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Phytochemical Screening, Antifungal and Antibacterial Effect of *Zanthoxylum zanthoxyloides* and *Zanthoxylum macrophylum* Used in Traditional Medicine in Yamboro (Central African Republic)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EKK, LAT and IT designed the study, performed the statistical analysis wrote the protocol and the first draft of the manuscript. Authors LAT and EKK managed the analyses of the study. Authors IZ and JLSM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The knowledge of traditional medicine has always guided the search for new cures. *Zanthoxylum zanthoxyloides* and *Zanthoxylum macrophylum*, (Rutaceae) are used as medicinal plants in Central African Republic for bacteria and fungi treatment**.** The aim of this study is to investigate *in vitro* antibacterial and antifungal activities of water-alcohol extract of leave, bark and root of *Z. zanthoxyloides* and *Z. macrophylum* and the phytochemistry group of some secondary metabolic*.*
The results of analysis of variance on the antibacterial and antifungal effect of extracts of leaves of *Z. zanthoxyloides* and *Z. macrophylum* with high concentration (4 mg/disc) showed the effect of treatment was a very highly significant variation according to the plant, bacteria and fungi ($P < 0.001$). Extract of leaves *of Z. zanthoxyloides* is very effective on *Klebsiella pneumonae* with high concentration (4 mg/disc)*.* Extract of leaves *of Z. macrophylum* is very effective on *Candida albicans* with high concentration (4 mg/disc)*.* Extract of bark *of Z. zanthoxyloides* is very effective on *Candida albicans* with high concentration (4 mg/disc)*.* Extract of root *of Z. zanthoxyloides* is very effective on *C. albicans* with high concentration (4 mg/disc)*.*
Preliminary phytochemical screening reactions of the crude extracts of leaves, bark and root of *Z. zanthoxyloides* and *Z. macrophylum* showed the presence of tannins, alkaloids, saponines and flavonoids.

Keywords: Z. zanthoxyloides; Z. macrophylum; phytochemical; antifungal; antibacterial; yamboro.

1. INTRODUCTION

Traditional medicine based on plants is an alternative form of therapy and has become the mainstream throughout the world due to the growing resistance of pathogens to conventional medicine [1,2,3].

The search for a therapy which can help to overcome bacteria and fungi effectively remains a major concern for modern medicine [4,3]. The numerous cases of resistance to the conventional medicine products, environmental hazards and insufficient financial resources in Central African Republic (CAR) have drawn attention to safe means of controlling bacterial and fungal infections [5,4,3,6] Inferences from indigenous traditional practices have uncovered plant chemicals that are useful in traditional therapy [7,8,9] and Who [10] encouraged African countries of which Central African Republic is et part, to develop regional strategies on traditional medicine in order to begin research on medicinal plants and to promote their optimal uses in health service systems.

The screening of plant extracts and plant products for their antimicrobial activity has in most cases involved higher plants, many of which have shown clinical relevance as sources of potential chemotherapeutic agents [11,3,12]. The development of herbal products is dependent on local botanical flora. Medicinal plants are distributed worldwide and many abound in tropical countries. CAR has a rich variety of medicinal plants distributed in the different geoecological regions of the country. The Rutaceae constitutes the largest group of anparasitic plants with about 950 distributed in 77 genera [13]. The leaves and young twigs of the plants have been used in medicine to treat different diseases such as circulatory and respiratory disease problems, malaria, diabetes, and hypertension [13]. There is paucity of reports on the antimicrobial properties of these plants and no report exists on whether the hosts on which they are found could influence their antimicrobial property. The anti- bacterial and anti-fungal plants are not fully documented despite their wide use in traditional medicine in CAR.

The aim of the study was to evaluate the antimicrobial activity of extract of *Z. zanthoxyloides* and *Z. macrophylum*, medicinal plants used in traditional medicine in CAR for bacterial and fungal treatment**.**

2. MATERIALS AND METHODS

2.1 Site Description

The survey was carried out at Yamboro (Fig. 1). The climate in Yamboro is sub-humid with a bimodal rainfall distribution. This allows for the production of most annual crops during both the long (March–August) and the short rains (September–October). Altitude ranges between 1200 and 1500 masl. In fact, Yamboro is rich in biodiversity of plants and this site was chosen to represent a range of environments and management practices in traditional medicine systems in CAR.

