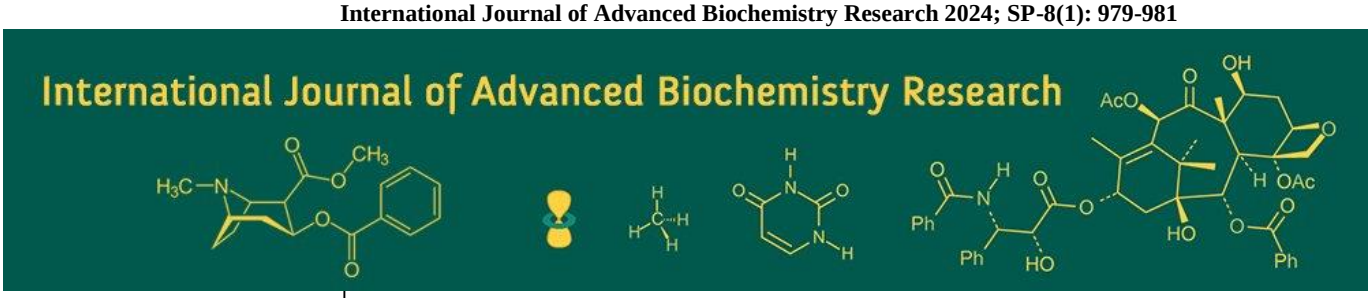

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Antimicrobial activity of herbal feed additives in animal nutrition

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Abstract
The present study was conducted to estimate *in-vitro* antimicrobial activity of six herbal plant extracts (Black pepper, Fennel, Turmeric, Black cumin, Ginger, Bay leaf) by using well diffusion method. It was determined that both methanolic and ethyl acetate extracts of these herbal additives possessed considerable antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi* and *Escherichia coli*. The results suggested the potential use of Black pepper, Fennel, Turmeric, Black cumin, Ginger and Bay leaf as phytogenic growth promoter or feed additive in animals which can be further explored for better production.

Keywords: Additive, antimicrobial, herbal, phytogenic

Introduction
Nowadays, use of feed additives in animal nutrition has gained prime importance and become obligatory as it improves feed efficiency and growth rate which generally results in lowering the production cost and better performance. Besides improving nutritive value of feed, feed additives tend to enhance animal performance with reduction in morbidity and mortality in birds. Being an eco-friendly alternative of antibiotics, natural growth promoter or phytogenic feed additives in poultry diet has received much attention in the recent years (Botsoglou *et al*., 2004) [5] to enhance production and prevent disease conditions (Steiner and Syed, 2015). Phytogenic feed additives or natural feed additives comprised of a broad variety of substances, mainly extracts from plant materials, such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots (Burt, 2004) [6]. The active principles or bioactive constituents present in them have been responsible for a wide range of beneficial properties and physiological effects (Lee *et al*., 2004) [10]. Due to concerning drug resistance and residual effect problem associated with antibiotic growth promoters, these herbal plant extracts have been screened for their potential use as effective substitutes for the treatment of certain infections, improving animal performance and safe meat production for consumers.
Among various herbal extracts and essential oils being used in feed industry, some common indigenous herbs or spices are Black pepper, Fennel, Turmeric, Black cumin, Ginger and Bay leaf, respectively. In order to explore more regarding the use of these herbs, the aim of this present study is to estimate the antimicrobial properties of these natural feed additives which eventually allow the determination of its suitability as an alternative to antibiotic growth promoters in livestock and poultry production.

Materials and Methods
Antibacterial activity
The dried samples of Black pepper seeds, Fennel seeds, Turmeric rhizome, Black cumin seeds, Ginger rhizome, Bay leaf were ground and crude extract were prepared by Soxhlet extraction method using methanol. Five grams of powdered sample of these herbs were filled in thimble directly, was placed in Soxhlet apparatus, and extracted using ethyl acetate or methanol for 24 hrs or until the solvent in siphon tube of the extractor become colorless. The extracts were then concentrated in pre weighted vials on a rotary evaporator below 50 ˚C. Dried extracts were weighed and reconstituted with a known volume of solvent and were stored in vials at 4 ˚C for further experimental studies. Antibacterial activities of various herbal extracts were examined by the well diffusion method (Murray *et al*., 1995) [11]. The test organisms used are

Staphylococcus aureus, Pseudomonas aeruginosa, *Proteus vulgaris*, *Salmonella typhi* and *Escherichia coli.* Stock cultures were maintained at 4 °C in nutrient broth. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth for bacteria that were incubated without agitation for 24 h at 37 °C. Media was prepared by dissolving 0.5% Peptone, 0.3% beef extract/yeast extract, 1.5% agar, 0.5% NaCl in 100 ml distilled water and autoclaved at 121 °C for 15 min. Wells were prepared on agar plates to which 100 µl extract and solvent in control well was inoculated and the plates were kept for incubation at 37 °C for 24 h. At the end of incubation, inhibition zones (mm) formed around the wells were measured with a transparent ruler.

