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Isolation of Lupeol from *Terminalia arjuna* Bark

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Abstract

A pure compound lupeol [*lup-20(29)-en-3β-ol*] was isolated from the diethyl ether extract of *Terminalia arjuna* bark and investigation of the antimicrobial effect of different extracts of the bark was performed. The pure compound was separated using chromatographic methods including column chromatography, thin layer chromatography, preparative thin layer chromatography and the structure of the compound was elucidated on the basis of FTIR and ^{1}H-NMR spectroscopic data. The antimicrobial activity of diethyl ether, chloroform, ethyl acetate and ethanol fractions was studied against 16 microorganisms and the ethanol and diethyl ether extracts showed moderate antibacterial and antifungal activity.

Keywords : Terminalia arjuna, Lupeol, Antimicrobial activity, Chromatography, NMR.

1. Introduction

Terminalia arjuna Linn (family: Combretaceae) is a well known large tree distributed throughout Bangladesh. It is 20-35m high tree and a commonly occurring medicinal plant. It has effective values in Ayurveda for its various therapeutic values [1]. Chemical constituents of different classes such as hydrolysable tannins [2], triterpenoid acids and their glycosides [3,4], flavonoids [5], Phenolics [6] were reported from stem bark portion of *Terminalia arjuna* species. Additionally, Arjunglucoside I-III, aujunic acid, arjunetin, arjunolic acid, and terminoic acid also form group of important constituents of the bark [7].

The bark of *T. Arjuna* is used in Bangladesh as cardioprotective agent in hypertension and ischaemic heart diseases [8]. It is rich in various chemicals such as polyphenols (about 60-70%), phenyl propanoids, tannins (20-24%), flavones, and flavonols and thus it finds useful applications in the treatment of diseases like ulcers, blood diseases, anemia and asthma. It also finds potential applications in curing hepatic, congenital, venereal and viral diseases. The bark powder is reported to show hypocholesterolaemic and antioxidant effect [9]. Various pharmacological studies deciphered usefulness of bark against angina pectoris, congestive heart failure, cardiovascular disorder, coronary heart disease, artheriosclerosis and myocardial necrosis [10,11]. Besides, the medicinal use of bark as fungicidal, antimicrobial, antibacterial, antifertility and anti-human immune deficiency virus induced diseases has also been evaluated [12, 13]. A number of terpenoid saponins (arjunic acid, arjunolic acid, arjunetin, arjunglycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid and phytosterol have been isolated from the bark [14-16].

In the present work, a pure compound is isolated from *Terminalia arjuna* bark and investigation of the antimicrobial activity of ethanol, diethyl ether, ethyl acetate and chloroform extracts of the bark was done using disc diffusion method.

2. Materials and Methods

General Experimental Procedure

The ^{1}H-NMR was acquired on a Bruker DPX-400 spectrometer operating at 400.13MHz. IR spectroscopy (in KBr) was performed on an IR Prestidge-21 FT-IR spectrometer. Column chromatography was carried out using silica gel (column grade, 60-120 mesh). Thin layer chromatography was performed using Merck pre-coated silica gel (G254). The spots were observed under UV lamp and iodine vapor and spraying with vanillin–sulfuric acid reagent, followed by heating.

Plant Material

The plant species used in the present investigation was collected from Jhenidah, Bangladesh and was identified by a taxonomist from the Department of Botany, University of Dhaka. The bark of *Terminalia arjuna* were at first sun dried for five consecutive days and finally was ground into a coarse powder using a grinding machine.

Extraction and Isolation

The powdered bark (1 Kg) was immersed in ethanol (2.5 L) at room temperature for 48 hours. The extract was collected and the process of extraction was repeated five times with fresh ethanol. The extracts were combined, filtered using Whatman no. 1 filter paper and concentrated by removal of the solvent in a rotary evaporator under reduced pressure at a temperature below 45^{0}C. A brownish gummy mass was obtained and was denoted as D. The concentrated ethanol extract D was fractioned by the modified Kupchan partitioning method into petroleum ether, diethyl ether, ethyl acetate and chloroform soluble fractions. Evaporation of the solvents afforded petroleum ether, diethyl ether, ethyl acetate and chloroform extract.

