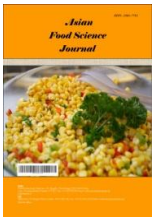

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Effects of *Garcinia mongostana, Lycium barbzarum, Momordica grosvenori*, and *Psidium guajava* on the Growth of *Lactobacillus* spp. and *Streptococcus thermophilus*

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Authors' contributions

This work was carried out in collaboration among all authors. Author EB designed the study, performed the statistical analysis and wrote the protocol. Author ABS wrote the first draft of the manuscript and managed the literature searches. Author ASB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

There are an increasing number of fermented beverage using herbal extract as a natural ingredient. The growth of bacteria in four plant water extract (*Lycium barbarum, Psidium guajava, Momordica grosvenori,* and *Garcinia mongostana*) as measured by the increase in turbidity of MRS and M17 growth medium containing *Lactobacillus* spp. and *Streptococcus thermophilus* respectively was investigated during 30 minutes of incubation at 37°C. The stimulatory effects on *Lactobacillus* spp. growth was tremendously enhanced ($p<0.05$) by *L. barbarum* (3.0%; OD=0.1.1) and *P. guajava* (1.5% (OD=0.5) and 3.0% (OD=0.6); respectively) compared to control (0%) after 30 minutes. In addition, inclusion of *P. guajava* (1.5%) shorted incubation time to reach plateau at 5 minutes. The inclusion of *M. grosvenori* and *G. mangostana* water extract at 0.30% increased *Lactobacillus* spp. growth by 2 and 14 fold respectively which higher than control. There was a

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dose response effect of all plant water extracts except for *M. grosvenori* on the growth of *S. thermophilus.* All the three concentrations of *L. barbarum* and *P. guajava* stimulated the growth of *S. thermophilus* which reach 3 fold higher than control at 1.5% *L. barbarum*. On the other hand, *M. grosvenori* water extract showed inhibitory effects on the growth of *S. thermophilus* at all doses but after the first 15 min at 3.0% the inhibitory effects were lost ($p<0.05$). *G. mangostana* water extract at 3.0% increased *S. thermophilus* growth 10 fold higher than control after 30 minutes. In conclusion, all plant water extract samples except *M. grosvenori* could be a good vehicle for carrying *Lactobacillus* ssp. and *S. thermophilus* while *M. grosvenori* could enhance the growth of *Lactobacillus* spp. but not *S. thermophilus.*

Keywords: Lactobacillus spp; S. thermophilus; plants extract; bacterial growth; optical density.

1. INTRODUCTION

Malaysia is one of the countries in Asia that is endowed with highly diverse biological resources. Natural phytochemical antioxidants, particularly in local fruits, have gained increasing interest among consumers and the scientific community [1]. This is because epidemiological studies have reported that frequent consumption of fruits is associated with a healthy lifestyle [2].

Momordica grosvenori or Luo Han Guo is cultivated for its fruit in the southern part of China and is used for the treatment of pharyngitis or pharyngeus pain, and antitussive medicine in China and Japan. The fruit is also consumed for its anti-inflammatory, antioxidant, anti-diabetic and nephroprotective properties [3,4].

The essential parts in *Psidium guajava* are the leaves which are used for medicinal and health care purposes [5]. The extract of leaves are used to cure gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, respiratory disease, as well an anti-inflammatory medicine [5,6].

Lycium barbarum belongs to the Solanaceae plant family. The red fruit of *L. barbarum* was used for thousands of years as traditional Chinese medicinal plant [7,8] with a wide variety of biological activities and pharmacological functions and play an important role in the prevention and treatment of various chronic diseases such as hyperlipidemia, diabetes, cancer, hypo-function immunity, hepatitis, thrombosis, and male infertility [8,9].

Garcinia mangostana fruit can be cultivated in tropical countries throughout Asia, e.g. Thailand, India, Malaysia, Vietnam and the Philippines. The white part of *G. mangostana* (aril) is edible portion of fruit that is soft and slightly have sour taste [10]. *G. mangostana* fruits are a rich source of phenolic acids, xanthones, anthocyanins, and condensed tannins i.e. proanthocyanidins [10,11].

