



Research article

Isolation and characterization of a flavone from acacia orfota (forssk.) schweinf and biological activity of total extract

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ABSTRACT

Information on the constituents of medicinal plants used in Sudanese ethnomedicine is very scarce. Hence, this study was set to investigate the phenolics of the medicinally important species *Acacia orfota* which is widely used in ethnomedicine to treat a wide array of human disorders. A Flavone was isolated from the leaves and its structure was partially elucidated on the basis of its spectral data (UV, ¹HNMR and MS). Antimicrobial activity of the total extract was screened in vitro against a panel of Gram positive and Gram-negative bacterial pathogens and fungi, in comparison with control drugs and significant results were obtained.

Keywords: *Acacia orfota*, Isolation, Flavone, Antimicrobial Activity.

INTRODUCTION

Acacia genus (Family Fabaceae) involves about 1350 species. These species are considered as a rich source of gallic and ellagic acids. *Acacia* species are characterized by the presence of flavonoids and phenolics. Some *Acacia* species find wide applications in ethnomedicine as antiarrhoeic, antidiabetic, antiamebic, anti-inflammatory and hypotensive. Many *Acacia* species were found to exhibit antimicrobial activity.

In Sudanese ethnomedicine *Acacia nilotica* is used as a remedy for malaria, sore throat, cough, intestinal worms and wounds. The plant is used commercially in Sudan for leather tanning. The gum from *Acacia seyal*- Gum Arabic- is considered as a safe dietary fiber by the United States Food and Drug Administration since 1970s. Although its effects were extensively studied in animal models, there is paucity of data regarding quantified use in humans. *Acacia* gum has been used in pharmaceuticals as demulcent. It is also used topically for healing wounds and has been shown to inhibit periodontic bacteria and early deposition of plaque.

The ethanolic extract of the leaves of *Acacia nilotica*, which is rich in phenolics, showed potent antioxidant activity against stable DPPH radical. Also in DPPH bioassay, Duduku et. al. evaluated the antioxidant capacity of the medicinally important species *Acacia auriculiformis*.

Little information is available about *Acacia orfota* (Forssk.) Schweinf., growing in Sudan. The present study deals with the isolation and identification of flavonoids of *Acacia orfota* leaves as well as the antimicrobial activity of the total extract ^[1, 2].

MATERIALS AND METHODS

General

An Agilent Cary Series Spectrophotometer was used to obtain UV spectra. NMR spectroscopy was carried out using a Bruker 400 MHz in MeOH-d₄

Plant material

The leaves of *Acacia orfota* were collected from Kordofan, west Sudan in March 2016 and identified by direct comparison with a herbarium sample.

Extraction and isolation of flavonoids

Powdered shade-dried leaves of *Acacia orfota* (1.5Kg) were exhaustively extracted by 70% methanol. The dry extract was suspended in water and fractionated successively with n-hexane, chloroform,

ethyl acetate and n-butanol. The n-butanol fractions were evaporated to dryness, yielding 34.5g. Two dimensional paper chromatography of n-butanol fractions revealed the presence of several purple and blue bands for phenolic compounds. Further fractionation on a polyamide 6S column eluted by water/methanol in decreasing polarity gave 30 fractions which were finally collected to seven major fractions after examination by PC. Purification on a Sephadex LH-20 column gave compound I in chromatographically pure form.

In-vitro antimicrobial activity

The methanol extract of *Acacia orfota* was screened in vitro against a panel of Gram-positive and Gram-negative bacterial pathogens, and fungi, in comparison with control drugs: Thiophenicol (Thiamphenicol, Sanofiaventis, France) as an antibacterial agent, and Treflucan (Fluconazole, Egyptian International Pharmaceutical Industries Company- EIPICO) as an antifungal agent, by the agar diffusion technique.

Five different concentrations of the extract were prepared and individually tested against Gram-positive bacteria (*Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC29213), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC27953), and fungi (*Candida albicans* ATCC 10321, *Aspergillus niger* NRRL-363, *Fusarium oxysporium* NRC23, *Alternaria alternata* NRC43 and *Alternaria tenuissima* KM651985). All microorganisms used were obtained from the culture collection of the Department of Chemistry of Natural and Microbial Products, National Research Centre, Cairo, Egypt.

Preparation of the discs

Different concentrations of the extract were mounted on paper discs prepared from blotting paper (5 mm diameter) on a concentration of (2.0, 1.0, 0.5, 0.25 and 0.125 mg/5 μ L DMSO/disc). Thiophenicol and Treflucan were used as positive controls for antibacterial and antifungal activity, respectively, in a concentration of 100 μ g/disc. DMSO showed no inhibition zones and was used as a negative control.

Preparation of agar plates and inoculation procedure

Agar plates were prepared by using 100 mL of suspension containing 1 x10⁸ CFU/mL of pathological test bacteria and 1 x10⁶ CFU/mL of fungi spread on nutrient agar (NA) and potato dextrose agar (PDA) respectively. After the media had cooled and solidified, the discs were applied on the inoculated agar plates and incubated for 24h at 30 °C for bacteria and 72 h at 28 °C for fungi. After incubation time, antimicrobial activity was evaluated by measuring the zone of

inhibition around the disc in millimeters (mm) and compared with that of the controls^[3].

