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EFFECT OF ORGANIC COATING ON PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY AND SHELF LIFE OF FRUITS AND VEGETABLES

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ABSTRACT

The present study was carried out in microbiological laboratory of Sardar Patel Mahavidyalaya Chandrapur during the year 2022. Organics like *Azadirachta indica, Aloe barbadensis* Miller and *Ocimum tenuiflorum* were collected from nearby nursery. Phytochemical and antimicrobial activity of organic samples were studied against fruits and vegetables spoilage bacteria. Phytochemicals like alkaloid, flavonoid, glycosides, steroid, terpenoid, saponin, tannin, reducing sugar, protein and phenol except terpenoid were found by the application of *Azadirachta indica* (Neem), except glycoside, steroid and protein by the application of *Ocimum tenuiflorum* (Tulsi), except flavonoid, steroid, saponin, tannin and reducing sugar by the application of *Aloe barbadensis* Miller (Aloe vera) in fruits and vegetables. Antimicrobial activity of the organic samples were tested in laboratory. These organic samples were effective for antimicrobial activity organics like *Azadirachta indica, Aloe barbadensis* Miller and *Ocimum tenuiflorum* were found sensitive against *E. coli, B. subtilis, S. typhi* and *P. auroginosa* .These organics were sensetive to all organics but more sensitive against *E.coli* and showed the maximum zone of inhibition of 30 mm in *Aloe barbadensis* Miller, 26 mm in *Azadirachta indica*, and 27 mm in *Ocimum tenuiflorum*. Fruits and vegetables were applied organics and divided into four categories i.e. Control (without coating fruits and vegetable), *Azadirachta indica, Aloe barbadensis* Miller, and *Ocimum tenuiflorum.* Room temperature was maintain 27^0 C after applying organics to fruits and vegetables. Freshness, weight and colour remained as such for long time and no external injuries was noticed when organics were coated to fruits and vegetable like apple, banana, chikoo, tomato, capsicum, grapes, lady finger. There was no change in any quality of coated fruits and vegetables. The control fruits and vegetables were decayed earlier as compared to coated fruits and vegetables like apple (Control : Coated; 6-7:20-25), banana (2:4-5), chikoo (3-4:10), tomato (5-6:23-25), grapes (3-4:8-10), lady finger (3-4:6-8). Coating of *Azadirachta indica* was more effective than other two organic samples. Hence, this treatment can be considered as an effective treatment for fruits and vegetables to prevent post harvest losses.

(Key words: Organic coating, antimicrobial activity, shelf life, phytochemical test)

INTRODUCTION

Herbal medicinal plants are a common therapeutic option because of their reputation for safety and lack of negative effects. They have an edge over coating is made from a variety of herbal plants, including *Azadirachta indica* (Neem), *Aloe barbadensis* Miller (aloe vera) and *Ocimum tenuiflorum* (Tulsi). Since they all possess therapeutic qualities. *Azadirachta indica*, also referred to a neem, is well known for its medicinal plant and wide range of applications. Neem leaf and its components have been shown to have immunomodulatory, anti-inflammatory, antihyperglycemia, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, ad anticarcinogenic properties. Samrit *et al.* (2020) stated that botanical like neem is used in Indian agriculture for over a century to minimize losses caused by pest and diseases. In general it possess low mammalian toxicity thus constitute list or no health hazards environmental pollution. *Ocimum tenuiflorum,* often known as Tulsi, is a plant that is widely used in homes. Basil has special value because it is thought to be a holy herb in Indian mythology. May be the importance of herbs come from their actual health advantages. *Aloe barbadensis* Miller (Aloe vera) is an herb found all over the world. Aloe plant is a rich source of many natural phytochemicals possessing health-promoting effects like, anthraquinones, vitamins, minerals, polysaccharides, sterols, amino acids, saponins, salicylic acids. Phytochemical ingredients like alkaloid play an essential role of human medicine and in an organism's natural defense.

Edible coatings are thin layers that cover the food's surface and enhance product quality. They can be safely consumed along with the rest of the meal. Therefore, the ingredients in edible coatings must be of a food-grade or generally recognized as safe quality (Baldwin, 1994). Underutilized fruits are those which do have nutritional value but not grown widely at commercial level which are rarely

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found in big markets lack recognition and appreciation at national level and a crop is called underutilized only if it fulfills certain point as crop must have nutritional food value of scientific and indigenous use, cultivated in specific geographical area less than other convential crop, weak or no formal supply of planting material due to less attention from researchers, farmers etc. Fruits are those which are found only in the natural wild habitat. Various phytochemicals are present in wild fruits because of different genotypes and environmental concerns, thus they are often considered healthy (Li *et al*., 2016).

