Apple cider vinegar: Effective adjuvant treatment for aerobic vaginitis

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Abstract

Aerobic vaginitis (AV) is a vaginal infection caused mainly by overgrowth of Escherichia coli and a reduction of Lactobacilli in the vagina. The infection is linked with adverse pregnancy outcomes, such as preterm birth. The current medical therapy for AV with antibiotics is associated with adverse effects and necessitates the use of alternative treatments. Apple cider vinegar (ACV) is a natural fermented product produced from apples and is reported to exhibit potent antibacterial activity. ACV also contains lactic acid bacteria, which can act as probiotics. Symptoms of AV can be improved by restoring the disturbed microbial imbalance rather than exposing the vagina to synthetic drugs. Hence, an attempt was made to investigate whether ACV could support growth of the beneficial bacteria and inhibit growth of the pathogenic bacteria such as E. coli in the vagina. In vitro evaluation of the anti-bacterial potential of ACV against E. coli showed a potent antibacterial activity, recording a zone of inhibition diameter of 32.9 ± 0.5 mm; however, no zone of inhibition was observed against Lactobacillus acidophilus. Turbidimetric analysis was used to ensure growth of the bacteria in a simulated vaginal fluid (SVF) by using a nephelometer as a Nephelometric Turbidity Unit (NTU). Both E. coli and L. acidophilus were grown individually in SVF containing ACV. The ACV expressed antibacterial efficacy against E. coli and favoured growth of L. acidophilus in the SVF. Conclusively, ACV can help in growth of the beneficial bacteria and restore the natural microbiota of the vagina, thus proving to be beneficial in the management of AV.

Keywords: E. coli, L. acidophilus, Apple cider vinegar, Simulated vaginal fluid, Aerobic vaginitis, Turbidimetry
1. Introduction

Aerobic vaginitis (AV) is a vaginal infection caused due to disturbance in the common vaginal microbiota, specifically due to the decrease in the Lactobacilli as a dominant microflora (Donders et al., 2002; Donders, 2007). AV is characterized by an increase in the vaginal discharge that is sticky, yellowish or yellowish green in color, with foul smell and inflamed vaginal mucosa (Donders et al., 2017). In AV the vaginal pH is raised to ≥ 5. The occurrence of AV is associated with adverse pregnancy outcomes such as chorioamnionitis; preterm birth and miscarriage, as it is linked to the increase in the level of interlukins IL1β, IL-6 and IL-8 (Donders et al., 2011). Unlike the bacterial vaginosis, AV is characterized by inflammation of the vaginal mucosa (Geng et al., 2015), and hence it is more associated with the risk of sexually transmitted diseases and HIV. The infection is caused due to the overgrowth of certain bacterial pathogens such as E. coli and Staphylococcus aureus, which results in reduction of the number of Lactobacilli in the vagina (Wang et al., 2016; Rumyantseva et al., 2016).

Lactobacilli contribute to nonspecific antibacterial host defence in the normal vagina (Eschanbach et al., 1989). They inhibit adherence of the pathogenic microbes to the vaginal region and suppress their growth, by competing for binding onto the receptors located on the host vaginal epithelial cells (Marcone et al., 2010). Lactobacilli produce several antimicrobial substances such as lactocin, bacitracin, lactic acid and hydrogen peroxide (Mendling, 2016). Bacitracin and lactocin are the proteins that have bactericidal activity against E. coli (Kale et al., 2005; Turovskiy et al., 2009; Razzak et al., 2011). Lactobacilli can interact with the immune cells and stimulate the host immune response (Lebeer et al., 2008). Moreover, they are reported to inhibit the growth and biofilms formation by E. coli. According to Tempera et al., (2006), in patients suffering from AV; increased level of E. coli and decreased level of Lactobacilli have been reported. Growth of E. coli and the other aerobic microbes are reported to increase 3-5 folds in case of AV, and are associated with inflammation of the vaginal mucosa. Any changes in the microbiota of the vagina cause reduction in the Lactobacillus spp., thus favoring the growth of other pathogens such as E. coli that causes vaginal infection.

Treatment of AV requires the administration of local anti-bacterials and anti-inflammatory agents (Bertuccini et al., 2017). However, the chances of development of resistance to antibiotics and occurrence of re-infection limit the chemical antibacterial therapy. Furthermore, the risk of side effects development caused by the use of antibiotics along with eradication of the beneficial bacteria are the main reasons for the unsuccessful treatments of AV with the use of synthetic anti-bacterials and antibiotics (Eade et al., 2012; Deshkar et al., 2022). Hence, recently the health care system is more inclined towards the use of herbal and natural remedies for the management of AV.

