DEVELOPMENT OF NANO-CARRIER BASED ANTIBACTERIAL FORMULATION OF GREEN TEA AND SYNERGISTIC PHYTOCHEMICALS

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BACKGROUND AND RATIONALE

Natural compounds have been used as therapeutic agents since ages, owing to their wide spectrum of pharmacological properties [1]. The extensive research work carried out for developing these natural products as alternative therapeutic agents has presented a whole new array of drug discovery. Use of these natural compounds even at higher doses exhibited minimal toxicity and side-effects [2]. Increasing prescription of natural compound based products has extended the area and scope of natural therapy with supporting reports disclosed by World Health Organization (WHO). According to one of the latest reports, WHO confirmed a global surge in the commercialization of natural products. Approximately, 80% of patients all over the world comply with the use of natural compounds out of which 25% are from western countries [3].

Green tea is one of the most commonly consumed drink over the world not just because of its refreshing flavour but also for its diverse range of biological and pharmacological effects like anti-microbial, anti-inflammatory, anti-tumour, anti-ageing and many more [4]. Anti-oxidative potential of green tea is the central and multi-faceted feature that is found to be responsible for these pharmaco-therapeutic effects [5]. Catechins from green tea, a subclass of plant polyphenols is also accountable for anti-microbial activity [6]. Green tea catechins (GTCs) have been shown to be effective against both gram positive (*Staphylococcus aureus*, *Enterococcus* spp.) as well as gram negative bacteria (*Escherichia coli*, *Salmonella* spp.). Wide variety of fungal strains (e.g., *Candida albicans*) and viruses (e.g., HIV, herpes simplex and influenza) were also found to be sensitive to the use of green tea and its catechins [7]. GTCs bind to the outer cell membrane of mico-organisms and cause cell leakage leading to ultimate cell lysis/death. However, gram negative bacteria are less susceptible to GTCs due to the presence of an additional lipopolysachharide layer over their cell membrane. Binding of GTCs to the outer membrane of micro-organisms, avoid their adhesion to other surfaces or mammalian cells thereby preventing their colonization to form biofilm and further progression of infection or pathogenesis [8]. Cho et al, showed that treatment with green tea changed the regulation of 17 individual genes in *E. coli*. Nine genes were up-regulated and eight were down-regulated leading to damage to the bacterial cell membrane [9].

Anti-microbial potential of green tea and its catechins could be enhanced by exploring their synergistic relationship with other anti-microbial agents including standard antibiotics particularly against *Methicillin- resistant Staphylococcus aureus, S. epidermidis* and *E. coli*.

Synergistic relationship between GTCs and different antibiotics was investigated by different groups included: enhanced effect of GTCs upon addition of tetracycline against *S. aureus* and *S. epidermidis* [10, 11]; with penicillin against *S. epidermidis* [12]; with penicillin, oxacillin, ampicillin/sulbactam, and imipenem against MRSA [13, 14, 15]; and with chloramphenicol, ciprofloxacin, and cefotaxime against isolates of *E. coli* having various levels of resistance, including extended-spectrum beta-lactamase (ESBL) producers [12, 16, 17].

The therapeutic profile of these agents can be improved if more amount of bioactive is delivered to target site and delivery to non-target sites is minimized. Designing and testing of effective delivery systems for bio-actives can overcome the limitations of extracts, decoctions etc. prepared for traditional medicines. Novel delivery systems for drugs/ bio-actives are capable of enhancing the solubility of active, evading the enzymatic attack thus increasing the bioavailability and prolonging the shelf life by protecting against oxidation and hydrolysis [18].

Colloidal drug delivery comprises of vesicular or particulate systems encapsulating the drug and exhibit nano-metric size range. Some of the examples of these types of systems are nanoparticles, liposomes, nanoemulsions, microemulsions, nanospheres etc. Microemulsions (MEs) and Nanoemulsions (NEs) serve as a versatile carrier for drug delivery owing to their lipophillic, hydrophilic and amphiphillic phases. The drug molecules/bioactives can be encapsulated depending on their physicochemical properties in either of these phases. Oil-in-water type of emulsions are considered to be more stable *in vivo*, as their structure does not get disturbed upon dilution by body fluids [19].

