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RESEARCH ARTICLE

Isolation,Characterization and Biological Activity of a Dihydroflavonol From *Tamarix nilotica* (Tamaricaceae) Leaves.

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Abstract

The authors report on the isolation,characterization and antimicrobial potency of a dihydroflavonol isolated from the leaves of Sudanese *Tamarix nilotica*. The isolate was purified by different chromatographic techniques and identified via a combination of spectral tools(IR, UV, ^{1}HNMR and Mass spectroscopy). The isolated flavonoid , ethyl acetate and n-butanol fractions were evaluated(*in vitro*) for their antimicrobial potential against Gram negative *(Escherichia coli, Salomonella typhi*) and Gram positive (*Bacillus subtilis* , *Staphylococcus aureus)* bacteria and the fungi :*Candida albicans* and *Aspergillus niger* and promising results were obtained.The isolated dihydroflavonol seems to be a suitable candidate for future optimization.

Introduction:-

Flavonoids are formed in plants from the aromatic amino acids : phenyl alanine and tyrosine(Harborne,1986). The basic structure consists of a C_6 - C_3 - C_6 skeleton : two benzene rings joined by a linear 3 - carbon chain .

Over the past decade evidence accumulated that flavonoids are an important class of defense antioxidants(Halliwel,1997; Blake and Winfard,1995; Harman,1965; Halliwell and Gutteridge,1998;Wayner *et.al.*,1987; Warma *et.al*.,1995; Halliwel,1994) .Oxidative processes , *in vivo* , may generate reactive oxygen species (ROS) including superoxide , peroxyl , alkoxyl , hydroxyl and nitric acid radicals (Cavangh,1972) . These radicals are very reactive and rapidly attack nearby cells and damage them leading to many serious diseases like cancer(Mabry *et.al*.,1972). Excess dietary antioxidants are needed for diminishing the cumulative effects of oxidative damage over the lifespan(Harborne,1998).

Flavanoids , are secondary metabolites produced by plants and are found in a non- glycosylated form (aglycone) or attached to a sugar molecule (glycoside). Many flavonoids are endowed with biological activities, such as anti-inflammatory, antiallergic, antischemic, antiplatelet, immunomodulatory, and antitumor activity(Prior and Cao,2000;Jelp *et.al*.,2000; Craig,1999) Flavonoids has protective effects against many infections (bacterial and viral diseases) and degenerative diseases(Middleton,1998; Cook and Samman,1996; Rice-Evans *et.al.*,1995;Pandey,2007).

The genus *Tamarix* is composed of 50-60 species in the family Tamaricaceae native to drier areas of Asia and Africa(Quattrocchi,U.,2000). They are evergreen or deciduous shrubs that can grow to 18m tall.They can tolerate alkaline conditions and grow on saline soils.

Tamarix nilotica is distributed in Sudan, Egypt, Plestine, Lebanone, Ethiobia and Kenya. *Tamarix nilotica* can form dense thickets in suitable locations(Akhani,2014). In the Nile Valley in Egypt this species grows along irrigation channels and beside river bank.

Tamarix nilotica has been used in ethno-medicine as antiseptic, antipyretic,for treatment of headaches and inflammations. The plant also has a reputation as aphrodisiac(Akhan,2014).

In continuation of our interest in bioconstituents of plant species used in Sudanese traditional medicine , we planned to investigate the flavonoids of *Tamarix nilotica* which is used by local healers to treat an array of human disorders.

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 as KBr disks, using 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* :-

Powdered shade -dried leaves (1 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 column chromatography using a 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(100ml each). Fraction(3) showed two major spots on TLC .It was further subjected to flash column chromatography using($CHCl_3$-MeOH 3:2) 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 . Compound I was isolated in chromatographically pure form as yellow powder.

Antimicrobial assay:-

The isolated compound , ethyl acetate and n- butanol fractions of *Tamarix nilotica*, were screened for their antimicrobial activity against six standard human pathogens (*Bacillus subtilis, Staphylococcus aureus, Sallmonela typhi, Escherichia coli , Aspergillas niger ,candida albicans).*

Preparation of bacterial suspensions:-

One ml aliquots of 24 hours broth culture of the test organism were aseptically distributed onto agar slopes and incubated at 37^{o}C for 24 hours .

