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Research Article

Evaluation of the Herbal Extracts as Potential Prebiotics for the Growth of Probiotic Microorganisms

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Abstract

Probiotics are beneficial microorganisms that maintain and regulate bacterial balance within the digestive system, while prebiotics are non-digestible oligosaccharides that selectively stimulate these beneficial microbes. This study aimed to evaluate the efficacy of seven medicinal plant extracts as prebiotics for enhancing the growth of probiotic strains, including *Lactiplantibacillus plantarum* MYSDV2, *Lacticaseibacillus rhamnosus* ATCC 53103, and *Saccharomyces boulardii* CNCM I-1079. The herbal extracts were from flowers of *Hibiscus rosasinensis*, *Woodfordia fruticosa* L., *Rosa canina* L. and *Punica granatm*, Bark of *Mangifera indica*, Stem of *Bambusa vulgaris* and leaves of *Camellia sinensis*. The polyphenol content of these extracts were ranged between 43.2% and 58%. A culture medium supplemented with these extracts was used to assess the growth rates of the probiotic microorganisms. Amongst of all extracts tested, the hibiscus flower extract demonstrated the highest growth-enhancing effect, increasing the growth of *L. rhamnosus* by up to 126.45%, and e also promoting the growth of *L. plantarum* and *S. boulardii* by 75.52% and 70.14%, respectively. An *Escherichia coli* inhibition assay using a co-culture method revealed that *L. rhamnosus* and *L. plantarum* significantly inhibited the growth of *E. coli* in the presence of aqueous Hibiscus flower extract. These findings indicate that hibiscus flower extract is a promising prebiotic that can enhance probiotic growth and inhibit pathogenic bacteria like *E. coli*. However, further clinical studies are necessary to confirm these effects in human subjects, providing deeper insights into the therapeutic applicability of this extract.

Keywords: *Lactiplantibacillus plantarum*, *Lacticaseibacillus rhamnosus*, *Saccharomyces boulardii*, polyphenol, plant extracts.

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1. Introduction

Microbiota interactions with human health have become a most promising research field for three decades. Multiple *in-vitro* tests, clinical trials, and animal studies have contributed to determining the mystery of nutrition,

medication, and gut microbiota interactions (Kumar et al., 2019; Cardona et al., 2013). This is important because many health-related diseases, including colorectal cancer, obesity, diabetes, and inflammatory bowel diseases, have been coupled with a disproportion

in the composition of gut microbiota, i.e., dysbiosis (Anhe et al., 2015).

In recent years, in addition to these studies on gut microbiota and probiotics, there has been growing interest in using polyphenol-enriched extracts as potential prebiotics to modulate gut microbiota composition and promote gut health (Plamada and Vodnar, 2021). It comprises a novel group of prebiotics as defined by the “International Scientific Association for Probiotics and Prebiotics” (ISAPP) (Milenkovic et al., 2021). The polyphenols' interaction with gut microbes is promoted by interfering with the cell function of the cell membrane and also its metabolism (Catalkaya et al., 2020). In addition, polyphenols also inhibit the biofilm formation of pathogens by hindering quorum sensing and helicase activities (Barbieri et al., 2017; Morais et al., 2016). Many of these polyphenols such as procyanidins, anthocyanidins, flavanones and flavonols, have low bioavailability in the digestive tract, reaching the colon in unaltered form (Poschner et al., 2019; Shen et al., 2016). These substances stimulate, modulate, and release metabolites in a reciprocal relationship with the intestinal microorganisms in the colon (Espin et al., 2017). Moreover, polyphenols can help the body to use “short-chain fatty acids” (SCFAs) more capably, which can additionally support the health of the gut (Nash et al., 2018).

Although many medicinal plants have been used for the treatment of gastrointestinal disorders, the impact of polyphenol-rich extracts from therapeutic plants on the growth of gut microbiota has not been explored (Farhat et al., 2017; George et al., 2019). So, the present study evaluated the potential impact of seven polyphenols-rich medicinal plant-based extracts on the growth of beneficial gut bacteria.