A barand-new method for extending the shelf life of whole or freshly cut fruits and vegetables is the application of edible coatings. Fresh fruit and vegetables that stop changes in colour, scent, appearance, taste, texture and any external injuries. Polysaccharides, proteins, and lipids are frequently employed in the creation of edible coatings; these include starches and modified starches, chitosan, alginates, gums, cellulose derivatives. Edible coatings are thin films that enhance product quality, may be consumed safely as part of the product, and do not give the food any undesirable characteristics Baldwin (1994). By minimizing gas exchange, water loss, flavour and aroma loss, edible coatings prolong shelf life by acting as a barrier against the elements and extending shelf life (Benzie and Choi, 2014). The first type of edible coatings was water-wax micro emulsions, which have been employed as fungicide carriers and to brighten and color fruit since the 1930s. Another issue that can be managed with edible coatings is water loss. Additionally, edible waxes can provide storage protection from cold damage. Mummin *et al*. (2018) stated that the use of synthetic insecticides to manage insect pests has arguably been the mainstary of fruit crop production. However, the increasing demand for organically grown fruits in the face of environmental and health concerns has downplayed reliance on synthetic pesticides to manage pest and ecofriendly.

Post-Harvest losses are a severe issue in tropical regions due to the quick deterioration that occurs during handling, shipping, and storage (Yahia, 1998). It has some distinctive qualities, including high value chain waste, poor storage and transportation infrastructure, low-tech harvesting little usage in food processing, and very few exports. Large amounts of fruits and vegetables are therefore wasted annually. Nearly one-third of the population, the majority of whom are small holder farmers, are negatively impacted by the post-harvest losses, which range between 35-40% and are estimated to cost about $800 million yearly. In a location where capita consumption is only half of the recommended level, reducing these losses could both increase farmer incomes and stimulate higher consumption of this very nutritious fruit. During storage and retail sales following harvest, table grapes exhibit serious issues. Weight loss for the quality losses, which shorten shelf life. There are currently no economical, technologically feasible, or saleable solutions to extend post-harvest shelf life that have been developed through previous research. Many of these techniques are primarily intended to preserve the firmness of fruit like apples and pears. Thus, it has become crucial to find novel methods for decreasing post-harvest losses by lengthening the shelf life of fruits and vegetables while being transported and stored. That's way use of organics coating of fruits and vegetable had no side effect on fruits and vegetables and economical also benefited (Amit *et al*. 2003).

MATERIALS AND METHODS

Collection of the plant sample

The fresh leaves of *Azadirachta indica, Ocimum tenuiflorum* and *Aloe barbadensis* Miller were collected from surrounding area of Chandrapur region. The plant leaves were washed with tap water to remove soil and unwanted dust particles. Then the samples were dried in shades for 7-10 days and then powdered by using mechanical blender and stored in air tight bottles. This was used as the raw materials for the extraction, phytochemical analysis and antimicrobial activity.

Aqueous extract of *Azadirachtav indica* leaves

4.0 g of *Azadirachta indica* leaves powder was taken and added in 100 ml of distilled water and kept in mechanical shaker for 48 hours at room temperature. Extract was then filtered by using whatman filter paper no. 1. After filtration, the solvent was evaporated in boiling water bath. This extract was kept in refrigerator for further use.

Aqueous extract of *Ocimum tenuiflorum* leaves

4.0 g of *Ocimum tenuiflorum* leaves powder was taken and added in 100 ml of distilled water and kept in mechanical shaker for 48 hours at room temperature. Extract was then filtered by using whatman filter paper no. 1. After filtration, the solvent was evaporated in boiling water bath. In this evaporated solution 10 ml of 5% DMSO (dimethyl sulfoxide) was added. This extract was kept in refrigerator for further use.

Aqueous extract of *Aloe barbadensis* Miller leaves

4.0 ml of *Aloe barbadensis* miller was taken and added in 100 ml of distilled water and kept in mechanical shaker for 48 hours at room temperature.

