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Full Length Research Paper

Antioxidant and antimicrobial activities of *Canarium schweinfurthii* Engl. Essential oil from Centrafrican Republic

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The antioxidant activity of the essential oil was investigated using 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging assay and the β-carotene bleaching test. Butylated hydroxytoluene (BHT) was employed as a positive control. The essential oil showed antioxidant and DPPH radical scavenging activities, and it displayed the inhibition of lipid peroxidation. The antibacterial and antifungal activities of the essential oil of *Canarium schweinfurthii* from Centrafrican Republic were also evaluated against twelve strains of bacteria and three strains of fungi using agar diffusion and broth microdilution methods. The essential oil showed antimicrobial activity against almost the strains studied. The results suggest that *C. schweinfurthii* essential oil could be a natural antimicrobial and antioxidant agent.

Key words: Canarium schweinfurthii, Burceraceae, essential oil, antimicrobial, antioxidant.

INTRODUCTION

In the last years, scientists have focused on increasing human infections caused by pathogen bacteria, fungi and viruses. Microorganisms have unfavourable effects on the quality and safety of life. Synthetic chemicals are widely used against these microorganisms; unfortunately they develop resistance to many antibiotics due to the indiscriminate use of commercial antibiotics (Service, 1995; Mukherjee et al., 2002). In addition, these antibiotics sometimes cause allergic reaction and immunity suppression. Therefore the use of essential oils and plant extracts is less damaging to the human health and environment (Isman, 2000; Misra and Pavlovstathis, 1997). *Canarium schweinfurthii* Engl. (Burceracea) is a tree growing in the equatorial forest region from Cameroon, Centrafrican Republic, Gabon to Congo (Tchiégang, 2001; Tchouamo et al., 2000). The fruit pulp contains 30 to 50% of oil used for the manufacture of shampooing and bio fuel (Tchiégang, 2001; Ajiwe et al., 2000). The rhizhomes and leaves are used as stimulant and against fever, constipation, malaria, diarrhoea, sexual infections, post-partum pain and rheumatism (Koudou et al., 2005; Aké Assi and Guinko, 1991). Previous studies on the isolation of lipids and fatty acids from the fruit and the human food, the chemical composition and the significant analgesic effect of the resin essential oil of *C. schweinfurthii* have been reported (Koudou et al., 2005; Agbo et al., 1992). However there is so far no report about the antimicrobial activities. In other hand, the traditional use of the plant suggested an antioxidant activity.

The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of the many human diseases, including cancer, aging and atherosclerosis (Perry et al., 2000). Free radicals can also cause lipid peroxidation in foods that leads to their deterioration. Although there are some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), these compounds are associated with some side effects (Ito et al., 1983).

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There is no information in literature about the antioxidant activity of any *Canarium* species. So the works in the determination of natural sources of antioxidants and the antioxidant potential of plants is important.

As mentioned above, the antimicrobial and antioxidant activities of the *C. schweinfurthii* essential oil have not been studied to date. Therefore, the aim of the present study is to assess the antibacterial and antifungal activities of the resin essential oil and to determine its antioxidant activity.

MATERIALS AND METHODS

Plant material

The resin of *C. schweinfurthii* was obtained from the tree growing in the equatorial rain forest near Boukoko village (Centrafrican Republic) in July 2006. Voucher specimens have been deposited in Cerphametra, University of Bangui (Centrafrican Republic).

Isolation of essential oil

Essential oil of *C. schweinfurthii* Engl was obtained by hydrodistillation of resin. The chemical composition of essential oil has been reported (Koudou et al., 2005).

Determination of antioxidant activity

The antioxidant activity was evaluated by two different methods: DPPH radical scavenging activity and β-carotene-linoleic acid test.

