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Research Article

Antimicrobial Effect of the Chloroform Phase of *Praxelis clematidea* R.M. King & Robinson

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ABSTRACT

Medicinal plants constitute an arsenal of chemicals that could be exploited by human to prevent microbial invasion. *Praxelis clematidea* R.M. King & Robinson belongs to the family Asteraceae. Plants from this family have been extensively studied for the development of new drugs and insecticides. Based on this information, the chloroform phase of *Praxelis clematidea* was evaluated for antibacterial and antifungal activity. Six bacterial strains and six fungal strains were used in the study for activities. Microdilution method was used for antibacterial and antifungal assay of the chloroform phase. The results were also compared with the standard drug, Chloramphenicol (100 µg/mL) and Nistatin (100 UI/mL). The obtained results showed activity of the chloroform phase against *Candida* species, in particular against *Candida albicans*, which highlights the immense antifungal potential of this plant species.

Keywords: Chloroform phase, *Praxelis clematidea*, antimicrobial effect, *Candida* species

INTRODUCTION

The rise in antibiotic-resistant microorganism in recent years has led to an increasing search for new antibiotics[1]. In general, it has been possible to observe an increase in resistance of pathogenic viruses, bacteria, fungi and protozoa against known drugs[2]. To overcome the drawbacks of the current antimicrobial drugs and to obtain more efficacious drugs, an antimicrobial drug having a novel mode of action should be developed[2].

This increasing bacterial resistance is prompting resurgence in research of the antimicrobial role of herbs against resistant strains. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds[3,4].

Medicinal plants constitute an arsenal of chemicals that could be exploited by human to prevent microbial invasion[5]. Secondary metabolites produced by plants constitute a major source of bioactive substances. The scientific interest in these metabolites has increased today with the search of new therapeutic agents from plant source, due to the increasing development of the resistance pattern of microorganisms to most currently used antimicrobial drugs[6].

Medicinal plant extracts offer considerable potential for the development of new agents effective against infections that are currently difficult to treat[7,8]. Previous studies have shown that several substances such as peptides, unsaturated long chain aldehydes, essential oils and alkaloid constituents of plant extracts have potential therapeutic properties[9]. Therefore, assessment of such plants remains an interesting and useful task to find new promising agents against bacterial infections[10].

Praxelis clematidea R.M. King & Robinson belongs to the Eupatorieae tribe of the family Asteraceae, and consists of 2,400 species distributed in 170 genera[11]. Plants from this family have been extensively studied for their chemical composition and biological activity and some have led to the development of new drugs and insecticides[12,13,14].

In phytochemical studies with ethanolic extract of *Praxelis clematidea* was isolated six flavonoids[15]. This class is increasingly becoming an object of investigation, and many studies have isolated and identified flavonoids that possess antifungal, antiviral and antibacterial activities. In addition, various studies have demonstrated synergy between active flavonoids, and between flavonoids and conventional chemotherapeutic agents[16,17]. Based on promising source of antimicrobial effects provided by species of the family Asteraceae, in particular those containing species flavonoid as secondary metabolites. The aimed of the present study were to investigate the antimicrobial effects of Chloroform Phase of the aerial parts of *Praxelis clematidea* R.M. King & Robinson.

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Table 1- Antibacterial activity of the chloroform phase of *Praxelis clematidea.*

Bacterial strains/ Substance	*Staphylococcus aureus* ATCC 25923	*Staphylococcus aureus* ATCC 13150	*Pseudomonas aeruginosa* P 03	*Pseudomonas aeruginosa* ATCC 25853	*Escherichia coli* ATCC 25922	*Escherichia coli* 5
CFPC (1024 µg/mL)	-	-	-	-	-	-
Negative control	-	-	-	-	-	-
Positive control	+	+	+	+	+	+