The diethyl ether soluble fraction (6.54 g) was designated as DE. TLC in different solvent systems showed the fraction DE to be a mixture of several compounds. The resolution

was best found in a solvent mixture of petroleum ether: ethyl acetate (9:1), which showed three spots at R_f 0.75, 0.95 and 0.42 with long tailing. Mass DE was subjected to column chromatography for fractionation on silica gel (Kieselgel 60, mesh 70-230). The column was eluted with the solvent mixture of petroleum ether: ethyl acetate (9:1). Fractions of about 3 ml were collected in 60 test tubes and examined separately on TLC plates. Seven portions showing spots in same place in TLC were mixed and were named as DEF-1 to DEF-7. The fractions were kept undisturbed for a day for drying purpose. Preparative thin layer chromatography (stationary phase- silica gel F_{254}, mobile phase- 10% ethyl acetate in Petroleum benzene, thickness of plates 0.5mm) of fraction DEF-3 afforded three separate bands and were marked as DEF-3a, DEF-3b, and DEF-3c. TLC of DEF-3a (18 mg) gave single spot (R_f 0.75) while DEF-3b and DEF-3c gave multi spots.

lupeol [*lup-20(29)-en-3β-ol*]: IR (KBr) (ν, cm^{-1}): 3452.58 (OH); 2926.01 (CH_2); 1633.71 (C= C); 1458.26 (CH); 1375.25 (CH_3); 721.38 (CH_2).

^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.67 (1H, m, H-5), 0.70 (3H, s, H-24), 0.79 (3H, s, H-28), 0.90 (3H, s, H-25), 0.91 (3H, s, H-27), 1.05 (3H, s, H-23), 1.12 (3H, s, H-26), 1.60 (3H, s, H-30), 1.19 (1H, m, H-22a), 1.46 (1H, m, H-22b), 1.38 (1H, m, H-21a), 1.90 (1H, m, H-21b), 2.4 (1H, m, H-19), 3.19 (1H, dd, J = 13.5, 4 Hz, H-3), 4.69 (1H, m, H-29a), 4.73 (1H, m, H-29b).

Antimicrobial Activity

The antimicrobial activity of different fractions was determined using disc diffusion method [17]. Solutions of the test samples were made by dissolving 8 mg of the samples in 200μl methanol. Dried and sterilized filter paper discs (6 mm diameter) were impregnated with 10μl of the test substances and the concentration was 400μg/disc. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) were used as positive and negative control. These plates were kept at low temperature (4^{0}C) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and the test materials dissolve and diffuse out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel. As a result there is a gradual change in the concentration of test materials in the media surrounding the discs [17]. The plates were then incubated at 37^{0}C for 24 hours to allow maximum growth of the organisms. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition, surrounding the medium, expressed in millimeter.

Table 1: Comparison of ^{1}H-NMR data of lupeol isolated from *Terminalia arjuna* with previously reported data [18,19].

No.	^{1}H-NMR of the isolated compound, δ ppm	^{1}H-NMR, δ ppm (Lupeol) [18,19]
1	0.98, 1.64	0.95, 1.62
2	1.21	1.23
3	3.19 (dd, J = 13.5, 4 Hz)	3.16 (dd, J=11.2, 5.2 Hz)
5	0.67	0.65
6	1.25, 1.48	1.33, 1.48
7	1.26	1.36
9	1.18	1.23
11	1.30	1.33
12	1.55	1.54
13	1.67	1.59
15	1.46	1.49
16	1.26, 1.45	1.36, 1.44
18	1.17	1.28
19	2.4	2.25
21	1.38 (1H, m, H-21a), 1.90 (1H, m, H-21b)	1.23
22	1.19 (1H, m, H-22a), 1.46 (1H, m, H-22b)	1.18, 1.36
23	1.05 (s)	0.94 (s)
24	0.70 (s)	0.73 (s)
25	0.90 (s)	0.80 (s)
26	1.12 (s)	1.00 (s)
27	0.91 (s)	0.92 (s)
28	0.79 (s)	0.76 (s)
29	4.69(br s) , 4.73 (br s)	4.54 (br s), 4.66 (br s)
30	1.60 (s)	1.65 (s)

Table 2: Antibacterial activity of four partition fractions (ethanol, diethyl ether, ethyl acetate and methanol) of *Terminalia arjuna* bark and standard disk Cipro Floxacin (30μg/disc).

