results are compared for antimicrobial activity of all the four types of bacteria cited in the table with those obtained with standard drug chloramphenicol, it is observed that the best results is obtained for E. Coli.

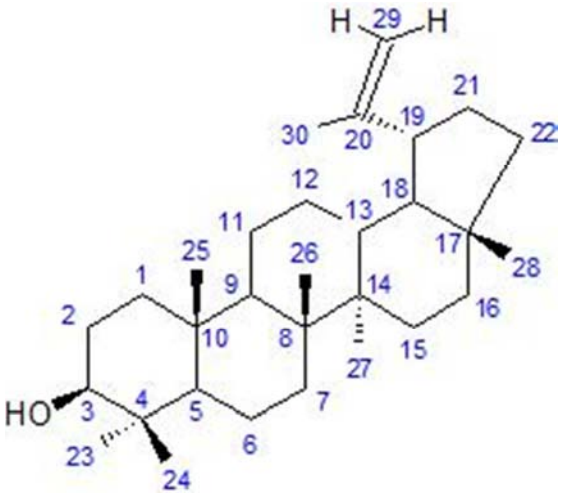

Figure 1. *Struture of Lupeol.*

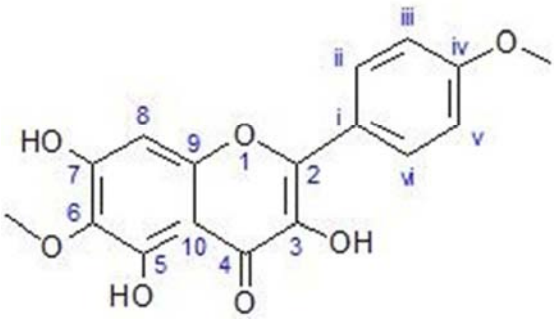

Figure 2. *Structure of 3, 5, 7 tri hydroxy IV, 6 dimethoxy flavone.*

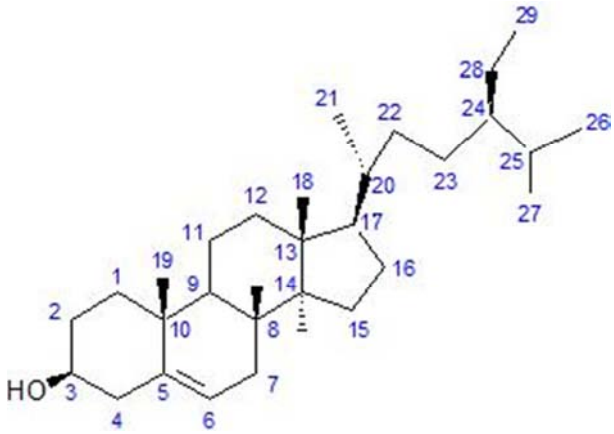

Figure 3. *Structure of Beta Sitosterol.*

6. Conclusion

The phytochemical analysis of the chloroform extract of *Abutilon indicum* supported by spectroscopic analysis has indicated the presence of lupeol, 3, 5, 7, trihydroxy iv, 6 dimethoxy flavones and β sitosterol. The antimicrobial testing on bacillus subtills, klebsiella pneumonia, Pseudomonas aeruginosa & E. coli bacteria showed that extract is best suited against Pseudomonas aeruginosa & E. coli.

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