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Research Article

**ISOLATION AND CHARACTERIZATION OF TWO NEW
COMPOUNDS FROM THE PLANT *BAUHINIA VARIEGATA***Bushra¹, Dil Faraz Khan¹, Hamid Ullah², Shafiullah Khan*¹¹Institute of Chemical Sciences, Gomal University, Dera Ismail Khan, KP, Pakistan²Department of Chemistry, Faculty of Arts and Basic Sciences, BUITEMS, Takatu Campus, Quetta-87300, Pakistan**Abstract:**

Bauhinia variegata plant is widely known and found throughout the world. It has extensive uses in our foods and also has a great medicinal importance which led the scientists to explore its responsible constituents. In this connection, in our studies on ethanolic extract of the *Bauhinia Variegata*, two new compounds were isolated and purified through chromatographic techniques. Then the molecular formula and characterization of these compounds were carried out with Mass spectral studies, and IR, UV and NMR spectral analysis. Consequently, to the new compounds Variegat A (1) molecular formula was assigned as $C_{25}H_{36}O$ and for the compound Variegat B (2) was assigned $C_{24}H_{32}O_3$ while the structural formulas for Variegat A (1) and Variegat B (2) was established as; 7-(tert-butyl)-6-ethoxy-1-(2-methylprop-1-en-1-yl)-3-(tert-pentyl) naphthalene and Methyl6-ethoxy-7-methyl-8-(2-methylprop-1-en-1-yl)-3-(tert-pentyl)-2-naphthoate respectively.

Key words: *Bauhinia Variegata*, Isolation, Characterization, Variegat A, Variegat B.***Corresponding author:****Dr. Shafiullah Khan,**

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INTRODUCTION:

Bauhinia Variegata is a species of flowering plants that is related to the family *Fabaceae*. The *B. Variegata* plant is locally recognised as *Kachnar* and originate in the tropical and subtropical parts of the world [1], including China, Pakistan, Sri Lanka, India, Hong Kong, US and Vietnam [2]. The tree is medium-sized deciduous [3, 4, 5, 6] and its height alters in between 6-12m. Branches of the plant grow 3 to 6m towards the outside [7]. Its bark exhibit pale-pink colour in the inside while on the outside show grayish brown colour and the external Leaves are 10 to 15 cm in length and width. When the plants get leafless, especially in January, the plant grows fragrant, white or purplish blossoms. Seeds are 10-15 in number [8]. *Kachnar* is eaten as vegetable and the buds are collected twice or thrice a year. An average plant yields flowers up to 25 kg. Variety of testy broths and pickles are prepared from Young buds of *Kachnar*. The buds have sufficient in phenolic contents which function as a source of dietary antioxidants. Its buds are referred in flour preparation, in curries, and also as flavouring agents [9]. When the plant remains leafless, usually in the period from January to April then its flowering starts in that period [10]. Almost all parts of the *Bauhinia Variegata* plant have been used with proven medicinal potential since ancient time. Its species are therapeutically referred for antimicrobial potential [2]. Its action against various diseases such as against cancer, ulcer and for wound healing. It also display great biological potential such as immunomodulatory, hypolipidemic, anti-inflammatory, insecticidal, antidiabetic, antibacterial, cytotoxic and other [11, 12, 13, 10]. Different parts of the *Kachnar* are applied as a part of culinary capacities [14].

Viewing the great importance of the *B. Variegata* plant in foods and medicines, it was necessary to further exploit its newly hidden chemical constituents, responsible for these effects.

With this aim, the present studies describe isolation and characterization of the new compounds through phytochemical studies on the ethanolic extract of the plant *B. Variegata*.