Moreover, nano-scale delivery systems have the ability to improve the pharmacokinetics and increase bio-distribution of therapeutic agents to target organs and have the desirable advantage of improving solubility of hydrophobic compounds in aqueous medium to render them suitable for administration [20]. Recent research work in this area is evident about the fact that NEs have a broad spectrum of activity against bacteria (both gram positive and gram negative), enveloped viruses, fungi and spores. These can be easily rescaled from laboratory to large scale production [21]. NE particles are thermodynamically driven to fuse with other lipid containing micro-organisms and their binding is further enhanced by the electrostatic interaction between them. A part of energy trapped within NE is released along with active ingredient when they come in contact with the micro-organism thereby causing cell lysis and death. Nano-biomaterials can be considered as next step in the class of anti-microbial and disinfecting agents [22, 23].

Aim of the present work was to prepare nano-carrier based formulations for Green tea catechins and other synergistic compounds for enhanced antibacterial activity.

OBJECTIVES

- To establish the antibacterial activity of Polyphenon 60 (Green Tea Catechins) and a synergistic phytochemical
- To develop and optimize a Polyphenon 60 and synergistic phytochemical loaded nanoemulsion
- To develop and characterize the Polyphenon 60 and synergistic phytochemical loaded nanoemulsion based gel
- *In vitro* and *in vivo* analysis of the optimized nanoemulsion based gel

THESIS CHAPTERS

Chapter 1: Introduction

Chapter 2: Literature Review

Chapter 3: Aims and Objectives

Chapter 4: Materials and Methods

Chapter 5: Development and Characterization of Green tea catechin (Polyphenon 60) loaded microemulsions as an antibacterial agent

Chapter 6: Microemulsion formulation for dual agents: Polyphenon 60 and caffeine

Chapter 7: Synergistic antibacterial action and optimization of Polyphenon 60 and Cranberry nanoemulsion

Chapter 8: *In vitro* and *in vivo* analysis of Polyphenon 60 and cranberry nanoemulsion based gel

Chapter 9: Discussion

Chapter 10: Conclusion and Future Recommendations

Appendix

OVERVIEW

Plant compounds like GTCs exhibit several pharmacological effects including antimicrobial activity that can be attributed to EGCG due to the presence of an extra 3-gallolyl moiety [24]. Caffeine and Cranberry are also reported for their anti-microbial action [25, 26].Cranberry is widely reported for its antibacterial action and proanthocyanidins in cranberry could be responsible for preventing *E. coli* adhesion [27]. Selected bacterial strains (*S. epidermidis, S. saprophyticus, E. coli* and *P. stuartii*) were screened for susceptiblility against Polyphenon 60 (P60), Caffeine (CAF) and Cranberry (CRB) via Kirby-Bauer disc diffusion assay. All the strains were found to be sensitive with larger zones of inhibition (ZOI) obtained for *S. epidermidis* (ZOI_{P60}=12.83±0.4 mm; ZOI_{CAF}=11.50±0.3 mm; ZOI_{CRB}=13.27±0.2 mm) and *S. saprophyticus* (ZOI_{P60}=14.50±0.3 mm; ZOI_{CAF}=12.43±0.2 mm; ZOI_{CRB}=14.33±0.2 mm) as compared to that of *E. coli* (ZOI_{P60}=11.83±0.7 mm; ZOI_{CAF}=10.17±0.3 mm; ZOI_{CRB}=12.00±0.3 mm) and *P. stuartii* (ZOI_{P60}=10.60±0.3 mm; ZOI_{CAF}=9.77±0.1 mm; ZOI_{CRB}=11.90±0.2 mm). Minimum inhibitory concentration (MIC) values were calculated using chequerboard microdilution assay in a 96-well micro-titre plate [28]. Results indicated that higher concentrations of P60, CAF and CRB were required for inhibition of *E. coli* (MIC_{P60}=3.30 mg/ml; MIC_{CAF}=6.60 mg/ml; MIC_{CRB}=10.00 mg/ml) and *P. stuartii* (MIC_{P60}=3.50 mg/ml; MIC_{CAF}=6.00 mg/ml; MIC_{CRB}=15 mg/ml) as compared to that of *S. epidermidis* (MIC_{P60}=1.65 mg/ml; MIC_{CAF}=3.30 mg/ml; MIC_{CRB}=6.50 mg/ml) and *S. saprophyticus* (MIC_{P60}=1.75 mg/ml; MIC_{CAF}=3.30 mg/ml; MIC_{CRB}=6.00 mg/ml). This variation in antibacterial potential of P60, CAF and CRB against different bacteria could be attributed to the variation in the composition of outer cell walls of gram positive and gram negative bacteria [22].

