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Phytochemical screening and antibacterial properties ofhydroalcoholic and ethanolic extracts from Brassica olerea L.

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ABSTRACT
Objective: *It has long been recognized that some plant materials exhibit antimicrobial properties. The objective of the present study is to explore the properties of Brassica olerea, a medicinal plant used in Congolese Medicinal Traditional, in treatment of burns and infectious diseases.*
Material and methods:*The leaves of Brassica olerea has submitted for chemical screening by conventional techniques focusing on color reactions and chemical precipitation. The susceptibility testing was realized and the extracts were tested against nineteen strains mainlyStaphylococcus pneumonae, Staphylococcus aureus(6 strains), Shigellaflexineri, Salmonelleenterica(3 strains), Pseudomonas aeruginosa, Klebsiellapneumoniae(3 strains), Eschericha coli (2 strains), Enterococcus fecalis, Hemophyllus influenza and Amoxicillinas reference drug. The macrodilution in liquid medium for 24 hours was used to determine the Minimal inhibition concentration.*
Results:*The phytochemical analysis showed the presence of polyphenols and saponins in aqueous extract. The value of Minimum Inhibitory Concentration (MIC) of all extracts wasmore than 50 mg/ml. The MIC value of the amoxicillin was superior (128 mg/ml) for fourteen strains. Greater diameter has obtained with alcoholic extract (Staphylococcus aureus NR46003, with 10 mm) however 27 mm with amoxicillin (Staphylococcus aureus NR46374).*
Conclusion:*These results suggested that the use of leaves from Brassica olerea, collected in Kibumba, must be study deeply before confirmed theirantibacterial activities.*
Keyword*: Brassica olerea, hydroalcoholic and ethanolic extracts, phytochemical screening, antibacterial activities.*

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I. INTRODUCTION

Infectious diseases are still a major health issue, especially in developing countries, leading to the death of millions of people, despite enormous improvement in health care systems. Attention is now being switch over to plants as they may present a new source of antibacterial, antifungal and antiviral agents (Amare*et al.* 2015).

Plants are oldest source of pharmacologically active compounds and have provided many medicinally useful compounds from centuries to the mankind. The research for natural products to cure diseases represents an area of great interest in which plants have been the most important source (Borate and Disale, 2013).

Brassica vegetables are a potent modulator of the innate immune response system with potent antiviral, antibacterial and anticancer activity (Chauhan*et al.* 2012). Some species of this family are of pharmacological interest. Several studies have revealed that they exhibit anti-inflammatory, antimycotic, photoprotective, antihyperglycemic, anticarcinogenic and antioxidant activities (Valéria Dal Prá*et al.* 2015). They are abundant of polyphenolic compounds and contain 15-20 different glucosinolates like compounds(Sikoraand Bodziarczyk, 2012). The secondary metabolite glucosinolate, are the characteristic compounds of the crucifer family (Talreja and Moon, 2014).

*Brassica oleracea*is a plant that has derivates from Europe and currently it is widely propagated all over the worldand it is one of the most important vegetables grown worldwide (Singh*et al.* 2006). This family

includes commonly available vegetables such as cabbage, sprouts, cauliflower. It belongs to the family Brassicaceae (Cruciferae) and, *Brassica oleracea*is a 6 species that includes Broccoli which is very nutritive, providing nutrients and health-promoting phytochemicals such as vitamins, carotenoids, fiber, soluble sugars, minerals, glucosinolates and phenolic compounds (Piruthiviraj*et al.* 2016).Studies have demonstrated the existence of antimicrobial compounds in vegetal materials. In addition, phenolic compounds have been studied due to their antioxidant and antimicrobial properties (Corrêa*et al.* 2014). Naturally occurring bioactive compounds from plant origin have greater antimicrobial activity than purified constituents (Sibi*et al.* 2013).With many problems of multidrug-resistance to antibiotics mainly synthetic drugs, we need to research a new alternative in order to resolve those problems.

The objective of this study was to assess the preliminary phytochemical composition and antibacterial activity of the variety of *Brassica olerea* used in the Eastern part of the Democratic Republic of Congo in many circumstances.

II. MATERIALS AND METHODS

2.1. Plant material

The leaves of *Brassica olerea* were collected in October 2016 at Kibumba; village localized about 15 kilometers from Goma city (East of the Democratic Republic of Congo, in North-Kivu Region). The botanical identification of the plant was done by Mr. Gentil IRAGI of Biological Department of the "Centre de Recherche en Sciences Naturelles de Lwiro", where the voucher specimens were conserved under the voucher specimen N°2102.

2.2. Preparation of extract

The leaves of *Brassica olerea* were collected, dried at room temperature then crushed using a mortar and pestle to obtain a powder. Three hundred grams of plant were introduced into each of the ethanol solvents and an ethanol-water mixture (70/30; v/v) and left to macerate for 24 hours. Duringmaceration, mixtures were stirred 3 times/day. It was made in two stages that is, during twosuccessive days with change of solvent every 48 hours (6000 = 3000 x 2). Each macerate was filtered using Whatman® N°1. The ethanol was evaporated at Rotavapor. The resulting solution was freeze-dried to remove residual water and get the crude extract. The extraction yields were respectively 0.02 % and 0.03%.

