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IN VITRO EFFICACY OF GARLIC, GLYCYRRHIZA AND NEEM AGAINST LOCAL ISOLATES OF MYCOPLASMA GALLISEPTICUM

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ABSTRACT

Minimum inhibitory concentrations (MICs) of methanol extracts of three herbal plants namely garlic, Glycyrrhiza and Neem against three local *Mycoplasma gallisepticum (*MG) isolates were determined using micro dilution method. A constant number (10^8CFU/ml) of three MG isolates were used after confirmation through polymerase chain reaction (PCR). A total of 50 mg/ml concentration of each extract was maintained in first well and then two folds serial dilutions were made. Mean values of MICs of glycyrrhiza and neem extracts were 6.25±0.000 against all of the three isolates showing non-significant differences statistically. While, garlic extract showed MIC 3.125±0.000 against two isolates and 6.25±0.000 for third isolate showing significant difference ($p \leq 0.05$). It was concluded that garlic is more effective than glycyrrhiza and neem in controlling the growth of MG and can be used as therapeutic agent in poultry to control MG infection, although field trials are needed.

Key words: *Mycoplasma gallisepticum*, Garlic, Glycyrrhiza, Neem, Minimum Inhibitory Concentration.

INTRODUCTION

Development of antibiotic resistance and phenotypic variation in *Mycoplasma gallisepticum* (MG) is major problem in its treatment and control. Trends of using plants as an alternate of antibiotics are increasing in many countries. Plants are reported to have antimicrobial agents such as emetine, quinine and berberine, tannins, terpenoids, alkaloids, and falvonoids (Marjorie, 1999). Eradication of Mycoplasma infection from animal, human host or cell culture is very difficult due to antibiotic resistance (Rbinson and Bebear, 1997). Keeping in view this scenario the current project was designed to evaluate the efficacy of three medicinal plants garlic (*Allium sativum*), glycyrrhiza (*Glycyrrhiza glabra*) and neem (*Azadirachta indica*) on the survival of local isolates of MG.

MATERIALS AND METHODS

Three medicinal plants garlic, glycyrrhiza and neem were used for determining Minimum inhibitory concentration against already characterized *Mycoplasma gallisepticum* isolates in University Diagnostic Lab, University of Veterinary and Animal Sciences, Lahore according to the protocol described by Momani *et al.*, (2007) with minor modification. Briefly: all three plants were shade dried at room temperature and grinded to powdered form and passed through 2 mm diameter mesh. The powdered form (10g) was soaked in 95% methanol (100 mL) for 24 hours at 25 °C. Subsequently, powder of each plant was separately mixed thoroughly by keeping on shaker at 200 rpm for 2 hours and mixture was centrifuged at 4000g for 10 minutes at 4°C. In order to get methanol extract, supernatant of centrifuged samples was filtered through Whatman No.4 filter paper in a beaker. Beaker was kept in rotary evaporator for methanol evaporation at 68 °C and dry crude methanol extracts of all three plants was collected for further analysis. All extracts were dissolved in dimethyl sulfoxide (DMSO) at concentration of 1100 mg/ml to prepare stock solution of each crude extract.

Minimum inhibitory concentration (MIC) of each extract was determined using sterile 96- well plate by using micro dilution method (Momani *et al.*, 2007). Frey's medium (180 µl) was added in 1st well to 12th well followed by adding stock culture of crude extract (10 µL) of each plant in 1st well. For two fold dilution 95 µL medium containing plant extract was transferred from 1st well to 2nd and dilute sample up to 9th well keeping 50 mg/ml in 1st well. After dilution of plant extracts, MG growth of 10 µL with same concentration 10^8CFU/mL was added in 1st to 10th well. Well number 10th, 11th and 12th were kept as positive control contains only MG and Frey's broth, DMSO without any extract and negative containing only Frey's broth respectively. Plate was incubated at 37 °C for 48 hours. After incubation plates were read on basis of color changing unit (CCU) and MIC was determined as last well having no color change from phenol red to yellow.

RESULTS

Minimum inhibitory concentration (MIC) of garlic was 3.125±0.000 mg/mL while Glycyrrhiza and neem showed 6.25±0.000 mg/mL. Growth inhibition of two MG isolates against garlic with MIC mean values of 3.125±0.000 and third isolate showed significant difference ($p \leq 0.05$) with MIC mean value of 6.25±0.000. In case of glycyrrhiza and neem, non-significant differences in growth inhibition of MG with MIC mean value of 6.25±0.000 as shown in Table 01.