2,2- Diphenylpicrylhydrazyl (DPPH) essay

The hydrogen atoms or electron-donating ability of the essential oil and BHT was determined from the bleaching of purple-colored methanol solution of DPPH. This spectrophotometric assay uses the stable radical DPPH as a reagent (Burits and Bucar, 2000). Experiments were carried out as described previously (Kordali et al., 2005). Briefly, 0.5 mM DPPH (Fluka) radical solution in methanol was prepared, and then 1 ml of this solution was mixed with 3 ml of the sample solution in ethanol. Various concentrations of extracts were obtained. BHT (Sigma) was used as a positive control at 100 $\mu g.ml^{-1}$ concentration. After incubation for 30 min in the dark, the absorbance was measured at 517 nm. Decreasing the absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. This activity is given as percent DPPH radical scavenging, which is calculated with the equation:

% DPPH radical scavenging = [(control absorbance- sample absorbance)/control absorbance] X 100

Control contained 1 ml of DPPH solution and 3 ml of ethanol. The measurements of DPPH radical scavenging activity were carried out for three sample replications, and values are an average of three replicates.

β-carotene-Linoleic acid assay

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide formation from linoleic acid oxidation (Dapkevicius et al., 1998). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade); 25 µl of linoleic acid and 200 mg of tween 40 were added as emulsifier because β-carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of distilled water saturated with oxygen was added with vigorous shaking at a rate of 100 ml/min for 30 min; 2500 µl of this reaction mixture was dispersed to test tubes, and 350 µl portions of extracts, prepared in 2 $g.l^{-1}$ concentrations, were added. The emulsion system was incubated for up to 48 h at room temperature. The same procedure was repeated with a positive control BHT and a blank. After this incubation time, the absorbance of the mixture was measured at 490 nm. Antioxidant capacities of extracts were compared with those at the BHT and the blank. Tests were carried out in triplicate. Inhibition of coloration of β-carotene in percentage (I %) was calculated as:

$I\%\ \text{inhibition} = [(A_{blank} - A_{sample})]/ A_{blank}] \times 100$

Where A_{blank} is the absorbance of the control reaction (containing all of the reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Bacterial and fungal strains

The micro organisms used were:

Reference bacterial and fungal strains: *Bacillus cereus* LMG 13569, *Enterococcus faecalis* CIP 103907, *Escherichia coli* CIP NCTC 11609, *Listeria innocua* LMG 1135668, *Salmonella enterica* CIP105150, *Shigella dysenteria* CIP 5451, *Staphylococcus aureus* ATCC9244, *Proteus mirabilis* 104588 CIP, *S. aureus* ATCC25293 BHI, *Staphylococcus camorum* LMG13567 BHI, *Candida albicans* ATCC10231 and *C. albicans* ATCC90028.

Hospital bacterial and fungal strains: *E. faecalis, Pseudomonas aeruginosa*, *S. aureus*, *Streptococcus pyogenes* and *C. albicans*. They were kindly provided by the St Camille Hospital of Ouagadougou, Burkina Faso.

Disk diffusion essay

The tests were performed using Miller-Hinton medium for bacterial strains and saboureaud dextrose agar for fungal strains using disk diffusion method following the National Committee for Clinical Laboratory Standards methods (Kiehlbauch et al., 2000). The sterile Petri dishes (90 mm diameter) containing solid and sterile Mueller-Hinton agar medium (Becton Dickinson, USA) was used. The oil absorbed on sterile Whatman paper disks (5 µl per disk of 6mm diameter), was placed on the surface of the media previously inoculated with 0.1 ml of microbial suspension (1 µg per Petri dish). One filter paper disk was placed per Petri dish in order to avoid a possible additive activity exhibited via the vapour phase of the components from more than one disk. Every dish was sealed with laboratory film to avoid evaporation, and then incubated aerobically at 30 or 37°C according to strain for 24 h. Positive and negative growth controls were performed for every test. The bacterial and fungal sensitivities to the essential oil were assessed by measuring the diameter of inhibition zone. The inhibition zones were compared with that of tetracycline and ticarcilline (Bio-Rad Marnes-la coquette-France), fluconazole and griseofulvin (Bio-Rad-la coquette-France). All tests were performed in triplicate.

Antimicrobial activity essay

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC),

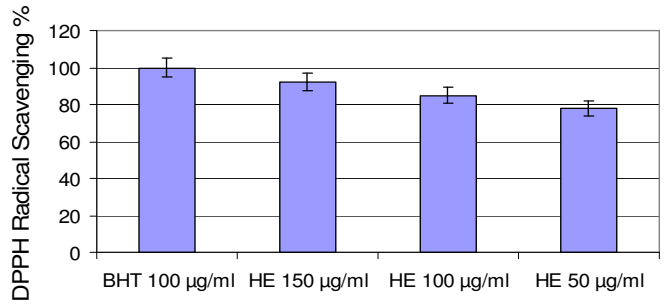

Figure 1. DPPH radical scavenging activity of *Canarium schweinfurthii* essential oil.

