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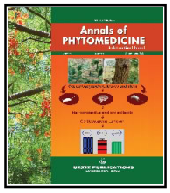

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Development and evaluation of topical ointment formulation containing gallic acid as an active pharmaceutical ingredient against bacterial infection and oxidative damage

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Abstract

Bacterial infections and oxidative damage are becoming more concerning health problems to be addressed by health professionals due to the multidrug resistance of pharmaceuticals. Despite, topical preference, oral administration compliance, and bioavailability berries, still remains the opacity to developed an alternative topical formulation with better compatibility. The study is aimed to prepare different topical ointment formulations containing gallic acid as an active pharmaceutical ingredient, followed by optimization of the best formulation. *In vitro* drug release profile was determined followed by antibacterial evaluation against *E. coli* and *S. aureus* viability. DPPH assay, *in silico* pharmacokinetic and network pharmacology analysis were performed to determine antioxidant potential, bioavailable response and multi-mechanistic therapeutic role of formulation/gallic acid in alleviation of bacterial infection even oxidative damage. The outcomes of the study showed that the formulation F5 was found as an optimized formulation which showed 5.76 ± 0.0207 pH, 1487.35 ± 4.868 Pas viscosity, 139.42 ± 1.665 g.cm/s spreadability index. In antibacterial activity, the average zone of inhibition was found 5.324 ± 0.458 mm and 5.324 ± 0.458 mm against the growth of *E. coli* and *S. aureus*, respectively. The developed formulation exhibited significant (p<0.001) antioxidant activity. *In silico*, pharmacokinetic showed good bioavailable response of gallic acid while network pharmacology analysis showed multi-mechanistic action of gallic acid in alleviation of inflammation and oxidative stress induced by the biological stimulus. Hence, it can be concluded that the developed optimized formulation can be used for the bacterial infection as well as oxidative damages induced by the biological stimulus.

1. Introduction

Infectious diseases are still a major health problem which accounts about 41% of the global disease burden measured in terms of disability-adjusted life years (DALYS). Microorganisms are the smallest or tiny living organisms found everywhere in our surroundings that are too small in shape which could not able to seen by the naked eye. Mostly, microorganisms live in water, ground soil with the highest density, and in the air. Among the high abundance of microorganisms, some of them are beneficial for humans and some of them harm human health, drastically. Furthermore, the human body is also acknowledged as the home of millions of microorganisms of different diversities (Venkatachalam *et al*., 2021; Cavicchioli *et al*., 2019).

World health organization (WHO) is an international body accountable for preserving the highest level of human health and well-being. As per WHO, microorganisms are one of the thought-provoking threats that are distressing global health, exponentially. Notably, less than 5% and perchance as little as 1% of all bacteria can be cultured in the laboratory (Aboudharam *et al*., 2010; Mourya *et al*., 2019). Most of the growing species of microorganisms in the laboratory are used to explore the determinant effect of natural or synthesized antibacterial agents. Other than, the advancement of pharmaceuticals criticized the necessity for those pharmaceuticals having antibacterial resistance (Clebak and Malone, 2018; Piddock, 2012). Antimicrobial resistance of contemporary medicine is being incessantly growing emergency accredited because of their overuse or misappropriation of such medications (Kiran *et al*., 2021). Besides, economic incentives and challenging regulatory necessities are the principal deficient reason which creates complication in the development of new antibacterial drugs by the pharmaceutical industry. For decades, bacterial resistance to the current pharmaceuticals is incessantly amplified, thus to curtail resistance and capitalize on the biological efficiency of antimicrobial agents. It is essential to reconnoiter the mechanism of antibacterial resistance through another biomolecular approach and disavow the efficiency of the therapeutic drug (Alshibl *et al*., 2020; Breijyeh *et al*., 2020).

Topical formulations are the type of pharmaceuticals which are available in different form such as creams, emulsions, foams, powders, liquids, gels, *etc*., containing one of more than one active pharmaceuticals for treating the affected site. Among them, ointments are the well-known pharmaceutical formulation due to

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their easy availability, accessibility and economic in nature. Several ointment formulations are available in market for the treatment of infectious disease, but they are still far from perfection due to antibacterial resistance and economic in nature. There is a paucity for the development of an alternative required to treat the bacterial infection and associated deleterious effects (Verma *et al*., 2013; Vogel *et al*., 2013).

Natural-derived active pharmaceuticals play a vital role in alleviating the several pathological onsets whether derived from microorganisms, drug toxins even any of the body disorders. Approx. or even more than 50% of drugs used in modern therapeutics are derived from natural products (Rana *et al*., 2021). Furthermore, several natural-derived constituents are widely used for their antimicrobial exploration and derived as the best pharmaceuticals to treat microbial infection (Meenakshi *et al*., 2018; Pan *et al*., 2013). Gallic acid is one of the most abundant naturally derived drugs used to treat several diseases such as diabetes, cancer, inflammation, *etc*. Despite its eminent therapeutic values, it is easily affordable, assessable, and economic. Being into consideration, the above facts, the study provoked us to develop an evaluation of topical ointment formulation containing gallic acid as an active pharmaceutical ingredient against bacterial infection and oxidative damage which could cover the existing pharmaceutical that has been exposed to the antibacterial drug resistance and least effective against the oxidative damage through bacterial infection. The study was explored through various preclinical approaches such as pre-formulation studies, antibacterial assessment, *in silico* approaches, *etc*.

