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Effects of Herbal Extracts Towards Microbicidal Activity Against Pathogenic *Escherichia coli* in Poultry

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Abstract: A total number of five plants were tested qualitatively to the presence of microbicidal activity by Kirby-Bour method in agar gel plates using poultry pathogenic *E. coli* as microbes. Among the five herbs used two herbs namely *Piper betal* and *Cassia auriculata* found to have microbicidal activity. The potency of microbicidal activity of that two herbs were tested quantitatively by the same method by using different dilutions and in different solvents, clinical experiments conducted in birds with infection and found that the plant *Cassia auriculata* have had more potent microbicidal activity when compare to *Piper betel* and screened for side effects, additional effects in birds.

Key words: Herbs, *E. coli*, poultry, microbicides

Introduction

Nowadays using antibiotics to subside infection produces adverse toxicity to host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonize with herbs (Lin and Song, 1989). Herbal molecules are safe, will over come the resistance produced by the pathogens since they are in combined form or in pooled form of more than one molecules in the protoplasm of the plant cell. Some herbs said to have prevent cancer (Sengupta *et al*., 2004). Some herbs have antibacterial and antifungal properties which will be useful to clinical use (Kalemba and Kunicka, 2003). Some *invitro* studies have been conducted that herbal oral liquids can be given to clinical drug resistant strains and different serotype strains of infection (Lu *et al*., 2002). Based on above information this experiment was designed to study the microbicidal activity of herbs against the *E. coli* in poultry.

Materials and Methods

Five herbs were selected namely *Ocimum sanctum, Piber betel, Acallypha indica, Jatropha curcuss,* and *Cassia auriculata,* all the herbs were dried in shadow in room temperature and powdered. Aquous extract were prepared to about 10 ml from each 10 grams of herbal powder. Filter paper discs were prepared from Whatmann No 1 filter paper using ordinary punching machine. 100 discs were soaked with 10 ml of aquous extract of each herb and kept it in incubator for about 48 hrs to get dried. This discs were used for this study.

Faecal materials of intestine and cloacae were collected from *E. coli* affected birds by post-morterm and the materials were prepared as inoculum and streaked in the plates containing selective media Mac Conkeys agar and kept it in incubator for *E. coli* colony production as per Michael *et al.* (1996). Then the *E. coli* were tested for pathogenicity as per Berkhoff and Vinal (1986), and few colonies were transferred to Mac Conkeys agar broth to culture *E. coli*. The cells concentration were checked as per Presscott (1999), the cells count were about $3x10^9$ /ml of broth. There are about 100 microlitre of *E. coli* were streaked in Mac Conkeys agar plate and

Table 1: Microbicidal activity of different herbs in aqueous extract (1gram/1millilitre)

Trail No	Ocimum sanctum	Piper betal	Acallypha indica	Jatropha curcus	Cassia auruculata
1.	00	10	00	00	11
2.	00	11	00	00	13
3	00	9	00	00	14
4.	00	9	00	00	11
5.	00	9	00	00	12
6.	00	9	00	00	12
7.	00	10	00	00	12
8.	00	9	00	00	13
9.	00	11	00	00	13
10.	00	10	00	00	12

Table 2: Microbicidal activity at different dilutions of aqueous extract of *Piper betal* and *Cassia auriculata*

Trail No	Piper betal 10^{-1}	Piper betal 10^{-2}	Cassia auriculata 10^{-1}	Cassia auriculata 10^{-2}
1.	6	2	8	4
2.	7	3	9	5
3.	6	2	8	3
4.	7	3	7	5
5.	5	2	9	5
6.	6	3	9	5
7.	7	4	8	4
8.	6	2	9	4
9.	6	3	9	5
10.	6	3	7	4

Table 3: Antimicrobial activity spectrum of Piber betal and cassia auriculata extracts in different solvants

Trail No	Piper betal			Cassia auriculata		
	Methanol	Ethanol	Acetone	Methanol	Ethanol	Acetone
1.	14	15	12	15	15	14
2.	13	14	13	14	15	14
3.	14	15	12	13	15	14
4.	14	14	15	15	14	15
5.	14	13	13	14	14	13
6.	13	13	13	13	14	14
7.	14	13	13	14	14	13
8.	14	13	13	14	14	13
9.	13	14	13	13	15	13
10.	14	13	13	14	13	13
Mean	13.6	13.8	13.1	14	14.3	13.6

discs were marked for identification and placed over the agar in equal distance and incubated it for 36 hrs and results were recorded. The experiments were repeated for about 10 times.

The herbs possessing the microbicidal activity were again tested with different dilutions and discs were prepared accordingly and the experiments were repeated and results were recorded. Then the same herbs were extracted by using different solvents in ethanol, methanol and acetone the above procedure was repeated and results were recorded.

The herbs which are possessing microbicidal action were fed at the dose rate of 10 grams per kg body weight to the chicks of 14 days old for about 10 days the trail group is 900 chicks and 100 chicks were control. After 10 days, 9 chicks both in control and trail were sacrificed and subjected for post morterm and observed for grass lesions and histological studies were done.

The same herbs were given to 35 weeks old layer birds at the dose rate of 100 mg per kg body weight to 900 birds as trail and 100 birds as control in two groups for about 10 days. After 10 days the egg production, body weight and feed intake were compared with before treatment data.

