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# Antibacterial effect of *Morinda citrifolia* fruit juice against mycoplasmas

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## ABSTRACT

*Mycoplasmas possess remarkable immunoregulatory properties and can potentially establish chronic latent infections with little signs of disease. Morinda citrifolia (noni) is largely used in traditional medicine. It was also reported that noni juice is typically applied for the treatment of cutaneous infections. In recent years, multiple resistances in human pathogenic microorganism have developed due to the indiscriminate used of antibiotic drugs commonly employed in the treatment of infectious diseases. The objective of the study was to evaluated antibacterial activity of the fruit juice of M. citrifolia against medically important mycoplasma. M. citrifolia fruit juice was subjected to screening against Mycoplasma pneumoniae, Mycoplasma penetrans and Mycoplasma fermentans. The antibacterial activity was assessed by the presence or absence of growth. M. citrifolia fruit juice produced the highest antibacterial activity against mycoplasmas tested. The activity of 100% fruit juice indicates that the active components are concentrated in this fraction. This it is the first report of antibacterial activity of M. citrifolia fruit juice against medically important mycoplasmas in comparison with other microorganisms.*

**Key words:** *Morinda citrifolia*, noni juice, antibacterial activity, human pathogens, mycoplasmas.

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## INTRODUCTION

The increase in antibiotic resistant bacteria is largely due to the widespread use of antibiotics in medicine, in animal care, and in agriculture. The problem is compounded by the lack of new antibiotics to attack bacteria in different ways to circumvent the resistant genes. Decreasing efficiency and resistant of pathogens to antimicrobial drugs made the search of a new antimicrobial agent an important strategy for the establishment of alternative therapies in difficult handling infections. *Staphylococcus aureus* and *Escherichia coli* are of the major causes of hospital-acquired infections. These organisms occur naturally in and on human body. However, certain strains can lead to infections and are becoming resistant to antibiotics [1-3]. In recent years, multiple resistances in human pathogenic microorganism have developed due to the indiscriminate used of antibiotic drugs commonly employed in the treatment of infectious diseases [4].

Mycoplasmas species lacking a cell wall are distributed among humans and other vertebrates. These bacteria are the smallest self-replicating, have a very small genome and they are thought to have developed from gram-positive bacteria by genome reduction. Mycoplasmas adhere to, invade, and fuse with host cells. They circumvent or modulate the host immune system and are thought to be causally involved in some chronic diseases, such as AIDS progression, rheumatoid arthritis, and oncogenic transformation of cells [5-7].

*Morinda citrifolia* Linn (Rubiaceae) also known as noni or Indian mulberry, is a small evergreen tree. The leaves are 8-10 inches long oval shaped, dark green and shiny, with deep veins. Is largely used in traditional medicine and has been heavily promoted for a wide range of uses; including arthritis, atherosclerosis, boils, burns, cancer, chronic fatigue syndrome, circulatory weakness, cold sores, congestion, constipation, diabetes, gastric ulcers, gingivitis, heart disease, hypertension and infections [8-12]. However, detailed studies highlighting the effects of different compounds isolated from the plant on the immune system are lacking [13]. Fruit juice of *M. citrifolia* is a well-known health drink and has various pharmacological properties including antioxidant and anti-inflammatory effects [14]. In this article, we report the antibacterial activity of *M. citrifolia* fruit juice against medically important mycoplasmas.

## MATERIALS AND METHODS

### Microorganisms

American Type Culture Collection (ATCC) and clinical isolates groups were used in this study. The ATCC strain was *Mycoplasma fermentans* ATCC 19989, the clinical isolates used were *Mycoplasma fermentans* P-140, *Mycoplasma pneumoniae* and *Mycoplasma penetrans* HF, isolated from hospitalized patients at the Hospital Universitario de Puebla and Instituto Mexicano del Seguro Social, and the clinical isolates were identified by PCR technique. The bacterial isolates were maintained on SP-4 agar plates, incubated at 37$^{o}$C for 24 h. All the cultures were kept at 4$^{o}$C until further used.

**Plant material and preparation**

*M. citrifolia* (noni) were collected in Veracruz-México and identified by Mrs. Allen Coombes, plant taxonomist and curator, Herbarium of Benemérita Universidad Autónoma de Puebla. The voucher specimen was deposited at the herbarium (Specimen number: 20253).

