

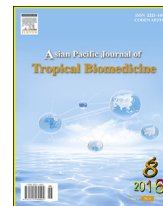
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ABSTRACT

Objective: To search for the most active antimicrobial and antioxidant sub-fractions related to traditional use of *Ziziphus oxyphylla* (*Z. oxyphylla*) and *Cedrela serrata* (*C. serrata*) in Pakistan against infectious and liver diseases.**Methods:** Fractions of different polarity were tested *in vitro* for their antiprotozoal, antimalarial, antibacterial and antifungal activity against different pathogens. Cytotoxicity on MRC-5 cell lines (human lung fibroblasts) as well as, *in vitro* radical scavenging activity was evaluated using the 1, 1-diphenyl-2-picrylhydrazyl radical assay.**Results:** The highest antiprotozoal activity was observed for the CHCl₃ fractions of *Z. oxyphylla* roots and leaves, and *C. serrata* bark. The CHCl₃ and EtOAc fractions of *Z. oxyphylla* roots, the CHCl₃ fraction of *Z. oxyphylla* leaves, the EtOAc and the residual MeOH: H₂O fraction of *C. serrata* bark showed antibacterial activity against *Staphylococcus aureus*. The same residual MeOH: H₂O fraction of *C. serrata* bark was active against *Candida albicans*. The highest antioxidant activity was observed for the more hydrophilic EtOAc fractions of *Z. oxyphylla* leaves, *C. serrata* bark and leaves, and the residual MeOH: H₂O fraction of *C. serrata* bark.**Conclusions:** This study supports at least in part the traditional uses of these plants for antimicrobial purposes and against liver diseases.

1. Introduction

Cedrela serrata (Meliaceae) (*C. serrata*) and *Ziziphus oxyphylla* (Rhamnaceae) (*Z. oxyphylla*) (sometimes spelled as

Zizyphus) are two plants indigenous to Pakistan, particularly the Swat area, used in traditional medicine. Both plants grow at a higher altitude and at cold rainy places. In an ethnobotanical survey on fuel wood and timber plant species from Pakistan, a decoction of leaves of *C. serrata* was reported for its use against diabetes [1]. Leaves and bark are used to treat fever, diabetes, dysentery, blood diseases, skin diseases *etc.* [2], indicating antimicrobial properties. An *in vitro* screening of leishmanicidal activity in Myanmar timber extracts revealed potent activity for *C. serrata* [3]. Also for *Z. oxyphylla* traditional antidiabetic activity has been reported [4], as well as its use against jaundice [5]. Various parts of this plant are used traditionally as remedy

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of pain, diabetes, allergy, fever, rheumatism and pain [6]. Various species of *Ziziphus* are traditionally used in Ayurveda against diabetes, fever, skin infections, urinary troubles, etc. [7]. Therefore, the plant parts from *C. serrata* and *Z. oxyphylla* that are traditionally used, i.e. leaves/stem bark and leaves/root, respectively, will be biologically evaluated in the present work in various assays related to some of their reported indications.

Fractions from both plant species extract will be evaluated in an integrated antimicrobial screen including selected protozoa, fungi and bacteria [8]. Bacterial and fungal infections remain important health issues all over the world, especially because of growing resistance against current antibiotics. Parasitic diseases such as trypanosomiasis and leishmaniasis, which are considered as neglected diseases, are still among the most lethal and major health problems, affecting the poor population of the world in developing countries. A large number of serious health issues are concerned with these organisms i.e. *Trypanosoma cruzi* (*T. cruzi*) is responsible for Chagas disease (American trypanosomiasis), and causes damage to various organs, more in particular the heart, lower intestines and esophagus. Similarly, *Trypanosoma brucei* (*T. brucei*), the causative agent for sleeping sickness (African trypanosomiasis), is associated with blood clotting and progresses towards chronic meningoencephalitis [9]. Malaria is considered as the most alarming parasitic disease affecting half of the population of the world. There were an estimated 219 million cases of malaria and 660 000 deaths in 2010 [10]. The most important parasite causing the malaria disease, *Plasmodium falciparum* (*P. falciparum*) has acquired resistance to most of the antimalarial drugs available nowadays.