Combination of two natural compounds is suggested to have different mechanisms on the bacterial growth thereby providing better antibacterial activity and less chances of development of resistance [29]. Antibacterial activity of Polyphenon 60+Caffeine and Polyphenon 60+Cranberry in combination were established against *S. epidermidis* ($FIC_{indexP60+CAF}$=0.5, Synergy; $FIC_{indexP60+CRB}$=0.25, Synergy), *S. saprophyticus* ($FIC_{indexP60+CAF}$=0.5, Synergy; $FIC_{indexP60+CRB}$=0.5, Synergy), *E. coli* ($FIC_{indexP60+CAF}$=1.0, Additive; $FIC_{indexP60+CRB}$=0.25, Synergy) and *P. stuartii* ($FIC_{indexP60+CAF}$=1.0, Additive; $FIC_{indexP60+CRB}$=1.0, Additive).

Though a large number of studies have proved the benefits of plant compounds, a vast difference was observed in the efficiency exhibited by their free form as compared to *in vivo* results. The short half-life was reported due to rapid systemic clearance resulting in poor oral bioavailability [30]. Various nano-encapsulation techniques for P60, CAF and CRB have been investigated that could sustain their functional stability. Furthermore, sustained release of these natural compounds from a nano-formulation would lead to a lower dose requirement due to decreased plasma fluctuations and, therefore, could be a valuable tool to limit the toxicity associated with a high dose of active natural products [31].

P60 loaded thermodynamically stable oil-in-water MEs (with a label claim of 20 mg/ml of ME) were prepared using labrasol as oil phase, cremophor EL as surfactant, glycerol as co-surfactant and water as aqueous phase. Malvern Zetasizer revealed that MEs droplets (placebo and P60 loaded) had nanometer size range (12.93 nm and 13.09 nm, respectively). All the ME formulations exhibited a narrow size distribution (range PI < 0.452). The mean droplet size of placebo ranged from 12.93 to 174.5 nm, which was lower as compared to the P60 loaded MEs (13.09 to 238.5nm). It is hypothetically described that at the optimum S_{mix} ratio (6:1), cremophor EL and glycerol lowered the interfacial tension between the oil and water molecules thereby stabilizing MEs and resulting in smallest droplet diameters (12.93 nm) with low PI value (0.171). P60 when encapsulated in ME form, further lowered the inhibitory concentration (two fold reduction) that is required to inhibit the growth of *E. coli*.

Based on preliminary studies, aforesaid combination of excipients (labrasol as an oil phase, cremophor EL as surfactant and glycerol as co-surfactant) was used for the preparation of P60+CAF loaded oil-in-water ME. Placebo and P60+CAF ME had PDI ranging from 0.179 to 0.229, indicating narrow size distribution. The average droplet size of placebo (12.78 nm) was lower as compared to P60+CAF ME (17.73 nm) indicating that the addition of P60 (7 mg/ml) and caffeine (13 mg/ml) increased the droplet size of ME. Combination of P60 and CAF further lowered the MICs of individual agents upon encapsulation in ME formulation. FIC_{index} value for *E. coli* which was found to be additive for aqueous form of P60+CAF (FIC_{index}=1.0) showed a synergistic relationship after encapsulation in ME formulation (FIC_{index}=0.5). This difference in the antibacterial spectrum of aqueous form of P60+CAF and its ME could be due to the basic difference in the morphology of gram positive and gram negative bacterial outer membrane. The outer membrane of gram negative bacteria is composed high lipopolysaccharide content of lipids thereby less susceptible to any of the