2.3. Preliminary Phytochemical screening

The phytochemical analyzes were performed, focusing on the color reaction and precipitate (Handa, 1995;Mangambu *et al.* 2013). The phytochemical screening summarized in the table below:

Table 1. Preliminary Phytochemical Screening of leaves from Brassica olerea

Metabolite	***Reagent Methods***	***Indicator***
Polyphenols	Ferricchloride	Greenishcolor.
Flavonoids	Zinc and Sulfuricacid	Yellow-orange color
Alkaloides	Dragendorff	Red-orangeprecipitate
Saponosids	Frothing test	Persistent frothing
Tannins	Copper sulphate/ammonia	Green, purple, blue or black color
Anthocyanins	Sulfuricacid/Ammonia	Purple-blue
Quinones	Ammonia	Pinkishredcolor
Reducing sugar	Fehling liqueur	Brick redcolor
Terpenoids	Acetic anhydride/sulfuricacid	Bleue color

2.4. Antibacterial activity

2.4.1. Sensibility Test

The method of Cheesbrough (2000) cited by Shiriki*et al.* (2015) was used. The various isolates were each inoculated and incubated at temperatures ranging from 28˚C to 42˚C and their growth and radial growth diameter were observed and measured.

2.4.2. Determination of Minimum Inhibitory Concentrations (MIC)

Macrodilution technique in liquid medium was used(Ngoupayo*et al.* 2015); Mueller Hinton broth (1000 µL) was introduced into 14 tubes making a range of dilution. The first volume of the extract was from a stock solution S, previously filtered on sterile membrane. Then 1000 µL of the stock solution S, being concentrated at 400 mg/mL, were introduced into the first tube of the dilution range. As a result, serial dilutions were in Mueller Hinton broth, so as to obtain a concentration range between 400 mg/mL and 195.10-3mg/ml of plant extract. Then 15 µL of bacterial inoculum was added to each tube of the dilution range (except the controls), and then incubated at 37 °C. After 18 to 24 hours, the turbidity was visually evaluated, and the tubes were centrifuged at a speed of 5000 revolutions/minute for 5 minutes. The MIC of each test sample was derived from the first tube of the range within which any visible growth has not occurred(Ngoupayo*et al.* 2016).

III. RESULTS

3.1. Preliminary Phytochemical analysis

Table 2. Preliminary Phytochemical Screening of leaves from Brassica olerea

Metabolite	***Aqueous extract***
Polyphenols	+
Flavonoids	-
Alkaloids	-
Saponosides	+
Tannins	-
Anthocyanins	-
Quinones	-
Reducing sugar	-
Terpenoids	-

Legend: +: Positive test -: Negative test

The preliminary phytochemical analysis of aqueous extract of leaves from *Brassica olerea*, collected in Kibumba city (North-Kivu, Republic Democratic of Congo) showed the presence polyphenols compounds, mainly saponosides. We observed absence of others secondary metabolites.

3.2. Antibacterial evaluations

Table 3. Minimum Inhibitory Concentration of extracts from Brassica olerea leaves

Microbialstrains	***MIC HA (mg/ml)***	***MIC EE (mg/ml)***	***Amoxicillin (128µg/ml)***
*Staphylococcus pneumonae*ATCC49619	>50	>50	>128
*Staphylococcus aureus*BAA 917	>50	>50	>128
*Staphylococcus aureus*ATCC43300	>50	>50	>128
*Staphylococcus aureus*NR45003	>50	>50	>128
*Staphylococcus aureus*NR46003	>50	>50	>128
*Staphylococcus aureus*CP7625	>50	>50	8
*Shigellaflexineri*NR518	>50	>50	>128
*Salmonelleenterica*NR4294	>50	>50	>128
*Salmonelleenterica*NR4311	>50	>50	>128
*Salmonelleenterica*NR13555	>50	>50	>128
*Pseudomonas aeruginosa*MC 592	>50	>50	>128
*Klebsiellapneumoniae*ATCC13883	>50	>50	>128
*Klebsiellapneumoniae*ATCC70603	>50	>50	16
*Klebsiellapneumoniae*NR41916	>50	>50	>128
Eschericha coli ATCC25922	>50	>50	16
Eschericha coli ATCC35218	>50	>50	>128
*Enterococcus fecalis*ATCC51219	>50	>50	16
*Staphylococcus aureus*NR46374	>50	>50	16
Hemophyllus influenza ATCC 49247	>50	>50	>128

***Legend: HA** (Hydroalcoholic extract), **EE** (Ethanolic extract)*

According this table 3, the value of Minimum Inhibitory Concentration in all extracts of the plant was superior of 50 mg/ml. the lowest values with drug reference were 8 and 16 *128µg/ml respectively for Staphylococcus aureus*CP7625 and *Klebsiellapneumoniae*ATCC70603, *Eschericha coli* ATCC25922, *Enterococcus fecalis*ATCC51219 and *Staphylococcus aureus*NR46374.