Phytochemical analysis

The prepared extract was analyzed for the presence of alkaloids, flavonoids, glycosides, steroid, terpenoids, saponins, tannins, reducing sugar, protein, carbohydrate and phenol.

Test for Alkaloid detection

Dragendorff's test: 1ml of extract was dissolved in 1 ml of 5 % HCl and few drops of dragendorff's reagent was added. Any precipitate or turbidity indicates the presence of alkaloids (Harborne, 1998).

Test for flavonoid detection

Ammonium test: 5 ml of dilute ammonia solution was added in 1 ml of extract followed by addition of 1 ml

concentrated H_2SO_4, yellow coloration indicates the presence of Flavonoids (Harborne, 1998).

Test for glycosides detection

1 ml of extract, 2 ml of glacial acetic acid and few drops of ferric chloride were added. Then 1 ml of concentrated H_2SO_4 was added. The appearance of brown ring at interface indicated the presence of glycosides (Harborne, 1998).

Test for steroid detection

2 ml acetic anhydride was added to 3 ml extract with the addition of 2 ml sulphuric acid. A colour change to green indicated the presence of steroid (Harborne, 1998).

Test for terpenoid detection

2 ml of extract was added in 2 ml of chloroform, and then few drops of H_2SO_4 were added.

Reddish brown coloration in the interface indicated the presence of terpenoids (Harborne, 1998).

Test for saponin detection

5 ml of distilled water was added to 2 ml of extract and then shaken vigorously.The formation of foam after shaking indicated the presence of saponins (Harborne, 1998).

Test for tannin detection

1ml of extract was mixed with 2 ml of ferric chloride. A greenish black coloration indicate the presence of tannins (Harborne, 1998).

Test for reducing sugar

1ml of plant filtrate in which Fehling's A and Fehling's B were separately added a brown colour with Fehlings B and green colour with Fehlings A indicate the presence of reducing sugar (Harborne, 1998).

Test for protein detection

1ml of extract treated with few drops of concentrated nitric acid, formation of yellow colour indicates the presence of protein (Harborne, 1998).

Study of antimicrobial activity

Preparation of inoculums broth

The loop full cultures of pathogenic organism like *Escherichia coli, Bacillus subtilis, Salmonella typhi,* and *Pseudomonas aeruginos*, were inoculated into the sterile nutrient broth and incubated at 37^0 C for 24 hours.

Detection of antibacterial activity of *Azadirachta indica, Ocimum tenuiflorum, Aloe barbadensis* Miller against pathogenic organism

Antibacterial activity against selected pathogens was performed by agar – well diffusion method. The petri plates and pipettes were sterilized by autoclaving at 15 lb presser for 15-20 minutes. Muller Hinton agar was sterilized by autoclaving at 15 lb presser for 15-20 minutes and after cooled to 40-50^0 C. The media was poured in petri plates and allowed to solidify, 0.2 ml broth culture of *Escherichia coli, Bacillus subtilis, Salmonella typhi,* and *Pseudomonas aeruginosa* on each plate were added separately and spread with L-shape spreader aseptically, after five minutes, plates were turned 60^0 angles, for removing the excess amount of broth from plates.

RESULTS AND DISCUSSION

Phytochemical studies

Phytochemical analysis of leaves extract of *Azadirachta indica, Ocimum tenuiflorum,* and *Aloe barbadensis* Miller extract was tested. The results of Phytochemical analysis are presented in Table 1.

The results showed that alkaloid, tannins, saponins, flavonoids, steroids, glycosidaes, reducing sugar, protein, terpenoids were detected in aqueous extract of organics.

Phytochemical and antimicrobial activity of organics samples were studied against fruits and vegetables spoilage bacteria. Phytochemical like alkaloid, flavonoid, glycosides, steroid, terpenoid, saponin, tannin, reducing sugar, protein and phenol except terpenoid were found by the application of *Azadirachta indica* (neem), except glycoside, steroid and protein by the application of *Ocimum tenuiflorum* (tulsi), except flavonoid, steroid, saponin, tannin and reducing sugar by the application of *Aloe barbadensis* Miller (Aloe vera) in fruits and vegetables.