MATERIAL AND METHODS:

Material of the whole *B. variegata* plant was collected from Gomal University, Dera Ismail Khan, Pakistan and Prof. Dr. Jamil Khan (Faculty of Agriculture,

Gomal University D.I.Khan) identified the plant. The plant material was dried under shade at ambient temperature for a period of two months. The obtained 3.5 kg of the dried material was crushed and extracted 3 times with ethanol for one week. The ethanolic extract was combined and was evaporated under reduced pressure which resulted into a gummy extract (95 g). This unrefined extract was put in ethanol and refined through water to get different fractions comprising F1 having 14.5 g with n-hexane, F2 (19.1 g) with CH_2Cl_2 , F3 (23.2 g) with EtOAc and F4 (15.4 g) with EtOH.

The CH_2Cl_2 fraction F2 (19.1 g) was transferred to the silica gel (70-230 mesh) column, and elution was carried out with n-hexane-DCM mixture in increasing order of polarity. Considering this column chromatographic studies, at n-hexane-DCM (2:8) eluting system and further purification through TLC analysis we obtained an unknown compound as yellow amorphous solid. Different spectroscopic techniques i.e. IR, NMR spectroscopy and Mass spectrometry were applied and detected a new compound as Variegat A (**1**). Additionally, at 100% dichloromethane (DCM) indicated one prominent spot along with some minor spots on TLC card. This portion was further repurified through silica gel column with eluting system EtOAc-DCM (1: 9) which resulted in another compound that was identified as Variegat B (**2**) after structural elucidation by applying different spectroscopic techniques.

RESULTS AND DISCUSSION:

Structural confirmation of the isolated compounds:

The extraction of ethanolic crude from whole plant of *B. variegata* by using different solvents (n-hexane, dichloromethane, ethyl acetate) furnished different fractions. Among the fractions, dichloromethane fraction furnished two new compounds as Variegat A (**1**) and Variegat B (**2**), through repeated column and thin layer chromatographic techniques (as described earlier in material and method section). Structures were assigned to the obtained compounds through different spectroscopic techniques i.e. IR, UV, ^1H and ^{13}C NMR and mass spectrometry. Structures of the compounds **1** and **2** are presented in figure 1. While the ^1H -NMR and ^{13}C -NMR data for the compounds are presented in table 1.

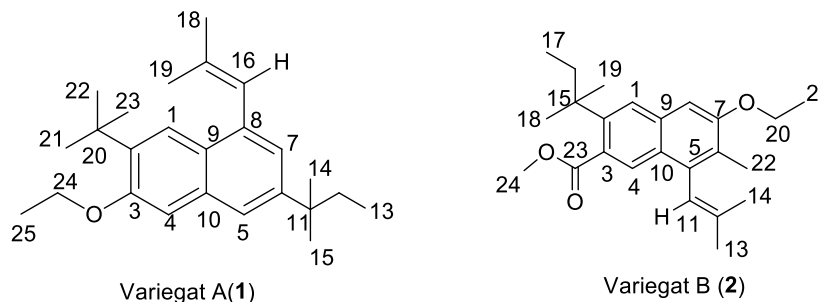


Fig. 1. Structures of the compounds Variegat A (1) and Variegat B (2)

Considering IR spectral data for the compound Variegat A (1), major signals were observed at 1610 cm^{-1} and at 1080 cm^{-1} indicated C=C of the aromatic system and the presence of ether group respectively. Additional signals at 3030 , 2970 and 2857 cm^{-1} appeared in the spectra corresponded to sp^3 and sp^2 – CH stretching vibrations respectively. The molecular formula was resolved through HREI-MS as $\text{C}_{25}\text{H}_{36}\text{O}$ ($m/z = 352.2766$; calcd. 352.2755), which determined 9 degrees of unsaturation. Eight of them represented 2 benzene cycles and one showed double bond due to alkene.

The ^1H NMR spectrum for the compound Variegat A (1) as shown in figure 2 and the relevant data in table 1, revealed a range of average asymmetrical peaks at $\delta = 8.13\text{--}7.02\text{ ppm}$ and $\delta = 7.55\text{--}7.29\text{ ppm}$ showed the existence of 2 benzene ring. A quartet appeared at $\delta = 3.78\text{ ppm}$ was attributed to the protons at C-24 and reveals the presence of ether group. A singlet peak $\delta = 5.33\text{ ppm}$ clearly indicated the vinylic proton at position C-16. The ethylene protons downfield value is due to the attached benzene cycle. The methylene protons at C-12 gives rise signal at $\delta = 1.29\text{ ppm}$ in the form of multiplet. Additionally, the multiple terminal methyl protons exhibited peaks at $\delta = 1.91$ to 0.91 ppm as a singlet and triplet, respectively.