Lactic acid bacteria (LAB) play an important role in fermented beverages. Fermented non-dairy beverages are produced using LAB (i.e. *Lactobacillus, Streptococcus*, *Leuconostoc*, and *Bifidobacterium*) and which leading to production of bioactive compounds with nutritional and therapeutic values [12,13]. Furthermore, some LAB possess probiotic features i.e. “living microorganisms, which on consumption in certain numbers exert health benefits beyond inherent basic nutrition” [13]. It is critical for LAB to be capable of growing increasingly in beverage and gives the final product its characteristic [14,15,16]. Therefore, it is important to inspect the differences of *G. mongostana, L. barbarum, M. grosvenori*, and *P. guajava* on the growth of *Lactobacillus* spp. and *S. thermophillus* during incubation at 37° C.

2. MATERIALS AND METHODS

2.1 Plant Materials

Four types of plant materials were used in the present studies. These were *Momordica grosvenori* (fruit), *Psidium guajava* (leaf), *Lycium barbarum* (fruit), and *Garcinia mangostana* (fruit). Dried fruits of *M. grosvenori* and *L. barbarum* were purchased from local Chinese medicinal shop. Partially dried *M. grosvenori* was subjected to further drying in the oven (50^0C) for 72 hrs. *Psidium guajava* leaves were harvested from a fruit orchard in Port Dickson, Negri Sembilan and these were initially washed clean of visible impurities followed by drying in the oven (50^0C) for 72 hrs. The dried *M. grosvenori* fruit and *P. guajava* leaves were ground to powder form. These were placed in airtight containers and stored at room temperature away from direct sunlight. *L. barbarum* (approximately 30g) was

meshed using mortar and pestle in the presence of double amount of distilled water in 60 ml to assist the formation of thick paste. The *L. barbarum* paste was then stored in the refrigerator (4° C) and used within 3 days. Fresh *G. mangostana* fruits were purchased from local fruit market. The fresh white pulp and the soft purple inner-skin were spooned outweighed and subsequently ground using blender in the presence of distilled water in ratio of 1: 1.

2.2 Preparation of Plant Water Extract

Plant suspension (6% w/v) was essentially prepared for using in optical density (OD) measurement. The materials from plants (leaves or fruits) were mixed in appropriate amount of water in ratio of 6: 100. The mixtures were incubated in water bath (70° C) overnight. The mixture was centrifuged (2000 rpm, 15 minutes, 4° C) and the clear supernatant obtained was used in the experiment.

2.3 Preparation of Bacteria Suspension

One milliliter of *S. thermophilus* or *Lactobacillus* spp. (*L. acidophilus* LA-5*, L. casei* LC-01, *L. delbruckii ssp bulgaricus*, *L. rhamnosus*) was initially diluted 10X by mixing in 9 ml sterile peptone water buffer *S. thermophilus* or *Lactobacillus* spp. was then cultivated on M17 and MRS enriched with lactose medium respectively [17]. Four types of plant extract at different concentration (0.75%, 1.5% and 3.0% w/v) was added to each broth and was then incubated at 37°C. Samples were poured into cuvette and then transferred to spectrophotometer for measuring optical density (OD) at 600nm, sample (0% plant extract) was used as control. OD was recorded every 5 minutes for 30 minutes to monitor the growth rate of bacteria.

2.4 Statistical Analysis

Assay was performed in triplicates and the results were expressed as mean ± S.E.M (standard mean error) values of the 3 batches (n=3). The statistical analysis was performed using one way analysis of variance (ANOVA, SPSS 19.0), followed by Duncan's post hoc test for mean comparison. The criterion for statistical significance was $p<0.05$.

