Isolation , Partial Characterization of a Flavone From Sudanese *Terminalia brownii* (Combretaceae) Root and Biological Activity of Ethanol Extract

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Abstract

Terminalia brownii is widely used in African system of medicine as a natural remedy for cough, tonsillitis, typhoid, tooth-ache, snake bite and rheumatic pain . Phytochemical screening of *Terminalia brownii* root revealed the presence of flavonoids , alkaloids,steroids, tannins, terpenes, saponins and coumarins. A flavonoid - compound I- was isolated from the ethanol extract by paper chromatography and its structure was partially characterized via some spectral data (UV and ^{1}HNMR).In the agar diffusion bioassay,the ethanol extract showed significant activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* It exhibited moderate activity against *Escherichia coli* and the yeast *Candida albicans* . It also showed moderate free radical scavenging capacity against stable DPPH radicals.

Keywords**:** *Terminalia brownii*, Isolation**,** Flavonoid, Antimicrobial Activity, Antioxidant activity.

Introduction

Recently , there have been an increasing interest in constituents of medicinal plants that could serve as leads for drug development and drug design , taking in consideration the global concern of microbial multi-drug resistance[1]. Herbal medicine is increasingly gaining popularity and a considerable number (about 25%) of available synthetic drugs are formulated either directly or indirectly from medicinal plants[1] .Herbal medicine plays an important role in primary health care in developing countries, where modern medicine is beyond affordability and has some adverse effects

Terminalia brownii is a medicinal plant of many attributes belonging to the family Combretaceae.The plant is widely distributed in Sudan, Tanzania, Ethiopia and Kenya where it grows along river banks ,dry areas and semi-arid regions[2,3]. It is widely used in African system of medicine[3]. *Terminalia brownii* is a natural remedy for cough, tonsillitis, typhoid, tooth-ache, snake bite and rheumatic pain[4-

[7] . Phytochemical screening affirmed the presence of flavonoids, terpenoids, steroids and saponins[8-12] . The anti-pyretic properties of the methanol extract of *Terminalia brownii* has been reported[13] and the antinociceptic activity of bark extract has been documented[14].

Materials and Methods

Plant material

Roots of *Terminalia brownii* were collected from the premises of Kordofan western Sudan.The plant was authenticated by Dr. Yahia (The Medicinal and Aromatic Plants Research Institute-Sudan).The plant material was shade - dried at room temperature and finally powdered.

Microorganisms

Organisms used for the antimicrobial assay are:

Gram +ve : *Bacillus subtilis and Staphylococcus aureus ;* Gram –ve *Escherichia coli, Pseudomonas aeruginosa* and the fungal strain *Candida albicans*

Media for bacterial growth:
Mueller Hinton agar

Media for fungal growth:
Sabouraud dextrose agar (Oxid, England)

Equipments

A Shimadzu UV spectrophotometer - model UV240 - was used for UV measurements. The ^{1}HNMR spectrum was obtained on a Joel- Nuclear Magnetic Resonance (NMR) spectrophotometer, (Brucker AC-250) operating at 500 MHz.

Methods

Extraction and isolation of flavonoids

Powdered plant material(1.5Kg) was macerated with 95% ethanol for 72h. at room temperature. The solvent was removed under reduced pressure and the dried extract of *Terminalia brownii* root was applied on Whatman 3mm papers (46×57 cm) as narrow strips and run in BAW(6:1:5, v:v:v).After the usual workup, a chromatographically pure flavonoid (compound I) was isolated.

Antimicrobial activity

The antimicrobial activity was evaluated using the cup plate agar diffusion assay. Briefly, holes (6 mm in diameter)were made in the seeded agar . Aliquots of test sample(100 mg/ml) were added into each well on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and then incubated at 37^{o}C for 24 h-for bacteria – and for three days at 25^{o}C for fungi. The assay was performed in duplicate and the resulting inhibition zones were measured in (mm) and averaged.

Results and Discussion

Phytochemical screening

Terminalia brownii was screened for major secondary metabolites and the results are displayed in Table 1.

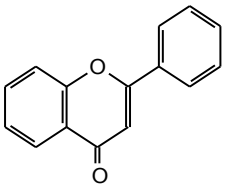
Table 1: Phytochemical screening of *Terminalia brownii* root

Secondary metabolite	occurrence
Saponins	+
Coumarins	+
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Terpenes	+

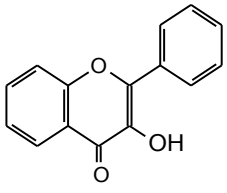
Characterization of compound I

From the ethanol extract of of *Terminalia brownii* roots a flavonoid –compound I has been isolated by paper chromatography.The structure of the isolated flavonoid has benn partially characterized by some spectral data(UV and NMR).