(-) No inhibition (+) inhibition

Table 2- Antifungal activity of the chloroform phase of *Praxelis clematidea.*

Fungal strains/ Substance	*Candida albicans* ATCC 90028	*Candida albicans* LM 109	*Candida tropicalis* ATCC 13803	*Candida tropicalis* LM 20	*Candida krusei* LM 13	*Candida krusei* LM 08
CFPC (1024 µg/mL)	+	-	-	-	-	+
CFPC (512 µg/mL)	+	-	-	-	-	-
CFPC (256 µg/mL)	+	-	-	-	-	-
CFPC (128 µg/mL)	+	-	-	-	-	-
CFPC (64 µg/mL)	+	-	-	-	-	-
CFPC (32 µg/mL)	+	-	-	-	-	-
Negative control	-	-	-	-	-	-
Positive control	+	+	+	+	+	+

(-) No inhibition (+) inhibition

MATERIALS AND METHODS

Preparation of plant extract

The aerial parts of *Praxelis clematidea* R.M. King & Robinson were collected in Lagoa do Paturi, a municipality of Santa Rita, in the state of Paraiba (Brazil), in May 2008. The identification of the botanical material was performed by Prof. Dr. Maria de Fatima Agra, Botany Sector, Laboratory of Pharmaceutical Technology/UFPB "Professor Delby Fernandes de Medeiros". Exsiccates of the plant are deposited in the Prof. Lauro Pires Xavier (JPB) Herbarium, Paraiba Federal University, under the code M. F. Agra et al. 6894 (JPB). Maia et al (2010) describe the method of obtaining the chloroform phase[15].

Bacterial and fungal strains

For antibacterial activity assays, were selected 6 strains of bacteria (*Staphylococcus aureus* - ATCC 13150, *Staphylococcus aureus* - ATCC 25923, *Pseudomonas aeroginosa* - P03, *Pseudomonas aeroginosa* - *ATCC 25853*, *Escherichia coli* - *ATCC 25922* and *Escherichia coli* - *5*) and for antifungal activity assays, were selected 6 strains of fungi (*Candida albicans* – ATCC 90028, *Candida albicans* – LM 109, *Candida tropicalis* - ATCC 13803, *Candida tropicalis* – LMP 20, *Candida krusei* – LM 13 *and Candida krusei* – LM 08). All the microorganism strains were obtained from the Laboratory of Mycology collection. Bacteria and fungi were kept on Nutrient Agar (NA) slants at 4 °C. Inocula were obtained from overnight cultures grown on NA slants at 37 °C and diluted in sterile saline solution (NaCl 0.85% w/v) to provide a final concentration of approximately 106 count forming unit per mL (cfu.mL^{-1}) adjusted according to the turbidity of 0.5 McFarland scale tube.

Antimicrobial and antifungal assay

The microplate bioassay was used to determine the minimum inhibitory concentration (MIC) of chloroform phase[18,19]. The antibacterial and antifungal activity was detected using the colorimetric method by adding 200 µL of resauzurin staining (0.1 g.100 mL-1) aqueous solution in each well at the end of the incubation period. MIC was defined as the lowest chloroform phase concentration able to inhibit the bacterial or fungi growth as indicated by resauzurin staining (dead cells were not able to change the staining color by visual observation – blue to red) [20]. All experiments were carried out at least twice with consistent results.

RESULTS

The results for antibacterial activity of the chloroform phase of *Praxelis clematidea* (CFPC) are show in Table 1. Moreover, the results for antifungal activity of the CFPC are show in Table 2. The activity, in both cases, was measured in terms of presence of microorganism growth. Results obtained from the *in vitro* antibacterial assay showed that the CFPC show no antibacterial activity against either gram (+) or gram (-) bacteria. However, results obtained from the *in vitro* antifungal assay showed that the CFPC show promising antifungal activity against *Candida albicans* (ATCC 90028) with MIC of 32 µg/mL, and low antifungal activity against *Candida krusei* (LM 08) with MIC of 1024 µg/mL.

DISCUSSION

Resistance to available antibiotics is increasing at a very alarming stage globally[21]. Efforts are urgently needed to replace current available antibiotics. In this context, the antibacterial activity of plants is continuously attracting global attention[22,23].

Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds that are part of the essential oils, as well as in flavonoid[24,25].

