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In vitro evaluation of antibacterial activity of flavonoids of *Thonningia sanguinea* Vahl. (Balanophoraceae)

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ABSTRACT

The purpose of this study was to evaluate antibacterial activity of flavonoids extracts of Thonningea sanguinea (Vahl) on the in vitro growth of bacteria implicated in gastroenteritis in infants. The antibacterial activity of flavonoids extracts of the plant was tested against three hospital clinical strains (Salmonella Paratyphi A, Salmonella typhi and Enterobacter aerogenes) and a reference strain (Escherichia coli ATCC25922), by well plate and microdilution methods. Selected antibacterial agents (ciprofloxacin, acid nalidixin, gentamicin and ticarcillin) were used as positive reference standards in the tests. The flavonoids extracts showed strong antibacterial activity agaisnt E. coli with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of 12.5 mg/mL and 25 mg/mL, respectively. For others strains tested, the MIC varied between 12.5 and 50 mg/mL and the MBC 50 to 100 mg/mL. Flavonoids of T. sanguinea was found to be a potential bactericidal against all trains tested. Therefore this secondary metabolite may be useful in the treatment of bacterial gastroenteritis.

Keywords: *Thonningea sanguinea*, Flavonoids, Antibacterial activity, Bactericidal.

INTRODUCTION

Thonningia sanguinea (Vahl) is a parasite plant found in the most of African tropical forests **[1]**. In traditional medecine this plant is used in the treatment of some diseases such as hemorroids, diarrhea, asthma, skin diseases, etc **[2-6].** Many works of our research team were carried out antibacterial **[7-10]** and antifungal **[11-13]** properties of differents extracts of *T. sanguinea*. Phytochemicals investigations showed that various groups of molecules particulary phenolics compounds are responsibles of antimicrobial activities of these extracts **[14, 15]**. To contribuate at the previous works of the antibacterial activities of *T. sanguinea* this study was aimed to teste it flavoinoids extracts on the *in vitro* growth of four bacteria strains involved in diarrheal infectious in infants.

MATERIALS AND METHODS

Plant material

Inflorescences of *Thonningea sanguinea* Vahl, (Balanophoraceae) were collected from Sandegue, locality of Bondoukou (north east region of Cote d'Ivoire) in August 2010. The plant was identified and authenticated by Pr Ake-Assi, of National Floristique Center of University Felix Houphouet Boigny of Cocody (Abidjan, Cote d'Ivoire) where a voucher specimen was deposited in the herbarium.

Extraction of plant flavonoids

The inflorescences of *T. sanguinea* were washed thoroughly with distilled water to remove dirt and air-dried at room temperature for 2 weeks, after which it was grinded to a uniform powder. Dry powdered plant material (100 g) was mixed with solvant constituate by 1 L of distilled water and 20 mL of acetique acid for 24 hours with constant stirring at room temperature for 48 hours. The extract was filtered twice through cotton wool, then through Whatman filter paper N°1. The filtrate was shaken three times successively with 300 mL of chloroform. The chloroform layer was evaporated to concentrate using a rotary evaporator (Buchi) before freeze-dried for better storage stability. The result powder was flavonoids extracts of *T. sanguinea* **[10]**.

Bacterial strains

In vitro antibacterial studies of flavonoids extracts of *T. sanguinea* were carried out on one reference strain (*Escherichia coli* ATCC 25922) and three gastroenteritis clinical isolates namely *Salmonella typhi* (05798A/10), *Salmonella paratyphi* A *(*05768A/10) and *Enterobacter aerogenes (*1114C10). They were all identified following NCCLS recommandations **[16]** by Bacteriological Laboratory of Pasteur Institute of Côte d'Ivoire.