2. MATERIALS AND METHODS

2.1. Plant Materials and Media

Flowers from *Hibiscus rosasinensis*, *Woodfordia fruticosa* L. (Dhataki), *Rosa canina* L. (rosehip) and *Punica granatum* (pomegranate). Bark from *Magnifera indica* (mango). Stem from *Bambusa vulgaris* (Bamboo) and leaves from *Camelia sinensis* (green tea) were collected from Ess Kay Herbs, Bangalore, India. Plants were identified by Dr. Syed Noorie, Senior Botanist, FRLHT, Bengaluru, India. Growth medium “De Man Rogosa and Sharpe” (MRS) and Yeast peptone dextrose broth (YPD) were purchased from “Hi Media Laboratories Pvt. Ltd.”, India. Other chemicals were purchased from SD Fine Chemicals, Pvt Ltd, Bangalore, India.

2.2. Probiotic Strains and Culture Conditions

The probiotic strain *Lactiplantibacillus plantarum* MYSDV2 was obtained from the “Applied Mycology Laboratory, Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore”. Yeast strain *Saccharomyces boulardii* CNCM I-1079S was obtained from La Renon Health Care Private Ltd. India and *Lactocaseibacillus rhamnosus* ATCC 53103 were obtained from “American type culture collection”

(ATCC). The probiotic bacteria (*Lactobacillus*) were grown overnight in an anaerobic chamber at 37°C for 24 h in MRS broth, while *S. boulardii* was grown in Yeast Peptone dextrose broth at 37°C for 24 h.

2.3. Preparation of extract: Seven plant extracts were prepared by placing 500 g of dried plant part in a round bottom flask and soaked in 2500 ml of water for 2 hours at 70-80°C. After 2 hours of extraction, the miscella was filtered via a filtration system fitted with Whatman No 1 filter paper. The collected filtrate was concentrated at 70 °C under reduced vapour pressure (680 mm Hg), and the free-flowing powder was stored at room temperature in an airtight container for phytochemical analysis.

2.4. Determination of Total Polyphenol Content

2.4.1. Qualitative analysis of total polyphenol: Qualitative phytochemical screening of the aqueous extracts was performed to analyse the presence of polyphenols. The preliminary screening of the selected herbal extracts was analysed by standard chemical tests as described by Patil and Murthy (2020).

2.4.2. Quantitative determination of total phenolic content: It was measured at 765 nm using the “Folin-Ciocalteu method” (Singleton and Rossi, 1965) and expressed as “gallic acid equivalent (GAE)/mg” ethanol extract using the formula.

2.5. Probiotic Activity

2.5.1. Screening

MRS (HiMedia) media was used for the growth of *L. rhamnosus* ATCC 53103, *L. plantarum* MYSDV2 and *S. boulardii* CNCM I-1079. The ability of *L. rhamnosus* and *S. boulardii* isolates to survive in prebiotics was investigated as described by Milutinović et al. (2021). The growth survivability of bacteria and yeast on basal MRS and YPD media, MRS media without carbohydrates, and with 1% prebiotics such as plant extracts (mango bark, hibiscus extract, dhataki extract, rosehip extract, pomegranate extract, green tea extract, bamboo extract) were determined. The media without prebiotics and sugar were taken as a positive control, i.e., the growth of the bacteria on MRS media containing 2 % glucose was considered as control. Same media condition was followed along with *S. Boulardii* with YPD media. The criteria for selecting a probiotic isolate were growth on the prebiotic that resembled the growth on MRS media. The survival rate was expressed by the population of viable cells grown in Petri plates supplemented with different media.

2.5.2. Procedure for probiotic assay

For the probiotic activity assay, the *Lactobacilli* and *S. boulardii* culture broth were tested. Approximately 100-200 µl of the diluted culture was added to the basal media containing 1% prebiotic plant sample mixture. The samples were then incubated at 37°C for 24 hours for *Lactobacilli* and 48 hours for *S. boulardii*. The survival rate was expressed by the population of viable cells grown in Petri plates supplemented with different media. Percentage was calculated by using the formula:

Survival rate (%) = [Biomass in test plate (T)/Biomass in control plate (C)]x100.)