2. Materials and Methods

2.1 Chemical and reagents

Gallic acid was purchased form sigma Aldrich (G7384), sodium lauryl sulfate, bentonite, methylparaben, white petrolatum, beeswax and soft paraffin were purchased from SRL Pvt. Ltd. Delhi. The solvents used in the experimental process were of analytical grade and purchased from SD Fine-Chem Limited, Mumbai.

2.2 Determination of absorption maxima and method validation analysis of gallic acid

The analysis for determination of absorption maxima of gallic acid was performed based on the described protocol (Kumar *et al*., 2010). Briefly, 1 mg/ml stock solution of gallic acid was prepared in methanol, followed by further dilution to the different concentrations ranging 200-1 µg/ml each prepared dilution was analyzed spectrophotometrically at 227 nm.

2.3 Development of topical ointment formulation

The ointment was prepared by fusion method using the described protocol with some modification (Bhagurkar *et al*., 2016; Verma *et al.*, 2021). In brief, different six batches of ointment were prepared using different concentrations of drug and base (water phase and oil phase). The water phase was prepared by heating sodium lauryl sulfate, bentonite and methyl paraben till the temperature attained 75°C to 80°C. The oil phase was comprised with white petrolatum, bees wax and soft paraffin by using temperature (75°C-80°C). Both the water phase and oil phase were mixed together into an ointment manufacturing vessel, followed by addition of gallic acid slurry with continues trituration after cooling the mixture. The obtained mixture was transferred to an ointment manufacturing vessel and homogenized for 1.5 h to obtain uniformity of ointment.

2.3.1 Selection of best formulation

The best formulation was selected based on free drug content in each developed formation. Briefly, 5 mg/ml methanolic solution of each developed ointment formulation was prepared using vertex, followed by centrifugation at 10000 rpm. The supernatant was analyzed spectrophotometrically at 227 nm. All the measurements were taken in triplicate (Bhagurkar *et al*., 2016; Verma *et al*., 2021).

2.4 Evaluation of ointment formulation

The best selected formulation was further evaluated for its physicochemical analysis. The parameters such as pH, viscosity, spreadability, uniformity of drug content, *etc*., were evaluated with the help of reference protocols.

2.4.1 pH measurement

The pH of optimized formulation was determined in three different samples withdrawn from the jar of optimized formulation. The pH of these solutions was measured using the pH meter (Kumar *et al*., 2020).

2.4.2 Determination of viscosity

The viscosity of the developed ointment was evaluated as per the standard protocol with some modifications as follows. In brief, the obtained ointment was evaluated based on physical appearance and Brookfield Viscometer was used to determine the viscosity of the developed ointment (Pandurangan *et al*., 2016).

2.4.3 Spreadability

Spreadability of the formulation was determined as per the reference protocol with some modification (Uprit *et al*., 2013). In brief, 1g of the formulation was placed at the middle center of acrylic plate and the second plate was concentrically placed above it. The width of the circle was estimated as per the rises in the diameter due to the dissemination of the ointment. Each measurement was taken in triplicate. The results were expressed as Mean ± SD, n=3.

2.4.4 Uniformity of drug content

5 mg of developed ointment was taken from six different regions of an ointment jar and placed to six different eppendorf's tubes. Each sample was dissolved in 1 ml of methanol. The obtained mixture was vortexed and centrifuged at 10000 rpm for 10 min. The supernatant form each tube was taken and measured spectrophotometrically at 227 nm. The content of drug was determined as µg/mg of the sample. Each measurement was taken in triplicate. The results were expressed as Mean ± SD using one-way ANOVA, followed by Tukey test (Bhagurkar *et al*., 2016; Verma *et al*., 2021).

2.5 *In vitro* drug release profile

The drug release profiling for optimized ointment formulation was performed with minor modifications in the method (Bhagurkar *et al*., 2016; El-Housiny *et al*., 2018). In brief, 1 g of sample was correctly weighed individually and placed on the respective cellulose dialysis membrane. The packed membrane was knotted using thread and placed in a flask containing phosphate buffer solution, pH 7.4 (500 ml). The flask was set in a position, so that a complete magnetic

field could be attained by the magnetic stirrer and operated to a constant 50 rpm at 37°C. Thereafter, an optimum amount of sample (1ml) was withdrawn from the flask on each interval of 0.25, 0.5, 01, 02, 03, 04, 06, 08, 12, and 24 h. The withdrawn amount of sample was replenished using the normal dissolution media at the same withdrawn time. The percentage release of gallic acid was determined spectrophotometrically at 240 nm in respect of blank. Each measurement was taken in triplicate. The results were expressed as Mean ± SD using one-way ANOVA, followed by Tukey test.

2.6 Antibacterial activity

In vitro antibacterial activity of the optimized formulation was performed through well-diffusion assay or cup plate assay against *S. aureus*, and *E. coli* bacterial strain using the standard protocol with some modification (Kumar *et al*., 2020). In brief, the bacterial inoculum from each secondary strain culture (prepared from 100 µl of primary suspension containing approx. 1×10^8 CFU/ml pathological tested bacteria) was evenly spread over the surface of the agar petri dishes plate using a sterile cotton swab. Thereafter, 5 mm diameter holes was made using a sterile tip, followed by addition of 10 µl of drug solution (5 mg/ml) was added into the wells and the controls well was treated with 10 µl of autoclaved double-distilled water as the control treatment. Each treated plate was incubated under aerobic conditions at 37°C temperature for 72 h. The antimicrobial effect was determined by the clear zone in the agar and measured as the efficacy of optimized ointment formulation.

