IJBPAS, June, 2017, 6(6): 1181-1194

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BIOLOGICAL ACTIVITY AND PHYTOCHEMICAL PROFILING OF *GREWIA TENAX* STEM BARK EXTRACTS

HIBA A. ALI[1] SALMA A, SALIH[1], OMIMA E. AHMED[1], ABDELHALIM A. HAMZA[1*]

Commission for Biotechnology and genetic Engineering, Department of Biochemistry, Sudan

Received 19th Oct. 2016; Revised 20th Dec. 2016; Accepted 17th March 2017; Available online 1st June 2017

ABSTRACT

The genus *Grewia* L. (Malvaceae) is native to Africa, Asia and Australia with 150 species. *G.tenax*(Forsk.) Fiori. (Gudiem) is found in Kordofan, Darfur, central and the southern part of the Sudan. *G. tenax*has been used traditionally as medicinal agents to treat various diseases such as trachoma, leucoderma, tonsillitis, hepatitis virus, diarrhea, asthma, jaundice, tuberclosis, tonsillitis and pulmonary infections employing different parts of the trees. The extracts and preparations from this folk medicine, which are expectantly safe, exhibited various biological effects, e.g. anti-oxidant, antibacterial, hepatoprotective, anti-inflammatory, anti-emetic, anti-malarial, analgesic, and anti-pyretic activities.

Air dried ground stem bark of *G. tenax* was extracted using 80% methanol. The methanolic extract was sequentially fractionated with petroleum ether, chloroform and ethyl acetate. The obtained extracts of *G. tenax* stem bark were screened for their antimicrobial activities against four standard bacteria (*Bacillus subtitles, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,* and two fungi *(Aspergillus niger* and *Candida albicans*) using *in vitro* agar diffusion method. The cytotoxicity evaluation of the bioactive fractions was carried out *in vitro* usingbrine shrimp lethality assay.

The methanolic extract of *G. tenax* stem bark possessed antibacterial activity against *Bacillus sublilis* bacteria with an MIC of 50 mg/ml and was safe to brine shrimp assay. TLC revealed the presence of terpenoids and phenolics in all tested fractions. Alkaloids were mainly accumulated in the ethyl acetate fraction and were unstable. Phenolics and alkaloids detected possessed antioxidant activity when sprayed with DPPH.

Key words: *Grewiatenax*, Phytochemical profiling, antimicrobial activity, brine shrimps lethality

INTRODUCTION

Medicinal plants have been attaining great recognition all over the globe. The World Health Organization (WHO, 2008), has estimated that 80% of the population of the developing countries relay in traditional medicine, mainly medicinal plants for their primary health care needs. 25% of prescription drugs in United State and up to 60% of those in Eastern Europe to consist of unmodified or slightly altered plant product, {1}.The search for new antimicrobial compounds from the indigenous plant used in treating bacterial related diseases with improved activity is necessary, {2}.

Grewiatenax is a tree spread in African and Southeast Asiatic continentsand in the in arid area such as sand and near mountains, especially in the Savanna plantation area of the Northern of Sudan {3}. *Grewiatenax*, locally known as "Guddaim" is one of the valuable plant species in Sudan. It belongs to the *Malvaceae* family. *G. tenax* (Gudiem) is wide spread in northern Kordofan, central Sudan and the southern part of the country. *G. tenax* plant contain protein, fat, fiber, ash, saccharides, essential amino acids and minerals like S, Na, Zn, K, Mg, Ca, Fe, Cu, Mnetc, {4}. The ethyl acetate fraction contained eleven terpenoides e.g. beta-sit sterol, beta-amyrin, betulin, stigma sterol, oleanolic acid etc. {5}.

There are many reported traditional uses of *G. tenax.* Decoction and fruits juice are used for their tonic and anti-anemic properties. In areas of Sudan the powdered roots are used for tonsillitis and throat infections. Root preparations are also used against tuberclosis, chest diseases, {1}.

The bark extract is purgative and is generally used as anthelmintic and against many intestinal parasites {6}. The bark was also reported to possess bactericidal activity and is used in the treatment of tuberculosis. To date very poor information about *G. tenax* secondary metabolites are reported. The plant has different traditional uses. *Grewia tenax* different extracts are expected to have antimicrobial activity against human pathogens{7}. Biologically guided fractionation will be adopted to identify polyphenols in active extracts. The aim of this study is to evaluate Phytochemical profiling, antimicrobial activity, antioxidant activity and brine shrimps lethality of *Grewia tenax* stem bark.

