Research Article

Antimicrobial, Antioxidant activities and Total phenolic Contents of *Chrozophora plicata* and *Croton zambesicus* crude extracts

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Abstract

The aim of this study was to evaluate the antimicrobial, antioxidant activities and quantitative determination of total polyphenol, flavonoids and tannins contents of four extracts from *Chrozophora plicata* and *Croton zambisicus* plants. Extracts from plants were prepared by sequential maceration. The antimicrobial activity was evaluated against Gram positive and Gram negative as well as two fungi by disc diffusion method. Antioxidant activity was assessed based on the scavenging activity of the stable 2,2-Diphenyl- 1-picrylhydrazyl free radical (DPPH). Total polyphenolic, flavonoids and tannins contents were determined by spectrophotometric assays. Generally, the highest results of antibacterial activity showed in methanolic extract and *C. plicata.,* against *Bacillus subtilis* and *Staphylococcus aureus* with (inhibition zone (IZ) = 17mm) respectively. While, the highest results of antifungal activity against *Candida albicans.,* was recorded in the methanolic extract of *C. plicata* and *C. zambicicus* (IZ) =20mm), and ethyl acetate extract of *C. zambicicus* gave the best activity (IZ =22mm) against *Candida albicans.,* from the disc diffusion method. The highest scavenging radical activity was obtained from the methanol extracts of *C. plicata* (90%), *C. zambicicus* (88%) and ethyl acetate of *C. plicata* (88%). Quantitative analysis revealed that the total polyphenolic content was highest value showed in methanolic extract of *C. plicata* (157.66mg gallic acid equivalents/g). The total flavonoids content was highest value recorded from the methanolic extract of *C. plicata* (865.31mg quercetin equivalents/g), while, highest value of total tannins content was found in the methanol extract of *C. plicata* (451.92mg tannic acid equivalents/g).

***Keywords*:** *Chrozophora plicata, Croton zambisicus,* total phenolic, antimicrobial, antioxidant

Introduction

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the system of useful drugs (Mustafa and El-kamali, 2020). According to WHO (World Health Organization), more than 80% of the world's population relies on traditional medicines for their primary health care needs (Mustafa and El-kamali, 2019). Sudan is located in tropical Africa and has high plant diversity and a multinational population. In Sudan and other developing countries traditional medicine plays a major role particularly in rural regions due to both economic and cultural reasons (Mustafa et al., 200). Euphorbiaceae, the spurge family, is a large family of flowering plants with 300 genera and around 7,500 species. Most spurges are herbs, but some, especially in the tropics, are shrubs or trees (Gibbs, 1974).

Chrozophora plicata Monoecious, annual to perennial herb up to 50 cm tall; stem angular, much-branched from

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the base, densely hairy with stellate hairs, yellowish or pinkish. In Sudan pounded stems or whole plants are applied to wounds to improve healing. The plant is also used medicinally in Saudi Arabia, Pakistan and India, e.g. against jaundice and to purify blood (Abde Alsiede et al., 2015). *C. plicata* possesses ulcer protective principles and flavonoids may be responsible for gastroprotective activity (Kadiri and Rao, 2013).

The *Croton zambesicus* Muell. Arg (Syn. Name: *C. amabilis* Muell. Arg.) (Family euphorbiaceae). It is a species of widely spread in tropical Africa (Abdalaziz et al., 2016). The root used for menstrual pain" (El-Hamidi, 1970). "Also it used as anti malarial and anti diabetic in Sudan (Okokon et al., 2006). "Moreover it used as anti diabetic and malarial remedy in Nigeria (El-Hamidi, 1970). In Sudan the seed decoction usually used to treat cough, malaria and to relieve menstrual pain. Hence, there is need to investigate the antimicrobial, antioxidant activity and total phenolic contents of *C. plicata* and *C. zambisicus* crude extracts.

Material and Methods

Plant Material

The leaves of *Chrozophora plicata* and Seeds of *Croton zambesicus* were collected in March, 2023 from Obeid, North Kordofan State, Sudan. The plants species was taxonomically identified by Dr. Mubarak Siddig Hamad, herbarium Department of Taxonomy and Phytochemistry, National Center for Research, Sudan. The plants were washed thoroughly under running water to remove contamination and shade dried with active ventilation at ambient temperature for 5 days; the dried leaves and flowers were to fine powder using pistil and mortar.

Preparation of extracts

Separately, 20 g of dried powdered of each species were extracted consecutively by maceration in hexane, chloroform, ethyl acetate and methanol (400 mL each) using a shaker apparatus, for about 24 h at room temperature, filtered and then solvents were evaporated under vacuum using a rotary evaporator. The resultant dry extracts from each samples were weighted and stored at 4°C until used.

