Pak. j. sci. ind. res. Ser. B: biol. sci. 2023 **66B**(2) 184-187

Short Communication

Analysis of Antioxidant and Antibiotic Potentiality of the Extract from Molten Butter Residue

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(received July 27, 2020; revised March 17, 2021; accepted March 26, 2021)

Abstract. This research work was drafted to explore the potential of the extract from dairy waste "Molten butter residue" as a source of the antioxidant agent. The dairy waste extract was subjected to estimation of total phenolic content, total anti-oxidation activity, % of DPPH radical scavenging activity and antibiotic activity. It exhibits total phenolic content of 60.11 mg/mL with maximum total antioxidant activity and 99.39% of DPPH scavenging activity when compared to ascorbic acid as a standard. It also shows antibiotic activity against micro-organisms like *Bacillus, Streptococcus* and *Staphylococcus*. The antioxidant and antibiotic potentiality of the extract from molten butter residue may be applicable in pharmaceutical field.

Keywords: antibiotic activity, anti-oxidation activity, DPPH, dairy waste, molten butter residue, phenolic content.

Anti-oxidizing agents inhibit the oxidation of other molecules and not only improve health they are effective in preventing diseases. Antioxidants may be hydrophilic or hydrophobic, depending upon their solubility in water. Water soluble antioxidants could react with oxidants lying in the cell cytosol and the blood plasma. Many plants and animals naturally synthesize antioxidants in the body and some are obtained in the human body through diet.

It is also possible to rationalize the use of antioxidants in the pharmaceutical formulations (Celestino *et al*., 2012). Antioxidants are active prophylactic and therapeutic agents which function in scavenging free radicals and prevent the damage caused by them. Antioxidants have wide application as manufactured phenolic cancer prevention agents butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT] and propyl gallate have restrained activity on or by the scavenging of free radicals (Babagana *et al*., 2019).

The phenolic and other bio-active compounds extracted from dairy waste (Khanam and Prasuna, 2017) were also subjected to the tests for antioxidant and antibiotic activity in this article.

Total phenolic content. Total phenolic content was determined by using Folin-Ciocalteu reagent spectrophotometrically. 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times with water) was added to a serially diluted sample of extract from molten butter residue (MBR) (200 µL-1000 µL).

After 3 min, 2 mL of sodium carbonate (Na_2CO_3) (75 g/L) was added. The sample was mixed. The control sample contained all the reaction reagents with water instead of the extract. After 30 min of incubation at room temperature, the absorbance was measured at 765 nm using a UV-VIS spectrophotometer. Total phenols were expressed as gallic acid equivalents/g dry weight of the sample. The extract from the dairy waste exhibits total phenolic content approximately 60.11 mg/mL (Fig. 1).

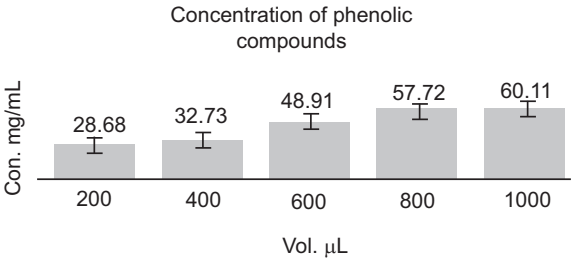

Fig. 1. Estimation of total phenolics: The concentration of phenolics estimated in a series of sample dilutions equivalent to the standard Gallic acid.

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The properties of phenolic compounds like antioxidant potential, reduction in risk of developing diseases like cardiovascular diseases, arteriosclerosis, cancer, diabetes, cataract, disorders of the cognitive function and neurological diseases, anti-aging, decrease in oxidative stress risk, anti-inflammatory (Minatel *et al*., 2017) may be obtained from the extract of the dairy waste.

Total antioxidant capacity assay. Aliquots of suitable working solutions (1-10 mg/mL) of the samples (MBR) were mixed with 1 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) and incubated at 95°C for 90 min (Nagare *et al*., 2015). The tubes were cooled to room temperature and the absorbance was measured at 695 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity was expressed as equivalents of ascorbic acid (Indumathy, 2016).

Antioxidant activity by DPPH radical scavenging assay. Different dilution of the extract (200, 400, 600 and 800 µg/mL) was prepared. DPPH solution was prepared by dissolving 6 mg of DPPH in 100 mL ethanol. 2 mL of the extract from each dilution was added into 2 mL of DPPH solution. Ascorbic acid was used as standard. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The scavenging activity of the extract was calculated using the formula: Scavenging activity % = 100 × (1– AE/AD), where, AE is the absorbance of the solution, when extracts have been added at a particular level and AD is the absorbance of the DPPH solution, without extract (control) (Gallotti and Lavelli, 2020).

It exhibits maximum total antioxidant property when compared to gallic acid as standard by using the phosphate molybdenum method (Fig. 2). It shows 99.39% of DPPH scavenging activity with a 200 µL/mL sample (Fig. 3).

It was reported that the antioxidant property of the phenolic compound influences the major components in foods like carbohydrates, proteins and fats and interact with minerals and vitamins too. (Alu'datt *et al*., 2017). For so long the studies on health benefits and applications of phenolic compounds obtained from various plant sources is numerous (Taamalli *et al*., 2019). Some of which are nutritious and commercially valuable. The application of phenolic compounds with great antioxidant activity from dairy waste may help to reduce the use of nutritious and commercially available food sources for the extraction of phenolic compounds.

