

Anti-fungal and Anti-Mycobacterial activity of plants of Nuevo Leon, Mexico

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Abstract: Severe fungal infections, particularly those caused by *Candida spp*, have increased in recent decades and are associated with an extremely high rate of morbidity and mortality. Since plants are an important source of potentially bioactive compounds, in this work the antifungal activity of the methanol extracts of 10 plants (*Acacia rigidula*, *Buddleja cordata*, *Cephalanthus occidentalis*, *Juglans nigra*, *Parkinsonia aculeata*, *Parthenium hysterophorus*, *Quercus canbyi*, *Ricinus communis*, *Salvia coccinea* and *Teucrium bicolor*) were evaluated. The activity was evaluated according to the micro dilution assay described in CLSI M27-A protocol using some clinical isolates of different species of *Candida* (*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. glabrata*). All extracts showed MIC values ≤ 31.25µg/mL against at least one of the strains used, which is very interesting because it was crude extracts. *Acacia rigidula* (0.93-3.75µg/mL) and *Quercus canbyi* (0.93-7.5µg/mL) had antifungal activity against 7 strains with MIC values <8µg/mL in all cases. Furthermore excerpts activity against *Mycobacterium tuberculosis* (strain H37rv) was evaluated. Only *Salvia coccinea* and *Teucrium bicolor* showed MIC values 125µg/mL by the method of MABA.

Keywords: *Acacia rigidula*; *Quercus canbyi*; *Candida spp*; *Mycobacterium tuberculosis*.

INTRODUCTION

Plants are the basis of systems of traditional medicine, which has existed for thousands of years and continue to provide new remedies to mankind. Modern allopathic medicine has its roots in ancient medicine. Following the guidelines provided by traditional knowledge and experience, many important remedies will be discovered and marketed in the future as it has been to date (Gurib-Fakim, 2006).

Biodiversity in Mexico is of global significance, it is estimated that ranks fourth in terms of abundance and diversity. The floristic richness of Nuevo Leon state may be the result of some evolutionary changes in plants influenced by climatic and geological changes. This diversity has enabled villagers traditionally use plants for generations (Alanís *et al.*, 2004). Natural products are very promising candidates for drug discovery and continue to play an important role in drug development programs with small organic compounds. (Gertsch, 2009).

The improper handling of antibiotics by human population has led to an increase in drug resistance microorganisms. Many researchers continue to work on finding better drugs. However, after some time of use,

new drugs are often ineffective or have side effects associated with them (Khan and Yadav, 2011).

Infectious diseases are a critical health problem and is a major cause of morbidity and mortality (Ladda and Magdum, 2012). Over the last three decades, has increased the frequency of severe infections caused by yeasts, especially *Candida spp*, this type infections are associated with an extremely high rate of morbidity and mortality. Although *Candida albicans* has been reported as the most commonly isolated species worldwide, in Mexico the frequency of candidemia generated by non-*C. albicans* accounts for 68% of the isolates. Among the most commonly isolated species are: *C. parapsilosis* (37.9%), *C. tropicalis* (14.8%) and *C. glabrata* (8%) (González *et al.*, 2008).

On the other hand, the resurgence of diseases thought eradicated, such as tuberculosis, has caused a global emergency in health systems. The development of anti-tuberculosis drugs in the last decades was limited. It should be noted that today there are about only 10 drugs in late stage clinical research. However, the emergence of strains resistant to available drugs is increasing and it has been reported that less than 5% of these cases received effective treatment. For this reason it is necessary to find new ways to treat this disease (Jimenez Arellanes *et al.*, 2010).

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In this paper, we report the activity of plants in the state of Nuevo Leon in Mexico, against five *Candida* species and *Mycobacterium tuberculosis*.

