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Phytochemical Screening and Antibacterial and Antifungal Activity of *allium sativum* Juice on Multi-Resistant Strains

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Abstract Among these plants are the species of the genus Allium which are unequally distributed in the world. There are more than 120 documented uses, but its chemical identity and antibacterial and antifungal properties (Agarwal K.C. et al. 1996,) are very poorly documented in the Congo. Material and Methods: We used fresh bulbs of Allium sativum grown in Congo, after grinding and filtration of garlic 120 ml of juice was obtained after. Phytochemical studies were carried out with determination of the content of flavonoids and on a young culture the antibacterial and antifungal activity in vitro of the aqueous extract of garlic was evaluated in the Bacteriology department, of the National Public Health Laboratory. Result: Allium Sativum harvested in Congo has a strongly positive flavonoid level. Gram-positive bacterial strains were more sensitive to the extract than gram-negative bacterial strains. The inhibitory effect of pure allium sativum extract was effective compared to conventional antibiotics. Conclusion. This study allowed us to justify the validity of the antimicrobial virtues granted to this plant.

Keywords *allium sativum*, Phytochemistry, Antibacterial, Antifungal

1. Introduction

Antibiotic resistance is a natural and predictable mechanism that refers to a situation where an antibiotic that should normally have stopped the development of a certain type of bacteria is no longer able to do so (Acar J.F et al., 2003). According to the WHO, with no response from the international community, more than 10 million people could die worldwide each year from resistant bacterial infections in 2050 (WHO, 2015). In Africa, many cases of multi-resistance have been reported particularly to beta-lactamases with rates ranging from 30% to 50% in enterobacteria or for resistance to meticillin in Staphylococcus aureus which is greater than 30% (Ouedraogo et al., 2017). In Congo, one study reported the prevalence of *H. pylori* infection of 75.52%, resistant to clarithromycin (4.2%) and tetracycline (1.2%) and levofloxacin (57%) (Ontsira et al., 2016). One solution is to explore medicinal plants, whose antibacterial and antifungal potency is believed to be due in whole and/or in part to the substances they contain (Dorant et al., 1996). Among these plants are the species of the genus Allium which are unequally distributed in the world. With each according to the location recovered the difference of taste, shape and color, but the biochemical identity remains close. There are more than 120 differents documented uses, however its antibacterial and antifungal properties (Adetumbi et al., 1993) are very poorly documented in the Congo. For example, the purpose of this study is to identify the different substances in the aqueous extract of garlic from Brazzaville, as most of its therapeutic properties are attributed to allicin (Sendl, 1995; Jiben et al., 2006) and to assess in vitro the antibacterial and antifungal potency of Allium sativum juice vis-à-vis «multi-resistant» clinical strains.

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2. Materials and Methods

2.1. Plant Material

We used the fresh bulbs of Allium sativum grown in Congo and present in the markets of Bacongo, Brazzaville (Congo) from 1 to 5 September 2018 and have been kept at

room temperature at the Laboratory of Pharmacology and Biochemistry of the Faculty of Health Sciences.

2.2. Preparation of Garlic Juice Extract

We obtained 120 ml of juice after grinding and filtration of garlic and their cloves; the filtrate was then centrifuged at 3000 rpm for 20 minutes and the supernatant was recovered and kept at +4℃ until use. To assess activity, dilutions of the juice in physiological water were prepared (100%, 80%, 50%, 20%, 10%).

2.3. Preparation of Solid Extractions

We impregnated the blotting paper discs (wattman) with garlic juice, of each previously prepared concentration for (07) seven hours.

2.4. Phytochemical Screening

This is a technique that allows the presence of groups of chemical families in active extracts of a plant substance to be demonstrated in order to evaluate the anti-bacterial activity. The results were presented as follows: (+++) Strongly positive, (++) Moderately positive, (+) Weakly positive and (-) Negative.

2.4.1. Flavonoids: Two (2) ml of the acquired extract have been evaporated and 5 ml of residue has been recovered in summer mixed with a few drops of magnesium, there is a heat release then a yellow coloration. this colouration confirmed the presence of flavonoids [Azzi R, 2012]. The Aluminum Trichloride method is used to quantify flavonoids in the extract. Absorbance is read at 415nm by a UV-visible spectrophotometer.