MATERIALS & METHODS

Plant material collection

Grewia tenax different parts (Leaves, stem, roots and bark) (Fig. 1) were collected from the Faculty of Agriculture, University of

Khartoum, Shambat, 2006. A voucher specimen was made for each sample and was taxonomically identified at the department of Botany, Medicinal and Aromatic plants Institute, National Center for research. The herbarium was deposited at the Department of Biochemistry, Commission of Biotechnology and Genetic Engineering, National Center for Research.

Plant material preparation and extraction

Barks collected were dried separately in shade and ground coarsely. 200 g of air dried ground bark of *E. alsinoides*were extracted using 80% methanol. The methanolic extract was sequentially fractioned with petroleum ether, chloroform, ethyl acetate. Extracts were obtained by removing solvents in vacuum. Extracts obtained was subjected to further biological and phytochemical analysis, (Fig. 2).

A **B**

C **D**

Figure 1: *Grewiatenax* parts studied; (A) *G. tenax*, Shambat 2006; (B) *G. tenax* bark (studied part); (C) *G. tenax* leaves and flower; (D) G. tenax leaves and stem

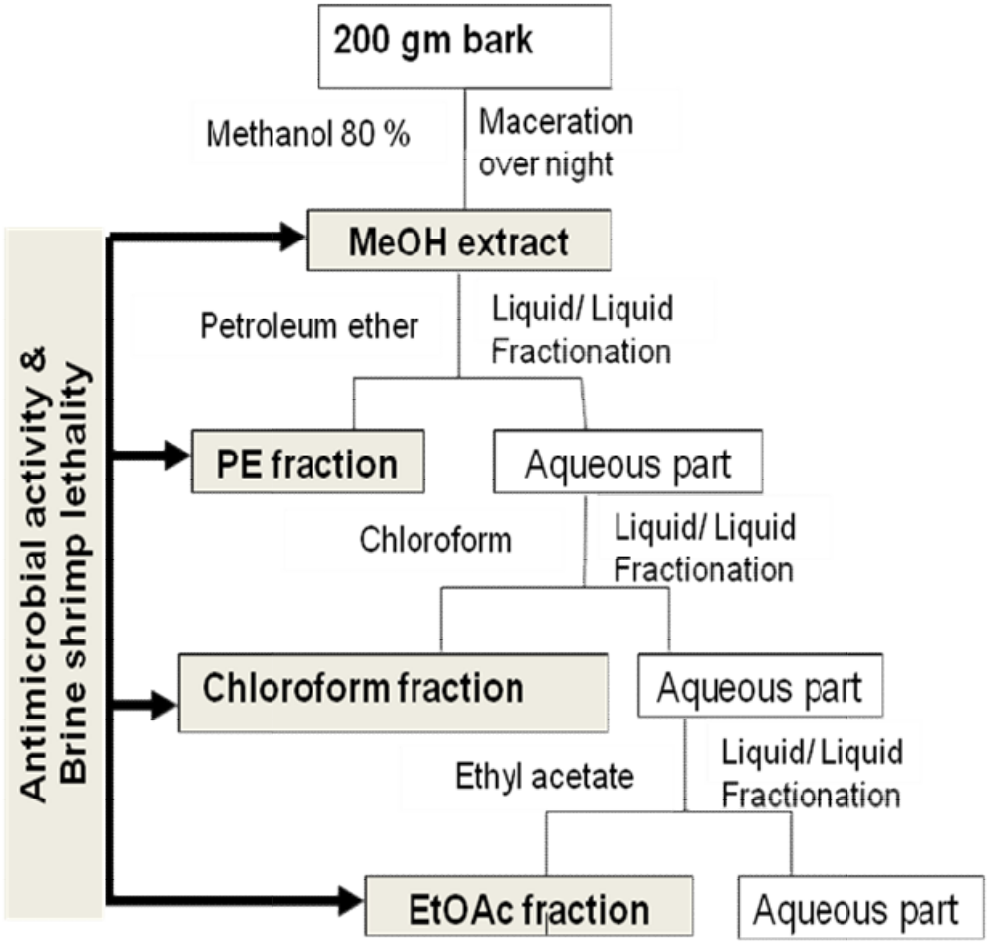

Figure 2: Extraction and fractionation of *G. tenax* bark

Chromatographical analysis

Thin Layer Chromatography (TLC)