With regard to the use of *C. serrata* and *Z. oxyphylla* against liver diseases and diabetes, reactive oxygen species are known to play an important role in these pathologies [11,12]. Therefore, the same fractions evaluated for their antimicrobial activity will be evaluated for their radical scavenging activity as well. Antioxidant and DNA protection activities have been reported in leaves extract and fractions of *C. serrata* [13].

2. Materials and methods

2.1. Plant material

Z. oxyphylla and *C. serrata* were collected from local mountain of Barikot, Swat, Khyber Pukhtoon Khwa, Pakistan. Leaves and roots of *Z. oxyphylla* and leaves and bark of *C. serrata* were collected in September and October 2009. Plants were identified by Prof. Dr. Mansoor Ahmad, University of Karachi and voucher specimens (0012–2009/AZ and 0013–2009/BC, respectively) were deposited at the Laboratory of Pharmacognosy, Research Institute of Pharmaceutical Sciences, University of Karachi, Pakistan. All plant material was cleaned with water and dried under shade for 10–12 days.

2.2. Extraction and fractionation

The dried plant parts were pulverized and about 1.5 kg of each part was macerated for 10 days in MeOH. Then MeOH was decanted and filtered through Whatman No. 1 paper. The filtered solutions were reduced to a semi-solid mass under reduced pressure at 40 °C to yield four different crude extracts viz. *Z.*

oxyphylla root (ZR) (508.7 g), *Z. oxyphylla* leaves (ZL) (600.3 g), *C. serrata* bark (CB) (482.4 g), and *C. serrata* leaves (CL) (640.5 g). One hundred grams of each crude extract was suspended in 80% MeOH (200 mL) and further partitioned with solvents of differing polarities (3 × 300 mL each): *n*-hexane (fraction H), CHCl₃ (fraction C), EtOAc (fraction E) and *n*-BuOH (fraction B); the residual MeOH-H₂O fraction was denoted as fraction M. The following subfractions were obtained with their respective yields: *Z. oxyphylla* roots: ZRM (65 g), ZRH (100 mg), ZRC (14 g), ZRE (10 g), ZRB (6 g); *Z. oxyphylla* leaves: ZLM (39 g), ZLH (22 g), ZLC (12 g), ZLE (14 g), ZLB (8 g); *C. serrata* bark: CBM (36 g), CBH (80 mg), CBC (10 g), CBE (30 g), CBB (21 g); *C. serrata* leaves: CLM (30 g), CLH (18 g), CLC (32 g), CLE (15 g), CLB (4 g).

2.3. Phytochemical screening

Phytochemical screening was carried out for each extract by thin layer chromatography on precoated silica gel plates (layer thickness 0.25 mm) using different chemical reagents and mobile phases for the identification of major phytochemical groups. Alkaloids were identified using Dragendorff's reagent [14], whereas flavonoids were determined by spraying 1% AlCl₃ [15].