The bacterial growth was harvested and washed off with sterile normal saline ,and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^4 colony forming units per ml . the average number of viable organism per ml of saline suspension was determined by means of surface counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0-.02ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates.The plates were allowed to stand for two hours at room temperature for the drop to dry and then incubated at 37^{o}C for 24 hours.

preparation of fungal suspensions:-
Fungal cultures were maintained on saturated dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Testing for antibacterial activity:-
The cup-plate agar diffusion method was adopted with some minor modification, to assess the antibacterial activity . (20ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes ,the agar was left to settle in each of these plates which were divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No. 4) .Each of the halves was designed for one of the test solutions. Separate Petri dishes were designed for standard antibacterial chemotherapeutic (ampicillin ,gentamycin and Clotrimazole).
The agar discs were removed and cups were filled with 0.1ml sample of each extract and pure compound using adjustable volume microtiter pipette and allowed do diffuse at room temperature for two hours .The plates were then incubated in the upright position at 37°C for 24 hours.

The above procedure was repeated for different concentration of the test solutions and standard antibacterial chemotherapeutics .After incubation the diameters of the resulting growth inhibition zones were measured in triplicates and averaged.

Testing for antifungal activity:-
The above mentioned method was adopted for antifungal activity ,but instead of nutrient agar saturated dextrose agar was used .Samples were used here by the same concentration used above .

Results and Discussion:-

Identification of compound I:-
Compound I was isolated from n-butanol fraction of *Tamarix nilotica* leaves and purified by a combination of chromatographic techniques.

The IR spectrum (Fig.1) displayed absorption bands at ν(KBr): 640 (C-H ,Ar.,bending) 1510 (C=C, Ar.) ,1650 (C=O), 2825 (C-H , aliphatic) and 3400cm^{-1} (OH) .

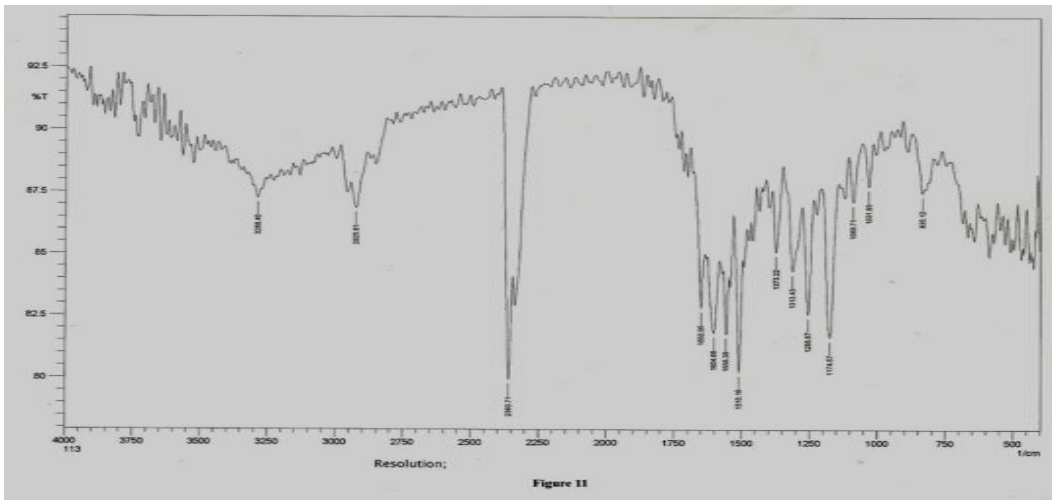


Fig.1: IR spectrum of compound I

The VU spectrum (Fig.2) showed λ_{max}(MeOH) 273 nm.No shoulder characteristic of isoflavones was detected in the range (300-340mn). The sodium methoxide spectrum revealed a 51 nm bathochromic shift with decrease in intensity(Fig.3). Such shift is indicative(Harborne,1998,Mabry,1976) of a C-3 OH function and compound I is consequently a dihydroflavonol.

