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# *In-vitro* antibacterial activity of *Zingiber officinale* (ginger) against bacteria isolated from reproductive tract of clinical endometritic Murrah buffaloes

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Endometritis involves disruption of epithelium, hypermia or edema in uterus, influx of inflammatory cells, mostly neutrophils, and lymphocytes (Singh and Sethi 2022). It results in poor conception rate, increasing calving interval, cost of treatment, and reduction in calf crop, milk yield, and increased culling rate (Noakes *et al.* 2002). Cervical mucus plays an essential role in the reproductive process and conception in mammals (Dalton 2022). Major uterine pathogens are *Staphylococcus* spp., *Streptococcus* spp., or non-*E. coli* aerobic gram-negative rods have also been isolated as additional flora (Osawa 2021).

Fourier Transform Infrared Spectroscopy (FTIR) is an analytic technique to obtain an infrared spectrum of absorption or emission of solids, liquids, or gases. Ginger is a perennial herb, with a leafy stem up to 60 cm. The rhizome is horizontal, branched, fleshy, aromatic, white, or yellowish to brown (Eltahir *et al.* 2018). According to Mao *et al.* (2019), rhizome is rich in secondary metabolites such as phenolic compounds (gingerols and shogaols), which work as antioxidant, anti-inflammatory, anti-lipid, anti-diabetic, analgesic, antipyretic, and anti-tumor.

Ginger roots were collected from a local market, cleaned, dried for three months, and ground into powder. Approximately 500 mg ginger powder was used for the extraction procedure as per WHO protocol, CG-04. Murrah buffaloes were screened and confirmed for endometritis based on per-rectal examination, ultrasonography of reproductive organs, white side test, *p*H, and appearance of vaginal mucus. Vaginal mucus samples were used for isolation and identification of bacterial pathogens.

A total of 42 cervicovaginal mucus samples were collected, cervical mucus from cyclic buffaloes was collected at 0 to 12 h after the onset of behavioral estrus mentioned by Tsiligianni *et al.* (2011). The vulvar and perineum region were washed with an antiseptic solution and wiped properly with absorbable sterile cotton. The internal genitalia were massaged (per rectum) and mucus flown out was collected in sterilized disposable petri dish. All the samples were transported to the laboratory on ice and subjected to isolation of major bacterial pathogens as per standard protocols and these isolates were characterized based on cultural, morphological, biochemical methods (Ema *et al.* 2022). Followed by, these major bacterial pathogens were confirmed by PCR-based amplification.

The functional groups were identified; the interaction between the components in the microcapsules was performed using FTIR (Fourier Transform Infrared Spectroscopy) using KBr pellets (Ellerbrock and Gerke 2021) in a spectrophotometer. *In vitro* antibacterial tests were conducted using ginger extracts at different concentrations on sterile discs. The antimicrobial sensitivity of the isolates was assessed using the modified disc diffusion method on Muller Hinton agar plates (Nassar *et al.* 2019). The diameter of the inhibition zone was measured.

The findings indicated the presence of *Staphyllococcus aureus*, *Pseudomonas spp*., *E.coli*, and *Campylobacter* spp. in the vaginal mucus samples of endometritic Murrah buffaloes. Out of four isolated bacterial pathogens, *E. coli* was identified in the highest number (n=23) of buffaloes, followed by *S. aureus* (n=21), *Pseudomonas spp*. (n=12) and *Campylobacter spp*. (n=11). This study corroborates with the findings of Azawi *et al.* (2007), who also reported *E. coli*, *Staphyllococcus* spp., and *Pseudomonas* spp. as a cause of toxic puerperal metritis. However, Onnureddy *et al.* (2013) also reported presence of *Campylobacter* spp. in endometritic water buffaloes.