2.4. Standard drug and strain used

All the fractions were tested against parasites *T. brucei*, *T. cruzi*, *Leishmania infantum* (*L. infantum*) and the chloroquine and pyrimethamine-resistant K1 strain of *P. falciparum*, and for cytotoxicity on MRC-5 cells as well as against bacteria *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and the fungi *Trichophyton rubrum* (*T. rubrum*), *Aspergillus fumigatus* (*A. fumigatus*) and *Candida albicans* (*C. albicans*) according to Cos et al. [8]. Standard drugs used during this assay as positive controls included chloramphenicol for *E. coli* (IC₅₀ 4.76 μmol/L), erythromycin for *S. aureus* (IC₅₀ 11.40 μmol/L), benzimidazole for *T. cruzi* (IC₅₀ 2.09 μmol/L), miltefosine for *L. infantum* (IC₅₀ 10.41 μmol/L), suramine for *T. brucei* (IC₅₀ 0.02 μmol/L), chloroquine for *P. falciparum* K1 (IC₅₀ 0.35 μmol/L), and tamoxifen for MRC-5 cytotoxicity (CC₅₀ 11.39 μmol/L), while miconazole was used for the fungi *T. rubrum* (IC₅₀ 2.08 μmol/L) and *A. fumigatus* (IC₅₀ 0.16 μmol/L), and the yeast *C. albicans* (IC₅₀ 5.04 μmol/L). All these reference drugs were obtained from Sigma–Aldrich or WHO-TDR. The strains used included *S. aureus* (ATCC 6538), *E. coli* (ATCC 8739), *P. falciparum* (K1), *L. infantum* (MHOM/MA(BE)/67), *T. brucei* (Squib 427) and *T. cruzi* (Tulahuen c12), while for the cytotoxicity assay the cell line used was MRC-5 (SV2).

Standard stock solutions (20 mg/mL) were prepared. Five dilutions were prepared from the stock solution, i.e. 64, 16, 4, 1 and 0.25 μg/mL. IC₅₀ values (inhibitory concentration 50%) were calculated.

2.5. Antiplasmodial activity

For antiplasmodial activity, testing a resistant strain of *P. falciparum* (K1 strain) was selected. The strain was cultured in RPMI-1640 medium supplemented with 4% human erythrocytes and 10% human serum [8]. This culture was maintained at 37 °C providing the microaerophilic requirements of 93% N₂,

4% CO₂, and 3% O₂ atmosphere. Assay was performed in 96 well plates in such a way that, each well contained the extract dilution along with the parasite inoculums (1% parasitaemia, 2% haematocrit). Plate was incubated for 72 h and frozen at –20 °C. After freezing, in a separate plate, 20 µL of the haemolysed parasite suspension from every well of the incubated and freeze thawed plate was mixed together with 100 µL of Malstat reagent and 10 µL of a same ratio solution of phenazine ethosulfate (2 mg/mL) and nitro blue tetrazolium (0.1 mg/mL). This plate was placed for 2 h in dark and then any change in color was measured at 655 nm on a spectrophotometer.

2.6. Antileishmanial activity

For antileishmanial activity, *L. infantum* inoculum was prepared, by harvesting the infected donor hamsters spleen amastigote. The inoculum obtained was used to infect the host cells, murine peritoneal macrophages. These peritoneal cells were harvested after 24–48 h of time lap. After harvesting, 10⁴ cells/well of the peritoneal cells were seeded in each well of 96 well microtiter plates. Amastigote (10⁵) were added subsequently at each well and incubated at 37 °C for 2 h. These plates after the addition of pre-diluted extracts of the tested plants were kept for 5 days incubation at 37 °C and 5% CO₂. After 5 days, the plates were observed for results by, measuring the parasite burdens (mean number of amastigote/macrophage) through, microscopic assessment after Giemsa staining of 500 cells. Results were expressed as percentage of the blank controls without plant extract [16].

2.7. Antitrypanosomal activity

Two different types of trypanosomes were studied which included *T. brucei* and *T. cruzi*.

Trypomastigote (blood stream form) of *T. brucei* were grown in HMI-9 medium [8], incubated at 37 °C, maintained at an atmosphere of 5% CO₂, and provided with a fetal calf serum (FCS) (10%). For assay to perform, 10⁴ parasites were kept in each well of the 96 well micro plates. Plate was incubated at 37 °C, for 4 days. After incubation period, resazurin was added for assessment of growth of the parasite by fluorimetric method [17].