The *Cassia auriculata* and *Piper betel* herbs were given to two different group of birds aged about 35 weeks old layer birds at the dose rate of 100 mg per kg body weight. In this group 70 birds were taken for trail, 15 birds were taken as positive control and 15 birds were taken as negative control. The infective dose of pure culture of pathogenic *E. coli* were added in boiled and cooled drinking water and given to trail and positive control birds. Before this the random sample of blood and droppings were checked and ensured that were free of *E. coli* from this all 100 birds. After the onset of mortality in trail and positive control group after three days of infection only in trail group the herbs were given at the above dose rate for about 10 days and mortality was recorded. From the dead birds the samples were collected and culture was done. The experiments were repeated and recorded.

Results and Discussion

Among five herbs tested for the presence of microbicidal property by Baur *et al.* (1966) method only two herbs namely *Piper betel* and *Cassia auriculata* shows the zone of clearence in the agar gel plates of Mac Conkey's media and the mean value of zone of clearence is about 9.7mm and 12.4mm respectively. The other herbs shows only nil value. According to Baur *et al.* (1966) method the microbicidal activity is classified into resistant if the zone of inhibition in millimeter is less than 8. If it is 9-11mm intermediate, if the inhibition is 12 or more it is sensitive. In this study *Piper betal* gives intermediate microbicidal activity and *cassia auriculata* gives sensitive microbicidal activity in the concentration of 1 gm crude herbal powder in 1 ml aquous base.

The mean value of *piper betel* in 10^{-1} dilution is 6.2mm and in 10^{-2} dilution is 2.6mm. Likewise the mean value of *Cassia auriculata* in 10^{-1}dilution is 8.3mm and in 10^{-2} dilution is 4.3mm. The above result indicates if it is diluted in aquous base more than the concentration of 10^{-1} the microbicidal activity of the above two herbs are getting lowered.

In the results of different solvant extracts in *piper betel* the mean value of methanol extract is 13.6 mm. Ethanol extract is 13.8mm. Acetone extract is 13.1mm. The mean value of different solvant extracts of *cassia auriculata* in methanol 14mm, Ethanol 14.3mm. Acetone 13.6mm. The results of solvant extracts reveals that the microbicidal activity of above two herbs are more in solvant extracts than in aquous extracts.

In 14 days old chicks at the dose rate of 10 gm per kilogram body weight, both the herbs were fed in separate groups. In post morterm grass lesions there was no lesions in the visceral organs and no histopathological lesions in spleen, liver and kidney, so we can assume the herbs do not have any adverse effects more over the *Piper betel* were traditionally used in most people's dinner.

In 35 weeks old layer birds only in *Cassia auriculata* treatment group there is increase in egg production about 5% when compare to control and other treatment group. The cassia auriculata herb may have other properties like nervine stimulant, immunostimulant and endocrine stimulant. If any one property present in that herb the egg production may increase because if any one of the above three system stimulated automatically other two systems stimulated. (Khansari *et al*., 1990).

Out of two different herbs trail groups infected with *E. coli* and treated only in trails with herbs, in the Caussia auricuala treated group the mortality was arrested with in three days and in *Piper betel* treated group the mortality was arrested with in five days and in positive control the mortality was continued. In negative control there was no onset of mortality.

Herbs are being recommended for ischemic heart disease(Gauthaman, 2005), tuberculosis (Mata *et al.,* 2004). Likewise this two herbs can also be recommended as microbicidal agents by conducting further research. The molecular biology and genetic engineering tools can be utilized to isolate active principle and to produce it in large quantities by secondary metabolities synthesis technology.

References

Baur, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standard single disk method. Am. J. Clin. Path., 45: 493-6.

Khansari, D.N., A.J. Murgo and R.E. Faith, 1990. Effects of stress on the immune system. Immunol., Today, 11: 170-5.

Gauthaman, K., S.K. Banerjee, A.K. Dinda, C.C. Ghosh and S.K. Maulik, 2005. Terminalia arjuna (Roxb.) protects rabbit heart against ischemic-reperfusion injury: role of antioxidant enzymes and heat shock protein. J. Ethnopharmacology, 96: 403-9. Epub 2004 Nov 21.

Berkhoff, H.A. and A.C. Vinal, 1986. Congo red medium to distinguish between invasive and non-invasive Escherichia coli pathogenic for poultry. Avian Dis., 30: 117-121.

Kalemba, D. and A. Kunicka, 2003. Antibacterial and antifungal properties of essential oils. Curr. Med. Chem., 10: 813-29.

Lin, W.S. and X.Z. Song, 1989. Clinical and experimental research on a kidney-tonifying prescription in preventing and treating children's hearing-loss induced by aminoglycoside antibiotic ototoxicity. Zhong Xi Yi Jie He Za Zhi. (Chinese article) 9: 402-4, 388.

Lu, Y., D.C. Chen, Q. Li, G.W. Xuan, B.J. Fan, J.W. Zhao and N. Wang, 2002. Antibacterial effect of niaoluqing oral liquid on clinical drug-resistant strains and different serotype strains of Ureaplasma urealyticum *in vitro*. Zhonghua Nan Ke Xue. (Chinese article); 8: 152-4.

Mata, R., I. Morales, O. Perez, I. Rivero-Cruz, L. Acevedo, I. Enriquez-Mendoza, R. Bye, S. Franzblau and B. Timmermann, 2004. Antimycobacterial Compounds from Piper sanctum. J. Nat. Prod., 67: 1961-1968.

Michael, J., J.R. Pelczar, E.C.S. Chan and Noel R. Krieg, 1996. Microbiology. Fifth edition.TATA McGRAW-HILL Publishing co. New Delhi, 133-147.

Prescott, H.K.,1999. Microbiology, Fourth edition.WCB McGraw-Hill, New York, 113-132.

Sengupta, A., S. Ghosh and S. Bhattacharjee, 2004. Allium vegetables in cancer prevention Asian Pac. J. Cancer Prev., 5: 237-45.