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Full Length Research Paper

A survey of the antibacterial activity of three plants used in the Congolese herbal medicine practiced by the healers in the city of Lubumbashi

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In order to promote Congolese folk medicine, which has been proven to be a viable alternative for our population confronted with health problems due especially to the lack of easy access to primary care and considering the multiplicity of diseases which daily torment them, a survey of the antibacterial activity of three medicinal plants used in herbal medicine by the healers in the city of Lubumbashi has been carried out. Tests of sensitivity of the microbes towards the plant extracts have been achieved in order to search for their therapeutic efficiency since according to the ethnobotanical information, they are used against many diseases. After analysis, the results showed that, as far as their inhibitory capacity on the microbes is concerned, the three plants (*Terminalia mollis*, *Diospyros batocana* and *Antidesma venosum*) were bioactive. *Proteus mirabilis* is the microbe more sensitive to the extracts of *T. mollis* whereas *Salmonella typhii* showed greater sensitivity to extracts of *D. batocana* and *A. venosum*. Concerning *Klebsiella pneumoniae pneumoniae*, no sensitivity was observed towards extracts of *A. venosum.*

Key words: Antibacterial activity, medicinal plants, microbe sensitivity.

INTRODUCTION

No one can minimize the important role played by the healers in the improvement of the health's system in many African countries (Barakat et al., 2013; Samy et al., 2013; Steenkamp et al., 2013). Recently, a lot of countries throughout the world have succeeded in integrating their traditional medicine into their health system in order to satisfy with efficiency the health needs expressed by their population. Indeed, modern medicine has showed its limits in some cases. This is the reason why, in a research on the healers and the medicinal plants in Lubumbashi published in 2004, Petit and Vakyanakazi (2004), indicated the importance of folk medicine and the fact that WHO have encouraged the Democratic Republic of Congo (DRC) to promote its herbal medicine as is the case in China, India, Argentina, Nigeria and Senegal which are now considered as examples of a succeeded cohabitation between the modern and the traditional medicines. In the DRC, the effort of integration of the traditional medicine into our health policy has been concretized in 2002 by the recognition of the Congolese Healers Association by the Ministry of Heath.

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It is true that folk medicine remains as an alternative for our population greatly impoverished and preoccupied by the problem of access to primary health care. To support this statement especially in the case of the city of Lubumbashi, one can note with bitterness, as Petit and Vakyanakazi (2004) did after their investigation, that the healers practice their professions successfully in the poor media of our city, that means the areas without hospitals and where the greatest part of our fellow citizens lives. Indeed, in our city the majority of inhabitants are confronted with serious heath problems. This is indicated when one consider the analysis of a small sample of sick persons which led to the identification of enterobacteria pathogens and their cure in the University Of Lubumbashi clinics (Cliniques Universitaires de Lubumbashi 2006). These statistics had revealed that, among the patients sampled, about 25% cases of gastro-enteritis, 25% of sharp enteritis, 15% of septicemia and 5% of cystitis, have been observed, respectively. Besides, these pathologies were generally observed during the rainy seasons.

Based on inquiries by Petit and Vakyanakazi (2004), it is established that these pathogens found among the population in the city of Lubumbashi are cured by modern physicians but also by the herbal medicine of the healers. However, it is true that an expert, involved in the health sector or its related field, will wonder about the rationality of the curative scheme and the efficiency of the therapeutic choice that the healer uses in their medicine. Here is the fundamental question to which this study would like to give answer. The present survey aims at evaluating the antibacterial activity of three medicinal plants used in the Congolese herbal medicine in view to check the therapeutic validity of the medicinal practices that the healers use in the city of Lubumbashi. It is based on a preliminary research consisting in tests of the sensitivity of three reference pathogenic microbes (*Klebsiella pneumoniae pneumoniae*, *Salmonella typhii* and *Proteus mirabilis*) towards extracts of *Terminalia mollis*, *Diospyros batocana* and *Antidesma venosum* since they are extensively used by the healers in the herbal medicine (Petit and Vakyanakazi, 2004). Indeed, based on the literature and the results from ethnobotanical investigations (Kitwa, 2002; Kruger, 2004; Petit and Vakyanakazi, 2004; Tshibangu, 1997), *T. mollis* is a plant of interest from a medical point of view because it acts against intestinal worms, diarrhea, tuberculosis, malaria, cholera, cancer and dysentery (Mustopha et al., 2000; Sanin et al., 2003; Sumesh et al., 1999). Literature also indicates the use of another plant species in the family, *Terminalia sericea*, against human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/AIDS), diarrhea, coughing and cutaneous infections (Bessong et al., 2004). Concerning *D. batocana*, it is in a family whose species have innumerable usages in phytomedecine (Borsub et al., 1976; Hostettmann et al., 2000; Khang and Timi, 1999(a-c); Khang et al., 1987; Mallavadhani et al., 1998; Moody and Roberts, 2002; Xing-Cong et al., 1998).