agents whilst preparation of MEs involves the use of surfactants that can effectively overcome the lipid barriers in bacteria [32].

The encapsulation of active into a ME system sustained its anti-oxidative potential and functional stability [33]. This was confirmed by carrying out the DPPH assay to check the anti-oxidative potential of P60+CAF in aqueous form and ME formulation at 7^{th} and 14^{th} day. Results indicated that ME system was effective as an anti-oxidative agents at least up to two weeks as compared to that of aqueous form. Results of MTT assay for cytotoxicity analysis on Vero cells showed lower cell viability for of P60+CAF ME formulation (~ 65%) and placebo (~39%) as compared to aqueous form of P60+CAF (~75%) that might be due to presence of surfactants which could be toxic to cells when present in high concentrations (in MEs and placebo).

Based on preliminary studies, aforesaid combination of excipients (labrasol as an oil phase, cremophor EL as surfactant and glycerol as co-surfactant) was used for the preparation of oil-in-water NE, with reduced concentrations of surfactant and co-surfactant. P60+CRB loaded oil-water NE was prepared in two steps. The first step was to prepare a coarse pre-emulsion by homogenization. A pre-emulsion was prepared by adding P60+CRB to the mixture of labrasol (10%), cremophor EL (10%), glycerol (3.52%) and water (76.48%). This pre-emulsion was then subjected to homogenization at 10,000 rpm for 15 min. Ultrasonic process (% amplitude= 60% and ultrasonic time=200 s) was used to further decrease the droplet size. PDI of developed NE ranged from 0.236 to 0.270, indicating narrow size distribution. The average droplet size of placebo (70.37 nm) was lower as compared to P60+CRB NE (89.47 nm) indicating that the addition of P60+CRB (label claim: P60=6.6 mg/ml; CRB=20 mg/ml) increased the droplet size of NE. The effect of NE on growth of *E. coli* indicated that the aqueous P60+CRB could inhibit the growth at 15 h while the NE formulation of P60+CRB showed the sharp drop in the turbidity of bacterial culture at the 5^{th} hour of inoculation. This difference in the activity of aqueous P60+CRB and its NE could be attributed to the fusion of small and negatively charged NE droplets with bacterial membrane leading to the cell lysis.

In order to further increase the label claim, an alternate P60+CRB loaded NE was prepared using oleic acid as an oil phase, tween 20 as emulsifier, and glycerol as co-surfactant as determined from solubility analysis. The formulation was optimized using response surface methodology via Box-Behnken design. All the emulsion composition variables (content of

oil, emulsifier and drug) and ultrasonication process variables (time of sonication and % amplitude) had significant effect on mean droplet size. Oil content and % amplitude significantly affected the zeta potential whereas no effect of variables was seen on polydispersity index. The optimized formulation showed mean droplet size of 58±1 nm, polydispersity index of 0.2±0.015 and zeta potential of -16±0.2 mV with a label claim of 41 mg/ml (P60=11 mg/ml; CRB=30 mg/ml) and was found to be stable at 4°C.

