Effects of Edible Coatings Containing *Ocimum sanctum* and *Aloe vera*on Shelf Life of Jaggery

Abstract

Ocimum sanctum, common name **"**Tulsi" and *Aloe vera* are generally used as antimicrobial food additives as they have numerous health benefits besides their known antimicrobial properties. The edible coatings containing antimicrobial herbs may extend the shelf life of Jaggery by providing a semi-permeable barrier to gases and water vapours and thus protecting it from deterioration. *Ocimum sanctum,* (OC), *Aloevera* (AC) and tulsi-aloe vera coated (TAC) jaggery were evaluated for physico-chemical properties, antioxidant activity, total viable count and antimicrobial activity and compared with non-coated control. Physico-chemical analysis revealed no significant difference between non-coated and coated Jaggery, however, the phenolics, tannins and flavonoids were preserved better in coated samples. Edible coating of Jaggery samples revealed significantly lesser ($p \leq 0.01$) microbial counts in comparison to non-coated. DPPH radical scavenging ability and reducing power potential of coated Jaggery exhibited better anti-oxidant activity in comparison to control. Tulsi and aloe vera coatings were effective in inhibiting the growth of Gram's positive as Gram's negative bacteria when compared with untreated Jaggery. The results suggest that herbal coating of Jaggery may be used to enhance the shelf life of Jaggery to maintain its quality as comparable to fresh Jaggery .

Objective: Jaggery is the common agriculture based cottage industry of western Uttar Pradesh and farmers are compelled to sell their product when fresh but at lower price. Hence, it was felt desirable to develop better methods of storage to enhance its shelf life. With this objective, the present study was designed to evaluate the effects of edible coatings of common Indian herbs -tulsi and aloe vera to find their potential to increase shelf life of Jaggery with comparable qualities to fresh Jaggery.

Methodology: Antimicrobial activity of tulsi and aloe vera coatings were determined using agar double diffusion method and the physioco-chemical characteristics were determined using standard protocol for analysis of reducing sugars, proteins, phenols, saponins, alkaloids and flavonoids.

Results: The experiments revealed that the edible coatings with tulsi and aloe vera enhance the shelf life of Jaggery during storage and no significant microbial spoilage was observed. The coatings with herbs also preserved the contents of phenolics, flavonoids and tannins resulting in better anti-oxidant activity in comparison to untreated control over a period of 6 months. Additionally, the edible coating's lower sugar content was better retained when turmeric and black pepper were added.

Key words: Jaggery, edible coating, tulsi, aloe vera, anti-oxidant activity, antimicrobial activity

1. Introduction

The sugar industry is the second largest agro-based industry in India and contributes significantly to the socioeconomic development of the rural population.Sugarcane industry provides employment to 0.5 million trained and semi-skilled workers, and supports 50 million farmers and their families. According to Quadri et al. [1] India presently produces 6.6 million metric tonnes of Jaggery and 27.7 million metric tonnes of sugar. In many traditional dishes, jaggery has been utilized for decades as a natural sugar substitute, flavour enhancer, and immune system booster [2,3]. It is extensively used throughout Africa, Latin America, the Caribbean, India, Pakistan, Sri Lanka, and other Asian nations. In Asia, jaggery is referred to by several names, including "Gur: India, Desi: Pakistan, Naam Taan Oi: Thailand, and Hakura: Sri Lanka" [4]. The numerous culinary and therapeutic applications of sugar cane jaggery are described in ancient literature. Many authors have assessed the nutraceutical profile of jaggery, which is regarded as one of the world's healthiest and most nutritious sugars [5-7]. Jaggery is cytoprotective, antibacterial, antioxidant, and anticancer properties [8-11]. Additionally, jaggery has a high dietary fibre content that promotes peristaltic movements in the gut, which has anti-inflammatory effects on the gut [12].

The physical and chemical makeup of the jaggery as well as its storage environment play a significant role in determining how well the product keeps. Jaggery essentially deteriorates in four ways while it is being stored: physically, chemically, biologically, and microbiologically. The two primary issues with solid jaggery storage are colour deterioration and running-off (liquefaction). Microbiological invasion and moisture

absorption are the causes of these issues [13]. There are many complexities associated with storing different food products, particularly jaggery, and there isn't a single, effective way to store food that works for all of India.