It is used to cure gonorrhea and infertility, particularly the azospermia. Talking about the medicinal use of *A. venosum*, as with other species of its family, it is a plant of great pharmacological interest (Buske et al., 2001, Bringmann et al., 2000, 2001) owing to its efficient action against gonorrhea, tooth decay, abdominal pains, gastritis, infertility, precocious abortion, threats of abortion and sexual impotence.

MATERIALS AND METHODS

Based on the ethnobotanical investigations, three plants used in Congolese phytomedicine were harvested and studied: *T. mollis* (Chrysobalanceae), *D. batocana* (Ebenaceae) and *A. venosum* (Euphorbiaceae). They have been selected in order to determine their antibacterial activity towards a group of well identified pathogenic microbes. The bioactivity tests of these plants have been achieved as described below:

Plant identification

Botanical identification and harvest of the studied plants was carried out by the Geomorphology and Botany laboratory, the Unit of Ecology and Environment (Department of Geography, Faculty of the Sciences) at the University of Lubumbashi (DRC). They come from the forest of the FIKUPA locality found in the mining hinterland of Katanga (DRC) and were identified under the following herbarium numbers: N° 4367 – *T. mollis Lacus* (Combretaceae), N° 4368 – *D. batocana Hiern.* (Ebenaceae), N° 4369 – *A. venosum* E.mey.ex Hoffn. (Euphorbiaceae).

Choice of the microbes of interest

The microbes that we used for the antibacterial tests were identified by their ATCC codes as described below and are usually used as reference for microbial testing in the Clinical Biology laboratory at the University of Lubumbashi Clinic. *P. mirabilis*: ATCC 35659; *K. pneumoniae pneumoniae*: ATCC 35657; *S. typhimurium:* ATCC 14028.

The preparation of the excerpts

For the preparation of the plant extracts, 50 g of plant material constituted of stem peels were dried, ground using an agate mortar and submitted to steeping for 72 h with 750 ml of methanol as solvent in view of extracting the bioactive molecules. The obtained alcoholic solution undergone filtration prior to the solvent removal by vacuum evaporation (40°C) using a BIBBY RE200B rotavapor connected to a KNF Neuberger LABOPORT vacuum pump. The preparation of the plant's extracts was done using 50 g of plant material and the following outputs were obtained: *T. mollis* (9.0%), *D. batocana* (15.6%) and *A. venosum* (8.1%). These plants' extracts were sampled and resuspended in distilled water to prepare solutions to be tested with bacterial strains of our choice.

Isolation of bacteria and inoculation of the plants extracts

The bacteria were cultured in liquid Mueller Hincton medium with a concentration equal to 2 units of Mac Farland (BioMérieux, 1996). This bacterial concentration corresponds to an optical density of 0.250 which was measured spectrophotometrically using the 550 nm wavelength (Spectrophotometer Genesis 20); 2 ml of this

Table 1. Result of microbe sensitivity tests towards *T. mollis* extracts.

Plant's extracts dilution (mg/ml)	*K. pneumoniae pneumoniae*	*S. typhii*	*P. mirabilis*	Number of positive tests	Proportion (%)
50	+	+	+	3/3	100
25	+	+	+	3/3	100
12.5	+	+	+	3/3	100
6.25	-	-	+	1/3	33.3
Number of positive tests	3/4	3/4	4/4	10/12	83.3
Proportion (%)	75	75	100		

+, Inhibition of the bacterial activity; –, failure or resistance to the extract action.

preparation were placed in tests tubes containing 2 ml distilled water containing the medicinal plant's extracts. These extracts were diluted with distilled water progressively in half of a mother solution with a concentration equal to 50 mg/ml to give extracts successively to concentrations equal to 50, 25, 12.5 and 6.25 mg/ml. For each test, three replicates were done per plant extract and concentration per bacterial strain. Incubation of the tests tubes was carried out for 24 h between 35 and 37°C in a stream room.