With the aim to enhance the residence time of the NE at the site of administration, it was decided to formulate a gel of the NE. Different concentrations of the gelling agents carbopol (1%, 2%, 3%, w/v) and chitosan (1%, 1.5%, 2%, w/v) were mixed in the water and lactic acid, respectively and kept overnight to ensure complete hydration. Chitosan gels were selected primarily based on their clarity and pH value (approximately 4.0) that is close to the physiological (vaginal) conditions. All three gels presented a non-Newtonian, pseudo-plastic behaviour, which is a characteristic of polymeric systems. No Newtonian flow regimen has been observed either at higher or at lower shear stress values within the considered shear rate interval. Higher elastic component (lower values of tan δ) observed for 1.5% gel, could favour its ability for prolonged residence time. Growth curve of *E. coli* indicated that the aqueous P60+CRB could inhibit the growth at 15 h while the NE formulation of P60+CRB and its NE based gel (NBG) showed the sharp drop in the turbidity of bacterial culture at the 5^{th} h of inoculation. This difference in the activity of aqueous P60+CRB and its NBG could be attributed to the presence of NH^{3+} groups on protonated chitosan which interact with negatively charged bacterial membrane resulting in formation of pores and ultimate cell lysis [34]. *Ex vivo* release profile of P60+CRB NBG was studied using porcine vaginal mucosa via Franz diffusion cell. Results confirmed sustained release potential of newly developed P60+CRB NBG throughout the assay with consistent increase in % release of both P60 and CRB. Maximum release of 90.92 ± 0.6% in 8 h was observed for P60, while CRB showed 99.39 ± 0.5% release within 6 h. The cell viability (%) of different test agents i.e., aqueous P60+CRB, its NE, placebo (P_{NE}), P60+CRB NBG and corresponding placebo (P_{NBG}) at their respective MIC values after being incubated with Vero cells for 24 h indicated the results of cell viability (%) in the following order: aqueous P60+CRB>NBG>NE>P_{NBG}>P_{NE} i.e., cell viability (%) in the presence of aqueous P60+CRB was highest (88.74%) as compared to its NE (74.95%), implying that the presence of surfactants could cause irritation to normal mammalian cells when exposed directly. However, the percentage of chitosan and the surfactants used in the formulation was below GRAS levels. Results confirmed that NBG of

plant P60+CRB exhibited better cell viability (84.95%) and comparable to its aqueous counter part due to the biocompatibility of chitosan with other biological systems [35]. Results of anti-adhesion assay indicated that the presence of P60+CRB either in aqueous form or NBG formulation, were able to inhibit the bacterial colonization/adhesion to the Vero cells. One of the possible mechanisms of inhibition of bacterial growth could be via alteration of bacterial membrane to prevent its binding to mammalian cells.

The drug is delivered in the vagina mainly via two routes: intra-vaginally to the vaginal epithelium or trans-vaginally through the vaginal mucosa to uterus and systemic circulation. Cicinelli et al, reported that the vagina has specific blood flow characterstics, either by a portal type circulation or by venous and lymphatic channels, that allow bypassing the GI tract absorption and liver detoxification and permit preferential transport of drug molecules from vagina to the uterus and systemic circulation [36]. Therefore, we proposed vaginal route of administration for the formulation as this route by passes the first pass effect and results in enhanced bioavailability. To assess the transport of the gel across the vaginal mucosa, Gamma scintigraphy studies were performed in Sprague-Dawley rats. P60+CRB were radiolabelled with ^{99m}Tc and tagging was optimized. NBG was prepared with ^{99m}Tc labelled P60+CRB. The female rats were divided into three groups (3 rats in each group). Group I: rats were administered with ^{99m}Tc-PC-NBG (NE based gel of P60+CRB, orally); Group II: rats were administered with ^{99m}Tc-PC-NBG (NE based gel of P60+CRB, vaginally) and Group III: rats were administered with ^{99m}Tc-PC (Aqueous form of P60+CRB, vaginally). Gamma imaging was done using Gamma Camera. Results of gamma scintigraphy images on Sprague-Dawley rats (at pre-determined time intervals) showed high radioactivity in the entire GI tract that might result in low bioavailability of formulation due to enzymatic degradation upon oral administration. While post vaginal administration, a high uptake of ^{99m}Tc-NBG into systemic circulation was observed as compared to ^{99m}Tc-PC (vaginal administration). Therefore, we can presume that vaginal administration of ^{99m}Tc-NBG could display better systemic absorption and bioavailability of P60+CRB that could be further quantified via *in vivo* bio-distribution studies.