3. RESULTS

3.1 Effects of Plant Water Extract on *Lactobacillus* spp. in MRS Growth Medium

The growth of bacteria in plant water extract as measured by the increase in turbidity of MRS growth medium containing *Lactobacillus* spp. are shown in Fig. 1. The growth of *Lactobacillus* spp. in the absence of plant water extracts increase with incubation time and generally began to plateau after about 10-20 minutes. The presence of plant water extracts had differential effects on the growth of *Lactobacillus* spp. Inclusion of *L. barbarum* at 0.75% or 1.5% had no effects whereas at 3.0% stimulated ($p<0.05$) the growth of *Lactobacillus* spp. (Fig. 1a). The growth of bacteria also began to plateau at much later time (i.e. after 30 mins) at 3.0% *L. barbarum* than that for control ($t_{plateau}$= 20 minutes; 0% *L. barbarum*). When *P. guajava* was added at 0.75% it stimulated the growth of *Lactobacillus* spp. (OD at plateau = 0.15, (Fig. 1b). The stimulatory effects on bacterial growth was tremendously enhanced ($p<0.05$) by 1.5% *P. guajava* inclusion (OD=0.5; T= 30 min) in addition to the shortening of time to reach plateau (5 mins). The addition of higher amount of *P.guajava* water extract (3.0%) also increased bacterial growth further (OD=0.6; T= 30 min; $p<0.05$) but plateau was reached at much later time (i.e. after 30 mins).

M. grosvenori water extract at 0.75% and 1.5% had little effect in stimulating the growth of *Lactobacillus* spp. ($OD_{plateau}$ = 0.07 and 0.10 respectively compared to control $OD_{plateau}$ = 0.06, Fig. 1c) at 30 minutes. *M. grosvenori* water extract at 0.30% increased bacterial growth 2 fold higher ($OD_{plateau}$ = 0.22; $p<0.05$) at 30 minutes. Similarly, *G. mangostana* water extract stimulated *Lactobacillus* spp. growth in the same manner as *M. grosvenori.* Inclusion of *G. mangostana* water extract at 0.75% had no effect on bacterial growth compared to control ($OD_{plateau}$ = 0.01, Fig. 1d) after 30 min. The addition of *G. mangostana* 1.5% increased the growth of *Lactobacillus* spp. by about $OD_{plateau}$ = 0.02 (T= 30 min). The inclusion of *G. mangostana* water extract at 3.0% increased bacterial growth 14 fold higher ($OD_{plateau}$ = 0.14; $p<0.05$) compared to control.

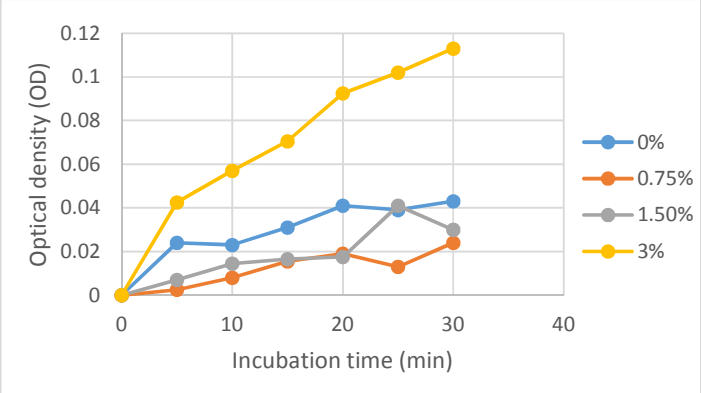

Fig. 1a. Effects of different concentration of *L. barbarum* on the changes in optical density (od) of *lactobacillus* spp. growing in MRS broth