2.7 *In vitro* antioxidant activity of developed ointment formulation against DPPH free radicals

The antioxidant activity of the prepared ointment formulation was determined by the described protocol with some modifications (Parveen *et al*., 2019). Briefly, 10 mg of the formulation was taken form jar and directly dissolved in 1 ml of methanol. The mixture was centrifuged and supernatant was placed to prepare different concentration dilutions ranging (1000-31.25 µg/ml). 20 µl of each dilution was mixed with 180 µl of DPPH (0.01 mM) solution prepared in methanol and the obtained mixtures were incubated for 30 min at room temperature. The measurements were taken spectrophotometer at 517 nm in triplicate. Vitamin C was used as standard drug.

2.8 *In silico* pharmacokinetic analysis

ADME computational analysis was performed for prepared ointment formulation through the "SwissADME (http://www.swissadme.ch/index.php)" *in silico* tool (Daina *et al*., 2017). The parameters such as topological polar surface area (TPSA) for drug integrity, Consensus log Po/w for drug lipophilicity and ESOL Log S values was predicted for physicochemical integrity of the metabolites. GI absorption, BBB permeant and log Kp (cm/s) (skin permeation) was predicted as the pharmacokinetic parameter for each metabolite. Besides, bioavailability score and synthetic accessibility was predicted to determine drug-likeness and medicinal chemistry. The prediction of these parameters was used to consider the optimistic idea of bioavailable or ADME response of gallic acid.

2.9 Network pharmacology analysis

Different targets or proteins were collected from genecard (https://www.genecards.org/) with their UniPort ID from the UniPort database (https://www.UniProt.org/uploadlists/) (Li *et al*., 2021). The ligation efficacy of each selected gene was predicted. Gene ontology (GO) analysis using the metascape gene analysis (metascape.org) tool was performed to evaluate multiple physiological roles of each gene in the regulation of inflammation and oxidative stress after the analysis of the compound-disease common target. Protein-protein interactions (PPI) network and compound-proteins interactions (CPI) were obtained based on the STRING platform (https://string-db.org/) and cytoscape (version 3.8.2) software. The target PPI network was constructed and integrated through cytoscape software, followed by the analysis of CPI for compound and targets interaction, biologically. The analysis covered all the nearly functional interactions among the expressed proteins-proteins and compound-proteins (Li *et al*., 2021; Yi *et al*., 2018).

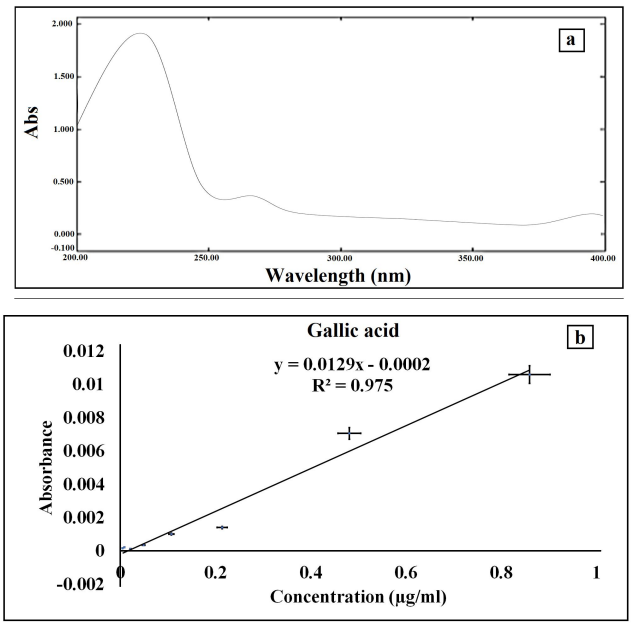

Figure 1: UV spectra of gallic acid (a) and calibration curve (b) for the validated method.

3. Results

3.1 Determination of absorption maxima and method validation analysis of gallic acid

Determination of absorption maxima and method validation analysis of gallic acid on different concentrations ranging 200-1 µg/ml was performed successfully using UV spectrophotometer. The outcomes of the study revealed the absorption maxima of gallic acid at 227 nm (Kumar *et al*., 2010). The validation analysis showed that the developed method was found linear, robust, accurate and precise under the different concentrations ranging 200-01 µg/ml. The calibration equation for developed method of gallic acid was found as y = 0.0043x + 0.0045. The limit of detection (LOD) and the limit of quantitation (LOQ) for gallic acid was found as 6.741 ± 0.359 and 20.430 ± 0.0852 ng/spot. The intra-day and inter-day precision were determined as percentage relative standard deviation (% RSD) or the coefficient of variation and the results showed intraday and interday precision 0.663-3.706 and 0.450-3.367, respectively. The

accuracy of the developed method was determined based on percentage drug recovery by percentage spiking with 0, 50, 100, and 150 % of the standard to the sample. The exhibited percentage recovery for gallic acid was found in the range of 98.546 - 98.860%. The UV spectra and calibration curve has been represented in Figure 1.