CONCLUSIONS

- Antibacterial activity of Polyphenon 60, Caffeine and Cranberry were established on *Staphylococcus epidermidis* (ZOI_{P60}=12.83±0.4 mm, MIC_{P60}=1.65 mg/ml; ZOI_{CAF}=11.50±0.3 mm, MIC_{CAF}=3.30 mg/ml; ZOI_{CRB}=13.27±0.2 mm, MIC_{CRB}=6.50 mg/ml), *Staphylococcus saprophyticus* (ZOI_{P60}=14.50±0.3 mm, MIC_{P60}=1.75 mg/ml; ZOI_{CAF}=12.43±0.2 mm, MIC_{CAF}=3.30 mg/ml; ZOI_{CRB}=14.33±0.2 mm, MIC_{CRB}=6.00 mg/ml), *Escherichia coli* (ZOI_{P60}=11.83±7 mm, MIC_{P60}=3.30 mg/ml; ZOI_{CAF}=10.17±0.3 mm, MIC_{CAF}=6.60 mg/ml; ZOI_{CRB}=12.00±0.3 mm, MIC_{CRB}=10.00 mg/ml) and *Providencia stuartii* (ZOI_{P60}=10.60±0.3 mm, MIC_{P60}=3.50 mg/ml; ZOI_{CAF}=9.77±0.1 mm, MIC_{CAF}=6.00 mg/ml; ZOI_{CRB}=11.90±0.2 mm, MIC_{CRB}=15 mg/ml) via disc diffusion assay and chequerboard microdilution assay, respectively.

- Antibacterial activity of Polyphenon 60+Caffeine and Polyphenon 60+Cranberry in combination were established against *Staphylococcus epidermidis* ($FIC_{indexP60+CAF}$=0.5, Synergy; $FIC_{indexP60+CRB}$=0.25, Synergy), *Staphylococcus saprophyticus* ($FIC_{indexP60+CAF}$=0.5, Synergy; $FIC_{indexP60+CRB}$=0.5, Synergy), *Escherichia coli* ($FIC_{indexP60+CAF}$=1.0, Additive; $FIC_{indexP60+CRB}$=0.25, Synergy) and *Providencia stuartii* ($FIC_{indexP60+CAF}$=1.0, Additive; $FIC_{indexP60+CRB}$=1.0, Additive).

- Oil-in-water microemulsion formulation was developed for Polyphenon 60 (label claim of 20 mg/ml) with mean droplet size ranging from 13.09 to 238.5 nm. Minimum inhibitory concentration of Polyphenon 60 (MIC_{P60}=3.30 mg/ml) upon encapsulation in microemulsion formulation ($MIC_{P60\,ME}$=1.65 mg/ml) was reduced by two fold against *E. coli.*

- Oil-in-water microemulsion formulation was developed for Polyphenon 60+Caffeine (label claim of 20 mg/ml; P60=7 m/ml, CAF=13 mg/ml) with mean droplet size of 17.73 nm. Fractional inhibitory concentration of Polyphenon 60+Caffeine ($FIC_{P60+CAF}$=1.0, Additive) upon encapsulation in microemulsion formulation

($FIC_{P60+CAF\ ME}$=0.5, Synergy) was reduced by two fold and exhibited synergistic effect against *E. coli.*

- Oil-in-water nanoemulsion formulation was developed for Polyphenon 60+Cranberry (label claim of 26.6 mg/ml; P60=6.6 mg/ml, CRB=20 mg/ml) with mean droplet size of 89.47 nm. The effect of nanoemulsion formulation of Polyphenon 60+Cranberry on the growth of *E. coli* indicated the inhibition of *E. coli* at the 5th hour of inoculation as compared to its aqueous counterpart (15 h).

