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**Original Article**

# Enhanced antibacterial activity of medicated and non-medicated toothpaste using *green tea* extract and nanoformulations: An *in vitro* mapping of nanophasic area

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## ABSTRACT

The objective of present investigation was to prepare *green tea* nanoformulation and evaluate its enhanced antimicrobial activity as well as hypocholesterolemic effect. Aqueous phase titration method was employed for constructing the phase diagram to localize nanophasic region in a pseudoternary phase diagram. Formulations were selected from optimized nanophasic regions followed by further screen using thermodyanamic and dispersion study. Optimized formulations were further characterized on drug release and percent transmittance studies. The constructed phase diagrams showed increase in nanophasic area upon increasing the surfactant contribution in comparison to co-surfactant concentrations and vice-versa. Thermodynamic study showed destabilization of the selected formulations leading to phase separation because of inappropriate emulsifier and formation of liquid crystalline regions. Formulation $NE^{Green\ Tea}5$ was optimized, containing Oil [10%, v/v; green tea (2.5%): capryol 90 (7.5%)], polysorbate 80 (24%, v/v), Sodium taurocholate (8%, v/v), and distilled water (58%, v/v), on the basis of better thermodynamic stability study, drug release and % transmittance. The optimized formulation $NE^{Green\ Tea}5$ was mixed with different medicated and non-medicated toothpaste to evaluate their comparative anti-microbial study. A significant enhanced zone of inhibition was observed in medicated ($p<0.005$) and non-medicated ($p<0.01$) toothpaste containing $NE^{Green\ Tea}5$. The increasing anti-bacterial activity observed was followed as non-medicated [$NE^{Green\ Tea}5$ (0%)] < medicated [$NE^{Green\ Tea}5$ (0%)] < *Green tea* extract <non-medicated [$NE^{Green\ Tea}5$ (50 *v/v*%)] < medicated [$NE^{Green\ Tea}5$ (50 *v/v*%)] < $NE^{Green\ Tea}5$ (100 *v/v*%). Anti-bacterial results concluded the significant role of *Green tea* nanoformulation and partial inability of marketed toothpastes to fight the oral microfloral growth have been observed.

**Key words:** Dental infection, Antimicrobial agents, Cup-plate method, Medicated tooth paste, Green tea.

## INTRODUCTION

Phytochemicals have gained increased acceptance in the recent years due to their implication in a number of pharmaceutical and cosmeceutical products. Due to their natural origin, immense therapeutic activity and minimum possible adverse drug reaction further attracted the global interest in the study of various medicinal plants. Green tea (GT) is believed to be one of the most accepted essential oil obtained from steam distilled leaves of *Camellia sinensis*. Green tea catechins have gained attention in many life science research studies due to positive physiological effects combined with antimutagenic, antibacterial [1, 2], antifungal [3,4], anti-inflammatory [5], cardioprotective [6, 7], anti-oxidant [2, 5], anti-cancer [8-11] and in various neurological disorders [12-16] and anticarcinogenic -antitumerogenic activities [17]. The green tea contains the group of flavonoids with the major catechins such as (-)-epigallocatechin, (-)-epigallocatechin gallate, (-)-epicatechin and (-)-epicatechin gallate [18]. These catechins of GT have strong antioxidant and free radical scavenging activity which leads their tremendous health benefit. Besides their tremendous therapeutic applicability of green tea, clinical benefits are still below optimum. This is because of poor dissolution, low permeability and extensive hepatic metabolism. Therefore, some novel therapeutic approaches are required to get their optimum clinical benefits.

Conventional delivery of these phytochemical didn't simulate *in vitro* experimental results obtained with the *in vivo* results. This may be because of insufficient drug concentration due to poor solubility, poor absorption, extensive metabolism, pre-systemic clearance and high fluctuation of plasma levels after per oral

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administration. A promising strategy to overcome these problems involves the development of suitable novel drug carrier. In recent years, the attention of many researchers have focused on nanolipidic carriers as an alternative to conventional drug delivery, because of combined advantages and minimal disadvantages of other colloidal carriers. Also, nanocarriers is one the most prevailing approach for the enhancement of topical permeation of essential oils [19-21] with the advantages of thermodynamic stability, protection of labile drugs from degradation, and excellent tolerability pattern [22-24]. Different formulations were prepared by aqueous titration method, employing capryol 90 as drug base, polysorbate 80: sodium taurocholate as surfactant mixtures ($S_{mix}$), and GT as a model drug. For analysis, reported HPLC–ESI-QTOF-MS [25, 26] was used and method validated in our laboratory.

