Enhancement of Refrigerated Shelf Life of Foods Against Microbial Spoilage Using Indian Spices

Abstract

AIMS: Evaluation of the effects of treatment of aqueous extracts and powder of spices on enhancement of refrigerated shelf life of vegetables.

Study Design: Aqueous extract and powder of turmeric *(Curcuma longa)*, black pepper *(Piper nigrum)*, garlic *(Allium sativum)*and cumin *(Cuminum cyminum)* were used to enhance refrigerated shelf life of potatoes (*Solanum tuberosum*), taro roots (*Colocasia esculenta*), bottle gourd (*Lagennaria siceraria*)and tomatoes (*Solanum lycopersicum*)

Place and Duration: The study was made at Shobhit Institute of Engineering & Technology, Meerut during the period of January to December, 2022.

Methodology: Microbial counts technique was used to evaluate the impact of spice treatment on boiled foods stored in the refrigerator.

Results: We found that aqueous treatment of spices was not as effective as treatment with powdered spices. Black pepper treatment enhanced the storage life of all four vegetables followed by turmeric, garlic and cumin. The mixed treatment with turmeric, black pepper, garlic and cumin was very effective and safe for consumption for up to 3 days when stored in refrigerator.

Conclusion: Unconsumed extra foods are commonly stored in refrigerator throughout the world and it is suggested that if the boiled vegetables are treated with a mixture of powder of turmeric + black pepper + garlic + cumin, they may be stored for longer time.

Keywords: Vegetables, Spices, Turmeric, Black pepper, Cumin, Garlic, Microbial Counts, Refrigerated Shelf life.

Introduction

Spices have been used as flavouring, colouring, and preservatives of foods and also as nutritional agents for human beings since early in the history [1]. Aromatic plan materials including spices have been used in food preparation, preservation as well as for embalming in several countries as Hindustan and Spice Islands [2, 3]. The spice trade in historic period was so crucial to economies of the countries that the rulers repeated mounted costly expeditions and Christopher Columbus undertook a hazardous voyages

to establish routes to spice growing countries-India. As per United States Food and Drug Administration (FDA), spice is an "aromatic vegetable compound in whole, broken, or crushed form, the main purpose of which in food is seasonings rather than nutrition" and from which "no component of any volatile oil or other flavoring method has been removed"[4].

Around the world, more than 100 different types of spices are produced. Asia is the primary producer of spices mainly cinnamon, pepper, nutmeg, cloves, ginger, turmeric, black pepper, cumin, fenugreek etc. while Europe generally grows basil, bay leaves, celery leaves, chives, coriander, dill tips, thyme, and watercress. Instead, pepper, nutmeg, ginger, allspice, and sesame seed are the most commonly grown spices in the United States [5]. While spices (primarily dried seed, fruit, root, bark, or plant products) have traditionally been used for rituals, skincare products, and fragrance, flavoring, coloring, and preservation characteristics have widespread applications in the traditional food preparations and the food industry [6].

Most spices contain dozens of chemicals as secondary metabolites which evolved in these plants to protect them against herbivorous insects and vertebrates, fungal, bacterial, viral pathogens and parasites as antimicrobial agents and repellents. Many chemicals extracted from spices have been demonstrated to exhibit antibacterial activity against food borne and Entropathogens [7,8]. They can stimulate saliva secretion, promote digestion, protect against colds and influenza, and reduce nausea and vomiting [9].

Food spoilage is a common phenomenon where food is permanently deterioratedand becomes tasteless or destroys its quality. These changes can be caused by a wide range of reasons, including physical (oxygen, temperature, and light) and biological (enzymatic activity and microbial growth and development). Despite current production-chain technology (such as storage in the freezer, pasteurization, drying, and preservatives), it appears difficult to totally eliminate the risk of food spoilage [10]. One of the most serious problems with food spoilage is the oxidation of lipids [11]. To prevent spoiling, food companies use antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), whereas their safety is being challenged, and customers are developing more interest in natural products [12]. Spices contain antioxidant properties, owing to the presence of phenolic substances [13] and possess antimicrobial properties with no side effects on our system rather they enhance the digestibility, improve taste and flavor including the pleasant colour. The primary constituents of all spices include phenolics compounds, flavonoids, saponins and terpenes, which are the foundation of the qualities of various of spices [8, 14]. These natural compounds extracted from spices also exhibit antimicrobial activity [15, 16]. Further these natural bio-preservatives can play an important role in overcoming challenges connected with microbial resistance while also keeping food products protected and safe without causing side effects that are harmful to customers [17]. These properties make spice extracts a potential substitute for currently available synthetic additives and their applications in food industry is being promoted further [18].