The mature fruit (1.5 kg) were washed with water and cut into small pieces and liquidized using an electrical blender, juice was obtained (about 500 ml/1.5 kg of fruit; 100% *M. citrifolia* fruit juice).

**Phytochemical screening methods**

Saponins: noni juice (10 ml) was boiled with 20 ml water for 2 min; the mixture was cooled and mixed vigorously for 3 min. The formation of frothing indicates the presence of saponins [15]. Tannins: to 10 ml of noni juice was added 20 ml of sodium chloride (2%), filtered and mixed with 10 ml 1% gelatin solution. Precipitation indicates the presence of tannins [15]. Triterpenes: noni juice (10 ml) was mixed with 20 ml chloroform and warmed at 80$^{o}$C for 30 min. Few drops of concentrated sulfuric acid was added and mixed well. The appearance of red color indicates the presence of triterpenes [16,17]. Alkaloids: noni juice (10 ml) was digested with 2M HCl, and the acidic filtrate was mixed amyl alcohol at room temperature. Pink color of the alcoholic layer indicates the presence of alkaloids [17,18].

**Antimicrobial assay**

The bactericidal activity of *M. citrifolia* juice was performed according to Doughari (2006), the initial inoculum was approximately 1X10$^{6}$ CFU/ml. A lop full of broth from the test tube that show no visible growth which regarded as the minimum inhibitory concentrations value was streaked onto the sterile SP-4 agar plate, and incubated at 37$^{o}$C for 24 h. The minimum bactericidal concentration was defined as the lowest concentration of *M. citrifolia* juice that completely prevented microbial growth and was determined by visible inspection of the SP-4 agar plates. The lowest concentration that inhibit the growth of bacteria were noted and considered as the minimum inhibition concentration value for each of the bacteria strain, assays were carried out in triplicates (Table 1).

**Statistics**

For statistical analysis SPSS software (version 2.0, Chicago, USA) was used, the *t* test was used to compare values of the experimental treatment and control group. A comparison was considered statistically significant if the *P* value was $< 0.05$.

## RESULTS AND DISCUSSION

The phytochemical analysis of the *M. citrifolia* fruit juice showed the presence of saponins, tannins, alkaloids and triterpenoids. The antibacterial activity of *M. citrifolia* fruit juice against *M. pneumoniae*, *M. penetrans* and *M. fermentans* was tested as presented in table 2 and figure 1. *M. citrifolia* fruit juice produced the highest antibacterial activity against mycoplasmas tested ($P<0.05$). This it is the first report of antibacterial activity of *M. citrifolia* fruit juice against medically important mycoplasmas in comparison with other microorganisms. Due to the lack of a cell wall, all mycoplasmas are innately resistant to all beta-lactams and glycopeptides. Sulfonamides, trimethoprim, polymixins, nalidixic acid, and rifampin are also inactive. Linezolid

is the prototype agent of the oxazolidinone class. These agents are much less active against *M. pneumoniae* than the other agents that inhibit proteins synthesis [20].

The present compound (1,8 dihydroxy-2-methyl-3,7 dimethoxyanthraquinone) in *Morinda angustifolia* demonstrated significant antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Micrococcus luteus*, *Sarcina lutea*, *Candida albicans* and *Saccharomyces sake* [21].

Koffi et al. (2010) demonstrated the most active against in vitro growth of *Vibrio cholerae* O:1 was the 70% ethanolic extract with a minimal bactericidal concentration of 5 mg/ml. the antibacterial properties of this medicinal plant can be of great benefit for management of cholera.

Seven anthraquinones isolated from in vitro cultured roots of *Morinda royoc* L. were tested for their antimicrobial activity against seven yeast and seven bacterial strains. The extract showed a minimum inhibitory concentration value of 15.6 microg/ml. against all species of *Candida* tested, and it inhibited the growth of oxacilin-resistant *Staphylococcus aureus* [23]. On the other hand, Kandaswamy et al. (2010) demonstrated that the propolis and *M. citrifolia* fruit juice were effective against *Enterococcus faecalis* in dentine of extracted teeth.

