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ANTIBACTERIAL ACTIVITY OF INDIGENOUS HERBAL EXTRACTS AGAINST UREASE PRODUCING BACTERIA

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ABSTRACT

Aqueous and alcoholic extracts of 14 local herbs (*Aloe Vera*, *Azadirachta indica*, *Allium sativum, Calotropis procera, Cannabis sativa, Carum capticum, Eucalyptus camaldulensis, Lantana camara, Mangifera indica, Mentha piperita, Nigella sativa, Opuntia ficus indica, Piper nigrum* and *Zingiber officinalis*) and four commercial products (Mentofin, Suduri, Safi, Yucca) were evaluated for their *in-vitro* antibacterial activity against *Proteus mirabilis* by serial dilution method. Complete random design (CRD) was followed. It was observed that with reference to rise in pH, Ammonia concentration and urease activity in aqueous and alcoholic extracts of *Allium sativum* (pH: 8.5560, 8.8480, Ammonia:4.42, 3.52 µg/mL, Urease: 0.009, 0.007 IU/mL respectively) had shown best results as compared to control positive (pH: 9.03, Ammonia: 6.7µg/mL, Urease: 0.013 IU/mL). Alcoholic extracts of *Mangifera indica* (8.8820, 5.42µg/mL, 0.010 IU/mL), *Mentha piperita* (8.8880, 4µg/mL, 0.008IU/mL) *Carum capticum* (8.9540, 4.84µg/mL, 0.009IU/mL) and aqueous extract of *Opuntia ficus indica* (8.8100, 5.22µg/mL, 0.010 IU/mL) had weak activity against *P. mirabilis*. Both aquous and alcoholic extracts of *Euclyptus camalduensis* (pH: 8.91, 8.96, Ammonia: 5.16, 5.06 µg/mL, Urease: 0.01, 0.01 IU/mL) had weak inhibitory effect. All commercial products had shown a strong antibacterial activity (pH: 4.8-6.8, Ammonia: 0µg/mL, Urease: 0 IU/mL). Results of remaining herbal extracts were not significantly different ($p< 0.05$) from positive control. It was concluded that all products had strong antibacterial activity against *P. mirabilis*. Mentofin at dilution rate of 1/1000 had shown the best results with optimum inhibitory concentration. Alcoholic extracts of few herbs had shown weak bactericidal activity. These herbs might be effective in *in-vivo* studies.

Key words: Antibacterial activity, Herbal extracts, Urease producing bacteria.

INTRODUCTION

Urease producing bacteria in poultry litter are mainly responsible for polluted environment of the sheds. Poultry birds are provided high-protein feed to meet their optimum requirements (Gay and Knowlton, 2005). Metabolism of the protein produces uric acid and urea as waste. This waste is eliminated along with intestinal microorganisms in droppings on litter. In presence of high humidity and ambient temperature, urease producing bacteria grow and convert the urea into ammonia. Ammonia is a health hazard for poultry and human (Nicholson *et al*. 2004). The ammonia often accumulates inside poorly ventilated or poorly managed poultry sheds particularly during winter season. Broilers in an environment having more than 25-50 ppm ammonia show reduced body weight, immunosuppression and enhanced susceptibility to respiratory pathogens. This polluted environment is serious health hazard for poultry workers as depicted by Occupational Safety and Health Administration (OSHA) (Gay and Knowlton, 2005).

Poultry litter is rich in protein, urea, uric acid and microbes. Poultry litter contains 38% crude protein, 52% fiber and minerals (Lanyasunya *et al.* 2006). Uric acid is 70% of total nitrogen in poultry litter. Litter microbes are responsible for conversion of uric acid to ammonia by secreting urease (Rothrock *et al.* 2008). Gram negative bacteria are mainly responsible for production of urease in litter. Most of these bacteria convert uric acid to urea. A few genera of these bacteria convert uric acid to ammonia (Kidd, 2011).

Yucca plant extract in poultry drinking water has ability to inactivate urease producing bacteria in intestine and inhibit ammonia emission from litter (Nazeer *et al*. 2002). Both *Aloe vera* leaf and gel has antibacterial activity (Agarry *et al.* 2005). More than 135 compounds have been isolated from different parts of neem tree, which have antibacterial activity (Biswas *et al.* 2002). *Allium sativum* has antibacterial activity against Gram positive and Gram negative bacteria (Iwalokun *et al.* 2004). *Calotropis procera* latex demonstrates strong inhibitory effect against microbes (Kareem *et al*. 2008). *Cannabis sativa* exhibited activity both against Gram-positive and Gram-negative bacteria and also against the fungi. The seeds of *Cannabis sativa* have remarkable impact on growth of broiler chicks and can help in alleviating feed expenditure incurred on raising broiler chicks (Khan *et al.* 2010). *Eucalyptus* extracts displayed broad antibacterial activity against gram positive and few gram negative bacteria (Cock, 2009). It was observed that *Lantana camara* was active against *M. tuberculosis*