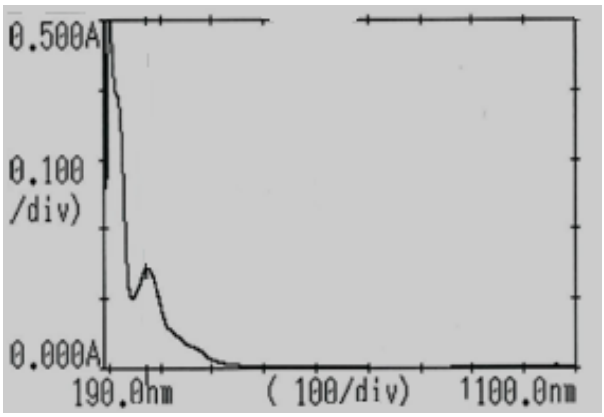


Fig.2: UV spectrum of compound I

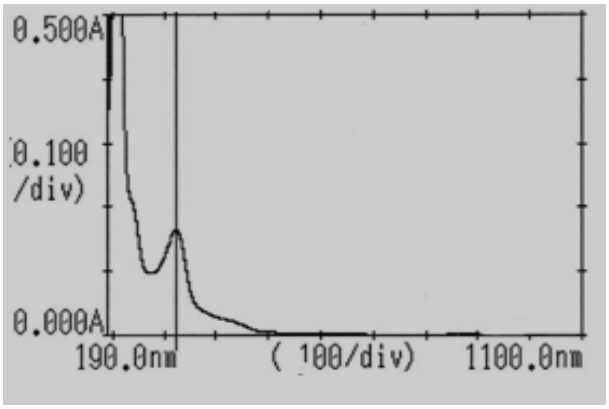


Fig.3: Sodium methoxide spectrum of compound I

No bathochromic shift was observed in the sodium acetate spectrum(Fig.4).. This indicates the absence(Mabry,1976) of a 7-OH function. Also the $AlCl_3$ spectrum(Fig.5) did not reveal any bathchromic shift indicating absence of a 3-, 5-OH groups and catechol moieties(Harborne,1998).

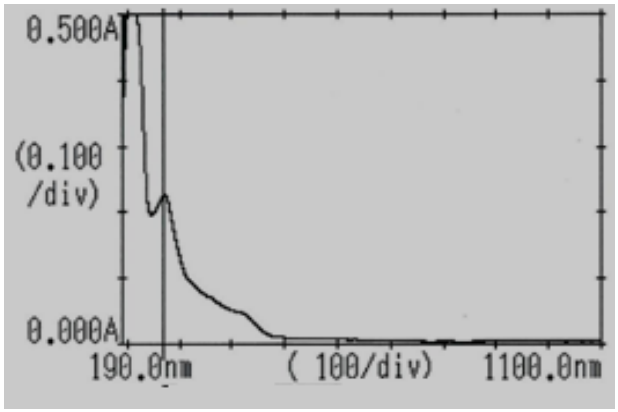


Fig.4: Sodium acetate spectrum of compound I

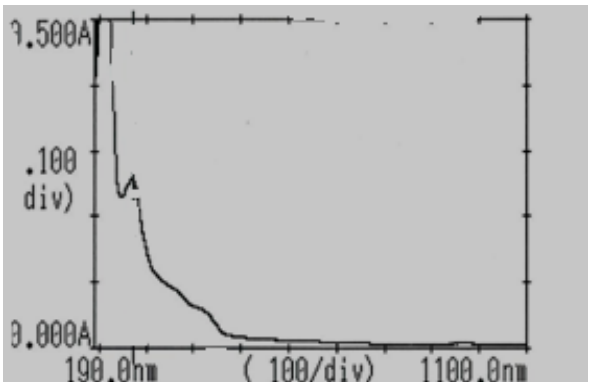


Fig.5: Aluminium chloride spectrum of compound I

The ^{1}HNMR spectrum (Fig.6) showed a signal at ∂ 0.9 (3H) assigned for a methyl group. The resonances at ∂3.70(3H), ∂3.90(3H) and ∂4.20(3H) account for three methoxyl functions. The signals at ∂ 6.2 (1H) and ∂ 6.4 (1H) were assigned for C_6 and C_8 protons respectively. Due to the deshielding influence of the oxygen atom at position 1,the C_8 proton invariably resonates at lower field relative to C_6 proton.

The double doublets at ∂7.6 (1H) and ∂7.65 (2H) were assigned to H-5' and the protons at $C_{2',6`}$ respectively. The latter protons resonate at lower field relative to H-5` due to the deshielding influence of the heterocyclic C ring.