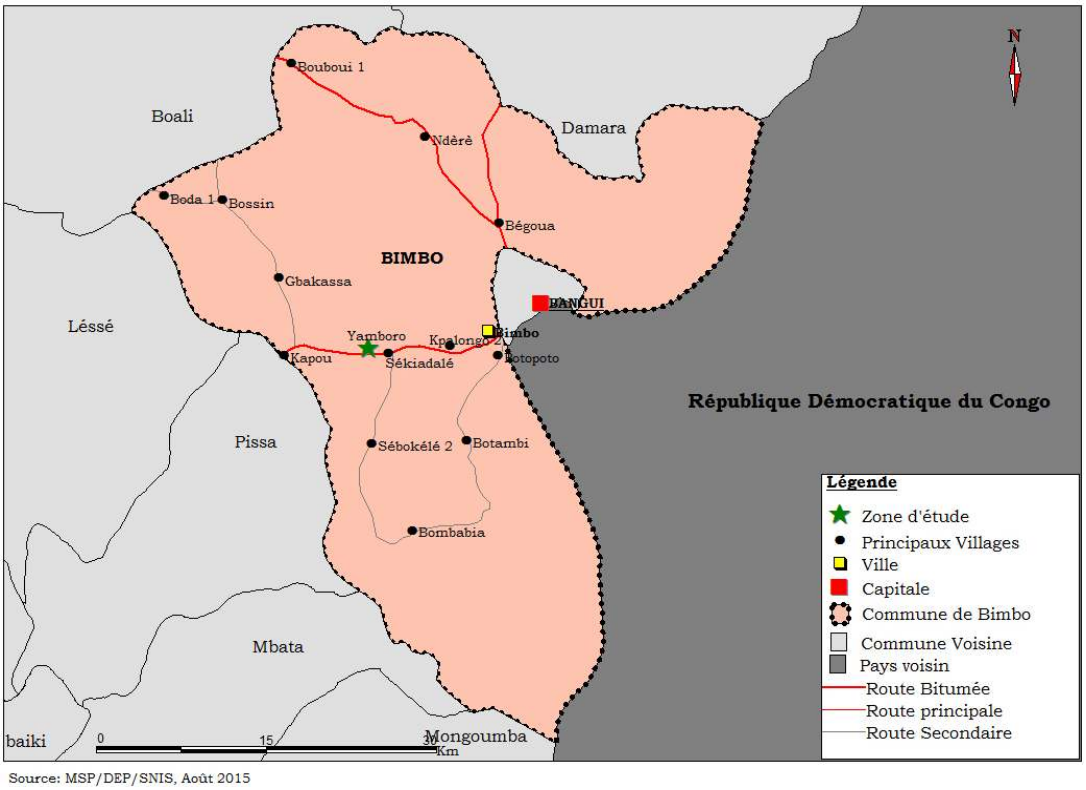

Fig. 1. Location of Yamboro in Central African Republic

2.2 Collection and Extraction of Plant Materials

Fresh leave, bark and root of *Z. zanthoxyloides* and *Z. macrophylum* were collected from Yamboro and the plants were authenticated by the taxonomist at the Department of Botany of University of Bangui. The identities of the plants were confirmed by comparing the voucher specimens of each plant with the herbarium specimens.