The methods of storage differ in different tracts according to climatic conditions, local customs and resources. The technologies adopted so far for the storage of various food products including jaggery with various edible coatings and also regarding use of multilayered or herbal based edible coatings to ensure more precise control of coating properties and functionality was shown in some studies. Some studies have shown beneficial effect of coating on jaggery during prolonged storage [8, 13-15]. Edible herbal coating involves the utilization of herbs either individually or in combination with other edible coatings. Widely used herbs in these coatings include Aloe vera gel, Neem, Lemon grass, Rosemary, Tulsi, and tulsi which are rich in vitamins, antioxidants, and essential minerals, herbs have antibacterial properties [16].

Tulsi (*Ocimum sanctum*) boosts natural immunity and prevents the spread of illnesses as it contains powerful antiviral, antifungal, and antibacterial effects that shield humans against a range of ailments [17]. Further, Tulsi has anti-diarrheal, anti-oxidant, antiinflammatory, hepato-protective, cardio-protective, reno-protective, analgesic and antipyretic qualities [18].

Aloe vera has been identified as having a multitude of biological properties, including the ability to combat fungal infections[19], bacteria, viruses, and antioxidants [20], wound healing [21], and skin diseases [22]. Both Tulsi and Aloe vera gel have gained widespread use in coating food due to its antimicrobial characteristics and its ability to reduce moisture and moisture loss [23].

Hence, the present investigation is undertaken to evaluate physico-chemical properties, microbial characteristics, antioxidant activity and antibacterial activity of carboxy methyl cellulose (CMC) based Tulsi and Aloe vera coating on jaggery.

2.Materials and Methods

2.1 Sample collection and materials

We obtained fresh jaggery samples (made from sugarcane variety 'Co 0238') from a nearby small-scale jaggery production facility in Muzaffarnagar, India. The local pharmacy supplied the aloe vera gel (BRM chemicals, India) and the tulsi liquid extract (*Ocimum sanctum*).

Himedia Laboratories, Mumbai, India, provided food-grade CMC (99.9%) with an average molecular weight of 41,000 $g.mol^{-1}$, analytical-grade glycerol, and other reagents. The jaggery was first analyzed in April 2023 and again in September 2023 following a six-month storage period.

2.2. Edible coating preparation and sample storage

The 1.5 g CMC were dissolved in 100 mL distilled water and stirred at a controlled temperature of 75°C until the mixture became clear. 5% glycerol was added as plasticizer. Then, solutions were cooled to 50°C and antimicrobial agents (Tulsi and Aloe vera extracts) was added with constant stirring (Mishra et. al., 2016). The amount of antimicrobial agent incorporated into the coating solutions was 2% wt. of CMC. Three types of coating solution was prepared: Tulsi coated (OC), Aloe vera coated (AC) and tulsi plus aloe vera (equal concentration) coated (TAC). Jaggery samples (100 g cubes) were coated by dipping them into the prepared coating solutions for 120 s at room temperature, then drying for 60s. Both non-coated (NC) and coated samples(OC, AC, TAC) were stored in aluminium pouches for six months for further analysis [24].

2.3 Physico-chemical characterization

Further analysis was performed on the coated and non-coated stored jaggery samples using Guerra and Mujica's [25] methodology for physico-chemical characterization (pH, colour, turbidity, filterability, insoluble particles, and water activity). Samples were dissolved in sterile water and the pH was noted down using digital pH meter (Labman, India). Using a visible Spectrophotometer (Labman, India), the colour of the 5% w/v solution of jaggery samples was measured by measuring the optical density (OD) at 540 nm. By putting the sample in a water activity metre (Labtron, India) and measuring the equilibrium relative humidity, the water activity was determined. A visible Spectrophotometer was used to measure the transmittance at 740 nm in order to determine the turbidity of the 5% w/v jaggery sample solution. The percentage of filterability (%) was determined by dividing the filtered volumes of 100 millilitres of each sucrose (280 Brix) solution and 5% w/v jaggery sample solution after three minutes of filtering through filter paper. The residue that remained on filter paper after filtering 1g of the jaggery sample solution was dried and weighed in order to calculate the quantity of insoluble particles.