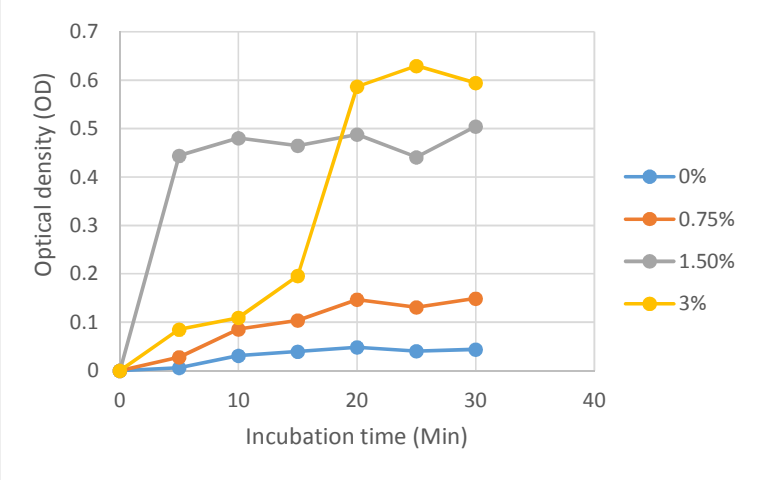

Fig. 1b. Effects of different concentration of *P. guajava* on the changes in optical density (OD) of *Lactobacillus* spp. growing in MRS broth

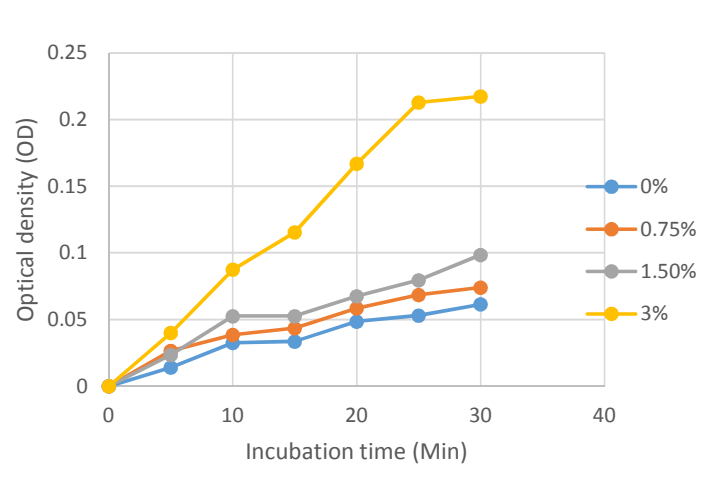

Fig. 1c. Effects of different concentration of *M. grosvenori* on the changes in optical density (OD) of *Lactobacillus* spp. growing in MRS broth

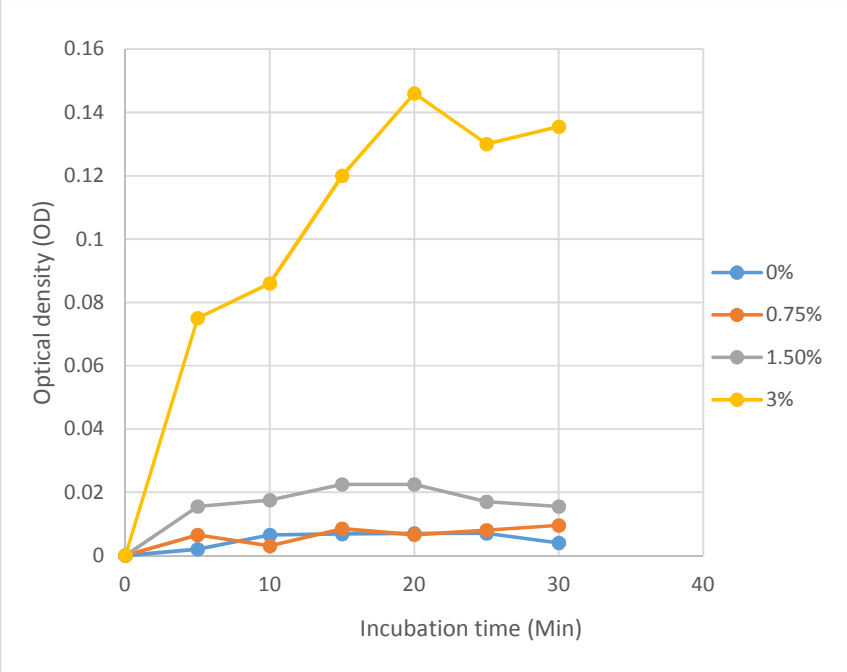

Fig. 1d. Effects of different concentration of *G. mangostana* on the changes in optical density (OD) of *Lactobacillus* spp. growing in MRS broth

