



ISOLATION, PARTIAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF A FLAVONOL FROM SUDANESE *INDIGOFERA OBLONGIFOLIA* (FABACEAE)

Prof. Abdel Karim M.^{1*}, Ibrahim H.¹, Salah El-Deen H.² and El-Hafez M.³

¹Sudan University of Science and Technology, Faculty of Science.

²Western Kordofan University, Faculty of Education.

³University of Gezira, Faculty of Education.

*Corresponding Author: Prof. Abdel Karim M.

Sudan University of Science and Technology, Faculty of Science.

Article Received on 07/08/2018

Article Revised on 28/08/2018

Article Accepted on 18/09/2018

ABSTRACT

Various *Indogifera* species have been investigated. *Indigofera oblongifolia* possesses, beside others, hepatoprotective, antimicrobial and lipoxygenase inhibition properties. In this study the heartwood of *Indigofera oblongifolia* was extracted with ethanol and the extract was purified by paper chromatography which gave a pure flavonol. The structure of this flavonoid was partially elucidated via some spectral tools (UV and ¹HNMR). The antimicrobial activity of the isolated flavonol was assessed via cup plate agar diffusion bioassay. Compound I showed good activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. It also showed weak activity against *Aspergillus niger* and weak anticandidal potency. The compound also exhibited partial activity against *Staphylococcus aureus*. The antioxidant properties of compound I has been studied and excellent free radical scavenging capacity has been detected.

KEYWORDS: *Indigofera oblongifolia*, Isolation ,Flavonol , Antimicrobial Activity, *aeruginosa*, *subtilis*.

INTRODUCTION

The role of plant kingdom in maintenance of life in our planet is well known. Different communities rely on herbal medicine in primary health care and medicinal plants are used extensively in pharmaceutical industries. Nowadays around 20% of modern drugs comes from plants.

The genus *Indigofera* belongs to the family Fabaceae, a family of about 650 genera and around 18000 species. Various *Indogifera* species have been investigated. It has been shown that *Indigofera oblongifolia* possesses hepatoprotective,^[1] antimicrobial^[2] and lipoxygenase inhibition properties.^[3] The snake –venom neutralizing bustle has been reported from *Indigofera pulehra*.^[4] Also it has been reported that *Indigofera tinctoria* has anti-dyslipidemic and antioxidant activities.^[5,6]

The antidiabetic properties of *Indigofera mysorens* has been demonstrated.^[7] This plant is also used traditionally against cough and hepatitis.^[3] The plant is claimed to be tonic and has antispasmodic effect.^[8] Roots are chewed for lethargy and toothache,^[9] while the plant extract inhibited the development of hypoglycemia in experimental models.^[10] Shoot extract showed antiinflammatory action and leaves exhibited antibacterial activity.^[8,11]

MATERIALS AND METHODS

Materials

Solvents

Analytical grade solvents (Loba Chemicals-India) were used. HPLC grade methanol was used for spectroscopic measurements (Sigma-Aldrich, England).

Plant material

Indigofera oblongifolia roots were collected from a forest reserve around Gezira state (Sudan). The plant was identified and authenticated by direct comparison with a herbarium sample.

Equipments

Ultraviolet absorption spectra were obtained in spectroscopic methanol on UV -Visible Spectrophotometer(Shimadzu). A Hanovia multiband UV lamp - λ_{max} 254 / 365 nm was used to visualize chromatograms in paper chromatography. ¹HNMR spectra were obtained on a Bruker AM 500 spectrophotometer (Germany) operating at 500 MHz in spectroscopic grade DMSO-d₆. The chemical shifts values were expressed in δ (ppm) units using (TMS) as an internal standard.

Test microorganisms

When assessing the antimicrobial activity, the standard microorganisms depicted in Table 1 were used:

Table 1: Test microorganisms.

Ser. No	Micro organism	Type
1	<i>Bacillus subtilis</i>	G+ve
2	<i>Staphylococcus aureus</i>	G+ve
3	<i>Pseudomonas aeruginosa</i>	G-ve
4	<i>Escherichia coli</i>	G-ve
5	<i>Candida albicans</i>	fungi
6	<i>Aspergillus niger</i>	fungi

Methods

Extraction and isolation of flavonoids

Powdered shade-dried *Indigofera oblongifolia* roots (1Kg) were macerated with 95% ethanol for 72 hours at room temperature. The extract was evaporated to dryness *in vacuo*.