Fresh leave, bark and root of *Z. zanthoxyloides* and *Z. macrophylum* were extracted in mixture solution water-alchool (50/50, alchool 95°) for 24 h and the extracts were filtered with Whatman no. 1 filter paper and concentrated to dryness at 40℃ using a rotary evaporator. The extracts were re-dissolved in the same solvent to the required concentrations for the antifungal and antibacterial studies. The extraction yield was calculated using the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of extract obtained}}{\text{Weight of plant powder used}} \times 100$$

2.3 Bioassay

Candida albicans, *Pseudomonas aeruginosa* and *Klebsiella pneumonae* were isolated at National Laboratory of Clinical Biology and Public Health in Bangui. The bacterial suspension turbidity adjusted to McFarland standard number 0.5, in Mueller Hinton. With a sterile cotton swab bacterial culture was streaked on previously prepared Mueller Hinton agar plate and *C. albicans* culture was streaked on previously prepared Sabouraud agar plate. Dried and sterilized paper discs were treated separately with desired concentration of previously prepared water-alchool solution of the bark, leaf and root extract using a micropipette dried in air under aseptic condition and placed at equidistance in a circle on the seeded plate. The concentrations of bark, leaf and root extract used were 2 mg/disc and 4 mg/disc. These plates were kept for 4-6 h at low temperature and the test materials diffuse from disc to the surrounding medium by this time. The plates were then incubated at 37℃ for 18 h. Each sample was used in triplicate for the determination of antibacterial and antifungal activity [12,14]. The diameter of zone of inhibition produced by bark, leaf and root extract was

compared with negative control (water-alchool (50/50, alchool 95°), bank disc impregnated with solvent water-alchool followed by drying off was used as negative control).

2.4 Chemical Assay

To identify the chemical constituents of plant extract standard procedures were followed. Freshly prepared crude extracts of *Z. zanthoxyloides* and *Z. macrophylum* were qualitatively tested for the presence of chemical constituents using the following reagents and chemicals, flavonoids with the use of Mg and HCl; Tannins with ferric chloride and potassium dichromate solutions and saponins with ability to produce stable foam and steroids with Libermann Burchard reagent, reducing sugars with Benedict's reagent and observed color change in respective samples.

2.4.1 Determination of alkaloids

Test of Mayer was used for the determination of alkaloids. To 0.5 g of powdered plant material was added 15 mL of 70% EtOH. After 15 mn of sonication and 20 h stirring, the extract is allowed to stand until complete settling, followed by filtration and evaporation to dryness. The residue is taken up in a few ml of 50% HCl. The yellowing precipitate after the addition of a few drops of the Mayer reagent (potassium mercuritetraiodide), testifies to the presence of alkaloids [15,16] .

2.4.2 Determination of saponosides

Dissolve a fraction of methanol extract in 2 mL of distilled water in a test tube for 15 mn. Stir for a few seconds in the direction of the length. The appearance of a column of water foam minus 1 cm high persisting for at least 15 mn indicates the presence of Saponosides [15].

2.4.3 Determination of tannins

A fraction of the methanolic and aqueous extract is dissolved in 1 mL of ethane and diluted in two mL of water. Add 2 to 3 drops of $FeCl_3$ solution 1% or 3% in the presence of tannins, a greenish or blue coloration develops [17].

2.4.4 Determination of flavonoids

Total flavonoids were estimated using the method of Ordonez [18]. To 0.5 mL of sample, 0.5 mL of 2% $AlCl_3$ ethanol solution was added. After 60 min at room temperature, the absorbance was measured at 420 nm. A yellow color indicated the presence of flavonoids [19].

2.5 Statistical Analyses

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to two way analysis of variance (ANOVA 2) and differences between samples were determined by Student test using the R (version 3.2.4.). P value= 0.00201 was used to determine the level of significance of the different treatments.

3. RESULTS

3.1 Parts of Plants Used in the Traditional Medicine

Leaves of *Z. zanthoxyloides* and *Z. macrophylum* are the part of the plants used in Yamboro in traditional medicine followed by bark plant parts (Fig. 2).

3.2 Extraction Yield of the Plant Extraction

The mixture solution water-alchool (50/50, alchool 95°) extraction yield obtained from cold maceration of 100 g of plant powder are presented in Table 1. The extraction yield varied from one part of the plant to another. Bark extracts respectively with extraction yield value of 19 and 16% of *Z. zanthoxyloides* and *Z. macrophylum* were high compared to the low yield 7.5% and 10% obtained respectively with the leaves of *Z. zanthoxyloides* and *Z. macrophylum*.