MATERIALS AND METHODS

Plant material

All plants were collected in Nuevo León State, México, in the summer of 2011. Plants were selected based on ethno pharmacological and chemotaxonomic criteria. Their common or reported uses are listed in table 1. The aerial parts of the plants were allowed to dry at room temperature in a ventilated room for three weeks and pulverized with a blender.

Extraction

The powdered material (100g) was subjected to extraction with 600mL of methanol (90:10) for two hours at room temperature with constant stirring. The solvent was evaporated in a rotary evaporator at controlled temperature (38°C). The extract was stored in amber vials under a nitrogen atmosphere and freezing until use.

Antifungal activity

Micro dilution assay was performed in 96 wells plate according to the protocol M27-A, the Clinical Laboratory Standards Institute (CLSI, formerly NCCLS). Strains used as a model for the study were the clinical isolates: *Candida albicans* (498, 501 and 53), *C. parapsilosis* (96), *C. tropicalis* (166), *C. krusei* (168), and *C. glabrata* (84). The inoculum for the assay was prepared by diluting each isolate to equal the turbidity of the standard 0.5 McFarland, continued with a 1:20 dilution and then 1:50 in RPMI-1640 medium. Working suspension is prepared immediately before inoculation in micro dilution plate. The extracts were tested in serial dilutions in a concentration range of 0.488µg/mL and 1000µg/mL. The plates were incubated at 37°C for 48 hrs. Each experiment was performed in duplicate and media sterility controls, positive controls and controls inhibit growth of strains used were included. The values of minimum inhibitory concentration (MIC) is visually determined as the concentration of extract that inhibited 80% or more of the growth of the strain with respect to growth control.

Antimycobacterial activity

The anti *Mycobacterium tuberculosis* activity was evaluated against *M. tuberculosis* H37Rv strain ATCC 27294 susceptible to the five first-line drugs; The strain was grown at 37°C in Middle brook 7H9 broth until it reached the logarithmic growth phase. The inoculum for the test was prepared by diluting the culture growing to equal the turbidity of the standard 1 of the McFarland scale, was followed with a 1:50 dilution in Middle brook 7H9 broth to obtain a working suspension having a concentration of 6×10^6 colony forming units per mL (CFU/mL). Working suspension is prepared immediately

before inoculation in micro dilution plate. The evaluation of the susceptibility was carried out using the test micro method of alamar blue (MABA) as previously described (Molina-Salinas *et al.*, 2006). The extracts were prepared to evaluate a concentration of 1mg/mL in 50µL of DMSO and 950µL of Middle brook 7H9 broth enriched. All solutions were sterilized by filtration using Millipore PTFE discs of 13mm diameter, 45µm. Extract concentrations evaluated were 125µg/mL to 7.8125µg/mL; results are reported as the values of minimum inhibitory concentration (MIC). All assays were performed in duplicate. Isoniazid and rifampicin were used as positive controls

RESULTS

Table 2 shows the results of antifungal activity. The better MIC values of antifungal activity were showed by *Acacia rigidula* and *Quercus canbyi* both of 0.9ug/mL against *Candida parapsilosis* and <8ug/mL against all other strains tested. *Parkinsonia aculeata* shows the lower activity against three strains tested. In general all the extracts displayed at least one MIC <32ug/mL against one of the strains evaluated. The anti tuberculosis activities of four extracts are shown in table 3.

DISCUSSION

The antifungal activity against six species of *Candida*, was evaluated (table 2). In some cases, we did not have enough sample to evaluate its activity against all strains or even, in some cases we could not get to set the MIC.

The results obtained in this research are very interesting. As reported by Cunha *et al.* (2013) the crude extract of a natural product, is considered promising when shows less than 500µg/mL MIC, however, our working group believes that MIC less than 100µg/mL is considered of particular interest to continue research (Salazar-Aranda *et al.*, 2013).