Table 01. Effect of selected herbal extracts on growth inhibition of MG isolates

Plants	MIC values of herbal plants mg/ml								
	Isolate 1			Isolate 2			Isolate 3		
	Replicates			Replicates			Replicates		
Garlic	3.125	3.125	3.125	3.125	3.125	3.125	6.25	6.25	6.25
Mean		3.125±0.000[1]			3.125±0.000[1]			6.25±0.000[2]	
Glycyrrhiza	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
Mean		6.25±0.000[2]			6.25±0.000[2]			6.25±0.000[2]	
Neem	6.25	6.25	6.25	6.25[2]	6.25	6.25	6.25	6.25	6.25
Mean		6.25±0.000[2]			6.25±0.000[2]			6.25±0.000[2]	

[1, 2,] in same row with different superscripts indicate significance difference

$P \leq 0.05$

Isolate 1 (breeder origin MG from Rawalpindi), isolate 2 (breeder origin MG from Rawalpindi) and isolate 3(breeder origin MG from Sheikhupura)

DISCUSSION

The results of current study showed that garlic extract has no deleterious effect on two MG isolates with MIC mean values of 3.125±0.000 while the third isolate showed significant difference ($p \leq 0.05$) with MIC mean value 6.25±0.000. Similar results were observed by Momani *et al.* (2007) who observed the antimycoplasma activity of *Allium sativum* (garlic) against six species of *Mycoplasmas* with MIC values range from 12.5 to 3.125 mg/ml. According to Ankri and Mirelman (1999) the MIC values of garlic against *Staphylococcus aureus*, *Escherichia Coli* (*E.coli*) and *Candida albican* is 26, 44 and 36 mg/ml, respectively. Garlic is very effective against gram negative and gram positive bacteria with MIC values 35.7 to 1.1 mg/ml and 142.7 to 35.7 mg/ml respectively as described by Bakri and Douglas (2005). According to Iwalokun *et al.* (2004) aqueous garlic extract has tremendous antibacterial effect against gram negative and gram positive bacteria with MIC values 22.9 to 37.2 mg/ml and 48.3 to 15.6 mg/ml respectively.

Results of the current study showed that glycyrrhiza and neem has non-significant differences on the growth inhibition of MG with MIC mean value of 6.25±0.000. Mahmood *et al.* (2010) documented that aqueous extract of neem seed has high efficacy against *Escherichia. coli*, *Pseudomonas aeroginosa*, *Streptococcus pyogenes* and *Staphylococcus aureus* with MIC values of 12.5, 25, 6.25 and 6.25 mg/ml respectively while hexane extract of neem seeds against same pathogens has MIC values 6.25, 12.5, 6.25 and 3.17 mg/ml, respectively. Abalaka *et al.* (2012) reported that MIC and minimum bacteriocidal concentration (MBC) of neem is 5 mg/ml and 50 mg/ml respectively against *Pseudomonas aeroginosa*, *Klebsiella ozanae*, *Staphylococcus aureus* and *E coli.* The MIC value of aqueous extract of roots of *Glycyrrhiza glabra* against *Candida albicans* is 625µg/ml as described by Irani *et al.* (2009). According to Meghashri and Shubha (2009) root extract of *Glycyrhiza glabra* has MIC value against *Candida albicans* and *Trichophytone rubrum* is from 200 to 0.8 mg/ml while 1.2 to 0.2 mg/ml against *Staphylococcus aureus*, *Listeria monocytogenes* and *E. coli.* The difference in these results might be due to use of different types of bacteria in the experiments to study the efficacy of glycyrrhiza and neem.

Conclusion: From the study it is concluded that garlic controls the growth of MG more effectively as compare to glycyrrhiza and neem, so garlic can be used as therapeutic agent for the control of MG infection in poultry in field conditions.

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REFERENCES

Abalaka, M., O.A. Oyewole and A.R. Kolawole (2012). Antibacterial activities of *Azadirachta indica* against some bacterial pathogens. Adva. Life. Sci. 2(2): 5-8.

Ankri, S. and D. Mirelman (1999). Antimicrobial properties of allicin from garlic. J. Microbes. Infect. 2:125-129.

Bakri, I.M. and C.W.I. Douglas (2005). Inhibitory effect of garlic extract on oral bacteria. J. Arch. Oral. Biol. 50: 645-651.

Irani, M., S. Maziyeh, B. Francoise, H.E.P. Gholam and S.B. Hossein (2009). Leaves antimicrobial activity of *Glycyrrhiza glabra L.* Iranian. J. Pharm.. Res. 9(4): 425-428.

Iwalokun, B.A., A. Oqunledun, D.O. Oqbolu, S.B. Bamiro and J.J. Omojola (2004). In vitro antimicrobial properties of aqueous garlic extract aginst multidrug resistant bacteria and candida species from Nigeria. J. Med. Food. 7(3): 327-333.

Majorie, M.C. (1999). Plant Products as Antimicrobial Agents. Clin. Microbiol. Rev. 12(4): 564.

Meghashri and G. Shubha (2009). Invitro antifungal and antibacterial activites of root extract of *Glycyrhiza glabra*. J. App. Sci. Res. 5(10): 1436-1439.

Momani, W.E., A.S. Janakat, A.J. Nicholas and R.D. Ayling (2007). In vitro antimycoplasmal activity of six Jordanian medicinal plants against three Mycoplasma species. Trop. Animal. Health. Prod. 39: 515–519.