The hydro-ethanolic dried extract of ginger was analyzed from the mid-IR spectrum (400-4000/cm). The wavelength of infrared spectrum for ginger dried powder ranged from 3361.89/cm to 664.26/cm (Table 1, Fig. 1) with eight functional groups, whereas in the hydro-ethanolic extract of ginger the wavelength was ranged from 3402.8/cm to 765.3/cm (Table 2, Fig. 2) with eleven functional groups. These findings are in agreement with the findings of Purnomo *et al.* (2010), who also reported 13 functional compounds on ginger rhizomes extracts. The

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Table 1. Wave numbers, vibration type, and functional compounds in *Zingiber officinale* (ginger) dried powder

| Wave length ($cm^{-1}$) | Vibration type | Functional compound | Appearance | Comment |
|---|---|---|---|---|
| 3361.89 | OH stretch, H- Bonded | hydrate ($H_2O$), hydroxyl (-OH), ammonium, or amino | Strong broad | Intermolecular bonded |
| 2926.28 | OH stretch, H-Bonded | Carboxylic acid (RCOOH) | Strong broad | Usually centered on 3000 $cm^{-1}$ |
| 1641.03 | C=C stretch | Alkenavenyl(-$CH_2$=$CH_2$) | Strong | Monosubstituted |
| 1455.88 | Ring aromatic stretch (4p) | C=C aromatic | - | - |
| 1216.46 | C-O-C stretch venyl ether | Ether (R-O-R) | Strong | - |
| 992.2 | C=C bending | Alkene | Strong | Monosubstituted |
| 767.5 | C-H bending | 1,2-disubstituted | Strong | - |
| 664.26 | C-Br stretching | Halo compound | Strong | - |

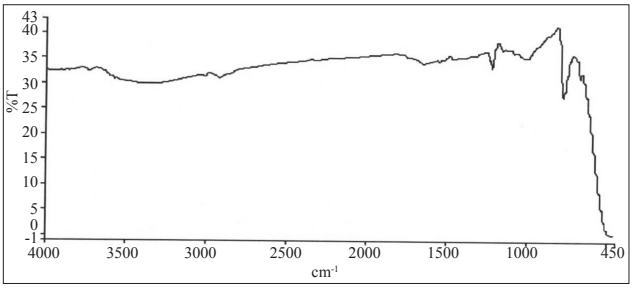


Fig. 1. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of *Zingiber officinale* (ginger) fine powder [transmittance (T%) at Y-axis; Wave number in $cm^{-1}$ at X-axis].

presence of broad bands at 3402.8/cm and 3361.89/cm can be attributed to (OH) stretching vibrations in both hydro-ethanolic ginger extract and dried ginger powder, respectively. Strong to medium intensities bands were also observed at 2979.15/cm and 2926.28/cm which confirms of carboxylic acid group. Other weak to strong intensity bands of the alkyne group were also observed at 2128.9/cm and 1647/cm in ginger extract and at 1641.03/cm in ginger powder. The infrared spectrum of hydro-ethanolic ginger rhizome extract had generated two functional compounds namely weak aromatic at 1270.04/cm and anhydride groups. The peaks were around 1085.77/cm in ginger extract and 1216.46/cm in ginger powder, which described the C-O stretch of C-O-C for both powder and hydroethanolic *Zingiber officinale* as similar to the findings of Norhidayah *et al.* (2013). However, peaks at 1603, 1190, 813, 850, 723/cm were strong indications of heterocyclic compounds such as flavonoids and alkaloids (Kumar *et al.* 2011). Moreover, it has been reported that standard gingerol has regular sharp peaks at position 680, 940, 1170, 1470, 1770 cm/cm wavenumbers (Meadows *et al.* 2004). Thus, the spectrum of the contents in the tested samples corresponded with standard 6-gingerol which indicated that the major component of the ginger is 6-gingerol (Norhidayah *et al.* 2013), which is a principal antimicrobial compound of *Zingiber officinale*.