Aluminium silica gel plates 60 F_{254} (Merck 5554) or pre-coated TLC plates were used in carrying out TLC of the different plants extracts. Standard chromatograms of the plant extracts were prepared by applying 20 µl solution (5 mg/ml) to a silica gel plate and developing it in Toluene: EtoAc: HCOOH (5:4:1) solvent system. Chromatograms were detected under UV light (254 and 366) and sprayed with diagnostic reagents which include: vanillin-H_2SO_4 reagent, Dragendorff, DPPH, and Natural Product Reagent (NPR).

Spray reagents

Dragendorff Reagent: Composed of two solutions:

- Solution A: 0.3 g bismuthsubnitrate in 1 ml of 25% HCL and 5 ml H_2O
- Solution B: 3 g potasium iodide in 5 ml H_2O

The spray reagent was composed of 5 ml (A) + 5 ml (B) + 5 ml of 12.5% HCL + 100 ml H_2O.

Vanillin-H_2SO_4: 0.5 g vanillin was dissolved in a ready prepared mixture of 85 ml MeOH, 10 ml acetic acid and 2.5 ml

conc. H_2SO_4. Sprayed TLC plates were examined after heating to 120 ^{0}C.

Natural Products (polyethylenglycol) (NP/PEG) Reagent: Plates were sprayed with 1% methanolicdiphenylboric acid (NP), followed by 5% ethanolicpolyethyleneglycol – 4000 (PEG) (10 ml and 8 ml, respectively).

2, 2-Diphenylpicrylhydrazyl (DPPH) Reagent: TLC plates were sprayed with 5% ethanolic 1,1-diphenyl-2-picrylhydrazyle.

Antimicrobial activity

The extracts of *Croton zambesicus* were tested *in vitro* for their antibacterial and antifungal activities against difference pathogenic organisms. Plant extracts were tested against *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) *Salmonella typhi* (ATCC 0650), *Staphylococcus aureus* (ATCC 25923), *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC 7596) using agar diffusion method {8} with minor modifications.

Minimum Inhibitory Concentrations (MIC) values define as theleast concentrations of the plant extracts that does not permit any visible growth of the inoculated test organism in the broth medium or the least concentration of the extract which inhibited the organism. MIC values weredetermined according to a modified method of the serial dilution {9}.The MIC of the extracts was determined by incorporating serial amounts (10, 5, 2.5, 1.25, 0.62, 0.31 mg/ml) of the extracts solutions. The estimation MIC of the extracts for each tested organism was in triplicate.

Brine shrimp lethality assay (Toxicity)

The bioactive extracts of *G. tenax* were tested for their toxicity using the brine shrimp *Artemiasalina* standard method as described by {10}, with a little lab required modification. The data were analyzed with finney computer program, and the lethal concentrations 50% (LD50) were determined.

RESULTS AND DISSCUSSION

Thin Layer Chromatography (TLC) of *Grewia tenax* stem bark extracts

There were many reported metabolites in Grewia e.g. triterpenoids , steroids, glycosides, flavones, lignanes, phenolics, alkaloids, lactones and organic acids have been isolated from various species of this genus, {11}.

TLC profiles of the different fractions of the methanolic extract of *G. tenax* stem bark are presented in Figures (3, 4) and Tables (1, 2, 3) Selection of a suitable stationary phase and solvents depends on the class of metabolites to be examined. Best separation was obtained using NP-TLC silica gel 60 (Merck).

Thin layer chromatography profiling of the chloroform fraction

Typical pink to purple colours were developed upon spraying with vanillin H_2SO_4 (heat 110˚C) by three compounds (Rf 0.017, 0.413, 0.620) (Fig.3). Vanillin H_2SO_4 is a universal reagent that detects components of essential oils, terpenoids, phenols etc. {12}. Five phenolic acids (Rf 0.017, 0.413, 0.551, 0.620, 0.965) were detected at UV 366 after spraying with NPR. Three of these compounds (Rf 0.017, 0.413, 0.551) reacted positive to the free radical DPPH indicating their antioxidant activity. No alkaloids were detected in this fraction.

