

Original Research Article

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## Evaluation of Antimicrobial and Antioxidant Properties of Phytogenic Feed Additives

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### ABSTRACT

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The present study was designed to examine *in-vitro* antimicrobial and antioxidant activity of Cinnamon bark and Ajwain seed extract by using well diffusion method and DPPH scavenging activity, respectively. It was determined that both Cinnamon and Ajwain possess considerable antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi* and *Escherichia coli*. In terms of DPPH activity, Cinnamon showed higher antioxidant power as compared to Ajwain. These findings suggested the potential use of Cinnamon and Ajwain as phytogenic growth promoter in feed industry which can be further explored for better production.

### Introduction

Herbs and plant extracts or natural feed additives used in animal feed, also referred as phytogenics feed additives, are defined as compounds of plant origin incorporated into animal feed in order to enhance performance and productivity by improving digestibility, nutrient absorption and elimination of undesirable gut pathogens (Athanasiadou *et al.*, 2007). Phytogenic feed additives have antimicrobial, antiviral, anti-parasitic, antifungal, antioxidant and anti-helminthic properties due to the presence of phytochemical or bioactive chemicals in

plants (Fulton *et al.*, 2002). Due to drug resistance and residual effect issue associated with antibiotics, these natural plant extracts have been screened for their potential uses as alternative medicines for the treatment of many infections and improving animal performance along with safe meat production for human consumption.

Cinnamon also known as “dalchini”, is a spice obtained from the inner bark of many tree species from the genus *Cinnamomum*, family Lauraceae, being widely used as condiment in India with many medicinal properties. Various parts of the cinnamon are

used in Ayurvedic and ethnomedicine. The active principle comprises of principal component or essential oil, cinnamaldehyde, as well as numerous other constituents, including eugenol. Another one is Ajwain or *Trachyspermum ammi*, also known as ajowan caraway, or carom is an annual herb in the family Apiaceae. It is one of the ancient medicinal, aromatic and a spice plant which has been used to cure several stomach disorders along with respiratory distress. Medicinal properties of the plant include antioxidant, antispasmodic, antimicrobial and antifungal (Zareshenas, 2013). Khajeh *et al.*, (2004) reported the active ingredients of the ajwain plant include six major chemical compounds including 49% thymol, 30.8%  $\gamma$ -terpinene, 15.7% p-cymene, 2.1% b-pinene, 0.8% myrcene and 0.7% limonene. In order to explore more regarding the properties of these herbs, the aim of this present study is to estimate the antimicrobial and antioxidant 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 sample of Cinnamon bark and Ajwain seeds was ground and crude extract was prepared by Soxhlet extraction method using ethyl acetate. Five grams of powdered sample of Cinnamon and Ajwain were filled in thimble directly, was placed in Soxhlet apparatus, and extracted using ethyl acetate 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). 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  $\mu$ l 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.

### **Radical DPPH scavenging activity**

The antioxidant activity of both extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH (2,2-diphenyl-1-picrylhydrazyl radical) method as modified by Sanchez-Moreno *et al* (1998). A methanolic solution (0.1 ml) of the sample at various concentrations (upto 150 $\mu$ g/ml) were added to 2.9 ml of DPPH (60  $\mu$ M) solution. When DPPH reacts with an antioxidant compound that can donate hydrogen, it gets reduced and the resulting decrease in absorbance at 517 nm was recorded at 10 min intervals up to 30 min using a UV-Vis spectrophotometer and the mean values were obtained from triplicate experiments. The remaining concentration of DPPH in the reaction medium was calculated from a calibration curve. Scavenging activity was calculated as:

{(Absorbance of control sample – Absorbance of sample with herb extract) / Absorbance of control sample} \* 100%.

## Results and Discussion

In the present investigation, *in vitro* antibacterial activity of crude extract of Cinnamon bark and Ajwain seeds was qualitatively assessed based on the zone of inhibition. The zone of inhibition (Table 1) for ethyl acetate extract of Cinnamon against *S. aureus* is more than that of Ajwain. Relatively larger zone of inhibition (mm) was observed for both herbal extracts against *P. aeruginosa*. Comparatively smaller zone of inhibition (mm) was obtained against *S. typhi*. Results of present study showed considerable antibacterial activity of Cinnamon and Ajwain against all tested organisms.