Good oral hygiene i.e. healthy teeth and gums is one of the most important issues now a day's. Dental pulp infection, as a result of caries, is the leading cause of odontogenic infection. The major pathogens identified in dental caries are members of viridens (alpha-hemolytic) streptococci family including *Streptococcus mutans*, *Streptococcus sobrinis* and *Streptococcus milleri*. Once bacteria invade the dental pulp, an inflammatory reaction results in necrosis and a lower tissue oxidation-reduction defence potential. There are numbers of medicated and non-medicated toothpastes are available for dental hygiene and care. Apart from that, the rate of dental infection is growing with their constant rate. Green tea is the richest source of catechins having strong antioxidant and free radical scavenging activity, and they have been tested as potent antimicrobial and antiviral agent apart from vivid therapeutic applicability [27]. Therefore, purpose of present research work was to study the enhancement of antimicrobial effect after incorporation of GT extract and their nanoformulation on available medicated and non-medicated toothpastes in the market for better oral healthcare.

## MATERIALS AND METHODS

**Material:** Green tea was purchased from local mall (Dawadmi, Saudi arabia). Polysorbate 80, Polysorbate 20, Polyethylene glycol (PEG-200), and Brij 32 were purchased from CDH (Mumbai, India). Capryol™ 90 (Propylene glycol monocaprylate), Carbitol (diethylene glycol monoethyl ether), Cremophor-EL (polyoxy-35-castor oil), Labrasol (caprylocaproyl macrogolglycerides), Labrafil (linoleoyl macrogolglycerides), and plurol oleique (polyglycerol oleate) were gift samples from Gattefosse (Saint Priest, Cedex, France). Rest of the required chemical were used of analytical grade which was obtained from local vendors (Riyadh, Saudi Arabia).

### Methodology

**Preparations of plant extracts:** Green tea (*Camellia sinensis*) leaves (50 g) was kept in a round bottom flask containing 200 ml of ethanolic distilled water (10% v/v) and connected with the distillation unit. The whole assembly was kept overnight for proper imbibitions. After 24 h, the assembly was put on distillation for extraction. The distillates were concentrated to get viscous plant extracts.

**Solubility study:** Solubility of GT extrats was determined in different oily lipids to choose the internal phase. An excess amount of extract was added in 1 mL of each excipient separately in 5 ml capacity stopper vials. These vials were then kept at 25 ± 0.5 °C in an isothermal shaker (Nirmal International, Delhi, India) for 72 hours to achieve equilibrium. The equilibrated vials were removed from shaker and centrifuged at 3000 rpm for 15 min using a centrifuge (Remi, India). The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of GT extracts was determined in different excipients by reported HPLC method using catechin and gallic acid as a marker compound [26, 28]. The mobile phase consisted of 0.1% acetic acid (Mobile phase A) and acetonitrile (Mobile phase B) and gradient elution was performed by varying A and B at 1.0 ml/min of total flow. The marker compound catechin and gallic acid were detected at UV 280 nm. The sample injection volume was used 10 µl. The surfactants and co-surfactants were optimized on the basis of the phasic behaviour with the selected oil.

**Selection of surfactant-co surfactant mixture ($S_{mix}$):** Possible combination of $S_{mix}$ was selected based on nanoemulsification performance. For this study, different surfactant-co surfactant mixtures (1:0, 1:1, 1:2, 1:3, 2:1, 3:1 and 4:1) were premixed with 1:9 to 9:1 ration of oil-$S_{mix}$ followed by aqueous titration with distilled water. The obtained nanophasic area was calculated using paper weight method. $S_{mix}$ compositions showing maximum nanophasic area were selected for formulation optimization.

**Nanophasic map construction:** In order to find out the concentration range of various components for the existence range of nanolipid carrier, pseudoternary phase diagrams were constructed using aqueous titration technique. The concentrated extracts (GT extract) was premixed with capryol 90 and combination of polysorbate 20 and Sodium taurocholate was selected as surfactant and cosurfactant respectively, on the basis of nanophasic performance. Distilled water (maintained at water bath; 25±0.2 °C) was used as an external media for titration. Fixed ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1) of surfactant and cosurfactant ($S_{mix}$) were used for aqueous titration. These $S_{mix}$ ratios were chosen in increasing concentration of co-surfactant with respect to surfactant and increasing the concentration of surfactant with respect to co-surfactant for comprehensive study. For each phase diagram, Oil phase and specific $S_{mix}$ ratio was mixed thoroughly in different volume ratios ranging from 1:9 to 9:1. Slow titration with aqueous phase (hot distilled water) was done for each combination of oil and $S_{mix}$ separately [19, 22, 23, 29]. The region of nanoemulsions was marked on a quarternary component based phase map with one axes representing the aqueous phase, oil phase, and the third representing a mixture of surfactant and co-surfactant at fixed volume ratios. The nanophasic areas of different phase diagram were calculated using paper weight method.

