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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF A FLAVONE FROM THE LEAVES OF *TAMARIX NILOTICA*(TAMARICACEAE).

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Abstract

The authors report on the isolation of a flavone (5,7,4`-trihydroxy-5`-methoxylflavone).This is a first isolation for such flavone from the Sudanese material of *Tamarix nilotica.* The flavonoid was isolated from the n-butanol fraction by column chromatography.The structure was elucidated by a combination of analytical tools (UV,IR,^{1}H NMR,^{13}C NMR,HMBC and MS).

Introduction:-

Flavonoids include a C_6-C_3-C_6 carbon framework, or more specifically a phenylbenzopyran functionality. They have two aromatic rings (usually designated by A and B) joined by a 3 carbon atom bridge usually forming a heterocyclic ring C (Grote,2006;Vallejo,2004).

Flavonoids are classified into : flavanones , flavones, dihydroflavonols , flavonols, flavanols, anthocyanidins, isoflavones, chalcones , aurones and proanthocyanidins. Flavonoids and their conjugates form a very large group of natural products. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs(Harborne and Williams,2000).

Flavonoid compounds are one of the most analyzed group of secondary metabolites in higher plants. The main reason for the interest in flavonoids is that they are major constituents of plant pigments. Anthocyanins, a flavonoid subclass, have been of special interest because of their ability to confer red, orange, blue, and purple coloration to leaves, flowers, and fruits(Mol *et.al.*,1998).

As pigments, flavonoids have facilitated the testing of hypotheses related to Mendel's law and transposable elements. Flavonoids have been the focus of attempts to modify flower color by genetic engineering(Tanaka *et.al.*,1998).

Some flavonoids have been reported to possess a variety of biological activities, including antiallergic, antiinnflammatory, antiviral, antiproliferative, and antitumor activities, in addition to having effects on mammalian metabolism(Zand *et.al.*,2002;Ren *et.al*.,2003) .

Flavonoids have received considerable attention because of their beneficial effects as antioxidants in the prevention of human diseases such as cancer and cardiovascular diseases, and some pathological disorders of gastric and

duodenal ulcers, allergies, vascular fragility beside viral and bacterial infections(Zand *et.al.*,2002) . They contribute to the antioxidant properties of green vegetables, fruits, olive and soybean oils, chocolate, and teas(Yao *et.al.*,2004). Several mechanisms by which flavonoids play an important role in cytotoxicity have been identified. Antitumour activity of several flavonoids (pinostrobin, quercetin, myricetin, morin) is attributed to their efficiencies to inhibit topoisomerase I and II(Sukardiman *et.al.*,2000;Constantinou *et.al.*,1995). Flavonoids might slow down cell proliferation as a consequence of their binding to estrogen receptor(Primiano *et.al.*,2001;Kohn *et.al.*,2001;Omiecinski *et.al.*,1999;Hodek *et.al.*,2002;Naczk and Shahadi,2004) .

There is also interest in using them as drugs or dietary supplements because of their strong antioxidant activities. In plants, flavonoids have several functions including attracting insects for pollination and dispersal of seeds, acting in defense systems (e.g., as UV-B protectants and phytoalexins), signaling between plants and microbes, and regulating auxin transport. Many of these functions can not occur unless flavonoids are properly localized within the cells(Winkel,2001; Bartel,2003) .

In continuation of our interest in constituents of Sudanese plants used in ethnomedicine ,this study was designed to investigate the flavonoids of the medicinally important *Tamarix nilotica*.

The genus Tamarix (tamarisk, salt cedar) is composed of about 50–60 species of flowering plants in the family Tamaricaceae, native to drier areas of Eurasia and Africa. The generic name originated in Latin and may have referred to the Tamaris River in Hispania Tarraconensis (Spain) (Quattrocchi,U.,2000).

They are evergreen or deciduous shrubs or trees growing to 1–18 m in height and forming dense thickets. The largest, *Tamarix aphylla*, is an evergreen tree that can grow to 18 m tall. They usually grow on saline soils, tolerating up to 15,000 ppm soluble salt and can also tolerate alkaline conditions.

T. nilotica is found in Lebanon, Palestine, Egypt, Sudan, Somalia, Ethiopia and Kenya. In the Nile Valley in Egypt, this tree grows beside the river and the irrigation channels. It can form dense thickets in suitable locations(Akhani,2014). *T. nilotica* has been used in traditional medicine as an antiseptic, an antipyretic, for alleviating headaches and reducing inflammation. It also has a reputation as an aphrodisiac(Akhani,2014).