Test Bacteria and Fungi	Standard disk CF (Cipro Floxacin)	Ethanol extract	Diethyl Ether extract	Ethyl Acetate extract	Chloroform extract
Gram Positive					
Bacillius cereus	46	09	10	-	-
Bacillius megaterium	46	08	11	-	-
Bacilliius subtilis	43	08	10	-	-
Staphylococcus aureus	43	07	09	-	-
Sarcina lutea	46	10	09	-	-
Gram Negative					
Escherichia coli	45	10	07	-	-
Pseudomonas aureus	46	10	08	-	-
Salmonella paratyphi	46	08	09	-	-
Salmonella tiphi	46	09	07	-	-
Shigella boydii	46	09	09	-	-
Shigella dysenteriae	45	10	08	-	-
Vibrio parahemolyticus	46	10	08	-	-
Vibrio mimicus	46	09	10	-	-
Fungi					
Candida albicans	46	10	08	-	-
Aspergillus niger	46	09	09	-	-
Saccharromyces cerevaceae	46	08	09	-	-

3. Results and Discussion

Analysis of the isolated compound and structural elucidation

The isolated compound (18 mg) appeared as white powder and was soluble in petroleum ether, chloroform and ethyl acetate. The IR spectrum of TE-1 showed absorption band at 3452.6 cm^{-1} for -OH stretching. Absorption band at 2926.0 cm^{-1} was due to presence of methylene C-H stretching. The absorption band at 1633.7 cm^{-1} was due to the presence of C= C stretching. The vibrations at 1458.2 cm^{-1} and 1375.2 cm^{-1} showed the presence of methylene C-H and methyl C-H bends. The absorption band at 721.4 cm^{-1} was due to rocking vibration methylene C-H.

The ^{1}H-NMR spectra revealed the presence of seven tertiary methyl protons at δ 0.70, 0.79, 0.90, 0.91, 1.05, 1.12 and 1.60 (integrated for 3H-each). A characteristic sextet of one proton of lupeol was found at δ 2.4 19β –H. The H-3 proton showed a multiplet at δ 3.19 while a pair of broad singlets at δ 4.69 and δ 4.73 (1H, each) was indicative of olefinic protons at (H-29 a & b). These assignments are in good agreement for the structure of lupeol (Table 1) [18,19].

Thus the compound was identified as lupeol [*lup-20(29)-en-3β-ol*], $C_{30}H_{50}O$ (Fig. 1.) by comparison with ^{1}H-NMR and IR spectral data with reported values [18,19].

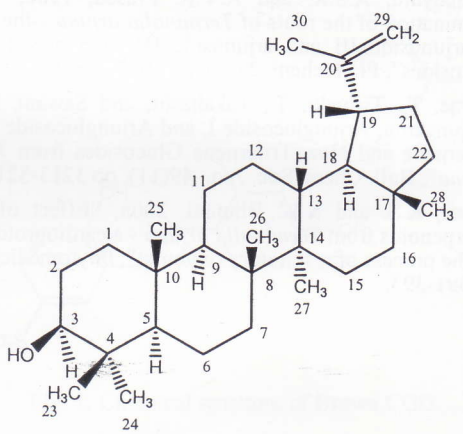

Fig. 1: Structure of separated compound lupeol [*lup-20(29)-en-3β-ol*].

Antimicrobial activity

The antimicrobial activity of the four partition fractions (diethyl ether, chloroform, ethyl acetate and ethanol) of *Terminalia arjuna* bark and standard disk CF (Cipro Floxacin) were investigated. The ethanol and diethyl ether extraction portions showed moderate antibacterial and antifungal activity. Other fractions did not show any activity. The results are shown in Table 2.

4. Conclusion

A new pentacyclic triterpinoid compound, lupeol [lup-20(29)-en-3β-ol], was successfully isolated from the diethyl ether extract of *Terminalia arjuna* bark. Antimicrobial screening of the extracted portions were done which showed moderate results with ethanol and diethyl ether fractions with the proof of the efficacy of medicinal value.

