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ISOLATION, PARTIAL STRUCTURE OF A DIHYDROFLAVONOL FROM SUDANESE *CASSIA TORA* SEEDS AND ANTIMICROBIAL ACTIVITY OF DIFFERENT FRACTIONS

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ABSTRACT

The flavonoids of the medicinally important species *Cassia tora* were investigated. The ethanol extract from seeds was initially purified by column chromatography and then via thin layer chromatography. In this way- a flavonoid was isolated in a chromatographically pure form. Its structure was partially elucidated on the basis of its spectral data (IR, UV, NMR, and MS). Different fractions of *Cassia tora* seeds were assessed for antimicrobial activity against six standard human pathogens .The ethyl acetate fraction showed excellent antibacterial activity against all test organisms and good antifungal activity. The n-butanol fraction exhibited excellent activity against *Escherichia coli* and *Pseudomonas aeruginosa*. Excellent activity against *Bacillus subtilis* was observed for the chloroform fraction. The hexane fraction gave significant activity against *Bacillus subtilis*, but it was inactive against other test organisms.

KEYWORDS: Isolation, Partial Structure, Flavonol, *Cassia tora*.

INTRODUCTION

Flavonoids are a major class of oxygen containing heterocyclic natural products that are wide- spread in the plant kingdom. Flavonoids have a three ring structure with diverse substitution pattern. In these compounds, two aromatic rings are linked via a three carbon bridge. Most flavonoids possess a carbonyl function located at one end of the bridge.[1,2] These phytochemicals play an important role in plant physiology and biochemistry, acting primarily as enzyme inhibitors, antioxidants and plant pigments.[3] Flavonoids act as growth regulators, plant growth hormones beside controlling respiration, photosynthesis, morphogenesis and sex determination. They also have a role in defense against infection.[4]

Flavonoids which are synthesized in plants via a phenyl propanoid pathway[5,6] are widely distributed in plants being found in vegetables, fruits, nuts, seeds, stems, flowers etc. These polyphenols are an integral part of our daily diet and dietary intake of flavonoids is estimated to be 1-2g/day.[7,8] Flavonoids prevent cell damage caused by the reactive oxygen species by various mechanisms including direct scavengng of the radicals.[9.10]

Flavonoids have gained recent interest because of their broad biological and pharmacological activities. Flavonoids have been reported to exert multiple biological effects including antimicrobial,[11] cytotoxicity,[12] anti-inflammatory[13] as well as antitumor activities.[14,15] Flavanones exhibit anti-oxidant, immunomodulatory and chemopreventive properties.[16] Flavanones with a hydroxyl functions at $C_{4'}$ and C_6 have shown significant cytotoxic and apoptotic effects against tumor cells, compared with other structurally related flavanones.[17] The hydroxylation at C_6 plays an important role in antioxidant activity of flavanones.[17]

Cassia tora belongs to family Leguminoseae .The genus *Tora* is an annual wild under shrub reaching up to 30- 90 cm in height.[18,19] In eastern Sudan it is known as "Sorib". It is widely distributed in Africa, America and Asia.[20] Leaves have high nutritive value.[21] The plant is rich in calcium and iron and is an excellent source of vitamin B. It also contains high levels of phosphorous, riboflavin and ascorbic acid.[21,22] In north Cameron *Cassia tora* leaves are sun dried prior to eating.[23] After fermentation, it is used in western and southern Sudan as a meat replacer.

In Sudanese system of medicine, fresh leaves of *Cassia tora* are used as laxative and antidiabetic while roots are used as diuretic and for treatment of insect bites. Root and leaves are also used to cure skin diseases.[24] Seed tea is drunk for headache, intestinal complains and fatigue.[25] However, some toxic constituents were reported from green leaves and the stems. In Chinese ethnomedicine the plant is claimed to be anticholesterolemic and hypotensive.

In continuation of our interest in plants used in Sudanese ethnomedicine, where information concerning their bioactive constituents is very scarce, we decided to investigate the constituents of the medicinally important species *Cassia tora.*

MATERIAL AND METHODS

Materials

Plant material

Cassia tora seeds were purchased from local market-Omdurman-Sudan. The plant was authenticated by The Institute of Aromatic and Medicinal Plants-Khartoum.

Instuments

UV spectra were run on a Shimadzu 2401PC UV-Visible Spectrophotometer. ^{1}HNMR spectra were measured on a Joel ECA 500MHZ NMR Spectrophotometer. Mass spectra were run on a Joel Mass Spectrometer (JMS- AX500).

