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Research Article

Isolation of a Flavone from the Sudanese Material of *Cassia Kordofania* (Leguminoseae)

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Abstract- The authors report on the isolation of a flavone (5,7,4`-trihydroxyflavone) from the Sudanese material of *Cassia kordofania*. The flavonoid was isolated from the ethyl acetate fraction by column chromatography.The structure was elucidated by sensitive analytical tools (UV, IR, ^{1}H NMR,^{13}C NMR and MS).

Index terms: *Cassia kordofania*, Isolation, Flavone, Characterization**.**

I. INTRODUCTION

Flavonoids encompass a large group of polyphenolic substances which are widely distributed in plants and foods of plant origin[1-4]. Flavonoids share a common basic skeleton consisting of two benzene rings joined by a linear three carbon bridge. They are divided into:flavans,flavones,flavonols,flavanones,isoflones,auron-es,chalcones,anthocyanins[1,2]. Recently,due to their health benefits, research on the flavonoids gained an increased pulse[2].

In vitro, flavonoids are effective scavengers of free radicals [5,6]. Several studies have examined the relationship between some measure of dietary flavonoid intake and coronary heart disease (CHD) risk [7-14].

It was found that intake of diet rich in flavonoids is associated with significant reduction in CHD risk [7,11-15].In general, the foods that contributed most to total flavonoids in these studies were: black tea, apples, and onions.

Different *In vitro* and *in vivo* studies revealed that some flavonoids exhibit antimicrobial potential[16-23],others exert antispasmodic activity [24].Several flavonoids have been shown to have potential as hepatoprotective agents [25].Flavonoids like gossypin and morin were found to show significant analgesic activity[26].

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Cassia kordofania belongs to the Leguminoseae family and the sub-family Caesalpiniaceae. It is Known as Egyption Senna, Tinnevelly Senna , East Indian Senna and Nubia Senna .It grows natively in all regions of Sudan specially in Kordofan-eastern Sudan. *Cassia Kordofania* is used in Sudanese ethnomedicine for treating an array of human disorders including allergic and inflammatory conditions[27]. Other species belonging to this genus are also medicinally important species. *Cassia obtusifolia*,which is used traditionally in the treatment of diabetes, showed promising antibacterial potential[28]. Aqueous and methanolic extracts of *Cassia fistula* (used in ethomedicine as anti-inflammatory,,laxative)were found to possess significant anti-inflammatory effect in both acute and chronic models [29].Also bark extracts showed significant radical scavenging by inhibiting lipid peroxidation initiated by CCl_4 and $FeSO_4$ in rat liver and kidney homogenates [29]. Ethanolic leaves extracts of *Cassia fistula* showed hepatoprotection against INH/RIF -induced hepatitis in rats [30] .It was shown that treatment with *Cassia auriculata* leaf extract has a lipid-lowering effect in rats with experimentally- induced, alcohol-related, liver damage[31]. It was also claimed that the *Cassia auriculata* flowers possess antihyperlipidaemic effect in addition to antidiabetic activity [32].Seeds of *Cassia roxburghii* , which is used in ethnomedicine for various liver disorders, exhibited significant hepatoprotective activity[33]. Different extracts of leaves of *Cassia occidentalis* L. were screened for their antimicrobial activity against seven human pathogenic bacteria and two fungal strains by disc diffusion assay with promising results [34].The plant also showed significant anti-allergic, anti-inflammatory and anti-lipid peroxidant effects *in vivo* [35].

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II. MATERIALS AND METHODS

Materials

Analytical grade reagents were used. The UV spectra were recorded on a Shimadzu 1601 Spectrophotometer and a UV lamp was used for localization of fluorescent spots on TLC plates. The IR spectra were recorded as KBr discs, using Shimadzu IR-8400 Spectrophotometer. Nuclear magnetic resonance spectra were recorded on a JEOL DELTA ESP400 MHZ NMR Spectrophotometer. Melting point were determined on a Kofler Hot-Stage apparatus and were uncorrected. Mass spectra were run on a VARIAN 450 GC -240 MS Spectrophotometer.