- An alternate oil-in-water nanoemulsion formulation was developed and optimized for Polyphenon 60+Cranberry (label claim of 41 mg/ml; P60=11 mg/ml, CRB=30 mg/ml) with mean droplet size of 58 nm using Box Behnken Design. Optimized formulation was found to be stable at 4°C.

- Chitosan based gel (1.5%) formulation was developed and characterized for Polyphenon 60+Cranberry nanoemulsion. Nanoemulsion based gel showed enhanced antibacterial activity (as compared to its aqueous and nanoemulsion counterparts) against *E. coli* as determined via growth curve and anti-adhesion assay. Mammalian cell viability (%) was found to be 84.95% that was comparable to its aqueous counterpart (88.74%).

- *Ex vivo* release studies of Polyphenon 60+Cranberry nanoemulsion based gel performed on porcine vaginal mucosa showed 99% release of Polyphenon 60 after 8 hrs while 90% Cranberry was released after 6 hrs.

- Gamma scintigraphy studies were performed by successful radiolabeling of Polyphenon 60+Cranberry. Radiolabelled nanoemulsion based gel was administered vaginally to Sprague Dawley Rats and images indicated the transport of gel from vaginal cavity into the systemic circulation

Nanoemulsion based gel for Polyphenon 60+Cranberry was developed and characterized for enhanced antibacterial activity that could be transported trans-vaginally from vaginal cavity to the systemic circulation

SUMMARY OF ANTIBACTERIAL STUDIES

Strains	MIC of Green tea (mg/ml)			Existing Antimicrobial Formulations	Formulation developed in the lab
	Reported in literature	Ref.	As established in the lab for P60[c]		
S. epidermidis ATCC 12228	~ 0.25[a]	[37]	1.65	Antiseptic creams, Liposomal hydrogel	Microemulsion with enhanced antibacterial activity against *E. coli* (MIC_{ME}=1.65 mg/ml)
S. saprophyticus ATCC 15305	~ 0.40[b]	[38]	1.75		
E. coli ATCC 25922	~ 4.00[b]	[39]	3.30		
P. stuartii NCTC 10318	N.R.	-	3.50		

NOTE a: Epigallocatechin gallate; b: Green Tea Extract; c: Polyphenon 60; N.R.: Not reported in literature to the best of our knowledge

Strains	Green tea + Caffeine		Formulation developed in the lab
	Reported in literature	As established in the lab	
S. epidermidis	N.R.	Synergistic	Microemulsion with enhanced antibacterial activity against *E. coli* (FIC_{index}=0.5, Synergy)
S. saprophyticus	N.R.	Synergistic	
E. coli	N.R.	Additive	
P. stuartii	N.R.	Additive	

NOTE N.R.: Not reported in literature to the best of our knowledge

Strains	Green tea + Cranberry		Formulation developed in the lab
	Reported in literature	As established in the lab	
S. epidermidis	N.R.	Synergistic	Nanoemulsion based gel inhibited the growth of *E. coli* within 5 h
S. saprophyticus	N.R.	Synergistic	
E. coli	N.R.	Synergistic	
P. stuartii	N.R.	Additive	

NOTE N.R.: Not reported in literature to the best of our knowledge

FUTURE RECOMMENDATIONS

- Antibacterial Potential of the formulation against BSL-2 strains of *E. coli*
- Exploration of Antifungal potential of the formulation
- Pharmacokinetic Studies of the formulation.

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PUBLICATIONS – RESEARCH ARTICLE

1. **S. Gupta,** R. Bansal, J. Ali, R. Gabrani, S. Dang. Development and characterization of polyphenon 60 and caffeine microemulsion for enhanced antibacterial activity. BioMed Research International. 2014; http://dx.doi.org+/10.1155/2014/932017.

Journal	Impact Factor	H5 Index	Fee for Publication	Peer Reviewed	Indexing	Publishers	Presented
BioMed Research International	**2.706**	51	Invited Article NIL	Yes	Scopus, PubMed	Hindawi Publishing Corporation, Egypt	No

2. **S. Gupta**, R. Bansal, D. Maheshwari, J. Ali, R. Gabrani, S. Dang. Development of a nanoemulsion system for polyphenon 60 and cranberry. Advanced Science Letters. 2014; 20: 1683-6.