Interestingly, both, *A. rigidula* and *Q. canbyi* were plants with remarkable activity against all species of yeast used, with MIC values between 0.93 and 7.5µg/mL. *A. rigidula*, is a shrub that grows in the Rocky Mountains of southwest and west Texas and northern states of Mexico, including Tamaulipas, Nuevo Leon, Chihuahua, San Luis Potosi and Jalisco. This plant is considered toxic to livestock, however is sold as a dietary supplement for weight loss in humans. For physical defense *A. rigidula* has sharp thorns and substantial levels of toxic alkaloids. Clement *et al.* (1998) isolated from leaves and stems 44 amines and alkaloids, including 29 are phenethylamine derivatives β, also contains horde nine, nicotine, tyramine, tryptamine, and catechol among others. The number and amount of phenolic amines found justify the toxicity of this plant. Furthermore, *Quercus canbyi* is distributed in

Table 1: Uses and reports of plants evaluated

Scientific name	Traditional use	Reported activity	References
<i>Acacia rigidula</i>	Reduces appetite	No report	
<i>Buddleja cordata</i>	Liver and kidney ailments, nosebleeds and rheumatism. Injury and inflammation of the skin or eyes, digestive disorders, ulcers, sore throat, antiseptic and diuretic.	Anti bacterial and Antimicobacterial	Ávila <i>et al.</i> , 1999 Ibarra-Moreno <i>et al.</i> , 2012 Jiménez Arellanes <i>et al.</i> , 2010
<i>Cephalanthus occidentalis</i>	To treat syphilis, skin infections, sore throats, colds, expectorant, laxative, diuretic, febrifuge, headache, dysentery, rheumatism, stomachache, toothache, diuretic, emetic, febrifuge, eye problems and stop bleeding.	No report	Smith HH, 1928 Moerman, 1998 Vestal and Evans, 1939
<i>Juglans nigra</i>	Eliminates pain, anti-inflammatory, astringent, blood purifier, detergent, laxative, anthelmintic and respiratory conditions tract.	Antimicrobial and antifungal	Rodriguez <i>et al.</i> , 2010 Amarowicz <i>et al.</i> , 2008
<i>Parkinsonia aculeata</i>	Febrifuge, sudorific, antiepileptic, abortifacient, antitussive and diabetes.	Antimicrobial and antifungal	Kamba and Hassan, 2010 Ali <i>et al.</i> , 1999
<i>Parthenium hysterophorus</i>	Fever, headache, aches, spasms, scabies, ringworm, herpes, regulates the menstrual cycle, diarrhea, neurological disorder, urinary tract infection, malaria, emmenagogue, inflammation, eczema, rashes, herpes, rheumatic pain, cold, problems cardiac and gynecological diseases.	Antimicrobial, antimicobacterial and antifungal	Patel, 2011 Madan <i>et al.</i> , 2011 Cantrell <i>et al.</i> , 1998 Malarakodiand Manoharan, 2013
<i>Quercus canbyi</i>	No report	No report	
<i>Ricinus communis</i>	Birth control to treat warts, cold tumors, induration of mammary glands, calluses, skin infections, antidote, antibacterial, arthritis, asthma, cholera, convulsions, dermatitis, epilepsy, inflammation, tuberculosis, wounds, cough and expectorant.	Antimicrobial, antitubercular and antifungal	Iqbal <i>et al.</i> , 2012 Ladda and Magdum, 2012 Poonamand Pratap, 2012 Khanand Yadav, 2011
<i>Salvia coccinea</i>	Diarrhea, lumbago, kidney disease, tuberculosis cough, varicose veins, blood clots and congested blood.	Antioxidant and antiinflammatory	Sierra Pérez <i>et al.</i> , 2011
<i>Teucrium bicolor</i>	No report	No report	

the Sierra Madre Oriental; Northeastern Mexico Coahuila, Nuevo Leon and Tamaulipas, are easily distinguished from other similar species by its annual fruiting. Are trees whose fruit is called acorn, bark is usually smooth in young specimens but be cracking with the maturity of age. Its species have presented great value to human communities, for their wood, bark and obtaining rich tannin tanning (Marroquin Flores, 1997). As far as we searched, we found no reports of antimicrobial activity of these two plants.