**Criteria of formulation development:** Different formulations were selected from the phase diagram showing nanoemulsion region on the following basis.

1. The oil phase should sufficient to dissolves the effective dose of *green tea* extract.
2. External phase concentration (water) must be always greater than the internal phase (oil phase).
3. For each percentage of oil selected, the emphasis was given to those compositions which contained minimum concentration of $S_{mix}$ to give nanoformulations and off course with all thermodyanamic stability.

**Physical stress tests**

***i. Centrifugation stress:*** Green tea nanoformulations ($NE^{Green\ Tea}$) were centrifuged using REMI International® (Mumbai, India) at 3500 rpm for 30 minutes. Those formulations which did not show any phase separation, precipitation and turbidity were taken for the Heating cooling cycle and those which didn't survived the stress were dropped from next study [24].

***ii. Freeze-thaw stress:*** The formulations were exposed to three freeze-thaw cycles at two different temperature conditions (-21±0.5 ºC and 25±0.5 ºC) using with storage at each temperature for not less than 48 hours [21].

***iii. Heating-cooling stress:*** Six cycles of heating and cooling were performed at 45±0.5 ºC and 4±0.5 ºC respectively with storage at each temperature for not less than 48 hours. Those formulations, which were stable at these temperatures, were subjected to freeze thaw testing [20, 29].

**Dispersibility/system dilutability:** Self-emulsification efficiency of nanoformulations (1 ml) was assessed using beaker (500 ml) filled with 200 ml of distilled water maintained at 37±1.0 ºC. *In vitro* performance of dispersibility was visually assessed by following grading system (Table 1).

**Table 1:** Dispersibility/dilutability performance and formulation grading system.

| Formulation (Grades) | Appearance | Dispersion time (second) |
|---|---|---|
| Grade A | Rapidly dispersing with a clear and transparent appearance | >30 |
| Grade B | Rapidly dispersing with transparent and bluish ting appearance. | ≥60 |
| Grade C | Slow dispersing with translucent and bluish appearance. | 60-90 |
| Grade D | Very slow dispersing with translucent and milky appearance | 90-120 |
| Grade E | Formulation, exhibiting either poor or minimal dispersion with large oil globules present on the surface. | ≥120 |

Formulations that passed thermodynamic stability as well as dispersibility test in Grade A were selected for further studies.

**Anti-microbial study**

***i.*** **Culture and isolation of bacterial strains:** The bacterial strains used in this study were isolated from early morning swab from patients with dental infection. The obtained mouth swab was stored in the normal saline (0.9% w/v) and later kept in BOD chamber maintained at 35±0.5 °C. Cultured strains were kept at a turbidity matching a 0.5 McFarland standard and placed in each well with a final concentration of $2.5\times10^5$ colony-forming unit (CFU) /ml.

***ii.*** **Agar well diffusion assay:** The antimicrobial activity of the green tea extract and nanoformulations was evaluated by agar well diffusion method. Bacteria were grown in Muller Hinton broth (HiMedia Laboratories Ltd., India) to match the turbidity of 0.5 McFarland standards to be inoculated on Muller-Hinton agar (HiMedia Laboratories Ltd., India by streak plate method. After inoculation, plates were stored at 37±0.5 °C for (15 min) for proper drying, and the wells (30 mm diameter) were created using sterile cork borers. The wells were filled with 100 µL of green tea extracts, nanoformulation and different compositions of $NE^{Green\ Tea}$ (0 & 50% v/v) with toothpastes. Commercially available gentamicin (10 µg/ml), (GENTAMICIN® (1.6 mg/ml%), B. BRAUN MEDICAL INC, USA) was used as a positive control in this study. All steps were performed aseptically to avoid any contaminations. Plates were incubated for 24 h at 37±0.5 °C. The diameters of the zone of inhibition (mm) for different formulations were measured. All experiments were done in triplicate and the average values were used (mean± sd).

**Statistical analysis:** All experimental results were presented as a means of triplicate observation (mean± sd) and the data in the tables and figures also followed the same (n=3). Statistical analysis was performed by one-way analysis of variance (ANOVA) and Dunnett test using Graph-Pad Prism version 4.0 (San Diego, CA, USA). Differences were considered significant at $P < 0.05$.

