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ISOLATION, CHARACTERIZATION OF LONG CHAIN FATTY ALCOHOL FROM EXTRACT OF *BRIDELIA RETUSA* (L) LEAVES.

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ABSTRACT

A compound was isolated from leaves of *Bridelia retusa* (Linn.) by column chromatography followed by preparative thin layer chromatography and crystallization. Infra red spectroscopy, Gas chromatography mass spectroscopy and nuclear magnetic resonance studies revealed that isolated compound was long chain of fatty alcohol. The isolated compound screened for antibacterial and antifungal activity done by paper disc method and showed significance antibacterial activity.

KEYWORDS: Phytochemical, *Bridelia retusa*, isolation, spectroscopy.

INDRODUCTION

Bridelia retusa Linn. commonly known as Asan, Aghan (hindi-Khaja) which is used in traditional system of medicine for treatment of various diseases and aliments. *Bridelia retusa* the medicinal plant species from family Euphorbiaceae is deciduous tree can grown 8 to 10 m in height found in warmer part of India. It is tall tree with irregular fissured, brownish black bark, leaves are elliptic oblong, obtuse, entire and tomentose beneath, symmetrical basically attach. Flowers are creamy white in terminal panicles of erect..[1,2] In ayurvedic medicine bark is given orally to women to develop sterility and as contraceptive. (Jain et al 2004) In rheumatism bark is used and bark of Bridella retusa is exhibited antiviral, hypoglycemic and hypotensive properties. Leaves are used to cure wounds (Ayyonar and lgnacimuthu 2005) and urinary tracts infection (Jain et al 2004) *Bridelia retusa* leaves in traditional medicine system as antibacterial.[3] Root of plant is purgative anstrigent and remedy for gonorrhea. One or two drops of fruit extract poured in ear to cure earache.[4] The plant also used in traditional system of medicine to cure dysentery and diarrhea.[5, 6] Bark juice taken internally in case of snake bite.

In southern India use of plant leaves along with leaves of curculigo orchiodes and castor and coconut oil to cure wounds (Ayyanar and Ignacimuthu, 2005). Literature survey revealed that no systemic and scientific study on isolation and characterization of phytochemicals from leaves of *Bridelia retusa* (L) so we have to done work on isolation and characterization of phytochemical from leaves of *Bridelia retusa* Linn. Results are being reported in this communication.

MATERIAL AND METHOD

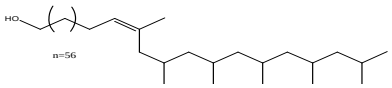
The leaves of *Bridelia retusa* were collected from hilly region Tornmal Dist. Nandurbar, state Maharastra, India. The plant was identified and authenticated by Dr. S.R. Kshirsagar, taxonomist department of botany L.K .P.R. Ghogrey science college of SSVP's, Dhule, Maharashtra, India. Voucher specimen was deposited in the department.

Extraction and isolation procedure

The leaves of *Bridelia retusa* were collected in summer season by hand picking method, dried in shade until easily broken by hand. Grinded to fine powder and stored in air tight bottle. 100gm powder of leaves extracted in Soxhlet extractor in solvent n-hexane at temperature below boiling point (60^{o}). Process of extraction was completed in 24 hours conform by applying TLC no spot chromatogram. Then solvent from extract was removed by distillation in rotary evaporator. Mark get after extraction was air dried and successively

extracted in solvent diethyl ether (25^{o}C) extraction was completed in 12 hours conform by applying TLC no spot on chromatogram. Solvent are removed by kept at room temperature in open container. Process of extraction was repeated 5 times get sufficient amount of extract. After extraction we get dark green sticky extract which shows presence of fats and triterpenes, small portion of extract dissolved in chloroform and solution was spotted on TLC plates. Plates run in specific solvent system better resolution in n-Hexane: ethyl acetate (9:1). TLC viewed in UV chamber followed by Iodine chamber.[7] Phytochemicals are separated by column chromatography using silica gel G of mesh size 60-120. Column was packed by silica gel G making slurry with n-hexane and after packing column kept overnight for better adsorption of solvent. 4 g of extract dissolved in chloroform and equal amount silica gel G mixing vigorously after evaporation of solvent making free flowing material which was loaded on top of the column. Top layer covered with the silica gel G to avoid disturbance of top of charge by solvent. After the set up cork of the column open and eluent collected in test tube 75 fractions are collected and observe TLC of each fraction. Fractions which showed similar compound are mixed after preparative thin layer chromatography and rerystallization we get one pure compound. The compound was labeled as NNP-6. A isolated compound have been characterized and their structure was identified by physical, chemical and spectroscopic techniques like IR, ^{1}HNMR, ^{13}C NMR and GCMS.