2.6 Inhibition of *E. coli* ATCC 25922 growth

The *in vitro* study was designed using Majeed et al., (2018) method to evaluate the inhibitory effect on the growth of Gram-negative pathogenic bacteria *E. coli* while growing on *Hibiscus* water extract. Trypticase soy agar (HiMedia) media was used to grow *E. coli* ATCC 25922. Three sets of experiment having *E. coli* ATCC 25922 with *Hibiscus* flower water extract (HWE), *L.*

rhamnosus + HWE and *L. plantarum* +HWE were incubated at 37 °C with 100 rpm for 24 h. Eosin methylene blue agar (EMB agar), was used to find viability of *E. coli* every 6 hrs interval, for 24 hours.

2.7. Statistical Analysis

The results were analyzed through One-way ANOVA (analysis of variance), and statistical analysis was performed. All the experiments were conducted in triplicates as independent runs.

3. RESULTS AND DISCUSSION

3.1 Polyphenol contents of seven medicinal plant extracts

Table 1: polyphenol content of plant extracts.

Serial Number	Extract Type	Raw Material (grams)	Yield (grams)	Yield (%) w/w	Polyphenol Content (%) w/w
1	<i>Rosa canina</i> L.	250	10	4%	43.2%
2	<i>Hibiscus rosasinensis</i>	250	25	10%	53.5%
3	<i>Mangifera indica</i> Extract	250	25	10%	57.5%
4	<i>Bambusa vulgaris</i>	250	10	4%	63.7%
5	<i>Camellia sinensis</i>	250	10	4%	58.0%
6	<i>Punica granatum</i>	250	27.5	11%	52.0%
7	<i>Woodfordia fruticosa</i> L.	250	37.5	15%	57.2%

The study noted that *Rosa canina* L. shows a low yield (4%) and polyphenol content (43.2%). *H. rosasinensis* has the highest yield (10%) and significant polyphenol content (53.5%). *Mangifera Indica* and *Punica granatum* also demonstrate high polyphenol contents of 57.5% and 52.0%, respectively, with moderate 10% and 4% yields. *Bambusa vulgaris* has a low yield (4%) but the highest polyphenol content (63.7%). *Camellia sinensis* and *Woodfordia fruticosa* L. provide balanced yields (4% and 15%) and high polyphenol contents (58.0% and 57.2%) (Table 1). This study found that polyphenol content can vary significantly across different medicinal plant species and even with in the same species depending on factors like genetic growth environment, harvest time and extraction methods. Gowd et al (2019) and Dias et al (2012).