## RESULTS AND DISCUSSION

**Solubility study:** The most important step for the preparation of nanoemulsions was the assortment of oils having requisite drug solubility justifying therapeutic dose. For present study, sefsol 218 (matrix) and capryol 90 possessing the highest solubilising power (11.95±1.18 mg/mL) was selected Fig. (1) as a matrix. After selecting the matrix, different surfactants, co-surfactants and their blends ($S_{mix}$) were tried to check the nanoemulsification efficiency. The nanophasic area of capryol 90 obtained after aqueous titration using equimolar ratio of different $S_{mix}$ is mentioned in Table 1. Maximum nanophasic area (73.12±4. 92 $cm^2$) was observed for Polysorbate 80 (*Surfactant*) and sodium taurocholate (*Cosurfactant*) (Table 1). Therefore, based on maximum nanophasic area polysorbate 20 and taurocholate were selected and different ratios of $S_{mix}$ (1:0, 1:1, 1:2, 1:3, 2:1, 3:1 and 4:1) were used in the development nanoformulation.

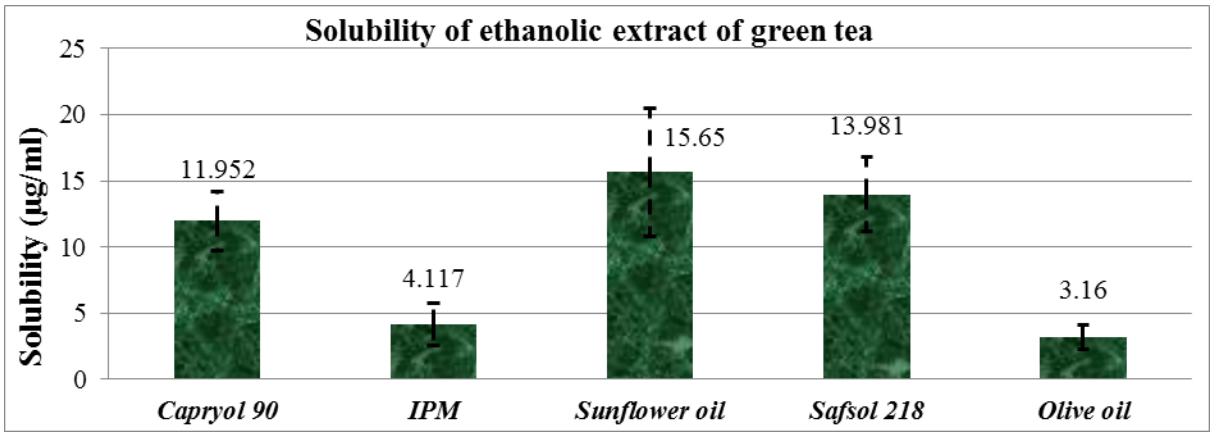


**Figure 1**: Solubility of ethanolic aqueous extract of green tea in different oil phases.

**Table 2:** Nanophasic performance of equimolar ratios of different $S_{mix}$

| Oil phases | Polysorbate 80-taurocholate | Polysorbate 20-taurocholate | Polysorbate 80-PEG 200 | Polysorbate 20-PEG 200 | Polysorbate 80-PEG 400 | Polysorbate 20-PEG 400 |
|---|---|---|---|---|---|---|
| **Capryol 90** | √ | √√ | x | √ | x | √ |
| **IPM** | x | x | √ | √ | x | x |
| **Sunflower** | x | √ | √ | √ | x | x |
| **Sefsol 218** | √ | √ | √ | √ | √ | **x** |
| **Olive oil** | √√ | √√ | x | √√ | **x** | **x** |

***Note:*** *Nanophasic area less than 50 mm$^2$ was not considered significant for selecting $S_{mix}$. Each* √ represents 50 *mm$^2$ where as x represents* <50 *mm$^2$.*

The results showed that polysorbate 20 and taurocholate (both are non-ionic surfactants) were found the most efficient in giving large nanophasic area for selected matrix capryol 90, therefore selected as a $S_{mix}$ system. Polysorbate 20, a non-ionic surfactants are often recommended in pharmaceutical formulation since these are less toxic and showed minimal variation of pH and ionic strength.