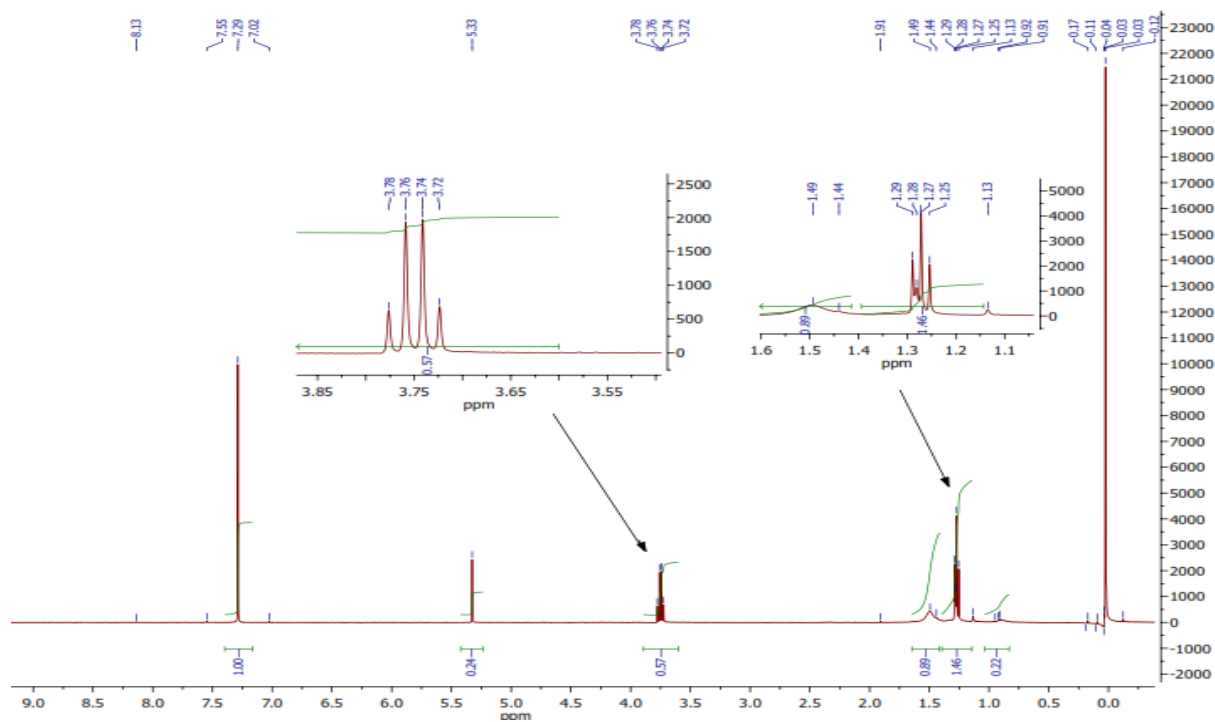


Fig. 2: ^1H NMR spectra of compound Variegat A (1), (500 MHz, CDCl_3).