Thin layer chromatography profiling of the ethyl acetate fraction

Alkaloids develop brown or orange visual day light zones immediately upon spraying with Dragendorff reagent {12}. Alkaloids were detected in the ethyl acetate fraction of the methanolic extracts of the bark after spraying with Dragendorff (Rf0.344, 0.426). Plants of the genus Grewia were reported to possess Harman alkaloids (Fig. 1). {13} reported that Harman alkaloids belong to the class of β-carbolines. This group of compounds has derived biological activity and pharmacological properties including sedative, antitumor, antiviral, antioxidants {14}.

Typical pink to purple colours were developed upon spraying with vanillin H_2SO_4 (heat 110˚C) in the ethyl acetate fraction (Rf0.573 , 0.655) (Fig.4). Vanillin H_2SO_4 is a universal reagent that detects components of essential oils, terpenoids, phenols etc. {12}.

TLC of the ethyl acetate fraction revealed the presence of flavonoids and phenolic acids. The presence of flavonoids was confirmed by their colour change from quenching fluorescence (366 nm) to yellow or orange colour (Rf 0.032, 0.049) and prominent blue colour in case of flavonoidal acids or other phenolic acids (Rf 0.032 ,0.049,0.508) at UV 366nm after spraying with natural product reagent (NPR) (Fig.4). Fluorescence behaviour of flavonoids in response to (NPR) is structure dependent, {12}. Flavonoids are important antioxidant as reported by {15}. The major structural characteristics which contribute to their reducing properties are in the B-ring, an unsaturated 2, 3 double bond and a 3-hydroxyl group in ring C.