Apple cider vinegar (ACV) is a natural apple product produced by the fermentation process, and consists of sugar, apple and yeasts (Del Campo et al., 2008; Medina and Pieper, 2016). The antibacterial potency of ACV on bacterial species such as E. coli is well established, and is also found effective against the Gram positive bacteria. This activity is attributed to the presence of a wide range of components such as organic acids; flavonoids, polyphenols, vitamins and minerals (Xia et al., 2020). There are two types of Lactic acid bacteria (LAB) most commonly identified in apple juice by-products mainly; Lactobacillus sp. and Oenococcus sp. (Cousin et al., 2017). Presence of these LABs in ACV will help to improve the dysbiosis condition in AV, which is caused by the increase in numbers of E. coli and the decrease in numbers of Lactobacilli. Moreover, acetic acid that is present in ACV is reported to have potent antibacterial efficacy.
Therefore, the objectives of the present work were to investigate the efficacy of Apple cider vinegar to promote growth of Lactobacilli and simultaneously inhibit *E. coli* in the vagina, in order to treat AV naturally.

2. Materials and methods

2.1. Materials

Bragg’s Apple cider vinegar was purchased from Organic India store, Pune, India. On the other hand, *L. acidophilus* and *E. coli* strains were provided by the National Collection of Industrial Microorganisms (NCIM), Pune, India.

2.2. Growth of *L. acidophilus* and *E. coli*

A single colony of *E. coli* was taken carefully and emulsified with 2 ml sterile saline in a test tube and its turbidity was adjusted equivalent to 0.5 McFarland. This bacterial suspension was further used to carry out the antibacterial activity using a cup plate assay (Balouiri *et al.*, 2016). This bacterial suspension of *E. coli* (1 ml) was inoculated aseptically into 10 ml of sterilized nutrient broth (NB) tubes, and incubated at 37 ºC for 24 h, for carrying out the turbidimetric analysis (Balouiri *et al.*, 2016).

*Lactobacillus acidophilus* is a normal Gram-positive anaerobic micro-organism, which grows at pH 4.5 at 37 ºC (Mousavi *et al.*, 2013). The bacterial suspension of *L. acidophilus* was prepared in the same manner as described earlier for *E. coli*. About 1 ml of this bacterial suspension was inoculated into 10 ml of sterile nutrient broth (NB) tubes, and grown anaerobically under aseptic condition in a desiccator at 37 ºC for 24 h, to carry out the turbidimetric analysis. Anaerobic condition in the desiccator was maintained by keeping a burning candle inside it. Combustion of the candle consumed the oxygen present in the desiccator and thus anaerobiosis was generated (Patore and Pandit, 2018).

2.3. Antibacterial activity

The antibacterial potential of ACV was determined using the cup-plate assay. Using the previously prepared bacterial suspensions of *E. coli* and *L. acidophilus* of $1.5 \times 10^8$ cfu/ml each, approximately (0.1 ml) of each bacterial suspension was individually inoculated into the surface of nutrient agar (NA) petri plates, and spread uniformly with the aid of a sterile glass spreader. Individual cups each of 6 mm diameter were punched in the NA plates using a sterile cork borer, and then 1 ml of ACV (5% acidity) was filled aseptically into these cups. Petri-plates were kept in refrigerator for 15 min. to diffuse the solution, and then incubated at 37 ºC for 24 h. The assay was carried out in triplicates. After incubation, the zone of inhibition was measured using a calibrated ruler (Balouiri *et al.*, 2016).

2.4. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of ACV

MIC is defined as the lowest concentration of an antibacterial agent that inhibits the growth of a bacterial strain. On the other hand, MBC is the lowest concentration of this agent required to kill or reduce the viability of an initial bacterial inoculum by ≥ 99.9% over a fixed period, and under a specific set of conditions. MBC is complementary to the MIC.