Although natural food preservative components are viewed as an alternative to synthetic preservatives, their use is considered to be causing some health issues, particularly when used excessively and over a long period of long time [19,20].

Materials and Methods

In this study, we designed the experiments to evaluate the microbial counts of foods treated with and without treatment of aqueous extracts and powdered spices separately. All selected spices turmeric *(Curcuma longa),* black pepper *(Piper nigrum),* garlic *(Allium sativum),* and cumin *(Cuminum cyminum) were* purchased from the local market of Meerut (U.P.), India and sorted out for any impurities. These were thoroughly washed under running water, wiped with tidy and clean dry cloths, then dried under sunlight, grounded into a fine powder and stored in an airtight container. All selected vegetables potatoes (*Solanum tuberosum*), taro root (*Colocasia esculenta*) and bottle gourd (*Lagennaria siceraria*) and tomatoes (*Solanum lycopersicum*) were also purchased from local market of Meerut; U.P. These vegetables were thoroughly rinsed under running water. These vegetables were boiled, peeled, and sliced into medium-sized pieces.

Treatment of Vegetables with Spices

5 g of each of the above 4 boiled vegetable was placed in 50 mL of aqueous extract of each of the test 4 spices individually as well as in combinations and their pH was determined using a glass electrode with a digital pH meter (Systronic- µ pH system 362). 100 g of each of the boiled vegetable was also treated with 200 mg of the dried powder of each of test spice - turmeric, black pepper, garlic and cumin separately and in combinations. To prepare the combination equal amounts of the spice were mixed and only 200 mg of the mixture was used for each of the 100 g of boiled vegetable. The powder of the spice was sprinkled over the surface of the vegetable uniformly so as to cover the whole surface. The control included untreated boiled vegetables. Twelve samples for each of the treatment and control were prepared and incubated glass jar in the refrigerator in the kitchen (where the temperature varied from 4-10°C) and the samples were drawn after every 24 h and processed for microbial counts.

Microbial counts from spice treated and untreated samples

Three samples of each treatment and control were collected aseptically for microbial examination immediately after treatment on zero day and after every 24 for 3 days from the refrigerated samples. Total bacterial counts as colony forming units were assessed from each of the three samples of each treatment and control separately by preparing a suspension of 1 g of f sample in 10 mL of sterile distilled

water from which the dilution series of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were prepared. 1mL aliquot of each of the suspension was poured into sterile 10 cm diameter Petri dish to which 20 mL of sterile nutrient agar medium was poured aseptically when it was cool but still molten [21]. These plates incubated at for 24-48 h at 28 and 37 ± 1°C and were examined for colony counts. The highest dilution which showed non-overlapping countable number of colonies was considered for calculations of microbial counts in terms of colony forming units.

$$\text{CFU/g of food} = \frac{\text{Total number of colonies} \times \text{Dilution Factor}}{\text{Volume of suspension/aliquot in the plate in mL}}$$

Results and Discussion

The antimicrobial activities of aqueous extracts of common kitchen spices - turmeric, black pepper, clove, cumin, cinnamon, garlic and fenugreek against the common food borne and Entropathogens *Pseudomonas aeruginosa* (ATCC 9027), *Bacillus subtilis* (ATCC 6633), *Shigella flexneri* (ATCC 12022), *Cronobacter sakazakii* (ATCC 29544), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6539), *Salmonella enterica* (ATCC 14028) and *Vibrio cholerae* (ATCC 3906) have earlier been demonstrated in our laboratories [8].