The Official AOAC techniques [26] were used to measure the moisture content, protein content, ash content, reducing sugars, and sucrose content of jaggery. 1g of jaggery sample was dried in a hot air oven for 24 hours, at which point the weight ratio was expressed to estimate the moisture content (%). Titrating the jaggery sample solution with a known volume of Fehling's solution allowed for the calculation of the reduction of sugars (%). Similar titration techniques were used to estimate the percentage of sucrose (%), but first the sample solution was inverted with acid and then neutralized with alkali. By burning 10g of jaggery sample in a muffle furnace and comparing the weight with air-dried jaggery sample, the ash content of the sample was ascertained. A mixture of 100 µL sample solution and 5 mL Bradford solution was used, and the mixture was incubated for 5 minutes to measure the protein concentration. Absorbance at 595nm was recorded and plotted with standard curve of bovine serum albumin.

2.4 Phyto-chemical characterization

The aluminium chloride method [27] and Folin-Ciocalteu's method [28] were used to determine the total flavonoid content, tannin content, saponins, total alkaloid, and total phenol content of jaggery, respectively. A 5% w/v solution of the jaggery samples was made for each analysis. 500 µl of the sample was combined with 2 ml of the Folin-Ciocalteu reagent and 2 ml of a 10% sodium bicarbonate solution. The mixture was then incubated for 1 hour at room temperature to determine the total phenol concentration. At 765 nm, absorbance was measured. The total phenol content was reported as mg of gallic acid equivalent (GAE)/gram of sample, with gallic acid serving as the benchmark. For total flavonoids, reaction mixture was prepared by adding 5ml of 10% aluminium chloride solution with 5ml of sample solution and absorbance at 415 nm was recorded after incubation for 30 min at room temperature. Catechin is used as standard and total flavonoid content is expressed as mg catechin per gram of sample (mg/g). The reaction mix for total alkaloids was made by mixing 100 µl of sample with 40 millilitres of 10% acetic acid in ethanol, and it was then allowed to sit at room temperature for four hours. Subsequently, ammonium hydroxide was gradually added to the mixture, and the residue was let a full hour to settle. After that, the residue was dried, weighed, and filtered. By mixing 1 ml of the sample with 7.5 ml of distilled water, 0.5 ml of the Folin-Ciocalteu reagent, and 1 ml of a 35% sodium carbonate solution, the total tannin concentration was ascertained. At 760 nm, absorbance was measured after one hour. Tannic acid equivalent, or mg/gram of sample, is the unit of

measurement for total tannin content. Saponin content was determined by purifying 5 ml of sample solution with ethanol and Di-ethyl ether and concentrating with n-butanol.

2.5 Anti-oxidant activity

The jaggery's capacity to scavenge DPPH radicals was assessed using the methodology outlined by Yamaguchi et al. [29]. 1 ml sample solution was mixed with standard BHT at varying concentrations. 3ml of DPPH was added to the mix and incubated for 30 mins in dark. The DPPH radical scavenging was expressed as I%= (A control-A sample)/A control * 100, and the absorbance was measured at 517 nm. Additionally, the 50% DPPH effective concentration (EC50) was computed. Additionally, jaggery's reducing power was ascertained using the previously published method [30]. The standard antioxidant Trolox was combined with varying concentrations of the jaggery sample solution, and an equal volume of 0.2M phosphate buffer and 1% potassium ferricyanide were added. The reaction mixture was incubated for thirty minutes at 500°C. After adding 10% trichloroacetic acid, the mixture was again centrifuged at 3000 rpm. The supernatant was gathered and combined with sterile water and a 1% ferric chloride solution. At 700 nm, absorbance was measured.

2.6 Microbiological and statistical analysis

Microbiological analysis for SAC, Yeast and Mould count and antibacterial activity was determined by agar well diffusion assay [31]. Data was subjected to statistical analysis for testing its significance by employing Analysis of Variance (ANOVA) technique.