The reading of the sensitivity of the microbes towards the plant extracts was achieved by visual observation of the turbidity development in the test tubes (Figure 1) and the obtained result validated using the Petri dishes technique for the numbering of the bacteria colonies (Ayres and Mara, 1996). The observation of the turbidity developed in the tests tubes has enabled us determine the minimum inhibitory concentration (MIC). As for the minimum bactericidal concentrations (MBC) in view establishing whether the studied extracts are bacteriostatics or bactericidal, it was determined through the seeding of bacteria, contained in the precipitate recovered from the test tubes, in the Petri dishes using the Mueller Hincton Agar.

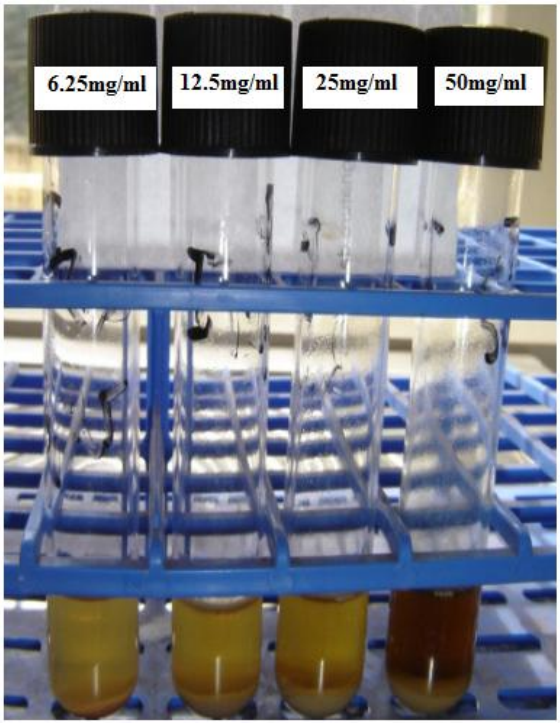

Figure 1. Tests of the sensitivity of *P. mirabilis* towards the extracts of *A. venosum.*

RESULTS AND DISCUSSION

The concerned results are related to the determination of sensitivity of *K. pneumoniae pneumoniae*, *S. typhii* and *P. mirabilis* towards the extracts of *T. mollis*, *D. batocana* and *A. venosum*. The active dilutions of the extracts which ranged from 50 to 6.25 mg/ml were incubated for 24 h at between 35 and 37°C.

Bioactivity of *T. mollis*

The sensitivity of the studied bacterial strains towards the extracts of *T. mollis* is shown in Table 1. It is obvious that *T. mollis* extracts have a substantial inhibitory action on all three microbes because about 83.3% of tests were successful. As a matter of fact, the bacterial activity was inhibited for all dilutions in 75 to 100% of the achieved bioassays. The sensitivity of *P. mirabilis* towards these extracts was the largest of all because the MIC of 6.25 mg/ml which corresponds to the greatest dilution of the extract. Concerning the sensitivities of *K. pneumoniae pneumoniae* and *S. typhii* towards the extracts, they seemed to be similar. The total inhibition of microbes was gotten at a dilution of 12.5 mg/ml. It is to this concentration that the microbes begin to resist the extracts inhibitory action. Additionally, the bioassays reveal the broadening of the antibacterial spectrum of the extracts of *T. mollis* when the concentration increases or surpasses the MIC which is below 6.25 mg/ml for *P. mirabilis*. Concerning *K. pneumoniae pneumoniae* and *S. typhii*, the MIC was 6.25 mg/ml of the extracts. As for the MBC, it is nil because the bacterial developed on the Mueller Hincton agar.

Bioactivity of *D. batocana*

The tests of sensitivity of the microbes towards the extracts of *D. batocana* led to the results in Table 2.

Table 2. Result of microbe sensitivity tests towards *D. batocana* extracts.

Plant's extracts dilution (mg/ml)	*K. pneumoniae pneumoniae*	*S. typhii*	*P. mirabilis*	Number of positive tests	Proportion (%)
50	+	+	+	3/3	100
25	+	+	+	3/3	100
12.5	-	+	-	1/3	33.3
6.25	-	+	-	1/3	33.3
Number of positive tests	2/4	4/4	2/4	8/12	66.7
Proportion (%)	50	100	50		

+, Inhibition of the bacterial activity; –,failure or resistance to the extract action.

Table 3. Results of microbe sensitivity tests towards *A. venosum* extracts.