The ^{13}C NMR spectral data for the compound Variogat A (1) (Table 1) revealed twenty five carbon atoms and their multiplicity were evidenced by DEPT ^{13}C NMR which showed nine CH_3 , two CH_2 , five methine and nine quaternary carbons. At $\delta = 148.6$ ppm, $\delta = 148.0$ ppm, $\delta = 134.3$ ppm, $\delta = 133.6$ ppm, and $\delta = 125.8$ ppm, the highly downfield peaks were appeared which confirmed the occurrence of quaternary carbons of the aromatic cycles. Carbon signals observed at $\delta = 124.4$ ppm, $\delta = 122.8$ ppm, and $\delta = 106.0$ ppm correspond to the presence of methine carbon of the aromatic rings, while at peak at $\delta = 65.0$ ppm was attributed to ether group. Signals at $\delta = 125.8$ ppm evidenced the presence of terminal methylene. The side chain of terminal methyl carbon gives rise peaks at $\delta = 31.7$ - 8.7 ppm. All the aforesaid data collectively identified Variogat A (1) as 7-(tert-butyl)-6-ethoxy-1-(2-methylprop-1-en-1-yl)-3-(tert-pentyl) naphthalene.

The IR spectral analysis for the compound Variogat B (2) evidence the presence of aromatic system as a signal appeared at 1610 cm^{-1} and ether group at 1080 cm^{-1} , while an intense solid peaks appeared at 1196

cm^{-1} confirmed the occurrence of ethoxy group. The Sp^3 and Sp^2 C-H stretches were confirmed by the signals appeared at 2852 , 2945 , and 3011 cm^{-1} . Furthermore, the vibrational signal for the ester carbonyl and ethylene C=C double bond were observed at 1743 cm^{-1} and 1658 cm^{-1} . Additionally, the substitution at benzene ring was also indicated by its bends appeared in the region from 860 - 750 cm^{-1} . The molecular formula was determined through HREI-MS as $\text{C}_{24}\text{H}_{32}\text{O}_3$ ($m/z = 368.2351$; calcd.368.2340), revealed ten degrees of unsaturation. Out of ten, eight of them were due to the substituted aromatic systems and one for double bonded ester carbonyl and one for double bonded ethylene carbon atom.

The ^1H NMR spectra of Variogat B (2) (Fig. 3) displayed signals at $\delta = 8.15$ - 7.02 ppm indicated the benzene ring. Two protons quartet at $\delta = 3.76$ ppm was attributed to methylene protons attached to oxygen while the singlet peak at $\delta = 4.25$ ppm was assigned to the methyl at position C-24. A strong singlet peak showed at $\delta = 5.33$ ppm was an indication of ethylene protons.