Extracts of *B. cordata*, *J. nigra* and *R. communis* showed good activity with MIC values between 31.25 and 62.5 µg/mL, and may be considered as attractive candidates for a bioassay-guided fractionation and its usefulness as an aid in the treatment of candidemia. The activity of the extract of *J. nigra* against strains of *Candida albicans*, *C. tropicalis* and *C. krusei* is consistent with that reported by Rodríguez *et al.* (2010) who reported the antifungal activity of the extract against *C. albicans*, *C. tropicalis*, *C. krusei*, *Cryptococcus neoformans*, *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis* and *M. gypseum*. Furthermore, Amarowicz *et al.* (2008) determined the activity of the plant against *Listeria monocytogenes*, *Escherichia coli*, *Brochothrix thermosphacta* and *Lactobacillus plantarum*. Recently Iqbal *et al.* (2012)

reported *R. communis* activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Shigella flexneri* as well as antioxidant activity. While leaves and root activity against *C. albicans* was reported by Khan and Yadav (2011). Poonam and Pratap (2012) demonstrated the activity of the seeds of the same plant of *C. albicans* and *C. glabrata*. All these reports are consistent with our results. Apparently this is the first time that the activity of *B. cordata* against *Candida* spp are reported, we did not find reports far as we could find. However Acevedo L *et al* in 2000 isolated of *R. communis* the compound 2 [4'-hydroxyphenyl]-ethyl lignocerate with antimicobacterial activity, likewise Ávila *et al.* in 1999 isolated a verbacoside with activity against *Staphylococcus aureus*.

Lower activity, but equally interesting was found with extracts *Cephalanthus occidentalis*, *Parthenium hysterophorus*, *Salvia coccinea*, and *Teucrium bicolor*, which showed activities between 31.25 and 125 µg/mL against different strains tested.

The activity against *Mycobacterium tuberculosis* (H37Rv strain) was performed only with four plants, the results are presented in table 3. *S. coccinea*, and *T. bicolor*, alone showed activity MIC values of 125 µg/mL. These results

Table 2: Minimum inhibitory concentration ($\mu\text{g/mL}$) of the plants tested against *Candida species*.

Plant	C.a 498	C.a 501	C.a 53	C.p 96	C.t 166	C.k 168	C.g 84
<i>Acacia rigidula</i>	3.8	1.9	3.8	0.9	7.5	3.8	1.9
<i>Buddleja cordata</i>	31.2	62.5	31.2	31.2	31.2	31.2	62.5
<i>Cephalanthus occidentalis</i>	31.2	NT	31.2	31.2	125	31.2	NT
<i>Juglans nigra</i>	62.5	NT	<31.2	<31.2	31.2	NT	62.5
<i>Parkinsonia aculeata</i>	31.2	31.2	500	500	62.5	31.2	1000
<i>Parthenium hysterophorus</i>	<31.2	62.5	NT	<31.2	125	NT	125
<i>Quercus canbyi</i>	3.8	7.5	7.5	0.9	7.5	7.5	3.8
<i>Ricinus communis</i>	<31.2	31.2	NT	<31.2	62.5	NT	62.5
<i>Salvia coccinea</i>	62.5	62.5	NT	31.2	125	NT	125
<i>Teucrium bicolor</i>	31.2	31.2	31.2	31.2	125	31.2	62.5
Fluconazole	1.9	0.48	15.6	1	2	4	31.2

C.a 498: *Candida albicans* strain 498; C.a 501: *Candida albicans* strain 501; C.a 53: *Candida albicans* strain 53; C.p 96: *Candida parapsilopsis* strain 96; C.t 166: *Candida tropicalis* strain 166; C.k 168: *Candida krusei* strain 168; C.g84: *Candida glabrata* strain 84. NT: Not tested.

confirm the traditional use of *S. coccinea*. Up where we look, we find no reports of antimycobacterial activity of these two plants.