3.2 Effects of Plant Water Extract on *S. thermophilus* Growth in M17 Growth Medium

The growth of *S. thermophilus* in the absence of plant water extracts increase with incubation time but plateau was not reached by the end of incubation (t=30 mins). Except for *M. grosvenori,* there was a dose response effects of plant water extracts for all plant studied on the growth of *S. thermophilus* (Fig. 2). Inclusion of *L. barbarum* at 0.75% and 3.0% stimulated the growth of *S. thermophilus* ($OD_{plateau}$ = 0.04 and 0.05 respectively compared to control $OD_{plateau}$ = 0.017 at the 30th minutes of incubation, (Fig. 2a). The inclusion of *L. barbarum* water extract at 1.5% did not only increase *S. thermophilus* growth by 3 fold higher ($OD_{plateau}$= 0.058; $p<0.05$) compared to control but also resulted in plateau in bacterial growth at t=10 mins. *P. guajava* water extract at 0.75% and 1.5% stimulated ($p<0.05$) the growth of *S. thermophilus* to similar extent ($OD_{plateau}$ = 0.24 and 0.21 respectively compared to control $OD_{plateau}$ = 0.10, (Fig. 2b) after 30 min of incubation. Increasing the water extract to 3.0% resulted in the highest OD achieved ($OD_{plateau}$ = 0.32; $p<0.05$) by the end of incubation. However, *M. grosvenori* water extract at 0.75 and 1.5% water extract inclusion showed inhibitory effects on the growth of *S. thermophilus* (Fig. 2c). The 3.0% water extract tested also showed inhibition but during the first 15 min, after which the inhibitory effects were lost. *G. mangostana* water extract stimulated *S. thermophilus* growth (Fig. 2d) in the same manner as *P. guajava.* Inclusion of *G. mangostana* water extract at 0.75% had a little effect of stimulating bacterial growth ($OD_{plateau}$ = 0.02) compared to control ($OD_{plateau}$ = 0.01) at 30 min. The addition of *G. mangostana* at 1.5% increased the growth of to $OD_{plateau}$ = 0.06 (T= 30 min). The inclusion of *G. mangostana* water extract at 3.0% increased *S.thermophilus* growth 10 fold higher ($OD_{plateau}$ = 0.10; $p<0.05$) compared to control after 30 min.

3.3 Comparison of *Lactobacillus* Spp. and *S. thermophilus* Density at 3% Plant Water Extract

The ratios of the effects of plant water extracts at 3.0% on OD of *Lactobacillus* spp. and *S. thermophilus* in relation to that of their respective controls with incubation time are presented in Fig. 3 and 4. Both *G. mangostana* and *L. barbarum* showed high OD ratios at t= 5 and 20-30 mins with a period of low OD ratios at t= 10-15 mins (Fig. 3). *M. grosvenori* and *L. barbarum* on the other hand showed consistent effects on *Lactobacillus* spp. growth with incubation time.

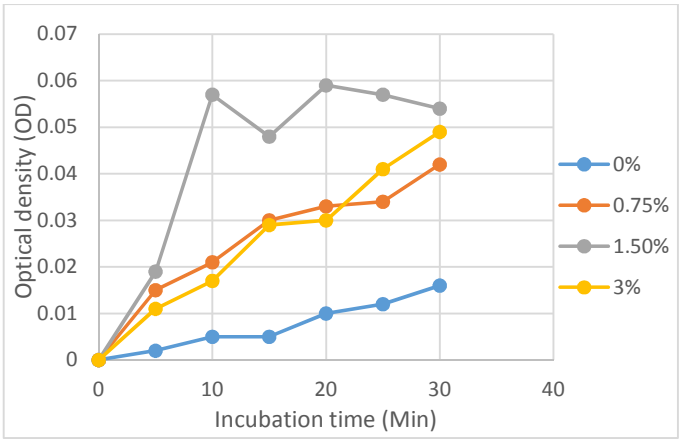

Fig. 2a. Effects of different concentration of *L. barbarum* on the changes in optical density (OD) of *S. thermophilus* growing in M17 broth