3.2 Study of Prebiotics activity

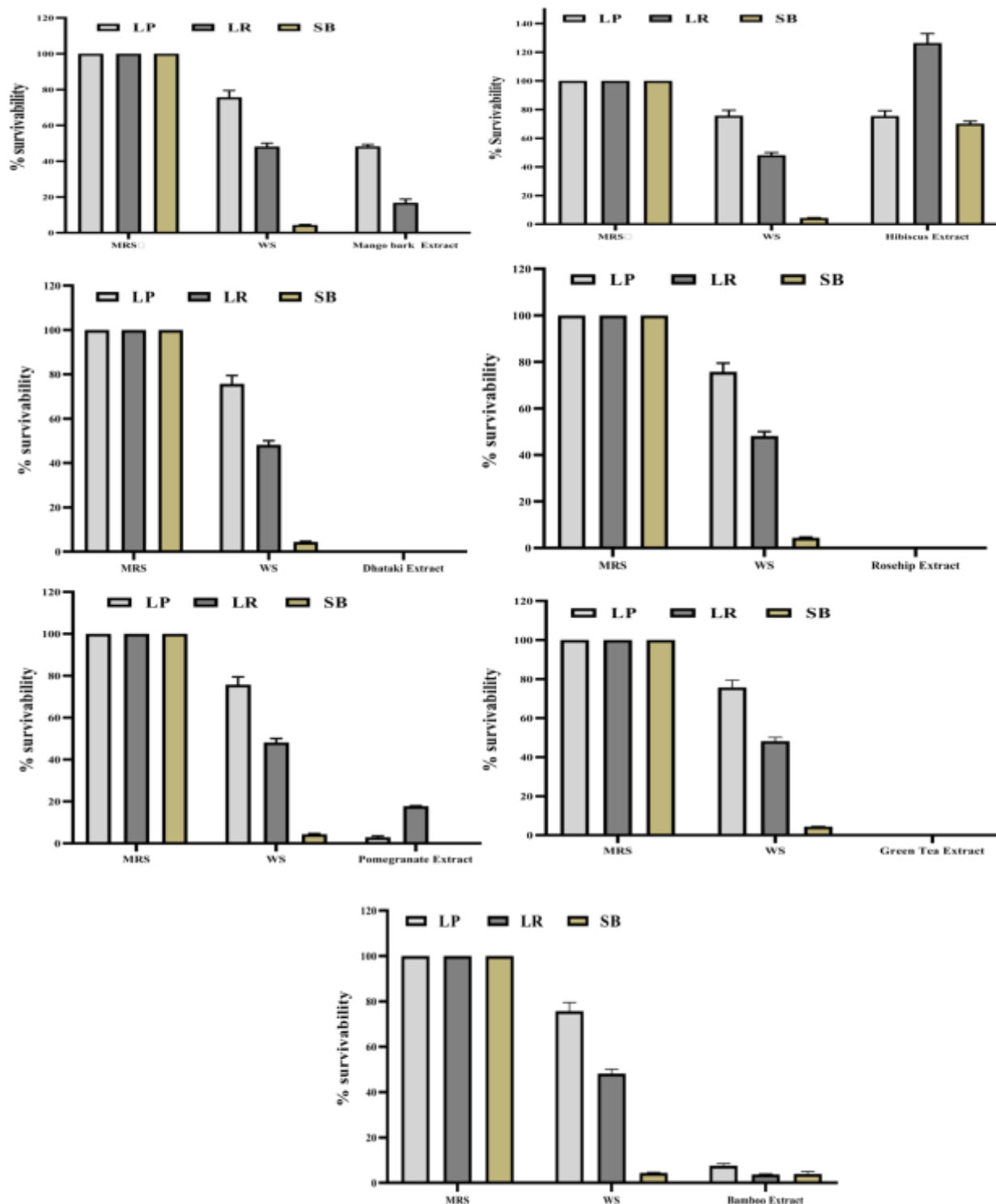
In the present study, among the seven plant extracts evaluated, the extract of *Hibiscus rosasinensis* demonstrated significant prebiotic activity (Fig 1), stimulating the growth of three probiotic strains: *Lactiplantibacillus plantarum* MYS2V2 (75.52%), *Saccharomyces boulardii* CNCM I-1079 (70.14%), and *Lactobacillus rhamnosus* ATCC 53103 (126.45%) (Fig 2-4). Similar findings were reported by Milutinovic et al. (2021), who also found that *Hibiscus rosasinensis* and

other herbal extracts were capable of enhancing the growth of probiotic strains. The polyphenolic compounds in *Hibiscus*, such as flavonoids and anthocyanins likely contribute to these prebiotic effects by providing selective substrates for probiotics, promoting their growth in a manner similar to other prebiotics like oligosaccharids. *S.boulardii* showed a 130-fold increase in growth at a 0.5 mg/ml concentration of a plant extract compared to the *Lactiplantibacillus plantarum* and *Lactobacillus rhamnosus* strains. In contrast, extracts such as mango bark inhibited the growth of *L. plantarum* and *L. rhamnosus* by 51.77% and 83.23%, while completely suppressing *S. boulardii*. Similarly, extracts from pomegranate and bamboo demonstrated substantial inhibitory effects on these probiotics, with inhibition rates reaching 97.1%, 82.25%, 100% and 92.4%, 96.31%, and 96.05%, respectively. All other extracts tested in the study completely inhibited probiotic growth compared to the control. Recent findings by Alexandra et al. (2022) further support these results, showing that *Hibiscus sabdariffa* extracts, particularly those rich in anthocyanins, can enhance the production of exopolysaccharides (EPS) in *Weissella confusa*, thereby improving probiotic efficacy. Additionally, red and white hibiscus extracts have been shown to reinforce the epithelial barrier, reduce inflammation, and modulate

tight junction proteins, indicating a promising prebiotic-like potential. The demonstrated capacity of *Hibiscus rosasinensis* to stimulate probiotic growth highlights its potential as a beneficial prebiotic source for gut health and probiotic support. The effectiveness of *Hibiscus rosasinensis* aligns with earlier findings by Dias et al. (2021) and Gowd et al. (2019), which indicated that

polyphenol-rich plant extracts can serve as prebiotic substrates, enhancing the growth of beneficial probiotic bacteria such as *Lactobacillaceae* and *Bifidobacteriaceae*. This effect is accompanied by increased production of secondary metabolites and the inhibition of pathogenic microorganisms.

