A REVIEW: ANTIMICROBIAL ACTIVITY OF LEMON BALM

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ABSTRACT

Yeast and bacteria that create acetic acid and ferment it produces kombucha, a traditional beverage. The agar-well diffusion method was used to assess the antibacterial activity of lemon balm Kombucha and certain specific control samples. Antioxidant a stable activity Radicals from the lemon balm compound 1,1-diphenyl-2-picrylhydrazyl the spectroscopy of electron spin resonance was used to identify kombucha and lemon balm tea. A significant amount of acetic acid's antibacterial action against bacteria was also present in samples of Kombucha and heat denaturized Kombucha. The fight against yeasts and molds, however, was inactive. In all applied sample quantities, kombucha demonstrated more antioxidant activity than tea sample.

KEYWORDS*: antimicrobial activity, antioxidant activity, lemon balm, Kombucha*

INTRODUCTION: -

The fermented beverage kombucha has been consumed for thousands of years in the Today, the phrase "East and yet" is extremely popular in the West. It has been asserted that the beverage is a as a diuretic in cases of edemas, arterosclerosis, gout, sluggish bowels, stones, etc., it is a prophylactic agent and beneficial to human health [1-3]. Furthermore, experience shown that Kombucha tea regulates the intestinal flora, builds cells, balances metabolism, acts as a natural antibiotic, and helps the body's pH, such as the acid-alkaline balance [3]. Still, many of these assertions need to be supported by evidence. Black tea that has been sweetened with sugar is traditionally used to make kombucha. Camelia sinensis L. Typically, cellulose pellicle formed in this medium is used as an inoculant. popularly referred to as a tea fungus during the previous cultivation and incubated statically under aerobic conditions for 7–10 days [2-4]. This 'tea fungus' is a symbiotic relationship between acetic acid bacteria (Acetobacter). Gluconobacter oxidants, Acetobacter acetic, and oxylium) [5] and yeasts (Saccharomyces species, Torulosis sp., Pichia sp., Zygosaccharomyces sp., and Brettanomyces sp.) [2,6]. The fungi the cultivation medium to convert the sugar into ethanol, which is then further oxidized by the microorganisms convert acetic acid to acetic acid. The medium's pH is lowered as a result. the last It is a tart, barely carbonated, acidic beverage made from organic sugars. acids, tea-related substances, vitamins, and minerals, like cider. Alcohols, aldehydes, ketones, esters, and amino acids are only a few of the flavoring substances that have been identified [1,7,8]. The necessary nitrogen for the tea fungus is provided by the tea in the growing medium. Black tea contains a variety of substances, the most abundant of which are purine derivatives (caffeine and theophylline) [7]. Due to this, sweetened black tea has become popular.

conventional and essentially the only suggested method for making kombucha. Green tea has more antioxidants than certain other production methods, according to studies. Black tea has a less stimulating effect on the Kombucha fermentation and takes longer to produce the fermentation product [9]. Green tea's energizing impact on Kombucha. An increased caffeine concentration relative to black tea was used to explain culture [10]. Sweetened Echinacea purpurea L. tea can be obtained and used to brew Kombucha as well. beverage has excellent antioxidant qualities [11]. The effectiveness of Echinacea spp. The historic use of herbal remedies and dietary supplements as immunostimulants in treatment for viral and inflammatory disorders. Additionally, peppermint tea can be successfully used to make kombucha [12]. The results of our earlier research indicate that drinking lemon balm tea (Melissa officinalis L.) as a substitute method, produces Kombucha beverage in less time than in the case of stout tea [13]. In addition, the well-known herb lemon balm is used to scent various food and beverage items. Additionally, it has been employed as a plant remedy for treatment for rheumatism, gastrointestinal issues, anxiety, and headaches. The As a well-known antibacterial and antifungal agent, essential oil is also for the plant's mildly depressed and spasmolytic qualities [14]. Because of these effects, lemon balm tea was used as a medium for Kombucha fermentation in the present work.

