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Investigations on *Terminalia Arjuna* Fruits: Part 1- Isolation of Comounds from Petroleum Ether Fractions

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Abstract

Arjun (*Terminalia arjuna*) is a source of many potent, biologically active compounds, planned all over Bangladesh. The chemical examinations of its fruits were taken up to isolate and identify active principles. For this purpose fresh fruits of *Terminalia arjuna* were extracted with rectified spirit. The extract was then triturated with petroleum ether (40-60^{O}C), which was then subjected to column chromatographic separation followed by PTLC. Such separation led to the isolation of some new pure compounds, TA-1 to TA-5. The structures of compounds were characterized through spectroscopic studies (IR, ^{1}H-NMR and ^{13}C-NMR).

Key words : Arjun, TA-1, TA-5, Spectroscopic studies

Introduction

Bangladesh being situated in the monsoon area of the world is a good treasure of indigenous plants of various families and they are grown widely in forests, jungles, hillocks and gardens. Different segments of the plants such as roots, stems, leaves, barks, flowers, seeds etc., may be an interesting and important research area for the natural product chemists. These segments are used directly by the Hakims, Kabirages and Vaidays as the medicine of various diseases, since the ancient times. But they are using the different parts of the plants without having enough scientific knowledge on them. Actually they do not know what are the constituents contained in the plants and which constituents are responsible for the cure of the diseases, or possesses harmful effects for the body. A number of plants used by the Hakims, Kabirages and Viadays may contain toxic constituents, which have some health hazard associated with them and may sometimes cause serious sufferings. In this situation a systematic investigation on the available plant resources is more important. Here one thing is very encouraging to note that our country abounds with a vast majority of medicinal plants and herbs. The availability

of medicinal plants demands a systematic investigation on the isolation, purification and characterization of physiologically active principles, which are actually useful for the treatment of various diseases. So, efforts have been made to investigate Terminalia arjuna, a medicinal plant available in Bangladesh. *Terminalia arjuna* belongs to the genus *Terminalia* (Ghani. 1998) and family Combretaceae and locally known as "Arjun gachh". Different parts of the plant are being used for the treatment of different diseases. Every part of the plant like fruits, barks and leaves contain various compounds like tannin, saponin, ester, sugar, steroids, acids and minerals (Kiritiker and Basu 1987, The Wealth of India, 1976). The present paper describes the isolation and characterization of petroleum ether triturate of *Terminalia arjuna* fruits.

Materials and Methods

Extraction of the fruits of Arjuna with rectified spirit

The powders of the fruits (4 Kg) were immersed in sufficient quantity of distilled rectified spirit at room temperature for 48 hours. The extract was collected and the process of extraction was repeated five times more with fresh rectified spirit until the extract become almost colorless. The extracts were combined and concentrated by removal of the solvent in a rotary evaporator under reduced pressure at a temperature below 45^{O}C. A greenish gummy mass was obtained and was denoted as R.

Preliminary fractionation of the crude extract, R

The concentrated rectified spirit extract; R was extracted with petroleum ether (200 ml). The petroleum ether layer was of greenish colour and separated with the help of a separatory funnel and extraction was repeated for five times more. The petroleum ether extract was freed from water using anhydrous Na_2SO_4 and evaporated to dryness under reduced pressure. A deep greenish gummy mass was obtained which was denoted as mass RP (12 g).

Examination of petroleum ether extract mass-RP

The deep greenish gummy Mass RP was completely soluble in petroleum ether and partially soluble in benzene, toluene, chloroform and ethyl acetate. TLC examination on silica gel plate in different solvent system showed the fraction to be a mixture of compounds. The resolution was best obtained in toluene: chloroform (1:1) which showed three spots at R_f 0.98, 0.61 and 0.48 with long tailing.

Column chromatographic separation of the mass RP

Mass RP (12 g) was dissolved in petroleum ether and then adsorbed in small quantity of silica gel. The adsorbed mass was evaporated to dryness under reduced pressure making

it completely free from solvent. It was then carefully poured on the top of a column of silica gel made in toluene. The column was eluted first with toluene followed by mixtures of toluene and chloroform. Fractions were collected in 100 ml conical flasks and examined by TLC.

