Antimicrobial activity of extracts of *Vernonia amygdalina* leaves from cultivated mother plants and progeny

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Abstract: The antimicrobial activity of *Vernonia amygdalina* chloroform ($CHCl_3$) and methanol (MeOH) leaves extracts were assayed against standard microorganisms. Two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), and one Gram negative (*Pseudomonas aeruginosa*) and two fungi (*Aspergillus niger* and *Candida albicans*). The leaves were harvested from mother plants and atissue culture raised progeny growing at the experimental area of Medicinal and Aromatic Plants and Traditional Medicine Research Institute. Discdiffusion method was used for determination of the inhibition zone as indicator of the antimicrobial activity. The extracts from *V. amygdalina* exhibited varying degrees of inhibition activity against the studied bacteria and fungi. The highest value of inhibition (30 mm) obtained was on *Staphylococcus aureus* using $CHCl_3$ extract, while the lowest activity was found in the MeOH extract of the Mother plants on tested organisms, comparison with the same extract of progeny. *B. subtilis* and *S. aureus* were highly susceptible to the$CHCl_3$ extracts of both mother plant and progeny, but *P. aeruginosa* was less sensitive to all different types of the studied extracts.Both types of extracts ($CHCl_3$ and MeOH) of the leaves from mother plants showed low to moderate activity against both fungi (*A. niger, C. albicans*) compared to the extracts from progeny. From this study it appears that the leaves extracts from *V. amygdalina*, either frommother plants or progeny had a comparable antimicrobial efficiency, which gives opportunities to propagation and commercial production of this multipurpose plant in Sudan. This result also suggested that *V. amygdalina*may have individual components that can be used as antibacterial and antifungal.

Key words: Antimicrobial, extracts, cultivated, tissue cultured leaves,*Vernonia amygdalina*

1. Introduction

Medicinal plants are known to produce bioactive compounds, which have been used traditionally

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to treat various ailments (Adebanjo*et al*. 1983). Most of the substances or chemical material which are used as antimicrobials,are produced by plants and microorganisms(Pelczar*et al*. 1993)**.***Vernoniaamygdalina* Del. (Asteraceae) is a small shrub about 5m high with dark green leaves about (5x15cm).The plant height is between 1m and 6m (Iwu and Kokwaro, 1996;Nwosu*et al*. 2013). The plant has different uses in traditional medicine and food. *V. amygdalina* leaves are consumed as vegetable due to their richness in minerals and vitamins (Nwosu*et al*. 2013;Sobukola*et al*. 2007). In Sudan, the water extract of *V. amagdalina*roots is used as an anthelmintic to treat stomach pain, skin infections, for swellings and a poultice (El Ghazali, 1987).

It has been used to treat amoebic dysentery, gastrointestinal disorders, as well as for its antimicrobial and antiparasitic activities (Akah and Ekekwe, 1995; Akinpelu, 1999;Moundipa*et al*. 2000; Hladik*et al*. 2005).This various uses have been referred to itsphytoconstituentssuch as alkaloids, saponins, flavonoids, terpenes, steroids, anthraquinone, lignans, phenolic acids, coumarins, xanthones, edotides and sesquiterpenes, (Izevbigie, 2003; Cimanga*et al*. 2004; Muraina*et al*.2010).More than thirty bioactive compounds have been isolated and identified from this plant, such as sesquiterpene lactones, steroidal saponins(vernoniosides), steroid glycosides and flavonoids (Kupchan*et al*. 1969; Jisaka*et al*. 1992; Igile*et al*. 1995;Erasto*et al*. 2006; Owoeye*et al*. 2010; Luo*et al*. 2011).The crude extracts from each parts of this plant are used to treat malaria and eczema due to a quinine substitute in leaves, stem, bark, androot (Challand and Willcox,2009).

In vitro and *in vivo*toxicology studies documented on*V. amygdalina* showed that this plant has no or low toxicity thereby (Awe*et al*. 1999; Ibrahim *et al*. 2001; Ekpo*et al*.2007; Ganfon*et al*.2008; Njan*et al*. 2008).Many researchers have studied the antimicrobial properties of tested plant extracts(Anibijuwon*et al*. 2012;Evbuomwan*et al*. 2018;Bamigboye and Ahmed, 2019), but there is no report for the study comparing different sources of the planti.e mother and progeny. Therefore, the aim of our study is to investigate theantimicrobial activity ofchloroform ($CHCl_3$) and methanol (MeOH)extracts of *V. amygdalina*leaves (figure 1:A.) obtained from two plants origin (mother and progeny) cultivated in Sudan.