During the present study, only turmeric, garlic, black pepper and cumin were evaluated for their potential to increase shelf life of stored vegetables in refrigerator as these are the most common spices used in all food preparations [22]. The pH of the aqueous extract of selected spices and the foods after treatment with species are shown in Table 1 which shows that except cumin extract all spices produced acidic values. Tomato paste was basically acidic in nature but after treatment with spices, its acid value decreased and cumin brought drastic change to make it alkaline [23, 24]. The alkaline property of cumin helps in digestion of food and reduces acidic waste in the body, provide antioxidant property and helps in prevention diseases like diabetes, liver cirrhosis, high blood pressure and cancer. Spices have been used in flavoring and coloring of foods since ages and they contain minerals, vitamins, high antioxidants, and anti inflammatory properties. They also helps in muscles cramps, tooth decay [25], boost immune system, strengthen blood vessels, reducing blood clots, and possess anti dandruff properties [26] and protect against skin diseases [27].

Table1: pH selected vegetable after treatment with aqueous extract of selected spices

Spice	Aqueous extract	Potatoes	Bottle gourd	Taro Roots	Tomatoes

Turmeric	5.9	6.38	6.8	6.14	4.9
Clove	4.6	6.02	6.8	6.06	4.7
Cumin	8.6	6.04	6.8	6.04	7.6
Cinnamon	7.4	6.08	6.7	6.30	6.7
Garlic	6.4	6.17	6.8	6.20	5.2
Black pepper	5.6	6.21	6.8	6.50	4.7
Mustard	5.4	6.25	6.5	6.5	4.9
Fenugreek	6.7	6.16	6.8	6.6	5.2

The majority of dietary pathogens are pH sensitive, low or extremely high pH values prohibit microbial development [28]. Food is digested by our intestines with the help of pancreatic organs that release digestive enzymes. The acidity and alkalinity of the original food is important when the digested food reaches the bloodstream through the gastrointestinal tract walls [29] and alkaline foods have numerous advantages.

Scientists are investigating the innovative methods to save the wastage of food by increasing better storage conditions and by increasing shelf life of stored food materials in the refrigerator. With this view point the antimicrobial activities of common Indian spices was used as a strategy to pre-treat the foods with selected spices before storage in the refrigerator and Table 2 shows that when aqueous extract turmeric, black pepper, fenugreek, garlic, mustard, clove, cumin and cinnamon were used to treat the boiled potatoes, taro roots, bottle gourd and tomatoes, it was found that all spices reduced the microbial counts of test foods, however, the highest reduction was observed by clove followed by cinnamon, turmeric and garlic Fig. 1, 2, 3).. Potato and taro roots showed almost similar effect while bottle gourds were less protected and the tomatoes were highly protected with treatment of aqueous extracts of the spices (Table 2, fig 1 and 2)). Food spoiling is a mechanism that occurs when a product appears undesirable or unhealthy for human consumption. The identification of spoiled food can be critical in preventing food borne infections, and different technologies have been developed to detect spoilage of food including the foul smell and visibility of softness of food tissues [30]. The protective value of aqueous extract of spices remained active till 3 days of incubation in the refrigerator, thereafter the food started giving foul smell.

The microbial colonies produced during storage with and without spice treatment were counted and the results are presented in Table 2, 3 and Fig 3. The treatment and untreated materials were also tested for foul smell and visible symptoms of spoilage and it was noticed that the powdered spice treated foods did not show any foul smell till 3 days while the untreated (control) exhibited the foul smell in 24-36 h. Hence, it is suggested that powdered spices may be used to enhance shelf life of foods in the refrigerator.

Table 2: Number of bacterial colony forming units (× 10^4/g fresh weight) isolated from aqueous extract spices-treated and untreated foods stored in refrigerator at 4 to 10° C temperature over a period of 3 days (each figure is an average of 3 independent replicate).