Test organisms

Cassia tora fixed oil was screened for antimicrobial activity using the standard microorganisms shown below:

Table 1: Test microorganisms.

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+ve
2	*Staphylococcus aureus*	G+ve
3	*Pseudomonas aeroginosa*	G-ve
4	*Escherichia coli*	G-ve
5	*Aspergillusniger*	fungus
6	*Candida albicans*	fungus

Methods

Extraction of flavonoids

Powdered seeds of Cassia tora (1Kg) were macerated with 5 L ethanol (95%) for 72 hr at room temperature with occasional shaking, and then filtered off. The extraction process was repeated two more times with the same solvent. Combined filtrates were concentrated until all ethanol was evaporated to dryness yielding a crude products.

Isolation of flavonoids

A silica gel(60-120 mesh) column(80x4cm) was used to purify the ethanol crude extract. Initially the column was eluted with hexane, and then successively with hexane-ethyl acetate: 2:1; 1:1(v:v), and finally 100% ethyl acetate. The ethyl acetate fraction was rich in phenolics. It was further purified by (TLC) using hexane: ethyl acetate: acetone (62:28:10, v:v:v) .The chromatograms were viewed under UV light using both short and long wavelengths. The chromatogram with R_f = 0.33 gave a chromatographically pure component which gave a positive test with vanillin/H_2SO_4 which is a specific qualitative test for flavonoids. The compound also reacted positively with ferric chloride. The structure of this isolate was partially elucidated on the basis of its spectral data **(**IR, UV, ^{1}HNMR, MS).

Antimicrobial assay

Preparation of microbial suspensions

Mueller Hinton and Sabouraud dextrose agars were the media used as the growth media for the bacteria and the fungi respectively. The media was prepared according to the manufacturer instructions: one ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37°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 10^8-10^9colony forming units per ml. The suspension was stored in the refrigerator at 4°C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique.

Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) 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°C for 24 hours.

Fungal cultures were maintained on Sabouraud dextrose agar incubated at 25°C for four days. The fungal growth was harvested and washed off 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 modifications, to assess the antibacterial activity of samples. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle. Each of these plates was divided into two halves. Two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for a sample. Separate Petri dishes were designed for standard antimicrobial chemotherapeutics.

The agar discs were removed, alternate cup were filled with 0.1 ml samples and allowed to diffuse at room

temperature for two hours. The plates were then incubated in the upright position at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

RESULTS AND DISCUSSION

Compound I

The IR spectrum of the compound I (Fig.1) showed: v (KBr) 673,703, 796 (C-H, Ar. bending); 1108 (C-O); 1388, 1463, 1514 (C=C Ar.); 1625 (C=O); 2856, 2927(C-H aliph.) and 3406cm^{-1} (OH).

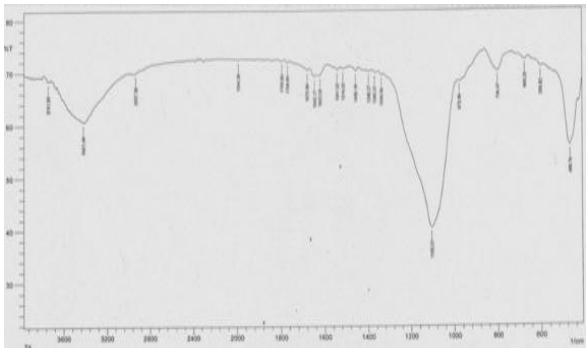

Fig. 1: IR spectrum of compound I.

The UV spectrum Fig.2 gave λ_{max}231, 282 nm which is a characteristic absorption of dihydroflavonols, isoflavones and flavanones.[1,2] No shoulder characteristic of isoflavones was detected in the UV spectrum in the range: 300-340nm. Also the ^{1}HNMR spectrum did not reveal a pair of multipletes at δ2.80ppm and 5.20ppm characteristic of the uneqivalent protons of the methylene function at C_3 and methine proton at C_2 of the flavanone. Compound I is thus a dihydroflavonol and the sodium methoxide spectrum (Fig. 3) revealed a 17nm bathochromic shift with decrease in intensity indicating the 3-OH function of dihydroflavonols.[1,2] A bathochromic shift diagnostic of a 7-OH function was detected in the sodium acetate spectrum (Fig. 4).The aluminum chloride spectrum (Fig.5) showed a bathochromic shift diagnostic of a 3--OH function.[1,2] The aluminium chloride spectrum was quite stable following acidification with HCl.