**Constructing nanophase diagram:** To found concentration range of various components and their existence range of nanophasic area, pseudoternary phase diagrams were constructed using low energy emulsification by aqueous titration technique at room temperature (27±0.5 °C). While constructing the phase diagrams, care was taken in order to ensure not to select metastable systems [30, 31]. Surfactant (polysorbate 20) and taurocholate were pre-mixed in different volume ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1) to get the maximum possibilities of required HLB value and hence nanophasic region. These $S_{mix}$ ratios were prepared on increasing concentration of co-surfactant with respect to surfactant and *vice versa*. Pseudoternary phase diagrams were constructed separately for each $S_{mix}$ ratio selected previously, so that O/W nanoregions could be defined (Figure 2). It was clear that, the compositions consisting of low oil phase and high surfactant phase give metastable nanogel region. Although, the obtained nanogel region spontaneously converted into nanoemulsion region, but sometime it leading to failure of the system. Therefore, we tried to discard the formulation coming in this region (Figure 1). Different formulation was selected from these nanoregions for formulation optimization. Following the phases diagrams consisting of $S_{mix}$ 1:1, 1:2 and 1:3, a decreasing nanophasic area was obtained. The phase diagram of $S_{mix}$ 3:1 showed, an increasing trend of nanophasic area. Although, the phase diagram of $S_{mix}$ 3:1 showed amplified nanophasic region incorporating large liquid crystalline (LC) region. These LC regions were found unable in breaking the interfacial tension between oil-water interfaces which is required to get the spontaneous nanoemulsification. Therefore nanophasic area obtained was less. But after adding co-surfactant ($S_{mix}$ 1:1 and 1:3), an increased nanophasic area was obtained with negligible LC region. The co-surfactant helped in making interfacial film more flexible leading to decreased in LC area appeared. A decrease in interfacial tension further increase the entropy of the system leading to spontaneous emulsification [32]. The increased in nanophase area was insignificant when surfactant concentration in $S_{mix}$ was increase from 1:1. This could be the metastable gel region generated by polysorbate 20 and it was continuously decreased after adding the sodium taurocholate. These results conclude that free energy of nanoemulsion formation is somehow dependent on the extent to which the surfactant and co-surfactants lower the interfacial tension of the oil–water interface [33]. The increase in free energy and dispersion entropy leading to the formation of spontaneous and thermodynamically stable nanomeulsion [33]. Therefore, while selecting the formulations composition from each phase diagrams, the care was taken to select those which could accommodate optimum quantity of oil phase by using lowest possible $S_{mix}$ to further avoid the possibility of liquid crystalline or metastable gel region.

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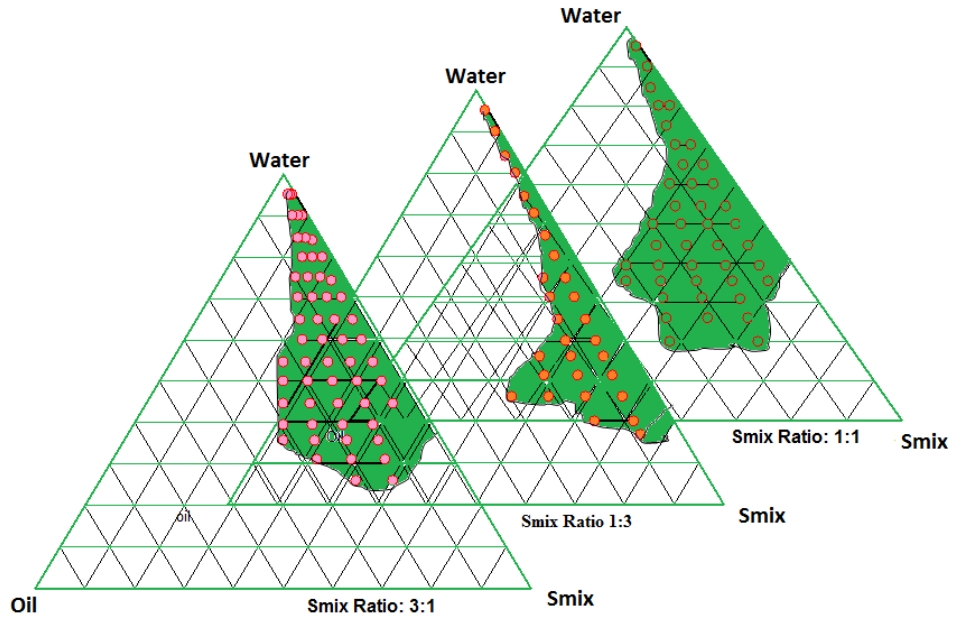


**Figure 2:** Phase diagram showing nanophasic region in different $S_{mix}$ (Polysorbate 20-taurocholate) ratios using capryol 90 as an oil phase containing green tea extracts.

Different formulations were selected at different points from the nanophasic area of different diagrams (1:0, 1:1, 1:2, 1:3, 2:1, 3:1 and 4:1 phases) which was sufficient to solubilised drug dose in their oils domain (Table 2). From each phase diagrams nanoformulations were selected at a difference of 2% oil phase (10, 12, 14, 16 and 20%). Almost in all cases, $S_{mix}$ concentration was kept below 40% and water phases' ≥49% of total formulation composition.