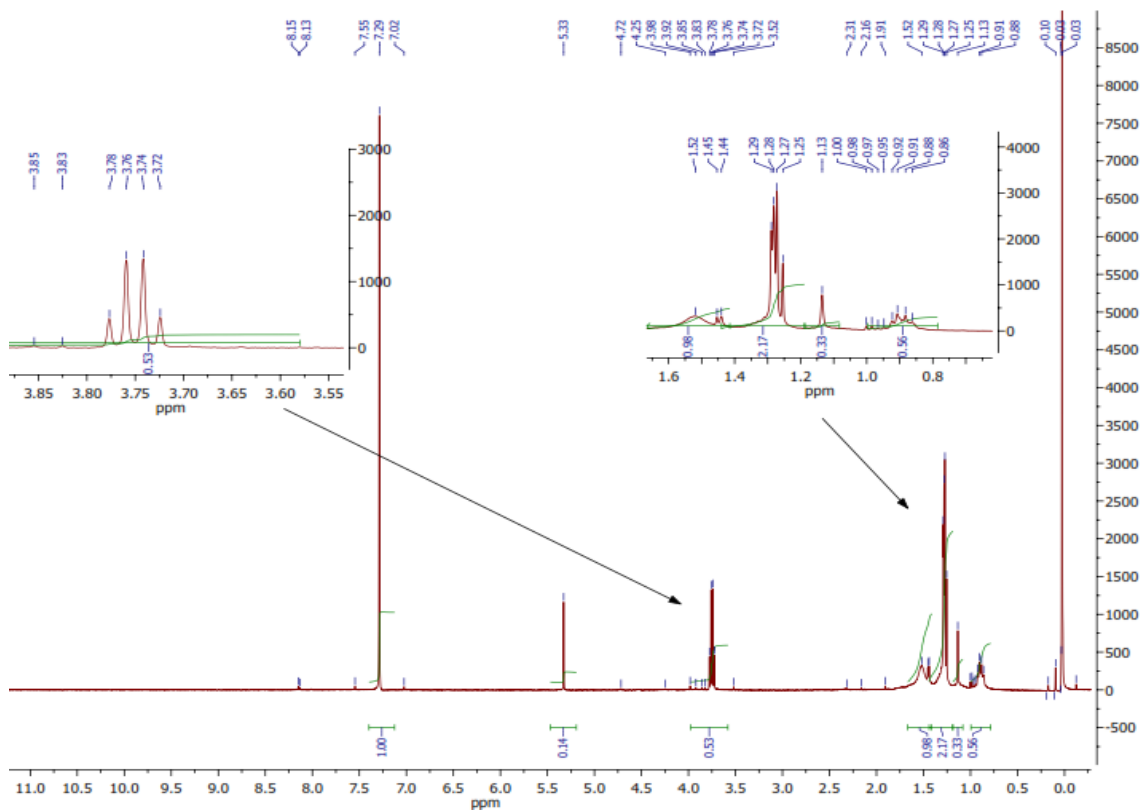


Fig. 3: ^1H NMR spectra of compound Variogat B (2), (500 MHz, CDCl_3).

Considering ^{13}C NMR spectra for the compound Variegat B (2), twenty four carbons were observed and their multiplicity through DEPT revealed ten quaternary carbons, two methylene, four methine and eight methyl carbon atoms. The resonating signals appeared at $\delta = 166.0$ ppm and $\delta = 147$ to 105 ppm indicated ester and aromatic groups, respectively. The

signals appeared at $\delta = 137.5$ to 33.4 ppm, were assigned as shown quaternary carbons. On the base of all the given and discussed data, the compound Variegat B (2) was recognized as Methyl 6-ethoxy-7-methyl-8-(2-methylprop-1-en-1-yl)-3-(tert-pentyl)-2-naphthoate.

Table 1: ^1H NMR (500 MHz, CDCl_3) and ^{13}C NMR (125 MHz, CDCl_3) data of Variegat A (1) and Variegat B (2), (δ =chemical shift in ppm)

Variegat A			Variegat B		
Position	δ (H)	δ (C)	Position	δ (H)	δ (C)
1	8.13s	122.8	1	7.55s	124.6
2	-----	140.6	2	-----	147.0
3	-----	148.6	3	-----	122.3
4	7.02s	106.0	4	8.15s	126.5
5	7.55s	124.4	5	-----	138.8
6	-----	148.0	6	-----	122.0
7	7.29s	142.1	7	-----	155.8
8	-----	134.3	8	7.02s	105.0
9	-----	125.8	9	-----	134.8
10	-----	133.6	10	-----	124.3
11	-----	40.6	11	5.33s	125.8
12	1.29m	38.1	12	-----	137.5
13	0.91t	8.7	13	1.52s	25.2
14	1.25s	28.9	14	1.91s	19.2
15	1.25s	28.9	15	-----	33.4
16	5.33s	125.8	16	1.28m	38.1
17	-----	137.5	17	0.88m	8.7
18	1.44s	25.2	18	1.13s	28.9
19	1.49s	19.2	19	1.13s	28.9
20	-----	31.2	20	3.76 q	65.0
21	1.13s	31.7	21	0.91m	14.8
22	1.13s	31.7	22	2.16s	8.7
23	1.13s	31.7	23	-----	166.0
24	3.78q	65.0	24	4.25s	51.5
25	0.91t	14.8			

CONCLUSION:

Phytochemical studies of the plant *Bauhinia Variegata* resulted in isolation of the two new constituents and structural confirmation of these compounds were carried out with the help of modern chromatographic and spectroscopic techniques. Since literature revealed that the plant *Bauhinia Variegata* and compounds isolated from this plant revealed great importance in our food and medicinal, therefore, to exploit its hidden medicinal importance, further studies on biological evaluation of the newly isolated compounds are in process.

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