3.2 Development of topical ointment formulation

Six different ointment formulations were prepared successfully as per the standard operating procedure. The method deals with numerous modified concentrations of base and drug for the development and optimization of best effective ointment at both treatment segments, *i.e.*, accelerated temperature and relative humidity. For the development of formulation, the temperature was controlled in both segments. During preparation, instant adding of base (o/w) with continuation mixing results in preservation of a viscous and uniform semisolid form of the preparation. However, the temperature was measured at the preliminary phase. The prepared ointments have been shown in Table 1. The content (w/w) released of the developed formulations have been summarized in Table 2. A best formulation was selected based on free drug content in each developed formation. The outcome of the study revealed that the ointment formulations (F5) was found as the optimized formulation which showed 4.555 ± 0.0211 % release of gallic acid after successful analysis using spectrophotometer. The figure of the developed gel formulation has been showed in Figure 2.

Table 1: Different developed ointment formulations

S. No	Formulation	Drug ratio (%, w/v)	Water phase	Oil phase
1.	F1	1	0.5	0.5
2.	F2	1	1	0.5
3.	F3	1	1	1
4.	F4	1	0.5	1
5.	F5	1	0.5	1.5
6.	F6	1	1.5	0.5

Table 2: Content of gallic acid in different developed formulations

Developed ointment formulations	Absorbance	Content of drug (µg/5 mg)	Content of drug (µg/mg)	Content of drug (%)
F1	0.246 ± 0.0123	102.674 ± 0.436	20.534 ± 0.127	2.053 ±0.0104
F2	0.534 ± 0.0205	123.139 ± 0.568	24.627 ± 0.234	2.462 ± 0.0117
F3	0.846 ± 0.0143	195.697 ± 0.885	39.139 ± 0.103	3.913 ± 0.0137
F4	0.421 ± 0.0186	166.627 ± 0.924	33.325 ± 0.168	3.332 ± 0.0345
F5	0.984 ± 0.0201	227.790 ± 0.889	45.558 ± 0.173	4.555 ± 0.0211
F6	0.855 ± 0.0185	197.797 ± 0.762	39.558 ± 0.129	3.955 ± 0.0185

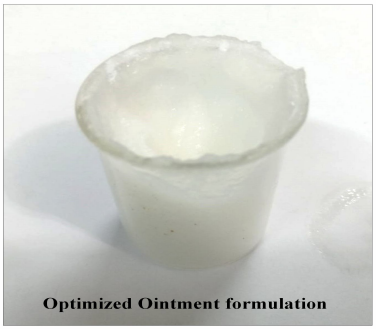

Figure 2: Optimized gel formulation (F5).

3.3 Evaluation of ointment formulation

The formulation F5 was evaluated for physicochemical characteristic. The parameters like pH, viscosity, spreadability, drug content uniformity, *etc.*, were evaluated with the help of reference protocols.The pH of formulation, F5 was determined in three different samples withdrawn from the jar of optimized formulation, successfully. The results showed and average pH of the formulation F5 was found as 5.76 ± 0.0207 (Kumar *et al.*, 2020). Viscosity is one of the imperative rheological parameters for the evaluation of semisolids pharmaceutical formulation for their better stability and compatibility. The viscosity of the developed ointment was evaluated based on physical presence and Brookfield Viscometer, successfully. The results showed that the viscosity of the developed ointment formulation was found as 1487.35 ± 4.868 Pas (Pandurangan *et al.*, 2016). Spreadability of the formulation F5 was determined to consider dissemination of the ointment. The analysis was determined based on cover slip method. The measurements were taken in triplicate and expressed statistically as Mean ± SD. The results revealed that the spreadability of formulation F5 was found as139.42 ± 1.665 g.cm/s. In a study conducted by Awad and his team reported the spreadability index

of optimized cream and ointment formulation of *Mangifera indica* extract under the range of 173.6-206.04 g.cm/s (El-Gied *et al*., 2015). Our finding strongly supports the past evidence reported on cream and ointments. Furthermore, assessment of uniformity of drug content in a pharmaceutical formulation describes to their perfections in development. In our study, uniformity of drug content was determined based on the selection of three samples from different location of jar and the content of gallic acid was determined spectrophotometrically at 227 nm. UV spectrophotometer is one of the simple and basic method to determine the uniformity of dug content throughout the wide sample of developed formulation (Chang *et al*., 2013). Based on the above facts, uniformity of drug content was determined based on the theoretical content present in the optimized formulation and the outcome of the study revealed 87.265 ± 6.023% (w/w).

3.4 *In vitro* drug release profile

The drug release profiling for optimized ointment formulation was performed, successfully (Bhagurkar *et al*., 2016), El-Housiny *et al*., 2018). 1 g of sample was used to determine the releasing rate of drug in the developed formulation while cellulose dialysis membrane was used as the media for drug release assessment. Phosphate buffer solution was used of pH7.4 to provide the favorable conditions to the membrane at 37°C. The sample group was matched with the control group to mimic any statistical fluctuations. The drug release profile was estimated on each sample withdrawn on 0.25, 0.5, 01, 02, 03, 04, 06, 08, 12, and 24 h. The results were expressed as Mean ± SD using one-way ANOVA, followed by Tukey test. The outcomes of the study revealed that the releasing rate was significantly ($p < 0.001$) higher till 3 h while no significant release in the concentration of gallic acid was found after 4 h of the process, which revealed that up to 90% of the drug release by 4 h of the time. The outcome of the study was matched by the previous studies reported on the same work objectives showed that releasing rate of gallic acid in the same proportion, approximately (de Moraes Alves *et al*., 2020). The outcome of the study has been summarized in Figure 3.