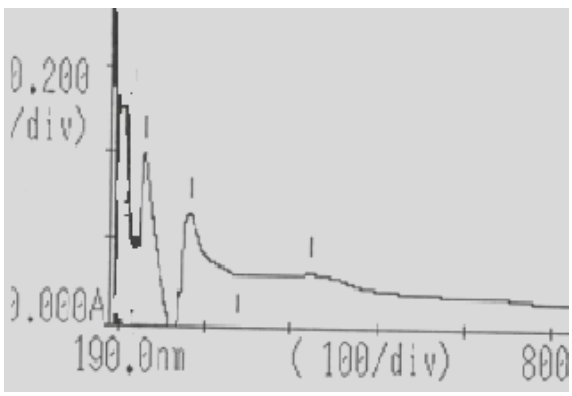

Fig. 2: UV spectrum of compound I.

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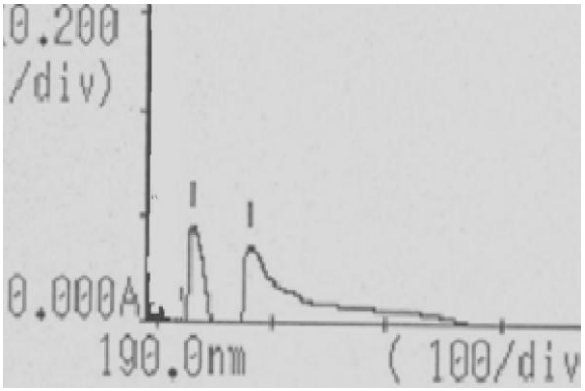


Fig. 3: Sodium methoxide spectrum of compound I.

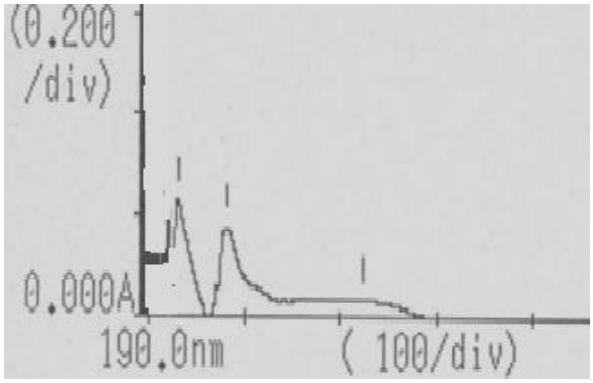


Fig. 4: Sodium acetate spectrum of compound I.

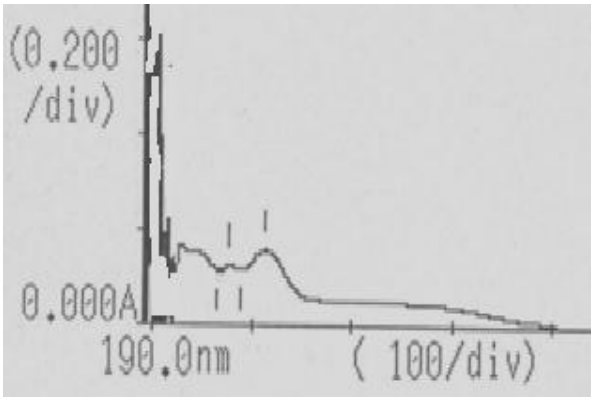
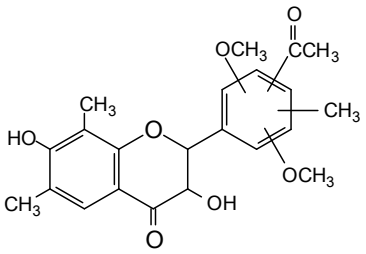


Fig. 5: Aluminium chloride spectrum of compound I.