Fig. 1: Prebiotic effects of seven plant extracts on probiotic strains in growth medium.



Note: LP- *L. plantarum*, LR- *L. rhamnosus*, SB- *S. boulardii*, MRS-MRS media, WS- MRS media without sugar

Fig 2: Growth of *Lactiplantibacillus plantarum* MYSDV2 in growth medium supplemented with different plant extracts.

***Lactiplantibacillus plantarum* MYSDV2**

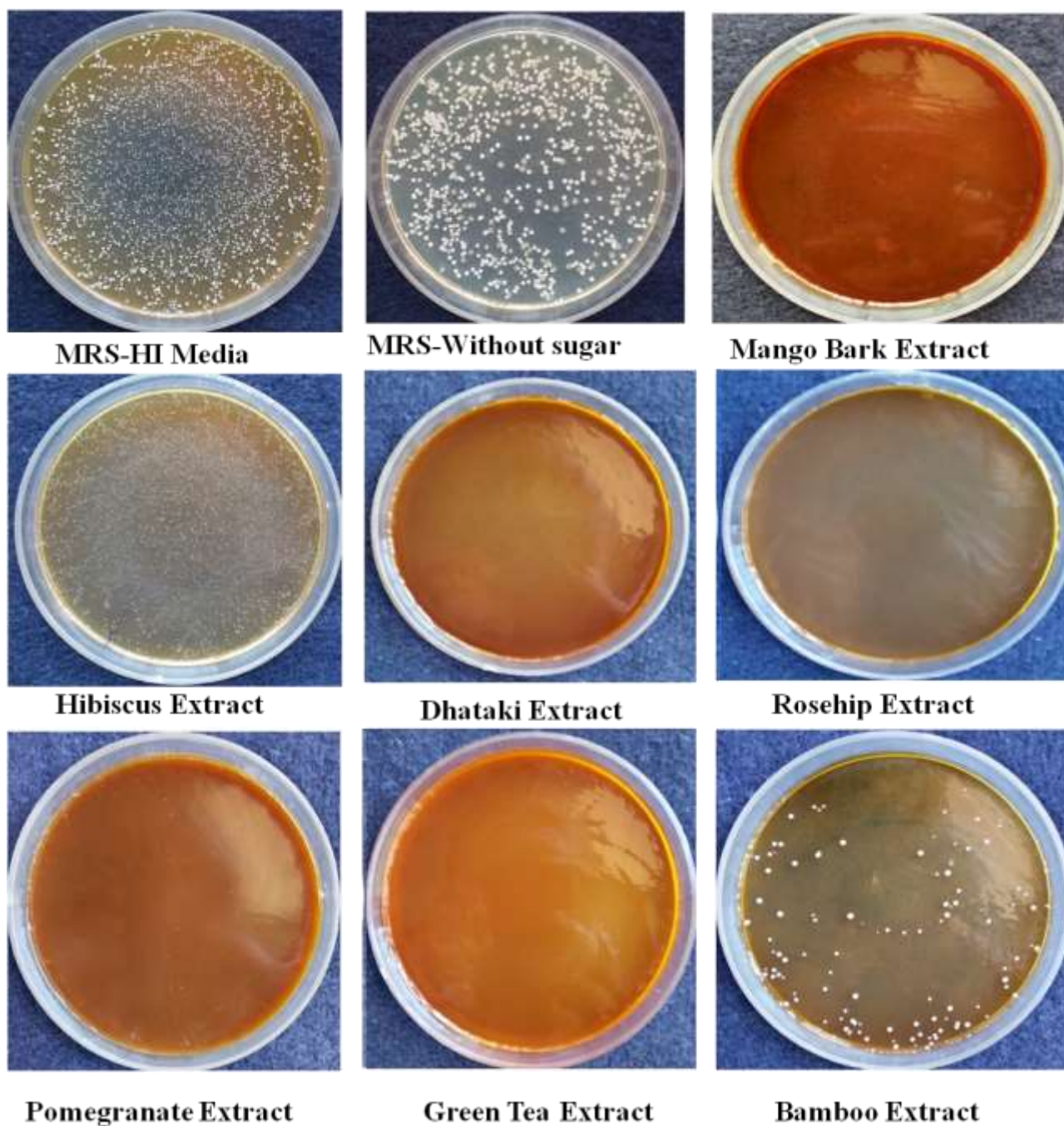


Fig. 3: Growth of *L. rhamnosus* ATCC 53103 in growth medium supplemented with different plant extracts.

Lacticaseibacillus rhamnosus ATCC 53103



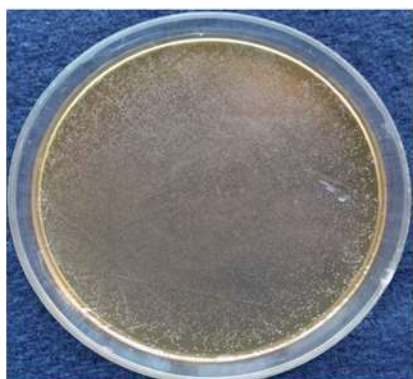
MRS-HI Media



MRS-Without sugar



Mango Bark Extract



Hibiscus Extract



Dhataki Extract



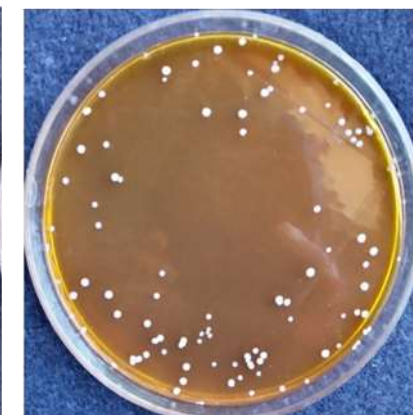
Rosehip Extract



Pomegranate Extract



Green Tea Extract



Bamboo Extract

Fig. 4: Growth of *S. boulardii* CNCM I-1079 in growth medium supplemented with different plant extracts.