Materials and Methods:-

Materials:-

Instuments:-

The UV spectra were recorded on a Shimadzu 1601 Spectrophotometer and UV lamp was used for localization of fluorescent spots on TLC . The IR spectrum was recorded by using a Shimadzu IR-8400 Spectrophotometer. Nuclear Magnetic Resonance spectra were run on a JEOL DELTA ESP-400MHZ NMR Spectrophotometer. Melting points were determined on a Kofler Hot-Stage Apparatus and were uncorrected. Mass spectra were measured on a Verian G-C450-MS-240 Spectrometer.

Plant material:-

The leaves of *Tamarix nilotica* were collected in August 2015 from Khartoum - Sudan.The plant was kindly authenticated by Department of Botany ,University of Khartoum.

Methods:-

Isolation of flavonoids from *Tamarix nilotica* extract:-

Powdered shade -dried leaves (1.00 Kg) were exhaustively percolated with 80% methanol (5L) at ambient temperature for 72hr . The solvent was evaporated under reduced pressure leaving a(50 g) residue. The residue was suspended in 300 ml of water and successively extracted with dichloromethane , ethyl acetate and n-butanol . Removal of the solvent under reduced pressure gave crude products which were manipulated further by chromatographic techniques.

The butanol fraction (9.0 g) was subjected to CC using Merck Silica gel with particle size 60 μm (70-230 mesh) as stationary phase, Successive elution with $CHCl_3$-MeOH in increasing order of polarity gave four fractions. The sub-fraction($CHCl_3$-MeOH:3:2;v:v) showed three major spots on TLC. It was further subjected to CC using($CHCl_3$-MeOH 7:3) to afford compound I, for more purification compound I was chromatographed on a sephadex LH-20

column(60 x1.5 cm) using methanol as an eluent . Identification of this compound was based on extensive UV shifting reagent studies and ^{1}H, ^{13}C NMR , 2D NMR and mass spectroscopy data.

Results and Discussion:-

Compound I(m.p.174^{o}C) was isolated from the butanol fraction of *Tamarix nilotica* leaves as yellow needles.The IR spectrum (Fig.1) displayed absorption bands at ν(KBr) : 656, 870(C-H , Ar. bending) ,1050 (C-O) ,1400 – 1500 (C=C , Ar. St.) , 1630 (C = O) , 2700 (C- H, aliphatic) and 3400cm^{-1} (OH).

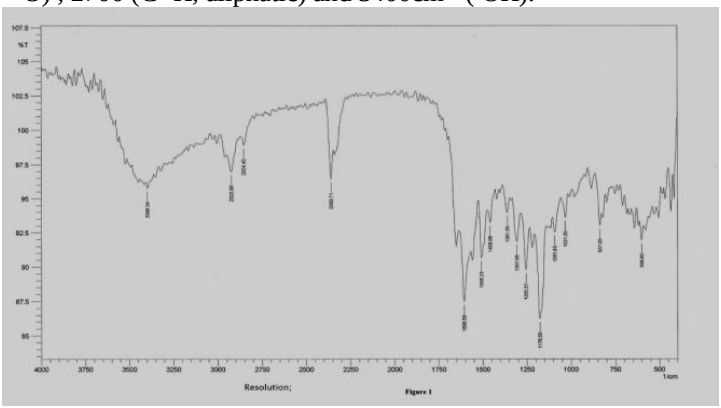


Fig.1: IR spectrum of compound I

The presence of a C=O function in the IR spectrum excludes the presence of (i)anthocyanins and (ii) catechins. The U V spectrum (Fig.2) showed λ_{max} (MeOH) 232, 348 nm which is characteristic of flavones(Harborne and Williams,2000). Band I in flavones is manifested in the range 304 – 350 nm.

The shift reagent sodium methoxide is a strong base . It is diagnostic of 3- and 4'- OH groups(Harborne and Williams,2000) .In both cases it gives a bathohromic shift but with decrease in intensity in case 3-OH. Addition of NaOMe to a methanolic solution of compound I caused(Fig.3) a bathochromic shift (72nm) in band I without decrease in intensity.This indicates the presence of a hydroxyl function at C-4'(Markham,1982).