T. cruzi the cause of Chagas disease, was studied by maintaining the Tulahuen strain of *T. cruzi* on MRC-5 cells, on minimal essential medium additionally supplemented with 16.5 mmol/L sodium hydrogen carbonate, 20 mmol/L L-glutamine and 5% FCS. For assay to perform, 96 well tissue culture plates were used. Each well was dispensed with pre-diluted plant extract along with 3 × 10⁴ MRC-5 cells and 3 × 10⁴ parasites. Plate was incubated for 7 days at 37 °C. After incubation period, plate was observed for the growth of parasite, by adding the β-galactosidase substrate (chlorophenol red-β-D-galactopyranoside). The color reaction was examined after 4 h at 540 nm. The absorbance values were calculated as percentage of the blank controls without the plant extract [18].

2.8. Antimicrobial activity

Antibacterial and antifungal study was performed for all the fractions. Antibacterial study was carried on *E. coli* and

S. aureus, while antifungal species consisted of *T. rubrum*, *A. fumigatus* and *C. albicans* [19]. Growth medium used for bacteria was Mueller–Hinton broth, while Sabouraud broth was used for fungi to be studied [20].

The inoculums used for this dilution method of microbes consisted of 10⁵ CFU/mL for bacteria and 10⁴ CFU/mL for fungi [21]. Inoculums were prepared from an overnight culture of the stock which was in the logarithmic growth phase.

Plant extracts in pre-prepared dilutions were mixed with the medium in the micro plates containing the inoculums previously added to this medium. Plates were incubated at 37 °C for specified length of period. Results were interpreted spectrophotometrically by plate reader after addition of MTT and resazurin as redox indicator [22,23].

2.9. Cytotoxicity against MRC-5 cells

For cytotoxicity assay, MRC-5 cells were cultivated in minimum essential medium provided with 5% FCS, 16.5 mmol/L sodium hydrogen carbonate and 20 mmol/L L-glutamine.

After cultivation, 10⁴ MRC-5 cells were dispensed into each well of micro plate containing pre-diluted plant extract. Plate was incubated for 4–7 days, at 37 °C and 5% CO₂ atmosphere. After incubation, resazurin was added into wells and cell viability or proliferation was studied after 4 h of incubation at 37 °C. Fluorescence was measured as excitation 550 nm, emission 590 nm, and the results were interpreted as % reduction in cell viability of extract treated well compared to control [24].

2.10. Antioxidant activity [1,1-diphenyl-2-picrylhydrazyl (DPPH) method]

Antioxidant (radical scavenging) activity was performed accordingly [25]. DPPH, 100 µmol/L solution, was prepared and its absorbance was measured by UV/vis spectrophotometer (GENESYS 10 UV, Thermo Scientific). The test samples were prepared by dissolving 1 mg in 3 mL as stock solutions, and then three dilutions were prepared with a concentration of 1/2, 1/4, and 1/8 from this stock solution. Absorbance was measured (250 µL of each dilution + 1.5 mL of 100% DPPH) at 517 nm. Quercetin was used as the positive control (IC₅₀ 3.6 µg/mL). IC₅₀ values were calculated from the three successive dilutions run for each fraction, and analyzed by ANOVA (*P* = 0.05).

3. Results

The results for the aforementioned activities were shown in Tables 1–5. With regard to antiplasmodial activity (Table 1), the highest activity was observed for the CHCl₃ fractions of *Z. oxyphylla* roots and leaves, and *C. serrata* bark. For *C. serrata* leaves, the *n*-hexane fraction was the most active one, although also in this case the CHCl₃ fraction showed antiplasmodial properties. The same observations could be made for the other parasites tested (Table 2). With regard to *T. cruzi*, *L. infantum* and *T. brucei*, the CHCl₃ fractions of *Z. oxyphylla* roots and leaves, and *C. serrata* bark were the most potent ones; for *C. serrata* leaves, the *n*-hexane fraction was most active. None of the fractions was active against *E. coli* (Table 3), whereas the CHCl₃ and EtOAc fractions of *Z. oxyphylla* roots,

Table 1IC₅₀ value for antiparasmodial activity (µg/mL).

