In Vitro Evaluation For Antibacterial Activity Of Dadimashtaka Choorna, An Ayurvedic Polyherbal Powder Formulation, Against Selective Bacterial Strains

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ABSTRACT
Dadimashtaka choorna is one of the classical ayurvedic polyherbal powder formulation explained in the context of Atisara chikitsa (treatment of gastrointestinal conditions such as diarrhoea), in the text of Astangahridaya. The formulation is mainly indicated for digestive disorders. Even though many researches have brought out the anti-bacterial activity of each component of the formulation, no studies were done to identify the activity of the compound formulation on any specific bacterial strains. Therefore, an attempt was made to accurately understand the action of the preparation against certain selected strains of bacteria. The formulation was prepared according to the classical method of preparation for choorna kalpana. As per the standard norms mentioned in Ayurvedic Pharmacopoeia of India, the concentrated extract of the medicament was prepared. The study was undertaken to evaluate the anti-bacterial activity of concentrated extract of Dadimashtaka choorna against selected microorganisms namely, Salmonella typhi (gram -ve), Escherichia coli (gram -ve), and Bacillus species (gram +ve), using agar well diffusion method and also to compare with the standard drug, ciprofloxacin. It was found that the concentrated extract of Dadimashtaka choorna has significant anti-bacterial activity against the bacterial strain, Bacillus (gram +ve). The study confirmed the anti-bacterial potential of the formulation Dadimashtaka choorna against Bacillus species(gram+ve), which supports the fact that the compound formulation is a promising remedy to curb the gastrointestinal disorders caused by the gram +ve bacterial strains.

INTRODUCTION
Any condition or a disease that occurs within the gastrointestinal tract is termed under the broad heading of Gastrointestinal disorders. Since the cause for gastrointestinal disorders can widely vary, the one with these disorders is diagnosed based upon the symptoms. Frequent digestive disorders such as chronic gastritis, irritable bowel syndrome (IBS), gastro-oesophageal reflux disease and duodenal gastric reflux disease are diagnosed based on the symptoms (Ling et al., 2015). An infectious gastrointestinal disorder is a dysentery which is depicted by inflammation of the intestines. It mainly advances through contaminated food, water and due to unhygienic condition. Certain bacteria like Escherichia coli (E.coli), Salmonella typhi, Bacillus and Shigella species are accountable for this (Internet, 2020). The prevalence of dysentery is hiked between the ages of 18 to 23 months (Henry, 1991). In devel-
oping countries, there are nearly 1.1 million deaths worldwide due to bacillary dysentery (Kotloff et al., 1999).

Gram-negative strains form a significant group of organisms which are responsible for dysentery and other GIT disorders. Escherichia coli strains usually don’t cause any harm, but they can cause pathogenic damage both in immune-compromised and debilitated individuals. Diarrhoeal strains have evolved through the accession and transfer of specific characteristics persisted in the host (Nataro and Kaper, 1998). Diarrhoeal disease is considered to be the second leading cause of death in children under five years of age, and also it is responsible for killing around 5,25,000 children every year. Globally, every year, there are nearly 1.7 billion cases of childhood diarrhoeal disease. Also, it is a leading cause of malnutrition in children under five years of age.

There are about three clinical types of diarrhoea:
1. Acute watery diarrhoea: It lasts for several hours or days, and it includes cholaera.
2. Acute bloody diarrhoea: It is also called as dysentery.
3. Persistent diarrhoea: It lasts for about 14 days or longer (WHO, 2019). The viral gastroenteritis infections can account for over 2,00,000 deaths of children per year worldwide, according to the Centers for Disease Control (Stuempfing and Seroy, 2020). The adherent invasive E.coli adhere to the intestinal epithelial cells and replicate intracellularly and thereby producing inflammation which can further bring about diseases like ulcerative colitis and Crohn’s disease (Palmela et al., 2018).

The transmission of Salmonella typhi is also through contaminated food and water, thereby invading the intestinal mucosa and interacting with the reticuloendothelial system and causing diseases (Das et al., 2014). These diseases are usually associated with diarrhoea, gastric irritation.

