International Journal of Research in Pharmacy and Pharmaceutical Sciences
ISSN: 2455-698X; Impact Factor: RJIF 5.22
Received: 26-11-2018; Accepted: 29-12-2018
www.pharmacyjournal.in
Volume 4; Issue 1; January 2019; Page No. 32-34

Isolation, Partial characterization and antimicrobial activity of a Dihydrochalcone From Sudanese *Bauhinia rufescens* Lam. (Fabaceae) Leaves

Abdel Karim M[1*], Haga S[2], Salah H[3], El-Hafez M[4]
[1, 2] Sudan University of Science and Technology, Faculty of Science, Sudan
[3] Western Kordofan University, Faculty of Education, Sudan
[4] Department of Chemistry, King Khalid University, Faculty of Science and Arts, Saudi Arabia

Abstract
Bauhinia species are trees up to 6-12m in height. Traditionally, leaves and stem barks have been used against pain, inflammation, dysentery, mycosis, diarrhea and diabetes. *Bauhinia rufescens* Lam. is a tropical forage up to 8 m in height. In African system of medicine, the plant has been used against gout, leprosy, and malaria.
This study was designed to investigate the major flavonoid of *Bauhinia rufescens* roots and to assess the antimicrobial activity of the crude ethanolic extract and the isolated flavonoid. The flavonoids were extracted with ethanol and the crude extract was purified by paper chromatography where a pure flavonoid (compound I) was isolated. The structure of this compound has been partially characterized by some spectral tools (UV, IR and ^{1}HNMR). In the antimicrobial assay, the crude ethanolic extract showed better inhibitory effect against a panel of human pathogens compared to compound I. While the crude extract showed significant antibacterial and antifungal properties, compound I exhibited moderate antibacterial activity and weak antifungal properties.

Keywords: *Bauhinia rufescens*, flavonoids, isolation, antimicrobial activity

Introduction
The genus *Bauhinia* (Fabaceae) is widely distributed in tropics. Bauhinia species are trees up to 6-12m in height [1].Traditionally, leaves and stem barks have been used against pain, inflammation, dysentery, mycosis, diarrhea and diabetes [2-6]. The antimicrobial activity of *Bauhinia variegate* has also been documented [7]. Also it has been reported that *Bauhinia racemosa* exhibited significant antimicrobial activity against a panel of human pathogens [8]. *Bauhinia manca* has shown significant inhibitory effect on phytopathogenic fungi on *Botrytis cinerea* and other species [6]. *Bauhinia rufescens* Lam. is a tropical forage up to 8 m in height [1]. In African system of medicine, the plant has been used against gout, leprosy, and malaria [2-4]. The plant is also used traditionally as remedy for dysentery, fibrosis, jaundice [9], diarrhea [10], mycosis [11] and gingivitis [12]. The inhibitory effect of stem bark and leaves extracts against lipoxyigenase and xanthine oxidase enzymes has been demonstrated [2]. *Bauhinia rufescens* contains tannins, sterols, saponins, triterpenes beside some phenolics [13, 14].

Materials and Methods
Plant Material
The roots of *Bauhinia rufescens* were collected from a forest reserve around Damazin, Sudan. The plant was authenticated by direct comparison with a herbarium sample.

Solvents
Analytical grade solvents were used. Methanol -HPLC grade- was used for spectroscopic purposes (Loba, India).

Equipments
Ultraviolet spectra were recorded in spectroscopic methanol on a Shimadzu UV -Visible Spectrophotometer.^{1}HNMR spectra were measured on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO-d_6.

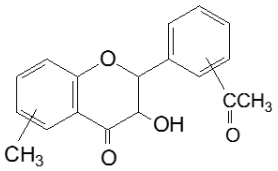
Methods
Isolation of flavonoids
Powdered shade – dried roots of *Bauhinia rufescens* (1Kg) were extracted with 95% ethanol for 48 hours. The solvent was evaporated to dryness under reduced pressure. The crude extract was applied on Whatman No. 3mm papers as narrow zones. The bands were developed with BAW(4:1:5;V:V:V). The developed chromatograms were air-dried and examined under UV light (λ_{max} 366nm).. The equivalent bands from each paper were combined, cut into small pieces and slurred with methanol. The solvent was evaporated to give a yellow powder -compound I.UV shift reagents (sodium methoxide, sodium acetate and aluminium chloride) were used as follows: the UV spectrum of compound I, in methanol, was first recorded; 3 drops of sodium methoxide reagent were added to the sample and the sodium methoxide spectrum was recorded ; 6 drops of $AlCl_3$ reagent were added to the fresh sample and the $AlCl_3$ spectrum was recorded ; powdered sodium acetate was then added to the fresh sample, the mixture was shaken and the sodium acetate spectrum was recorded.