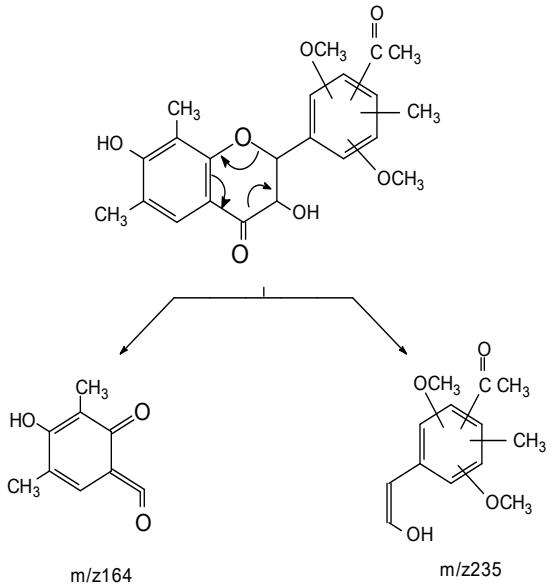
^{1}HNMR spectrum (Fig.6) showed δ 1.23(9H) due to three methyl groups. Two methyls were assigned to C_6 and C_8 of aromatic ring (A). This is supported by the absence of signals of C_6 and C_8 protons in the ^{1}HNMR spectrum (Fig.6). The retro Diels-Alder fission[2] also lends evidence for such substitution pattern .The signal at δ 2.23 (3H) was assigned for an aromatic acetyl group, while the resonances at 3.70(3H) and δ3.83(3H) account for two methoxyl functions.[1,2] The C_5 proton resonated well downfield at δ8.01ppm due to the electron-withdrawal effect of the 4-keto function. The B ring proton resonated at δ6.7 ppm. (The signals at δ2.50 and δ3.30 ppm are due to DMSO residual protons and DMSO residual water respectively). The mass spectrum (Fig. 7) gave m/z 398 for (M^+ - 2H).

Comparison of the above cumulative data with literature data, gave the following partial structure for compound I.



I

The retro Diels – Alder fission (Scheme I) lends additional evidence in favor of the substitution pattern of the A and B aromatic rings. A future 2D NMR (^{1}H- ^{1}H COSY NMR, HSQC and HMBC) would suggest a full structural elucidation.



Scheme I: Retro Diels-Alder fission of compound I.

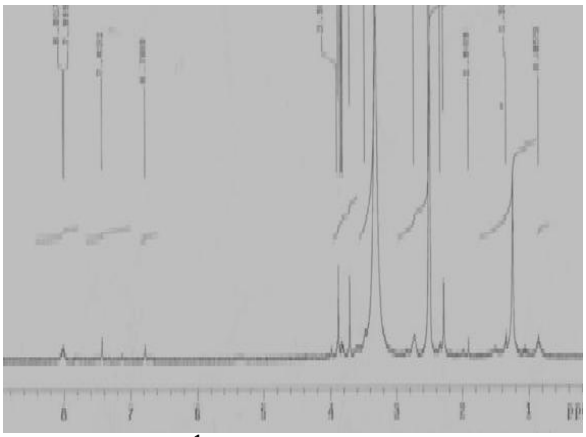


Fig. 6: ^{1}H NMR of compound I.

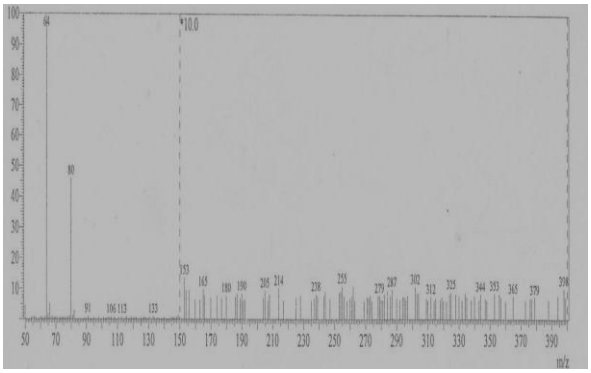


Fig. 7: Mass spectrum of compound I.

Antimicrobial activity

Different fractions of *Cassia tora* were screened for antimicrobial activity against six standard bacterial strains. The results are displayed in Table (2) .Results were interpreted in conventional terms: (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active). Tables (3) and (4) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 2: Antibacterial activity of *Cassia tora* fractions.

Fraction	Conc. (mg/ml)	Ec	Ps	Sa	Bs	Ca	An
Hexane	100	-	-	-	19	-	-
Chloroform	100	17	16	16	20	-	-
Ethyl acetate	100	20	21	22	20	15	15
n-Butanol	100	20	21	15	13	15	17
Aqueous	100	15	18	-	12	-	-

Table 3: Antibacterial activity of standard chemotherapeutic agents.