3. Results and Discussion

3.1 Physico-chemical Characterization of coated Jaggery

The results of physical properties of non-coated and coated jaggery are represented in Table 1. Guerra and Mujica [25], the pH range for coated and non-coated jaggery was 5.7–5.9. Jaggery's low pH could be the result of not adding enough lime when the juice was being clarified.

The main determinant of customer choice and marketability in jaggery is its colour, which is influenced by dark chemicals produced during processing. The Maillard reaction, phenolic chemical oxidation, alkaline breakdown of sucrose, or caramelization of sugars can all lead to browning of jaggery Damodaran [32]. Coated jaggery depicts elevated absorbance at 540 nm (TAC>OC>AC) compared to non-coated jaggery

(NC). NC has golden brown color, while, darkened color was resulted in all coated jaggery samples. Moisture content and water activity are two important parameters determining the quality, stability and shelf-life of foods during storage. TAC and AC showed a marked increase (0.9%) in moisture content but a very slight increase in moisture content observed for OC. This demonstrates that covering the jaggery samples assisted in preserving moisture content to a certain degree. Water activity (aw) controls microbial development and is a measure of the water status in the food chain [33]. The coating of the jaggery samples resulted in a considerable decrease in the values obtained for the non-coated and coated samples, indicating significant ($p \leq 0.01$) differences in water activity. The findings suggested that TAC and AC might provide jaggery with a longer shelf life and more promising quality when stored. However, aw in the range of 0.60–0.68 is found to be the ideal environment for the growth of xerophilic and osmophilic microorganisms like *Saccharomyces* and *Aspergillus*, which promotes their growth on jaggery and causes spoiling [33].

Turbidity of all coated jaggery showed a gradual increase (TAC<AC<OC) with respect to NC jaggery. About 7.5–8.8 %, increase in turbidity was observed between NC compared to AC and OC, respectively. Marginal increase in filterability is seen between NC and OC jaggery. However, results showed initial remarkable increased (7 and 16 %) filterability in AC and TAC, respectively, but the ash content was differed by 0.02 % in all coated jaggery.

The results of chemical properties are represented in Table 1. Sucrose and reducing sugar content of coated jaggery showed very marginal increase in coated jaggery (TAC>OC>AC). Protein content of OC and AC showed no significant difference over NC jaggery, but, TAC depicted increase of about 0.3 mg/g of protein content. Increase in total phenol, tannin and flavonoid contents was resulted in all coated jaggery (TAC>AC>OC). OC, AC and TAC exhibited increase in 12.0, 12.5 and 17 % phenol; 13.5, 14.0 and 16.0 % tannin and 9, 6.5 and 7.0 % flavonoid contents, respectively from NC jaggery. Because of their distinct functional groups, flavonoids are the most prevalent form of dietary polyphenols with antioxidant potential. Both flavonoids and total phenols are in accordance with the previously reported study [24,34,35]. Thus, our results depicted that edible coated jaggery may be used as a source of antioxidants. Saponin content and total alkaloid content values were not significantly different between samples and depicted only marginal increase in coated samples a compared to non-coated.

3.2 Antioxidant activity

Antioxidant activity of coated jaggery was measured by two in vitro assays, i.e., DPPH radical scavenging ability and reducing power assay.Stable free radical DPPH absorbs at 517 nm when in its radical form; absorption falls down when an electron or hydrogen atom is accepted from an antioxidant because DPPH-H, its non-radical form, is formed [36]. Test samples' antioxidant potential can be measured stoichiometrically by looking at the degree of DPPH decolorization. Table 2 displays the EC50 values for TAC, OC, and AC, which represent their scavenging abilities. The ability of coated jaggery to scavenge free radicals was concentration dependant. Compared to uncoated jaggery, coated jaggery had a lower EC50 concentration. Compared to 3.78 for NC, the EC50s for OC, AC, and TAC were 3.08, 3.076, and 3.045 mg/mL, respectively. The conventional BHT EC50 was 0.0075 mg/mL.Both coated and non-coated jaggery showed higher (450 folds) EC_{50} concentration than standard BHT. Results of DPPH radical assay showed a positive correlation (r = 0.94, 0.88 and 0.89) with total phenolics of OC, AC and TAC jaggery, respectively. High correlations between total phenolics and scavenging of DPPH radical indicated that polyphenols present in the coated jaggery are the main antioxidants.