Plant material

The leaves of *Cassia kordofania* were collected from Gazeira province –Sudan during November 2012 and authenticated by Dr. Jacob Thomas, Department of Botany and Microbiology,King Saudi University. A voucher specimen was deposited in the herbarium of this department .

Methods

Extraction and isolation of the active constituents

Powdered air -dried leaves of *Cassia Kordofania* (2.5Kg) were macerated with 95 % ethanol (5 L) at ambient temperature for 48hr.. The solvent was removed under reduced pressure and the residue (25g) was fractionated by column chromatography using silica gel (60-120 mesh). Sequential elution by chloroform, ethyl acetate and n- butanol was performed.The ethyl acetate fraction(8g) was then fractionated on a silica gel (60-120 mesh) column using MeOH : $CHCl_3$(1:1,v:v) as eluent.The column was monitored by TLC and five ml fractions were collected. Depending on their TLC pattern fractions **(**F_2 - F_{18}) were pooled together. Removal of the solvent under reduced pressure gave a pure component-compound I - which reacted positively with ferric chloride and vanillin-H_2SO_4, indicating its strong phenolic nature.Compound I(25mg) is a yellow powder, m.p.319^0(chloroform);UV λ_{max} (MeOH) 343, 265, 226, (MeOH-NaOMe) 248, 274, 392, (MeOH-NaOAc) 364, 374, (MeOH-NaOAc-H_3BO_3) 266, 342 ,(MeOH-$AlCl_3$) 266, 305, 397 (MeOH-$AlCl_3$-HCl) 266, 304 ,398 . IR (KBr): ν (cm^{-1}) : 638 , 723 , 1089 , 1614 ,1660 and 3319. GC-MS, m/z : 273(M^+ +3H), 152, 118, 124.

III. RESULTS AND DISCUSSION

The electronspray mass spectrum of (I) exhibited(Fig.1) a peak at m/z 273 corresponding to (M^++3H). Compound I gave a positive $FeCl_3$ and $AlCl_3$ tests indicating a phenolic nature.

In the UV it absorbs(Fig.2) at λ_{max}(MeOH) 265,343nm due to $\pi \rightarrow \pi^*$ and n $\rightarrow \pi^*$ transitions of benzoyl and cinnamoyl chromophores of flavonoid moiety. This absorption suggests a flavone skeleton[1,2]. Furthemore , the compound gave colour reactions characteristic of flavones[1]