The critical feature of bacillary dysentery is frequent and painful diarrhoea, and it is associated with fever and abdominal pain. It can also occur through contamination of food (Wikipedia contributors, 2018). All the three bacterial strains are almost related and belong to Gram-negative bacterial strains. The mode of treatment against these bacteria is considerably tedious because the outer membrane of the bacteria acts as a barrier for the compounds. Currently, the drug of choice available to treat is the spectra of antibiotics such as derivatives of oligo-acetyl-lysy1, polymyxin and cationic steroid (Zabawa et al., 2016).

While screening the traditional literature, one of the compounds which could be useful against such bacterial infections might be Dadimashtaka choorna, and the āyurvedic review of this formulation reveals its uses in the management of diarrhoea, dysentery, indigestion and IBS (Narang and Herswani, 2018). It is a powder formulation which includes the ingredients such as Punica granatum, Bambusa bambos, Cinnamomum zeylanicum, Cinnamomum tamala, Elettaria cardamomum, Mesua ferrea, Trachyspermum ammi, Coriandrum sativum, Cuminum cyminum, Piper longum, Piper longum root, Zingiber officinale, black pepper and sugar (Singh, 2017).

The main ingredient in this formulation is Punica granatum, and compounds such as gallic acid, myricetin, cyanidin -3-glucoside, quercetin and pelargonidin -3-galactoside were isolated from the fruit juice extract. Among all the compounds gallic acid was found to show potent anti-bacterial activity against Staphylococcus, Streptococcus, Bacillus subtilis, Shigella species, Salmonella, Vibrio cholera and E.coli (Naz et al., 2007).

Various spices used in the formulation have also shown anti-bacterial activity. Cinnamomum zeylanicum is proven to manifest anti-bacterial activity. The essential oil of cinnamon and component such as cinnamaldehyde showed inhibition against E.coli, Staphylococcus aureus (Liu et al., 2017).

The anti-bacterial activity of Cuminum cyminum essential oil was proved against E.coli, S.aureus, B.cereus. The major isolated compounds from essential oils are cuminaldehyde, cymene and terpenoids The volatile compounds responsible for antimicrobial activity in Zingiber officinale were camphene, borneol and linalool. From the rhizome of ginger two compounds namely: [12]-gingerol and [10]-gingerol were isolated, and they were proven to possess anti-bacterial activity against gram-negative bacterial strains (Park et al., 2008). The oil of Coriandrum sativum exhibited bactericidal activity against Staphylococcus aureus, E.coli and Salmonella typhimurium (Silva et al., 2011). The essential oil of black pepper is proven to have shown intense anti-bacterial activity(Diameter values of Zone of inhibition >20mm) against E.coli (Zhang et al., 2017). The significant compounds isolated from essential oil are caryophyllene, limonene, alpha-pinene may be responsible for this activity (Menon et al., 2003). Various components of essential oil of Elettaria cardamomum such as 1,8 cineole, linalool, alpha-terpineol possessed anti-bacterial activity against E.coli, Bacillus cereus, Salmonella typhi and Staphylococcus aureus (Singh et al., 2008). Acetone and hot water seed extracts of
Trachyspermum ammi showed anti-bacterial activity against E. coli, Salmonella typhi, Salmonella typhimurium, Shigella flexne and Staphylococcus aureus (Kaur and Arora, 2009).

Cinnamomum tamala is said to exhibit at least 25-29mm of a zone of an inhibition against gram positive and gram-negative multidrug-resistant bacterial strains (Rath and Padhy, 2014). The flower extract of Mesua ferrea is said to exhibit anti-bacterial activity against Salmonella typhimurium (Mazumder et al., 2005).

Majority of the components of the formulation is proven to possess anti-bacterial property. So here an attempt is made to verify the anti-bacterial effect of the compound formulation against the bacterial strains such as E. coli, Salmonella typhi and Bacillus species.