The overall study findings support the legitimacy of the use of the medicinal plant as medicine and imply that some plant extracts include chemicals with antibacterial activity which enhances the shelf life of fruits and vegetables. Organics extract may be employed in protective antioxidant supplements when the results of specific tests were analyzed. Following this investigation, it is anticipated that the extract will be used in powerful new formulations of natural antimicrobial medications and to enhanced quality and shelf life of fruits and vegetables.

Antibacterial activity

This study shows the significant antibacterial activity of *Azadirachta indica, Ocimum tenuiflorum* and *Aloe barbadensis* Miller, extracts against pathogens organism *Escherichia coli, Bacillus subtilis, Salmonella typhi* and *Pseudomonas aeruginosa*. These organic samples were effective against antimicrobial activity of organic samples but more sensitive against *E.coli* and showed the maximum zone of inhibion by the application of *Aloe barbadensis* Miller, *Azadirachta indica* and *Ocimum tenuiflorum* i.e 30, 26 and 27 mm respectiviy (Table 2).

Time duration of shelf life enhancement of fruits and vegetables showed that apple and tomato had large storage shelf life and they are healthy and edible. Freshness, weight and colour remained as such for long duration and no external injuries was noticed when organics were coated to fruits and vegetable like apple, chikoo, ladies finger, banana, grapes, tomato etc. The control fruits were decayed in short time period when compared with coated fruits and vegetable. Apple fruits were decayed within 6 to 7 days in control and coated apple remained as such upto 20 to 25 days, tomato decayed within 5 to 6 days in control and

coated tomato remained as such for 20 to 25 days, banana decayed within 2 days and coated within 4 to 5 days, chikoo decayed within 3-5 days in control and 10 days in coated, grapes decayed within 3-4 days in control and 8-10 days in coated and lady finger decayed within 3 days in control and 6-8 days in coated. *Azadirachta indica* was more effective organic samples, when compared with remaining two organics (Table3).

Present work was carried out to study the phytochemical and antimicrobial activity of organic samples and enhancement in self-life of fruits and vegetables. Padmaja and John (2014) studied that the wider utilization of Indian jujube hindered by the rapid deterioration of the fresh fruits and resulted in the striking decline in quality. Abdul Razack *et al*. (2013) studied that the EPS play an extensive role as biopolymers in the environment by replacing synthetic polymers as they are degradable, nontoxic, and produced by microorganism. Organic samples were effective in antimicrobial activity i.e. neem, tulsi and aloe vera. These samples were sensitive against *E. coli, B. subtilis, S. typhi* and *P. auroginosa*. These organics were more sensitive against *E. coli* (30 mm, zone of inhibition observed). The control fruits were decayed in short time of period as compared to coated fruits.

The present study was mainly based on determining the antimicrobial and phytochemical analysis of organic samples. It can be concluded that leaves extract of organic samples were highly effective against the fruits and vegetables from spoilage of bacteria. It can also be observed that organic samples were found effective in enhancement of shelf life of fruits and vegetables than the non-coated fruits and vegetables

Table 1. Phytochemical analysis of organic sample

Phytochemical tests	*Azadirachta indica*	*Ocimum tenuiflorum*	*Aloe barbadenis* Miller
Alkaloid	+	+	+
Flovonoid	+	+	–
Glycosides	+	–	+
Steroid	+	–	–
Terpenoid	–	+	+
Saponin	+	+	+
Tannin	+	+	–
Reducing sugar	+	+	–
Protein	+	–	+

[+ = Positive] [- = Negative]

Table 2. Antibacterial activity of *Azadirachta indica, Ocimum tenuiflorum* and *Aloe barbadensis* Miller in aqueous and by agar well diffusion method

Samples	***E.coli***	***B. Subtilis***	***S. typhi***	***P. auroginosa***	**Inferance**
Azadirachta indica	26 mm	27 mm	21 mm	13 mm	Sensitive
Ocimum tenuiflorum	27 mm	24 mm	15 mm	13 mm	Sensitive
***Aloe barbadensis* Miller**	30 mm	27 mm	29 mm	21 mm	Sensitive

Table 3. Time duration of shelf life enhancement of fruits and vegetables

Fruits	***Azardirachta indica***	***Aloe barbadensis* Miller**	***Ocimum tenuiflorum***	**Control**
Apple	25 days	20 days	25 days	6 to 7 days
Banana	5 days	4 days	5 days	2 days
Chikoo	10 days	10 days	10 days	3 to 4 days
Tomato	25 days	23 days	25 days	5 to 6 days
Grapes	10 days	10 days	8 days	3 to 4 days
Lady finger	8 days	7 days	6 to 7 days	3 to 4 days