Table 1. Extraction yield of the plant extraction

Plants	Parts used	Extraction yield (%)
Z. zanthoxyloides	Leave	7.5
	Bark	19
	Root	14.5
Z. macrophylum	Leave	10
	Bark	16
	Root	15

3.3 Bioassay

3.3.1 Bioassay with leaves of *Z. zanthoxyloides* and *Z. macrophylum*

With 2 mg/disc, extract of leaves of *Z. zanthoxyloides* and *Z. macrophylum* is not effective on *C. albicans*, *P. aeruginosa* and *K. pneumonae* (Fig. 3).

The results of analysis of variance on the antibacterial and antifungal effect of extracts of leaves of *Z. zanthoxyloides* and *Z. macrophylum* with high concentration (4 mg/disc) showed the effect of treatment is highly significant depending up on the plant, bacteria and fungi ($P < 0.001$) (Fig. 4). Extract of leaves *of Z. zanthoxyloides* is very effective on *Klebsiella pneumonae* with high concentration (4 mg/disc). Extract of leaves of *Z. macrophylum* is very effective on *Candida albicans* with high concentration (4 mg/disc).

3.3.2 Bioassay with bark of *Z. zanthoxyloides* and *Z. macrophylum*

With 2 mg/disc, extract of bark of *Z. zanthoxyloides* and *Z. macrophylum* is not effective on *C. albicans*, *P. aeruginosa* and *K. pneumonae* (Fig. 5).

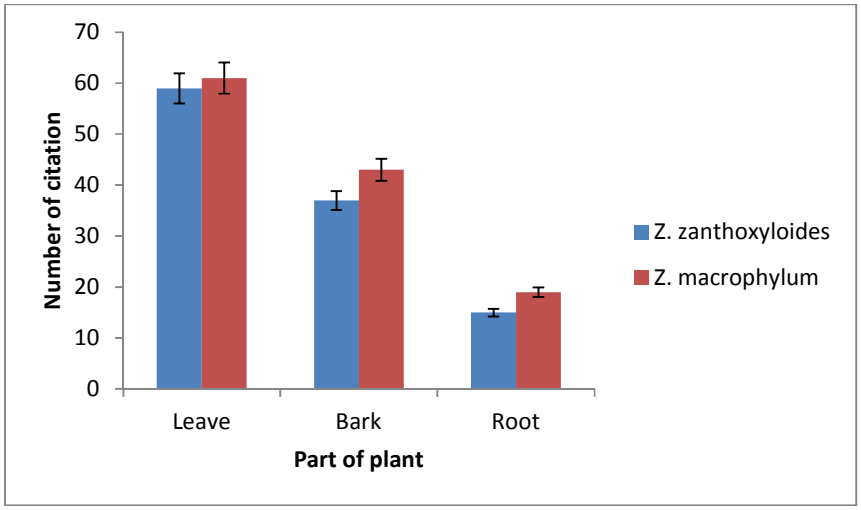

Fig. 2. Parts of plants used in traditional treatment of bacteria and fungi in Yamboro