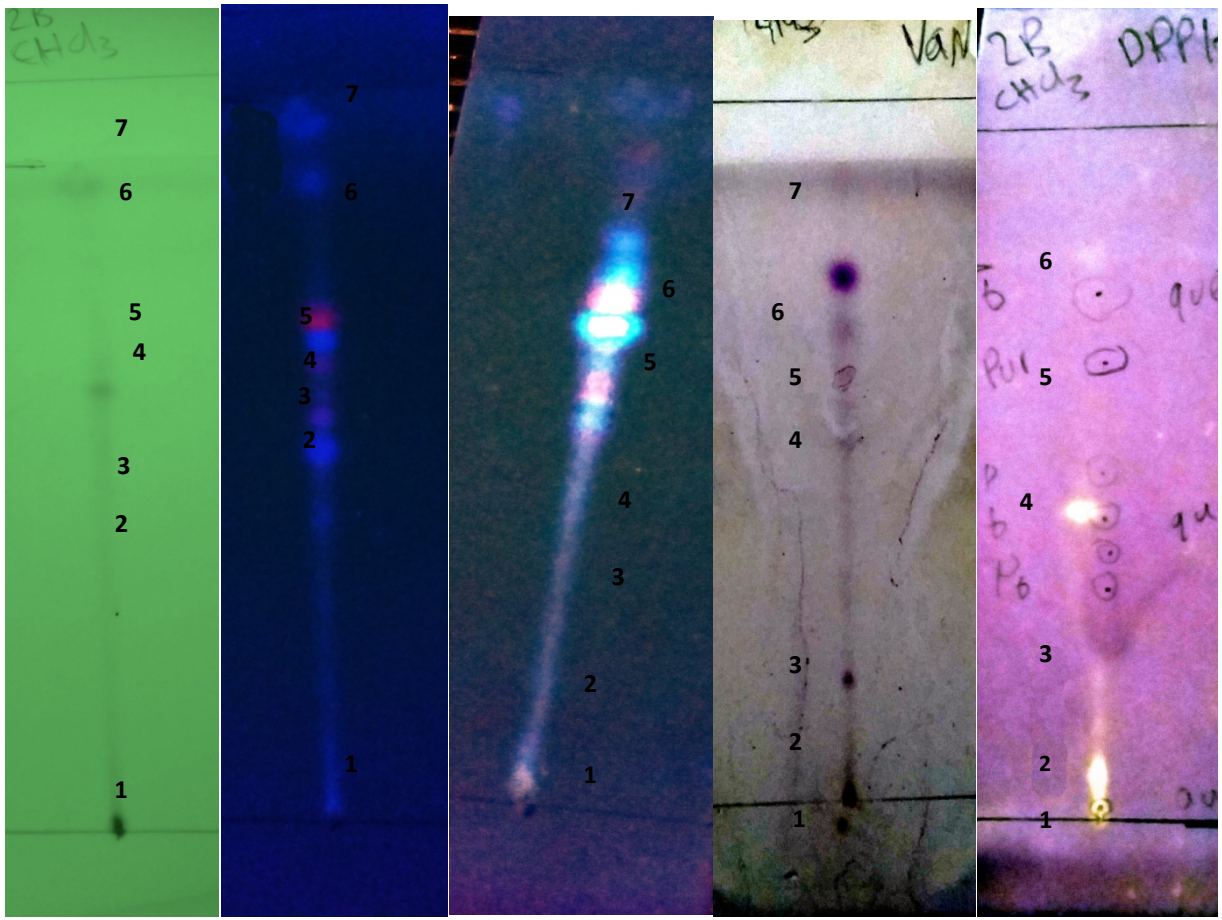

Figure 3: ***G. tenax*** **stem bark chloroform NP-TLC chromatograms using different diagnostic reagents and Toluene : EtOAc: Formic acid 5 : 4: 1 solvent system**

Table (1): TLC Profiles of chloroform fractions of *Grewia tenax* bark

Comp. spot	R_f Values	UV Reaction		Compounds Colour Reactions to Diagnostic Spray Reagents				Expected Metabolites & Antioxidant activity (+/-)
		254 Nm	366 nm	Vanillin H_2S0_4	NPR 366 nm	Dragendorff	DPPH	
1	0.017	Quenching	Blue	Dark blue	violet	-ve	yellow	Terpenoid , phenolic acid (+)
2	0.413	-ve	Violet	Blue	Dark blue	-ve	yellow	Terpenoid , phenolic acid (+)
3	0.482	-ve	Pink	-ve	Blue	-ve	-ve	Chlorophyll
4	0.551	Quenching	Blue	-ve	Bule white	-ve	yellow	Phenolic acid (+)
5	0.620	-ve	Dark blue	Dark violet	Blue	-ve	-ve	Terpenoid , phenolic acid (-)
6	0.827	-ve	Violet	-ve	pink	-ve	-ve	Chlorophyll
7	0.965	Quenching	Blue	-ve	blue	-ve	-ve	Phenolic acid (-)

Table (2): TLC Profiles of the ethyl acetate fractions of *Grewiatenax*stem bark

Compound spot	R_f Values	UV Reaction		Compounds Colour Reactions to Diagnostic Spray Reagents				Expected Metabolites & Antioxidant activity (+/-)
		254 nm	366 nm	Vanillin H_2S0_4	NPR 366 nm	Dragendorff	DPPH	
1	0.032	Quenching	Blue	-	Blue	-ve	Yellow	Phenolic acid (+)
2	0.049	Quenching	Yellow	brown	Faint Yellow	-ve	Yellow	Flavonoid (+)
3	0.344	Quenching	Blue	brown	-ve	Brown	Yellow	Alkaloid (+)
4	0.426	Quenching	Blue	-ve	Faint blue	Brown	Yellow	Alkaloid (-)
5	0.508	-ve	-ve	-ve	W. blue	-ve	Yellow	Phenolic (+)
6	0.573	-ve	Blue	purple	-ve	-ve	-ve	Terpenoid (-)
7	0.655	-ve	-	Dark purple	-ve	-ve	-ve	Terpenoid (-)

Thin layer chromatography profiling of the petroleum ether fraction

Terpenoids were recorded in the petroleum ether fraction of *G. tenax* (Rf 0.35, 0.61, 0.89) giving blue to violet colours with H_2SO_4.

Simple phenolic compounds and phenolic acid were also noted reacting yellow and *blue to NPR (Rf 0.19, 0.23, 0.89).*

No alkaloids were recorded in the petroleum ether fraction. Additionally none of the recorded metabolites possessed antioxidant activity.