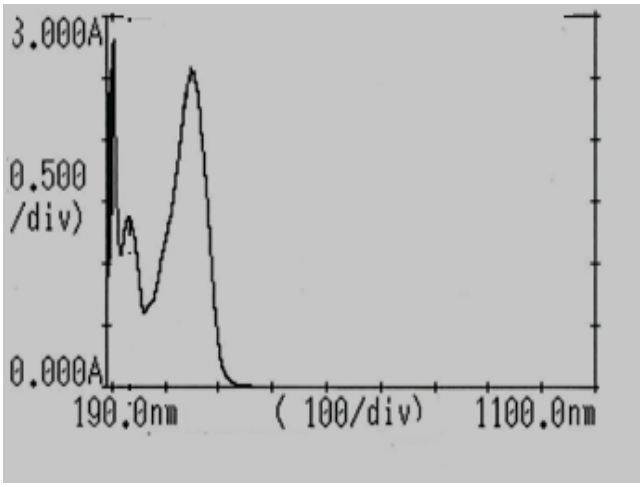


Fig.2: UV spectrum of compound I

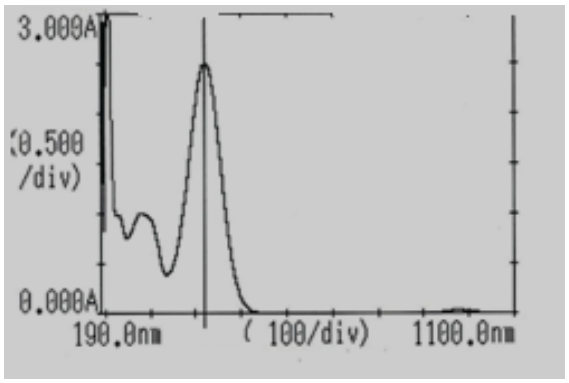


Fig.3: Sodium methoxide spectrum of compound I

Soudim acetate is a weaker base than sodium methoxide and as such ionizes only the more acidic hydroxyl group in flavonoids. It is particularly useful diagnostic reagent for the specific detection of a 7-hydroxyl function(Harborne and Williams,2000;Harborne,1976) .The sodium acetate spectrum (Fig.4) revealed a bathochromic shift (46 nm) in band II indicative of a 7-OH function.

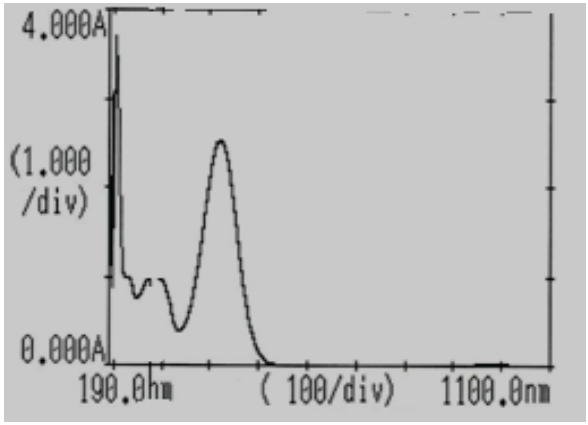


Fig.4: Sodium acetate spectrum of compound I

Aluminum chloride chelates with functional groups such as the 5-hydroxy-4-keto-,3-hydroxy-4-keto and this is evidenced by bathochromic shifts of one or both bands in the spectrum .It also chelates with catechol systems(Harborne,1976). The aluminium chloride spectrum revealed a bathochromic shift (67nm) in band I(Fig.5). However , magnitude of the shift is indicative(Markham,1982) of a 5-OH function.

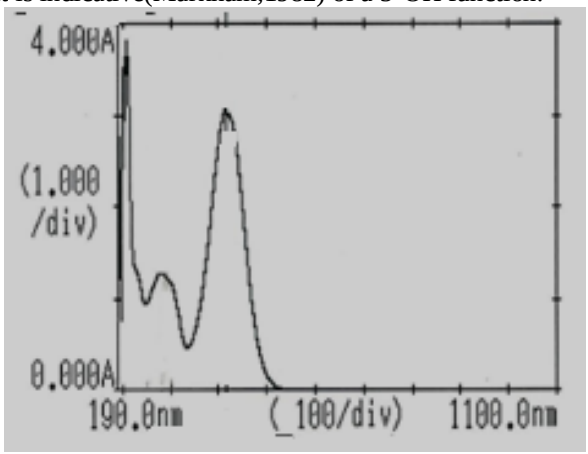


Fig.5: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Fig. 6) showed a signal for a methoxyl function at ∂ 3.9(s,3H) and two *meta* coupled aromatic protons resonating at ∂ 6.19 (s,1H) and 6.33 (s,1H) assignable to H-6, H-8 respectively. The C_8-H usually resonates at

lower field relative to C_6 -proton due to the deshielding influence of the oxygen atom at position 1(Harborne and Williams,2000;Markham,1982). The B-ring protons appeared as a doublet at ∂ 7.05(2H)- due to H-2',H-3 '- and singlet at ∂ 7.9 (1H) due to the H-6'.