Plant	Part	Fraction	<i>P. falciparum</i>
<i>Z. oxyphylla</i>	Roots	Methanol: water	> 64
		Chloroform	5.25
		Ethyl acetate	13.2
		<i>n</i> -Butanol	> 64
<i>C. serrata</i>	Bark	Methanol: water	> 64
		Chloroform	22.8
		Ethyl acetate	> 64
		<i>n</i> -Butanol	> 64
<i>Z. oxyphylla</i>	Leaves	Methanol: water	> 64
		<i>n</i> -Hexane	24.1
		Chloroform	8.8
		Ethyl acetate	> 64
<i>C. serrata</i>	Leaves	Methanol: water	> 64
		<i>n</i> -Hexane	4.39
		Chloroform	29.3
		Ethyl acetate	> 64
		<i>n</i> -Butanol	> 64

the CHCl₃ fraction of *Z. oxyphylla* leaves, the EtOAc and the residual MeOH: H₂O fraction of *C. serrata* bark showed antibacterial activity against *S. aureus*. The same residual MeOH: H₂O fraction of *C. serrata* bark was active against *C. albicans*, being the only fraction affecting this yeast (Table 4). None of the fractions showed activity against *A. fumigatus*, whereas only the CHCl₃ fraction of the *C. serrata* leaves was active against *T. rubrum*.

Apparently, the CHCl₃ fractions of *Z. oxyphylla* roots and leaves, and the CHCl₃ fraction of *C. serrata* bark, also were the more cytotoxic ones against MRC-5 cells; only for *C. serrata* leaves, the *n*-hexane fraction looked more cytotoxic than the CHCl₃ fraction (Table 5).

Table 2IC₅₀ value for other antiparasitic activities (µg/mL).

Plant	Part	Fraction	Parasites		
			<i>T. cruzi</i>	<i>L. infantum</i>	<i>T. brucei</i>
<i>Z. oxyphylla</i>	Roots	Methanol: water	> 64	> 64	> 64
		Chloroform	1.84	32.4	21.1
		Ethyl acetate	6.06	> 64	59.1
		<i>n</i> -Butanol	> 64	> 64	> 64
<i>C. serrata</i>	Bark	Methanol: water	48.5	> 64	> 64
		Chloroform	1.37	> 64	34.8
		Ethyl acetate	28.5	> 64	> 64
		<i>n</i> -Butanol	33.7	> 64	> 64
<i>Z. oxyphylla</i>	Leaves	Methanol: water	> 64	> 64	> 64
		<i>n</i> -Hexane	22.9	> 64	29.7
		Chloroform	19.7	> 64	23.5
		Ethyl acetate	> 64	> 64	> 64
<i>C. serrata</i>	Leaves	Methanol: water	> 64	> 64	> 64
		<i>n</i> -Hexane	2.24	32.4	8.17
		Chloroform	31.6	> 64	> 64
		Ethyl acetate	> 64	> 64	> 64
		<i>n</i> -Butanol	> 64	> 64	> 64

Table 3IC₅₀ values for antibacterial activity (µg/mL).

Plant	Part	Fraction	Bacteria	
			<i>E. coli</i>	<i>S. aureus</i>
<i>Z. oxyphylla</i>	Roots	Methanol: water	> 64	> 64
		Chloroform	> 64	15.5
		Ethyl acetate	> 64	22.4
		<i>n</i> -Butanol	> 64	> 64
<i>C. serrata</i>	Bark	Methanol: water	> 64	12.3
		Chloroform	> 64	> 64
		Ethyl acetate	> 64	26.2
		<i>n</i> -Butanol	> 64	> 64
<i>Z. oxyphylla</i>	Leaves	Methanol: water	> 64	> 64
		<i>n</i> -Hexane	> 64	> 64
		Chloroform	> 64	54.8
		Ethyl acetate	> 64	> 64
<i>C. serrata</i>	Leaves	Methanol: water	> 64	> 64
		<i>n</i> -Hexane	> 64	> 64
		Chloroform	> 64	> 64
		Ethyl acetate	> 64	> 64
		<i>n</i> -Butanol	> 64	> 64