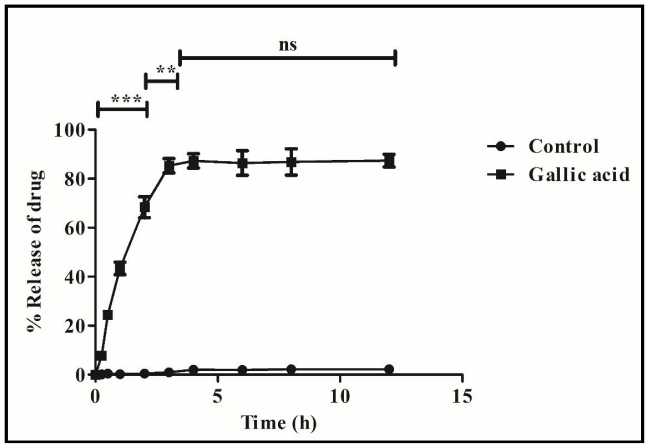

Figure 3: Percentage of drug release from developed formulation.

3.5 Antibacterial activity

In vitro antibacterial activity of the formulation F5 was achieved through well-diffusion assay or cup plate assay against *E. coli* and *S. aureus* bacterial strain, successfully. The study was performed based on the treatment of each strain with the constant concentration. The antimicrobial effect of developed formulation was determined by the clear zone appeared on plate against the viability of the bacteria. The outcome of the study showed an average zone of inhibition exhibited by developed formulation against the growth of *E. coli* was found as 5.324 ± 0.458 mm while the average zone of inhibition exhibited by developed formulation against the growth of *S. aureus* was found as 5.324 ± 0.458 mm. The agar developed plate has been summarized in the Figure 4.

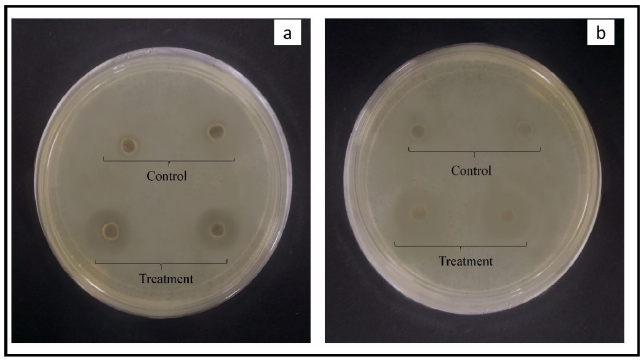

Figure 4: Antibacterial activity of developed ointment formulation, Figure (a) represents the activity of developed formulation against *E. coli* bacterial strain, Figure (b) represents the activity of developed formulation against *S. aureus* bacterial strain. The antibacterial activity of formulation was determined based on the clear zone of inhibition.

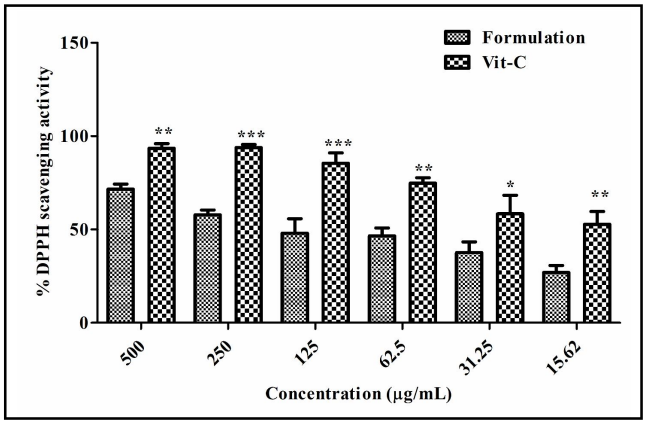

Figure 5: DPPH scavenging activity of developed ointment formulation in comparison of Vit-C. The measurements were taken in triplicate and the data was expressed statistically as Mean ± SD using one-way ANOVA, followed by Tukey. Test the comparisons were made between test sample and standard drug (Vit-C) while significance level was considered at $p < 0.05$.

3.6 *In vitro* antioxidant activity of developed ointment formulation against DPPH free radicals

The antioxidant activity of the developed ointment formulation was determined using DPPH scavenging method, successfully. The outcome of the study was expressed statistically as Mean ± SD using one-way ANOVA, followed by Tuckey test. The results

showed that the developed formulation significantly scavenging the free radicals of DPPH, and the activity was found in dose dependent manner. High concentration showed high scavenging activity while lowest concentration showed lowest scavenging activity. Vitamin C was used as positive control which was found most significant active in comparison of the developed formulation. The results of DPPH scavenging activity have been summarized in the Figure 5.