***Thermodynamic stability and system dilutability:*** In the search of a robust formulation and to eliminate the problems of metastable formulation, thermodynamic stability/ physical stress tests (centrifugation, heating–cooling cycle and freeze–thaw cycle) were performed as already discussed in our previous study [20]. In physical stress testing real fate of the nanoformulations can be observed as shown in Table 3.

**Table 3:** Different physical stress tests of nano-formulations selected from phase diagrams.

| $S_{mix}$ **ratio (S:CoS)** | **Formulation code** | **Compositions (v/v)** | | | **Stress stability tests** | | | **Dilutability** |
|---|---|---|---|---|---|---|---|---|
| | | **Oil** | **$S_{mix}$** | **Water** | **H/C** | **Cent** | **Freez/Thaw** | |
| 4:1 | $NE^{Green\ Tea}$ 1 | 10 | 32 | 58 | √ | √ | √ | Grade A |
| | $NE^{Green\ Tea}$ 2 | 12 | 35 | 53 | √ | x | - | - |
| | $NE^{Green\ Tea}$ 3 | 14 | 37 | 49 | √ | x | - | - |
| 3:1 | $NE^{Green\ Tea}$ 4 | 10 | 32 | 58 | √ | √ | √ | Grade A |
| | $NE^{Green\ Tea}$ 5 | 12 | 35 | 53 | √ | √ | √ | Grade A |
| | $NE^{Green\ Tea}$ 6 | 14 | 37 | 49 | √ | x | - | - |
| 2:1 | $NE^{Green\ Tea}$ 7 | 10 | 32 | 58 | √ | √ | √ | Grade A |
| | $NE^{Green\ Tea}$ 8 | 12 | 35 | 53 | √ | √ | √ | Grade A |

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| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $NE^{Green\ Tea}$ 9 | 14 | 37 | 49 | √ | √ | √ | Grade B |
| 1:3 | $NE^{Green\ Tea}$10 | 10 | 32 | 58 | √ | √ | √ | Grade B |
| | $NE^{Green\ Tea}$11 | 12 | 35 | 53 | √ | x | - | - |
| | $NE^{Green\ Tea}$12 | 14 | 37 | 49 | x | - | - | - |
| 1:2 | $NE^{Green\ Tea}$13 | 10 | 32 | 58 | √ | √ | √ | Grade B |
| | $NE^{Green\ Tea}$14 | 12 | 35 | 53 | √ | √ | √ | Grade B |
| | $NE^{Green\ Tea}$15 | 14 | 37 | 49 | √ | x | - | - |
| 1:1 | $NE^{Green\ Tea}$16 | 10 | 32 | 58 | √ | √ | √ | Grade A |
| | $NE^{Green\ Tea}$17 | 12 | 35 | 53 | √ | √ | √ | Grade B |
| | $NE^{Green\ Tea}$18 | 14 | 37 | 49 | √ | x | - | - |
| 1:0 | $NE^{Green\ Tea}$19 | 10 | 32 | 58 | √ | x | - | - |
| | $NE^{Green\ Tea}$20 | 12 | 35 | 53 | √ | √ | **x** | - |
| | $NE^{Green\ Tea}$21 | 14 | 37 | 49 | √ | - | - | - |

During thermodynamic stability and system dilutability study, some formulations became turbid and in some case phase separation was observed. One reason of this instability may be due to Ostwald ripening in which molecules move as a monomer and coalescence of smaller droplet took place, resulting in the formation of large droplets by diffusion process driven by the loss in surface free energy. Wennerstrom and Olsson (2009) prove the role of temperature in Ostwald ripening [34]. During stress stability study, temperature quenches leading instability of nanoformulation due to separation of oil phase and droplet distribution of smaller size which favours changes in curvature free energy. After thermodyanamic stability study, formulations which showed no phase separation, creaming, cracking, coalescence and phase inversion were selected. The compositions of these selected formulations are shown in Table 3. Selected formulations were also evaluated for their infinite dilutability (Table 3). Previous research work suggested that, formulations with Grade A & B with no any precipitation showed their size in nanometric range [20, 21]. Therefore, in the present study, formulations with grade A were included. The droplet size distribution will be ascertained by electron microscopic study and particle size distribution study which is under progress.

