Antibacterial activity assessment of different crude extracts obtained from the leaves of *Caesalpinia decapitala* grown in Rwanda

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Abstract:

In this study, both crude extracts and essential oils extracted from ***Ceasalpinia decapitala*** *leaves have been chemically analysed and their biological activity evaluated. Phyto-chemical screening showed that leaves of this plant contain flavonoids, saponins, tannins and terpenoids. Coupled gas chromatography/mass spectroscopy (GC/MS) analysis indicated that essential oil of this plant was rich in both α and β-pinene (25.5 and 8.4%)), α-phellandrene (4.5%), β-ocymene (31.6%), caryphyllene (7.5%) and geraniol (5.9%). The evaluation of antibacterial activity of different organic crude extracts, against the growth of* ***Escherichia coli, Salmonella typhimurium, Listeria innocua and Staphylococcus aureus*** *showed that only methanol crude extract is active against the growth of* ***Staphylococcus aureus*** *and* ***Salmonella thyphimurium****. After further analysis of this active extract, it was found that tannins could be responsible of this antibacterial activity. All bacteria tested in this study were sensible to essential oil extracted from* ***Ceasalpinia decapitala*** *leaves.*

Key words: *Antibacterial activity,* ***Caesalpinia decapitala*** *leaves, crude organic extracts, essential oil*

1. Introduction

Nowadays, the world is confronting not only to the problem of microorganisms resistance towards conventional drugs (Le Loir et al., 2003; Indu et al., 2006; Oussalah et al., 2007; Prazak, 2004; Korsac, 2004; Corrégé, 2001), but also to the remarked toxicity of some used biocides (Cooper, et al., 2008; INERIS, 2007 and 2005; GTIF, 2003; Kamrin, 1998; FAO/UNEP, 1996; Hughes, 1996). For this reason, scientists are working hard to discover other efficacious and environment friendly drugs to fight against harmful micro-organisms. Plant materials are good candidate to achieve this goal due to their uses for a long time as antimicrobial agents. They have

used by traditional healers in all over the world to treat both human and animals diseases and have showed their efficacy. However due to the development of modern medicine, the use of plant as curative means became unfamiliar in many countries and this progressively led to their disappearance. Furthermore, the lack of sufficient scientific knowledge and proof on their curative efficacy constitute a barrier to their wide use. Considering that plants can constitute the main starting material to discover new needed drugs, efforts and policy to preserve and to valorise them should be quickly taken to avoid their extinction. This study intended to contribute in this way and aimed to conduct a scientific study on *C. decapitala* leaves in order to prove its efficacy for combating against harmful microorganisms, especially those contaminating food.

2. Material and methods

Healthy *C. decapetala* leaves were collected from Cyarwa, Ngoma Sector, Huye District in the Southern Province of Rwanda at the beginning of rain season, precisely in the month of April. They were identified by a botanist of the Faculty of Science, Dr. Elias BIZURU. Extraction of crude extracts was firstly done by fractionation using soxhlet extractor apparatus and three solvents with different polarities such us petroleum ether, diethyl ether and ethanol and secondly by maceration with methanol. Standard methods for phytochemical screening (Trease and Evans, 2002; Harborne, 1993) were used to identify the main groups of compounds in the leaves of *C. decapitala* and to partially characterise the active ones. Furthermore, preparative thin layer chromatography (PTLC, Silica gel 60 F254, Germany) was used to fractionate methanol extract and this using a mixture of petroleum ether: ethyl acetate (7:3) as mobile phase. In this study, essential oil from *C. decapetala* leaves was also obtained through hydro distillation method by Clevenger type apparatus. The chemical components of this oil were known using coupled gas chromatography/mass spectroscopy (GC/MS) analysis (Muhizi et al., 2011). The antibacterial activity of all compounds was evaluated by coating and disk diffusion methods (Muhizi et al., 2009) using *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium* and *Listeria innocua* as microorganisms.

2. Results

Phytochemical screening realized in this study showed that leaves from *C. decapetala* contain flavonoids, saponines, tannins and sterol terpenoids, while alkaloids, anthocyanins and quinones, were not found. The extraction done by soxhlet led to petroleum ether (PE), diethyl ether (DE) and methanol (ME1) extracts with yields of 2.5%, 2% and 5.2% while ME2 was obtained through maceration method. The essential oil from fresh leaves of *C. decapitala* was obtained with a yield of 0.2% and 23 different natural compounds were identified and quantified from this oil (table 1). The antibacterial activity study of ME1, DE, PE and ME2 crude extracts at the concentration of 1 mg/mL showed that three crude extracts, ME1, DE and PE, did not exhibit any activity against the growth of *E. coli*, *S. aureus*, *L. innocua* and *S. thyphimurium*; while ME2 extract was effective against the growth of *L. innocua*, *S. aureus*. No significant antibacterial activity was observed against *E. coli* and *S. thyphimurium*. Gentamycin, used as positive control drug, was completely inhibited the growth of all bacteria (table 2).