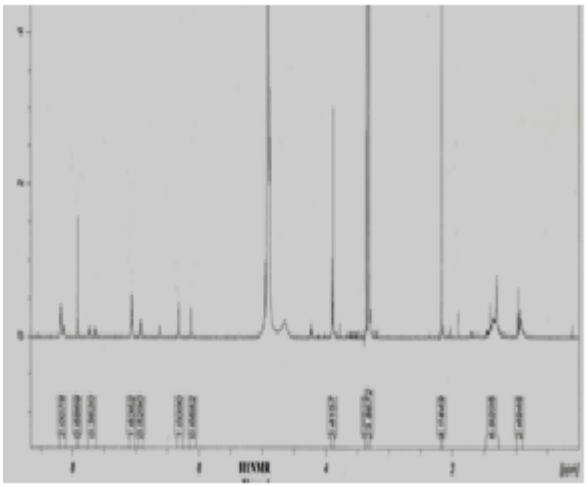


Fig.6: ^{1}HNMR spectrum of compound I

The HMBC spectrum revealed(Fig.7) a long range coupling between the methoxyl resonance(∂3.9ppm) and C-4`($\partial_c$ 160ppm).Hence the methoxyl function was assigned for $C_{5`}$ (δ_c131ppm) .The ESI-MS(Fig. 9) showed a molecular ion peak at m/z 300 [M^+] .Other important fragments,resulting from retro Diels-Alder fission , were shown at m/z 152, 119.

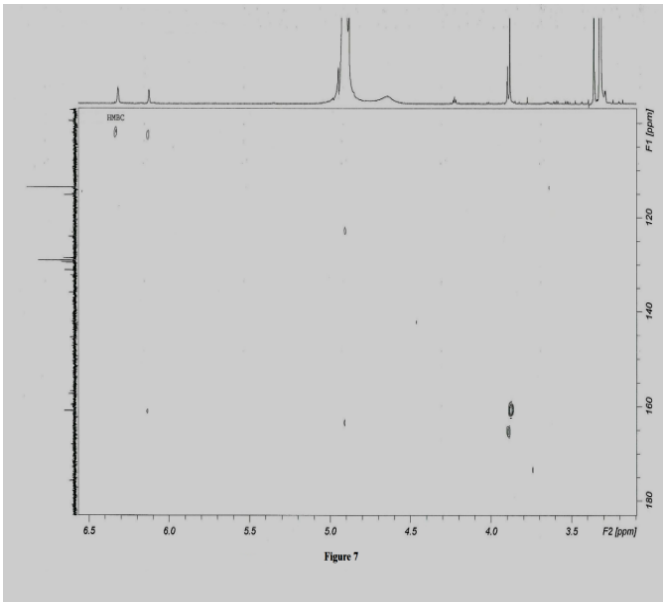


Fig.7: HMBC spectrum of compound I

The ^{13}C NMR(Fig. 8) revealed a pattern characteristic of a C_{16} compound. The assignment of the chemical shifts (∂_c) is depicted in Table 1.

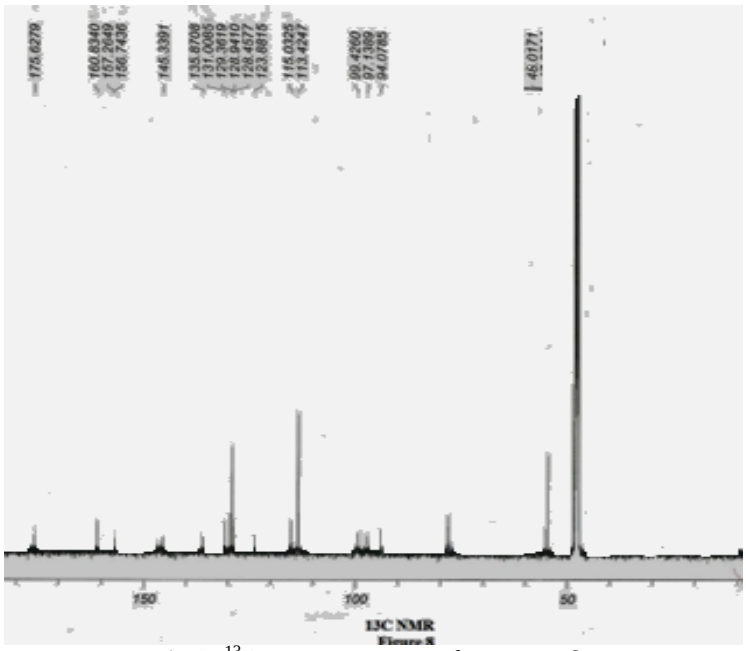
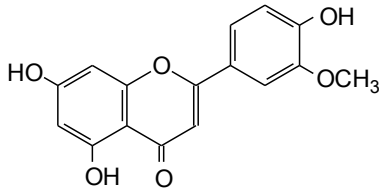