Mycoplasmas are known primarily as mucosal pathogens that reside extracellularly on epithelial surfaces. However, during the past few years, the potential for several mycoplasmal species to fuse with and enter host cells that are not normally phagocytic has been demonstrated. Such an occurrence should not be unexpected for microorganisms lacking a rigid cell wall that are typically closely associated with host cell surfaces [25]. An intracellular existence that sequesters mycoplasmas could facilitate the establishment of latent or chronic states circumvent mycoplasmacidal immune mechanisms, facilitate its ability to cross mucosal barriers and gain access to internal tissues, and impair the efficacy of some drugs therapies, accounting for difficulty in eradicating the mycoplasmas under clinical conditions [26]. Fusion of the mycoplasmal cell membrane with that of the host may also result in release of various hydrolytic enzymes produced by the mycoplasma as well as insertion of mycoplasmal membrane components into the host cell membrane, a process that could potentially alter receptor recognition sites and affect cytokine induction and expression [25].

Mycoplasmas were detected in the airways of humans in absence of symptoms of acute infection, and incidence was greater in asthmatics [27]. Using sensitive PCR-based detection methods, high incidences of *M. fermentans* positivity have been noted in saliva, blood and urine from apparently normal healthy subjects [28-31]. Data regarding the presence of *M. fermentans* within the human lung or its ability to establish chronic “symptomless” pulmonary infection, however, are severely limited.

This study shows the antibacterial effect of *M. citrifolia* fruit juice against mycoplasmas clinical isolates and type strain. A possible explanation the mechanism of the antimicrobial effects of fruit juice could be the pH value (3.5), exhibited various biological activities including therapeutic benefits in wound healing, inhibition of xanthine oxidase and scavenging of superoxide anions and toxics and antioxidants properties [32-34].

In conclusion, *M. citrifolia* fruit juice has been shown here to have good *in vitro* activity against all the mycoplasmas isolates examined, and so presents an interesting possibility for the future. However, the fruit juice needs investigating further, and more *in vitro* and *in vivo* experiments against medically important mycoplasmas are necessary to provide detailed information on the potential of this natural product as an antimicrobial drug.