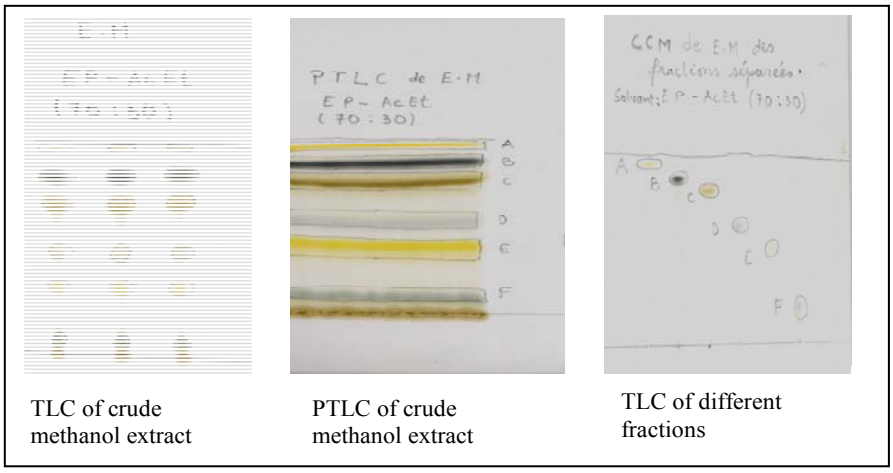
Table 1: Chemical composition of essential oil of *C. Decapitala*

Component	**Quantity (%)**	**Component**	**Quantity (%)**	**Component**	**Quantity (%)**
α-pinene	25.5	p-cymene	1.2	Humulene	1.1
Bicyoclo (3.1.0)hex-2-ene,4-methylene-1-(1-methylethyl)-	0.1	Limonene	1.4	Citronellyl acetate	0.1
Camphene	0.1	Eucalyptol	0.2	Cryptone	2.4
β-phellandrene	0.3	β-ocymene	31.6	Bornyl acetate	0.3
β-pinene	8.4	3-carene	5.6	Geraniol	5.9
(Z)-hex-3-enyl acetate	1.0	Terpinolene	0.4	Spathulenol	0.72
β-Myrcene	Tr	Longifolene	0.1	Eudesm-11-en-1-ol	0.52
α-phellandrene	4.5	Caryophyllene	7.5	***Total peak area***	***99.91***

Table 2: Effect of crude methanol extract (ME2) and fractions D and F on the growth of bacteria

Drugs at 1 mg/mL	**Microorganisms and percentage of inhibition (Mean ± SEM)**			
	E. coli	*S. thyphimurium*	*S. aureus*	*L. innocua*
ME2	9.0 ± 0.1	13.0 ± 0.3	40.0 ± 0.0	52.0 ± 0.1
Fraction D	31.3 ± 0.1	38.0 ± 0.0	67.0 ± 0.0	73.0 ± 0.4
Fraction F	38.3 ± 0.1	40.0 ± 0.0	77.0 ± 0.0	83.0 ± 0.4
Gentamycin	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

In addition to these results, ME2 was also fractionated with preparative layer chromatography (PTLC) into six different fractions named A, B, C, D, E and F, which had respective retention factors of 0.98, 0.80, 0.64, 0.48, 0.38 and 0.08 (figure 1).



TLC of crude methanol extract | PTLC of crude methanol extract | TLC of different fractions

Figure 1: Chromatograms of crude methanol extract before and after fractionation by PTLC

The assessment of their antibacterial activity showed that only fractions D and F were significantly effective against the growth of all bacteria and this in comparison with control drug (table 2). Two Gram negative bacteria, *S. aureus* and *L. innocua*, were the more sensitive towards these fractions. The phytochemical screening done on fraction F showed that it contains tannins while the active fraction D was not identified due to its small quantity. Meanwhile, the antibacterial activity of *C. decapitala* essential oils (Eos) was evaluated by disk diffusion method and all bacteria were sensitive towards this essential oil (table 3).

Table 3: Antibacterial activity of *C. decapitala* essential oil

Drugs tested	**Diameter of inhibition (Mean ± SEM) in mm**			
	E. coli	***S. typhimurium***	***S. aureus***	***L. innocua***
Eos	14 ± 1	17 ± 2	14 ± 1	18 ± 2
Gentamycin	22 ± 3	21 ± 1	25 ± 1	26 ± 1

4. Discussion and conclusion

In spite of its curative utility (Christian, 2011), *C. decapitala* is only used as hedge in Rwanda. Thus, we conducted this study to contribute to its possible valorisation. A phytochemical screening showed that leaves of this plant contain flavonoids, saponines, terpenoids and tannins. Biological activity of these compounds are known, thus they can be further isolated for their interest (Pamplona, 2001; Guignard, 1996; Bruneton, 1987; Dubé, 1978). The antibacterial activity assessment of ME1, DE and PE extracts did not show any efficiency while ME2 was effective against the growth of bacteria. Furthermore, fractions isolated from ME2 were more effective than this crude extract. The lack of efficiency for the first crude extracts compared to ME2 extract could be due to the heating method used during extraction, which method could decompose the active components. The higher antibacterial activity remarked from fractions D and F could be due to negative synergic effects caused by some compounds which were eliminated by PTLC. Furthermore, fraction F was found to contain tannins and this was strongly supported by previous reports about the antibacterial activity of this group of natural compounds (Pamplona, 2001; Bruneton, 1987). The analysis of essential oil from the plant by GC/MS indicated 23 components in which α-pinene and β-ocymene were in high amount (table 1). Furthermore, this essential oil inhibited the growth of bacteria with the inhibition diameters varying from 14 mm to 18 mm (Table 3), which is significant compared to the positive control and means its effectiveness (Johnson and Case, 1995). The main components of this oil can partially explain this since their antiseptic, antibacterial and antinociceptive properties were well known and reported (Liapi et al, 2008; Rondon et al., 2006). Differences noted between sensibilities of bacteria towards drugs can be partially explained by chemical compositions of their cell walls which are also

different depending on the type of bacteria (Muhizi, 2008; Muhizi et al., 2009). In conclusion, tannins and essential oil from *C. decapitala* showed more pronounced antibacterial activity against bacteria contaminating food and can be further studied for their possible application in this domain. Isolation of pure active compounds and the study of their toxicological profile will be of interest.

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