The same standard concentration of $1 \times 10^7$ cfu/ml of both *E. coli* and *L. acidophilus* was used to assess their susceptibilities to the different concentrations of ACV, according to Edris *et al.*, (2017). To define the MIC of ACV against both of the tested bacterial strains, different sets of NB treatments were prepared individually. The sets included negative control tubes (NB without *E. coli* or *L. acidophilus* and without of ACV); positive control tubes (NB tubes inoculated individually only with *E. coli* or *L. acidophilus* without ACV); and the treatment tubes (NB tubes inoculated individually with *E. coli* or *L. acidophilus*, and containing different concentrations of ACV, mainly; 0.5, 1, 1.5 and 2 ml of ACV). The MBC was defined by sub-culturing 1 ml of the tube that represented MIC of each bacterium individually into...
the surface of NA petri plates, and then spread uniformly using a sterile glass spreader (Edris et al., 2017). Plates were incubated at 37°C for 24-48 h. After incubation, the number of bacteria (cfu/ml) or (cfu/g of sample) was evaluated as follows:

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\text{cfu/ml} = \frac{\text{Number of colonies}}{\text{Dilution} \times \text{volume of inoculum}}
\]

MBC was recorded if no growth was observed on the NA plates, which confirmed that the effect of AVC on the tested bacterial strains was bactericidal and not bacteriostatic (Edris et al., 2017).

2.5. Measurement of turbidity using Nephelometer

The turbidimetric assay is a simple and rapid quantitative determination method of the bacterial population (Metzger et al., 2018). In Nephelometry, the particle density is a function of the scattered light directed to the detector. The amount of scattered light is directly proportional to the amount of insoluble particles in a solution. The higher the amount of particles scattering the light beam, the more light reaches the detector, the more Nephelometric Turbidity Units (NTU) is recorded (Dalgaard et al., 1994). The turbidimetric analysis was carried out to assess the individual growth of E. coli and L. acidophilus in the simulated vaginal fluid (SVF) medium, as when the bacterium grows in the SVF medium, more amount of bacterial cells were detected in this medium. When these treated SVF were subjected to turbidimetric analysis, more amount of light was scattered reflecting the increase in the bacterial population, and hence indicated the growth of E. coli and L. acidophilus. Aerobic vaginitis occurs due to the imbalance between the levels of E. coli and L. acidophilus in the vagina. Therefore, growth of these bacterial strains was studied in SVF. Simulation of the vaginal environment was created using SVF medium for growth of the anaerobic bacterium (Borges et al., 2012). The SVF was prepared as follows: 3.51 g/ l sodium chloride, 1.40 g/ l potassium hydroxide, 0.222 g/ l calcium hydroxide, 0.018 g/ l bovine serum albumin (BSA), 2 g/ l lactic acid, 1 g/ l acetic acid, 0.16 g/ l glycerol, 0.4 g/ l urea and 5 g/ l glucose, in reference to Owen and Katz, (1999).

The effect of ACV on the growth of E. coli and L. acidophilus in SVF was studied using turbidimetric analysis. In this study, E. coli (10^7 cfu/ml) and L. acidophilus (10^7 cfu/ml) were grown individually in 10 ml of SVF containing 1 ml of ACV. Control treatments were also used in absence of ACV. Different dilution groups were made namely Groups I to IV. The composition of Group I: 10 ml SVF+ 1 ml of NB culture of E. coli (10^7 cfu/ml); Group II: 10 ml SVF + 1 ml of NB culture of L. acidophilus (10^7 cfu/ml); Group III: 10 ml SVF + 1 ml of NB culture of E. coli (10^7 cfu/ml) + 1 ml ACV; Group IV: 10 ml SVF + 1 ml of NB culture of L. acidophilus (10^7 cfu/ml) + 1 ml ACV. All the test tubes were incubated at 37°C for 24 h. The developed turbidity due to bacterial growth was measured at 600 nm using nephelometer (331, Electronics, India), and was recorded as a Nephelometric Turbidity Unit (NTU). This assay was carried out in triplicates.

2.7. Statistical analysis

The antibacterial effect of AVC on E. coli and L. acidophilus was assessed by measuring the zone of inhibition, where the results were expressed as the mean value of three independent replicates ± the standard deviation (SD) The data derived from the turbidimetric analysis was subjected to statistical analysis using Graph pad prism version 7 (student t-test), to find the statistical significance of the effect of ACV on growth of E. coli and L. acidophilus.