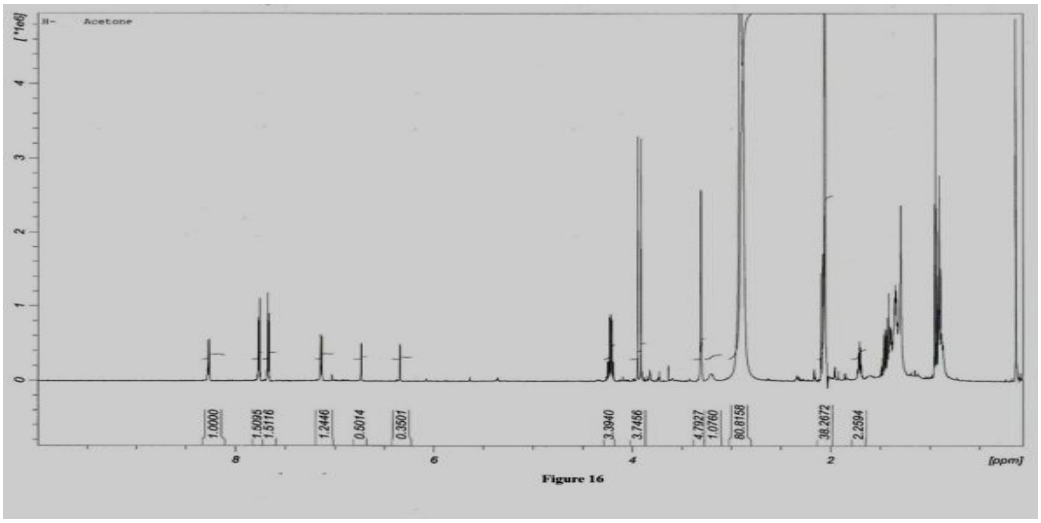


Fig.6: ^{1}H NMR spectrum of compound II

The ^{13}C NMR(Fig.7) revealed the signals δ_c: 167.13(C-4), 67.41(C-2), 54.92(C-3) 91.88(C-5),97.50(C-6),132.55(C-7), 97.58(C-9), 113.98(C-10),129.45(C-1`),128.75(C-2`), 131.16(C-3`) ,128.75(C-4' , C-5',C-6`)-see also Fig. (8).

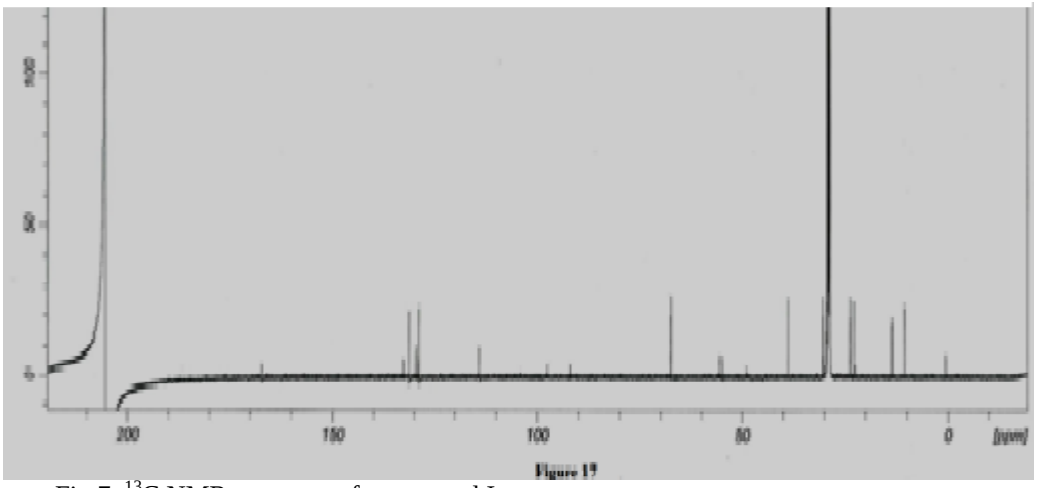


Fig.7: ^{13}C NMR spectrum of compound I

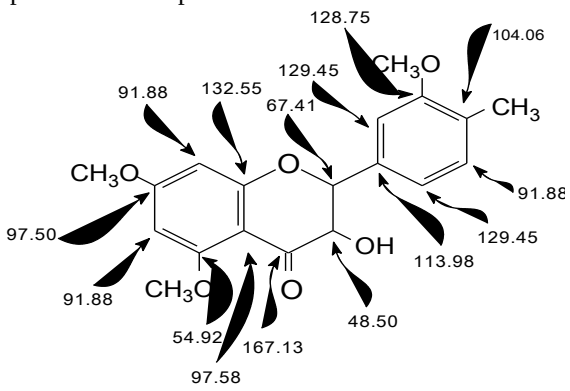
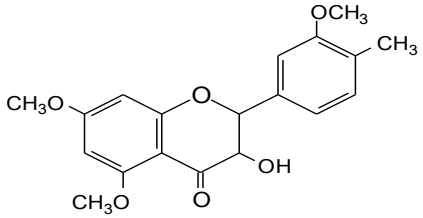


Fig. 8:Assignment of δ_c

The mass spectrum (Fig.9) gave the peak m/z 342 for $[M^+ - 2H]$.Other important fragments resulting from retro Diels – Alder fission (Scheme I) were shown at m/z180 and m/z150. Such fragments originate from intact A and B rings respectively and provide additional evidence in favor of the following proposed structure for compound I.