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Advanced Science Letters	**1.253**	19	NIL	Yes	Scopus	American Scientific Publishers, U.S.	Poster

3. **S. Gupta**, J.K. Sahni, J. Ali, R. Gabrani, S. Dang. Development and characterization of green tea loaded microemulsion for vaginal infections. Advanced Material Letters. 2012; 3(6): 493-7.

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Advanced Material Letters	-	14	NIL	Yes	Scopus	VBRI Press, India	Poster

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   *** Both the authors have equally contributed in the research work**

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Food Chemistry	**3.259**	83	NIL	Yes	Scopus	Elsevier, U.K.	No

PUBLICATIONS – REVIEW ARTICLES

1. **S. Gupta**, A. Jain, M. Chakraborty, J.K. Sahni, J. Ali, S. Dang. Oral delivery of therapeutic proteins and peptides: a review on recent developments. Drug Delivery. 2013; 20(6): 237-46.

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Drug Delivery	**2.202**	20	NIL	Yes	Scopus	Informa Healthcare, New York	No

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Recent Patents on Drug Delivery and Formulations	-	18 (H)	NIL	Yes	Scopus, PubMed	Bentham Science Publishers, U.A.E.	No

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Recent Patents on Drug Delivery and Formulations	-	18 (H)	NIL	Yes	Scopus, PubMed	Bentham Science Publishers, U.A.E.	No

BOOK CHAPTER

S. Dang, **S. Gupta**, R. Bansal, J. Ali, R. Gabrani. "Nano-encapsulation of a natural polyphenol, green tea catechins: way to preserve its anti-oxidative potential" In: Free radicals in human health and diseases. Springer, Germany 2015, pp 397-415. Online ISBN 978-81-322-2035-0

COMMUNICATED RESEARCH PAPER

S. Gupta, R. Bansal, D. Sharma, J. Ali, R. Gabrani, S. Dang. "Synergistic antibacterial activity of Cranberry and Polyphenon 60 encapsulated in an optimized nanoemulsion" in Journal of Microencapsulation, Informa Healthcare.

ABSTRACTS

1. **Sonal Gupta**, Rakhi Bansal, Dipika Maheshwari, Javed Ali, Reema Gabrani and Shweta Dang. Development of a nanoemulsion system for Polyphenon 60 and Cranberry. National conference on Nanotechnology and Renewable Energy (NCNRE-14) at Jamia Millia Islamia, New Delhi from 28-29th April, 2014.

2. **Sonal Gupta**, Rakhi Bansal, Dipika Maheshwari, Javed Ali, Reema Gabrani and Shweta Dang. Development and characterization of cranberry powder loaded microemulsion against uropathogenic E. coli. International conference on Drugs for future:Infectious diseases- 2014, Antimicrobial Drug Discovery: Challenges and Perspectives at NIPER, Hyderabad from 27-28th March, 2014.

3. **Sonal Gupta**, Javed Ali, Reema Gabrani and Shweta Dang. Transmucosal Drug Delivery-Vaginal Route for Biopharmaceuticals. IPGA sponsored National Seminar on Expectations of Pharmaceutical Industry from Pharmacy Graduates at PDM College of Pharmacy, Bahadurgarh on 8th September, 2012.

4. **Sonal Gupta**, Reema Gabrani and Shweta Dang. Development and characterization of green tea loaded microemulsion for vaginal infections. International Conference on Nanomaterials and Nanotechnology at Delhi University, New Delhi form 18-21st December, 2011.

5. Ramya Jain, **Sonal Gupta**, Pramod K, Javed Ali, Reema Gabrani and Shweta Dang. Development and characterization of transdermal microemulsion for alendronate. AICTE sponsored national seminar on New Horizons in Drug Discovery and Development at Jamia Hamdard, New Delhi from 17-18th September, 2011. (Best Poster Award).

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