**Anti-microbial study:** The formulations which passed thermodyanamic stress tests and infinite dilutability tests with grade A were selected for further study. Phases with high concentration of surfactant (4:1, 3:1, 2:1) showed good thermodynamic performance. The phase consists of high concentration of co-surfactant showed poor thermodyanamic performance and dilutability. Therefore formulations from these phase diagram were not included for further study. Similarly, phase diagram consists of $S_{mix}$ 1:0 and 4:1 showed very high liquid crystalline region which may lead to metastability. Therefore, formulation from $S_{mix}$ 3:1 and 2:1 were selected for their anti-microbial study. Form preliminary anti-microbial study, $NE^{Green\ Tea}$ 4 was selected from phase diagram ($S_{mix}$ 3:1). Comparative antimicrobial study of available medicated and non-medicated toothpastes along with green tea extract and their nanoformulations were performed as shown in Figure 3 and 4. The antimicrobial results showed a significant role of green tea extracts ($p<0.05$) in countering oral bacterial growth compared to medicated toothpastes. The enhanced antimicrobial properties of green tea formulations could be as a result of epigallocatechin gallate present which is acting as an antibacterial agents may be because of protein precipitation and various other mechanism [35, 36]. The anti-microbial potential of green tea was significantly high ($p<0.001$) after converting into nanoformulations. The comparative difference in zone of inhibition by medicated toothpastes after mixing with green tea nanoformulatiosn (1:1 v/v) was found similar with the standard Gentamycin (10 µg/ml). The zone of inhibition by medicated toothpastes after mixing with green tea extract showed compromised activity. This might be the consequences of compromised permeability of crude extract compared to its nanoformulations. Similarly, the antimicrobial potential of medicated and non-medicated tooth pastes got enhanced after formulating with green tea extract and nanoformulations

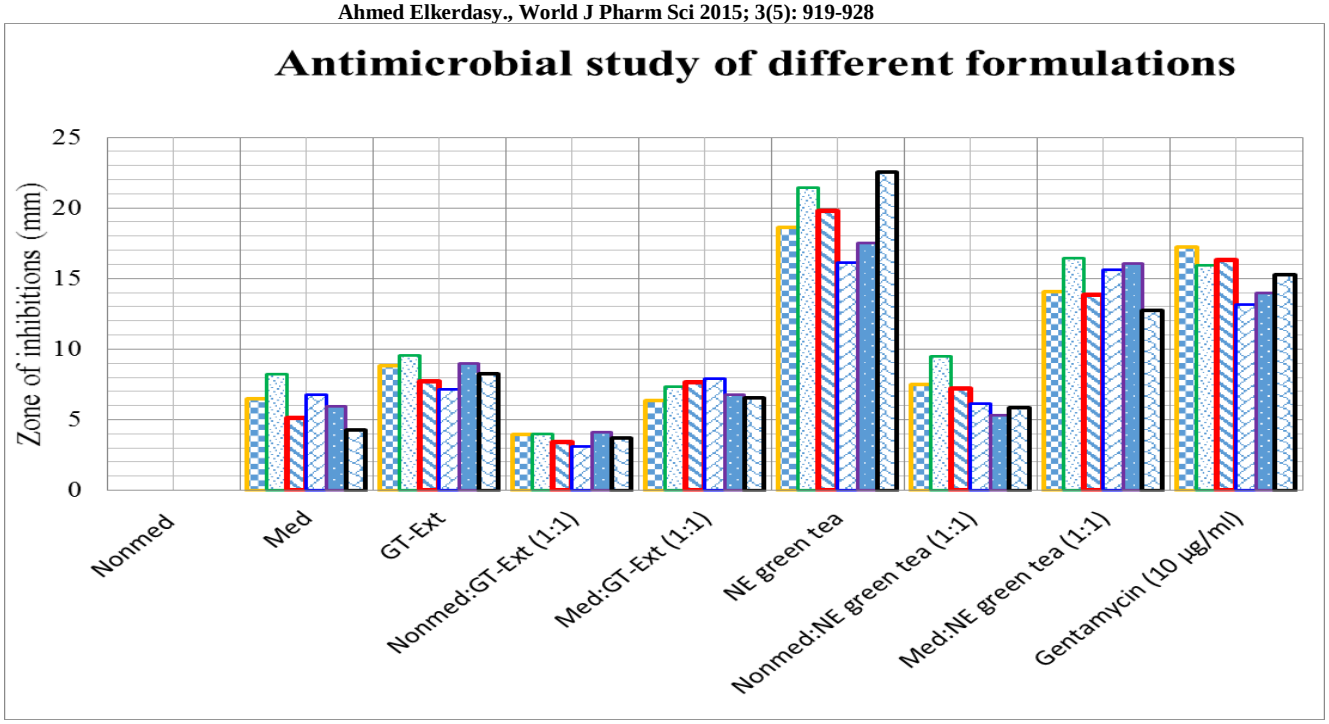


**Figure 3:** Individual plate performance of medicated and non-medicated toothpastes, Green tea extract, Green tea nanoformulation and their combinations. Abbreviations used were: Med (Medicated toothpaste), Nonmed (Non-medicated toothpastes), GT-Ext (Green tea extract), NE green tea (Green tea nanoformulation).