***Saccharomyces boulardii* CNCM I-1079**



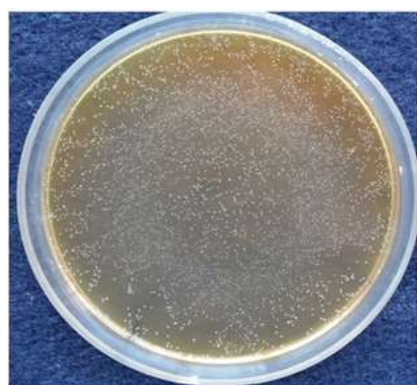
MRS-HI Media



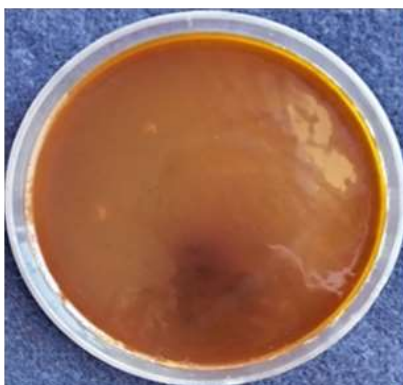
MRS-Without sugar



Mango Bark Extract



Hibiscus Extract



Dhataki Extract



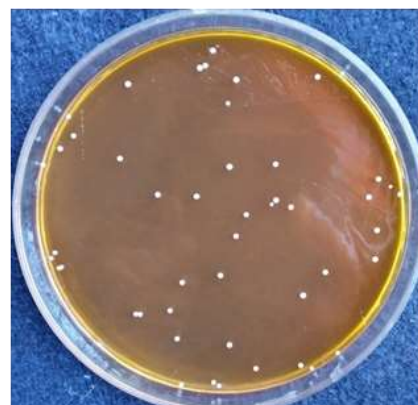
Rosehip Extract



Pomegranate Extract

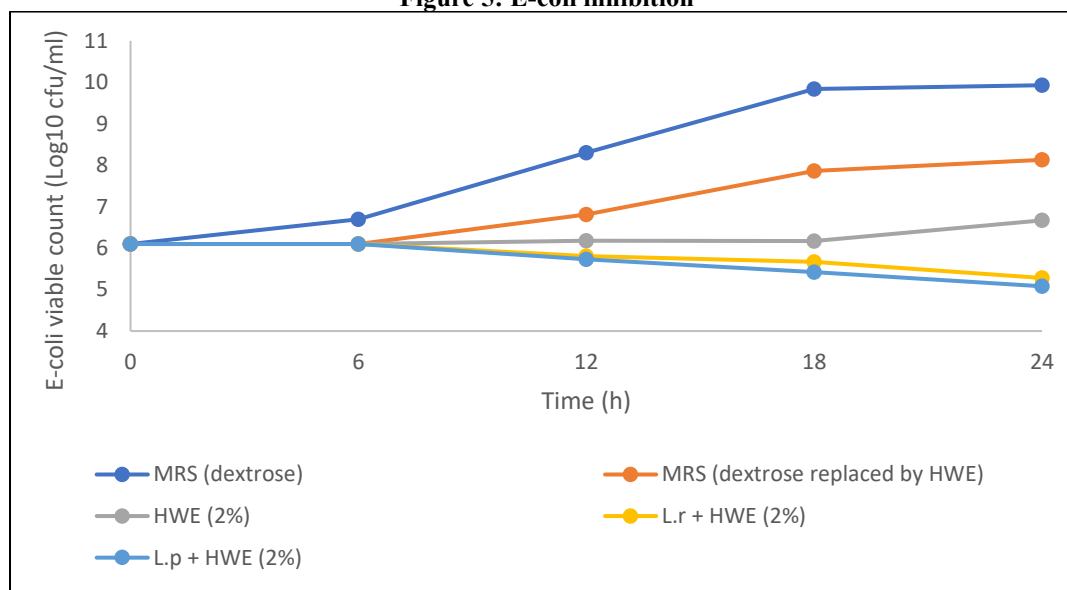


Green Tea Extract



Bamboo Extract

Figure 5: E-coli inhibition



3.3 Growth inhibition of *E. coli*

The effect of *L. rhamnosus* ATCC 53103 and *L. plantarum* MYSDV2 on *E. coli* ATCC 25922 growth was investigated in a coculture model when growing in HWE. A significant difference in *E. coli* count was noticed when cocultured with *L. rhamnosus* ATCC 53103 and *Lactiplantibacillus plantarum* MYSDV2 in the presence of HWE compared to MRS and HWE alone. This might be due to the effective disruption of *E. coli* biofilm formation through various mechanisms by prebiotic bacteria (Ahmed et al., 2018). This suggested that the HWE supported the growth of the probiotic strain *L. rhamnosus* ATCC 53103 and *Lactiplantibacillus plantarum* MYSDV2 when cocultured with *E. coli* ATCC 25922 (Fig. 5).

4. CONCLUSION

The present study noted the effect of seven polyphenol-rich medicinal herb extracts on the growth of probiotic microorganisms and noted *Hibiscus* flower extract as the most powerful prebiotic. We further studied the effectiveness of these probiotic microorganisms in inhibiting the growth of pathogenic *E. coli* and found them effective in the presence of *Hibiscus* flower water extracts. The results suggest that *Hibiscus* flower water extracts can improve the growth and development of intestinal microbiota members *L. plantarum* MYSDV2, *S. boulardii* CNCM I-1079, and *L. rhamnosus*. However, validation in preclinical studies supporting the prebiotic effect of *hibiscus* flower extract is required.

DECLARATIONS

Ethical Approval: This article contains no studies with human or animal subjects.

Conflict of Interest: The authors declare no competing interests.

The authors have no relevant financial or non-financial interests to disclose.

Funding: This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability: All data generated or analyzed during this study were included in this article. Sufficient data has been given in the form of tables and figures.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

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