Isolation , Partial Characterization of a Dihydroflavonol From Sudanese *Wetheria indica* L.(Sterculiaceae) Stems and Biological Activity of the Ethanol Extract

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Abstract

Phytochemical screening of *Wetheria indica* stems revealed the presence of flavonoids, tannins steroids , saponins and alkaloids. A flavonoid - compound (I)- was isolated from ethanol extract by thin layer chromatography and its structure was partially characteized on the basis of its spectral data (IR, UV, NMR). The ethanol extract of *Wetheria indica* stems was evaluated for antimicrobial activity and it showed moderate activity against all test organisms. Also the extract exhibited significant free radical scavenging capacity.

Keywords**:** *Cistanch phelypea* , Isolation, Dihydroflavonol, Antibacterial Activity, Antioxidants Activity.

Introduction

For centuries and long before the discovery of

modern medicines , plants and plant-derived formulations have been used by humans to fight different diseases[1-5]. In developing countries, where modern drugs are beyond affordability, herbal remedies are the major therapeutic resources for the majority of communities and ethnic groups[6]. In Sudan,traditional healers are still playing a vital role in primary health care specially in rural areas.

Wetheria indica L. (also called monkey bush, buff coat, marsh-mallow) is a plant in the family Sterculiaceae. The plant is native to Florida and Texas and widely distributed along tropics and worm tropics. In ethnomedicine, *Wetheria indica* is a plant of many attributes. It has been used in folklore medicine for the remedy of many pathologies in Hawaii[7] ,India[8] ,South[9] America[10] ,South Africa[11] ,East Africa[12] and West Africa[13] . *Wetheria indica* is used as tonic, analgesic, astringent, purgative and febrifuge[14].Various extracts of *Wetheria indica* have been used traditionally against cough, skin diseases and infertility[15]. The plant is also used as immunomodalatory[16] and as a natural remedy for internal hemorrhage, syphilis[15] , inflammation and circulatory problems[18,19].

Materials and Methods

Materials

Plant material

Leaves of *Waltheria indica* were collected from Nyala- west Sudan. The plant was identified and authenticated by the department of phytochemistry and taxonomy, Aromatic and Medicinal Plants Research Institute, Khartoum, Sudan.

Instruments

IR spectra were run on a Shimadzu IR spectrophotometer , UV spectra were measured on a Shimadzu 2401PC UV-Visible Spectrophotometer. NMR spectra were measured on a Joel ECA 500MHZ NMR Spectrophotometer.

Test of organisms

The following standard microorganisms were used to assess the antimicrobial activity : *Bacillus subtilis* (Gram +ve) , *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* (Gram –ve) , *Escherichia coli* (Gram –ve) and the fungal species *Candida albicans* .

Methods

Preparation of plant extract for phytochemical screening

(150 g) Of powdered air- dried leaves of *Waltheria indica* were extracted with 95% aqueous ethanol by maceration. This prepared extract (PE) was used for phytochemical screening. Phytochemical screening was accomplished according to the method described by Harborne[16].

Extraction and isolation of flavonoids

(1 kg) of powdered air-dried stems of *Waltheria indica* was macerated with 95% ethanol (5L) for 48hr at room temperature with shaking .The extraction process was repeated two more times

with the same solvent. Combined filtrates were concentrated under reduced pressure at 40^{o} C yielding a crude product.

The crude extract was applied on TLC plates as concentrated spots. The spotted (TLC) plates were developed with methanol:chloroform(1:19 ; v:v) . The chromatograms were then viewed in UV light using both long and short wavelengths (λ_{max} 366 nm and λ_{max} 254 nm) with and without exposure to NH_3 vapour. After the usual workup, a chromatographically pure flavonoid-compound I -was isolated.

Biological activity

Antimicrobial assay

The agar diffusion bioassay was used with some minor modifications to assess the antimicrobial activity of different fractions of the studied plant.