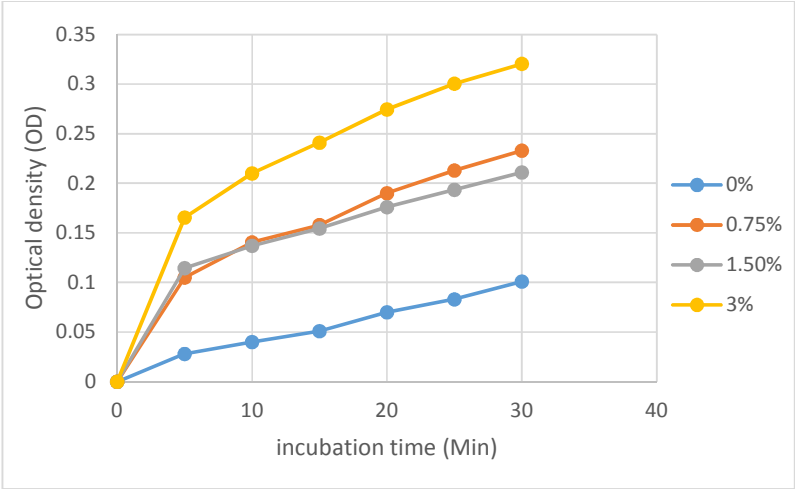

Fig. 2b. Effects of different concentration of *P. guajava* water extract on the changes in optical density (OD) of *S. thermophilus* growing in M17 broth

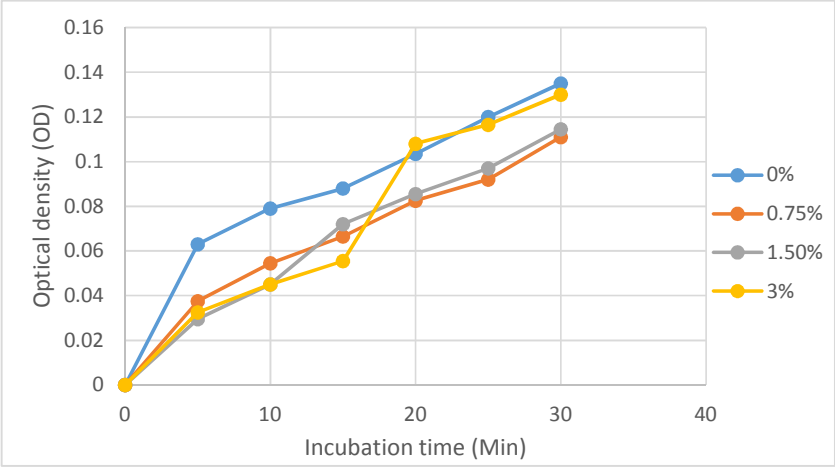

Fig. 2c. Effects of different concentration of *M. grosvenori* on the changes in optical density (OD) of *S. thermophilus* growing in M17 broth