Figure 1. Organic samples of Neem, Tulsi and Aloe vera

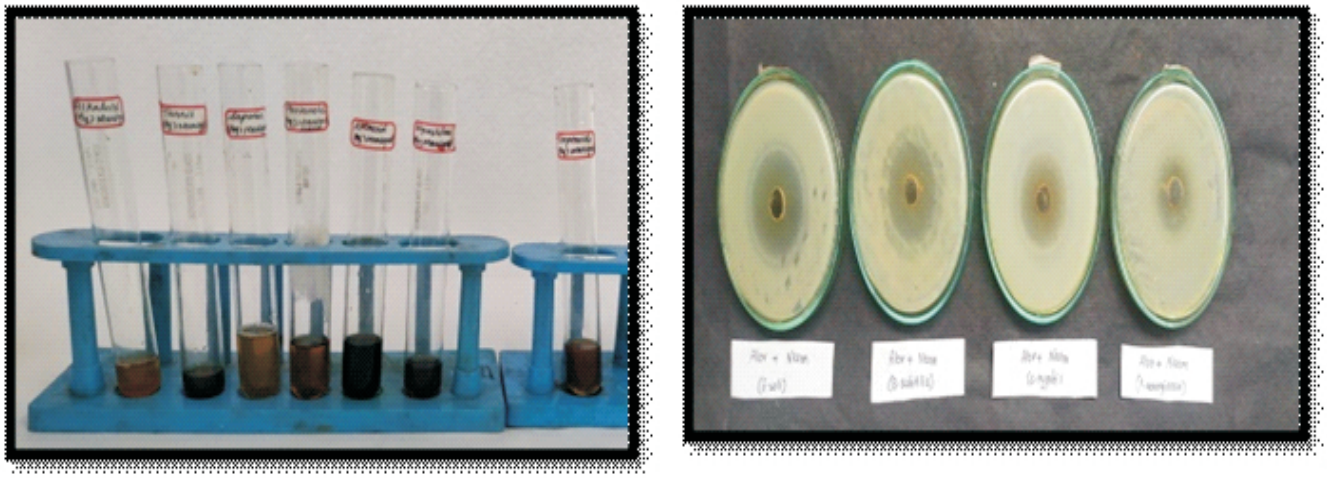

Figure 2. Phytochemical andAntimicrobial activity of organic sample against *E. coli, B. subtilis, S.typhi* and *P. auroginosa*

Figure 3. Fruits and Vegetables sample

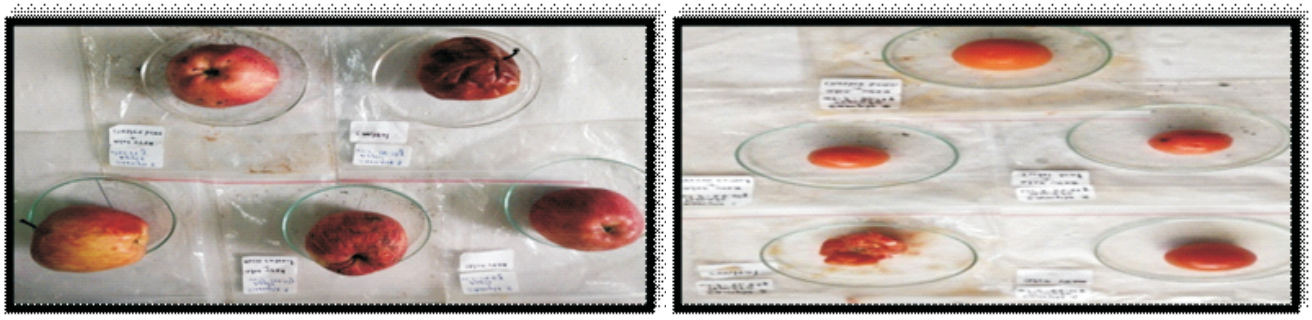

Figure 5 .5 to 10 days coated and control fruits and vegetable (Banana and Grapes)

Figure 6.8 to 10 days coated and control fruits and vegetables (Lady finger and Chikoo)

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