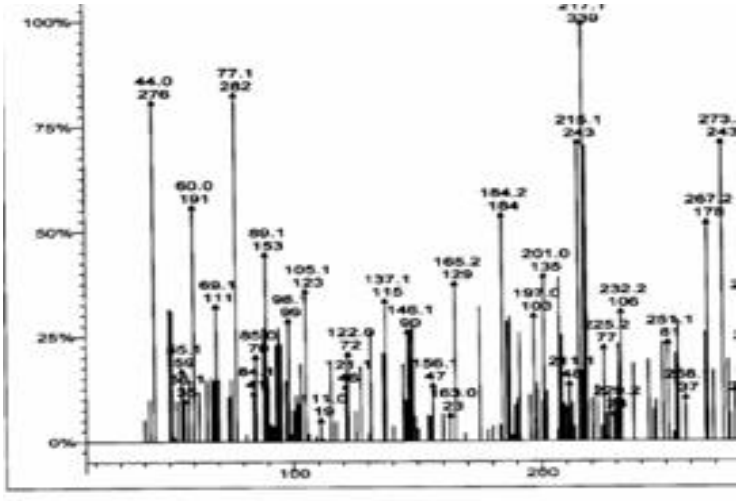


Fig.1: Mass spectrum of compound I

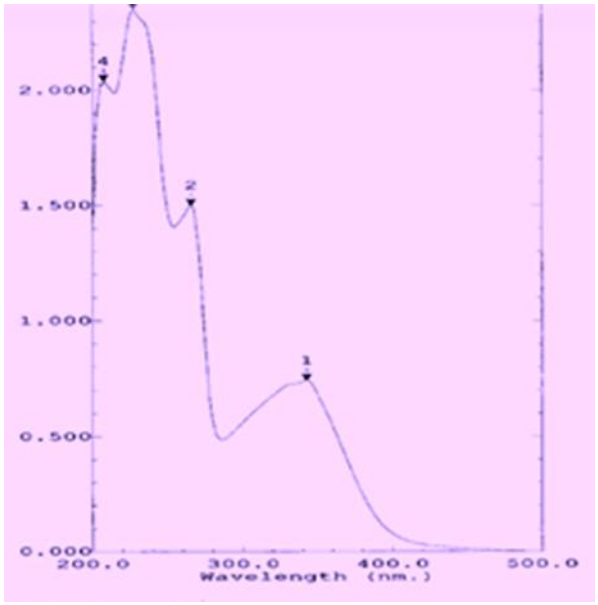


Fig.2: UV spectrum of compound I

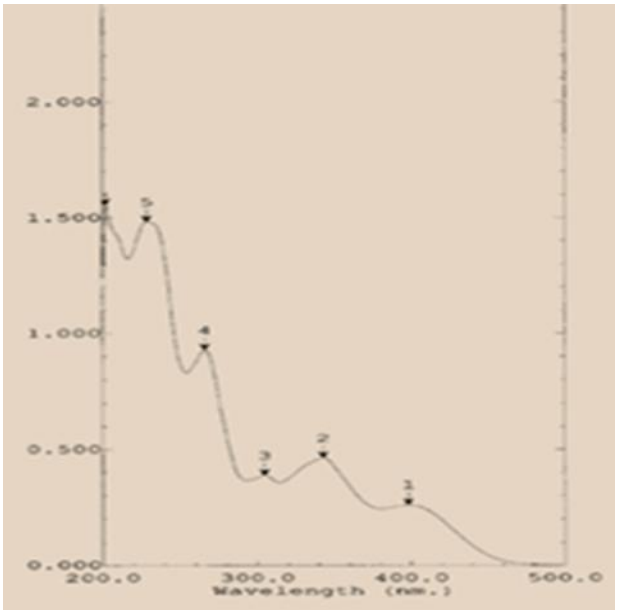


Fig.3: Aluminium chloride spectrum of compound I

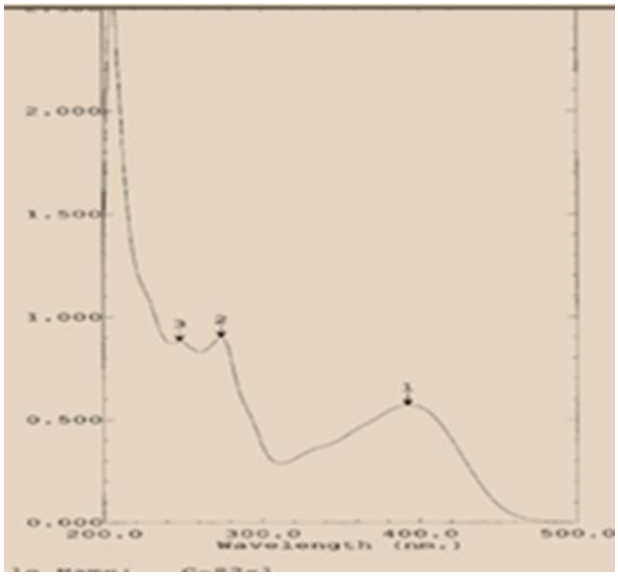


Fig.4: Sodium methoxide spectrum of compound I

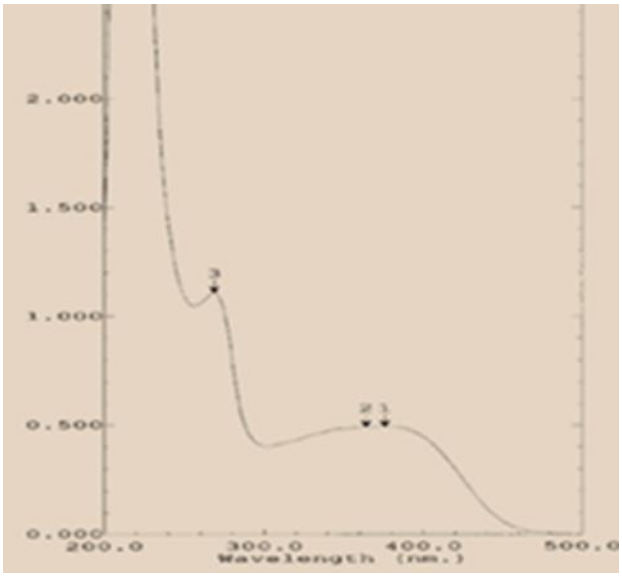


Fig.5: Sodium acetate spectrum of compound I

The aluminium chloride spectrum(Fig.3) of (I) gave a 54nm bathochromic shift without degeneration on treatment with HCl indicating a 5-OH function[2]. The sodium methoxide spectrum(Fgi.4) gave a 49 nm bathochromic shift in band I without decrease in intensity and this is diagnostic of a 4`-OH[1,2]. The shift reagent sodium acetate gave a 9nm bathochromic shift(Fig.5) which is indicative[2] of a 7-OH function. No bathochromic shift indicative of catechol moieties [1] was observed in the boric acid spectrum(Fig.6).