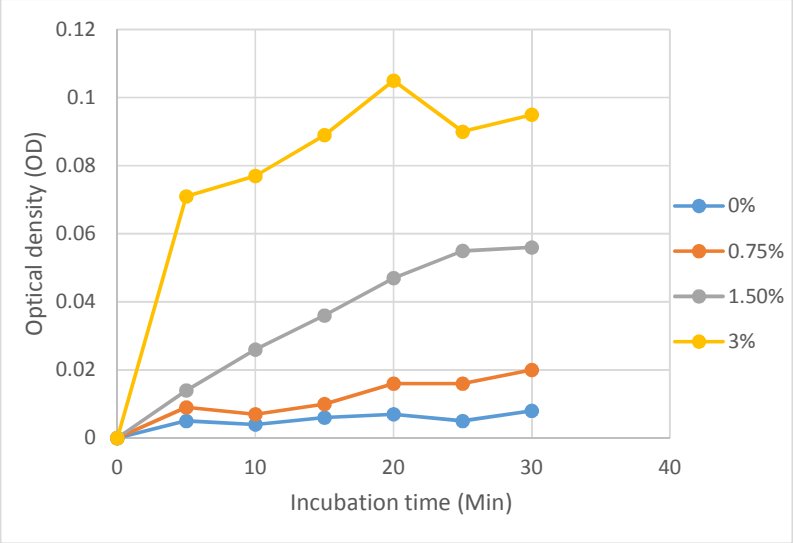

Fig. 2d. Effects of different concentration of *G. mangostana* on the changes in optical density (OD) of *S. thermophilus* growing in M17 broth

G. mangostana had highest OD ratio for *S. thermophilus* growth and this occurred at t = 10 and 25 mins of incubation (Fig. 4). Both *P. guajava* and *L. barbarum* had maximal OD ratio at t = 5 min but the ratio decreased with incubation time. *M. grosvenori* water extract showed the lowest OD ratio but it showed consistent increase in OD ratio with incubation time which reached its plateau at t = 20 mins (Fig. 4).

4. DISCUSSION

Optical density (OD), measured in a spectrophotometer, can be used as a measure of bacteria mass in a suspension. As visible light passes through a cell suspension the light is scattered. Greater scattering indicates that more bacteria or other material is present [18]. The amount of light scattered can be measured in a spectrophotometer. Typically mid log-phase of bacteria growth is measured by measuring absorbance at 600nm (OD600), but time course measurement of OD may also be used to estimate the rate of microbial growth in a medium suspension [18]. In the present study, the growth of *S. thermophilus* and *Lactobacillus* spp. in all the concentrations of the four plant water extracts was higher than control (0%). It can be assumed that plant water extracts enriched the growth medium which then enhanced the bacterial growth. Moreover, although most of the effects of plant water extracts on bacterial growth were dose-dependent for *Lactobacillus* spp. several showed inhibition of bacterial growth when a higher amount of plant water extracts were added into the growth medium. This was seen for the growth of *S. thermophilus* which increased during 30 minutes incubation at 1.5% but not at 3.0% for *L. barbarum* (Fig. 2a). Sustained inhibitory effects on the growth of *S. thermophilus* also occurred in the presence of *M. grosvenori* (Fig. 2c). The antibacterial activity of some phytochemicals commonly found in medicinal plants could have affected *S. thermophilus* growth [19]. The apparent inhibitory effects could be a methodological flaw associated with the limitation of OD measurement. This is because the sensitivity of this method is limited to about 10^7 cells per ml for most bacteria [20] which may be appropriate for *Lactobacillus* spp. but not for *S. thermophilus.* However, this method which compares dose-response effects on cell mass, allows quick appreciation of the growth of bacteria in the presence of potentially complex modulators present in plant water extracts.

Several factors contributed to the changes in OD during incubation and this includes pH and temperature [21], glucose [22], and metabolic stress factors (lactic acid, acetic acid, and hydrogen peroxide; [23,24]). Natural plant dyes were found directly affect the colorimetric absorbance [25]. In addition, the plant water extracts consist of different amounts of phytochemicals which may impact the growth and metabolism of *Lactobacillus* spp. and *S. thermophilus.*