Further, reducing capacity assay provides a measure of compound's ability to donate electrons and reduce the oxidized intermediates formed in peroxidation process. The assay is based on the reduction of Fe^{+3}-ferricyanide complex that is monitored at 700 nm. A rise in absorbance signifies a rise in reductive capacity [37]. Since reducing power of a compound serves as a significant indicator of its antioxidant activity [38], coated jaggery assayed for reducing power ability. In table 2, coated jaggery exhibited in-vitro ferric reducing potential in increasing manner. The absorbance of coated jaggery at 700 nm had increased than non-coated jaggery. The standard, trolox, has an absorbance of 1.37 at 50 lg/mL. Compared to non-coated jaggery, the lowering potential of OC, AC, and TAC rose by 23.00, 25.00, and 24.55%, respectively.

Natural antioxidants have a significant impact on the food system's acceptability and safety as well as its ability to prevent and intercept oxidative damage. By preventing microbial growth, they function as a strong preservative and keep the food stable against oxidation. Antioxidants have many health benefits, including preserving biological function and guarding against diseases like cirrhosis, diabetes, heart disease, gastropathy, chronic renal disease, and cancers [39]. In addition, antioxidant activity of plant is often associated with polyphenols that with hydrogen donating capacity inhibits free radical induced oxidation [30]. The phenolic compounds of sugarcane juice exhibited antioxidant

potential [40] and conferred various biological activities. The antioxidant compounds extracted from jaggery showed stronger antioxidant potential than BHT in earlier reports [24, 35]. In present investigation, aloe vera and tulsi edible coating resulted synergistic increase in both total phenolic content and anti-oxidative potential of jaggery, and hence the combination of nutritional and medicinal benefits determines them as a functional food.

3.3 Microbial characterization

The total viable count (TVC) in cfu/g (Colony Forming Units per gram) in NC, TAC, OC, AC jaggery after six months of storage were $4x10^3$, $2.0x10^3$, $1.5\ x10^3$ and $3.8x10^3$, respectively. Coating of jaggery samples with edible coating significantly ($p \leq 0.01$) affected microbial counts as shown by marked difference in TVC obtained for uncoated and coated samples. This depicts that coating the jaggery samples with edible coating may reduce the microbial deterioration of jaggery to some extent. Similar conclusion were reported previously [13, 15, 24, 41].

3.4 Antibacterial activity

The antibacterial activity of non-coated and coated jaggery was determined by measuring the diameter of inhibition zone as shown in Table 3 and Figure 1. Among the coated jaggery, only TAC and AC were effective in inhibiting the growth of gram-positive bacteria compared to NC jaggery. Against gram-negative bacteria, TAC, AC and OC jaggery significantly inhibited growth compared to NC jaggery. Based on the diameter of inhibition zone, it was observed that Gram-positive bacteria were more sensitive to the coated jaggery samples than gram-negative bacteria. Polyphenols and antioxidant properties of the jaggery may be responsible for the antibacterial activity [42]. Vitamin C, antioxidants, antiseptic, and antiviral activities are abundant in tulsi. Tulsi is used as a natural hand sanitizer because of its antibacterial properties [43]. Molluscides such as ursolic acid, luteolin, apigenin-7-Oglucuronide, luteolin-7-O glucuronide, orientin, and molludistin were also isolated from the leaf extract. Additionally, it contains a variety of monoterpenes and sesquiterpenes, including cholesterol, stigmasterol, bornyl acetate, and camphene [44]. Tulsi is a potent herb with many medicinal applications and health benefits. This easy-to-grow plant strengthens immunity and fights off dangerous bacteria and viruses.

Aloe vera gel contains a few significant polysaccharides that have been shown to have antibacterial action against both Gram (+Ve) and Gram(-Ve) microorganisms. *Aloevera* contains compounds called saponins and anthraquinones that are utilised to fight bacterial infections [19]. *Aloevera*gel works against a variety of bacterial species, including *Streptoccocusfaecalis*, *Shigella flexneri*, and *Streptoccocus pyogenes*.Acemannan prevents *Pseudomonas aeruginosa* from attaching to human lung epithelial cells, and it is utilized as a disinfecting agent against the pathogen [20]. Flavonoids, alkaloids, terpenoids, and anthraquinone found in aloe vera latex have been shown to be strongly connected with antibacterial action [45].The results of this study also showed that the antibacterial activity of coated jaggery is directly correlated with its flavonoid and phenolic component concentrations, and *vice versa*.