The *in vitro* antibacterial activity of ginger extract was tested at various concentrations (2.5, 5, 10, 20, 30, 40 and 50 mg/ml) against *Staphylococcus aureus*, *Pseudomonas*

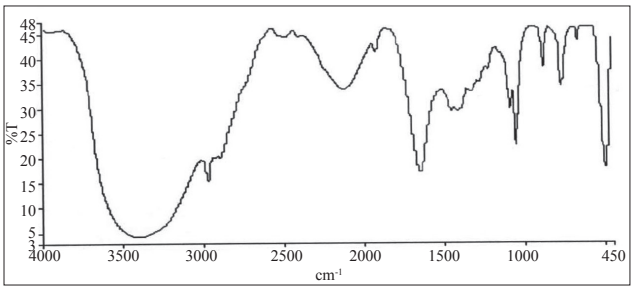


Fig. 2. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of *Zingiber officinale* (ginger) dried hydro-ethanolic extract [transmittance (T%) at Y-axis; Wavenumber in $cm^{-1}$ at X-axis].

Table 2. Wave numbers, vibration type, and functional compounds in *Zingiber officinale* dried hydroethanolic extract

| Wave length ($cm^{-1}$) | Vibration type | Functional compound | Appearance | Comment |
|---|---|---|---|---|
| 3402.8 | OH stretch, H- Bonded | hydrate ($H_2O$), hydroxyl (-OH), ammonium, or amino | Strong broad | Intermolecular bonded |
| 2979.15 | OH stretch, H- Bonded | Carboxylic acid (RCOOH) | Strong broad | Usually centered on 3000 $cm^{-}$ |
| 2497.54 | - | - | - | - |
| 2128.97 | C≡C weak | alkyne | Weak | Monosubstituted |
| 1922.2 | C-H bending | Aromatic compound | Weak | Overtone |
| 1647 | C=C stretch | Alkene | Medium | - |
| 1452.79 | Ring aromatic stretch (4p) | C=C aromatic | - | - |
| 1085.77 | C-O-C stretch akkyl-aryl ether | Ether (R-O-R) | - | - |
| 1046.89 | CO-O-CO | Anhydride | Strong broad | - |
| 880.02 | C-H bending | 1,2,4-trisubstituted | Strong | - |
| 765.35 | C-H bending | 1,2-disubstituted | Strong | - |

Table 3. Antibiotic susceptibility of *Staphylococcus aureus, Pseudomonas, E. coli* and *Campylobacter* at different *Zingiber officinale* (ginger) concentrations

| Ginger Con. (mg/ml) | *Staphylococcus aureus* (21) | | *Pseudomonas* spp. (12) | | *E. coli* spp. (23) | | *Campylobacter* Spp.(11) | |
|---|---|---|---|---|---|---|---|---|
| | No. of sensitive | No. of resistant | No. of sensitive | No. of resistant | No. of sensitive | No. of resistant | No. of sensitive | No. of resistant |
| 2.5 | 12 | 09 | 00 | 12 | 00 | 23 | 00 | 11 |
| 5 | 15 | 06 | 00 | 12 | 00 | 23 | 02 | 09 |
| 10 | 18 | 03 | 02 | 10 | 00 | 23 | 02 | 09 |
| 20 | 18 | 03 | 05 | 07 | 00 | 23 | 03 | 08 |
| 30 | 18 | 03 | 06 | 06 | 02 | 21 | 04 | 07 |
| 40 | 18 | 03 | 06 | 06 | 02 | 21 | 04 | 07 |
| 50 | 18 | 03 | 06 | 06 | 02 | 21 | 04 | 07 |

spp., *E. coli*, and *Campylobacter* spp (Table 3, Fig. 3). The ethanolic extract of *Zingiber officinale* exhibited moderate to strong antibacterial effects and demonstrated stronger inhibition against Gram-positive bacteria, consistent with the findings of Kaushik *et al.* (2011) and Nikolic *et al.* (2014). Notably, *Staphylococcus aureus*, a Gram-positive bacterium, displayed the highest sensitivity to ginger extract, aligning with the results of Bashir *et al.* (2015), although Hasan *et al.* (2012) reported increased sensitivity at higher concentrations (50-25 mg/ml).