METHODALOGY: -

Cultural conditions of the tea fungus

The tea fungus's environmental circumstances to create the substrate for Kombucha fermentation, 70 g/l of commercial adding 5 g/l of dry, crushed lemon balm leaves to boiling tap water after adding sucrose (Melissa the addition of officinalis L.). After 15 minutes, the tea leaves were extracted by filtration. once it has cooled to about 300 C, the inoculum (old-fashioned kombucha) A 10% (v/v) addition of process) was made. Afterwards, 0.33 l of the prepared medium was put into tiny flasks with a capacity of 0.72 l and incubated at 280 C° under aerobic conditions Using an electronic pH-meter,

Chemical Analysis: -

The pH of samples of fermented liquid was measured (HI 9321). After removing the CO2, samples of fermented beverages were potentiometrically titrated with NaOH, c = 0.1 mol/l, to evaluate their total acidity [15].

Sample: -

Following samples were evaluated for determination antibacterial activity:

- Kombucha beverage (total acidity: 4.56 g/l after three days of fermentation),

- an acetic acid solution with the same 4.56 g/l concentration as fermented tea,

- a sample of unfermented tea (5 g/l), a sample of neutralized kombucha (made by Kombucha beverage's pH being adjusted with 0.1 M NaOH).

- Kombucha that has been heated (100°C for 10 min.).

To remove cells, samples were run through a sterile 0.22 m microfilter. microbiological resistance

Test microorganisms

Microbes are tested. Gram-negative microorganisms: Pseudomonas aeruginosa (ATCC) Escherichia coli (ATCC 25922), Proteus mirabilis (ATCC 35859), Erwinia carotovora (NCPPB 595), and Gram positive bacteria Staphylococcus aureus (ATCC 25923), Sarcina lutea (ATCC 9341), Saccharomyces cerevisiae (112, Hefebank Weihenstephan), Candida pseudotropicalis, and Bacillus cereus (ATCC 10876) are examples of yeasts (clinical isolate), Rhodotorula genus Molds include Penicillium aurantiogriseum (natural isolate), and Aspergillus flavus (natural isolate), Aspergillus niger (natural isolate), and other aspergilli were employed. as research organisms 167.

ANTIMICROBIAL ACTIVITY: -

Agar-well diffusion was used to evaluate antimicrobial activity. method. The strains were raised on either Sabouraud Dextrose or Mueller-Hinton (bacteria). (Molds and Yeasts) slopes 24 hours at 37 or 25 0 C and had its purity verified. following incubation After being removed from the agar surface, the cells were suspended in sterile physiological solution. 1107 cells were counted in 1 ml of the inoculation suspension using a Mc Farland nefelometer cfu ml 1. This suspension was homogenized with 19 ml of melted (45 0) in a volume of 1 ml. C) Mueller-Hinton or Sabouraud Dextrose Agar and poured into Petri dishes. With a sterile metal tube, wells of 9 mm in diameter were created. utilizing a vacuum pump. Then, sterile samples (100 l) were placed in the wells of agar plates with test microorganisms injected. At 37°C, plates were incubated (bacteria) or 25°C for 24 hours (yeasts and molds), and the diameter of the halo zones was measured. The Three iterations of the antimicrobial activity evaluation of the samples were completed. In comparison to control samples, lemon balm Kombucha exhibits antibacterial activity.

RESULT AND DISCUSSION

The three-day fermentation process for the kombucha included the following conditions: Titratable acidity is 4.56 g/l and pH are 2.89 + 0.05. It was decided to end on the third day. the process since Kombucha's optimum consuming acidity (3.5-) was reached on that day. 4.5g/l) (16). (16). g/l) (16). (16). demonstrates that acetic acid solution, Kombucha, and heat denaturized Kombucha has powerful antibacterial properties. Determined was the antimicrobial activity. toward Sarcina lutea as well, but nothing happened. Acetic acid is the most powerful activity against all germs, however there aren't many distinctions between them (the halo zone's diameter is in comparison to the other samples, around 2-3 mm larger). Erwinia was visible in the biggest halo zones. the tiniest Bacillus cereus, and Carotovora. Only Escherichia coli exhibited bacteriostatic action in neutralized Kombucha (30 0.00 mm halo zone). Heat denaturing Kombucha was tested to see if the active antibacterial ingredients are still present. to determine whether the active ingredients are big proteins that are thermostable. Samples included examined yeasts (Saccharomyces cerevisiae, Candida pseudotropicalis, etc.) are not inhibited by it. Rhodotorula sp.) and molds (Aspergillus niger, Aspergillus flavus, and Penicillium aurantiogriseum), possibly because yeasts and molds are more abundant in acidophilic environments. organic acid resistant Therefore, potential

risk of contamination with Molds might develop when Kombucha is grown at home. Even though there are many Despite claims that tea's polyphenols and tannins can inhibit a wide range of Gram-positive and Gram-negative bacteria [17], unfermented tea did not exhibit any antibacterial activity in this investigation. antibacterial activity of any kind toward the test organisms. Most likely because of the tea broth's 0.5% concentration and the polyphenol/tannin level's extremely low concentration tea was not likely to hinder the growth of the test bacteria. Namely, the inhibitory effects of Kombucha increased with the content of tea, according to research by Greenwalt et al. (9) Previous research have examined the traditional Kombucha (made from black tea antibacterial)'s properties. tea) and comparison samples was made [18].