The ethanolic crude extract was applied on paper sheets (Whatman No. 3) as narrow strips. The bands were irrigated with BAW(5:1:4;V:V:V). The developed chromatograms were air-dried and examined under both visible and UV light (λ_{max} 366, 245nm). . The equivalent bands from each paper were then cut into small pieces, combined and slurred with methanol. After several hours of contact, with occasional shaking, the liquid was evaporated *in vacuo* to dryness. In this way compound I(R_f 0. 50) was isolated in pure form. Purity was checked by TLC experiments in different solvent systems.

Antimicrobial test

Compound I was screened for antimicrobial activity against six standard human pathogens: Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and the fungal species: *Candida albicans* and *Aspergillus niger*. The cup plate agar diffusion bioassay was used.

Preparation of microbial suspensions

Aliquots(1ml) of a 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 100 ml sterile normal saline solution to produce a suspension containing about 10^8 - 10^9 C. F. U/ ml. The suspension was stored in the refrigerator at 4° C till used. The average number of viable organisms 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 solution and 0. 02 ml volumes of the appropriate dilution were transferred by micro pipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drops to dry and then incubated at 37 °C for 24

hours. After incubation, the number of developed colonies in each drop was counted. The average number of colonies per drop (0. 02 ml) was multiplied by 50 and by the dilution factor to give the viable count of the stock suspension, expressed as the number of colony forming units per ml suspension.

Each time a fresh stock suspension was prepared. All the above experimental conditions were maintained constant so that suspensions with very close viable counts would be obtained.

Fungal cultures were maintained on dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100ml of sterile normal saline and the suspension was stored in the refrigerator until used.

Testing of antimicrobial susceptibility

The cup plate agar diffusion bioassay was used to assess the antimicrobial activity of the sample and performed by using Mueller Hinton agar (MHA).

Standardized bacterial stock suspension(2ml) was mixed with (200 ml) of sterile molten nutrient agar which was maintained at 45°C. (20 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes. The agar was left to settle. Each plate was divided into two halves. In each half two cups (10mm in diameter) were cut using sterile cork borer (No 4). Each half was designed for a test solution.

Agar discs were removed, alternate cups were filled with (0. 1 ml) samples of each test solution and allowed to diffuse at room temperature for two hours. The plates were then incubated at 37°C for 24 hours. After incubation, the diameters of the resultant growth inhibition zones were measured in duplicates and averaged.

The above mentioned method was adopted for antifungal activity, but instead of agar, dextrose agar was used. Samples were used here by the same concentrations used above.

RESULTS AND DISCUSSION

Identification of compound I

Compound I absorbs (Fig. 1) in the UV at λ_{max} 284, 362nm. Such absorption is characteristic of flavonols which are distinguished from flavones by band I (arising from cinnamoyl chromophore).

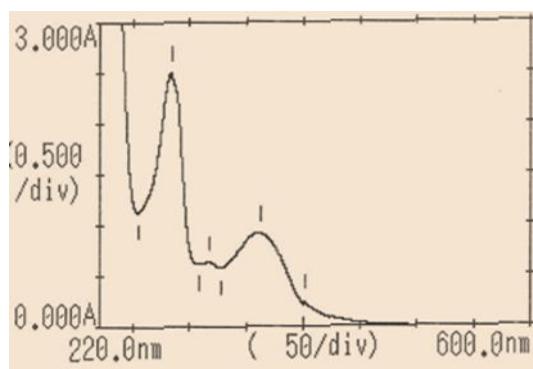


Fig. 1: UV spectrum of compound I.

The hydroxylation pattern of this flavonol was investigated by using different UV shift reagents, namely, sodium methoxide, sodium acetate, aluminium chloride and boric acid.

The UV shift reagent-sodium methoxide revealed a bathochromic shift diagnostic of 3-OH function. Bathochromic shift with decrease in intensity was observed (Fig. 2).

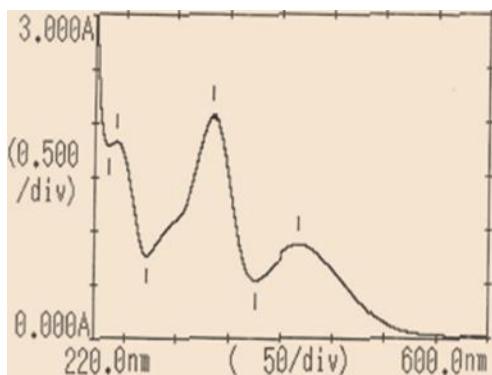


Fig. 2: Sodium methoxide spectrum of compound I.