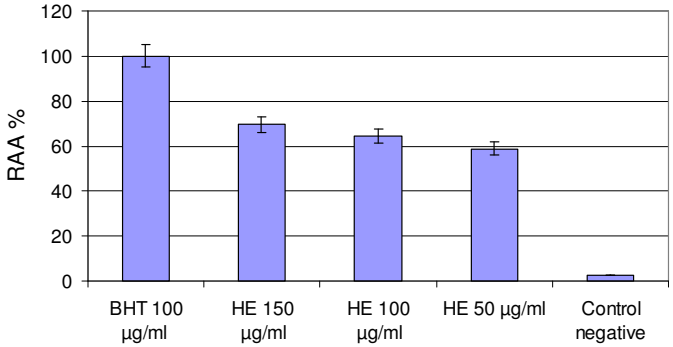

Figure 2. β-carotene bleaching test of *Canarium schweinfurthii* essential oil.

(Bassolé et al., 2003). All tests were performed in Mueller-Hinton Broth (Becton Dickinson, USA).

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity of *C. schweinfurthii* essential oil was investigated with two different methods: 2,2-diphenylpicrylhydrazyl radical scavenging assay and β-carotene bleaching test. The DPPH radical scavenging activity of essential oil was high but relatively lower than that of BHT (Figure 1). The ability of essential oil to inhibit the lipid peroxidation and evaluated by β-carotene bleaching test (Figure 2) showed that the peroxidation of lipids was effectively inhibited by *C. schweinfurthii* essential oil. The essential oil showed higher values than the negative control and a strong activity, but this activity remained weak in comparison to the activity of a 100 $\mu g.ml^{-1}$ concentration of BHT.

Antimicrobial activity

The results showed that almost all of bacterial strains were sensitive to the essential oil (Table 1). Only *P. mirabilis* CIP 104588 was not sensible (zone of inhibition 9 mm). The best sensitivity to essential oil was respectively obtained on *S. enterica* CIP 105150 (27 mm), *S. pyogenes* (25 mm) and *S. aureus* (24 mm). The other strains tested had sensitivities between 14 – 22 mm. Following the results in Table 1, the different strains were more sensitive to essential oil than tetracycline, but were less sensitive to essential oil than tircacilline. The essential oil exhibited more activity on *S. enterica* CIP 105150 (27 mm) than tetracycline (*S. enterica* CIP 105150, 16 mm).

The essential oil was tested against *Candida albicans* as pathogenic fungal species in human body and compared with fluconazole and griseofulvin. The result showed that the growth of fungal species was significantly inhibited by the essential oil (Table 2). Clinic origin *C. albicans* was more sensitive to the essential oil (23 mm) than reference *C. albicans* strain. It was also interesting to find that the inhibition effect of the oil against *C. albicans* (23 mm) were higher than that of fluconazole (*C. albicans*, 9 mm) and griseofulvin (*C. albicans*, 11 mm).

The MICs, MBCs and MFCs of the essential oil for all the strains tested are presented in Table 2. The essential oil failed to inhibit *E. coli* CIP NCTC11602 and *P. aeruginosa* obtained from hospital at the highest concentration (8%). *L. innocua* LMG 1135668, *S. aureus* ATCC9244, *S. camorum* LMG13567 BHI, *S. aureus* (clinic strain), *C. albicans* ATCC90028, *C. albicans* (clinic strain) were inhibited at the lowest MIC of 0.25%. The results of MBC and MFC demonstrated a bactericidal and fungicidal effect. The essential oil was bactericidal for *E. faecalis*, *L. innocua*, *S. enterica*, *S. aureus*, *S. camorum* (reference strains) and *S. aureus* (clinic strains). Furthermore the oil was fungicidal for *C. albicans* ATCC10231 and *C. albicans* (clinic strain). The MIC and MBC values showed that the essential oil was most effective against Gram-positive bacteria than Gram-negative bacteria. Previous reports show that the presence of oxygenated monoterpenes as 1,8-cineole, linalool, α-terpineol, nerolidol, spathulenol in high proportions exhibits antibac-terial and antifungal activities (Chalchat et al., 1997; Kordali et al., 2005; Setzer et al., 2004; Yoshihiro et al., 2004). *C. schweinfurthii* essential oil was composed of relatively lower proportions of these compounds and had antimicrobial activity. These reports are compatible with our results in the present study. Furthermore the essential oils consist of complex mixtures of numerous constituents. Possible synergistic effects of compounds in the essential oil should also be taken into consideration.