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 4: Antifungal activity of 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*
Pa.: *Pseudomonas aeruginosa*
An.: *Aspergillus niger*
Ca.: *Candida albicans*
Bs.: Bacillus subtilis

The ethyl acetate fraction showed excellent antibacterial activity against all test organisms and good antifungal activity. The n-butanol fraction exhibited excellent activity against *Escherichia coli* and *Pseudomonas aeruginosa*.Excellent activity against *Bacillus subtilis* was observed for the chloroform fraction. The hexane fraction gave significant activity against *Bacillus subtilis*, but it was inactive against other test organisms

REFERENCES

1. Harborne, J. B. "Phytochernical Methods", Chapman and Hall Ltd, London, 2001.
2. Mabry, T., Markham, K. R., and Thomas, M. B., "The Systematic Identification of Flavonoids" Springer-Verlag, New York, 1970.
3. MC Clure J.W., Physiology of flavonoids in Plants flavonoid in Biology and medicine: Biochemical Pharmacology, and structure activity relationship, New York, 1986; 77-85.
4. Smith D.A., and Banks S.W., Formation and Biological properties of isoflavonoid phyto alexins, in plant flavonoids in biological and medicine, biochemical pharmacological and structure activity relationship, New York, 1986; 113-124.
5. Mahomoodally, M.F., Gurib. F.A., and Subratty, A.H., pharmaceutical Biology 43, 237- 242 (2005). Pandey, A.K., National Academy science letters, 2007; 30: 383-386.
6. Halliwell B., "How to charectrize an antioxidant biochemical society symposium", 1995; 61: 73-101.
7. Peterons J., Dwyer M.S.J., flavonoids dietary occurrence and biochemical activity, *Nutrition Research*, 1998; 18: 1995-2018.
8. Tsuchiya H., Structure dependent membrane interaction of flavonoids associated with their bioactivity, *food chemistry*, 2010; 120: 1089-1096.
9. Van Acker S.A., TrompM.N., Haenen G.R., Vander V.W.J., and Bast A., "Flavonoids as scavenger of nitric oxide radical. Biophysical research communication", 1995; 214: 755-759.
10. Zafar R., "Medicinal plant for India" CBS publisher and distribution, New Delhi, 1st ed, 1994; 37-42.
11. Yenjai, C., Prasanphen, K., Daodee, S., Wongpanich, V., Kittakoop, P., *Fitoterapia*, 2004; 31: 89.
12. Furuta, T., Kimura, T., Kondo, S., Wakimoto, T., Nukaya, H., Tsuji, K., And Tanaka, K., *Tetrahedron*, 2004; 11: 9375.
13. Xia, Y., Yang, Z.Y., Xia, P., Bastow, K.F., Nakanishi, Y., and Lee, K.H., *Bioorganic and Medicinal Chemistry Letters*, 2000; 30: 699.
14. Aura, A.M., Martin-Lopez, P., O'Leary, K.A., Williamson, G., Oksman-Caldentey, K.M., Poutanen, K., Santos-Buelga, C., *European journal of nutrition*, 2005; 3: 133.
15. Graf, B.A., Milbury, P.E., Blumberg, J.B., *J. Med. Food*, 2005; 8: 281.
16. Harborne, J. B. "Phytochernical Methods", Chapman and Hall Ltd, London, 2001; 111.
17. Ko, C.H., Shen, S., Chen, Y., *Free Radic. Biol. Med.*, 2004; 36: 897.
18. Nadlkarni A.K., "Indian Materia Medica", popular prakashan Pvt ltd, 3rd ed, 1982; 1: 634-635.
19. Dirar. The indigenous fermented food of the sudan, CAB international, Cambridge UK, 1993.
20. Dirar H.A., Kawal, meat substitute from fermented cassia obtusifolia leaves, *Economic Botany*, 1984; 38: 342-349.
21. Tawel M.M.A., Effect of possessing on in vitro protein digestibility and anti nutritional factor of chickpea, M.SC U of K, Sudan, 2003.
22. Ousman A., Ngassoum M., and Kange, Chemical composition of cassia obtusifolia leaves, *food technology*, 2005.
23. Anon., Medical and aromatic plant of the sudan, Kartoum trading and project company, kartoum, sudan, 1982.
24. Tubiana, The Zaghawa from an ecological perspective, A.A.A., 1977.
25. Anon. Poison plant of southern U.S. the cooperative Extension Service. U of Georgia College of agriculture, 1980.