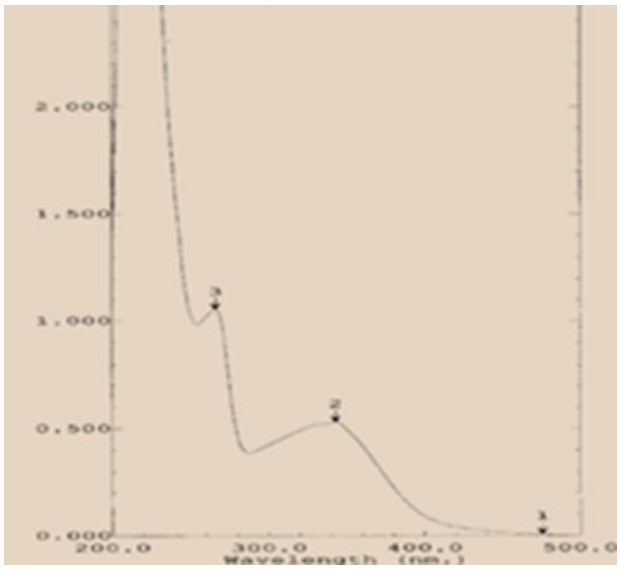


Fig.6: Boric acid spectrum of compound I

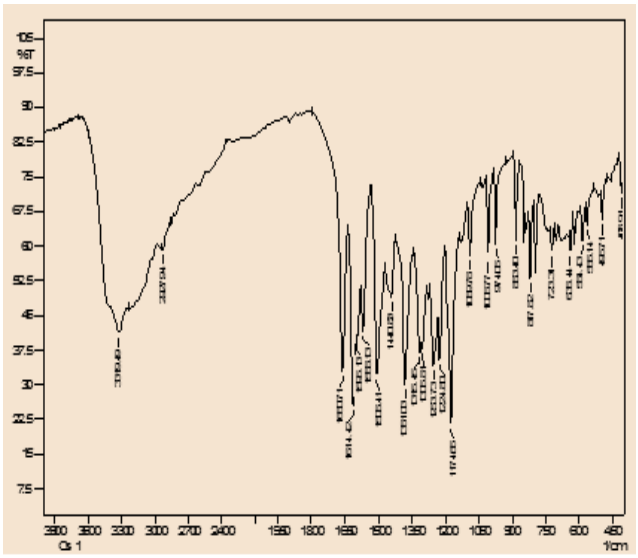


Fig.7: IR spectrum of compound I

The IR spectrum (Fig7) showed absorption bands at ν(KBr) : 3410 (OH), 1655 (α,β-unsaturated carbonyl group), 1614 (aromatic C=C) cm^{1}functionalities. The ^{1}H NMR spectrum (Fig.8)) exhibited resonances at: δ6.22 (d,2H) and δ 6.46(d,2H) accounting for C_6- and C_8-protons respectively. In flavonoids the C_6- proton usually resonates at higher field relative to C_8- proton[36].The doublet at δ 6.95(2H) accounts for $C_{3'}$- and $C_{5'}$- protons, while the resonance at δ 8.03(d,2H) is characteristic of $C_{2'}$- and $C_{6'}$- protons. The latter protons resonate downfield relative to $C_{3'}$- and $C_{5'}$- protons due to the deshielding effect of the neighbouring heterocyclic C ring [36].

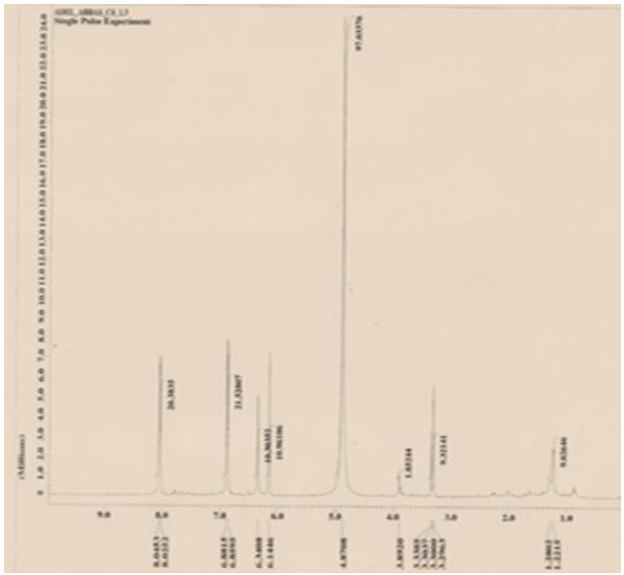


Fig.8:^{1}H NMR spectrum of compound I

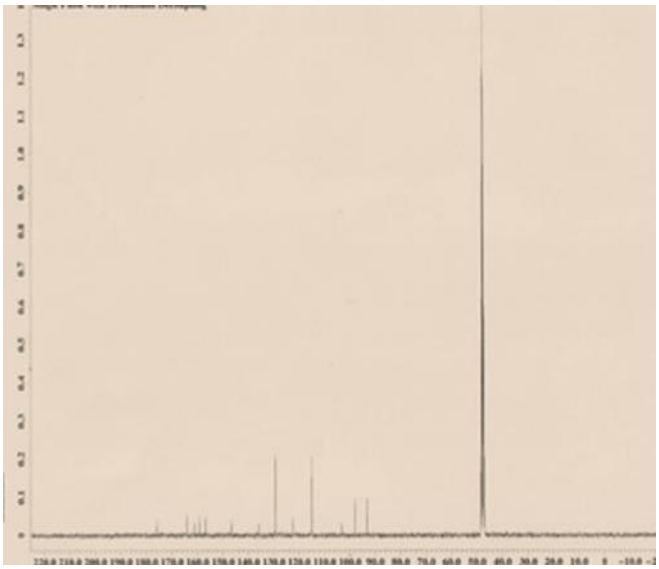


Fig.9: ^{13}C NMR spectrum of compound I

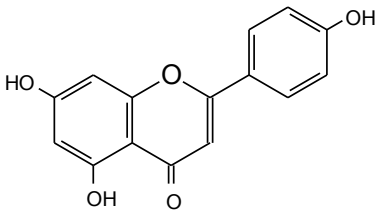
The structure was further supported by the retro Diels –Alder fission depicted in scheme (I) and by its ^{13}C NMR spectrum(Fig.9) which revealed a pattern characteristic of a C_{15} skeleton[37,38] . The chemical shifts (δ_c) are depicted in table (1)