Minimal inhibitory concentration (MIC) measurement

The minimal inhibitory concentration (MIC) of the extract was evaluated at the final concentrations; 1000, 500, 250, 125 and 62.5 μ g/disc. The lowest concentration showing inhibition zone around the disc was taken as the minimum inhibitory concentration^[4, 5].

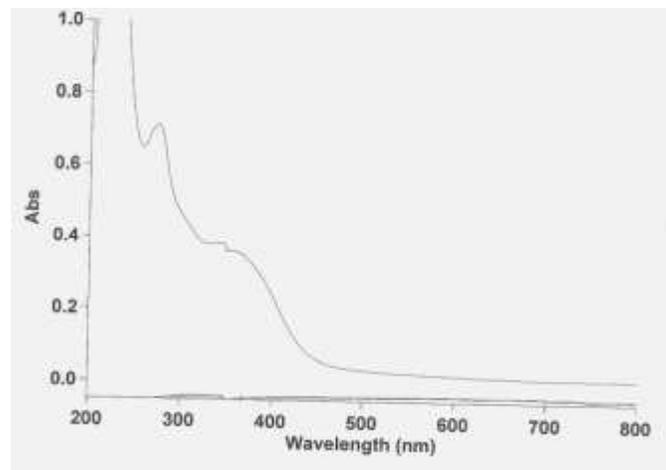
RESULTS AND DISCUSSION

Sequential solvent extraction followed by polyamide and Sephadex columns allowed isolation of a chromatographically pure flavonoid- compound I from *Acacia orfota* leaves. The structure of the isolate was partially elucidated via a combination of spectral techniques (UV, ¹HNMR and MS). The total extract was evaluated for *in vitro* antimicrobial activity against a panel of human pathogens.

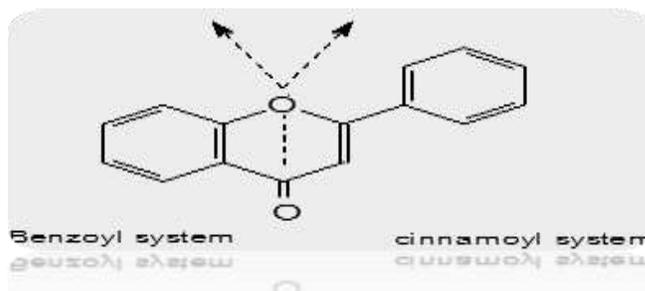
Compound I

In UV, compound I absorbs (Fig.1) at λ_{max} (MeOH) 268,331nm. Such absorption indicates conjugation between the 4 keto function and the B aromatic ring of the flavonoid nucleus. It is characteristic of flavones.

Figure 1: UV spectrum of compound I



In their UV spectra flavones give both band I (due to cinnamoyl chromophore) and band II (due to benzoyl chromophore), a feature which is shared by flavonols, chalcones and aurones. Other classes: isoflavones, flavanones, dihydrochalcones and dihydroflavonols afford only one peak originating from the benzoyl system. Band I, usually 300 – 400nm and band II, usually 240 – 280 nm^[26-27].



CONCLUSION

The results of the present study indicate that leaves of this plant exhibits potent inhibitory activity on salivary alpha amylase enzyme. IC50 value of methanolic and steroid extracts of leaves are lowest than other extracts of leaves, indicating high inhibitory potential of these extracts. Thus, these extracts might be helpful in identification of new potent lead molecule for natural amylase inhibitors.

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REFERENCES

1. Domig J K, Mayrhofer S, Zitz U, 2007. *Indian Farming*. Int. J. Food Microbiol. Page 191.
2. Chowdhury JA, Islam MS, Asifuzzaman SK, 2009. Antibacterial and cytotoxic activity screening of leaf extracts of *Vitex negundo* (Fam: Verbenaceae). *J Pharm Sci Res*. 1(4), Pages 103-8.
3. Eichler HG, Korn A, Gasic S, Prison W, Businger J, 1984. The effect of new specific α -amylase inhibitor on post-prandial glucose and insulin excursions in normal subjects and Type 2 (non-insulin dependent) diabetic patients. *Diabetologia*. 26(4), Pages 278-81.
4. Gopikrishna AV, Kandaswamy D, Jeyaval Rajan K. 2006. Comparative evaluation of the antimicrobial efficacy of five endodontic root canal sealers against *Enterococcus faecalis* and *Candida albicans*. *J Cons Dent*. 9, Pages 2-11.
5. Xiao Z, Storms R, Tsang A, 2006. A quantitative starch-iodine method for measuring alpha- amylase & glucoamylase activities. *Analytical Biochemistry*. 351, Pages 146-8.
6. Miller GL, 1959. Use of dinitrosalicylic acid reagent for determination of reducing suger. *Analytical chemistry*. 31, 426-8 Matsui T, Tanaka T, Tamura S, 2007. Alpha-Glucosidase inhibitory profile of catechins and theaflavins. *J Agric Food Chem*. 55, Pages 99-105
7. Mertes G, 2001. Safety and efficacy of acarbose in the treatment of Type 2 diabetes: data from a 5-year surveillance study. *Diabetes Research and Clinical Practice*. 52, Pages 193-204.