The sodium acetate spectrum (Fig. 3) did not afford any bathochromic shift indicating absence of a 7-OH function (Fig. 3).

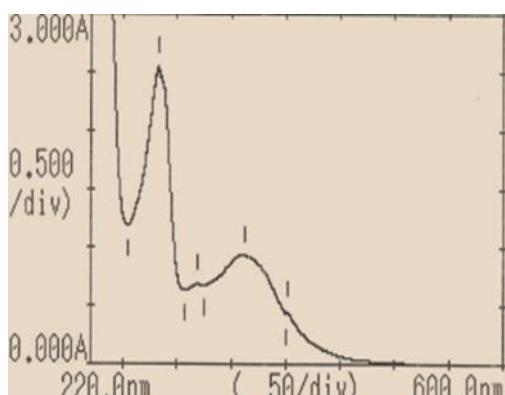


Fig. 3: Sodium acetate spectrum of compound I.

Aluminium chloride is another useful shift reagent. It is diagnostic of 3- and 5-OH functions as well as catechol systems (catechol moieties are also detectable by the shift reagent-boric acid). In all cases this reagent

produces a bathochromic shift but with decrease in intensity in case of a 3-OH function. The 3- and 5-hydroxylation patterns are distinguishable from catechol systems by their aluminium chloride spectrum which is stable in acidic media unlike the catechol spectrum.

The aluminium chloride spectrum revealed a bathochromic shift indicative of a 5-OH function (The spectrum is stable in acidic media) (Fig. 4). The boric acid spectrum did not reveal any bathochromic shift indicating absence of catechol systems (Fig. 5).

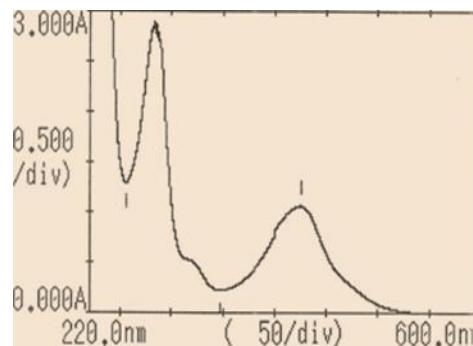


Fig. 4: Aluminium chloride spectrum of compound I.

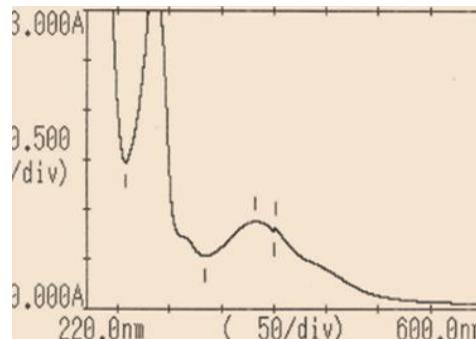


Fig. 5: Boric acid spectrum of compound I.

The ^1H NMR spectrum (Fig. 6) showed a signal at δ 1.20 assigned for a methyl group. The resonance at δ 2.10 accounts for an acetyl function. The aromatic protons resonated at δ 7.65 and δ 8.25 ppm. The resonances at δ 2.50 and δ 3.34 ppm are due to the solvent (DMSO-d_6) residual protons and residual water respectively.

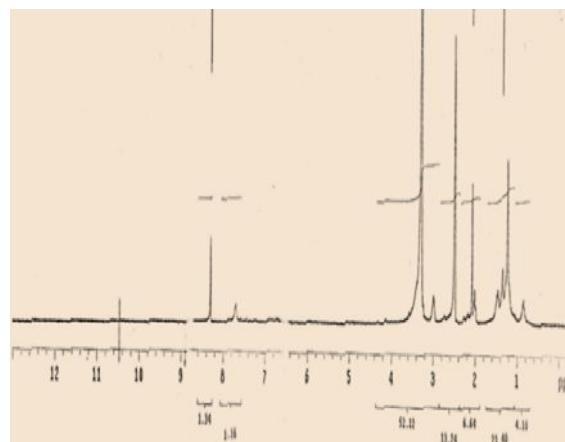
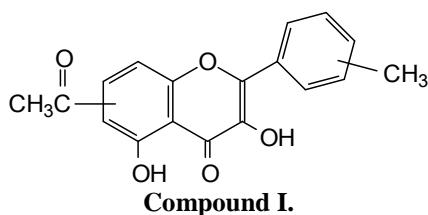


Fig. 6: ^1H NMR spectrum of compound I.