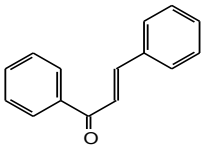
The UV spectra of flavonoids provides important spectral data for the characterization of flavonoids . Most flavonoids are unsaturated at C_2-C_3 link and exhibit two absorption bands in the UV-referred to as band II(in the range 230-285nm) and band I (in the range 300-400nm) . Band I is associated with the absorption of the cinnamoyl chromophore and band I is due to the benzoyl chromophore.Four classes of flavonoids are known to give two UV bands: the flavone, flavonols, chalcones and aurones.



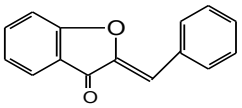
Flavone



Flavonol

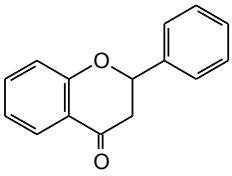


Chalcone

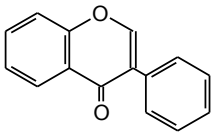


Aurone

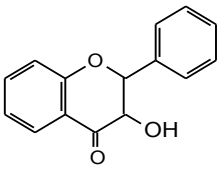
On the other hand, those flavonoids which are characterized by loss of conjugation between the two aromatic rings(A and B) of flavonoids exhibit only one absorption band – band II which accounts for the absorption of the benzoyl chromophore .These are : the flavanones, iso flavone, dihydrochalcones and dihydroflavonols.



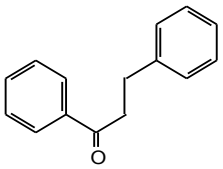
Flavanone



Isofalvone



Dihydroflavonol



Dihydrochalcone

In the UV , compound I absorbs(Fig.1) at λ_{max} 220,354nm. Such absorption is given by : flavone, flavonols, chalcones and aurones. Chalcones give a dominant band I absorption, while aurones have band I beyond 400nm. This indicates that the isolated flavonoid is either a flavone or a flavonol. Flavonols absorb in the range 358-400nm(band I), while flavone absorb in the range 300-356nm.Consequently the isolated flavonoid is a flavone.

Next different UV shift reagents(sodium methoxide, sodium acetate and aluminium chloride) have been used to establish the hydroxylation pattern on the nucleus of the flavonoid.The shift reagent sodium methoxide induces a bathochromic shift in presence of a 3- or 4`-OH functions, while sodium acetate gives bathochromic shifts diagnostic of a 7-OH group. Aluminium chloride is used in the chemistry of flavonoids for the specific detection of 3-,5-OH groups as well as catechol moieties. The sodium methoxide spectrum (Fig. 2) of compound I did not reveal any bathochromic shift suggesting absence of 3- and 4`-OH functions. Also the aluminium chloride spectrum(Fig.3) failed to show a bathochromic shift indicating absence of 3- and 5-OH groups as well as catechol systems.The sodium acetate spectrum (Fig.4) eshibited a bathochromic shift indicative of a 7-OH function.