In conclusion, this study shows *in vitro* high antimicrobial activities and low antioxidant activity of the *C. schweinfurthii* essential oil. It was bactericidal and fungicidal for most of the reference strains and some clinic strains tested. Its effect is most effective against Gram-positive bacteria than Gram-negative bacteria tested. The essential oil exhibits also antioxidant activity. These results indicate that the essential oil of *C. schweinfurthii*

Table 1. Diameter of inhibition zone (mm) of *Canarium schweinfurthii* essential oil on microorganism growth.

Reference strains	Origin	*C. s.*[a]	Te[b]	Ti[b]
Bacillus cereus LMG13569	LMG	18	18	50
*Enterococcus faeca*l*is* CIP103907	CIP	14.	19	30
Escherichia coli CIP NCTC11602	CIP	22	22	8
Listeria innocua LMG1135668	LMG	21	14	50
*S*a*lmonella enterica* CIP105150	CIP	27	16	50
Shigella dysenteria CIP5451	CIP	22	21	31
Staphylococcus aureus ATCC9244	ATCC	18	17	48
Staphylococcus camorum LMG13567	LMG	22	20	19
Proteus mirabilis CIP 104588	CIP	9	15	16
Hospital strains				
*Enterococcus faeca*l*is*	Foecal	21	20	28
Pseudomonas aeruginosa	Vaginal liquid	21	21	19
Staphylococcus aureus	Vaginal liquid	24	21	27.66
Streptococcus pyogenes	Vaginal liquid	25	20	24.66
Fungal strains			Fluconazole	Griseofulvin
Candida a*lbicans* ATCC10231	ATCC	13	13	15
Candida a*lbicans* ATCC90028	ATCC	17	13	10
Candida a*lbicans*	vaginal liquid	23	9	11

Each value represents mean of three different observations.
[a]*Ca. s. Canarium schweinfurthii*,
[b] Te: tetracycline, Ti: ticarcilline.

Table 2. Minimum inhibitory concentration, minimum bactericidal concentration, minimum fungicidal concentration data (% v/v) of *Canarium schweinfurthii* essential oil obtained by microdilution method.

Strain	Origin	MIC	MBC
Bacillus cereus LMG13569	LMG	4	4
*Enterococcus faeca*l*is* CIP103907	CIP	0.5	0.5
Escherichia coli CIP NCTC11602	CIP	8	8
Listeria innocua LMG1135668	LMG	0.25	0.25
*S*a*lmonella enterica* CIP105150	CIP	0.5	0.5
Shigella dysenteria CIP5451	CIP	1	4
Staphylococcus aureus ATCC9244	ATCC	0.25	0.5
Staphylococcus camorum LMG13567	LMG	0.25	0.5
Hospital strains			
*Enterococcus faeca*l*is*	Foecal	1	4
Pseudomonas aeruginosa	Vaginal liquid	8	8
Staphylococcus aureus	Vaginal liquid	0.25	0.5
Streptococcus pyogenes	Vaginal liquid	4	4
Fungal strains		MIC	MFC
Candida a*lbicans* ATCC10231	ATCC	0.5	0.5
Candida a*lbicans* ATCC90028	ATCC	0.25	1
Candida a*lbicans*	vaginal liquid	0.25	0.25

Each value represents mean of three different observations.

could be used as a natural antimicrobial agent for human and infectious diseases and in food preservation. Furthermore, the development of natural antimicrobial agents will help to decrease negative effects (pollution of environment, resistance) of synthetic chemicals and drugs.

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