(Kirimuhuzya *et al.* 2009), Gram positive *Bacillus cereus* and Gram negative *Salmonella typhi* (Pour *et al*. 2010). Both seed and leaves of Mango (*Mangifera indica*) have antimicrobial activity (Doughari *et al.* 2008; Sairam *et al.* 2003). *M. piperita* leaves showed the highest zone of inhibition (17.24 mm) while stem showed least (15.82 mm) against 56 Gram negative bacteria (Sabahat and Parween, 2005). Black pepper showed activity against 75% bacteria from Oral isolates (Nazia and Tariq, 2006). *Opuntia* leaves inhibited intracellular virus replication and inactivate extra cellular virus (Ahmed *et al*. 1996). In the present study Extracts (aqueous and alcoholic) of indigenous herbs were prepared and their antibacterial activity against urease producing bacteria was determined.

MATERIALS AND METHODS

Isolation and identification of bacteria: Urease producing bacteria was isolated and identified as *Proteus mirabilis* from fresh poultry droppings (Bergay and Holt, 1994). Twenty four hours old culture at dose rate of 10^6 Colony Forming Unit (CFU)/mL was used as inoculum (Babayi *et al*, 2004).

Extracts of herbs: Aqueous and Alcoholic extracts of local 14 herbs were prepared. These herbs were *Aloe Vera*, *Azadirachta indica*, *Allium sativum, Calotropis procera, Cannabis sativa, Carum capticum, Eucalyptus camaldulensis, Lantana camara, Mangifera indica, Mentha piperita, Nigella sativa, Opuntia ficus indica, Piper nigrum* and *Zingiber officinalis.*

Fresh leaves, rhizome and dry seeds of herbs (100 gm each) were homogenized in 50 mL distilled water, macerated and centrifuged. It was used as aqueous extract (Babayi *et al.* 2004). Dried and powdered leaves, rhizome and seeds of herbs (5gm each) were homogenized in 20 mL Alcohol. They were macerated for 2 days and then filtered. The filtrate was evaporated in water bath at 70 °C. It was reconstituted in 10 mL distilled water and used as Alcoholic extracts (Joe *et al.* 2009). Mentofin (Ewabo), Yucca, Suduri and Safi (Humderd) were procured from local market. Antibacterial activity of the herbs was determined by 10 fold dilution of the herbal extracts/products in Urea broth containing 10^5 CFU/ml of the bacteria. After 72 hours of incubation at 37 °C, change in pH, Activity of bacteria (growth: Yes/No), Ammonia concentration (µg/mL) and urease activity (I.U/mL) were observed. For estimation of Ammonia and urease activity, Colorimetric method and Nessler, s reagent was used, respectively (Massmann, 1962). Optimum inhibitory concentration of herbs and products were determined. Antibacterial activity of the herbal extracts and 4 herbal products were estimated and results were obtained. The data thus obtained was analyzed using ANOVA and Duncan's multiple range tests by SPSS statistical program version 13.0.

RESULTS AND DISCUSSION

Urease producing bacteria such as *Proteus marabilis* grew well in urea broth and showed the enhanced urease activity that resulted in enhanced pH and ammonia concentration. Aqueous and alcoholic extract of *Allium sativum*[a] had shown weak but the best antibacterial activity as compared to all other extracts in the broth. Aqueous extract of *Opuntia ficus indica*[b] and alcoholic extracts of *Carum capticum*[b], *Mentha piperita*[b] *Piper nigrum*[b], *Mangifera indica*[c] and both the extracts of *Euclyptus camaldulensis*[c] showed poor antibacterial activity. Either of the commercial products did not support the growth of *Proteus mirabilis*. The pH of Yucca (4.8972[a]) is less than the pH of the control negative (6.820). The pH of the medium could be low because of low pH of commercial product (Yucca: pH 3). The pH of control positive is high (9.0280) due to absence of any herbal product and urease activity of the bacteria. Ammonia concentration and urease activity in the broth containing herbal commercial products was zero. All the remaining alcoholic and aqueous extracts failed to show antibacterial activity against *Proteus mirabilis.* Both extracts of *Allium sativum* showed weak antibacterial activity against *P. mirabilis*. Aqueous extracts of garlic shows antibacterial activity against multi drug resistant human enteric pathogenic bacteria and alcoholic extracts of *Carum capticum, Euclyptus camaldulensis, Mentha piperita, Mangifera indica* and *Piper nigrum* show weak effect against the bacteria (Iwalokun *et al.,* 2004, Joe *et al*., 2009 and Ross *et al*. 2001). Aqueous extract of *Euclyptus camaldulensis* had inhibited growth of *Bacillus subtilis* and *Staphylococcus aureous* (Babayi *et al.* 2004). Moreover, leaves of Mint has antibacterial activity against *P. vulgaris* and *P. mirabilis* (Sabahat and Parween, 2005). Alcoholic extract of *Opuntia ficus indica* and *Piper nigrum* did not show antibacterial activity and hence did not interfere the urease activity such as increased production of Ammonia in the broth. Both the aqueous and alcoholic extracts of *Aloe vera, Azadirachta indica, Calotropis procera, Cannabis sativa, Lantana camara, Nigella sativa* and *Zingiber officinalis* did not show any antibacterial activity against *P. mirabilis.* However, extracts of *Allium sativum, Mentha piperita, carum capticum, Eucalyptus camaldulensis, Opuntia ficus indica Mangifera indica* and *Piper nigrum* might be effective against urease positive bacteria *in-vivo*. Mentofin (Ewabo) showed strong antibacterial activity even at 1:1000 dilution. It stopped the bacterial growth thus no production of urease. This resulted in decrease pH and no production of ammonia. It has ethereal oils of Mint and Eucalyptus that might have shown antibacterial activity against urease