3. Results and Discussion

3.1. Study of antibacterial potential

The ACV produced a zone of inhibition diameter of 32.9 ± 0.5 mm against E. coli (Fig. 1a). Thus, ACV demonstrated good antibacterial efficacy against E. coli, the causative agent of AV. The presence of phenolic compounds and organic acids in ACV contribute to its antibacterial properties (Halstead et al., 2015; Du et al., 2019; Lagana et al., 2019; Ousaaid
The antibacterial effect of ACV against *E. coli* is attributed to the presence of several phenolic compounds including; vanillic acid, chlorogenic acid, caffeic acid, gallic acid, catechin, epicatechingallate and phlorizin; however, the most prominent being gallic acid and tannins (Yagnik *et al*., 2018). Gallic acid and tannins express antibacterial potential by binding to the bacterial cell membrane ergosterol, which is responsible for maintaining the integrity of the microbial cell wall, and also by inhibiting the activity of the catalase enzyme (Lagana *et al*., 2019; Ousaaid *et al*., 2021). Moreover, ACV possess organic acids such as acetic acid and lactic acid, which release protons into the bacterial cell wall and decrease its intracellular pH, causing destabilization of the bacterial cell wall and eventually leads to the cell death (Lourenço *et al*., 2019). Furthermore, the organic acids decrease the raised vaginal pH > 5 and restore it back to its normal level (Lourenço *et al*., 2019). Lactic acid is reported to exert antibacterial effect specifically against the Gram-negative bacteria (Zhang *et al*., 2021). As being a fermented food product, ACV contains both the lactic acid and acetic acid bacteria, which produce lactic acid and acetic acid, respectively (Aumiller *et al*., 2021). Lactates and acetates enzymes are known to promote the growth of Acetobacters and Lactobacilli, respectively (Aumiller *et al*., 2021). Acetobacters have the characteristics of probiotics. Thus, Acetobacters and Lactobacilli share a synergistic relationship and mutually promote the growth of one another through cross feeding (Aumiller *et al*., 2021; Guine *et al*., 2021). Thus, ACV did not present antibacterial potential against *L. acidophilus*; however, it promoted *L. acidophilus* growth, where no zone of inhibition was observed on the treated NA plates (Fig. 1.b).

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**Fig. 1:** a)-Antibacterial effect of ACV against *E. coli* (zone of inhibition was observed), b)-Antibacterial potential of ACV against *L. acidophilus* (no zone of inhibition)
3.2. Determination of the MIC and MBC

The absence of bacterial growth in the MIC test and then its growth on the NA plates indicates that although a particular antibacterial agent caused inhibition; however, further plating of the bacteria onto NA still result in bacterial proliferation, which is attributed to the bacteriostatic mode of action of this antibacterial agent (Edris et al., 2017). Results of MIC and MBC are summarized in Table (1). Therefore, the MBC was performed to get the exact antibacterial dose of the ACV. Turbidity was observed in all the tubes containing the tested concentrations of ACV against L. acidophilus (Fig. 2). Results of MBC demonstrated that L. acidophilus expressed growth on the NA plates when taken from all the tested ACV concentrations (0.5, 1, 1.5 and 2 ml). However, in the case of E. coli (Fig. 3), not all tubes expressed turbidity. Thus, the recorded MIC of ACV tested against E. coli was 0.5 ml. Thereafter, the MBC of ACV at a concentration of 0.5 ml was tested against E. coli, which did not present any growth. Accordingly, these results revealed the bactericidal action of ACV against E. coli; however, no such activity was detected against L. acidophilus. These results are in line with the study conducted by Yagnik et al., (2018), reporting the antibacterial potency of ACV against E. coli. Meanwhile, the previous study of Cousin et al., (2017) confirmed the presence of lactic acid bacteria in the ACV, which could have supported the growth of L. acidophilus. These results were recorded from the current MIC and MBC studies.

Table 1: Determination of MIC for both E. coli and L. acidophilus induced by ACV

| Test Tube | Volume of nutrient broth medium (ml) | Concentration of ACV (ml) | Visual results |
|-----------|-------------------------------------|---------------------------|---------------|
|           |                                      |                           | L. acidophilus |
| a         | 5.0                                  | 2                         | Clear         |
| b         | 5.0                                  | 1.5                       | Clear         |
| c         | 5.0                                  | 1                         | Clear         |
| d         | 5.0                                  | 0.5                       | Clear         |
| e         | 5.0                                  | 0                         | Turbid        |
| f         | 5.0                                  | 0.0                       | Clear         |

| Test Tube | Volume of nutrient broth medium (ml) | Concentration of ACV (ml) | Visual results |
|-----------|-------------------------------------|---------------------------|---------------|
|           |                                      |                           | E. coli       |
| g         | 5.0                                  | 0                         | Clear         |
| h         | 5.0                                  | 2                         | Turbid        |
| i         | 5.0                                  | 1.5                       | Turbid        |
| j         | 5.0                                  | 1                         | Turbid        |
| k         | 5.0                                  | 0.5                       | Turbid        |
| l         | 5.0                                  | 0.0                       | Turbid        |