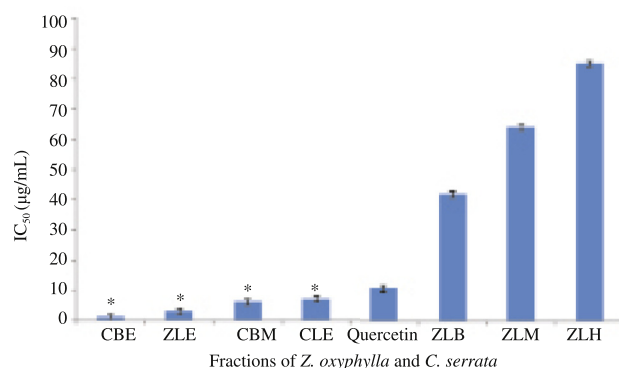
The highest antioxidant (radical scavenging) activity in the DPPH assay was observed for the EtOAc fractions of *Z. oxyphylla* leaves, and *C. serrata* bark and leaves, showing an IC₅₀ < 10 µg/mL (Figure 1). Also the residual MeOH: H₂O fraction of *C. serrata* bark was active in the same range. The DPPH scavenging activity of all fractions displaying an IC₅₀ < 100 µg/mL was shown in Figure 1; all fractions with an IC₅₀ > 100 µg/mL were summarized in Figure 2. The results indicated that fractions CBE, ZLE, CBM, and CLE had similar DPPH scavenging activity when compared with quercetin (*P* = 0.05).

Table 4IC₅₀ value for antifungal activity (µg/mL).

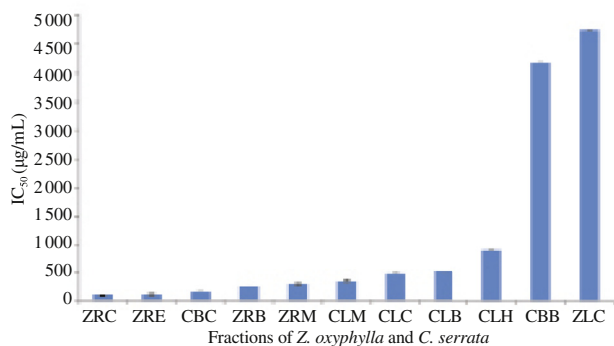
Plant	Part	Fraction	Fungi		
			<i>T. rubrum</i>	<i>A. fumigatus</i>	<i>C. albicans</i>
<i>Z. oxyphylla</i>	Roots	Methanol: water	> 64	> 64	> 64
		Chloroform	> 64	> 64	> 64
		Ethyl acetate	> 64	> 64	> 64
		<i>n</i> -Butanol	> 64	> 64	> 64
<i>C. serrata</i>	Bark	Methanol: water	> 64	> 64	39.3
		Chloroform	> 64	> 64	> 64
		Ethyl acetate	> 64	> 64	> 64
		<i>n</i> -Butanol	> 64	> 64	> 64
<i>Z. oxyphylla</i>	Leaves	Methanol: water	> 64	> 64	> 64
		<i>n</i> -Hexane	> 64	> 64	> 64
		Chloroform	> 64	> 64	> 64
		Ethyl acetate	> 64	> 64	> 64
<i>C. serrata</i>	Leaves	Methanol: water	> 64	> 64	> 64
		<i>n</i> -Hexane	> 64	> 64	> 64
		Chloroform	25.9	> 64	> 64
		Ethyl acetate	> 64	> 64	> 64
		<i>n</i> -Butanol	> 64	> 64	> 64

Table 5CC₅₀ (µg/mL) value for cytotoxicity (MRC-5 cells).