**Table 1. Work scheme for evaluation the antibacterial activity of *M. citrifolia* fruit juice against mycoplasmas.**

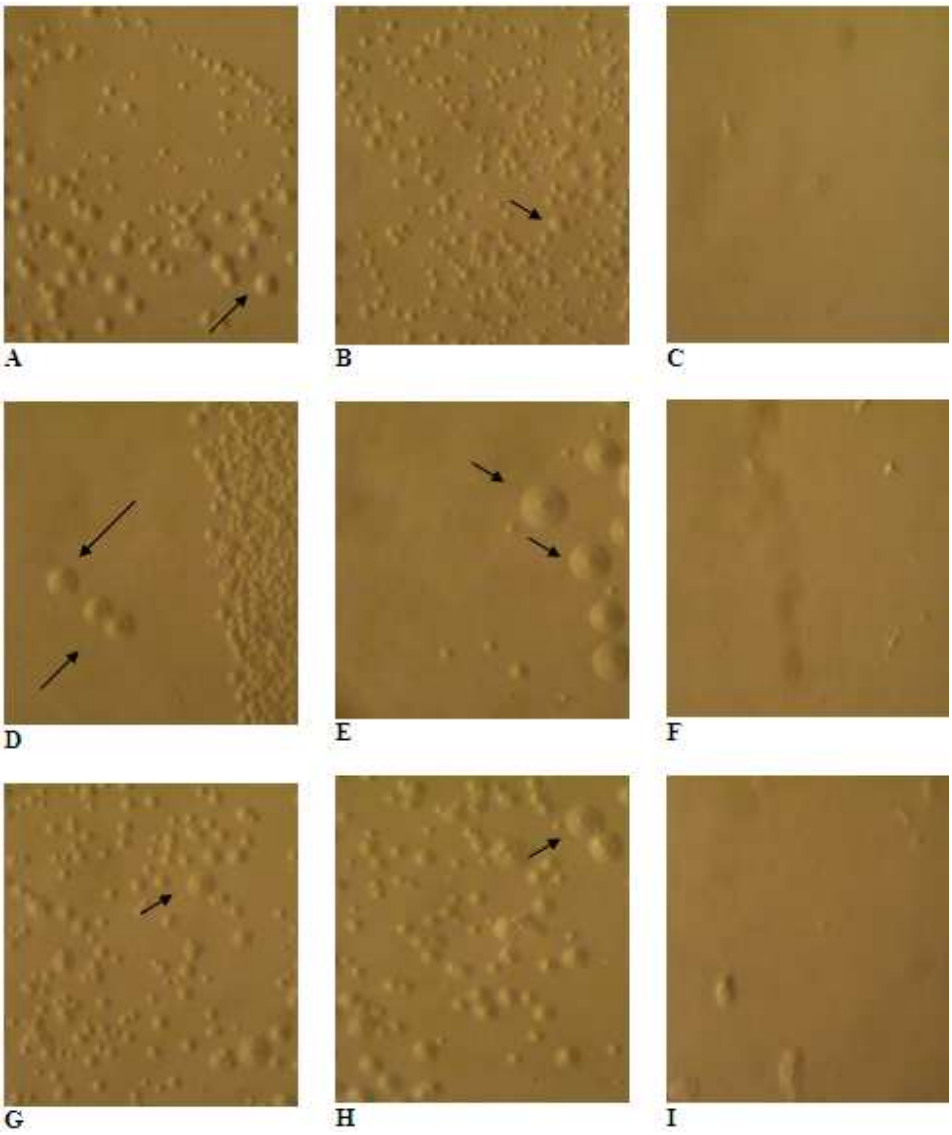
| Experimental treatments | Evaluation |
|---|---|
| 800 µl *Mycoplasma pneumoniae*<br>100 µl SP-4 broth<br>100 µl noni juice | 5 µl was inoculated onto SP-4 agar<br>(24, 48 and 72 hours post inoculation)<br>the microorganism was identified by stereoscopic microscopic |
| 700 µl *Mycoplasma pneumoniae*<br>100 µl SP-4 broth<br>200 µl noni juice | 5 µl was inoculated onto SP-4 agar<br>(24, 48 and 72 hours post inoculation)<br>the microorganism was identified by stereoscopic microscopic |
| 600 µl *Mycoplasma pneumoniae*<br>100 µl SP-4 broth<br>300 µl noni juice | 5 µl was inoculated onto SP-4 agar<br>(24, 48 and 72 hours post inoculation)<br>the microorganism was identified by stereoscopic microscopic |
| 500 µl *Mycoplasma pneumoniae*<br>100 µl SP-4 broth<br>400 µl noni juice | 5 µl was inoculated onto SP-4 agar<br>(24, 48 and 72 hours post inoculation)<br>the microorganism was identified by stereoscopic microscopic |
| 400 µl *Mycoplasma pneumoniae*<br>100 µl SP-4 broth<br>500 µl noni juice | 5 µl was inoculated onto SP-4 agar<br>(24, 48 and 72 hours post inoculation)<br>the microorganism was identified by stereoscopic microscopic |
| Controls | Evaluation |
| 500 µl SP-4 broth<br>500 µl noni juice | 5 µl was inoculated onto SP-4 agar<br>(24, 48 and 72 hours post inoculation) |
| 500 µl *Mycoplasma pneumoniae*<br>500 µl SP-4 broth | 5 µl was inoculated onto SP-4 agar<br>(24, 48 and 72 hours post inoculation)<br>the microorganism was identified by stereoscopic microscopic |

*Clinical isolates and strain ATCC had the same treatment.*

**Table 2. Antibacterial activity dates of *M. citrifolia* fruit juice against mycoplasmas tested.**

| Time/Strain | Clinical isolate *Mycoplasma fermentans* | Clinical isolate *Mycoplasma penetrans* | Clinical isolate *Mycoplasma pneumoniae* | Type strain *Mycoplasma fermentans* ATCC 19989 |
|---|---|---|---|---|
| 0 h (Exp) | +++ | +++ | +++ | +++ |
| 24 h (Exp) | + | + | + | + |
| 48 h (Exp) | - | - | - | - |
| 72 h (Exp) | - | - | - | - |
| 24 h (Control) | +++ | +++ | +++ | +++ |

*Activity is classified according to the intensity of the growth in SP-4 agar (+++: > $1X10^6$ CFU/ml; +: < $1X10^2$ CFU/ml; -: without growth). Data presented are means of three independent experiments. $P<0.05$ between the experimental and control group after 24 hours.*

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