Table 1 : ^{13}CNMR data of compound I

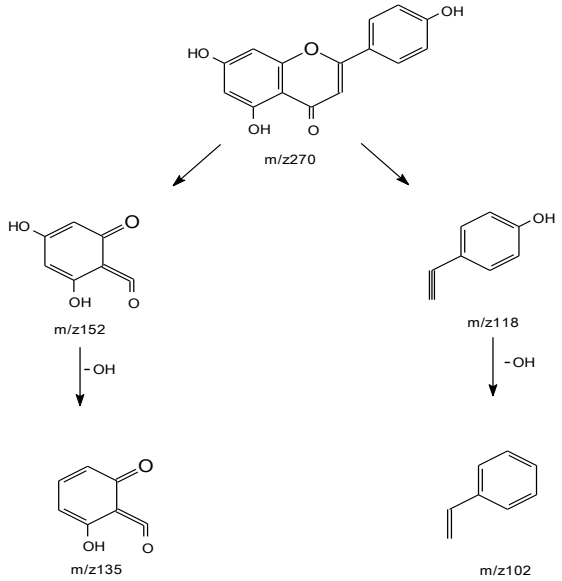
Carbon No.	δ_c (ppm)
2	164
3	136
4	175
5	161
6	99
7	161
8	93
9	159
10	103
1`	122
2`	129
3`	115
4`	159
5`	114
6`	122

The retro Diels-Alder cleavage gave evidence for the proposed structures since the ions. M/z102,118,135,152 which correspond to intact aromatic rings were detected in the electron beam.

The above cumulative data limits the structure of compound I to the following:



The retro Diels-Alder cleavage is shown in scheme (I).



Scheme I : Retro Diels-Alder fission of compound I

REFERENCES

[1] Markham, K.R., "Techniques of Flavonoids Identification" Academic Press, London(1982).

[2] Harborne, J.B. (Ed.) : Phytochemical Methods: A guide to Modern Techniques of Plant Analysis, Chapman and Hall, London(1973)

[3] Nuutila, A.M., Kammiovirta, K.M., Oksman-Caldentey, K.M., *J. Food Chem*. ,**76**, 519(2002).

[4] Argaez, B., Flowers, B.A., Gimenez-Turba, A., Ruiz, G., Waterman, P.G., Pena-Rodriguez, L.M., *J. Phytomed*. **11,** 372**(**2007)

[5] Heijnen ,C.G., Haenen, G.R., van Acker, F.A, van der Vijgh,. W.J., Bast ,A. , *Toxicol. In Vitro.* ,**15(1),**3(2001).

[6] Cao, G., Sofic,E.,Prior,R.L., *Free radical Biol. Med*., **22,**749(1997).

[7] Geleijnse J.,M., Launer, L.J., Van der Kuip, D.A., Hofman ,A, Witteman,. J.C. , *Am. J. Clin. Nutr*. , **75(5),**880(2002)

[8] Hertog ,M.G, Feskens,. E.J., Kromhout, D. , *Lancet* , **349(9053),**699(1997)

[9] Hirvonen ,T., Pietinen P., Virtanen, M., *Epidemiology*,**12(1),**62(2001).
[10] Knek,t P., Kumpulainen, J, Jarvinen,. R., *Am. J. Clin. Nutr*. ,**76(3),**560(2002)
[11] Yochum, L., Kushi ,L.H., Meyer, K., Folsom, A.R. , *Am. J. Epidemiol*., **149(10**),943(1999)
[12] Hertog ,MG.., Sweetnam ,P.M., Fehily ,A.M., Elwood, P.C., Kromhout, D. ,*Am. J. Clin. Nutr*. ,**65(5),**1489(1997)
[13]Rimm, E.B., Katan, M.B., Ascherio, A., Stampfer, M.J., Willett, W.C. , *Ann Intern Med*.,**125(5),**384(1996).
[14]Sesso, H.D., Gaziano ,J.M., Liu ,S., Buring, J.E. , *Am. J. Clin. Nutr*. ,**77(6),**1400(2003).
[15] Mink, P.J., Scrafford ,C.G., Barraj, L.M., *Am. J .Clin. Nutr.*, 85(3),895(2007).