Compound NNP6 was isolated as white crystalline solid, m. p. 98^{o}C yield 50 mg R_f-0.87 IR(KBr, νmax.Cm^{-1})-3487.70, 2928.12,1654.17,1497.57,1438.30, 1385.18, 1255.09, 1090.20, 1062.73. ^{1}H NMR(500MHz,$CDCl_3$)-δ1.11to1.01(t,17 H) 1.38to 1.17 (m, 129H broad peak) , 1.56(m, 5H) (5.28 (s, 1H). ^{13}C NMR(100MHz, $CDCl_3$)-δ14.08(C-1), 22.67 (C-2) 29.68 to29.34(t, CH_2 long chain broad peak due to overlapping C3-C61) 31.90(s,C68-C71)



Fatty alcohol

Antibacterial and antifungal activity

Antibacterial antifungal Screening

Compound	Zone of inhibition			
	E. Coli	P. aeruginousa	A. niger	C. albicans
1	16.56	15.67	5.30	-
Chlorampinicol	25.30	30.50	-	-
Nystatin	-		9.30	9.68

The isolated compound was stored in screw capped bottle at -4^{o}C Antibacterial and antifungal activity were performed by paper disc method .Petri dishes were filled with (5cmdiameter) filled with sterile nutrient agar for bacteria and yeast for fungi. A sterile paper disk of 6mm preloaded with 100 mcg of compound dissolved in DMSO and blank disc of DMSO solvent in the centre of nutrient agar plates of bacteria MAYP plates of fungi. Two bacterial and fungal inculums were placed upside down at the quarter circle point 20mm radius and blank disc treated with chloromphenicol for bacteria and Nyastatin for fungal. The petri plates were incubated at 37± 1^{0}C for 24 hours for bacterial screening and at 25^{0}C for 2 to 7days for antifungal screening. The diameter of zones of inhibition of each disk was recorded.

RESULT AND DISCUSSION

A isolated compound having m.p. 98^{o}C and white crystalline solid compound IR spectroscopy showed intensively broad band at 3487.70 cm^{-1} assigned as –-OH group band at 2928.12 cm^{-1} and moderately intense band at 1438.30 cm^{-1} indicated stretching and bending vibration of methyl part. Very intense band at 1654.17 cm^{-1} and weak band at1497.57 cm^{-1} indicated- C=C-stretch. Moderately intense band at 1385 cm^{-1} and 1090.20 cm^{-1} weak band at 1255 cm^{-1} conforming presence of OH stretch of CH_2-OH group.[8,9]

From ^{1}H NMR spectral data it appeared that spectrum at δ 5.28 indicated C=C-CH_3 triplet at δ 1.54 to 1.38 of five hydrogen at C62,C64, C66, C68 and C70, broad spectrum at δ 1.28to 1.17due to CH_2 C3toC57 and C59,C61,C63, C65and C67; triplet at δ1.11to 1.o1 terminal methyl groups.[10] In ^{13}C NMR, spectrum at δ 14.08 C1 carbon atom and spectrum at δ 22.67 at C2 carbon and broad spectrum at δ 29.68to 29.34 triplet due toCH_2 groups. In GCMS data indicating retention time 13.8 and base peak at 57 indicating long chain of aliphatic hydrocarbon. All evidence and data showing molecular formula of compound is $C_{77}H_{152}O$ and compound is long chain fatty alcohol.

CONCLUSION

A compound isolated from leaves of *Bridelia retusa* Linn. Spectral data of the compound was showed molecular formula $C_{77}H_{152}O$ characterized as long chain fatty acid. In the list of phytochemicals present in *Bridelia retusa* Linn. leaves is long chain fatty alcohol was, thus, included. The Compound exhibited significance antibacterial activity but lowest antifungal activity against A.niger.

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