Antimicrobial activity of lemon balm Kombucha (diameter (mm) of the halo zone mean including well (9 mm) ± SD)

MICROORGANISM	Kombucha		Acetic acid C=4.56 g/l		Heat denaturated Kombucha	
	A	B	A	B	A	B
Salmonella enteritidis	13.85±0.54	28.12±1.2	17.23±0.36	Ø	15.25±0.32	28.67±0.89
Escherichia coli	13.67±1.54	30±0.0	16.67±0.58	Ø	14.4±0.89	30±0.0
Proteus mirabilis	15±1.0	Ø	17.75±0.96	Ø	17.0± 0.82	Ø
Pseudomonas aeruginosa	14.4±0.89	Ø	17.0±0.71	Ø	16±0.00	Ø
Staphylococcus Aureus	16.0±1.22	Ø	16.8± 2.17	Ø	15.8±1.64	Ø
Bacillus cereus	14.33±1.54	Ø	15.0±1.73	Ø	14.25±1.7	Ø
Erwinia carotovora	17.83±1.18	Ø	22.8±0.84	Ø	21.6±0.89	Ø

A – microbicidal activity; B – microbiostatic activity; Ø – no activity (growth inside the wells);
+/- -boundary antimicrobial activity (without growth inside and on brim of wells, zone about 9 mm)

Eight days were spent cultivating the Kombucha, which had a pH of 2.87 0.01 and was titratable.

pH = 3.55 0.03 g/l. The outcome is displayed in Table 2.

Table 2. Antimicrobial activity of traditional Kombucha (diameter of the halo $zone_{mean}$ (mm) including well (9 mm) ± SD)

Microorganism	Kombucha		Acetic acid c=3.55 g/l		Kombucha pH=7		Heat denaturated Kombucha
	A	B	A	B	A	B	A
Salmonella enteritidis	12.33±0.58	29±1.73	13±0.58	Ø	Ø	24.67±0.58	12.67±0.92
Escherichia coli	13.67±0.58	Ø	13±0.5	Ø	Ø	Ø	13.25±1.12
Proteus mirabilis	Ø	15.67±0.58	Ø	17.33±0.58	+/-	20±0.0	Ø
Pseudomonas aeruginosa	12±0.0	Ø	12±0.0	Ø	Ø	Ø	11.33±0.0
Staphylococcus aureus	12.33±0.58	Ø	Ø	14±0.0	Ø	Ø	13.2±0.63
Bacillus cereus	9.33±1.53	10.33±0.71	10.33±1.5	10.67±0.58	9.33±1.35	Ø	9.2±1.23
Erwinia carotovora	14.33±1.23	19.3±0.0	15.45±1.25	Ø	9.55±1.05	22.33±0.0	14.5 ±0.5
Penicillium aurantiogriseum	+/-	Ø	+/-	Ø	Ø	Ø	Ø

A– microbicidal activity; B – microbiostatic activity; Ø – no activity (growth inside the wells); +/- - boundary antimicrobial activity (without growth inside and on brim of wells, zone about 9 mm)

As shown in Table 2, acetic acid solution, heat-denaturized kombucha, and

The most noticeable antibacterial action is found in kombucha. Their actions are comparable. except for Staphylococcus aureus, where acetic acid solution is ineffective only be bacteriostatic, not bactericidal. While neutralized Kombucha (pH=7) only displays bacteriostatic action against Salmonella enteritidis and Proteus mirabilis, it has bactericidal activity toward Bacillus cereus and Erwinia carotovora. None of the samples have any ability to fight Sarcina lutea's microbes. Samples don't impede the growth of Rhodotorula species and yeasts (Saccharomyces cerevisiae, Candida pseudotropicalis, Except for Penicillium aurantiogriseum, where borderline microbicide activity of Kombucha and acetic acid was obtained, molds (Aspergillus niger and Aspergillus flavus) were resistant. Unfermented tea exhibits no antibacterial action when tested with test organisms. It is evident that lemon balm tea derived Kombucha has greater action toward all than Kombucha made with black tea. Kombucha made from lemon balm tea also appears. Proteus mirabilis was killed by bactericidal action, whereas Staphylococcus aureus was destroyed by an acetic acid solution.