The results of the chromatographic separation are shown in the Table I.

Purification of fraction RPF_1

It was purified by passing through a small column of silica gel made in petroleum ether and eluted with same solvent and showed a

Table I. Column chromatographic separation of fraction RP

Collection. Nos	Eluting solvent	TLC examination	Observation	Fraction nos.
1-3	Toluene 100%	One single spot at R_f 0.98 with small tailing near baseline	One pure compound with impurities	RPF_1
4-7	Toluene 100% 0.92 and 0.78	Three spots at R_f 0.98, . three compounds.	Mixtures of at list	RPF_2
8-10	Toluene : Chloroform (10:1)	One single spot at R_f 0.78	One pure compound	RPF_3
11-17	Toluene : Chloroform (5:1)	Tailing from the base line	No good resolution	RPF_4
18-25	Toluene : Chloroform (1:1)	Tailing from the base line	No good resolution	RPF_5
25-31	Toluene : Chloroform(1:1)	One spot at R_f 0.61 with tailing.	One compound with impurities	RPF_6
32-37	Toluene : Chloroform (2:3)	Spot near base line	No good resolution	RPF_7
38-45	Toluene: Chloroform (1:3)	Tailing from the base line Spot near base line	No good resolution	RPF_8
46-50	Chloroform 100% with small tailing	One spot at R_f 0.37	One compound with impurities	RPF_9
51-55	Chloroform : Ethyl acetate (10:1)	Tailing from the base line	No good resolution	RPF_{10}
56-60	Chloroform : Ethyl acetate (8:1)	Tailing from the base line	No good resolution	RPF_{11}
61-65	Chloroform : Ethyl acetate (5:1)	One major spot at R_f 0.48	One compound with impurities	RPF_{12}

single spot at R_f 0.98 in petroleum ether (40-60^{O}C). It was a white waxy solid and melted at 65-67^{O}C. The compound was soluble in petroleum ether, toluene, benzene and chloroform. It was designated at TA-1.

Similarly, the other fractions, RPF_3, RPF_6, RPF_9 and RPF_{12} were purified and the isolated compounds were denoted as TA-2, TA-3, TA-4 and TA-5 respectively.

Results and Discussion

Study on the fraction RPF_1 (compound TA-1)

The compound TA-1 was a waxy solid, melted at 65-67^{O}C and soluble in petroleum ether, chloroform and ethyl acetate.

The IR Spectrum of the compound TA-1 showed sharp bands at 2920 and 2848 cm^{-1} for C-H stretching vibration, 1470 and 1378 cm^{-1} for C-H bending vibration and for rocking vibration. These absorptions are characteristics for long alkyl chains. The hydrocarbon nature of the compound was further confirmed by ^{1}H-NMR spectrum, which showed a triplet at δ 0.89 for the terminal methyl protons and a huge unsplit singlet at δ 1.27 for methylene protons of long alkyl chain. The high intensity at δ 1.27 suggested the presence of a long alkyl chain. The high intensity at δ 1.27 suggested the presence of large number of methylene protons. It may therefore be concluded that TA-1 is a hydrocarbon. The m.p. of TA-1, 65-67^{O}C is more close to that of Hentriacontane (Ahmad *et al.* 1982). The structure of Hentriacontane is shown below:

$$CH_3\text{-}\ (CH_2)_{29}\ \text{-}CH_3$$

Study on the fraction RPF_3 (compound TA-2)

The compound TA-2 was an amorphous solid, melted at 70-72^{O}C and soluble in petroleum ether, chloroform and ethyl acetate. It responded to hydroxamic acid test of ester. The IR spectrum of the compound TA-2 showed sharp absorption band at 2918 and 2848 cm^{-1} due to presence of C-H (aliphatic) asymmetric and symmetric stretching vibrations respectively. The absorption band at 1712 cm^{-1} was due to >C=O group. Absorption band at 1461 cm^{-1} was due to -CH_3 bending vibration. It also showed C-O stretching at 1217 cm^{-1}. From this information the compound TA-2 was assumed to be an ester. The compound TA-2 was isolated in too small quantity to carry out further investigation, on it and was not possible to elucidate the structure of the compound.