Fig.8: ^{13}CNMR spectrum of compound I

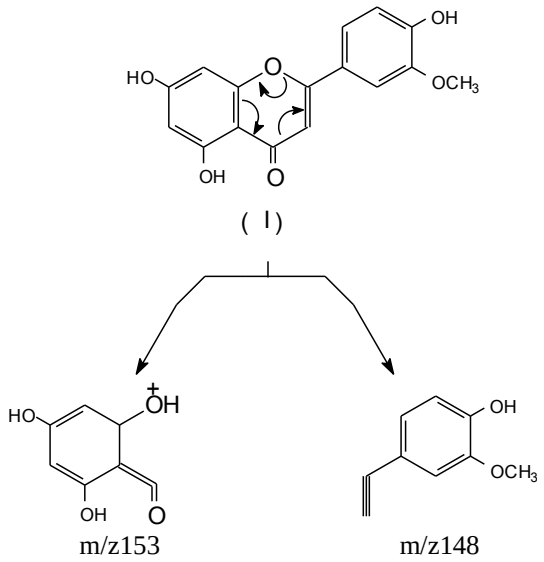
Table 1: Assignment of ($\delta_{c.}$)

δ_c	Assigment
175	C-4
160	C-4`
157	C-5
156	C-2
145	C-7
135	C-9
131	C-5`
129	C-6`
128	C-3
128	C-1`
123	C-2`
115	C-2`
113	C-10
99	C-6
94	C-8
54	OCH_3

Comparison of the above cumulative data with data published in the literature revealed that compound I is a derivative of apignine with the following structure:



The following retro Diels- Alder fission(Scheme I) provides additional evidence in favor of the proposed structure:



Scheme I: retro Diels-Alder fission of compound (I)

References:-

1. Akhani, H. (2014), "*Tamarix nilotica*", Version 2013.2, International Union for Conservation of Nature.
2. Bartel, B., and Matsuda, S. ,*Plant physiol*., **143**,299(2003) .
3. Constantinou, A., Mehta, R., Runyan, C., Rao, K.,Vaughan, A., Moon, R. (1995), *J. Nat. Prod*., **58**, 217.
4. Grote,E. (2006) "The Science of Flavonoid Compounds" Springer Verlag, New York ,P47 .
5. Harborne, J. B., and Williams, C. A. (2000), *Phytochemistry*, **55,**481.
6. Harborne, J. (1976) "Phytochemical Methods" , Chapman and Hall, New York .
7. Hodek, P., Trefil, P.,Stiborova, M. (2002), *Chem.-Biol. Interact*., **139**, 1.
8. Kohn, M.C., Walker, N.J., Kim, A.H., Portier, C.J. (2001), *Toxicology*, **162**, 193.
9. Mol, J., Grotewold, E., and Koes, R. ,(1998), *Trends Plant Sci*., **3**, 212.
10. Markham, K.R., (1982) "Techniques of Flavonoid Identification", Academic Press, London .
11. Naczk, M., Shahidi, F. (2004), *J. Chromatogr.* **A**, 1054.
12. Omiecinski, C.J., Remmel, R.P., Hosagrahara, V.P. (1999), *Toxicol. Sci.*, **48**, 151.
13. Primiano, T., Yu, R., Kong, A.N.T., *Pharm. Biol*., **39**, 83(2001).
14. Quattrocchi, U. (2000), CRC World Dictionary of Plant Names, 4 R–Z, Taylor and Francis, US, p. 2628.
15. Ren, W., Qiao, Z.H.,Wang,H.W. (2003), *Med.Res. Rev*.**,23,**519 .
16. Sukardiman,D., A., Tanjung, M., Darmadi, M.O. (2000), *J.Clin. Hemorheol. Microcirc*., **23**, 185.
17. Tanaka, Y., Tsuda, S., and Kusumi, T. (1998), *Plant Cell Physiol*., **39**, 1119.
18. Vallejo,F. (2004), *J Chromatogr.A*., **1(2**),181.
19. Winkel-Shirley, B. , Plant Physiology, **126**, 485(2001).
20. Yao, L.H., Jiang, Y.M., Shi, J. (2004), *Plant Foods for Human Nutrition*,**59**,113.
21. Zand, R.S.R., Jenkins, D.J.A., Diamandis, E.P. (2002), *J. Chrom*. **777**,219.