Plant	Part	Fraction	MRC-5
<i>Z. oxyphylla</i>	Roots	Methanol: water	> 64
		Chloroform	5.1
		Ethyl acetate	19.99
		<i>n</i> -Butanol	> 64
<i>C. serrata</i>	Bark	Methanol: water	43.71
		Chloroform	23.78
		Ethyl acetate	38.91
		<i>n</i> -Butanol	52.86
<i>Z. oxyphylla</i>	Leaves	Methanol: water	> 64
		<i>n</i> -Hexane	> 64
		Chloroform	18.22
		Ethyl acetate	> 64
<i>C. serrata</i>	Leaves	Methanol: water	> 64
		<i>n</i> -Hexane	23.99
		Chloroform	50.8
		Ethyl acetate	> 64
		<i>n</i> -Butanol	> 64

**Figure 1.** IC₅₀ values (< 100 µg/mL) for different fractions (mean ± SD).

*: Denote fractions with significantly similar activity to the positive control (quercetin).

**Figure 2.** IC₅₀ value (> 100 µg/mL) for different fractions (mean ± SD).

4. Discussion

Fractions of different polarity from roots and leaves of *Z. oxyphylla*, and bark and leaves of *C. serrata* were tested for antimicrobial activity against various organisms including bacteria, fungi and parasites, and for cytotoxicity against MRC-5 cells.

The results of the antimicrobial screening demonstrate that in general the lipophilic fractions, more in particular the CHCl₃

fractions, are the most promising in order to isolate active constituents. The phytochemical screening revealed that the CHCl₃ fractions contained alkaloids, which may be the reasons for antimicrobial activity. *Z. oxyphylla* is known to contain cyclopeptide alkaloids, such as oxyphylline A, nummularine C, nummularine R and hemsine A [7,26,27]. Antimicrobial activity has been observed for many constituents of this class [28]. Antibacterial cyclopeptide alkaloids were reported from *Condalia buxifolia* and *Scutia buxifolia* [29,30]. Ziziphine N and Q, isolated from *Ziziphus oenoplia* var. *brunoniana* showed antiplasmodial activity in the range of 3–4 µg/mL [31]. Mauritine K from *Ziziphus mauritiana* exhibited antifungal properties [32]. More recently, antiplasmodial and antimycobacterial cyclopeptide alkaloids have been isolated from the root of *Ziziphus mauritiana* with IC₅₀ values in the micro molar range [33]. Therefore, it can be hypothesized that the antimicrobial activity of the more lipophilic fractions of *Z. oxyphylla* is due to the cyclopeptide alkaloids. This study can be supported by the phytochemical screening as mentioned [34] which reports the presence of different phytochemical groups in these two plants. On the other hand, no particular hypothesis can be raised about the chemical nature of the active constituents of *C. serrata*, since no phytochemical studies have been reported yet.

The extracts contained flavonoids and therefore, it may be hypothesized that the antioxidative/radical scavenging activity of the more hydrophilic fractions is related to the presence of phenolic compounds. At least for the leaves, these results are in line with those obtained by Perveen *et al.* [13], who have reported antioxidant and DNA protection activities in leaves extract and fractions of *C. serrata*. This result also supports the study carried out for the antiglycation and antioxidant potential of the fraction of these two plants [34,35] as well as the presence of flavonoids phytochemical present as reported [34,36].

The results are in line with the already published literature on the two plants reporting the agar well diffusion antibacterial and antifungal studies [34,35].

The results give an idea to consider the fractions especially CHCl₃ fractions and the EtOAc fractions for further research studies *i.e.* isolation, structure determination and establishment of pharmacological activities based on the considerable results revealed in our present study. Similarly, the leaves, *n*-hexane fractions, also showed prominent results as mentioned in respective tables and thus can be considered for further research studies.

Altogether, these results support at least in part the traditional uses of these plants for antimicrobial purposes and against liver diseases. Hence, they should be further investigated to isolate and identify the active constituents.

Conflict of interest statement

We declare that we have no conflict of interest.

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