Table 3: Minimum Inhibitory Concentration ($\mu\text{g/mL}$) of the plants tested against *M. tuberculosis* ($\mu\text{g/mL}$).

Plant	MIC
<i>Acacia rigidula</i>	>125
<i>Quercus canbyi</i>	>125
<i>Salvia coccinea</i>	125
<i>Teucrium bicolor</i>	125

Our results are highly relevant due to the high activity of some of the extracts against *Candida spp*, regard that we worked with crude extracts. So we can consider them as good candidates to further guided fractionation in order to improve the activity or isolate the active compounds, thus having a potential alternative for treating *Candida* infection in Mexico.

CONCLUSION

We can say that some plant species that grow in Nuevo León state show activity against some *Candida* species which currently represent a health risk for the population.

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REFERENCES

Acevedo L, Martínez E, Castañeda P, Franzblau S, Timmermann BN, Linares E and Bye Ry Mata R (2000) New phenylethanoids from *Buddleja cordata* subsp. *Cordata*. *Planta. Médica.*, **66**(3): 257-261.

Alanís GJ, Velazco CG, Foroughbakhch R, Valdez Vy and Alvarado MA (2004). Diversidad florística de nuevo León: especies en categoría de riesgo. *Ciencia UANL* **7**(2): 209-218.

Ali MS, Azhar I, Amtul Z, Ahmad VU and Usmanghani K (1999). Antimicrobial screening of some Caesalpiniaceae. *Fitoterapia.*, **70**(3): 299-304.

Amarowicz R, Dykes GA and Pegg RB (2008). Antibacterial activity of tannins constituents from *Phaseolus vulgaris*, *Fagopyrum esculentum*, *Corylus avellana* and *Juglans nigra*. *Fitoterapia.*, **79**(3): 217-219.

Ávila JG, de Liverant JG, Martínez A, Martínez G, Muñoz JL, Arciniegas A and Romo De Vivar A (1999). Mode of action of *Buddleja cordata* verbascoside against *Staphylococcus aureus*. *Journal of Ethnopharmacology*, **66**(1): 75-78.

Cantrell CL, Fischer NH, Urbatsch L, McGuire MS and Franzblau SG (1998) Antimycobacterial crude plant extracts from South, Central and North America. *Phytomedicine*, **5**(2): 137-145.

Clement BA, Goff CM and Forbes DA (1998). Toxic amines and alkaloids from *Acacia rigidula*. *Phytochemistry*, **49**(5): 1377-1380.

Cunha MG, Franchin M, Galvão LCC, TascaGóis de Ruiz AL, Carvalho JE, Ikegaki M, Alencar SM, Koo H and Rosalen PL (2013). Antimicrobial and antiproliferative actives of stingless bee *Meliponas cutellaris* geopropolis. *Complementary & alternative Medicine*, **13**(1): 13-23.

Gertsch J (2009) How scientific is the science in ethno pharmacology? Historical perspectives and epistemological problems. *Journal of Ethnopharmacology*, **122**(1): 177-183.

González GM, Elizondo M and Ayala J (2008). Trends in species distribution and susceptibility of bloodstream isolates of *Candida* collected in Monterrey, Mexico, to seven antifungal agents: Results of a 3-Year (2004 to