The results obtained from the chloroform phase showed a significant and important antifungal effect against *Candida albicans*. Conventionally, treatment for candidiasis is usually done with the topical and oral administration of antifungal azole and polyene, but has been making frequent presence of such resistance to these microorganisms drugs because thaeir heir inappropriate use. Furthermore, these drugs can cause toxic effects and considerable side, the decreases patient acceptance. Thus, the use of medicinal plants as medicine traditional proves to be quite attractive as an alternative therapy, requiring studies science on the subject, which are still insufficient[26].

In addition, the different behavior observed between strains of the same species could be justified by the existence of genetic variability among different strains[27]. This antifungal activity against *Candida albicans* of CFPC has been observed in other studies with extracts of plant species of the family Asteraceae[28,29] and is showed next to the results obtained with the ethanol extract of the same plant[30].

CONCLUSION

Based on these results it can be stated that the CFPC has an important antifungal activity against Candida species, which highlights the need for further studies with other fungal species to investigate the immense therapeutic potential of this plant species and with his isolated secondary metabolites.

REFERENCES

1. O'Donnell F, Smyth T, Ramachandran V, Smyth W. A study of the antimicrobial activity of selected synthetic and naturally occurring quinolines. *Int. J. Antimicrob. Agents*. 2010; 35:30-8.
2. Orhan D, Özçelik B, Özgen S, Ergun F. Antibacterial, antifungal, and antiviral activities of some flavonoids. *Microbiol. Res*. 2010; 165:496-504.
3. Ríos JL, Recio MC. Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.* 2005; 100(1–2):80-84.
4. Kokoska L, Polesny Z, Rada V, Nepovim A, Vanek T. Screening of some Siberian medicinal plants for antimicrobial activity. *J. Ethnopharmacol*. 2002; 82(1):51-53.
5. Kuete V, Fozing D, Kapche W, Mbaveng A, Kuiate J, Ngadjui B, *et al*. Antimicrobial activity of the methanolic extract and compounds from *Morus mesozygia* stem bark. *J. Ethnopharmacol.* 2009;124:551-5.
6. Mbosso E, Ngouela S, Nguedia J, Beng V, Rhomer M, Tsamo E. *In vitro* antimicrobial activity of extracts and compounds of some selected medicinal plants from Cameroon. *J. Ethnopharmacol.* 2010; 128:476-81.
7. Khan R, Islam B, Akram M, Shakil S, Ahmad A, Ali SM, Siddiqui M, Khan AU. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules*. 2009, 14(2):586-597.
8. Adeniyi BA, Groves MJ, Gangadharam PRJ. In vitro anti-mycobacterial activities of three species of Cola plant extracts (Sterculiaceae). *Phytother. Res*. 2004; 18(5):414-418.
9. Cowan MM: Plant products as antimicrobial agents. *Clin. Microbiol. Rev*. 1999; 12(4):564-582.
10. Naik SK, Mohanty S, Padhi A, Pati R, Sonawane A. Evaluation of antibacterial and cytotoxic activity of *Artemisia nilagirica* and *Murraya koenigii* leaf extracts against mycobacteria and macrophages. *BMC Complement. Altern. Med*. 2014; 14:87.
11. Hsu TW, Peng CI, Wang CM. *Austroeupatorium inulifolium* (Kunth) King & Robinson (Asteraceae), a Nwely Naturalized Plant in Taiwan. *Taiwani*. 2006, 51: 41-46.
12. Gasparetto JC, Campos FR, Budela JM, Pontarolo R. *Mikania glomerata* Spreng. and *M. laevigata* Sch. Bip. ex Baker, Asteraceae: Agronomic, genetic, anatomical, chemical, pharmacological, toxicological studies and its use in herbal therapy programs in Brazil. *Rev. Bras. Farmacog.* 2010; 20: 627-640.
13. Khan AL, Hussain J, Hamayun M, Gilani SA, Ahmad S, Rehman G, Kim YH, Kang SM, Lee IJ. Secondary metabolites from *Inula britannica* L. and their biological activities. *Molecules*. 2010; 15: 1562-1577.
14. Lima Silva F, Fischer DCH, Tavares JF, Silva MS, Athayde-Filho PF, Barbosa-Filho JM. Compilation of secondary metabolites from *Bidens pilosa* L. *Molecules.* 2010; 16: 1070-1102.
15. Maia GLA, Falcão-Silva VS, Aquino PGV, Araújo-Júnior JX, Tavares JF, Silva MS, Rodrigues LC, Siqueira-Júnior JP, Barbosa-Filho JM. Flavonoids from *Praxelis clematidea* R.M. King and Robinson