2.4.2. Tannins: Highlighted by adding to 1 ml of water extract, 1 ml of water and 1 to 2 drops of 1% diluted Fecl3 solution The appearance of different colours corresponds to a specific type of tannin.

2.4.3. Saponins: a series of 5 numbered tubes, 10ml of the solution to be analysed prepared by decoction in an aqueous medium is introduced followed by an adjustment of the with distilled water. Shake each tube lengthwise for 15 seconds at 2 agitations per second. Let stand 15 min and measure the height of the foam produced in each tube.

2.4.4. Tri-terpenes and Sterols: they are sought by the Liebermann reaction.

2.4.5. Alkaloids: In order to check their presence, a precipitation reaction with Dragendorff reagent is required. [Azzi R, 2012].

2.5. Selection of Microbial Strains

To assess the antimicrobial activity of allium sativum, clinical strains with lethal resistance of at least four (04) conventional antibiotics and/or antifungals were selected. Micro organisms from urine (Candida.albicans, Candida.spp, Staphylococcus.aureus, Klebsiella.oxytoca, Enterobacter.spp), stool (Eschéricha.coli) and vaginal collection (Staphylococcus. non coagulase Klebsiella.pneumonia).

2.5.1. Antimicrobial Activity

The method of diffusion on disk was used from 19 to 22 September 2018 to evaluate the antibacterial and antifungal activity in vitro of the aqueous extract of garlic in the department of Bacteriology, of the National Laboratory of Public Health. On a young crop, we sampled a colony, suspended 10ml of physiological water, and then inoculated on the surface of a Muller Hinton agar can (MH) by swabbing. Then, sterile blotting paper discs (Wattman N°1, 6 mm in diameter) impregnated with each garlic juice dilution and an additional disc reserved for the negative control (distilled water) were placed with a sterile clamp on the surface of the M.H petri dishes previously seeded with the bacterial and fungal strains to be tested. Petri dishes were left at 37℃ for 30 minutes to allow the juice to be distributed. They were then incubated at 37℃ for 18-24 h. Inhibition diameters were expressed in mm. The tests were duplicated and the results were expressed as averages.

The T Student test was used to calculate the mean inhibition diameters of bacterial and fungal strains.

3. Results and Discussion

3.1. Phytochemical Study

The presence in strong or small quantities of each chemical fraction was judged by the degree of staining:

Table 1. Results of phytochemical analysis of aqueous allium extracts

	Chemical groups				
	Alkaloids	Tanins	Flavonoids	Tri-terpenes and sterols	Saponosides
Allium Sativum L	++	++	+++	+ ++ +	-

Figure 1. Result after mixing

The determination of flavonoids was carried out using the Alcl3 method. Our reference was Quercetin. The unit of content was mg Quercetin equivalent per mL of extract (mg EQ/mL E). the appearance of the yellow colour as indicated above revealed the presence of flavonoids. The level of recovered flavonoids of 0.06 0.002 was strongly positive.

This result is partly in line with that of Moumen (2016), who found a moderately positive presence. This difference may be related to a difference in culture method. However, overall, the families present in our study were found in the literature (Derridj et al., 2013). The level of flavonoids found is lower than those found in Algeria. This difference can be related to both countries' climate and conservation (Bouyha et al, 2016).

3.2. Anti-microbial Activity

We observed that, gram-positive bacterial strains were more sensitive to the extract compared to gram-negative bacterial strains. This observation is consistent with that reported by Benzeggout et al., (2005) which reported the susceptibility of gram-positive bacteria to garlic juice with diameters between 20 mm and 22 mm for Staphylococcus (Gram+). Other species including E. coli ATCC 25922 and Enterobacter sp (Gram-) were less sensitive with inhibition diameters between 13mm and 15mm. This could be due to the chemical composition of the wall of gram-negative bacteria that exhibit a lypopolysaccharide structure (Amagase H., et al., 2003; Ackermann TC, 2001).

The inhibitory action of the allium sativum extract decreased with dilutions. This could be explained by several factors including the mode of extraction and the concentration of volatile active ingredients (Thangara et al 2000). The inhibitory effect of pure allium sativum extract was effective compared to conventional antibiotics used in the present study with inhibition zones between 15mm and 22mm.