Where; the tested concentrations of ACV were represented using the following letters; a: 2 ml; b: 1.5 ml; c: 1 ml; d: 0.5 ml of ACV; e: positive control; f: negative control (tested against L. acidophilus). On the other hand, g: negative control; h: 2 ml; i: 1.5 ml; j: 1 ml; k: 0.5 ml of ACV and l: positive control) tested against E. coli. *Negative control refers to NB tube without E. coli or L. acidophilus and without of ACV, while, Positive control refers to NB tube inoculated individually with E. coli or L. acidophilus and without ACV
3.3. Detection of growth of the tested bacterial strains in SVF using turbidimetric analysis

The turbidity observed for *E. coli* in SVF (Group I) was 1500 NTU, while that for *L. acidophilus* in SVF (Group II) was 1578 NTU. However, the recorded turbidity in Group III was reduced to 900 NTU, which was statistically significant (*p* < 0.001) when compared to Group I (Fig. 2). These results demonstrate the antibacterial action of ACV on *E. coli*. Furthermore, the turbidity observed in Group IV was slightly increased to 1659 NTU, which was statistically insignificant when compared to Group II. Thus, no growth inhibition was recorded by *L. acidophilus* in SVF. However, ACV slightly favored growth of *L. acidophilus*, which was evident from the slight increase in the recorded turbidity of Group IV. The turbidometric analysis further supported the efficiency of ACV in slightly promoting the growth of *L. acidophilus* and inhibiting the growth of *E. coli* (Table 2).

![Graph showing turbidity levels in different groups](image)

**Fig. 2.** Effect of ACV on growth of *E. coli* and *L. acidophilus*. Error bars represent the standard error (SE). Data was analyzed by Graph pad prism version 7. (Where; student t test (unpaired) **** (*p* < 0.001) ns: - non –significant). Groups I and III represent the treatment groups for *E. coli*, and Groups II and IV represent the treatment groups for *L. acidophilus*.
Table 2: Measurement of *E. coli* and *L. acidophilus* bacterial growth in SVF using turbidity analysis

| Group | Dilution fluid + Bacterial culture | Turbidity (NTU) |
|-------|-----------------------------------|-----------------|
| I     | SVF + 1 ml of NB culture of *E. coli* | 1500 ± 0.5      |
| II    | SVF + 1 ml of NB culture of *L. acidophilus* | 1578 ± 0.6      |
| III   | SVF + 1 ml of NB culture of *E. coli* + ACV | 900 ± 0.2       |
| IV    | SVF + 1 ml of NB culture of *L. acidophilus* + ACV | 1659 ± 0.5      |

Where; (±): indicates standard error. SVF: simulated vaginal fluid, NB: Nutrient broth. Results are means of 3 replicates.

The turbidometric results indicate that ACV could help to restore the disturbed vaginal microflora by inhibiting the growth of *E. coli* as observed by reduction in turbidity in Group (III), in accordance with the previous study of *Yagnik et al.,* (2018). Meanwhile, the possible reason for the slight increase in turbidity observed in group (IV) could be attributed to the presence of acetic acid in ACV, which was reported previously to enhance growth of the lactic acid bacteria (*Safari et al.,* 2017), thereby promoted growth of the *L. acidophilus* in SVF.

### Conclusion

AV is a vaginal infection due to imbalance in the number of bacteria that reside in the vagina. Therefore, the line of treatment was to maintain bacterial balance. ACV served dual functions by reducing the growth of *E. coli*, and simultaneously enhancing the growth of *L. acidophilus*. Thus, ACV plays an important role in maintaining the normal flora in the vagina. ACV showed good antibacterial activity against *E. coli*, while favored the growth of *L. acidophilus* in the SVF. Finally, Apple cider vinegar is regarded as a good adjuvant treatment to maintain the microbiota of the vagina, thus could be used effectively to avoid the excessive use of the synthetic anti-bacterials, steroids and antibiotics, which are currently manipulated to treat AV.