Antimicrobial activity

The bacterial cultures used were *Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 10145. The used fungi cultures were *Aspergillus niger* ATCC 9763 and *Candida albicans* ATCC 7596. Each extract (10 mg/disc) was tested using the disc diffusion method as described by Mbavenge and coworkers (Mbavenge et al., 2008).

Antioxidant activity

The antioxidant activity of the extracts was evaluated using the *in vitro* 2,2-Diphenyl- 1-picrylhydrazyl (DPPH) radical scavenging method (Grochowski et al., 2017)

Determination of total polyphenols content

Total polyphenolic content was determined by adopting the method described by Wolfe *et al.* (2003).

Determination of total flavonoids content

The total flavonoid content was determined by adopting the method described by Ordonez *et al.,*(2006).

Determination of total tannins content

Total tannins content was determined according to the procedure reported by Sun *et al*., (1998).

Statistical analysis

All the procedures for the descriptive analysis (mean and standard deviation) was used to discuss the results, assuming the normal distribution of the studied variables.

Results and discussion

Antimicrobial activity

Plants extract was studied displayed variable antimicrobial activity. The highest antibacterial activity was recorded against *B. sabtilis* and *S. aureus* exerted by methanolic extract of *C. plicata* (17mm) and methanolic extract against *E. coli* of *C. plicata* with inhibition zone of 15 mm in Table1. The highest antifungal activity against *C. albicans* was recorded from the ethyl acetate extract of *C. zambisicus* (22mm) while both the methanolic extract of *C. plicata* and *C. zambisicus* (20 mm). Also the methanolic extract of *C. zambisicus* (18 mm) gave the highest antifungal activity against *A. niger* followed by the n-hexane extract (15mm) of *C. zambisicus*. Saleh *et al.* (2009) evaluated the antibacterial effect of ethanolic and water extracts of *C. plicata* stems and leaves at different concentrations against four endemic bacteria *E coli*, *S. aereus*, *P. aeroginesa* and *P. mirabilis*.

Antioxidant activity

The highest scavenging radical activity was exerted by the *C. plicata* with the methanol extract gave highest activity (90%) followed by the ethyl acetate and methanolic extracts of *C. plicata* and *C. zambicicus* (88%) respectively. A previous study by Kumar *et al.,* (2013), on the antioxidant activity of *C. plicata* found that polar extracts from the leaves displayed higher antioxidant activity supported the results obtained in the present study.

Total polyphenolic, flavonoids and tannins contents

Methanol extracts from the *Chrozophora plicata* accumulated the highest total polyphenolic content (157.66±0.03mg GAE/g), while highest polyphenolic form

Table 1. Antimicrobial activity of *C. plicata* and *C. zambesicus*

Botanical name	Parts	Extract	Inhibition zones diameter (IZD) in (mm)					
			B. s	***S. a***	***E. c***	***P. a***	***A. n***	***C. a***
C. *plicata*	Leaves	N- hexane	NA	NA	NA	NA	NA	11±2.5
		Chloroform	9±0.58	12±6.93	NA	NA	11±1.53	9±1.53
		Ethyl acetate	NA	13±5.56	NA	NA	9±5.19	13±1.74
		Methanol	17±0.5	17±1.89	15±3.22	11±2.65	10±0.58	20±0.58
C. *zambesicus*	Seeds	n- hexane	13±1.7	12±2.52	11±1.16	12±2.64	15±4.17	12±2.09
		Chloroform	NA	11±1.00	NA	NA	8±5.85	9±5.51
		Ethyl acetate	7±6.08	10±1.16	NA	NA	10±5.78	22±6.81
		Methanol	13±1.1	12±0.58	14±1.74	9±0.58	18±5.51	20±4.17
Gentamicin*	SD	10µg/disc	15±04	130±1	17±24	14±01	NA	NA
Nystatin*	SD	10µg/disc	NA	NA	NA	NA	22±03	20±00

NA: not active, positive control (10µg/disc) B.s = *Bacillus subtiles.* S.a = *Staphylococcus aureus,* E.c = *Escherichia coli,* P.a = *Pseudomonas aeruginosa,* A.n = *Aspergillus niger,* C.a = *Candida albicans.* IZD (mm): > 18mm: Sensitive: 14-18mm : intermediate : < 14mm: Resistant.