Antibacterial assay

Antibacterial activity of flavonoids extracts of *T. sanguinea* was performed with Mueller Hinton (Biorad, France) agar by well plate method **[18, 19]**. Nutrient agar plates were seeded with overnight cultures of the test organisms. Wells, 6mm wide, were cut in the agar plates with cork borer and 80 µLs of extracts at 200 mg/mL were pipetted and carefully added. A control well for each bacterial strain was added with 80 µL of mixture containing DMSO/sterile distilled water (V/V) **[20]**. The nutrient agar plates were incubated right side up at 37°C for 24 hours. The zones of inhibition were then measured and the mean of two replicates recorded. Ciprofloxacin (5 µg/mL), Nalidixin acid (30 µg/mL), Gentamicin (100 µg/mL) and Ticarcillin (75 µg) were used as standards antibiotics.

Determination of antibacterial parameters of flavonoids extracts

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was determined by tube dilution method for each of the test organism in triplicates **[21]**. To 0.5 mL of varying concentrations of the extracts (6.5 mg/mL – 400 mg/mL), 2 mL of nutrient broth was added and then a loopful of test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the tubes **[22]**. The procedures were repeated on the test organisms using standard antibiotics (Ciprofloxacin, Nalidixin acid, Gentamicin and Ticarcillin). A tube containing nutrient broth only seeded with the test organisms was served as control. Tubes containing cultures were then incubated at 37°C for 24 hours. After incubation the tubes were examined for bacterial growth observing the tubidity.

To determine the MBC for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar by streaking. Plates inoculated with bacteria were then incubated at 37°C for 24 hours. After incubation the concentration at which no visible growth was seen was noted as MBC **[23]**.

Statistical analysis

The data are presented as mean ± SEM. All the data were analyzed by one-way ANOVA and differences between the means were assessed with Neuman-Keuls's multiple comparison tests. Differences were considered significant *at* $p < 0.05$. All analyses were carried out using Graph Pad software, version 5.01 (USA).

RESULTS

The *in vitro* antibacterial activity of flavonoids of *T. sanguinea* against the four strains were shown in Table 1. Comparatively to standard antibacterial agents the flavonoids extracts have shown significantly ($p<0.05$) minimum zone of inhibition against all tested bacteria varying 10 to 13mm. Gentamicin gave significantly the lowest activities against *S. typhi* (25±0.33 mm) and *S. paratyphi* A (23±0.33 mm) whilst Ciprofloxacin produced maximum zone diameter of 30±0.33 mm against *E. coli*. Except Ciprofloxacin (13±0.16 mm) the others standards antibiotics have shown any activities against *E. aerogenes*.

The results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were shown in Table 2. Flavonoids extracts were found effective against all strains tested. The values showed that flavonoids of *T. sanguinea* is highly sensitive against *E. coli* strain (MIC 12.5±0.9 mg/mL and MBC 25±2.4 mg/mL), moderately sensitive to *S. typhi* (MIC 50±1.2 mg/mL and MBC 100±2.5 mg/mL) and *S. paratyphi* A (MIC 50±0.7 mg/mL and MBC 100±1.8 mg/mL) whilst less sensitive to *E. aerogenes* (MIC 100±0.3 mg/mL and MBC 200±3.1 mg/mL).

For all strains tested flavonoids have shown bactericidal activity according values of MBC per MIC (MBC/MIC < 4) **[24]**.

Table 1: Sensitivity of flavonoids extracts and standars antibiotics on bacterial strains
Zone of inhibition diameters (mm)

Bacterial strains	Flavonoids extracts (200mg/mL)	Standards antibiotics CI (5µg/mL)	AN (30µg/mL)	GE (100µg/mL)	TI (75µg/mL)
S. typhi	13±0.16	21±0.33	22±0.16	25±0.33	23±0.16
S. paratyphi A	11±0.33	21±0.16	22±0.16	23±0.33	21±0.16
E. coli	12±0.16	30±0.33	21±0.33	21±0.16	29±0.33
E. aerogenes	10±0.33	13±0.16	0	0	0