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### Conflict of interest

The authors declare that they have no conflict of interests.

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### Ethical approval

Not applicable, because no patient's samples were involved.

### 4. References

Aumiller, A.; Stevens, E.; Scheffler, R.; Güvener, Z.T.; Tung, E.; Grimaldo, A.B.; Carlson, H. K.; Deutschbauer, A.M.; Taga, M.E.; Marco, M.L. and Ludington, W.B. (2021). A chemically-defined growth medium to support *Lactobacillus- Acetobacter* community analysis. Microbiology. 05(12): 443930. [https://doi.org/10.1101/2021.05.12.443930](https://doi.org/10.1101/2021.05.12.443930)

Balouiri, M.; Sadiki, M. and Ibsouida, S.K. (2016). Methods for *in vitro* evaluating antimicrobial activity:
A review. Journal of Pharmaceutical Analysis. 6(2): 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Bertucciini, L.; Russo, R.; Iosi, F. and Superti, F. (2017). Effects of *Lactobacillus rhamnosus* and *Lactobacillus acidophilus* on bacterial vaginal pathogens. International Journal of Immunopathology and Pharmacology. 30(2): 163-167. https://doi.org/10.1177/0394632017697987

Borges, S.; Silva, J. and Teixeira, P. (2012). Survival and biofilm formation by Group B Streptococci in simulated vaginal fluid at different pHs. Antonie Van Leeuwenhoek. 101(3): 677-82. https://doi.org/10.1007/s10482-011-9666-y

Cousin, F.J.; Le Guellec, R.; Schlusselhuber, M.; Dalmasso, M.; Laplace, J.M. and Cretenet, M. (2017). Microorganisms in Fermented Apple Beverages: Current Knowledge and Future Directions. Microorganisms. 5(3): 39. https://doi.org/10.3390/microorganisms5030039

Del Campo, G.; Berregi, I.; Santos, J.I.; Dueñas, M. and Irastorza, A. (2008). A development of alcoholic and malolactic fermentations in highly acidic and phenolic musts. Bioresource Technology. 99(8): 2857-2863. https://doi.org/10.1016/j.biortech.2007.06.007

Deshkar, S.S.; Ahire, K.S. and Mahore, J.G. (2022). *In vitro* and *in vivo* evaluation of prebiotic potential of pectin on vaginal lactobacilli. Journal of Applied Biology and Biotechnology. 10(1): 103-111. https://doi.org/10.7324/JABB.2021.100113

Donders, G.G. (2007). Definition and classification of abnormal vaginal flora. Best Practice & Research Clinical Obstetrics. 21: 355-73. https://doi.org/10.1016/j.bpoobgyn.2007.01.002

Donders, G.G.; Bellen, G.; Grinceviciene, S; Ruban, K. and Vieira-Baptista, P. (2017). Aerobic vaginitis: no longer a stranger. Research in Microbiology. 168(9-10): 845-858. https://doi.org/10.1016/j.resmic.201

Donders, G.; Bellen, G. and Rezeberga, D. (2011). Aerobic vaginitis in pregnancy. British Journal of Obstetrics and Gynaecology. 118: 1163-70. https://doi.org/10.1111/j.1471-0528.2011.03020.x

Donders, G.G.; Vereecken, A.; Bosmans, E.; Dekeersmaecker, A.; Salembier, G. and Spitz, B. (2002). Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. British Journal of Obstetrics and Gynaecology. 109: 34-43. https://doi.org/10.1111/j.1471-0528.2002.00432.x

Du, G.; Zhu, Y.; Wang, X., Zang, J.; Tian, C.; Liu, L.; Meng, Y. and Guo, Y. (2019). Phenolic composition of apple products and by-products based on cold pressing technology. Journal of Food Science and Technology. 56(3): 1389-1397. https://doi.org/10.1007/s13197-019-03614-y

Eade, C.R.; Diaz, C.; Wood, M.P.; Anastos, K.; Patterson, B.K.; Gupta, P.; Cole, A.L. and Cole, A.M. (2012). Identification and characterization of bacterial vaginosis-associated pathogens using a comprehensive cervical-vaginal epithelial coculture assay. PLoS One. 7 (11): e50106. https://doi.org/10.1371/journal.pone.0050106