2.Materials and methods

2.1. Plant material

Seedlings of *Vernoniaamygdalina* were initially supplied from the origin habitat (Rashad, South Kordofan- Sudan) and raised as mother plants in an experimental field of Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI), Khartoum state. A tissue culture technique has been used to establish a population of progenies which are kept at the experimental farm of MAPTMRI. Leavesfrom both mother plants and progeny were collected separately and air dried under room condition before extraction.

2.2. Preparation of plants extracts

The extracts were prepared in different types of solvents, namely chloroform and methanolat room temperature by simpleextraction method (Sukhdev*et al*. 2008), hence,leaves sample from the mother and progeny (100g each)were placed in separate sterile glass flasks.500 ml of chloroform was added to each flask, then left tomacerate for three days at room temperature, then after this extraction period, extracts werefiltered through Whatman No. 1 filter paper, and these filteredextracts were concentrated under reduced pressure using a rotaryevaporator. Solvent (chloroform) were removed from the concentratesby incubated them into room temperature at 37°C for 24 h. after fulldried extracts from each plant, only 2 g from each plant extractwere added to 2 ml of distilled water (0.1ppm). The same steps were used for 80% methanol extraction.

2.3. Preparation of bacterial and fungal suspension

Standard microorganisms of bacteria (*Bacillus subtilis*(NCTC 8236),*Staphylococcus aureus*(ATCC 25923)and *Pseudomonas aeruginosa*(ATCC 27853)) and fungi (*Aspergillusniger*(ATCC 9763)and *Candida albicans*(ATCC 7596)) were obtained from the Department of Microbiology, Medicinal and Aromatic Plants and Traditional Medicine Research Institute (MAPTMRI, NCR. Khartoum. Sudan).They were routinely subcultured in appropriate agars for the purpose of purity and maintained at 4ºC until further use.

2.4. Biological assay of antimicrobial activity

The determination of the biological activity of chloroform and methanol crude extracts of cultivated and tissue cultured leaves of *V.amygdalina*were tested according to the disc diffusion method(NCCLS,1999; Duru *et al*. 2003).Bacterial and fungal suspensions were diluted with sterile physiological solution to 10^8CFU/ ml (Turbidity = McFarland standard 0.5). Tested bacterial and fungal suspensions were swabbed uniformly onsurface of nutrient agar for bacteria and Sabouraud dextrose agar for fungi, sterilized filter paper discs(Whatman No.1, 6 mm in

diameter) were placed on the surface of each plate and soaked with 20 µl of a solution ofeach type of extracts separately. Then inoculated plates were incubated into roomtemperature in the inverted position to allow growth of tested organisms,and for each treatment were used in triplicate, then after 24 h,the diameters around thefilter paper disc with plant extract were measured, as the diameters of clear zones (mm).

2.5. Statistical Analysis

Statistical analyses of inhibition of microbial growth were subjected to one way analysis of variance (ANOVA) using MSTATCsoftware (Michigan state University, 1991).

3. Results andDiscussion

The antimicrobial activity was assayed and data on effect of *V.amygdalina*chloroform and methanol extracts on the growth of bacteria (*Bacillus subtilis,Staphylococcus aureus*and *Pseudomonas aeruginosa*) and fungi (*Aspergillusniger* and *Candida albicans*)were presented in Table (1). The mean diameter of inhibition zones (MDIZ) produced by the candidate crude extracts against the studied organisms is presented as screening antimicrobial activity (100 mg/ml). The results were expressed in terms of the diameter (mm)of the growth-inhibition zone (clear zones) according to Mukhtar and Ghori(2012). The extracts of studied plant exhibited varying degrees of inhibition activity against studied bacteria and fungi ranging from 11 to 30 mm. Moreover,the inhibition activity value of the same crude extract from the same source of planthas changed according to the tested organisms.

The both types of the leaves extracts reduced the growth of both bacteria and fungi studied. Where, inhibition zones of 30 mm and 11 mm were recorded as the maximum and the minimum diameters of inhibition obtained.The $CHCl_3$ extract of leaves obtained from mother plant has the highest value (30 mm)of inhibition zoneon *S.aureus*, while the lowest diameter of inhibition zone was found in the methanolic extract of the leaves of mother plant on *S.aureus*in comparison with the same extract of progeny.

Gram positive bacteria *B.subtilis*and *S.aureus*were highly sensitive to the$CHCl_3$ extracts of both mother plant (28 mm, 30mm) and progeny (25 mm, 28 mm).But Gram negative*Ps.aeruginosa*was less sensitive to all different types of the studied extractsranging (11-13 mm).This variation with two bacteria was due to their relative composition of cell wall components (Evbuomwan*et al*. 2018).