Modulate Bacterial Drug Resistance. *Molecules*. 2011; 16: 4828-4835.
16. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents*. 2005; 26: 343-356.
17. Mota KSL, Dias GEN, Pinto MEF, Luiz-Ferreira A, Souza-Brito ARM, Hiruma-Lima CA, Barbosa-Filho JM, Batista LM. Flavonoids with gastroprotective activity. *Molecules*. 2009; 14: 979-1012.
18. Viljoen A, Vuuren SV, Ernst E, Lepser M, Demirci B, Baser H, Van Wyk BE. *Osmitopsis astericoides* (Asteraceae) – the antimicrobial activity and essential oil composition of a Cape-Dutch remedy. *J. Ethnopharmacol*. 2003; 88: 137-143.
19. Sahin F, Güllüce M, Daferera D, Sökmen A, Sökmen M, Polissiou M, Agar G, Özer H. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. *Food Control.* 2004; 15: 549-557.
20. Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Lett. Appl. Microbiol*. 2003; 36:162-167.
21. Stuart BL, Bonnie M. Antibacterial resistance worldwide: causes, challenges and responses. *Nat. Med.* 2004; 10:S122-S129.
22. Rukayadi Y, Lee K, Han S, Yong D, Hwang J-K. *In vitro* activities of panduratin a against clinical staphylococcus strains. *Antimicrob. Agents Chemother*. 2009; 53:4529-4533.
23. Guzman JD, Gupta A, Evangelopoulos D, Basavannacharya C, Pabon LC, Plazas EA, Muñoz DR, Delgado WA, Cuca LE, Ribon W, Gibbons S, Bhakta S. Anti-tubercular screening of natural products from Colombian plants: 3-methoxynordomesticine, an inhibitor of MurE ligase of *Mycobacterium tuberculosis*. *J. Antimicrob. Chemother*. 2010; 65:2101-2107.
24. Jansen AM, Cheffer JJC, Svendsen AB. Antimicrobial activity of essencial oils: a 1976-1986 literature review. Aspects of test methods. *Planta Medica*. 1987; 40: 395-398.
25. Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, Ohyama M, Tanaka T, Iinuma M. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. *J. Ethnopharmacol*. 1996; 50: 27-34.
26. Batista RSA, Silva GS, Machado SEF; Vieira KVM. Atividade antifúngica de alecrim-pimenta (Lippia sidoides Cham.) sobre Candida spp. *Agrotec.* 2013; 34 (1): 40-49.
27. Lubian CT, Texeira JM, Lund RG, Nascente PS, Del Pino FAB. Atividade antifúngica do extrato aquoso de *Arctium minus* (Hill) Bernh. (Asteraceae) sobre espécies orais de *Candida. Rev. Bras. Plantas Med*. 2010; 12(2): 157 – 162.
28. Pereira JV, Bergamo DCB, Pereira JO, França SC, Pietro RCLR, Silva-Sousa YTC. Antimicrobial activity of *Arctium lappa* constituents against microorganisms commonly found in endodontic infections. *Braz. Dental J.* 2005; 16 (3): 192 – 196.
29. Holetz FB, Pessini GL, Sanches NR, Cortez DAG, Nakamura CV, Dias-Filho BP. Screening of Some Plants Used in the Brazilian Folk Medicine for the Treatment of Infectious Diseases. *Mem. Inst. Oswaldo Cruz*. 2002; 97(7): 1027-1031.
30. Oliveira-Filho A A, Fernandes HMB, Sousa JP, Maia GLA, Silva DF, Barbosa-Filho JM, Oliveira TL, Lima EO, Pêsoa HLF. Antibacterial and Antifungal Potential of the Ethanolic Extract of *Praxelis clematidea* R.M. King & Robinson. *Int. J. Pharmacog. Phytochem. Res*. 2013; 5(3): 183-185.