The advantage of edible coatings is their ability to incorporate beneficial components including flavours, antioxidants, and antimicrobials. Food value, stability, functionality, and safety can all be improved with this ability.When tulsi and aloe vera were applied to jaggery samples, the results showed a considerable inhibition of microbial growth and an elevated capacity for antioxidants compared to uncoated jaggery [24]. Applying an edible coating based on proteins to jaggery, vacuum-packaging it, and keeping it in a controlled environment with regulated temperature and relative humidity can help prevent moisture absorption and microbiological attack [13]. When compared to uncoated jaggery, the overall effects of edible coating during storage were favourable, leading researchers to conclude that edible coating made of whey protein concentrate may help preserve the quality and extend the shelf life of jaggery [15]. According to Mishra et al. [14], coating a jaggery sample could help it preserve the ideal moisture content to a certain level. It can also improve the quality of the jaggery by applying an edible coating made of whey protein concentrate and carboxymethyl cellulose.

Conclusions

Edible coatings have been used by the food industry as a food storage solution for many years. These coatings are made of a variety of components, including as proteins, waxes, and hydrocolloids. It has been demonstrated that edible coatings increase the shelf life of fresh product, reduce moisture loss, slow down the ripening process, and successfully stop the growth of microorganisms, especially in food. Herbal

edible coatings are a recent development in edible coatings that have shown improved results and health advantages.Edible coatings made of herbs preserve nutrients in food while also having therapeutic benefits, which is an added benefit. The current study's findings suggest that tulsi-aloe vera and aloe vera coated jaggery may provide a longer shelf life and more promising quality during storage because they partially inhibit the microbial destruction of the fruit. Additionally, adding these herbal extracts to jaggery boosts its phenolic content and antioxidant capacity. Therefore, jaggery coated with tulsi and aloe vera may be used in place of conventional jaggery and have added health benefits.

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Table 1: Physico-chemical properties of non-coated and coated stored jaggery

Property	Non-coated Control	Tulsi coated (OS)	Aloe vera coated (AV)	Tulsi + Aloe vera coated (OSAV)
pH	5.7	5.8	5.8	5.9

Color (OD=450nm)	1.2	1.2	1.4	1.3
Turbidity (%T at 720 nm)	19.5	20.5	21.4	23.6
Filter-ability (%)	64.5	78.54*	79.52*	83.30*
Insoluble solids (%)	7.45	8.47	9.52*	9.67*
Water activity (aw %)	0.68	0.68*	0.63	0.62
Moisture (%)	4.7	5.85*	5.8	6.34*
Ash (%)	0.03	0.04	0.04	0.04
Sucrose (%)	73	73.66	73.75	74.00*
Reducing Sugar (%)	17.32	17.32	17.43	17.72
Protein (mg/g)	1.21	1.22	1.55	1.85*
Phenols (mg/g)	3.66	4.18	4.85*	5.13
Flavanoids (mg/g)	0.65	0.75	0.85*	0.83
Saponin (mg/ml)	30.38	34.11	35.45	35.18
Alkaloids (mg/ml)	2.14	2.20	1.88	2.30

Values are expressed as mean (n=3). * depicts significant difference among means (P <0.05). NC= non-coated jaggery; OC= tulsi coated jaggery; AC= aloe vera coated jaggery; TAC= tulsi plus aloe vera coated jaggery