The mechanism of action of phytochemical additive depends on their chemical composition, and their antimicrobial activity is not attributable to a single mechanism but is instead a cascade of reactions involving the entire bacterial cell (Nazzaro *et al.*, 2013). However, it is accepted that the antimicrobial activity depends on the lipophilic character of the components. The components permeate the cell membranes and mitochondria of the microorganisms and inhibit the membrane

bound electron flow hampering energy metabolism. High concentrations may also lead to lysis of the cell membranes and denaturation of cytoplasmic proteins (Nazzaro *et al.*, 2013). The present findings of antibacterial property of Cinnamon falls in agreement with those of Goni *et al* (2009) who described the antibacterial activity of cinnamon and other essential oils against Gram-positive organisms (*Listeria monocytogenes*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus*), as well as against Gram-negative bacteria (*Salmonella choleraesuis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Yersinia enterocolitica*). Likewise, Hameed *et al* (2016) evaluated antibacterial activity of *Cinnamomum zeylanicum* against *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and observed zone of inhibition as 4.92, 3.99, 5.39, 6.12 and 5.19 mm, respectively. The current observations regarding antibacterial activity of Ajwain is also supported by results of Salma and Ramakrishnan (2018) who observed zone of inhibition against *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella typhi* and *Proteus vulgaris* to be 10, 11, 9 and 13, respectively. Phenol, alcohols, ketones and aldehydes present in herbs are mainly associated with antibacterial actions.

**Table.1** Antibacterial and antioxidant activity of Cinnamon and Ajwain extracts

Microorganisms	Zone of inhibition (mm)	
	Cinnamon	Ajwain
<i>S. aureus</i>	12	10
<i>S. typhi</i>	7	7
<i>P. aeruginosa</i>	16	15
<i>P. vulgaris</i>	9	8
<i>E. coli</i>	10	11
<b>DPPH activity (%)</b>	77.05	68.26

The radical scavenging activity (DPPH) of methanolic extract of Cinnamon and Ajwain was found to be 77.05 and 68.26%, respectively (Table 1). Cinnamon showed more antioxidant activity as compared to Ajwain. In accordance with current findings, Asimi *et al* (2013) observed % inhibition (scavenging activity) of cinnamon to be 86.40 at 25mg/ml. Higher free radical scavenging activity of cinnamon (IC<sub>50</sub> 13.1µg/ml) was reported due to presence of higher amount of phenolic compounds (El-Baroty *et al* 2010). Cinnamaldehyde and other flavonoids isolated from cinnamon possessed free-radical-scavenging activities and antioxidant properties (Okawa *et al.*, 2001). In another study it was found that that cinnamon oil potentially exhibits superoxide-dismutase like activity as revealed by the inhibition of the inhibiting capacity of pyrogallol autoxidation (Kim *et al.*, 1995). Significant radical scavenging activity was evident at all the tested concentrations of cinnamon bark and the EC<sub>50</sub> value was found to be 4.21 µg/ml which was inversely proportional to the antioxidant capacity (Matthew and Abraham, 2006).

The results of antioxidant activity of Ajwain falls in agreement with that of Chatterjee and coworkers (2012) who revealed that at 240µg/ml concentration of methanolic ajwain seed extract, the DPPH free radical scavenging activity was observed to be 83.42%. Saxena *et al* (2012) also found radical scavenging activity of Ajwain seed extract to be 68.07 %. Ethanol extract of ajwain oil quenched 73.41% of DPPH radicals (Mazahir *et al.*, 2015). Ajwain comprised carotenoids (β-carotene and lutein), flavonoids and phenolic compounds (thymol) which provides powerful antioxidant protection. Due to presence of phytoantimutagens, there is inhibition of free radicals formation.

Concluding these results we can say that Ajwain and Cinnamon both exhibited considerable antibacterial and antioxidant activity which can be further explored to use them as an alternative to antibiotic growth promoters in animal feed for getting better production.

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