Each of the microbial strain was inoculated into 9.0ml of nutrient broth agar using sterile wire loop. The cultures were incubated at 37^{o} C for 24h. Each of the Nutrient agar plates was divided into two halves and each half was designed for one of the standard organisms. A 4mm sterile cork borer was used to bore a well in each

half.The Nutrient agar plates were then inoculated with the cultured microbes using sterile cotton swab sticks. The test samples were aseptically dispensed into the wells. The plates were allowed to stand in the work bench for 30 minutes and then incubated at 37ºC for 24 hours (for bacteria) and for 72 hours at 25ºC for fungi. After incubation the plates were observed for zones of inhibition. Tests were performed in duplicates and the inhibition zones were measured and averaged as indicators of activity.

Antioxidant activity

The DPPH radical scavenging assay

The antioxidant potential of the ethanol extract of *Waltheria indica* stems was evaluated using the method described by Shimada et.al.[20]. The ethanol extract of *Waltheria indica* stems was dissolved in DMSO while DPPH was prepared in ethanol[23]. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by sample was determined in comparison with a DMSO treated control group.All tests and analysis were run in triplicate.

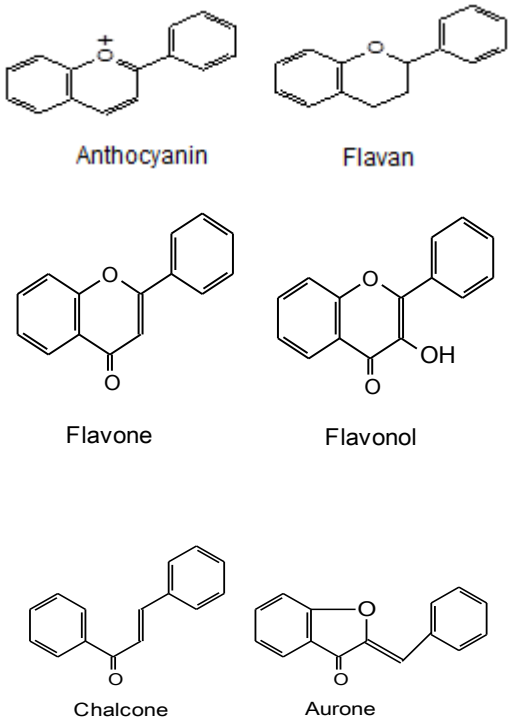
Results and Discussion

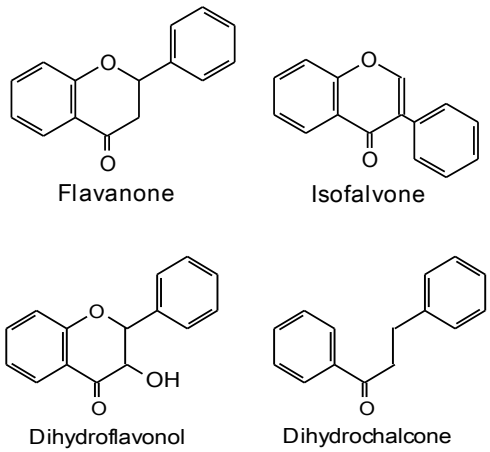
Characterization of compound I

Phytochemical screening of the leaves of *Waltheria indica* showed the presence of tannins, flavonoids , saponins, steroids , and alkaloids. From the ethanol extract of *Waltheria indica* , a flavonoid- compound I- was isolated by thin layer chromatography and its structure was elucidated partially via a combination of spectral techniques (UV, IR, ^{1}HNMR).

The IR spectrum of compound I showed a pattern characteristic of flavonoids. It revealed ν (KBr): 829 (C-H, Ar. ,bending),1174(C-O) ,1560,1598 (C=C, Ar.),1699 (C=O) , 2804 (C-H,aliph, stretching) and 3733cm^{-1}(OH).

Flavonoids are classified into : flavones, flavonols, chalcones , aurones, flavanones, dihydroflavonols, dihydrochalcones ,isoflavones, anthocyanins and flavans.