Compound I

The citation of two methoxyl groups at postions 5 and 7 of ring (A) was based on ^1H-^1H COSY NMR experiments which indicated long range coupling between a methoxyl function and C_6 –H, and another long range coupling between a methoxyl group and the protons at C_6 and C_8. The third methoxyl function was assigned position 3` due to long range coupling between a methoxyl function and $C_{2`}$–H. A long range coupling between a methyl group and the $C_{5`}$ proton suggests methylation at position 4`.

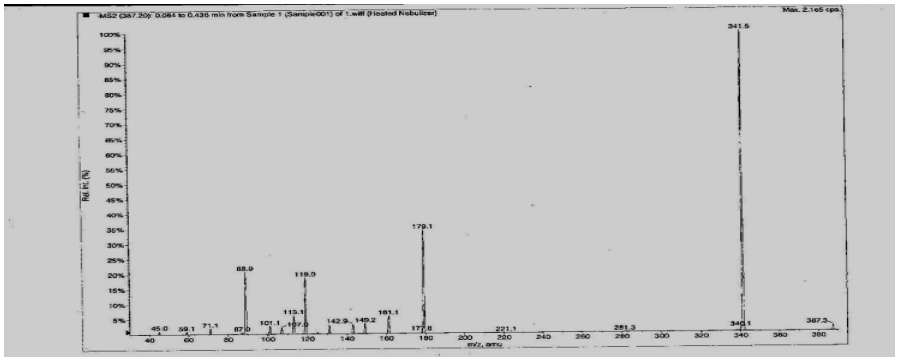
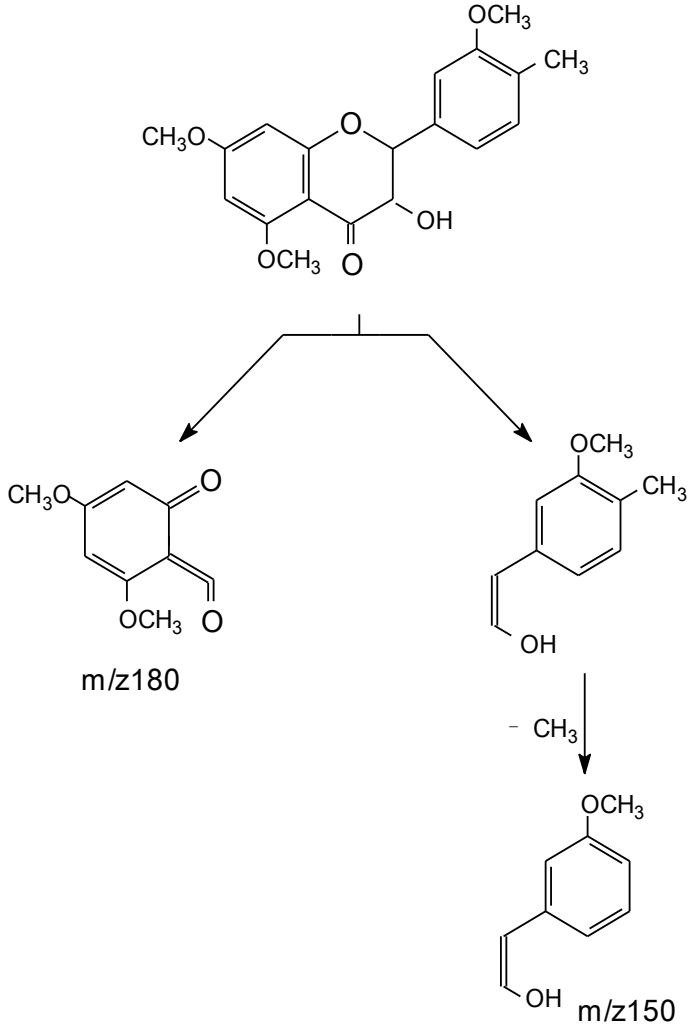


Fig.9: Mass spectrum of compound I



Scheme 1: Retro Diels-Alder fission of compound I

Antimicrobial activity:-

Compound I and two fractions from *Tamarix nilotica* (ethyl acetate and n-butanol) were evaluated for potential antimicrobial activity against six standard human pathogens.