Edris, H.; Makhdoumi, P.; Taha, P.; Hossini, H.; Pirsaheb, M.; Omid, S.; Stelling, R. and Stelling, J. (2017). A review of available techniques for determination of nano-antimicrobials activity. Toxin Reviews. 36(1): 18-32. https://doi.org/10.1080/15569543.2016.1237527
Eschanbach, D.A.; Davick, P.R.; Williams, B.L.; Klebanoff, S.J.; Young-Smith, K.; Critchlow, C.M. and Holmes, K.K. (1989). Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. Journal of Clinical Microbiology. 27: 251-6. https://doi.org/10.1128/jcm.27.2.251-256.1989

Geng, N.; Wu, W.; Fan, A.; Han, C.; Wang, C.; Wang, Y. et al. (2015). Analysis of the risk factors for aerobic vaginitis: A case-control study. Gynecologic and Obstetric Investigation. 81: 148-54. https://doi.org/10.11159/000431286

Guine, R.P.F.; Barroca, M.J.; Coldea, T.E.; Bartkiene, E. and Anjos, O. (2021). Apple Fermented Products: An Overview of Technology, Properties and Health Effects. Processes. 9(2): 223. https://doi.org/10.3390/pr9020223

Halstead, F.D.; Rauf, M.; Moiemen, N.S.; Bamford, Amy.; Wearn, C.M.; Fraise, A.P.; Lund, P.A.; Oppenheim, B.A. and Webber M.A. (2015). The antibacterial activity of acetic acid against biofilm-producing pathogens of relevance to burns patients. PLoS ONE. 10(9): e0136190. https://doi.org/10.1371/journal.pone.0136190

Kale, V.V.; Trivedi, R.V.; Wate, S.P. and Bhusari, K.P. (2005). Development and evaluation of a suppository formulation containing Lactobacillus and its application in vaginal diseases. Annals of the New York Academy of Sciences 1056: 359-365. https://doi.org/10.1196/annals.1352.017

Lagana, P.; Anastasi, G.; Marano, F.; Piccione, S.; Singla, R.K.; Dubey, A.K.; Delia, S.; Coniglio M.A.; Facciola, A.; Di Pietro, A.; Haddad, M.A.; Al-Hiary, M. and Caruso, G. (2019). Phenolic Substances in Foods: Health Effects as Anti-Inflammatory and Antimicrobial Agents. Journal of AOAC International. 102(5): 1378-1387. https://doi.org/10.5740/joaacint.19-0131.

Lebeer, S.; Vanderleyden, J. and De Keersmaecker, S.C. (2008). Genes and molecules of lactobacilli supporting probiotic action. Microbiology and Molecular Biology Reviews. 72: 728e64. https://doi.org/10.1128/MMBR.00017-08

Lourenço, A.; Pedro, N.A.; Salazar, S.B. and Mira, N.P. (2019). Effect of Acetic Acid and Lactic Acid at Low pH in Growth and Azole Resistance of Candida albicans and Candida glabrata. Frontiers in Microbiology. 9: 3265. https://doi.org/10.3389/fmicb.2018.03265

Marcone, V.; Rocca, G.; Lichtner, M. and Calzolari, E. (2010). Longterm vaginal administration of Lactobacillus rhamnosus as a complementary approach to management of bacterial vaginosis. The International Journal of Gynecology & Obstetrics. 110: 223Y6. https://doi.org/10.1016/j.ijgo.2010.04.025

Medina, E. and Pieper, D.H. (2016). Tackling threats and future problems of multidrug-resistant bacteria. Current Topics in Microbiology and Immunology. 398: 3-33. https://doi.org/10.1007/82_2016_492

Mendling, W. (2016). Vaginal microbiota. Advances in Experimental Medicine and Biology. 902: 83-93. https://doi.org/10.1007/978-3-319-31248-4_6

Metzger, N.; Konrad, A.; Blendinger, F.; Modler, A.; Meixner, A.J.; Bucher, V. and Brecht, M. (2018). Low cost GRIN- lens based Nephelometric turbidity sensing in the range of 0.1-1000 NTU. Sensors. 18(4): 11-15. https://doi.org/10.3390/s18041115

Mousavi, Z.E.; Mousavi, S.M.; Razavi, S.H.; Hadinejad, M.; Emam-Djomeh, Z. and Mirzapour, M. (2013). Effect of fermentation of pomegranate juice by Lactobacillus plantarum and Lactobacillus acidophilus on the antioxidant activity and metabolism of sugars, organic acids and phenolic
compounds. Food Biotechnology. 27(1): 1-13. https://doi.org/10.1080/08905436.2012.724037