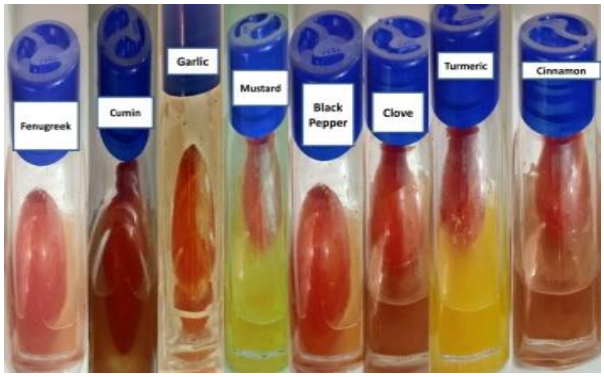
Vegetable / Spices	Potatoes						Taro Root						Bottle gourd						Tomatoes					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3		Day 1		Day 2		Day3		Day 1		Day 2		Day3	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Turmeric	128	67	525	171	1002	325	124	59	506	118	989	339	228	98	528	224	1104	431	96	43	178	86	356	112
Black pepper	128	82	525	176	1002	368	124	76	506	126	989	327	228	103	528	243	1104	424	96	24	178	52	356	86
Fenugreek	128	94	525	189	1002	380	124	84	506	134	989	389	228	102	528	236	1104	446	96	56	178	89	356	130
Garlic	128	92	525	259	1002	330	124	64	506	124	989	346	228	108	528	214	1104	424	96	29	178	62	356	104
Mustard	128	98	525	274	1002	367	124	92	506	190	989	398	228	112	528	232	1104	471	96	32	178	68	356	114
Clove	128	58	525	169	1002	315	124	56	506	106	989	326	228	92	528	212	1104	419	96	22	178	48	356	96
Cumin	128	97	525	254	1002	334	124	68	506	154	989	372	228	104	528	237	1104	441	96	52	178	88	356	140
Cinnamon	128	69	525	174	1002	320	124	79	506	129	989	346	228	106	528	226	1104	453	96	47	178	74	356	115

Abbreviation used: C = Control; T=treated with spice;

(A). Potatoes

(B). Taro root

(C). Bottle gourd



(D). Tomatoes

Fig 1: Aqueous extract spices-treated and untreated foods (A). Potatoes (B). Taro root (C). Bottle gourd and (D). Tomatoes.

In second set of experiment the treatment was given with dried powder of the four most commonly used spices-turmeric, black pepper, garlic and cumin and their mixtures. Surprisingly, it was observed that the number of microbial counts in control as well as treated foods dropped down 10 fold when compared with aqueous extracts. Black pepper was highly active in control of microbial counts in all four vegetables - potatoes, taro roots, bottle gourd and tomatoes followed by garlic turmeric and cumin powder (Table 3). When the mixture of turmeric + black pepper; turmeric + garlic, turmeric + cumin and the mixture of turmeric + black pepper + garlic + cumin were used it was found that the mixture of all four spices was very effective in protection of foods against spoilage and from the microbial counts (Table 3).

Table 3: Number of bacterial colony forming units (× 10^3/g fresh weight) isolated from powdered spices-treated and untreated foods stored in refrigerator at 4 to 10° C temperature over a period of 3 days (each figure is an average of 3 independent replicates).

Vegetable	Potatoes						Taro Root						Bottle gourd						Tomatoes					
	Day 1		Day 2		Day 3		Day 1		Day 2		Day 3		Day 1		Day 2		Day3		Day 1		Day 2		Day3	
Spices	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T
Turmeric	168	45	489	120	670	289	158	54	466	136	868	312	256	76	490	128	864	336	98	0.86	236	24	560	86
Black pepper	168	23	489	68	670	188	158	36	466	85	868	186	256	42	490	76	864	230	98	0.24	236	10	560	44
Garlic	168	36	489	85	670	294	158	48	466	112	868	226	256	58	490	114	864	268	98	0.54	236	18	560	58
Cumin	168	56	489	134	670	306	158	68	466	146	868	246	256	64	490	156	864	290	98	0.68	236	38	560	72
Turmeric+ Black Pepper	168	0.65	489	20	670	89	158	0.74	466	36	868	62	256	0.78	490	48	864	110	98	0.14	236	0.56	560	0.92
Turmeric + Garlic	168	0.86	489	34	670	110	158	0.93	466	49	868	86	256	0.86	490	54	864	136	98	0.36	236	0.88	560	10
Turmeric + Cumin	168	0.96	489	45	670	156	158	0.98	466	58	868	94	256	0.96	490	62	864	180	98	0.58	236	0.92	560	18
Turmeric + Black pepper +Garlic +Cumin	168	0.22	489	09	670	46	158	0.34	466	14	868	30	256	0.38	490	21	864	56	98	0.09	236	0.12	560	0.34