[16]Ng,T.B., Ling,J.M.L. and Wang,Z.T., *J.Gen. Pharmac.* , **27(7),**1237(1996).
[17]Galvez,J.Zarzuelo,A. and Crespo,M.E., *Planta Medica*,**59**,333(1993).
[18]Alzand,K.M.,Ph.D. Thesis, Chemistry Dept.,Faculty of Science, Sudan University of Science and Technology(2011).
[19] M. Abdel Karim, A. A. Abdalla and A. Ali , *OJPS* , **1(4)** , 47 (2008).
[20] Khir –Alla, F.A., M.Sc. Thesis , Chemistry Dept.,Faculty of Science, Sudan University of Science and Technology (2011).

[21] Inas,O.K., Ph.D. Thesis, Chemistry Dept., Faculty of Science,Sudan University of Science and Technology (2010).
[22]Nosaiba,K.H, , M.Sc. Thesis, Chemistry Dept., Faculty of Science,Sudan University of Science and Technology (2011).
[23] Seham,H.B., Ph.D. Thesis, Chemistry Dept., Faculty of Science,Sudan University of Science and Technology(2011).
[24] Lin,Y.M. and Flavin,M.T., *Bioorganic and Medicinal Chemistry*,**10(8),**2795(2002).
[25] Bergendorff ,O. and Sterner,O.,*Planta Medica*,**61**,370(1995).
[26] Xu,Q.,Yuan,K.and Wang,R.,*Pharmacol. Research*,**36**,402(1997).
[27] Gamal,E.B., Mahgoub,S.,Awatif,A.,Wail,S. and Mohamed,M.G. "Medicinal Plants of the Sudan",Omdurman Islamic University Press,Khartoum(1997).
[28] Um Hassan,T.,Ph.D. Thesis , Chemistry Dept.,Faculty of Science, Sudan University of Science and Technology (2011).
[29] Iavarasan,R., Malika,M. and Venkataraman,S.,*Afr.J.Trad.Med*.,**2(1),**70(2005)30-Jehangir,A., Nagi,A.H., Shahzad,M. and Zia,A., *Biomedica*,**20**,25(2010).

[31] Kumar,R.S.,Ponmozhi,M.,Viswanathan,P. and Nalini,N.,*Asia Pacific J. Clin. Nutr*.,**11(2),**157(2002).
[32] Pari,L. and Latha,M.,*Singapore Med. J.*,**43(12),**617(2002).
[33]Arulkumaran,K.,Rajasekaran,A.,Ramasamy,R.,Jegadeesan,M., Kavimani,S. and Somasundaram,A.,*Inter. J. of PharmTech. Res*.,**1(2),**273(2009).
[34] Arya,V.,Yadav,S.,Kumar,S. and Yadav,J.P., *Life Science and Medicine Research* , Published online, April,2010.
[35]Streejith,G.,Latha,P.G.,Shine,V.J.,Anuja,G.I.,Suja,S.R.,Sini,S. and Shyamal,S., *Indian Journal of Experimental Biology*,**48,** 494(2010)

[36] Harborne,J.B.,Mabry,T.J. and Mabry,H." The Flavonoids" Pt.I, Academic Press,New York(1975)
[37] Ito,C.,Muzono, T.,Matsuoka, M., Kimura, Y., Sato, K., kajiura,l., Omura, M., Ju-Ich,M.,Furukawa, H.,*Chemistry Pharmaceutical Bulletin*,3292(1988)
[38] Agrawal,P.K. "Carbon-13 NMR of Flavonoids" Elsevier, Amsterdam(1989).