MATERIALS AND METHODS

Aim
1. To check whether the concentrated extract of Dadimashtaka choorna has anti-bacterial activity against the following bacteria:
   - Salmonella typhi (gram -ve)
   - Escherichia coli (gram -ve)
   - Bacillus species (gram +ve)
2. To compare the potential anti-bacterial activity with the standard drug – Ciprofloxacin.

Preparation of Dadimashtaka choorna

Dadimashtaka choorna is one of the ideal preparation explained in the context of atisara chikitsa (treatment of gastrointestinal conditions such as diarrhoea) in the text of Astangahridaya. The preparation was made as per the classical norms of preparation, as mentioned in choorna kalpana (herbal powder preparation method) (Murthy, 2003). Table 1 enlists the ingredients of Dadimashtaka choorna, the polyherbal formulation.

The above ingredients were separately powdered and sieved. The resultant fine powders were mixed together and again sieved to obtain a uniform powder mixture. The final product was weighed, and properly stored in air tight container.

Preparation of extract

10g of the herbal powder was added with 100 ml of distilled water in a ground conical flask for 24 hours. It was shaken frequently during the first 6 hours and allowed to stand for 18 hours. It was then filtered rapidly, taking precaution against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow flask and dried at 105°C. The resultant was weighed. The percentage of water-soluble extractive regarding the herbal powder was calculated (Department of Ayush, 2008).

The concentrated filtrate thus obtained was then used as the sample for the study.

Materials Required

Nutrient Agar (Merck, Germany), Micropipettes, Autoclaved Pipette tips, Petri plates, Conical Flask, L-rod, Spirit, Bunsen burner, Ciprofloxacin (100mg/ml), Autoclaved MilliQ water, Discard beaker, Autoclaved microcentrifuge tubes.
Table 1: Ingredients of Dadimashtaka choorna, the polyherbal formulation

| S No. | Sanskrit names of drugs used | Botanical name                     |
|-------|-----------------------------|-----------------------------------|
| 1.    | Dadima                      | Punica granatum Linn.             |
| 2.    | Tawakshiri                  | Bambusa bambos Druce.             |
| 3.    | Twak                        | Cinnamomum zeylanicum Blume.      |
| 4.    | Ela                         | Elettaria cardamomum Maton.       |
| 5.    | Patra                       | Cinnamomum tamala Nees.           |
| 6.    | Nagakesara                  | Mesua ferrea Linn.                |
| 7.    | Ajamoda                     | Apium graveolens Pers.            |
| 8.    | Dhanyaka                    | Coriandrum sativum Linn.          |
| 9.    | Granthi                     | Piper longum Linn.                |
| 10.   | Shunti                      | Zingiber officinale Rosc.         |
| 11.   | Maricha                     | Piper nigrum Linn.                |
| 12.   | Pippali                     | Piper longum Linn.                |
| 13.   | Sita                        | Cane sugar                        |

Table 2: Diameter of zone of inhibition of concentrated filtrate of Dadimashtaka choorna against various bacterial strains

| Organism    | Concentrate filtrate(test drug)(mm) | Ciprofloxacin(mm) |
|-------------|--------------------------------------|-------------------|
| Salmonella typhi | Nil                                  | 45                |
| E. coli     | Nil                                  | 45                |
| Bacillus    | 30                                   | 53                |

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**Place of study**

The anti-bacterial study was conducted at Amrita Centre For Advanced Research in Ayurveda (ACARA), Amrita School Of Ayurveda Campus, Kollam.

**Anti-bacterial study**

The in vitro anti-bacterial activity was assayed by using agar well diffusion method.

The Nutrient Agar Media plates were prepared and inoculated with the test microorganism (bacteria) by spread plate method.

Agar wells of approximately 10mm in diameter were made and filled by adding the sample under test to the concerning wells.

Each well was made in such a way that the anti-bacterial zone of the drug added was easily visible in the agar surface and distributed evenly so that they were no closer than 24 mm from each other, centre to centre.

In each sample test, one well was consigned to a known sensitive antibiotic (positive control, here ciprofloxacin), which will show a zone after incubation.
tion and was then incubated at 37°C. After 24 hours of incubation, all plates under test were examined.