Antimicrobial activity of Grewiatenax stem bark extracts (MIC)

The antimicrobial activities of the different extracts of *G. tenax* (stem bark) are presented in Table (4). None of the tested extracts and fractions possessed antifungal activity, Table (4). The methanolic extract of *G. tenax* (stem bark) showed bactericidal activity. The MIC of the *G. tenax* (stem bark) crude extract was 50 mg/ml against *Bacillus subtitles*. After fractionation of the *G. tenax* (stem bark) methanolic extract a rather weak antibacterial activity was mainly accumulated in the ethyl acetate phase against *Bacillus subtitles* and *E. coli*. The genus Grewia was reported to have anti-microbial in addition to its pharmacological activity, {16, 17} tested the biological activity of G. Erythraea Schwein f. against 12 fungal and 12 bacterial strains by agar well diffusion and disk diffusion assays *Grewi aerythraea* methanol extract showed good activity only against *Pseudomonas aeruginosa*.. The antibacterial activity of *G. occidentalis* was also reported by, {18}. The methanol extracts of *G. occidentalis* showed significant inhibition against gram-positive and gram-negative bacteria. The methanolic extract of *G. bicolor* showed activity against gram-positive and gram-negative organisms, {19}.

Brine shrimp lethality assay (Toxicity) of *Grewia tenax* stem bark extracts

In vitro toxicity activity of the methanolic crude extract of the *G. tenax* stem bark against the brine shrimp (Artemiasalina) was assessed. It was found to be non toxic (LD50....) against *A. salina*.