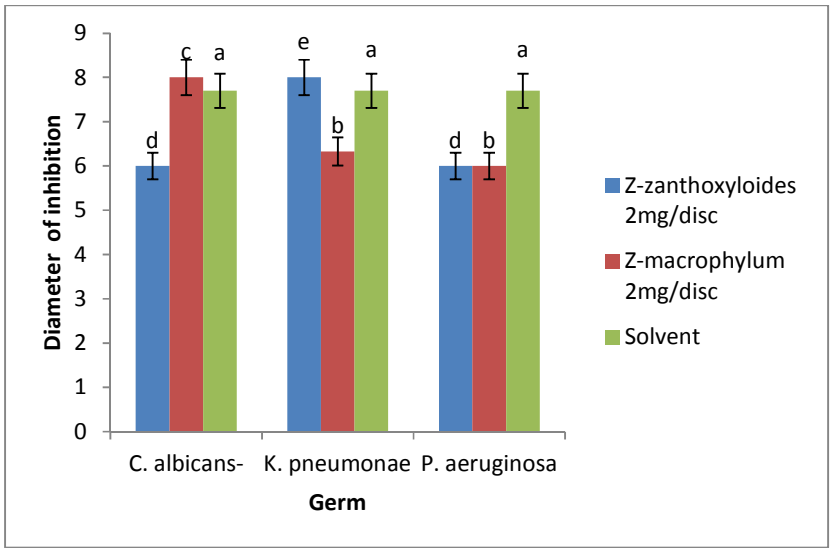

Fig. 3. Bioassay with leave extract for 2 mg/disc on bacteria and fungi a,b, c, d, e mean values on the same row with different superscripts differ significantly (P<0.01)

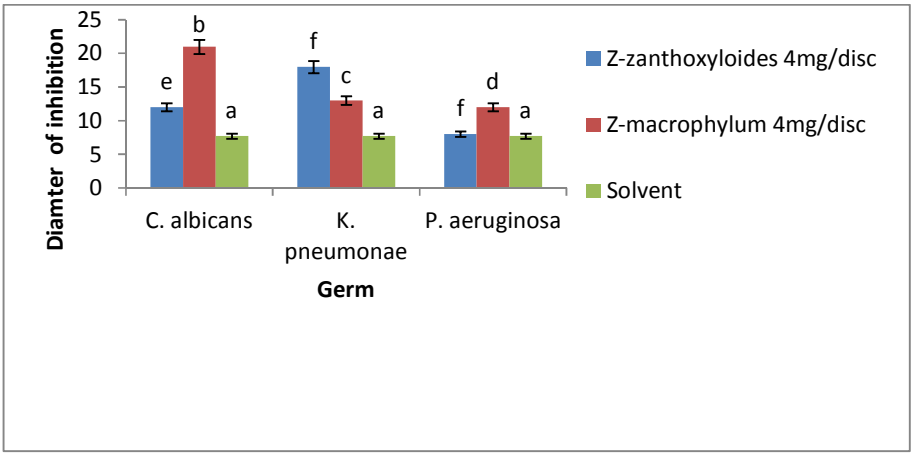

Fig. 4. Bioassay with leave extract for 4 mg/disc on bacteria and fungi a,b, c, d, e, f, g mean values on the same row with different superscripts differ significantly (P<0.01)

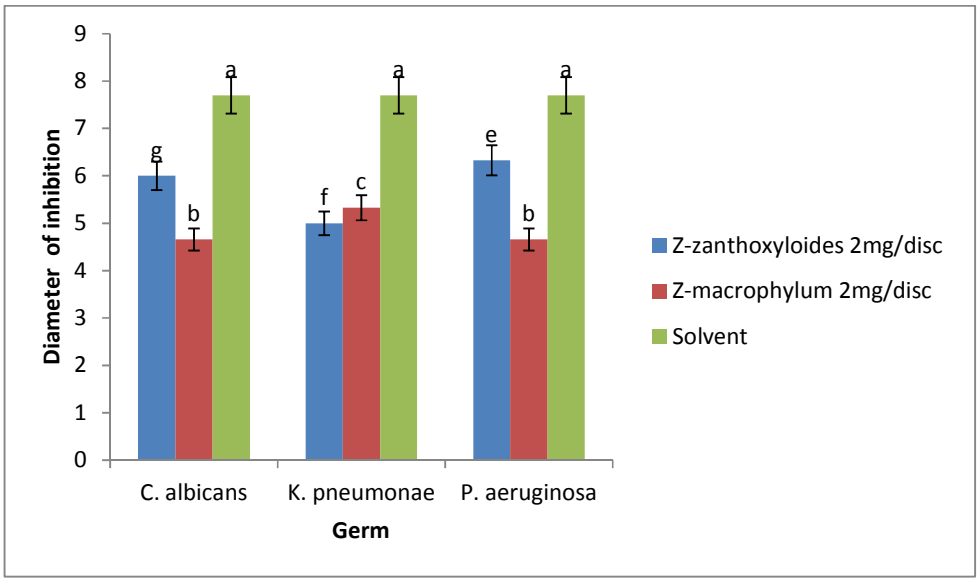

Fig. 5. Bioassay with bark extract for 2 mg/disc on bacteria and fungi a,b, c, d, e, f, g mean values on the same row with different superscripts differ significantly (P<0.01).