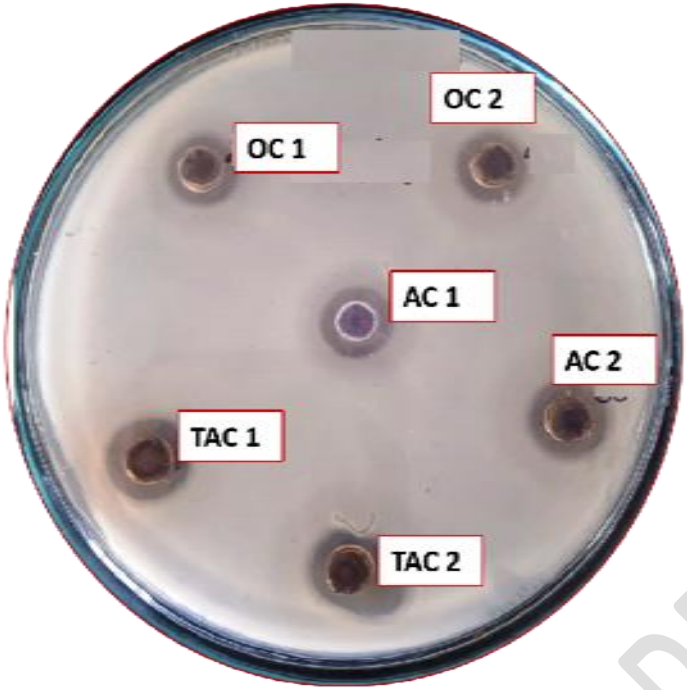
Table 2: DPPH radical scavenging activity (standard BHT) and reducing power (standard Trolox) of non-coated and coated jaggery

EC50 (mg/ml)	BHT	Non-coated control	Tulsicoated	Aloe vera coated	Tulsi + Aloe vera coated
	0.0075	3.78	3.08	3.076*	3.045**
Absorbance (700 nm)	**Trolox**	**NC**	**TC**	**PC**	**TPC**
	1.37	0.46	0.49	0.55**	0.60**

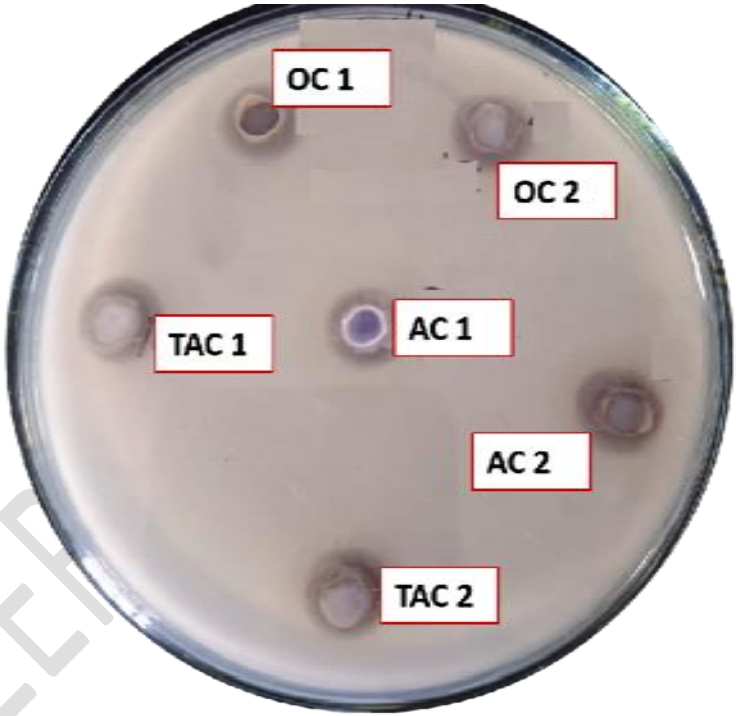
(*P <0.05; **P<0.01, ***P<0.001)

Table 3: Zone of inhibition (mm) of coated and non-coated jaggery against selective bacterial strains (values are shown in mean of 2 replicates each)