Antibiotic activity-disc method. Sterilized cellulose paper discs were immersed in 100 µL of the sample and allowed to get absorbed for few hours. Agar plates inoculated with the respective microbial cultures-*Bacillus, Streptococcus* and *Staphylococcus* were prepared. The discs with the impregnated sample were carefully placed in the centre of the petri-dish and incubated for 24 h at 37°C. After incubation, the plates were observed for the zone of inhibition.

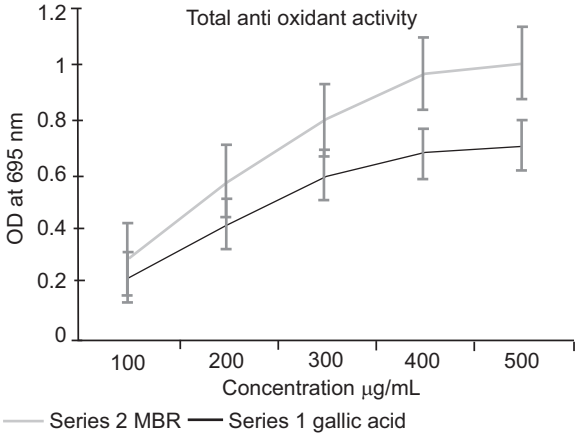

Fig. 2. Estimation of total antioxidant activity: The total antioxidant activity of the sample extract was compared to that of Gallic acid.

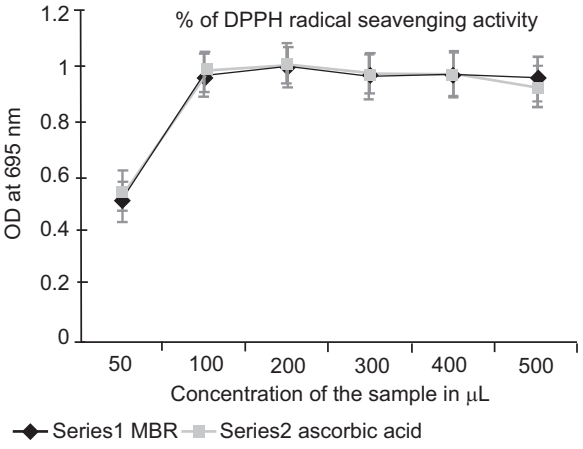

Fig. 3. Percentage of DPPH radical scavenging activity: The percentage of DPPH radical scavenging activity of the sample was compared to that of standard ascorbic acid.

After 24 h of incubation, a prominent zone of inhibition was observed in the petri dish inoculated with the respective bacteria *Bacillus, Streptococcus* and *Staphylococcus* (Fig. 4).

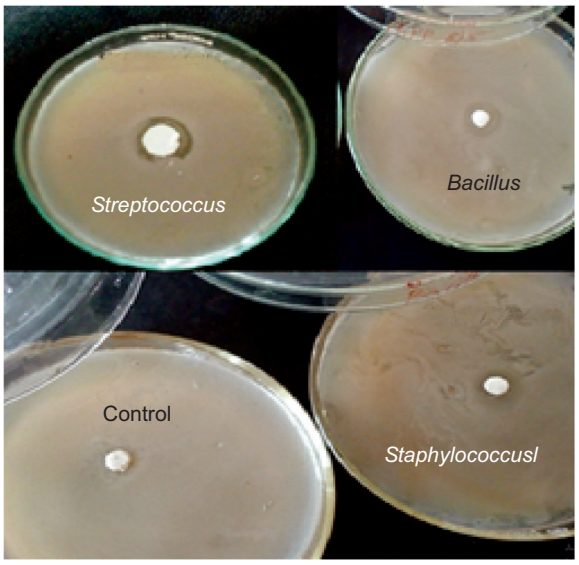

Fig. 4. Antimicrobial activity against *Bacillus, Streptococcus* and *Staphylococcus*: Approximately 100 µL of the sample in each disc of sterile cellulose paper shows prominent zone of inhibition.

In addition to antioxidant activity, the extract from dairy waste exhibits antimicrobial activity against bacteria (*Bacillus, Streptococcus* and *Staphylococcus*) which are commonly found as normal flora and in food spoilage, responsible for food-borne diseases. This property of phenolic compounds has been used as a natural preservative (Chibane *et al*., 2019). The extract from dairy waste may be applicable as an alternative to synthetic food preservatives.

Conclusion

The radicals produced due to a chain of unintentional reaction at the cellular level may cause harm to basic cell biomolecules which are exceedingly dangerous and produce oxidative stress causing oxidative harm to DNA (Sindhi *et al*., 2013). Many medicinal plants and the secondary metabolites obtained from various parts of plants have been reported to be potent free radical scavengers (Aydemir and Yemenicioğlu, 2014; Osuna-Martínez *et al*., 2014). The extract from molten butter residue may be applicable in the pharmaceutical area as it exhibits effective antioxidant and antimicrobial properties. Additionally, a commercial value can be added to the molten butter residue resulting an increase in economic status in dairy industries.

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