producing bacteria. Similarly, Suduri and Safi (Humderd) had also given same effect at 1:10 dilution and Yucca showed antibacterial activity at 1:10 dilution. It shows better results *in-vivo* study (Saif-ur-Rehman and Muhammad, 2011)

It is concluded that alcohol or water extracts of the indigenous herbs have antibacterial activity against urease producing bacteria. A commercial product can therefore be prepared from locally available herbs to control environmental pollution in and around poultry sheds.

Table #1: Effect of herbal extracts on pH, ammonia and urease activity of avian strain of *Proteus mirabilis.*

Name of Herbs	pH 0f culture	Ammonia Concentration µg/mL	Urease activity I.U/mL
Aloe vera (w)	9.0160 ^{e}	5.24 ^{e}	0.010^{c}
A. vera (a)	9.0500 ^{e}	6.90 ^{e}	0.013 ^{c}
Aza. indica (w)	8.9400 ^{e}	5.90 ^{e}	0.011 ^{d}
Aza. Indica (a)	9.0220 ^{e}	5.62 ^{e}	0.011 ^{d}
Allium sativum (w)	8.5560 ^{a}	4.42 ^{c}	0.009 ^{b}
Allium sativum (a)	8.8480 ^{c}	3.52 ^{a}	0.007 ^{a}
Calo. procera (w)	9.0140 ^{e}	6.00 ^{e}	0.011 ^{d}
Calo. procera (a)	9.0139 ^{e}	6.01 ^{e}	0.011 ^{d}
Cannabis satival (w)	8.9440 ^{e}	5.24 ^{e}	0.010 ^{c}
Cannabis satival (a)	9.00 ^{e}	5.00 ^{d}	0.010 ^{c}
Carum capticum (w)	8.9440 ^{e}	5.32 ^{e}	0.010 ^{c}
Carum capticum (a)	8.9540 ^{e}	4.84 ^{d}	0.009 ^{b}
E.Camaldulensis (w)	8.9100 ^{e}	5.16 ^{e}	0.010 ^{c}
E. Camaldulensis (a)	8.9580 ^{e}	5.06 ^{e}	0.010 ^{c}
Lantana camara (w)	8.9520 ^{e}	5.32 ^{e}	0.010 ^{c}
Lantana camara (a)	9.0520 ^{e}	5.94 ^{e}	0.011 ^{d}
Mangifera indica (w)	8.9600 ^{e}	5.18 ^{e}	0.010 ^{c}
Mangifera indica (a)	8.8820 ^{d}	5.42 ^{e}	0.010 ^{c}
Mentha piperita (w)	9.0120 ^{e}	5.16 ^{e}	0.010 ^{c}
Mentha piperita (a)	8.8880 ^{d}	4.00 ^{b}	0.008 ^{a}
Nigella sativa (w)	9.0060 ^{e}	6.32 ^{e}	0.0118 ^{e}
Nigella sativa (a)	8.9780 ^{e}	5.30 ^{e}	0.010 ^{c}
O. f. indica (w)	8.8100 ^{b}	5.22 ^{e}	0.010 ^{c}
O. f. indicab (a)	9.0360 ^{e}	6.30 ^{e}	0.012 ^{e}
Piper nigrum (w)	8.9900 ^{e}	5.08 ^{e}	0.0102 ^{c}
Piper nigrum (a)	8.9080 ^{e}	4.80 ^{d}	0.009 ^{b}
Zin. Officinalis (w)	9.0260 ^{e}	6.36 ^{e}	0.0118 ^{e}
Zin. Officinalis (a)	9.0263 ^{e}	6.33 ^{e}	0.011 ^{e}

Figures in the column having similar superscripts are not significantly different, ($p<0.05$).
W: Aqueous extracts, a: Alcoholic extracts.

Table #2: Effect of Commercial products on pH, Ammonia and Urease activity of avian strain of *Proteus mirabilis*.

Name	pH 0f culture	Ammonia Concentration µg/mL	Urease activity I.U/mL
Mentofin	6.8280^{b}	0	0
Suduri	6.80600^{b}	0	0
Safi	6.81000^{b}	0	0
Yucca	4.8972^{a}	0	0
Positive control	9.0280	6.70	0.013
Negative control	6.8200	0	0

Figures in the column having different superscripts are significantly different, ($p<0.05$).

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