Study on the fraction RPF_6 (compound TA-3)

The compound TA-3 was a waxy solid, melted at 60-62^{O}C and soluble in petroleum ether, chloroform and ethyl acetate. The IR spectrum of the compound TA-3 showed absorption band at 3490 cm^{-1} due to -OH group. Absorption bands at 2917 and 2848

cm^{-1} due to aliphatic -CH asymmetric and symmetric vibrations. Absorption band at 1738 cm^{-1} due to >C=O group. Weak absorption band at 1652 cm^{-1} due to isolated C=C band. Absorption band at 1462 and 1378 cm^{-1} due to $-CH_2-$ and $-CH_3$ bending.

The ^{1}H-NMR spectrum and ^{13}C-NMR spectrum of the compound TA-3 could not be analyzed with confidence. Therefore characterization of this compound was not further pursued.

Studies on the fraction RPF_9 (compound TA-4)

The compound TA-4 was a white needle shaped crystalline substance. It melted at 136-137^{O}C.and was soluble in chloroform, ethyl acetate and acetone. It positively responded to Salkowsky and Libermann-Burchard colour reaction. The IR spectrum of TA-4 showed an absorption band at 3500 cm^{-1} due to -OH group. Absorption bands at 2931 and 2850 cm^{-1} due to aliphatic -CH asymmetric and symmetric vibrations. Absorption band at 1710 cm^{-1} was due to >C=O group. Weak absorption band at 1653 cm^{-1} was due to isolated C=C band. Absorption band at 1464 cm^{-1} was due to $-CH_2-$ bending.

The ^{1}H-NMR spectrum of compound TA-4 in $CDCl_3$ has two sharp singlet at 1.00 (3H, s) and 1.25 (3H, s) ppm. These indicated the presence of two angular methyl protons at C-18 and C-19 respectively. Three doublets at d 0.93 (6H, d, J=8.0 Hz), 0.68 (3H, d, J=7.26 Hz) and 0.81 (6H, δ, J=4.8 Hz), were for the methyl protons of C-21, C-26 and C-27 respectively which were attached to methine carbons. A multiplet at δ 0.84 ppm was accounted for methyl protons of C-29 were attached to a methylene group. The ^{13}C-NMR spectrum of the compound TA-4 (Fig. 4.15 to 4.18, Table-4.2) in $CDCl_3$ exhibited the signals at δ 140.91 and 121.87 ppm were due to olefinic carbons in the ring system. The most deshielded chemical shift at δ 140.91 ppm was due to the quaternary carbon.

The signal at δ 71.96 was assigned to oxymethine carbon. The signals of δ 12.01 (C-18), 19.54 (C-19), 18.93 (C-21), 19.84 (C-26), 19.05 (C-27), 12.28 (C-29) ppm were due to six methyl groups of compound TA-4.

The deshielded signals at d 36.30 (C-10) and δ 42.33 (C-13) ppm were due to quaternary carbons (>C<).