Key: *CI: Ciprofloxacin, AN: Nalidixin acid, GE: Gentamicin, TI: Ticarcillin. Values are mean ±S.E.M. of 3 replications.*

Table 2: Antibacterial parameters values of flavoinoids of *T. sanguinea* (Vahl)

Bacterial strains	Antibacterial parameters MIC	MBC	MBC/MIC	Antibacterial activity
S. typhi	50±1,2[a]	100±2,5[a]	2	Bactericidal
S. paratyphi A	50±0,7[a]	100±1,8[a]	2	Bactericidal
E. coli	12,5±0,9[b]	25±2,4[b]	2	Bactericidal
E. aerogenes	100±0,3[c]	200±3,1[c]	2	Bactericidal

Note: *Values are the mean of three tests ±S.E.M.*
[a-c]*Mean values with the same superscript within a row do not differ significantly ($p < 0.05$).*

DISCUSSION

In this study antibacterial activity of flavonoids of *Thonningia_sanguinea* (Vahl) was tested against four bacteria implicated in diverses human pathologies particulary gastroenteritis in infants. The results of this study showed that flavonoids have varied bactericidal activities against all tested bacteria strains. In order effectiveness *E. aerogenes* has shown highest resistance and *E. coli* was found the most sensitive strain whilst the two others strains (*S. paratyphi* A and *S. typhi*) were moderate sensitives to flavonoids. Reports have shown that flavonoids are secondary metabolites recognized for their high antibacterial potency **[25, 26]**. This propertie of flavonoids extracts of *T. sanguinea* was clearly demonstrated with some strains of Staphylococcus by Bagre *et al* **[10]**. These authors have shown that at 200 mg/mL total aqueous extract of the plant have a real inhibitory activity against *Staphylococcus aureus* MR a strain which resisted to Oxacilline and Cefoxitin respectively at 5 µg/mL and 30 µg/mL. However inhibitory potency of flavonoids of *T. sanguinea* tested in this study was more low (MIC 12.5 – 50 mg/mL) than it total aqueous extract used by Bagre *et al* (MIC 1.56 – 6.25 mg/mL) against the same bacteria strains **[27]**. Additionally, these authors have shown that the antibacterial activities of flavonoids and alcaloids of *T. sanguinea* were similars according values of their MBC (50 - 100 mg/mL). Phytochemical screening revealed that aqueous extracts of *T. sanguinea* contained alcaloids, flavonoids, tannins, saponins and quinones **[28]**.These compounds have been reported to possess antibacterial properties **[26, 29, 30, 31].** Particulary antibacterial activity of alcaloids of the plant was prouved by Ouattara et *al* in previous study **[27]**. But aqueous extract of *T. sanguinea* presented highly antibacterial activity than it two secondary metabolites which are flavonoids and alkaloids. Thus this high effectiveness activity of aqueous extract can be explained by a synergie of actions beetwen these two secondary metabolites and the others compounds of *T. sanguinea*. It is interesting to note that *E. aerogenes* have shown the highest resistance to standards antibiotics whilst *E. coli* was found most sensitive. The effectiveness of flavonoids of *T. sanguinea* against *E. aerogenes* which most resisted to standards antibiotics used in this study can be a real hopes in research of novel broad spectrum antibacterial agent.

CONCLUSION

The bacteria strains used in this study are mostly responsibles of gastroenteritis. However, treatments of these infections were some times ineffective. This study has shown that flavonoids of *T. sanguinea* were effectiveness against the four strains tested. These flavonoids can be one of compounds implicated in antibacterial activity of aqueous extract of the plant. Thus this study has revealed the scientific basis for therapeutic use of *T. sanguinea* in

traditionnal pratice for the treatment of gastroenteritis in infants. This work is a contribution for reaserch of novel broad spectrum antibacterial compounds. There is need for further investigation to purifyed flavonoids extracts of *T. sanguinea* in order to maximised it antibacterial activity such as this of standards antibiotics.

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