3.7 *In silico* pharmacokinetic analysis

ADME computational analysis was performed for gallic acid through the "SwissADME (http://www.swissadme.ch/index.php)" *in silico* tool, successfully. The parameters such as TPSA, consensus Log Po/w, ESOL Log S values, GI absorption, BBB permeant and log Kp (cm/s) (skin permeation) were predicted to determine the ADME response of gallic acid. The outcomes of the study showed that TPSA for gallic acid was found as 97.99. Polar surface area (PSA) of any drug is calculated using the fragmental technique TPSA. It is one of the specific parameter which acts as a convenient descriptor to estimate some ADME properties of drug molecules concerning absorption and brain access (Daina and Zoete, 2016). The consensus log Po/w is the arithmetic mean of the values anticipated by the five proposed methods of lipophilicity which represents the lipophilicity of anticipated molecules. The classical descriptor for lipophilicity is generally by partition coefficient between n-octanol and water (log Po/w). Swiss ADME dedicated this section due to the critical importance for the assessment of physicochemical property for pharmacokinetics drug discovery using computational tools. The models accelerate the prediction accuracy for physicochemical property through consensus log Po/w (Mannhold *et al.*, 2009). The outcomes of the study revealed a 0.21 value for logP means the compound poses both hydrophilic and lipophilic nature. Moreover, a high positive value for logP represents lipophilicity of the molecule. Potts and Guy provide the model to predict the skin permeability as skin permeability coefficient (Kp). It was found that Kp linearly correlated with lipophilicity and molecular size of the molecule. The more negative the log Kp (with Kp in cm/s), revealed the less skin permeant of the molecule (Potts and Guy, 1992). Our findings suggested gallic acid possess high skin permeability as its log Kp value was found as - 6.84.

Blood brain barrier (BBB) permeant affinity of the molecules depends on consensus log Po/w and TPSA, which represents for apparent polarity and lipophilicity. In case, if the egg-shaped molecular classification plot covers the yolk, it means that the molecule exhibits physicochemical nature for highly probable BBB permeation while it remains within the range of the white which represents the physicochemical space for highly probable HIA absorption. Besides, both compartments are not reciprocally exclusive by the molecule and remains outside grey region represents the molecules implying low absorption and partial brain penetration (Daina *et al.*, 2017). The outcomes of our study suggest gallic acid has BBB permeant affinity excluding other ones. Further, the outcomes of ADME analysis have been summarized in Figure 6.

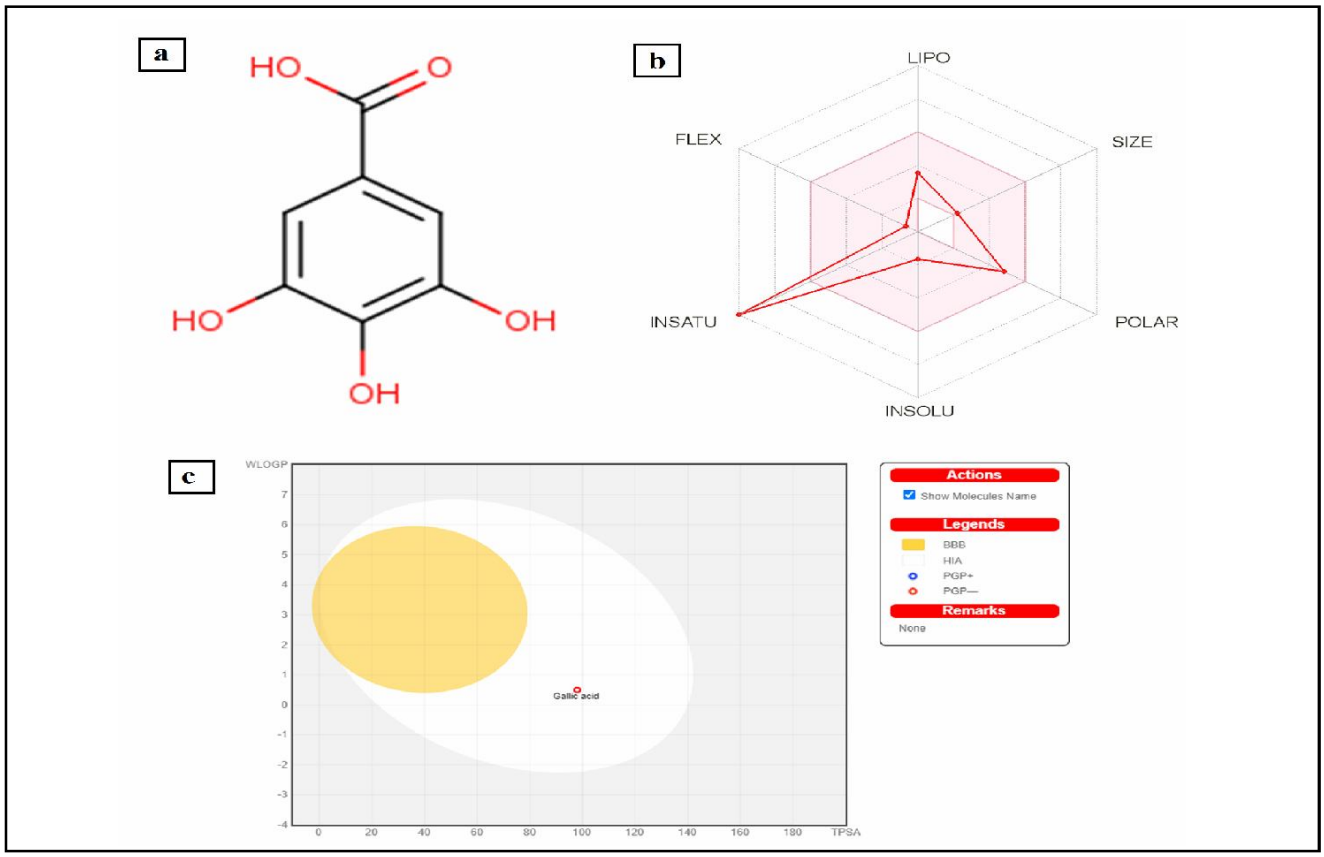

Figure 6: ADME analysis of gallic acid, Figure (a) represents the chemical structure of gallic acid, Figure (b) represents the radar plot of physicochemical properties of gallic acid while Figure (c) represent the boiled egg plot of gallic acid.