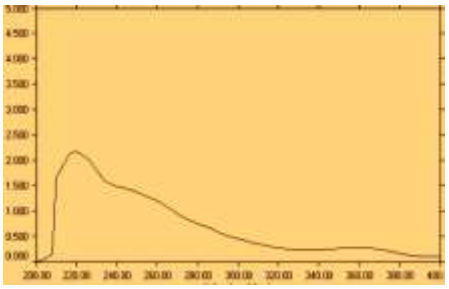


Fig. 1: UV spectrum of compound I

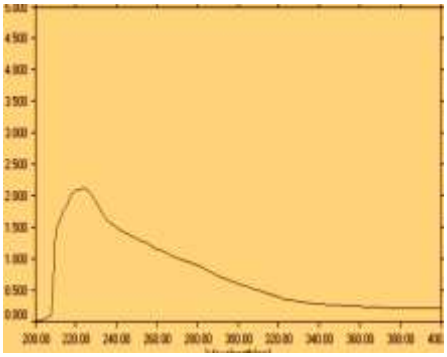


Fig. 2: Sodium methoxide spectrum of compound I

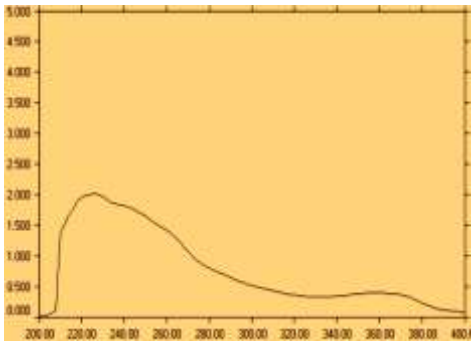


Fig. 3: Aluminium chloride spectrum of compound I

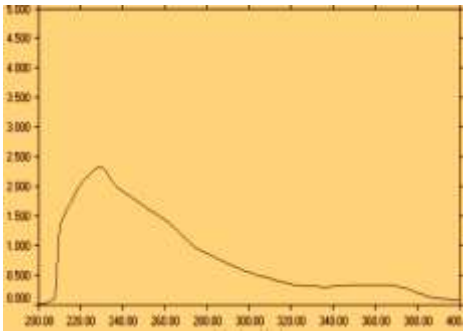
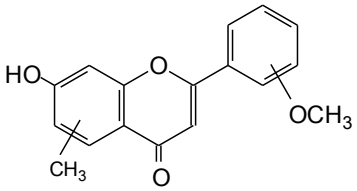


Fig. 4: Sodium acetate spectrum of compound I

The ^{1}HNMR spectrum (Fig. 5) showed δ(ppm) : 1.20(assigned for a methyl group) ; 4.40,4.85,5.40 assigned for sugar protons (other sugar protons overlapped the solvent-DMSO- residual water protons which resonated within the multiplet : δ3.00-3.85). This sugar was not identified in this study. The resonance at δ4.10 accounts for a methoxyl function. The aromatic protons appeared at δ6.70ppm. The signal at δ2.50 is due to the solvent

residual protons.On the basis of the above argument, the following partial structure was proposed for the aglycone of compound I :



Compound I

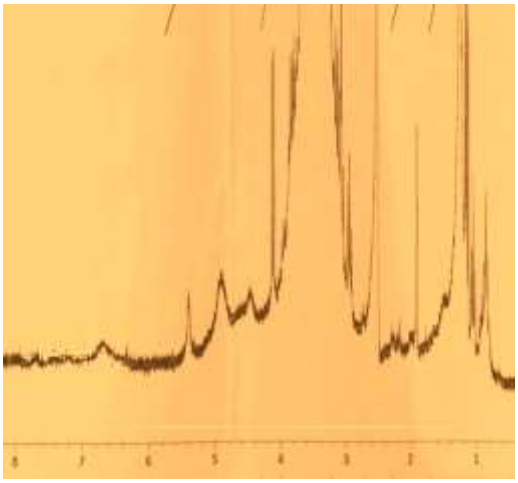


Fig. 5:[1]HNMR spectrum of compound I

Antimicrobial activity

The ethanol extract of *Terminalia brownii* root was screened for antimicrobial activity against five standard human pathogens(Table 2). The results are presented in Table 3. The results were interpreted as follows: (<9mm: inactive; 9-12mm: partially active; 13-18mm: active; >18mm: very active). Ampicilin, gentamycin and clotrimazole were used as positive controls(Tables 4 and 5).

The extract showed significant activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* It also exhibited moderate activity against *Escherichia coli* and the yeast *Candida albicans* **.**

Table 2: Test organisms

No	Micro organism	Type	Source
1	*Bacillus subtillus*	G+ve	ATCC 2836(*)
2	*Staphylococcus aureus*	G+ve	ATCC 29213
3	*Pseudomonas aeroginosa*	G-ve	NCTC(**) 27853
4	*Escherichia coli*	G-ve	ATCC 25922
5	*Candida albicans*	fungi	ATCC 7596

* NCTC. National collection of type culture, Colindale. Englan *ATCC. American type culture collection, Maryland, USA

Table 3: Antimicrobial activity of ethanol extract

Sample	Ec	Ps	Sa	Bs	Ca
Ethanol extract	15	20	25	-	15

Table 4: Antibacterial activity of standard drugs

Drug	Conc. (mg/ml)	Bs	Sa	Ec	Ps
Ampicilin	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

Sa. *Staphylococcus aureus*
Ec.: *Escherichia coli*
Pa.: *Pseudomonas aeruginosa*
Bs: *Bacillus subtilis*
Ca: *Candida albicans*

Antioxidant activity

The antioxidant capacity of the ethanol extract of *Terminalia brownii* root was carried out by measuring the capacity of the test sample against stable DPPH radical. The change in color is measured spectrophotometrically at 517 nm. As shown in (Table 6) the extract exhibited moderate antioxidant activity. Propyl gallate was used as positive control.

Table 5: Antifungal activity of standard drug

Sample	Antioxidant activity
Propyl gallate	92.00%
Linum usitatissimum oil	48.00%

Table 6: Antioxidant activity of ethanol extract

Drug	Conc.(mg/ml)	Ca
Clotrimazole	30	38
	15	31
	7.5	29

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