(A)Potatoes with turmeric

(B).Taro root with turmeric

(C) Bottle gourd with turmeric

(D).Tomatoes with turmeric

(E)Potatoes with black pepper

(F) Taro root with black pepper

(G) Bottle gourd with black pepper

(H) Tomatoes with black pepper

(I) Potatoes with garlic

(J) Taro root with garlic

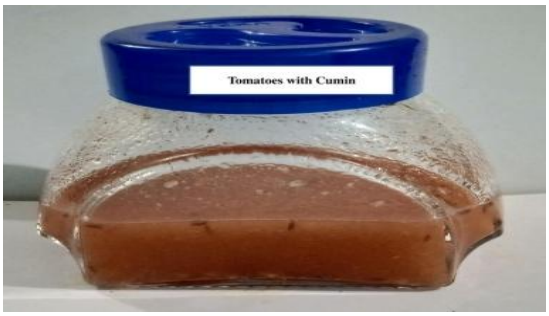
(K) Bottle gourd with garlic

(L) Tomatoes with garlic

(M) Potatoes with cumin

(N) Taro root with cumin

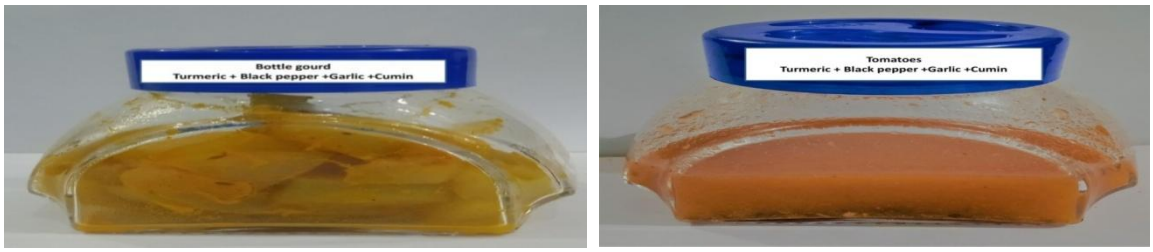
(O) Bottle gourd with cumin



(P) Tomatoes with Cumin

(Q) Potatoes with garlic, turmeric, cumin and black pepper

(R) Taro root with garlic,turmeric,cumin and black pepper



(R) Bottle gourd with garlic, turmeric, cumin and black pepper

(S) Tomatoes with garlic, turmeric, cumin and black pepper

Fig 2. Powdered spices-treated and untreated foods (A) Potatoes with turmeric (B).Taro root with turmeric (C) Bottle gourd with turmeric (D).Tomatoes with turmeric,(E). Potatoes with black pepper (F). Taro root with black pepper (G) Bottle gourd with black pepper (H) Tomatoes with black pepper (I) Potatoes with garlic (J) Taro root with garlic (K) Bottle gourd with garlic (L) Tomatoes with garlic (M) Potatoes with cumin (N) Taro root with cumin (O) Bottle gourd with cumin (P) Tomatoes with Cumin (Q) Potatoes with turmeric+black pepper+garlic+cumin (R) Taro root with turmeric+black pepper+garlic+cumin (S).Bottle gourd with turmeric+black pepper+garlic+cumin (T) Tomatoes with turmeric+black pepper+garlic+cumin.