3.8 Network pharmacology analysis

Network pharmacology analysis was done to investigate molecular mechanism of gallic acid *via* their interaction by the genes associated in inflammation, immune response and oxidative stress. In this study, a total of 21 genes were selected based on their interaction with the compound and further evaluated by STING and cytoscape gene analytical tool. Protein-protein interaction (PPI) network was established to determine the interrelation of each target. The results of the study showed that the generated network exhibited the significant interaction among each gene. Each gene found to be interacted with each other which represents their strong even partial interaction among each other and played their biological role in inflammation, immune response and oxidative stress. In the constricted network possess number of nodes: 20, number of edges: 55, average node degree: 5.5, average local clustering coefficient: 0.729, expected number of edges:14 and the PPI enrichment *p*-value was found as 3.33e-16. The PPI network of the selected targets has been summarized in Figures 7 and 8.

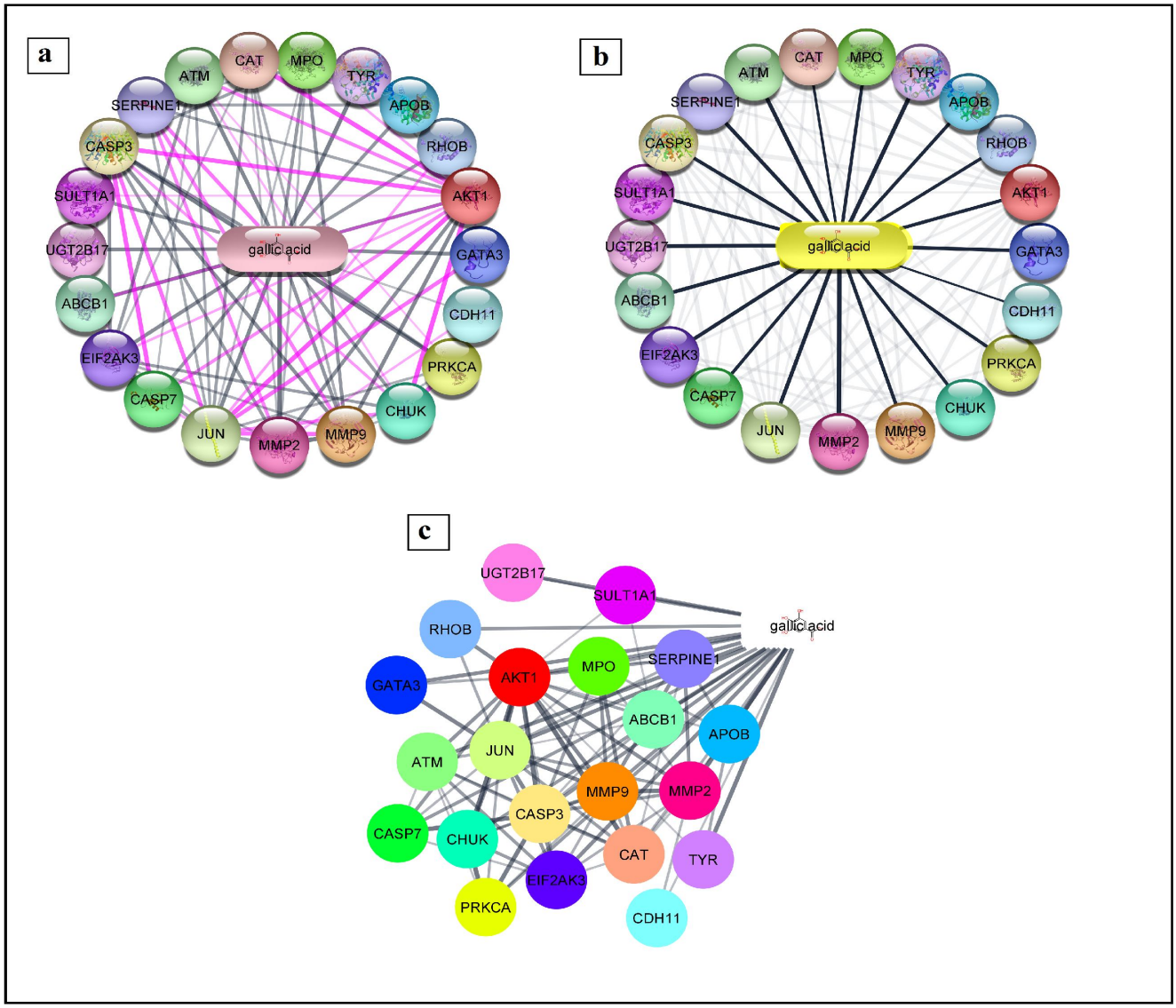

Figure 7: Network pharmacology analysis of gallic acid, Figure (a) represents the PPI network screened from the experimental data resources, Figure (b) represents the CPI network showed active nods interacted with the gallic acid while Figure (c) represents the combined PPI and CPI cluster of screened targets and gallic acid.