- 2007) Surveillance Study. *Journal of Clinical Microbiology*, **46**(9): 2902-2905.
- Gurib-Fakim A (2006) Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*, **27**(1): 1-93.
- Ibarra-Moreno S, Ibarra-Velarde F and Ávila-Acevedo JG (2012). *In vitro* evaluation of Fasciolicide activity with hexane, methanol and ethyl acetate with extracts processed and obtained from some Mexican plants used in traditional medicine based on ethno botanical studies. *American Journal of Plant Science*, **3**(1): 506-511.
- Iqbal J, Zaib S, Farooq U, Khan A, Bibi I and Suleman S (2012). Antioxidant, antimicrobial and free radical scavenging potential of aerial parts of *Periploca aphylla* and *Ricinus communis*. International Scholarly Research Network. doi 10.5402/2012/563267.
- Jiménez Arellanes MA, Cornejo Garrido J y León Díaz R. (2010) Las plantas medicinales mexicanas como fuente de compuestos antimicrobianos. *Revista Mexicana de ciencias farmacéuticas.*, **41**(1): 22-29.
- Kamba AS and Hassan LG (2010) Phytochemical and microbial screening of *Parkinsonia aculeata* L. leaves. *International Journal of Drug Development & Research*, **2**(1): 1-7.
- Khan JA and Yadav KP.(2011) Assessment of antifungal properties of *Ricinus communis*. *Journal of Pharmaceutical and Biomedical Sciences*, **11**(2): 1-3.
- Ladda PL and Magdum CHS (2012) Evaluation of anti-tubercular activity of *Ricinus communis* Linn by proportion, NRA and BACTEC/Alerth methods. *International Journal of Pharmacy and Pharmaceutical Sciences*, **4**(3): 474-478.
- Madan H, Gorgia S and Sharma S (2011) Antimicrobial and spermicidal activities of *Parthenium hysterophorus* Linn and *Alstonia scholaris* Linn. *Indian Journal of Natural Products and Resources*, **2**(4): 458-463.
- Malarakodi E and Manoharan A (2013) Antifungal activity of *Parthenium hysterophorus* L. *Journal of Chemical and Pharmaceutical Research*, **5**(1): 137-139.
- Marroquín Flores RA (1997). Algunos aspectos sobre la fenología, producción de bellota y propagación de seis especies de encino *Quercus* L. del estado de Nuevo León. Tesis de Maestría en ciencias forestales.
- Moerman DE (1998). Native American Ethnobotany. Timber Press, Portland, pp.643-750.
- Molina-Salinas GM, Ramos-Guerra MC, Vargas-Villarreal J, Mata-Cárdenas BD, Becerril-Montes P and Said-Fernández S (2006), Bactericidal activity of organic extracts from *Fluorensia cernua* DC against Stains of *Mycobacterium tuberculosis*. *Archives of Medical Research*, **37**: 45-49.
- Patel S (2011) Harmful and beneficial aspects of *Parthenium hysterophorus*: up date. *Biotech.*, **1**(1): 1-9.
- Poonam K and Pratap SK. (2012) Antimicrobial activities of *Ricinus communis* against some human pathogens. *International Research Journal of Pharmacy*, **3**(7): 209-210.
- Rodriguez L, Gonçalves M, Amaral M and Batista M (2010). Antifungal activity in *Juglans nigra* green husks. *Planta Medica.*, **76**(12): 444.
- Salazar-Aranda R, Pérez-López LA, Rivas-Galindo V and Waksman de Torres N (2013). Applications of medicinal plants, Chapter 7 “Antimicrobial activity of plants used in México for gastrointestinal and respiratory disorders. *Springer*, pp.131-188.
- Sierra Pérez R de la C, González Canavaciolo VL, Merrero Delange D and Rodríguez Leyes EA. (2011) Análisis fitoquímico de la *Salvia coccínea* que crece en Cuba. *Revistacubana de Plantas medicinales*, **16**(1): 54-59.
- Smith HH (1928). Ethnobotany of the Meskwaki Indians. *Bulletin of the Public Museum of the City of Milwaukee*, **4**(2): 175-326.
- Vestal PA and Evans Schultes R (1939). The Economic Botany of the Kiowa Indians: As it relates to the History of the Tribe. Botanical Museum, Cambridge, Massachusetts, pp.51-52.