The average of the diameters of the growth inhibition zones are shown in Table (1) .The results were interpreted in terms of the commonly used terms ; <9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active) .

Diameters of the growth inhibition zones for standard chemotherapeutic agents are depicted in Tables (2) and (3).

Table 1: Antimicrobial activity of compound I and *Tamarix nilotica* extracts

Microorganism	Gram	ET*	nB*	compound I (100mg/ml)
Bacilus subtilis	+ve	25	32	15
Staphylococcus aureus	+ve	24	27	17
Escherichia coli	-ve	27	33	16
Salomonella typhi	-ve	16	30	14
Candida albicans	Fungus	17	19	16
Aspergillus niger	Fungus	14	15	20

* ET = Ethyl acetate fraction
nB = n=Butanol fraction

Table (2) : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Sa.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	-
	20	22	18	18	-
	10	17	14	15	

Table (3.5) : Antifungal activity of a standard chemotherapeutic agent

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- Sa.*: Staphylococcus aureus*
- Ec.*: Escherichia coli*
- Sa.: *Salomonella typhi*
- An.: *Aspergillus niger*
- Ca.: *Candida albicans*
- *Bs.: Bacillus subtilis*

From table (1) it seems that the antimicrobial activity of the ethyl acetate and n-butanol fractions surpass that of the pure compound. But compound (I) showed more potency against the fungus: *Aspergillus niger* than both fractions.. However, compound I is regarded as a suitable candidate for future optimization.

References:-

1. **Akhani,**H.(2014) "Tamarix nilotica" , Version 2013-2,International Union **for Conservation of Nature.**
2. **Blake** , D. , Winfard , P.G.(1995) In : Imminopharmacology of Free Radical Species , Academic Press , New York.
3. **Craig**, W.J.(1999), *Am J Clin Nutr*.**,70**,491.
4. **Cook**, N . C. and Samman, S.(1996) , *Journal of Nutritional Biochemistry*, **7**(2) , 66 (1996).
5. **Harborne** , J. B.(1986), In : "Plant Flavonoids in Biology and Medicine" , P 15 , John Willey and Sons, New York (1986).
6. **Halliwell** , B.(1997), *Nutr. Rev.* , **S44** , 55 .
7. **Harman** , D.(1965) , *J. Gerontology* , **11** , 289 .
8. **Halliwell** , B. and Gutteridge , J.(1998) , In : "Free Radicals in Biology and Medicine" , Oxford University Press , Oxford .
9. **Halliwell** , B.(1994) , *Lancet* , **344** , 721.
10. **Harorne**,J.B.(1998) " Phytochemical Methods" , Chapman and Hall, London,UK.
11. **Ielpo**, MTL., Basile, A., Mirando, R.(2000) , *Fitoterapia* , **71**,101.

12. **Kavangh**,F.(1972) " Analytical Microbiology" ,Academic Press, New York , P11.
13. **Mabry**,T.J., Markham,K.R. and Thomas,M.B.(1972) " The Systematic Identification of the Flavonoids " ,Springer-Verlag, Berlin .
14. **Middleton**, E.J(1998). , *Adv Exp Med Biol.,* **439**,175.
15. **Quattrocchi**,U.(2000), CRC World Dictionary of Plant Names,4R-Z,Taylor and Francis,US.
16. **Pandey**, A. K(2007) , *National Academy Science Letters*, **30**(11),383.
17. **Prior**, RL., Cao, G.(2000) *Nutr Clin Care* , **3**,279.
18. **Rice-Evans**, C.A., Miller, N. J., Bolwell, P. G., Broamley, P. M. and
19. Pridham, J. B.(1995) *Free Radical Research*, **22**(4), 375.
20. **Wayner** , D. , Burton , G. W. , Ingold , K. U . , Barclay , L.R . , Locke , S.(1987) , *Biochem . Biophys. Acta* **, 924** , 408.
21. **Warma** , S. D. , Devamanoharan , P. S. , Morris , S. M .(1995) , *Crit. Rev. Food Sci. nutr.* , **35** , 111.