Results and Discussion

In the present investigation, *in vitro* antibacterial activity of crude extract of six herbal extracts was qualitatively assessed based on the zone of inhibition. The zone of inhibition (Table 1) for methanolic extract of Black pepper was found to be comparatively more than others against various bacteria. Among five bacteria tested, Black pepper showed highest zone of inhibition against *E.coli* while lowest against *S. typhi*. In case of turmeric extract, relatively larger zone of inhibition (mm) was observed against *S. aureus* and *E.coli* as compared to others. For Black cumin, Fennel and Ginger, more antibacterial activity was seen against *S. aureus* while least was observed against *P. vulgaris.* No zone of inhibition was observed for Bay leaf methanolic extract against *P. aeruginosa*, *P. vulgaris*, *S. typhi*.

The ethyl acetate extracts of herbs in study showed relatively lesser antibacterial activity than methanolic extract against tested microorganisms (Table 2). The zone of inhibition varied from 9 mm (*S. typhi*) to 14 mm (*S. aureus*) in case of Black pepper extract. The turmeric extract revealed no activity against *S. typhi* and *P. vulgaris*. Further, larger zone of inhibition was exhibited by Black cumin and Ginger extracts against *S. aureus* and by Fennel seed extract against *E.coli*. Also, the bay leaf extract demonstrated certain antibacterial activity against *S. aureus*, *P. aeruginosa* and *E.coli*. On comparing the antibacterial activity of above mentioned herbal extracts, Black pepper and Black cumin showed relatively more pronounced activity as compared to others.

The mechanism of action for various properties like antimicrobial activity of essential oils or herbs depends on their chemical composition and is not attributable to a single mechanism but a cascade of reactions involving the entire bacterial cell (Nazzaro *et al*., 2013) [12]. Further, it has been reported that the antimicrobial activity depends on the lipophilic character of the components which permeate the cell membranes and mitochondria of the microorganisms and hinder the membrane bound electron flow hampering energy metabolism of bacterial cell causing lysis of the cell membranes and denaturation of cytoplasmic proteins (Nazzaro *et al*., 2013) [12].

The properties of herbs or spices or essential oils such as antibacterial, antifungal or anti-inflammatory varies from one plant to another in different regions which is attributed to many factors such as effect of climate, soil composition, age, stage, on the quality, quantity and composition of extracted product, different bacterial strains (Angioni, 2006) [3]. Also, type of solvent and method of extraction affect the quantity of essential oil and its extent of activity (Bedi, 2010) [4].

The findings of present study collaborates well with those of Pundir and Jain (2010) [13], Qadir *et al*. (2017) [1], Algabri *et al.* (2018) [2], Joe *et al.* (2009) [8] and Kumar *et al.* (2019) who reported considerable antibacterial activity of herbal extracts or essential oils against various micro-organisms. Phenol, alcohols, ketones and aldehydes present in herbs are mainly associated with antibacterial actions. The presence of phytochemicals like sabinene, β-pinene, limonene, terpinene, borneol, carvone, carvacrol, 1, 8-cineol and linalool in Black pepper have contributed to its antimicrobial activity (Harold, 2004) [7]. Similarly, the active constituents exerting several beneficial properties to these herbs are present as zingiberol, zingiberine and bisabolene in Ginger, trans-anethole and estragole in Fennel, flavonoids, alkaloids, eugenol and linalool in Bay leaf, curcumins and other curcuminoids in Turmeric and p-cymene, carvacrol, thymohydroquinone in Black cumin, respectively.

It can be concluded that these herbal extracts possess appreciable antibacterial activity against tested organisms. Further, considering the safe meat production with no issue of drug residue and better animal performance, the herbal growth promoters could replace antibiotics in feed industry but these herbal products have numerous components and properties which need to be fully explored in order to get maximum benefits for optimum production.

Table 1: Antibacterial activity of methanol extracts of various herbal plants

Microorganisms	Zone of inhibition (mm)					
	Black pepper	Turmeric	Black cumin	Fennel	Ginger	Bay leaf
S. aureus	18	17	15	16	14	12
S. typhi	13	7	12	-	8	-
P. aeruginosa	15	11	13	11	10	-
P. vulgaris	14	-	9	7	-	-
E. coli	19	18	14	12	11	13

Table 2: Antibacterial activity of ethyl acetate extracts of various herbal plants

Microorganisms	Zone of inhibition (mm)					
	Black pepper	Turmeric	Black cumin	Fennel	Ginger	Bay leaf
S. aureus	14	13	13	11	15	10
S. typhi	9	-	10	-	9	-
P. aeruginosa	12	10	11	12	9	8
P. vulgaris	10	-	-	-	-	-
E. coli	11	14	12	14	10	11

Conclusion

The above findings shows the potential of these herbal feed additives to be used in poultry feed in order to replace antibiotic growth promoter.

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