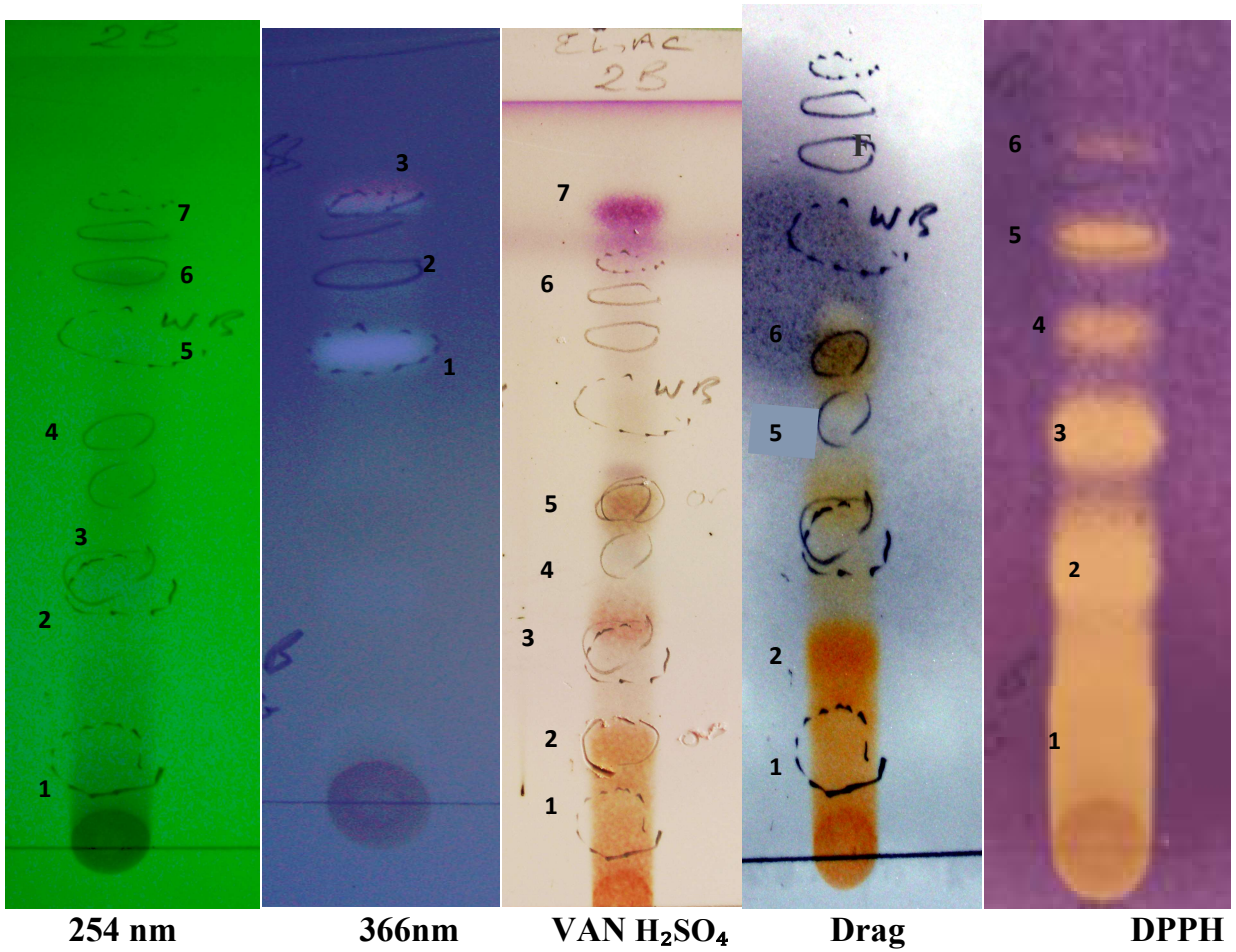

Figure 4 : G.tenax stem bark ethyl acetate NP-TLC chromatograms using different diagnostic reagents and Toluene : EtOAc: Formic acid 5 : 4: 1 solvent system

Table (3): TLC Profiles of the petroleum ether fractions of Grewiatenax stem bark

Comp. spot	R_f	UV Reaction		Compounds Colour Reactions to Diagnostic Spray Reagents				Expected Metabolites & Antioxidant activity (+/-)
		254 nm	366 nm	Van. H_2S0_4	NPR 366 nm	Dragendorff	DPPH	
1	0.19	-ve	Blue	-ve	Yellow	-ve	-ve	Phenolic (-)
2	0.23	-ve	Blue	-ve	yellow	-ve	-ve	Phenolic (-)
3	0.32	-ve	pink	-ve	-ve	-ve	-ve	Chlorophyll (-)
4	0.35	-ve	pink	Faint blue	-ve	-ve	-ve	Terpenoid (-)
5	0.61	-ve	violet	Faint blue	Yellow	-ve	-ve	Terpenoid (-)
6	0.89	quench.	blue	violet	Blue	-ve	-ve	Terpenoid (-) phenolic acid (-)

Table (4) Antimicrobial activities of the extracts of *Grewiatenax* stembark

Extract	Measurement of inhibition zones diameter (MIZD) in (mm)						MIC mg/ml
	*Bacteria				*Fungi		
	S. a	B. s	P. a	E. c	A. n	C. a	
Methanolic	_	17	_	12	_	_	50
PE	_	11.5	_	_	_	_	_
CHCL3	_	_	_	_	_	_	_
EtOAc	_	12	_	11.5	_	_	_

B. s = *Bacillus subtitles.* S. a = *Staphylococcus aureus,*E.c= *Escherichia coli,* P. a = *Pseudomonas aeruginosa,* A. n = *Aspergillusniger,* C. a = *Candida albicans
MIZD (mm): > 18 mm: Sensitive; 14— 18 mm: intermediate; < 14 mm: Resistant

CONCLUSIONS AND RECOMMENDATIONS

Grewia spp. was reported to have antioxidant, anti-microbial against different fungi, gram-positive and gram-negative bacteria, in addition to other pharmacological activity. Among the important metabolites in the genus Grewia flavonoids and β-carboline alkaloids are the most important. Biological activities of β-carboline alkaloids include antioxidant, inhibition of platelets aggregation. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, antiinflammatory, vasodilating actions. These pharmacological effects are generally linked to the antioxidant properties of these molecules.

G. tenax (Gudiem) is wide spread in northern Kordofan, central Sudan and the southern part of the country. To-date no phytochemical report is available for G. tenax. Plant studied stem bark extracts were subjected to biological and chemical

screening implementing different analytical methods.

CONCLUSIONS

- None of the screened extracts were active against the tested fungi. Additionally the methanolic extract possessed antibacterial activity against Bacillus sublilis bacteria with an MIC of 50 mg/ml.
- TLC revealed the presence of terpenoids and phenolics in all tested fractions. Alkaloids were mainly accumulated in the ethyl acetate fraction and were unstable.
- Coumarines and flavonoides were mainly detected in the chloroform and ethyl acetate fractions using TLC with NPR reagent.
- Phenolics and alkaloids detected possessed antioxidant activity when sprayed with DPPH
- All extracts were quite safe in brine shrimp lethality assay

Recommendations

- Subjecting the different extracts obtained from Grewiatenax bark to other biological activities e.g. antiparasitic, antimycetoma, etc.
- Identification of compounds in the active extracts

REFERENCES

[1] El Gazali GEB, 1997. Medicinal plant of the Sudan. In: Medicinal plants of northern kordofan. Omdurman Islamic university printing and publishing house, Omdurman.