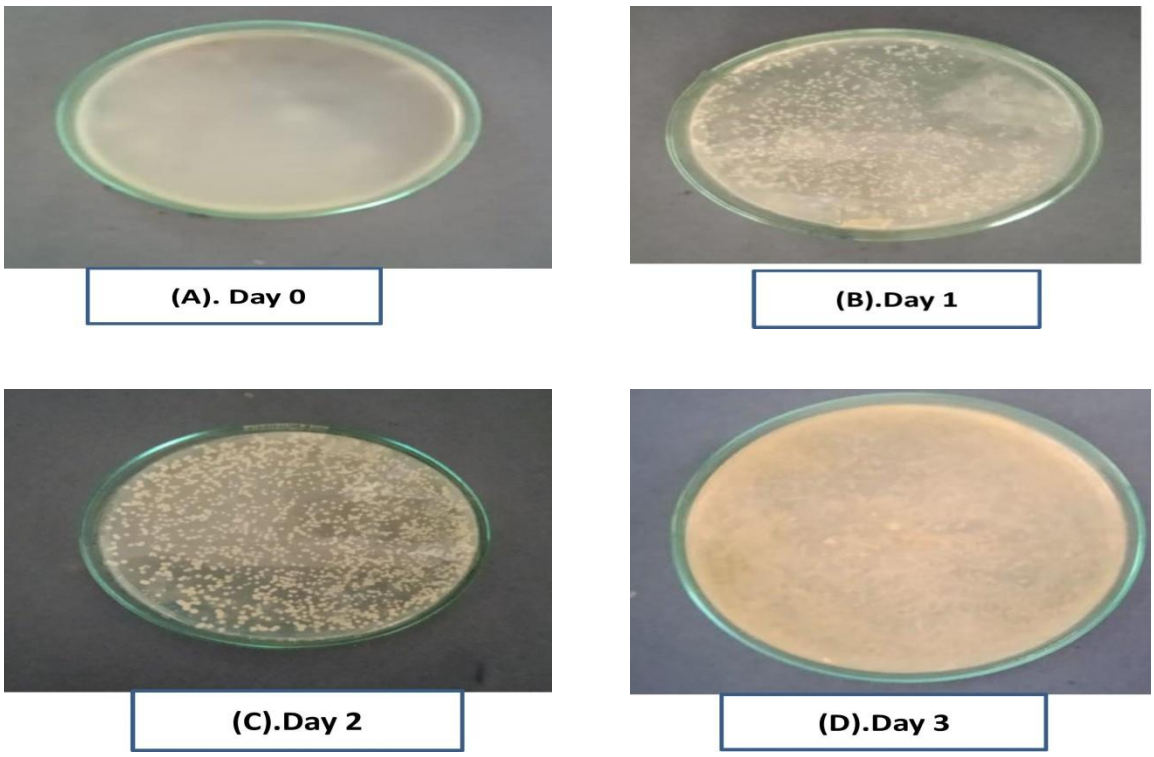


Fig 3: Bacterial colonies formed on spices-treated and untreated foods stored in refrigerator at 4 to 10° C temperature over a period of 3 days (A) Day 0, (B) Day1, (C) Day 2 and (D) Day 3.

Mostly food borne pathogens caused severe diseases like typhoid, diarrhea, and cholera etc [31]. Normally cooked food eliminates microorganisms on food before it reaches our plate while uuncooked

foods are typical sources of food illness since they do not pass through high temperature [32, 33]. Microbes can contaminate food in a variety of ways, including growth, nutrient consumption, enzymatic alterations, unpleasant flavors, chemical breakdown, and the production of chemicals. Food deterioration affects the human health and cause disease like diarrhea, cholera, vomiting, nausea and stomach pain [34, 35, 36]. An increasing percentage of consumers especially after Covid-19 pandemic prefer minimal processing of foods, without artificial preservatives. Many of these processed foods and novel food types represented innovative food system in terms to health hazard and nutritional risk [37, 38]. In considering this, and depending on greater awareness of the complex nature of microbial interactions, recent strategies focus increasingly on the potential that biological preservation offers. The antimicrobial properties of common spices were used in this study to evaluate their potential to increase shelf life of refrigerated foods without any chemical preservatives. It is a common practice in Indian, Asian and western kitchen including Nordic countries the extra foods are stored in the refrigerator for further use but many times it develop foul smell and get deteriorated due to microbial growth. Our studies shows that if the extra food is treated with dried powder of mixture of turmeric + black pepper + garlic + cumin before storage in the refrigerator, it can save the food from spoilage, can add to the flavor and aroma of the food and is safe for consumption.

Conclusions

Our study showed that Indian spices may be used to increase the shelf life of refrigerated foods and the treatment of boiled vegetable with dried powder of turmeric + black pepper + garlic + cumin within eatable limits (200 mg/100 g) can enhance shelf life of refrigerated food by protecting them from microbial growth and contamination. Additionally, they add flavor, aroma and color to the food. The spices are safe to be used for human consumption. They have numerous health benefits.

Competing interest

The authors declare that they have no conflict of interest.

Authors Contributions:

Priya conducted all experiments and collected the literature while APG designed the experiments, conceptualized the idea and wrote the manuscript.

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