The results of analysis of variance on the antibacterial and antifungal effect of extracts of leaves of *Z. zanthoxyloides* and *Z. macrophylum* with high concentration (4 mg/disc) showed the effect of treatment is very significant depending up on the plant, bacteria and fungi ($P < 0.001$) (Fig. 6). Extract of bark *of Z. zanthoxyloides* is very effective on *C. albicans* with high concentration (4 mg/disc).

3.3.3 Bioassay with root of *Z. zanthoxyloides* and *Z. macrophylum*

With 2 mg/disc, extract of roots of *Z. zanthoxyloides* and *Z. macrophylum* is not effective on *C. albicans*, *P. aeruginosa* and *K. pneumonae* (Fig. 7).

The results of analysis of variance on the antibacterial and antifungical effect of extracts of roots of *Z. zanthoxyloides* and *Z. macrophylum* with high concentration (4mg/disc) showed the effect of treatment to be highly significant depending up on the plant, bacteria and fungi ($P < 0.001$) (Fig. 8). Extract of root *of Z. zanthoxyloides* is very effective on *C. albicans* with high concentration (4 mg/disc).

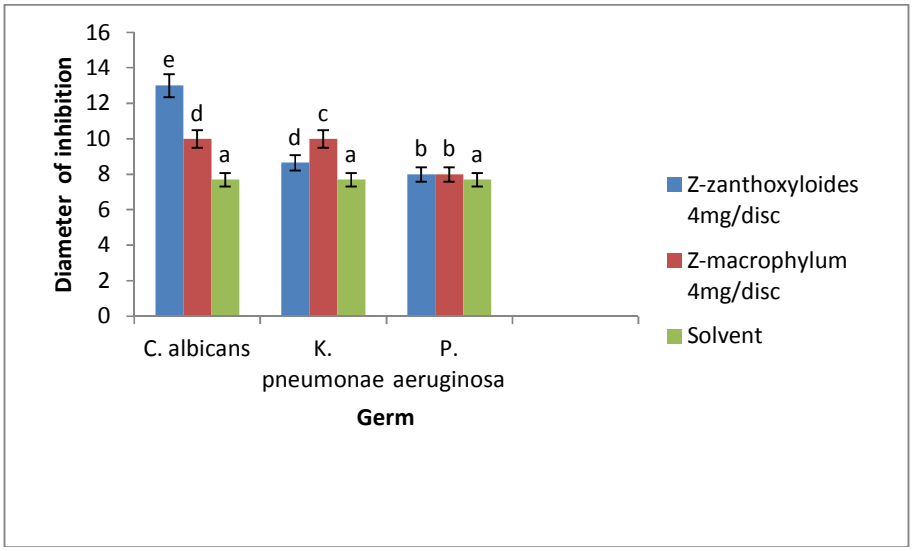

Fig. 6. Bioassay with bark extract for 4 mg/disc on bacteria and fungi a,b, c, d, e mean values on the same row with different superscripts differ significantly (P<0.01)