The resulting zones of inhibition were uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition, including the diameter of the control zone were measured.

RESULTS AND DISCUSSION

This study was undertaken to evaluate the antibacterial activity of Dadimashtaka choorna extract against the selective pathogenic agents for gastrointestinal disorders viz. Salmonella typhi (gram -ve), Escherichia coli (gram -ve), and Bacillus species (gram +ve), using agar well diffusion method. Table 2 shows the diameter of zone of inhibition of concentrated filtrate of Dadimashtaka choorna against various bacterial strains.

The following images shows the antibacterial study report of the samples against different bacteria. The Figure 1, Figure 2, Figure 3 shows the antibacterial study reports of concentrated filtrate against Salmonella typhi, Escherichia coli and Bacillus species respectively.

The above data indicates that the concentrated extract of Dadimashtaka choorna has an antibacterial effect against gram +ve strain of bacteria, Bacillus. The bacterial strains can be divided into gram +ve and gram –ve formed on the existence of the outer membrane and the thickness of a layer called peptidoglycan. The above selected bacterial strains that are E.coli and Salmonella typhi comes under the gram –ve strains and those of Bacillus is included under gram +ve class of bacteria. These gram +ve strains do not contain the lipopolysaccharide outer membrane like that of gram –ve strains, instead they contain teichoic acids and lipoteichoic acids. The thickness of the peptidoglycan layer is also more in Gram +ve strains compared to other groups owing to its serotype and attachment roles. Even though the thickness varies both the strains of bacteria have a similar structure (Auer and Weibel, 2017). Compared to +ve strains, –ve strains possess an extra covering of membrane, making it more resistant.

Considering the –ve strains it is established that the lipoteichoic acids are responsible for cell division and these are attached to the peptidoglycan layer, so the blockage of synthesis of peptidoglycan layer makes the cells liable to osmotic lysis. So the components which can block this synthesis may act as anti-bacterial agents (Schirner et al., 2009).

One of the main ingredients of this formulation is Punica granatum. By using various analytical techniques, it has been identified that the pericarp possesses multiple constituents such as flavonoids like quercetin, other compounds like ellagitannins, proanthocyanidin compounds and minerals like calcium, potassium, sodium and magnesium (Al-Huqail et al., 2018). The synergetic activity of quercetin as an inhibitor on the synthesis of peptidoglycan and on damaging the cell membrane has been established (Siriwong et al., 2015), validating its role as anti-bacterial agents. Other phenolic compounds present were gallic acid, and due to their lipophilic action, they inhibit the electron transfer chain of the plasma membrane and thereby exhibiting anti-bacterial property (Kubo et al., 2004).

The hydrophobic nature of phenolic compounds present in the formulation can be explained in such a way that these compounds are reported to hinder the generation of spore due to the interaction of hydrophobic region and compounds which are closely associated with L-alanine receptor site on the spore, thereby preventing further germination (Yasuda-Yasaki et al., 1978).

The gram-positive bacterial cell wall has a structure which easily allows the penetration of hydrophobic molecules. Essential oils present in the drug contain phenolic compounds, and owing to the hydrophobic property of phenolic compounds, it possesses anti-bacterial property against gram positive bacterial strains (Nazzaro et al., 2013). So, in general, we can say that the components with essential oil may contribute to the anti-bacterial property. A component named Trans –cinnamaldehyde(TC) has been isolated from cinnamomi cortex which shows antibacterial property with an acrolein group which is vital for this property (Bae et al., 1992).TC contains a phenol group and shows hydrophobic interaction and also constitute a small part of essential oils (Hyldgaard et al., 2012). The phenolic containing compounds can pass through the bacterial cell wall possessing phospholipid bilayer and harm the regular functions (Juven et al., 1994).

The essential oil constituents isolated from Trachyspermum ammi are thymol, beta-cymene and eugenol and the accumulation of these constituents in the cytoplasmic membrane resulted in the loss of integrity of the membrane attributing to an increase in extracellular and decrease in intracellular ATP pool of the membrane. As a result, there occurred ATP leakage, proving its anti-bacterial effectiveness (Dubey et al., 2011).