Among these classes, anthocyanis and flavans are easily detected via their IR spectra. These classes lack the 4- keto function which distinguishes the 8 other classes.

Isoflavones, flavanones, dihydrochalcones and dihydroflavonols all give similar UV spectra as a result of having little or no conjugation between the A- and B-rings. In their UV spectra, they typically exhibit an intense Band II absorption with only a shoulder or low intensity peak representing Band I. Due to effective conjugation between the two aromatic rings of flavonoids(rings A and ring B), the flavones, flavonols,chalcones and aurones show both band I and II. In flavonoids band I(in the range:300-400nm) is associated with the absorption of the cinnamoyl chromophore , while band II(the range:235-290nm) originates from absorption of the benzoyl chromophore.

The UV spectrum of compound I showed λ_{max} 276nm, i.e. it gave only one band-band II. Such absorption is characteristic of flavanones, dihydroflavonols, dihydrochalcones and isoflavones.

The band II absorption of isoflavones usually occurs in the region 245 - 270 nm with a characteristic shoulder in the UV range : 300-340nm. However, such shoulder was not detected in the UV spectrum of compound I (Fig.1).

The UV shift reagent : sodium methoxide is used in the chemistry of flavonoids for the specific detection of 3- and 4`-OH functions. In the presence of both groups it exhibits a characteristic bathochromic shift but with decrease in intensity in case of a 3-OH substituent.However , the sodium methoxide spectrum (Fig. 2) revealed a bathochromic shift with decrease in intensity.Such shift is diagnostic of a 3-OH function.Hence the isolated flavonoid is a dihydroflavonol.

Next the hydroxylation pattern on the nucleus of the flavonoid was investigated using different UV shift reagents : sodium acetate(diagnostic of a 7-OH) and boric acid (diagnostic of catechols).

The sodium acetate spectrum (Fig.3) showed a bathochromic shift characteristic of a 7-OH function. However,,the boric acid spectrum (Fig.4) failed to afford any bathochromic shift indicating absence of catechol systems.