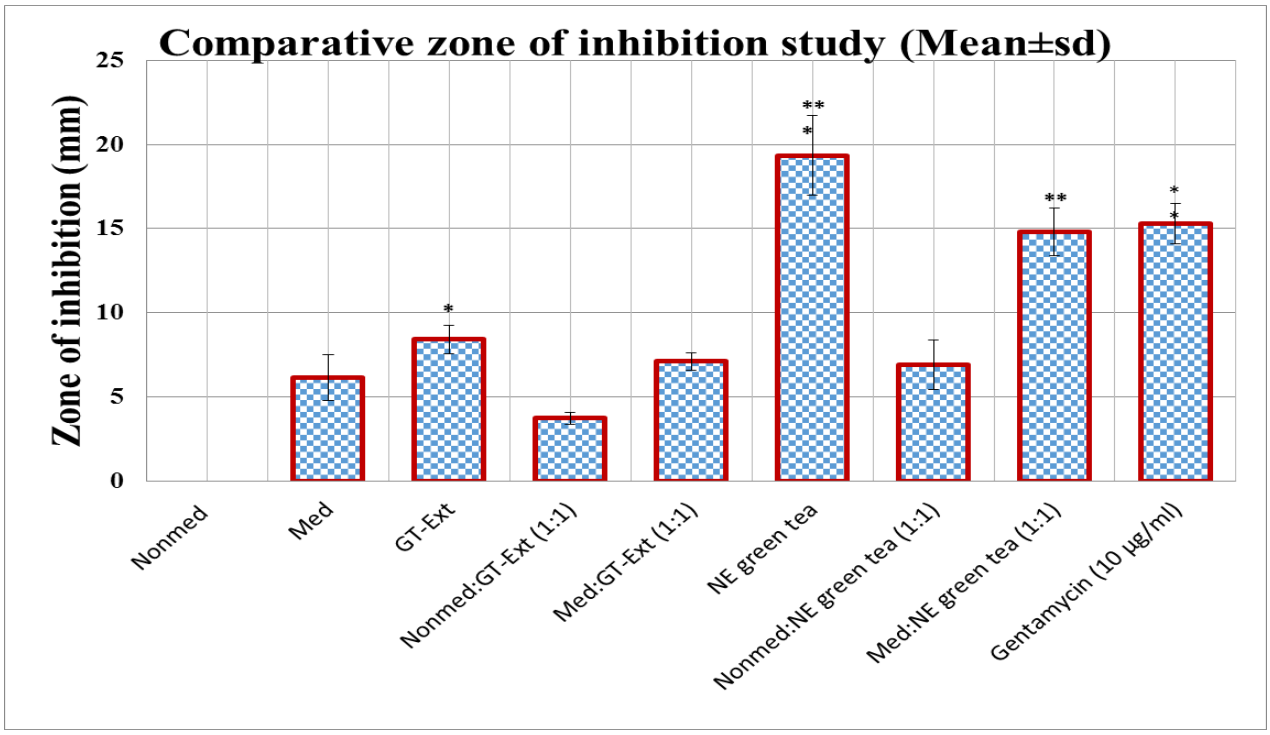


**Figure 4:** Comparative antimicrobial study of medicated and non-medicated formulations and their combinations with Green tea extract and nanoformulations ($NE^{Green\ Tea}$). Data represented as the mean ± standard deviation of three replicates. Here *p*<0.05*, *p*<0.01* and *p***<0.001*.Abbreviations used were: Med (Medicated toothpaste), Nonmed (Non-medicated toothpastes), GT-Ext (Green tea extract), NE green tea (Green tea nanoformulation).

## Conclusion

An ethanolic extract of green tea and its nanoformulation showed excellent anti-bacterial activity. The larger zone of inhibition for $NE^{Green\ Tea}$ proved their better penetrating and permeation potential in comparision to crude green tea exract. The anti-bacterial property of medicated toothpastes was significantly high where as non-medicated toothpastes showed inability. After mixing the optimized $NE^{Green\ Tea}$ with medicated and non-medicated toothpastes showed multifold increase in the anti-bacterial activity because of synergistic effect. Therefore it is concluded that, green tea nanoformulation might be a potential component of medicated tooth paste to enhance antimicrobial activity for better oral hygiene.

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