ethyl acetate *C. zambicicus* (85.16±0.02 mg GAE/g) in Table3.The results were also in agreement with those obtained by Alsiede, *et al.* (2015). The highest total flavonoids content was obtained from the methanolic and ethyl acetate extracts of *C. plicata* (865.31±0.08 mg QE/g, 760.25±0.03 mg QE/g) respectively. It was reported that the concentration of flavonoids in plant extracts and nature of extracted flavonoids depends on the polarity of solvents used in the extract preparation (Tran et al., 2020). While the total tannins content in these two species was also in high abundance methanolic extract from *C. plicata* and *C. zambicicus* (451.92±0.09 and 181.11±0.09mg TAE/g respectively). A previous study by (Khurm et al., 2020) on the total tannins content of *C. plicata* reported values ranged between 64.57143 to 2533.213 mg/l for leaves. Variation in polyphenolic and falvonoids contents of the studied species from values reported for the same studied species in the literature could be attributed to different factors like geographical areas and climatic conditions for the growth of the plant (Tran et al., 2020).

Pearson content correlation analysis revealed that the scavenging activity of the studied plant extracts was mainly attributed to the total phenolics (R_2= 0.997) and tannins (R_2=0.998) content rather than their total flavonoids content (R_2 = 0.09915). Several researchers reported significant correlation between the phenolic content and antioxidant activity of extracts (Sanoria et al., 2020). Thus the highest

Table 2. Antioxidant activity of *C. plicata* and *C. zambesicus*

Plant name	Organ	Extracts	%RSA±SD (DPPH)
C. plicata	Leaves	N- hexane	25±0.05
		Chloroform	67±0.09
		Ethyl acetate	88±0.02
		Methanol	90±0.01
C. zambesicus	Seeds	N- hexane	38±0.12
		Chloroform	23±0.01
		Ethyl acetate	28±0.15
		Methanol	88±0.00
Standard	SD	Propyl gallate	94±0.01

RSA=Radicals scavenging; DPPH= 2,2, Diphenyl-1- Picrylhydrazzyl.

Table 3. Total phenol content, total flavonoids and total tannin of *C. plicata* and *C. zambesicus*

Botanical name	Extracts	Total phenol content (Y=0.005X+0.000) R_2= 0.998	Total flavonoids content (Y=0.0012X+0.0958) R_2= 0.09915	Total tannine content (Y=0.002X+0.591) R_2= 0.997
Chrozophora plicata	N- hexane	81.19±0.01	500.37±0.03	156.96±0.11
	Chloroform	52.13±0.01	433.22±0.05	30.67±0.01
	Ethyl acetate	115.51±0.01	760.25±0.03	133.48±0.02
	Methanol	157.66±0.03	865.31±0.08	451.92±0.09
C. zambesicus	N- hexane	62.59±0.01	212.54±0.00	0
	Chloroform	94.35±0.23	150.88±0.00	41.74±0.02
	Ethyl acetate	85.16±0.02	249.39±0.00	118.85±0.06
	Methanol	78.13±0.08	208.69±0.06	181.11±0.09

GAE: Gallic acid equivalent; QE: Quercitin equivalent; TAA: Tannic acid equivalent

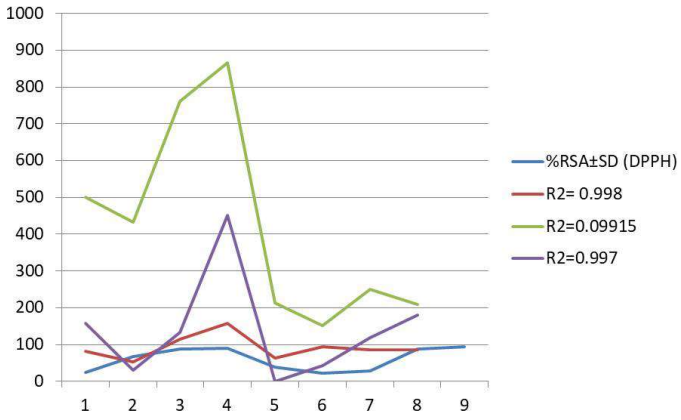

Figure 1. Correlation analysis between scavenging activity and the total polyphenolics, flavonoids and tannins contents. Red line = total polyphenolic content / scavenging activity; Green line = total flavonoids content / scavenging activity; Violet line = total tannins content / scavenging activity

content of polyphenolic and flavonoids in the polar extracts of *C. plicata* and *C. zambicicus* supported their contribution in their antiradical activity.

Conclusions

The inhibitory zones of different extracts varied with the type of microorganism tested. Generally, extracts of the plants exhibited better antifungal activity than antibacterial one with highest antifungal activity against *C. albicans* and *A. niger* was recorded from the ethyl acetate extract of *C. zambicicus*. The highest scavenging radical activity was exerted by the two plants species. The majority of extracts were rich in flavonoids while the polyphenols were mainly accumulated in the two polar extracts. Therefore, these plants could a very beneficial source of natural bioactive agents.

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