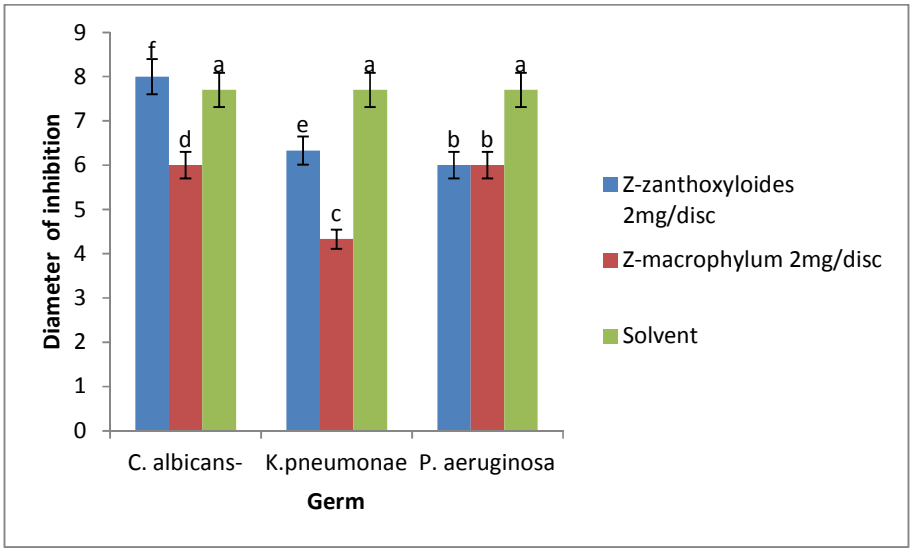

Fig. 7. Bioassay with root extract for 2 mg/disc on bacteria and fungi a,b, c, d, e, f mean values on the same row with different superscripts differ significantly (P<0.01)

3.4. Phytochemical Screening

Principal chemical groups identified are presented in the Table 2. The result showed that alkaloids, saponosides flavonoids and tannins are present in extract water-alchool (50/50, alchool 95°).

4. DISCUSSION

Traditional medical practitioners use a variety of herbal preparations for either the treatment or management of different kinds of microbial infections. The family of Rutaceae contains metabolites with various biological properties [20] *Z. zanthoxyloides* and Z. *macrophylum* belong to the family of Rutaceae and are such plants used by the traditional healers for the treatment or management of microbial infections in Central African Republic.

The water-alcohol (50/50, alcohol 95°) extract of different part of *Z. zanthoxyloides* and Z.

macrophylum showed varying degrees of antimicrobial activities against the test organisms. Extract of leaves *of Z. zanthoxyloides* is very effective on *Klebsiella pneumonae* with high concentration (4 mg/disc). Extract of leaves of *Z. macrophylum* is very effective on *C. albicans* with high concentration (4 mg/disc). The results of analysis of variance on the antibacterial and antifungal effect of extracts of leaves of of *Z. zanthoxyloides* and *Z. macrophylum* with high concentration (4 mg/disc) showed the effect of treatment to be highly significant depending up on the plant, bacteria and fungi ($P < 0.001$) (Fig. 6). Extract of bark *of Z. zanthoxyloides* is very effective on *C. albicans* with high concentration (4 mg/disc). The results of analysis of variance on the antibacterial and antifungal effect of extracts of roots of *Z. zanthoxyloides* and *Z. macrophylum* with high concentration (4 mg/disc) showed the effect of treatment to be highly significant depending up on the plant, bacteria and fungi ($P < 0.001$) (Fig. 8). Extract of root *of Z. zanthoxyloides* is very effective on *C. albicans* with high concentration (4 mg/disc).

The antibacterial activity of another species of Rutaceae, *Clausena lansium* was tested in China. In Traditional Chinese Medicine, the leaves of *C. lansium* are used for cough, asthma, viral hepatitis, dermatological, and gastrointestinal diseases. Different parts of this plant are used as folk medicines for treatment of acute and chronic viral hepatitis in China [21-23]. It was reported that carbazoles and coumarins from *C. lansium* exhibited a variety of bioactivities such as antimicrobial China [24-30].

Preliminary phytochemical screening of ethanol leaf extract of *Z. zanthoxyloides* and *Z. macrophylum* showed the presence of tannins, saponins, flavonoids, steroids, phenolics, anthraquinonnes, glycosides, cardiac glycosides and alkaloids. Phytochemical constituents such as flavonoids have been found *in vitro* to be effective against a wide range of microorganisms [31,17]. Tannins and saponins have been reported to prevent the growth of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them [32,33]. Studies have also shown that the growth of many fungi, bacteria and viruses are inhibited by tannins [32] and the presence of flavonoids, tannins and saponins in the ethanol leaf extract could therefore be responsible for the antimicrobial properties of the *Z. zanthoxyloides* and *Z. macrophylum*.