[2] Angeh ,J.E, (2005).Isolation and characterization of antibacterial compounds present in member of Combretum section Hypocrateroptis .Phd Thesis University of Pretoria

[3] FAO (1988). Traditional Food Plants: A Resource Book for Promoting the Exploitation Consumption of Food Plant in Arid, Semi-arid and Sub-humid Lands of Eastern Africa. Rome: FAO Food and nutrition paper 42.

[4] Elhassan Mohammed GO, Yagi SM 2010. Nutritional Composition of Grewia Species (Grewiatenax (Forsk.) Fiori, G. flavescensJuss and G. VillosaWilld) Fruits. *Advance Journal of Food Science and Technology; 2 Suppl* 2010; 3: 159-162.

[5] Ahmed E, Sharif A, Hussain S, Malik A, Hassan Mu, Munawar MA, Nagra SA, Anwar J, Ashraf M, Afza N, Athar M. Phytochemical and Antimicrobial Studies of Grewiatenax. J.Chem.Soc.Pak 2011; 33Suppl 5: 676-681.

[6] Neuwinger, H.D, African traditional medicine.Adictionary of plant use

and application Med. Pharm. Press .Stuttgart , Germany: 2000; 46.

[7] El-Kher YM and Salih MH. Investigation of certain plants used in Sudanese folk medicinal Fitoterabia, 1980; 51:143.

[8] Kavanagh, 1972. , VoI. 11. Academic press, New York, London.

[9] Adeleye, I.A.; Ogunniyi, A.A. and Omonigbehin,E. A., Antimicrobial activity of some local herbs on common skin pathogens. *Bioscience Research Communication, 2003;* 15 (3): 231-236.

[10] Meyer, BN.; Ferrigni, NR; Putnam, JT.; Jacobsen, LB.; Nichols, DE.; McLaughlin, JL. Brine Shrimp: A convenient general bioassay for active plant constituents. PlantaMedica, 1982; 45: 31-34.

[11] Patil P, Patel MM, Bhavsar CJ. Preliminary Phytochemical and Hypoglycemic Activity of Leaves of GrewiaAsiatica L. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2011; 2 Suppl 1: 516-520.

[12] Wagner, H.; Bladt, S. and Zgainsk, E. M. (1984). Plant Drug Analysis a Thin Layer Chromatography, Springer Verlgc, Heidelberg New York.

[13] Kumar GS, Swamy BMV, Babu VLA, Sridhar Chandanam Rao TS, Tarakaram K . HPTLC estimation of harmine from the stem bark of SymplocosracemosaRoxb. Asian J Chem. 2006; 18(4):2851–2855.

[14] Berlin, J, Rugenhagen, C, Kuzovkina, I, Fecker, L, Sasse, F. "Are tissue cultures of Peganumharmala a useful model system for studying how to manipulate the formation of secondary metabolites?." Plant Cell, Tissue and Organ Culture, 1994; 38:289-297.

[15] Rice-Evans C. A., Miller J.M., and PagangaG. Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Radic. Biol. Med. 1996; 20,933_956.

[16] Mohammed, A., A.B. Adelaiye, A.G. Bakari and M.A. Mabrouk. Antidiabetic and some hematological effects of ethyl acetate and n-butanol fractions of Ganoderma lucidum aqueous extract in alloxan-induced diabetic wistar rats. International Journal of Medicine Sciences, 2009; 1 (12): 530-535, 2009.

[17] ZaidiMA,CrowSAJr. . Biologically active traditional medicinal herbs from Balochistan ,Pakstain . J. Ethonpharmacol . 2005; 96 (1-2):33 1-4 .

[18] Grierson DS ,Afolayan AJ . Antibacterial activites of some indigenous plants used for the treatment of wounds in the Fastern cape , South Africa .J Ethnophamacol1999;66(1):103-6.

[19] Jaspers MW,Bashir AK ,Zwaving JH , Malingre TM . Investigation of grewiabiolorJuss.J Ethno pharmacol 1986.; 17 (13) :205 - 110.