Oussaid, D.; Laaroussi, H.; Bakour, M.; Ennaji, H.; Lyoussi, B. and El-Arabi, I. (2021). Antifungal and Antibacterial Activities of Apple Vinegar of Different Cultivars. International Journal of Microbiology. 1-6. https://doi.org/10.1155/2021/6087671

Owen, D.H. and Katz, D.F. (1999). A vaginal fluid simulant. Contraception. 59: 91-95. https://doi.org/10.1016/s0010-7824(99)00010-4.

Patole, V.C. and Pandit, A.P. (2018). Mesalamine-loaded alginate microspheres filled in enteric coated HPMC capsules for local treatment of ulcerative colitis: in vitro and in vivo characterization. Journal of Pharmaceutical Investigation. 48(3): 257-267. https://doi.org/10.1007/s40005-017-0304-1.

Razzak, M.S.A.; Al-Charrakh, A.H and Al-Greitty, B.H. (2011). Relationship between lactobacilli and opportunistic bacterial pathogens associated with vaginitis. The North American Journal of Medical Sciences. 3: 185-92. https://doi.org/10.4297/najms.2011.3185

Rumyantseva, T.A.; Bellen, G.; Savochkina, Y.A.; Guschin, A.E. and Donders, G.G. (2016). Diagnosis of aerobic vaginitis by quantitative real-time PCR. Archives of Gynecology and Obstetrics. 294: 109-14. https://doi.org/10.1007/s00404-015-4007-4.

Safari, R.; Hoseinifar, S.H.; Nejadmoghadam, S. and Khalili, M. (2017). Apple cider vinegar boosted immunomodulatory and health promoting effects of Lactobacillus casei in common carp (Cyprinus carpio). Fish and Shellfish Immunology. 67: 441-448. https://doi.org/10.1016/j.fsi.2017.06.017.

Tempera, G.; Abbadessa, G.; Bonfiglio, G.; Cammarata, E.; Cianci, A.; Corsello, S.; Raimondi, A.; Ettore, G.; Nicolosi, D. and Furneri, P.M. (2006). Topical kanamycin: An effective therapeutic option in aerobic vaginitis. Journal of Chemotherapy. 18: 409-14. https://doi.org/10.1179/joc.2006.18.4.409.

Turovskiy, Y.; Ludescher, R.D.; Aroutcheva, A.A.; Faro, S. and Chikindas, M.L. (2009). Lactocin 160, a bacteriocin produced by vaginal Lactobacillus rhamnosus, targets cytoplasmic membranes of the vaginal pathogen, Gardnerella vaginalis. Probiotics and Antimicrobials Proteins 20(1): 67-74. https://doi.org/10.1007/s12602-008-9003-6.

Wang, Z.L.; Fu, L.Y.; Xiong, Z.A.; Qin, Q.; Yu, T.H.; Wu, Y.T.; Huaa, Y.Y. and Zhanga, Y.H. (2016). Diagnosis and micro-ecological characteristics of aerobic vaginitis in outpatients based on preformed enzymes. Taiwanese Journal of Obstetrics and Gynecology. 55: 40-4. https://doi.org/10.1016/j.tjog.2015.06.012.

Xia, T.; Zhang, B.; Duan, W.; Zhang, J. and Wang, M. (2020). Nutrients and bioactive components from vinegar: A fermented and functional food. Journal of Functional Foods. 64: 103681. https://doi.org/10.1016/j.jff.2019.103681.

Yagnik, D.; Serafin, V. and Shah, A. (2018). Antimicrobial activity of apple cider vinegar against Escherichia coli, Staphylococcus aureus and Candida albicans; downregulating cytokine and microbial protein expression. Scientific Reports. 8(1): 1732. https://doi.org/10.1038/s41598-017-18618-x.

Zhang, H.; HuangFu, H.; Wang, X, Zhao, S.; Liu, Y.; Lv, H.; Qin G.Y. and Tan, Z. (2021). Antibacterial Activity of Lactic Acid Producing Leuconostoc mesenteroides QZ1178 against Pathogenic Gallibacterium anatis. Frontiers in Veterinary Science. 8: 630294. https://doi.org/10.3389/fvets.2021.630294.