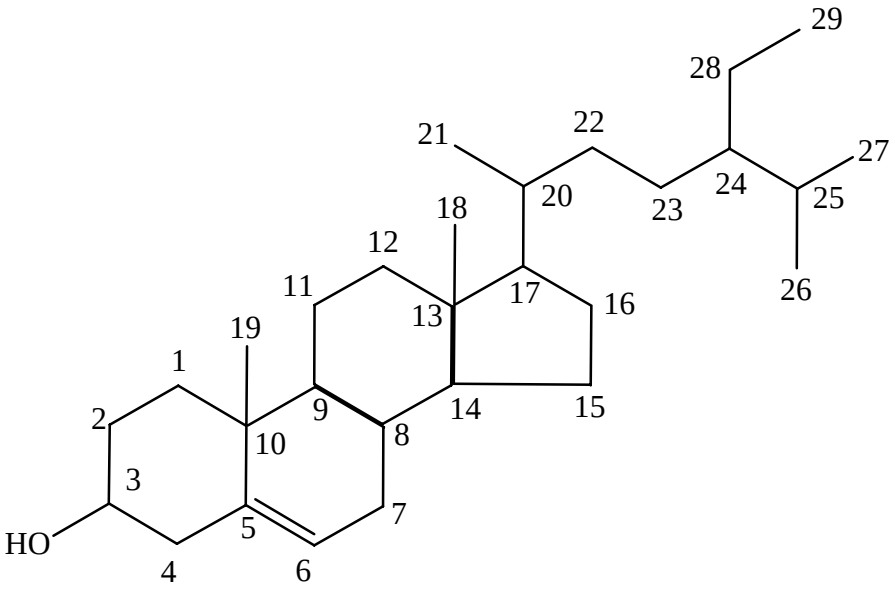
The DEPT-135 spectra displayed eleven methylene carbons at δ 37.41 (C-1), 31.82 (C-2), 42.25 (C-4), 31.94 (C-7), 21.10 (C-11), 39.93 (C-12), 24.33 (C-15), 28.27 (C-16), 33.96 (C-22), 26.08 (C-23), 23.08 (C-28); nine methine carbons at δ 140.91 (C-4), 121.87 (C-5), 31.94 (C-8), 50.15 (C-9), 56.78 (C-14), 56.22 (C-17), 36.17 (C-20), 45.85 (C-24) and 29.16 (C-25).

A comparison of ^{13}C-NMR spectral data of TA-4 and that reported for ß-sitosterol (Ahmed. 2002) is shown in Table II.

Table II. A comparison of ^{13}C-NMR spectral data of TA-4 and that reported for ß-sitosterol

Carbon number	Types of Carbon	Chemical shift in ppm	
		TA-4	ß-sitosterol
1	$-CH_2-$	37.41	37.33
2	$-CH_2-$	31.82	31.62
3	>CH-OH	71.96	71.73
4	$-CH_2-$	42.25	42.42
5	>C=	140.91	140.78
6	=CH-	121.87	121.69
7	$-CH_2-$	31.94	31.91
8	>CH-	31.94	31.91
9	>CH-	50.15	50.16
10	>C<	36.30	36.48
11	$-CH_2-$	21.10	21.12
12	$-CH_2-$	39.93	39.82
13	>C<	42.33	42.33
14	>CH-	56.78	56.79
15	$-CH_2-$	24.33	24.32
16	$-CH_2-$	28.27	28.26
17	>CH-	56.22	56.12
18	$-CH_3-$	12.01	11.89
19	$-CH_3-$	19.54	19.44
20	>CH-	36.17	36.17
21	$-CH_3-$	18.93	18.85
22	$-CH_2-$	33.96	33.98
23	$-CH_2-$	26.08	26.13
24	>CH-	45.85	45.58
25	>CH-	29.16	29.18
26	$-CH_3-$	19.84	19.84
27	$-CH_3-$	19.05	19.07
28	$-CH_2-$	23.08	23.09
29	$-CH_3-$	12.28	12.33

One the basis of the comparison of the various physical properties and spectral comparison of TA-4 and ß-sitosterol, the compound TA-4 was identical to be ß-sitosterol. The structure is represented bellow :



ß-Sitosterol

Study on the fraction RPF_{12} (compound TA-5)

Compound TA-5 was colourless crystalline compound. It melted at 108-110^{o}C and was soluble in petroleum ether, chloroform and ethyl acetate.

The IR Spectrum of the compound TA-5 showed absorption bands at 3063 and 3031 cm^{-1} due to (aromatic) C-H. Absorption bands at 2925 and 2854 cm^{-1} were due to (aldehyde) C-H. Absorption band at 1698 cm^{-1} was due to C=O conjugated. Absorption bands at 1602 and 1584 cm^{-1} were due to C=C group (aromatic). Absorption bands at 806 and 711 cm^{-1} were due to -CH (aromatic).

The compound TA-5 was isolated in too small quantity to carry out further investigation, on it. So, it was not possible to elucidate the structure of the compound.

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