On the basis of the above cumulative spectral data, the following partial structure was proposed for compound I:



Antimicrobial assay

Compound I has been screened for antimicrobial activity against six human pathogens. The results are depicted in Table 2, Tables 3 and 4 display the antibacterial and antifungal activity of standard drugs respectively.

Table 2: Antimicrobial activity of compound I.

	Ec	Ps	Sa	Bs	Ca	An
Compound I	15	15	11	15	13	13

Ec = *Escherichia coli*

Pa = *Pseudomonas aeruginosa*

Sa = *Staphylococcus aureus*

Bs = *Bacillus subtilis*

Ca = *Candida albicans*

An = *Aspergillus Niger*

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

Compound I showed good activity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. It also showed weak activity against *Aspergillus niger* and weak anticandidal potency. The compound also exhibited partial activity against *Staphylococcus aureus*.

Antioxidant assay

In vitro antioxidant assay for compound I was conducted. Evaluation of the antioxidant activity was carried out by measuring the capacity of the test sample against stable DPPH radical. The change in colour is measured spectrophotometrically at 516nm. As depicted in Table

(5) compound I exhibited significant anti- oxidant activity.

Table 5: Radical scavenging activity of compound I.

Sample	Antioxidant activity (%)
Trolox	96. 50
Compound I	82. 02

REFERENCES

- Shinwari, Z. K., Watanabe, T., Rehman, M. and Youshikawa, T., A Pictorial Guide to Medicinal Plants of Pakistan, KUST, Kohat, Pakistan, 2006.
- Dahot, M. U., Antibacterial and antifungal activity of small protein of *Indigofera oblongifolia* leaves, *J. Ethnopharmacol.*, 1999; 64: 277-282.
- Shahjahan, M., Vani, G., and Devi, C. S., Protective Effect of *Indigofera oblongifolia* in CCl4-induced hepatotoxicity, *J. Med. Food*, 2005; 8: 261-265.
- Abubakar, M. S., Balogun, E., Abdurahman, E. M., Nok, A. J., Shok, M. M. and Garba, M., Ethnomedical treatment of poisonous snakebites: Plant extract neutralized *Naja nigricollis* venom, *Pharm Biol.*, 2006; 44: 343- 348.
- Prakash, D., Suri, S., Upadhyay, G. and Singh, B. N., Total phenol, antioxidant and free radical scavenging activities of some medicinal plants, *Int. J. Food Sci. Nutr.*, 2007; 58: 18-28.
- Waako, P. J., Katuura, E., Smith, P. and Folb, P., East African medicinal plants as a source of lead compounds for the development of new antimalarial drugs, *Afr. J. Ecol.*, 2007; 45: 102–106.
- Chakrabarti, R., Damarla, R. K. B., Mullangi, R., Sharma, V. M., Vikramadithyan, R. K. and Rajagopalan, R., Insulin sensitizing property of *Indigofera mysorensis* extract, *J. Ethnopharmacol.*, 2006; 105: 102-106.
- Hamayun, M., Khan, A. and Khan, M. A., Common medicinal folk recipes of District Buner, NWFP, Pakistan. *Ethnobot. Leafl.*, 2003; 1: 14.
- Gamble, J. S. A., Singh, B. and Singh, M. P, Manual of Indian Timbers, 1972.
- Nyarko, K., Alexander, A., and Rchibald, S., *Indigofera arrecta*: Safety evaluation of an antidiabetic plant extract in non-diabetic human volunteers, *J. Phytother. Res.*, 1998; 12: 52-54I.
- Amala, B. E., Ganga, N. and Arivudainambi, R., Anti-inflammatory activity of *Indigofera aspalathoides* Vahl, *Ind. J. Med. Res.*, 1982; 76: 115-121.
- Umar, M., Antibacterial and antifungal activity of small protein of *Indigofera oblongifolia* leaves, *J. Ethanopharmacol.*, 1999; 64: 277-282.