Plant's extracts dilution (mg/ml)	*K. pneumoniae pneumoniae*	*S. typhii*	*P. mirabilis*	Number of positive tests	Proportion (%)
50	-	+	+	2/3	66.7
25	-	+	+	2/3	66.7
12.5	-	+	+	2/3	66.7
6.25	-	+	-	1/3	33.3
Number of positive tests	0/4	4/4	3/4	7/12	58.3
Proportion (%)	0	100	75		

+, Inhibition of the bacterial activity; –, failure or resistance to the extract action.

These results indicate that *S. typhii* showed a very great sensitivity (100%) towards the excerpt of *D. batocana* because the minimal inhibitory concentration is the lowest possible for this microbe since it resists even at the greatest used dilution. For the other microbes, *K. pneumoniae pneumoniae* and *P. mirabilis*, displayed a similar behavior (50%) towards these extracts to which they resisted for dilutions below 25 mg/ml. Their MIC is equal to 12.5 mg/ml of the extracts of *D. batocana*. Globally, the inhibitory action of the extracts on the microbes is 50%. However, it is worth mentioning that the MBC is also nil in this case.

Bioactivity of *A. venosum*

The test of sensitivity of the microbes on *A. venosum* extracts is shown in Table 3. These results show that *S. typhii* is more sensitive to the extracts of *A. venosum* because for this microbe, the proportion of positive tests is equal to 100% whatever may be the dilution. As far as the sensitivity of the other microbes towards the extract is concerned, one can notice that *P. mirabilis* (Figure 1), with 75% of positive tests, came second in position. On the other hand, the resistance shown by *K. pneumoniae pneumoniae* to the plant extract was the largest noted while analyzing the bioassay results. Nevertheless, the inhibitory action on the microbes shown by the extracts of *A. venosum* is smaller than for *T. mollis* and *D. batocana* because only 58.3% positive tests were recorded. The MIC for *S. typhii* is below 6.25 mg/ml of the extracts of *A. venosum* contrarily to *P. mirabilis.* However, the MBC is nil due to the growth of bacteria on the Mueller Hincton Agar.

Globally, it was deduced from the results that the three plants, according to their inhibitory action on the microbes, may be classified as follows: *T. mollis*, *D. batocana* and *A. venosum*. On the other hand, one can see that *P. mirabilis* is more sensitive to the extracts of *T. mollis* whereas *S. typhii* showed a bigger sensitivity towards *D. batocana* and *A. venosum*. With regard to *K. pneumoniae pneumoniae* , it did not have any sensitivity to the extracts of *A. venosum*. Its sensitivity to those of *T. mollis* was more pronounced than it demonstrated towards the extracts of *D. batocana*. To all dilutions and whatever may be the excerpt, the sensitivities of the microbes were classified in the following order: *S. typhii* with 91.7% of the positive tests, *P. mirabilis* with 75% and *K. pneumoniae pneumoniae* with 41.7%, respectively. Considering that the MBC is nil in all the cases, it can concluded that the plants' extracts tested against the bacterial strains of our interest are bacteriostatics. Based on the sensitivity shown, at different dilutions, by the studied bacterial strains towards the plants' extracts, only the result related to the action of the extracts of *A. venosum* against *K. pneumoniae pneumoniae* shows a difference statistically significant ($p = 0.021$). No significant difference is observed with regard to the results

given by the sensitivity of bacterial strains towards the remaining plants' extracts ($p > 0.05$).

Conclusion

On the antibacterial efficacy standpoint, the three plants used in the Congolese folk medicine can be classified as follows: *T. mollis*, *D. batocana* and *A. venosum*. Their use by the healers against illnesses such as diarrhea, gastroenteritis, urinary infections, typhoid fever and dysentery is well justified. *P. mirabilis* is the microbe most sensitive to the extracts of *T. mollis* whereas *S. typhii* showed more sensitivity to *D. batocana* and *A. venosum*. However, *K. pneumoniae pneumoniae* showed a strong resistance to the extracts of *A. venosum.* The sensitivity of the germs to the extracts of *T. mollis* was bigger than the one shown towards those of *D. batocana*. To all dilutions of the extracts, sensitivity of the microbes was classified as follows: *S. typhii*, *P. mirabilis* and *Klebsiella pneumoniae pneumonia*. Only the extracts from *T. mollis* and *D. batocana* possess antibacterial activities enabling them to be used as sources of new drugs. The plants extracts tested against the reference bacteria of our interest are bacteriostatic.

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ABBREVIATIONS

DRC, Democratic Republic of Congo; **HIV/AIDS,** human immunodeficiency virus/ acquired immunodeficiency syndrome; **MIC,** minimum inhibitory concentration; **MBC,** minimum bactericidal concentrations.

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