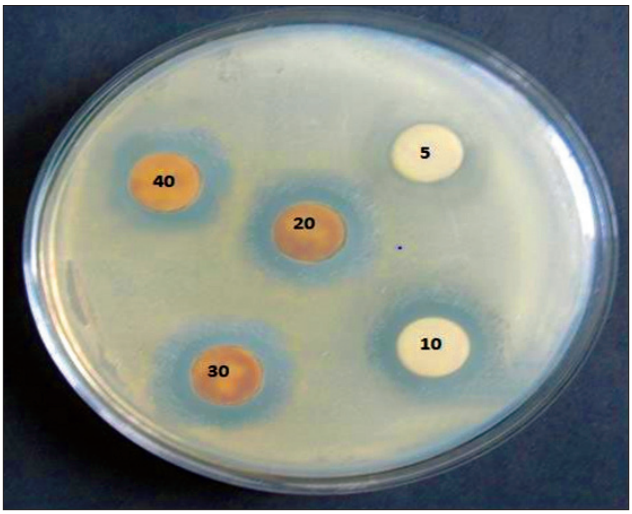


Fig. 3. Antibacterial activities of *Zingiber officinale* (ginger) extract at different concentrations against *Pseudomonas* spp. (concentration mg/ml).

In contrast, *E. coli* isolates in this study exhibited significant resistance to ginger extract, possibly due to the presence of antibiotic resistance genes on plasmids, as described by Fulgueiras *et al.* (2012), and the known propensity of *E. coli* to rapidly develop antibiotic resistance (Aypak *et al.* 2008). *Pseudomonas* species displayed moderate sensitivity to ginger, with 5 out of 12 samples showing sensitivity at the higher concentration of 20 mg/ml. These findings correspond with Socransky and Haffajee (2002), although Nikolic *et al.* (2014) reported sensitivity at lower concentrations (2.5-10 mg/ml). In the antibiotic sensitivity test, *Campylobacter* spp. also exhibited a moderate level of sensitivity, with four samples showing inhibition zones. These results are in line with the findings of Luc *et al.* (2022), who discussed the antibacterial effects of plant essential oils on *Campylobacter* spp.

This study reveals that *Zingiber officinale* (ginger) possesses significant antibacterial potency against multidrug-resistant uterine pathogens. Ginger exhibits greater inhibitory effects on Gram-positive bacteria compared to Gram-negative ones. The extract of ginger can serve as an economical solution for the therapeutic treatment of endometritis in buffaloes and can find utility in managing uterine infections in animals raised for organic milk production programs.

## SUMMARY

Endometritis, an inflammation of the innermost uterine layer, is caused by a variety of bacteria, including Gram-positive and Gram-negative aerobes and anaerobes. This study aimed to identify the active compounds in *Zingiber officinale* (ginger) and assess its effectiveness against common bacterial pathogens found in Murrah buffaloes suffering from endometritis. The infrared spectrum of ginger powder displayed a range of wavelengths from 3361.89/cm to 664.26/cm, indicating the presence of eight functional groups. In the hydro-ethanolic ginger extract, the spectrum ranged from 3402.8/cm to 765.35/cm, revealing 12 functional groups. A total of 42 vaginal mucus samples were collected, with *E. coli* being the most prevalent in 23 samples, followed by *S. aureus* in 21 samples, *Pseudomonas* spp. in 12 samples, and *Campylobacter* spp. in 11 samples. To evaluate the *in vitro* antibacterial activity of ginger extracts, antimicrobial susceptibility tests were conducted at concentrations of 2.5, 5, 10, 20, 30, 40, and 50 mg/ml. *Staphylococcus aureus* exhibited the highest sensitivity to the ginger extract, while *E. coli* showed substantial resistance. In conclusion, ginger demonstrates potent antibacterial activity against multidrug-resistant uterine pathogens and could serve as an alternative therapy to mitigate the risk of drug resistance in treating uterine infections.

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