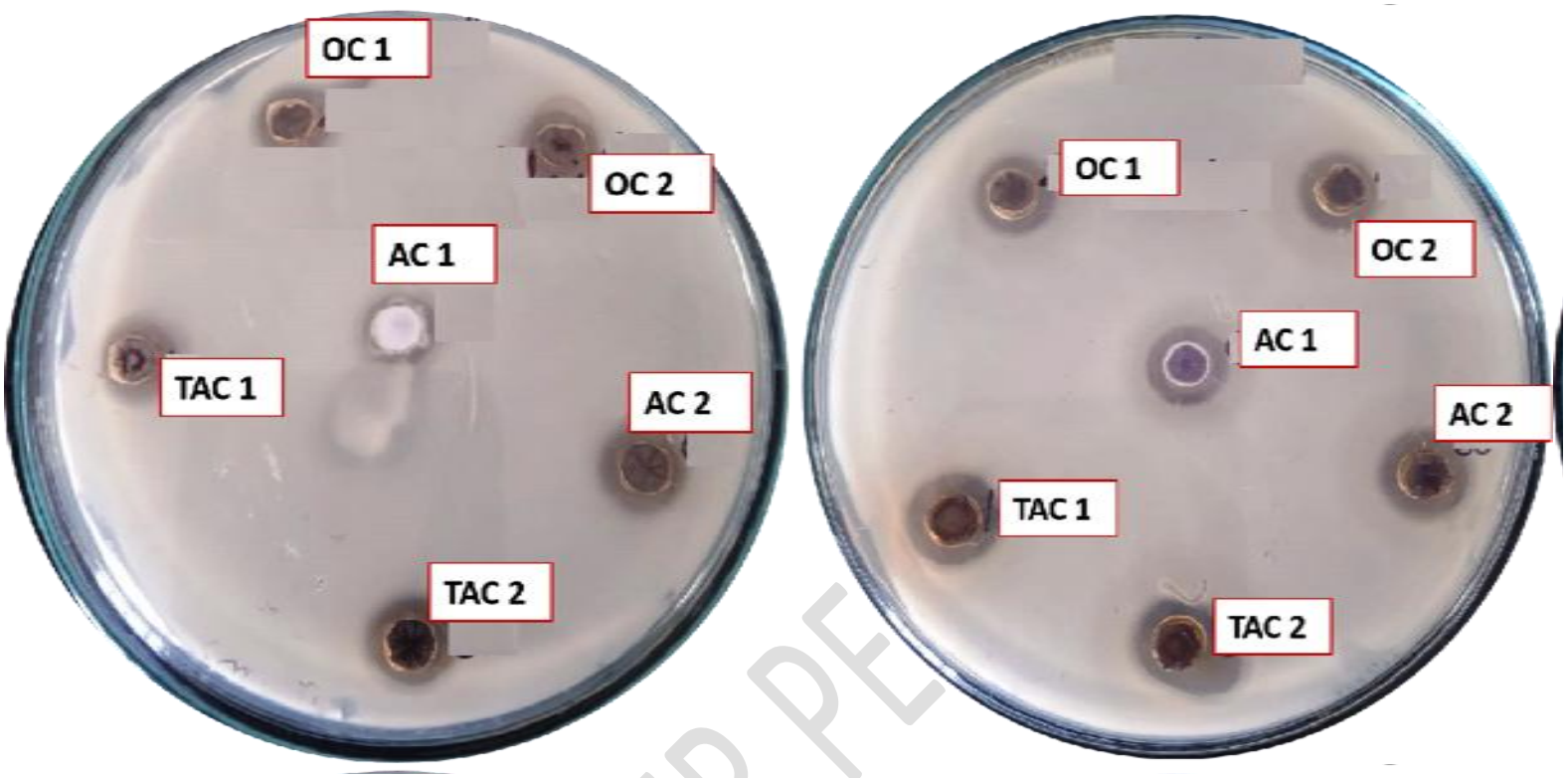
Sample	Zone of Inhibition (mm)			
	Gram +ve		Gram -ve	
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonasaeruginosa***
Non-coated (NC)	-	-	7.5	8.5
Tulsi coated (OS)	12.25	7.25	12.50	21.50
ALoe vera coated (AV)	14.5	8.75	11.50	24.50
Tulsi + Aloe vera coated (OSAV)	15.0	8.25	10.50	27.50



Staphylococcus aureus



Bacillus subtilis



Escherichia coli *Pseudomonas aeruginosa*

Fig. 1: Inhibition zone of OC, AC and TAC (in 2 replicates each) against *Staphylococcus aureus* and *Bacillus subtilis* (Gram's positive) and *Escherichia coli* and *Psuedomonas aeruginosa* (Gram's negative)

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