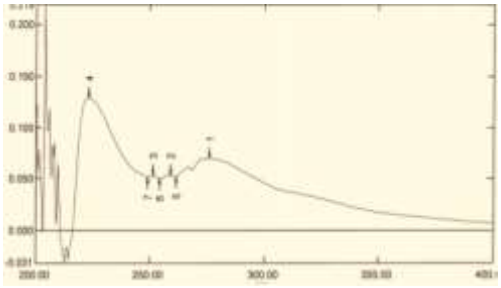


Fig. 1: UV spectrum of compound I

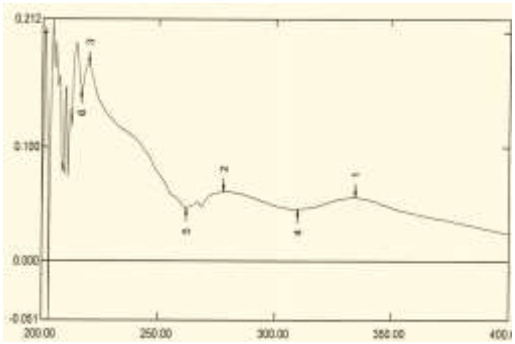


Fig.2: Sodium methoxide spectrumof compound I

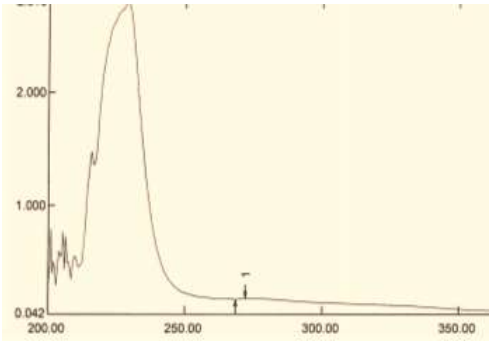


Fig.3: Sodium acetate spectrum of compound I

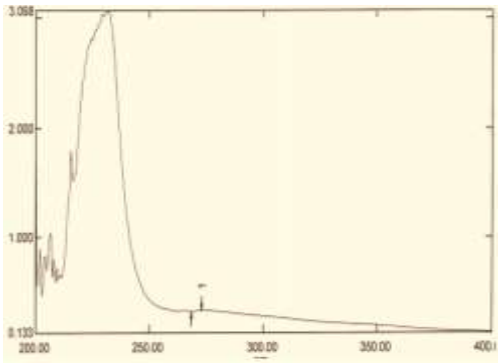
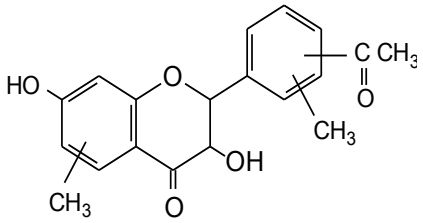


Fig. 4: Boric acid spectrum of compound I

The ^{1}HNMR spectrum(Fig.5) showed , δ(ppm : 1.23,1.62 - assigned for two methyl groups. The signal at δ2.00ppm was assigned for an acetyl group. The resonance at δ6.61 , 7.20 and 8.50 ppm accounts for the aromatic protons. The signals at δ2.50 and δ3.40 ppm are due to the solvent(DMSO) residual protons and residual water respectively. On the basis of the its spectral data , the following partial structure was propososed for compound I:



Compound I

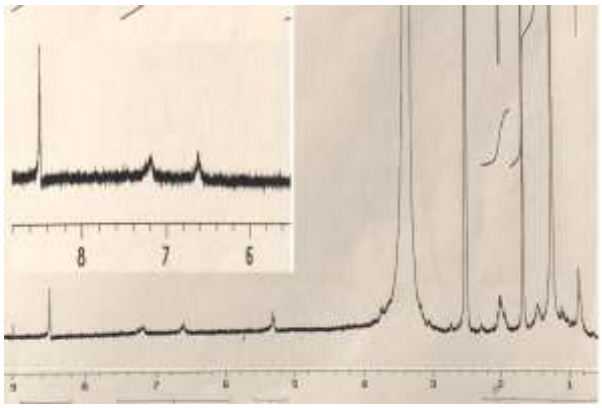


Fig. :^{1}HNMR spectrum of compound I

Antimicrobial activity

The ethanolic extract of *Wetheria indica* stems was screened for the antimicrobial activity against five standard microorganisms.The inhibition zones are shown in Table (2) .Results were interpreted as follows : (>9mm: inative;9-12mm:partially active;13-18mm: active;<18mm:very active) . The ethanolic extract showed moderate antibacterial and antifungal activity against test organisms(Table 2). Ampicilin , gentamycin and clotrimazole were used as positive controls(Tables 3 and 4).

Table 1: Test organisms

Ser.No.	Moicroorganism	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 type culture, Colindale. England

*ATCC. American type culture collection, Maryland, USA

Table 2 : Antimicrobial activity of ethanolic extract

Extract	Conc.(mg/ml)	**Sa**	Bs	Ec	Ps	Ca
Ethanolic extract	100	15	14	14	14	13

Drug	Conc.(mg/ml)	An	Ca
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

Table 3 : Antibacterial activity of standard chemotherapeutic agents

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	2	1	1	1
	10				

		2 1 7	8 1 4	8 1 5	5 1 2

Table 4 : Antifungal activity of standard chemotherapeutic agent

Antioxidant activity

The antioxidant capacity of the ethanol extract of *Wetheria indica* stems has been measured. Evaluation of the antioxidant activity was carried out by measuring the capacity of the test extract against stable DPPH radical. The change in color was measured spectrophotometrically at 517 nm. As displayed in (Table 5). The extract exhibited significant free radical scavenging capacity.

Table 5: Radical scavenging activity of ethanol extract

Sample	Antioxidant activity
Propyl gallate	92.00%
Ethanol extract	89.02%

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