***Table 4.*Zone of inhibition of *Brassica olerea* extracts with test organisms in mm**

Microbialstrains	***HA (50mg/mL)***	***EE (50mg/mL)***	***Amoxicillin (128µg/mL)***
*Staphylococcus pneumonae*ATCC49619	9	0	20.7
*Staphylococcus aureus*BAA 917	0	0	0
*Staphylococcus aureus*ATCC43300	0	0	8.7
*Staphylococcus aureus*NR45003	0	0	24.2
*Staphylococcus aureus*NR46003	**6**	**10**	14.7
*Staphylococcus aureus*CP7625	0	**7**	11.2
*Shigellaflexineri*NR518	0	0	21.7
*Salmonelleenterica*NR4294	0	0	0
*Salmonelleenterica*NR4311	**8**	0	7
*Salmonelleenterica*NR13555	0	0	10.7
*Pseudomonas aeruginosa*MC 592	0	0	17.2
*Klebsiellapneumoniae*ATCC13883	0	0	23.7
*Klebsiellapneumoniae*ATCC70603	0	0	12.5
*Klebsiellapneumoniae*NR41916	0	0	16.5
Eschericha coli ATCC25922	0	0	18.5
Eschericha coli ATCC35218	0	0	4
*Enterococcus fecalis*ATCC51219	0	0	21.7
*Staphylococcus aureus*NR46374	**6**	0	27.5
Hemophyllus influenza ATCC 49247	0	0	22.5

***Legend: HA** (Hydroalcoholic extract), **EE** (Ethanolic extract)*

IV. DISCUSSION

This phytochemical analysis of *Brassica olerea* leaves (were shade dried followed by hot air oven drying at 50° centigrade) showed the presence of polyphenols compounds as saponosides. This result is near of this of Chauhan and Singh (2016) that showed the presence of saponosides in the same sample. According Šamec*et al.* (2014), Brassica vegetables are rich in polyphenolic compounds that have recently receive considerable attention as potential protective factors against cancer and heart diseases, particularly because of their antioxidative properties. In addition to polyphenolics, carotenoids are another group of bioactive components highly abundant in cabbage. The Analysis by CG-MS allowed the identification of many compounds as phenolic (Owis, 2015). Contrary to our present study, a qualitative phytochemical analysis showed the absence of phenolic compounds but presence of alkaloids, flavonoids and terpenoids (Suganya*et al.* 2016).

Sanz-Puig*et al*. (2014) reported the correlation between functional properties attributed to these vegetables and their polyphenol contents. The phytochemical contents of the leafy vegetables serve as supplements for food and also have the potential to improve the health status of its users as a result of the presence of various compounds vital for good health.

In the present study, the antimicrobial activity of two extracts (hydroalcoholic and ethanolic)of *Brassicaceae*leaves was evaluated using the macrodilution in liquid medium for 24 hours to determine the Minimal inhibition concentration against nineteen pathogenic strains like *Staphylococcus aureus*(6 strains), *Shigellaflexineri, Salmonelleenterica*(3 strains), *Pseudomonas aeruginosa, Klebsiellapneumoniae*(3 strains), *Eschericha coli* (2 strains), *Enterococcus fecalis*, *Hemophyllus influenza* and Ampicillinas reference drug. These two extracts showed an MIC value superior to 50 mg/ml at all strains. Diameter of inhibition did not show a conclusive result except for two cases with ethanolic and hydroalcoholic extracts at *Staphylococcus aureus*NR46003 (respectively 10 and 8 mm). Others cases have obtained with ethanolic extract to *Staphylococcus aureus*CP7625 (7mm) and hydroalcoholic extract to *Salmonelleenterica*NR4311 (8 mm). This diameter was greater than observed with drug reference (7 mm). In another study, Ethanol, acetone, chloroform extracts from red cabbage showed least inhibition against *strains (*Ayshwarya and Rameshwari, 2015). However, the ethanol extracts of all the plant parts were found to be highly effective whereas the petroleum ether, methanol and ethyl acetate extracts of root, stem and leaves of *Brassica campestris*respectively exhibited a good antibacterial activity against all bacterial strains (Prasad, 2014).

It is widely accepted that the antimicrobial component may be different between ethanolic extracts and aqueous extracts of the plants (Peng*et al.* 2014). However, this study a fermeted juices indicated that the strongest inhibitory activity, after 21-day fermentation (Gogo et al. 2010).

Many studies reported showed the antibacterial and fungicidal activities of Brassica species in different solvent mainly acetone, chloroform, ethyl acetate,methanol, petroleum ether. In addition, antimicrobial activity of *Brassica oleracea*varieties were reported (Begum and Poonkothai, 2013; Sibi*et al.* 2013; Amare*et al.* 2015; Satya*et al.* 2015;

Thus it is very important to examine all of those solvent and select the best for deeper studies.

V. CONCLUSION

Hydroalcoholic and ethanolic extracts of *Brassica olerea* leaves by agar well diffusion method, against nineteen strains selected revealed none antibacterial properties. Further research, including phytochemical analysis, with others extracts, in order to precise its properties.

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