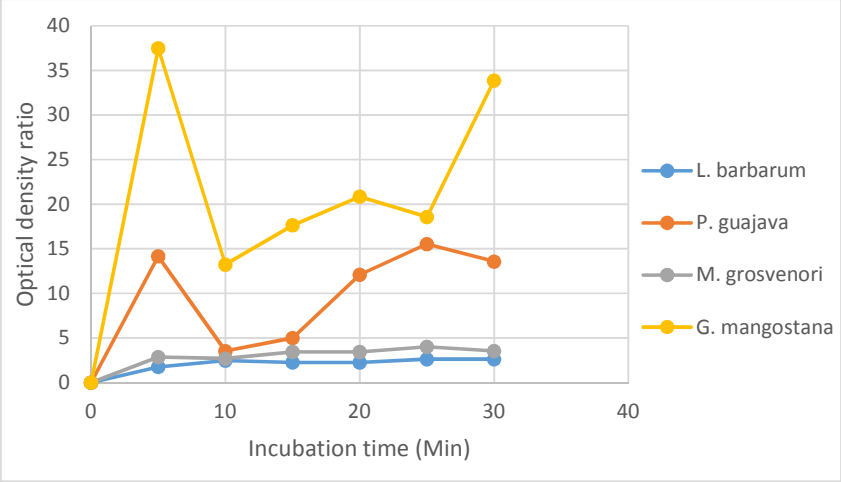

Fig. 3. Changes in the optical density (OD) ratio with time for *Lactobacillus* spp. grown in 3% plant water extracts in relation to their respective control

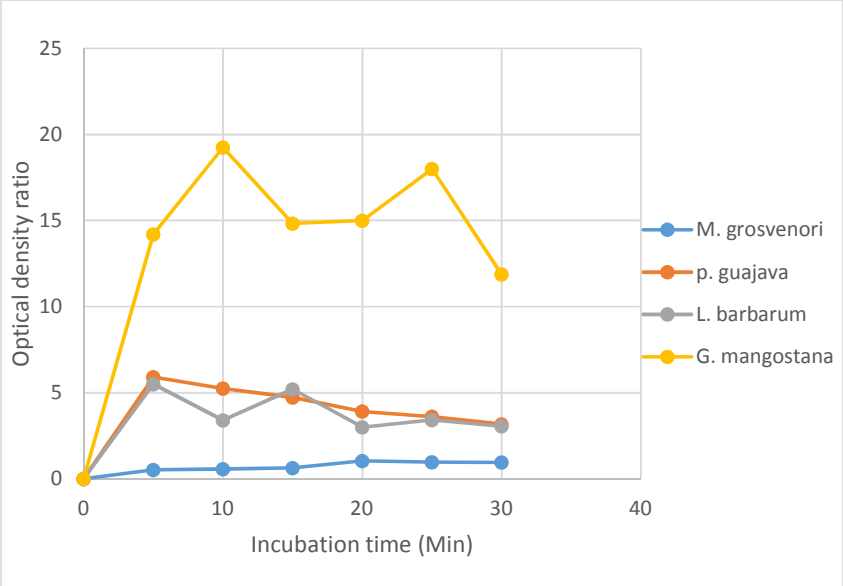

Fig. 4. Changes in the density (OD) ratio with time for *S. thermophilus* grown in 3% plant water extracts in relation to their respective control

5. CONCLUSION

The growth of *Lactobacillus* spp. and *S. thermophilus* in the absence of plant water extracts increase with incubation time. Generally, *Lactobacillus* spp. began to plateau after about 10-20 minutes whereas *S. thermophilus* plateau was not reached by the end of incubation (t=30 mins). The presence of plant water extracts had differential effects on the growth of *Lactobacillus* spp. and *S. thermophilus*. There was a dose response effects of all plant water extracts on the growth of *bacteria* except for *M. grosvenori* that showed inhibitory effects on *S. thermophilus* growth. However, the stimulatory effects on *Lactobacillus* spp. and *S. thermophilus* growth was tremendously enhanced by 1.5% *P. guajava* and 1.5% *L. barbarum* compared to their respective 3.0% dose. All plant water extract samples except *M. grosvenori* could be a good vehicle for carrying *Lactobacillus* ssp. and *S. thermophilus* while *M. grosvenori* could

enhanced the growth of *Lactobacillus spp.* but not *S. thermophilus.* Further study is needed to investigate the changes in pH level of the bacterial growth medium in the presence of these four plant extracts. In addition, the growth rate of *Lactobacillus* spp. in the presence of different plant extracts needs to be investigated during 24 hours.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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