Acetic acid might be presumed to be the main antibacterial agent in both situations. In Traditional Kombucha can occasionally be more active than acetic acid, which suggests the big proteins and the presence of an antibacterial substance other than acetic acid. in each in certain instances, there are no impacts on the yeasts and molds. Black tea derived Kombucha's antibacterial properties were identified by Greenwalt et al. Bactericidal effects on Staphylococcus aureus, Escherichia coli, and Bacillus sp. compared to our findings for lemon balm Kombucha, were significantly greater (3-5 times).

It most likely is since their Kombucha had an acetic acid concentration of 7 g/l, whereas lemon had a concentration of the overall acidity of balm Kombucha was 4.56 g/l, and it is well known that acetic acid is the primary anti-microbial substance If the black tea Kombucha's acetic acid content was 8.5 g/l, just We measured bacteriostatic activity [17]. When compared to lemon balm Kombucha Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus are more active lower in comparison to Bacillus sp. Using the ESR technique in the DPPH model system, the antioxidant activity of lemon balm tea and lemon balm Kombucha was examined. The steady DPPH ESR spectrum radicals produced in the presence of 100 l of lemon balm and DPPH radicals.

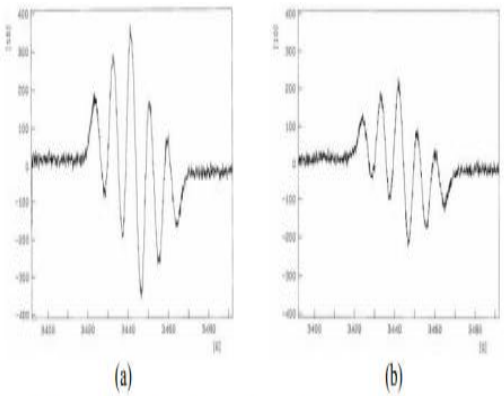

Fig. 1. ESR spectra of stable DPPH free radicals: a) blank and b) in the presence of 100 µl of lemon balm Kombucha.

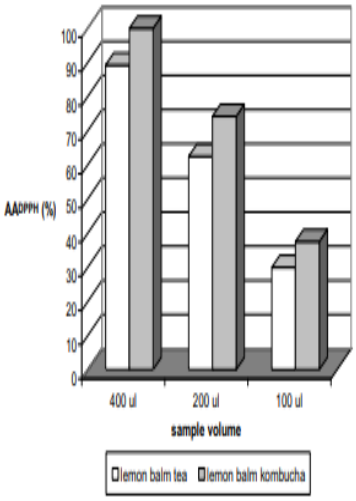

Fig. 2. Antioxidant activity of lemon balm tea and lemon balm Kombucha on stable DPPH radicals

With the growing sample size for Kombucha samples and tea samples, it is evident Additionally to volume, antioxidant activity is rising. Kombucha had stronger antioxidant activity than lemon balm tea, regardless of sample amount. Most likely because of some metabolic by-products produced by fermentation (such as vitamins C and B). Antioxidant properties of echinacea tea based Kombucha and their effects on DPPH radicals Control samples and black tea have both been determined earlier [19]. In the Using the identical sample volumes (100 l), echinacea tea and black tea Kombuchas have greater activity than lemon balm Kombucha (AADPPH is

roughly 80%). Samples of tea, nevertheless Compared to black tea, lemon balm tea was more active, but not as much as echinacea tea.

CONCLUSION

Samples of kombucha, acetic acid, and heat-denaturized kombucha revealed except for Sarcoma lutea, there was substantial antibacterial action against all microorganisms, although There was no activity to combat yeasts and molds. Kombucha neutralized and unfermented tea showed no antibacterial action when evaluated against test pathogens. samples of lemon balm tea and Kombucha samples demonstrated anti-DPPH radical antioxidant efficacy in all used volume samples. Samples of kombucha demonstrated stronger antioxidant activity than samples of tea.

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