Results and Discussion
Compound I was isolated as yellow powder from roots of *Bauhinia rufescens* using paper chromatography. The UV spectrum of compound I gave (Fig.1) λ_{max}(MeOH) 261 nm. The appearance of only one band- band II suggests: (i)a flavanone, (ii) an isoflavone, (ii)a dihydoflavanol or (iv) a dihydrochalcone. Isoflavones usually reveal a shoulder in the range 300-340nm.Such shoulder was not detected in the UV spectrum of compound I(Fig.1).Also the sodium methoxide spectrum(Fig.2) did not show any bathochromic shift

indicating absence of 4`- and 3-hydroxyl groups(the 3-OH is a characteristic feature of dihydroflavonols). Flavanones are characterized by a double multiplets around 2.80 and 5.20ppm.These multipletes which appear due to mutual splitting of C_2 –H and the magnetically unequivalent C_2 protons were not detected in the ^{1}HNMR spectrum of compound I. The above argument suggests that compound I is a dihydrochalcone. The sodium acetate spectrum (Fig.3) did not reveal any bathochromic shift suggesting absence of a 7-hydroxylation pattern. Also the aluminium chloride spectrum (Fig.4) failed to show a bathochromic shift indicating absence of 3-, 5-OH functions as well as catechol systems.

The ^{1}HNMR spectrum (Fig.5) gave δ(ppm): 1.35(assigned for a methyl group); 1.78(acetyl group) : 8.45(aromatic protons). Signals at δ2.50 and δ3.40 are due to solvent residual protons and residual water respectively.

On the basis of its spectral data, the following partial structure was suggested for compound I:



Compound I

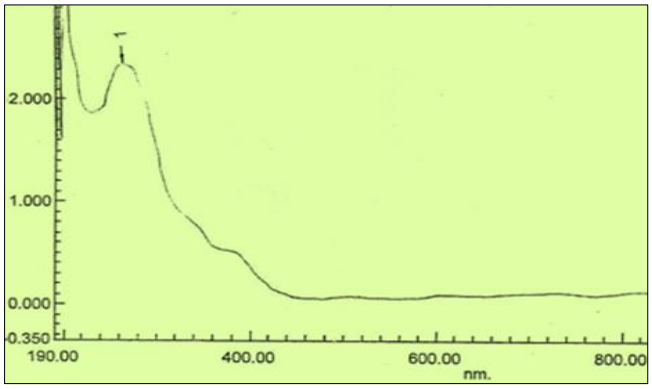


Fig 1: UV spectrum of compound I

Antimicrobial assay

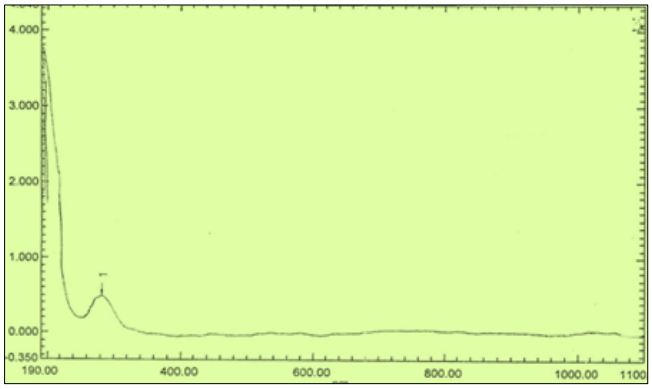


Fig 2: Sodium methoxide spectrum of compound I

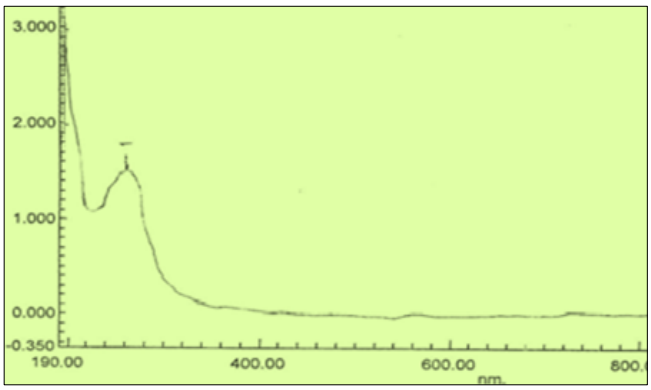


Fig 3: Sodium acetate spectrum of compound I

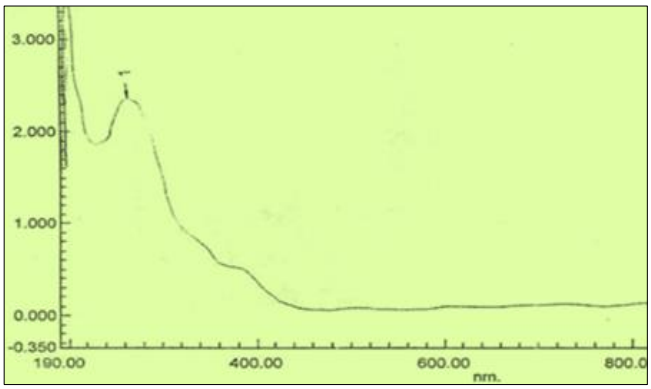


Fig 4: Aluminium chloride spectrum of compound I

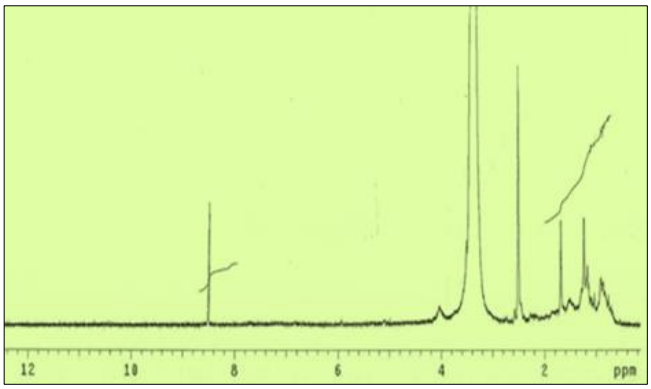


Fig 5: 1HNMR spectrum of compound I

Both of the crude ethanolic extract of *Bauhinia rufescens* and chromatographically pure flavonoid isolated from this species were assessed for their antimicrobial activity against six standard human pathogens. Ampicilin, gentamycin and clotrimazole were used as positive controls.

The diameters of inhibition zones are listed in Table 1. The crude extract showed better inhibitory effect compared to compound I. While the crude extract showed significant antibacterial and antifungal properties, compound I exhibited moderate antibacterial activity and weak antifungal properties.

Table 1: Diameters of inhibition zones(mm)

Organism	Inhibition growth zone diameter at 100 mg/ml	
	Crude extract	Comp. I
Bacillus subtitles	19	15
Staphylococcus aureus	20	16
Escherichia coli	22	15
Pseudomonas aeruginosa	20	15
Aspergillus niger	18	12
Condida albacans	18	14

***** *Activity:*
10 mm – 13 mm → weak.
14 mm – 18 mm → medium.
18 mm - over → high.

References

1. Balogun RO, Jones RJ, Holmes JHG. Digestibility of some tropical browse species varying in tannin content. Anim Feed Sci Tech. 1998; 76:77-88.
2. Compaoré M, Lamien CE, Lamien-Meda A, Vlase L, Kiendrebeogo M, Ionescu C, *et al*. Antioxidant, xanthine oxidase and lipoxygenase inhibitory activities and phenolics of *Bauhinia rufescens* Lam. (Caesalpiniaceae). Nat Prod Res. 2011; 26:1069-74.
3. Tapsoba H, Deschamps JP. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. J Ethnopharmacol. 2006; 104:68-78.
4. Maillard MP, Recio-Iglesias MC, Saadou M, Stoeckli-Evans H, Hostettmann K. Novel antifungal tetracyclic compounds from *Bauhinia rufescens* Lam. Helv Chim Acta. 1991; 74:791-9.
5. Silva KLD, Filho VC. Plants of the genus Bauhinia: chemical composition and pharmacological potential. Química Nova. 2002; 25:449-454.
6. Cechinel Filho V. Chemical composition and biological potential of plants from the genus Bauhinia. Phytother Res. 2009; 23:1347-1354.
7. Parekh J, Karathia N, Chanda S. Evaluation of antibacterial activity and phytochemical analysis of Bauhinia variegata L. bark. Afr J Biomed Res. 2006; 9:53-56.
8. Kumar RS, Sivakumar T, Sunderam RS, Gupta M, Mazumdar UK, Gomathi P, *et al*. Antioxidant and antimicrobial activities of Bauhinia racemosa L. stem bark. Braz J Med Biol Res. 2005; 38:1015-1024.
9. Abubakar MS, Musa AM, Ahmed A, Hussaini IM. The perception and practice of traditional medicine in the treatment of cancers and inflammations by the Hausa and Fulani tribes of Northern Nigeria. J Ethnopharmacol. 2007; 111(3):625-629.
10. Adamu HM, Abayeh OJ, Agho MO, Abdullahi AL, Uba A, Dukku HU, *et al*. An ethnobotanical survey of Bauchi state herbal plants and their antimicrobial activity. J Ethnopharmacol. 2005; 99(1):1-4.
11. Inngjerdingen K, Nergard CS, Diallo D, Mounkoro PP, Paulsen BS. An ethnopharmacological survey of plants used for wound healing in Dogonland, Mali, West Africa. J Ethnopharmacol. 2004; 92(2-3):233-244.
12. Tapsoba H, Deschamps JP. Use of medicinal plants for the treatment of oral diseases in Burkina Faso. J Ethnopharmacol. 2006; 104(1-2):68-78.
13. Aliyu AB, Ibrahim MA, Musa AM, Ibrahim H, Abdulkadir IE, Oyewale AO. Evaluation of antioxidant activity of leave extract of *Bauhinia rufescens* Lam. (Caesalpiniaceae). J Med Plant Res. 2009; 3(8):563-567.
14. Compaoré M, Lamien CE, Lamien-Meda A, Vlase L, Kiendrebeogo M, Ionescu C, *et al*. Antioxidant, xanthine oxidase and lipoxygenase inhibitory activities and phenolics of *Bauhinia rufescens* Lam. (Caesalpiniaceae). Nat Prod Res. 2011; 26(11):1069-1074.