The essential oil constituent of Cuminum cyminum namely cuminaldehyde is shown to exhibit mild
cytoplasmic changes in the Bacillus species (Pajohi et al., 2011) so thereby it may cause alteration on the membrane and cause harm to the bacteria. Coriandrum sativum also possess essential oils such as linalool and decanol, which destroy the cytoplasmic membrane leading to its bactericidal property (De et al., 1999).

The formulation undertaken for the study consists of two piperine containing drugs. Piperine is an alkaloid which can enhance the bioavailability of other drugs. (Shoba et al., 1998). Reports suggest that piperine can improve the amino acid metabolism, which further increase the activation of mammalian target of rapamycin complex 1 (mTORC1) (Pan et al., 2015). For the proper response of amino acid, mTORC1 is a significant contributing factor; as it is necessary for detecting the sufficiency of nutrition and regulates various metabolic process (Hara et al., 1998). The activation of mTORC1 intensifies the ability of macrophages such as its phagocytic property which further augmented bacterial dispensation implicating the destructive nature of piperine on bacteria.

The critical component isolated from Zingiber Officinalis is [10]-Gingerol (13). Gingerol is reported to have the capacity to damage the cell membrane due to its hydrophobic property and hence may act as a bactericidal agent (Nagoshi et al., 2006). Terpenoids are phenolic components present in Elettaria cardamomum which possess hydroxyl group and delocalised electrons which interact with the bacterial membrane and impart anti-bacterial activity. Such a structure with delocalised electrons and hydroxyl group helps in proton exchange and thereby diminishing the cytoplasmic membrane gradient which eventually leads to decrease in ATP pool owing to the lysis of cell (Arfa et al., 2006).

The polarity of the solvent used in the extraction also plays a vital role in its anti-bacterial activity. The non-polar solvents were found more efficacious than polar solvents while analysing the anti-bacterial property of Messua ferrea extracts (Chanda et al., 2013).

Most of the ingredients present in the formulation have specific bactericidal activity ascribing to the presence of various phenolic components and essential oil present in the drug. The probable antibacterial activity may be due to enzyme inhibition or interaction with various components of the cell membrane, which can be attributed only against Gram-positive bacteria rather than to the negative strains. The disability of components to act against Gram-negative strains may be due to the presence of extra outer membrane and a peptidoglycan layer which provides antagonist action against the formulation.

The other critical gram-positive strains include streptococcus and staphylococcus species. The enterotoxins produced by staphylococcus strains cause severe symptoms like diarrhoea, vomiting etc. because these enterotoxins can easily pass through the stomach and cause Gastrointestinal symptoms (Boyce and Havill, 2005). By considering the probable mode of action, Dadimashtaka choornan can be a drug of choice for all infections caused by Gram-positive strains either for controlling or preventing the infection.

CONCLUSION

The results obtained in this study is suggestive of the fact that the concentrated extract of dadimashtaka choorna showed significant anti-bacterial activity against Bacillus species (gram +ve), among the three strains of bacteria selected for the study viz., Salmonella typhi (gram –ve), Escherichia coli (gram –ve) and Bacillus species (gram +ve), which serves as the causative organisms for gastrointestinal disorders. The study confirmed the anti-bacterial potential of the polyherbal powder formulation Dadimashtaka choorna against Gram-positive strains, which supports the fact that the compound formulation is a promising remedy to curb the gastrointestinal disorders caused by these bacterial strains, highlighting the specificity of the choorna (powder formulation) on specific strains. Since the study showed significant anti-bacterial activity against Gram-positive strain of bacteria rather than Gram-negative strains, further studies can be conducted on different Gram-positive strains, to get a broader view of the activity of the medicament. Also, extract prepared with a different solvent system can be analysed, to access the anti-bacterial activity.

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Conflict of Interest

The authors would like to declare that there are no conflicts of interest.

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