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References

1. Nadkarni, K.M., 2000, "The Indian Materia Medica", Popular Prakashan, India, 1, pp 1198 – 1202.
2. Kandil, F.E. and M.Y. Nassar, 1998, "A tannin anti-cancer promotor from *Terminalia arjuna*", Phytochem. 47(8), pp 1567-1568.
3. Tripathi, V.K., Pandey, V.B., Udupa, K.N. and G. Rucker, "Arjunolitin, a triterpene glycoside from *Terminalia arjuna*", Phytochem. 1992, 31, pp 349–351.
4. Patnaik T., Dey R.K., and P. Gouda, 2007, "Isolation of Triterpenoid Glycoside from Bark of *Terminalia arjuna* using Chromatographic Technique and Investigation of Pharmacological Behavior upon Muscle Tissues", E-Journal of Chemistry, 4,(4), pp 474-479.
5. Patil, U.H. and D.A.K. Gaikwad, 2011, "Pharmacognostical Evaluation of Stem Bark of *Terminalia arjuna*", Int. J. Pharm. Pharm. Sci. 3, pp 98-102.
6. Anjaneyulu, A.S.R. and A.V.R. Prasad, 1982, "Chemical examination of the roots of *Terminalia arjuna*—the structures of arjunoside III and arjunoside IV, two new triterpenoid glycosides", Phytochem. 21(8), pp 2057–2060
7. Murae, T., Tsuyuki, T., Takahashi, and Sawani, M., 1976, "Arjungenin, Arjunglucoside I, and Arjunglucoside II. A New Triterpene and New Triterpene Glucosides from *Terminalia arjuna*", Bull. Chem. Soc. Jpn., 49(11), pp 3213-3218.
8. Pawar, R.S. and K.K. Bhutani, 2005, "Effect of oleanane triterpenoids from *Terminalia arjuna* - a cardioprotective drug on the process of respiratory oxyburst", Phytomedicine, 12(5), pp 391-393.
9. Gupta, R., Singhal, S., Goyle, A. and V.N. Sharma, 2001, "Antioxidant and Hypocholesterolaemic Effects of *Terminalia arjuna* Tree-Bark Powder: A Randomised Placebo-Controlled Trial", J. Asso. Physicians. India. 49, pp 231-235.
10. S. Dwivedi, 2007, "*Terminalia arjuna* Wight & Arn.-A useful drug for cardiovascular disorders", J. Ethnopharmacol. 114(2), pp 114-129.
11. Asha, S. and G. Taju, 2012, "Cardioprotective Effect of *Terminalia arjuna* on Caffeine Induced Coronary Heart Disease", IJPSR, 3(1), pp 150-153. 43(11).
12. Chouksey, B.K., and S.K. Srivastava, 2001, "Antifungal agent from *Terminalia arjuna*", Indian J Chem, 40B, pp 354–356.
13. Buckheit Jr., R.W., White, E.L., Fliakas-Boltz, V., Russell, J., Stup, T.I., Kinjerski, T.I., Osterling, M.C., Weigand, A. and J.P. Bader, 1999, "Unique anti-human Immunodeficiency virus activities of the nonnucleoside reverse transcriptase inhibitors calanodile A, costatolide, and dihydrocostatolide", Antimicrob. Agents Ch. 43(8), pp 1827-1834.
14. Kuscemoto, I.T., Nakabayashi, T., Kida H., Miyashiro, H., Hattori, M., Namba, T and K. Shimotohno, 1995, "Screening of various plant extracts used in ayurvedic medicine for inhibitory effects on human immunodeficiency virus type 1 (HIV-1) protease", Phytother. Res., 9, pp 180-184.
15. Ali, A., Kaur, G., Hamid, H., Abdullah, T. and M. Ali, 2003, "Terminoside A, a new triterpene glycoside from the bark of Terminalia arjuna inhibits nitric oxide production in murine macrophages", Nat. Prod. Res. 5(2), pp 137-142.
16. Chopra, R.N., Nayar, S.L. and I.C. Chopra, 1956, "Glossary of Indian Medicinal plants", C.S.I.R publication, New Delhi, India, pp 241-245.
17. Bauer, A.W., Kirby, W.M.M., Sheris, J.C., and M. Turuk, 1966, "Antibiotic Susceptibility Testing by a Standard Disc Method", Am. J. Clin. Pathol. 45(4) pp 493-496.
18. Chowwanapoonpohn, S., Buddhasukh, D. and M. Garson, 2005, "Isolation of Pentacyclic Triterpenoid Compounds from *Lithocarpus elegans* (BL.) Hatus. Ex Soep. by Electrocoagulation", Acta Horticulturae, 677, pp 19-26.
19. Baek, M.Y., Cho, J.G., Lee, D.Y., Ahn, E.M., Jeong, T.S. and N.I. Baek, 2010, "Isolation of Triterpenoids from the Stem Bark of Albizia julibrissin and their Inhibition Activity on ACAT-1 and ACAT-2", J. Korean Soc. Appl. Biol. Chem. 53(3) pp 310-315.