Both types of extracts($CHCl_3$ and MeOH) of the leaves from mother plants showed low to moderate activity against both fungi (*A.niger, C.albicans*) compared to the extracts from progeny. The methanol extractof theleaves from progeny had a good activity than mother plants, and the same result found in extracts of chloroform.

From the Statistical analyses of inhibition of microbial growth, the chloroform extract of leaves obtained either from mother plants or its progeny showed the best inhibition of the bacterial growth (28 mm &30 mm), while the methanol extract showedmoderate activity (13 mm to 17 mm).

In the previous studythe extracts of *V. amygdalina*showed antibacterial activity against several types of bacteria such as Gram positive (*Staphylococcus aureus,Streptococcus mutans, Bacillus subtilis, Bacillus megaterium, and Serratiamarcescens*), and Gram negative (*Escherichia coli, Pseudomonasaeruginosa*and *Klebsiellapneumoniae*), and antifungal activity against (*Aspergillusniger, Candida albicans, and Penicilliumchrysogenu*)(Anibijuwon*et al*. 2012;Evbuomwan*et al*. 2018;Bamigboye and Ahmed, 2019). However, in the present study, the chloroform and methanolic extracts of *V. amygdalina*also showed a good antimicrobial activity regarding to the different sources of leavesand extracts.

The antimicrobial activity of *V. amygdalina*seemed to be more dependent on the solvent used for extraction and this result is in agreement with earlier studies work by (Evbuomwan*et al*. 2018). From our study it is clear that the tested standard microorganisms (bacteria and fungi) were differentially affected by the methanol and chloroform extracts.

Chloroform extracts were observed to have more antimicrobial activity compared to the methanol extracts. The same results have been reported by Habtamu*et al.*(2018), they attributed their results to presence of vernolide,which has previously been confirmed in aerial parts of *Vernonia amygdalina* (Zdero*et al*. 1991; Jisaka*et al*. 1993), The structure of this compound has been established with NMR and MS spectroscopic techniques, found as white needle crystals, and has mono-hydroxylated compound belong to elemanolide lactone system(Erasto*et al*. 2006), vernolide has been reported to have antibacterial activity and antifungal activity (Erasto*et al*. 2006; Luo*et al*. 2017). Furthermore, vernolide has been considered to possess antitumor activity (Alara*et al*. 2017).

Previous research and evaluation of antimicrobial activity of *V. amygdalina* was done using leaves from wildly growing plant (Magboul*et al.*1997). This is first report on antimicrobial activity of leaves extracts from cultivated plants (progeny) of *V. amygdalina*.

4. Conclusion

Based on the results in our study it seems that *V. amygdalina*from mother plant or progenyhad a comparable antimicrobial efficiency,which gives many opportunities for propagation and commercial production of this threatened plant species in Sudan using tissue culture techniques. The results also support the traditional uses of *V. amygdalina*and further investigation is needed to standardize and scale up the extract to develop new antimicrobial formula from this plant.

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Table 1. Antimicrobial activity of *V. amygdalina* chloroform and methanol extracts against tested microorganisms.

Family/Botanical /Vernacular name	**Source used (leaves)**	**Types of extracts**	**Mean of Diameter Inhibition Zone (mm)**				
			B.s	***S.a***	***Ps.a***	***A.n***	***C.a***
Asteraceae, *Vernonia amygdalina* Garb al-wadi	Mother plant	$CHCl_3$	28	30	13	12	11
		MeOH	13	11	11	12	12
	Progeny	$CHCl_3$	25	28	12	15	14
		MeOH	17	14	12	13	15

Key: *B.s*: *Bacillus subtilis*, *S.a*: *Staphylococcus aureus*, *Ps.a*: *Pseudomonas aeruginosa*, *A.n*:*Aspergillusniger*, *C.a*: *Candida albicans*.

Concentration used 100 mg/ml, disc diameter: 6 mm

The antimicrobial activity was expressed in term of the diameter of zone of inhibition: < 9 mm zone considered as inactive; 9-12 mm as low activity; 13-18 mm moderate activity; and >18 mm high activity.

Figure 1: *Vernonia amygdalina*, A: Progenyplant; B: Leaves; C: Stem;D: Flower.

Figure 2: Preparation of plants extracts, A: Leaves with solvent; B: Filtered extract; C: Rotary evaporator (source: appletonwoods.co.uk. 2021);D: Crude extract.

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