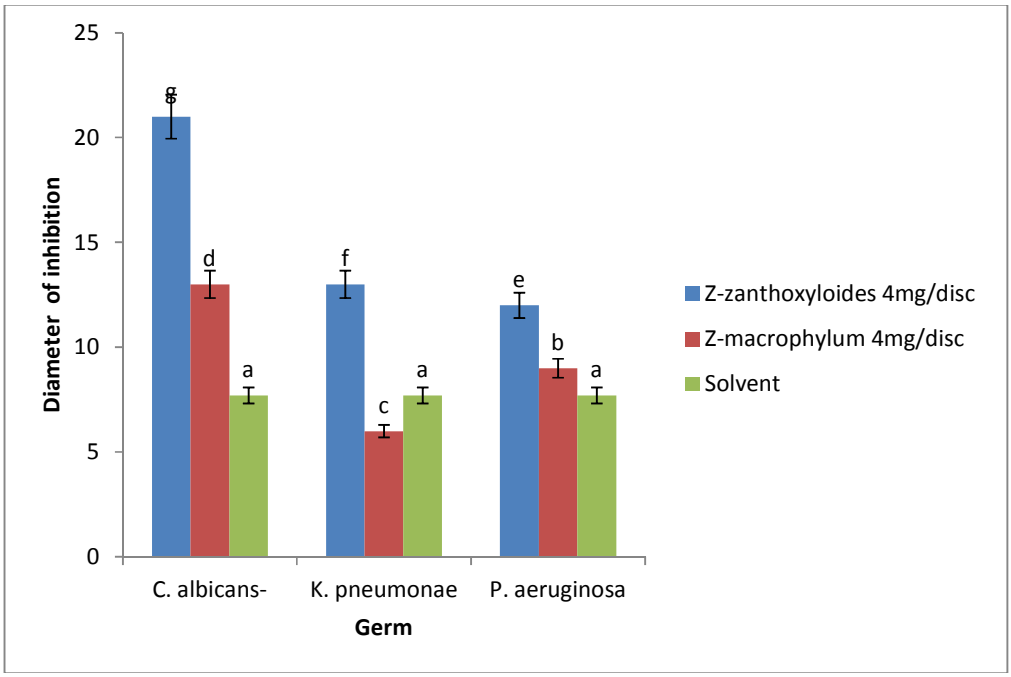

Fig. 8. Bioassay with root extract for 4 mg/disc on bacteria and fungi a,b, c, d, e, f, g mean values on the same row with different superscripts differ significantly (P<0.01)

Table 2. Principal chemical groups

Chemicals groups	*Z. zanthoxyloides*			*Z. macrophylum*		
	Leave	**Bark**	**Root**	**Leave**	**Bark**	**Root**
Alkaloids	+++	+++	+++	+	-	+
Saponosides	+++	-	+++	-	++	-
Tannins	+++	-	+++	+++	+++	++
Flavonoids	+++	+++	+++	++	++	++

+++ = highly present; ++ = moderately present; + = present; - = absent

Z. zanthoxyloides and *Z. macrophylum* can be a potential source of antibactericide and antifungicide molecules.

5. CONCLUSION

Extract of leaves *of Z. zanthoxyloides* is very effective on *K. pneumonae* with high concentration (4 mg/disc). Extract of leaves *of Z. macrophylum* is very effective on *C. albicans* with high concentration (4 mg/disc). Extract of bark *of Z. zanthoxyloides* is very effective on *C. albicans* with high concentration (4 mg/disc). Extract of root *of Z. zanthoxyloides* is very effective on *C. albicans* with high concentration (4 mg/disc).

Phytochemical screening of leaf, bark and root extact of *Z. zanthoxyloides* and *Z. macrophylum* revealed the presence of tannins, alkaloids, saponines and flavonoids. The chemical profil of *Z. zanthoxyloides* and *Z. macrophylum* may be responsible for antibacterial and antifungal properties and may justify use of the plant for treatment of microbial infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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