Table 2. Mean D.I of the different dilutions of A.S on bacterial strains

Bacterial strains	Inhibition diameter (mm)									
	Extract from A.S						**TCC**	**VEN**	**IPN**	**AZM**
Dilution	**100%**	**80%**	**50%**	**20%**	**10%**	**T-**	**10ug**	**10ug**	**10ug**	**15ug**
Gram -										
K.pneumonia	**20**	17	15	10	0	0	-	-	**19**	-
K.Oxytoca	**20,5**	**17**	14	9	0	0	-	**16,5**	-	-
Enterobacter.spp	**18**	14	9,5	0	0	0	-	**17**	-	-
E.coli	**19,5**	16,5	12	0	0	0	-	-	**18,5**	-
P. aerogenosis	**22**	**19,5**	15,5	8	0	0	**18,5**	-	-	-
Proteus.spp	**19**	15	8	0	0	0	-	-	**19,5**	-
S.typhi	**19,5**	15	10	0	0	0	-	-	-	**19**
Shigellas	**17,5**	15	10	0	0	0	**17**	-	-	-

D.I: Inhibition Diameter, TCC: Ticarcilin+Clavunalic acid, GEN: Gentamycin, IPN: Imipenen, AZM: Azithromycin, T: Negative control, A.S: Allium Sativum

Table 3. Mean D.I of the different dilutions of A.S on bacterial strains

Bacterial strains	**Inhibition diameter (mm)**								
	Extract from A.S						**VA**	**GEN**	**E**
Dilution	**100%**	**80%**	**50%**	**20%**	**10%**	**T-**	**30ug**	**10ug**	**15ug**
Gram +									
S.aureus	**20,5**	**19**	**15**	10	0	0	**15**	-	-
S.non coagulase	**22**	**20**	14	9	0	0	-	**18**	-
Streptococcus.spp	***20,5***	**17,5**	10	0	0	0	-	-	**17,5**
S. α- hemolytic	***20***	**18**	10	7	0	0	-	-	**18**

D.I: Inhibition Diameter, VA: Vancomycin, GEN: Gentamycin, E: Erythromycin
T- Control negative, A.S: Allium Sativum

Table 4. Mean of D.I of the different dilutions of A.S on fungal strains

Fungal strains	Inhibition diameter (mm)							
	Excerpt from **A.S**						**IT**	**NY**
Dilution	**100%**	**80%**	**50%**	**20%**	**10%**	**T-**	**10ug**	**50ug**
Candida. Albicans	**17,5**	15	11	09	0	0	**16,5**	-
C. non albicans	**21**	**18**	12	10	0	0		**19**

3.3. Antifungal Activity of Garlic Juice

Allium sativum juice showed antifungal activity. Many studies have reported that the inhibitory action of allium sativum juice on fungi may be due to the formation of hydrogen bonds between the hydroxyl group of phenolic compounds and the active sites of the target enzymes (Yin and Tsao et al., 1999). Other authors have shown that the extract acts on mycelium hyphae, causing the loss of stiffness and integrity of the cell wall, resulting in its collapse and death of the mycelium (Sheela C.G. et al., 1992). An effective inhibitory action of pure extract against species of C.non albicans and C.albicans was observed. Our results are similar to those of Yamada et al., 1997, which had shown in a study that pure allicin was very effective against species of Candida, Crytococcus, Trichophyton, Epidermophyton with a MIC of 1,57 to 6, 25µg/ml. This inhibitory action of the extract decreased as a function of the dilutions achieved. This could be explained by the method of extraction and the presence of chemicals at different concentrations of the extract (Irkin et al., 2007). The inhibitory effect of pure allium sativum extract was effective compared to the conventional antifungals used in this study with inhibition zones between 17.5 mm and 21mm.

4. Conclusions

The chemical identity of allium sativum grown in the Congo is very close to that described in the literature. The sensitivity of the different strains tested to allium sativum juice and the antibiotic and/or fungal control is of great importance in the treatment of the associated pathologies. This study allowed us to justify the validity of the antimicrobial virtues granted to this plant.

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