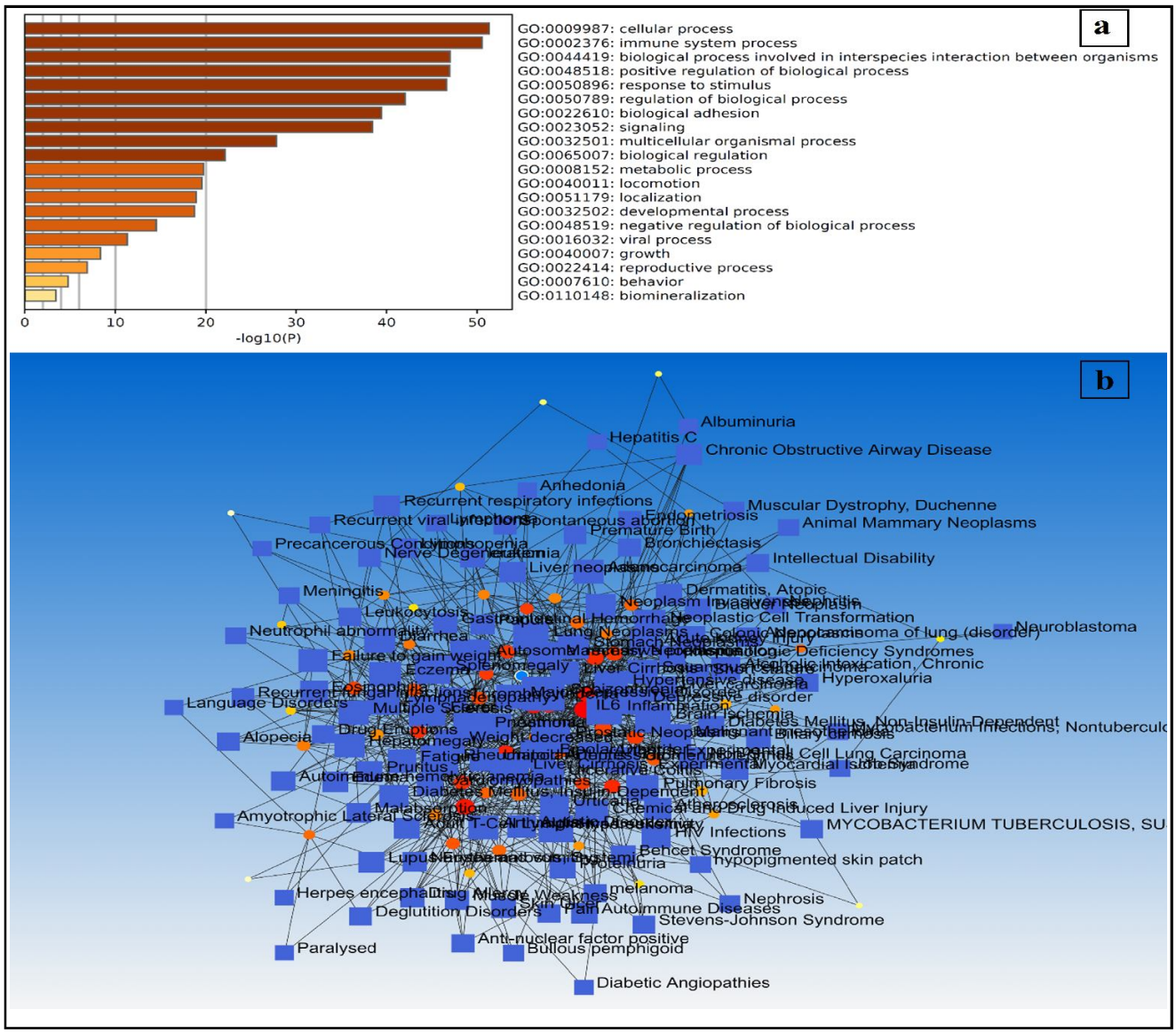

Figure 8: Represent the multiple physiological role of selected targets in alleviation of inflammation and oxidative stress, Figure (a) Gene ontology (GO) analysis performed through metascape and Figure (b) represents the targets-disease association analysis performed through network analyst.

4. Discussion

Topical ointment formulation was developed and optimized based on the drug content compatibility, successfully. The best formulation was screened based on content (w/w) released after final development of the formulation. The formulation which showed high content release profile was considered as the best and optimized formulation. Physiochemical based assessment of the pharmaceuticals is one of the essential quality step to validate the pharmaceuticals based on their quality and efficacy (Gdula-Argasińska *et al.*, 2009; Yarnykh *et al.*, 2012). Post-formulation studies were conducted to determine the pH, viscosity, spreadability and percentage drug release. The outcome of the study was found favorable to validate it as the optimized developed formulation. The previous studies conducted on the developed gel formulation support the present study (Kumar *et al.*, 2020; Pandurangan *et al.*, 2016; Chang *et al.*, 2013). Antibacterial activity of the developed formulation was evaluated against gram-positive and negative bacterial strains. The finding suggested that the developed formulation exhibited a potent antibacterial effect against survivability of the bacterial strains. In a study reported by Borges and his team showed antibacterial effect of gallic acid and its mechanism of action against the *via*bility of several bacterial strains including *E. coli* and *S. aureus.* The outcomes of the study revealed minimum inhibitory concentration (MIC) for gallic acid found as 1500 µg/ml for *E. coli*, 1750 µg/ml for *S. aureus*. Gallic acid led to irreversible changes in membrane properties such as intra and extracellular permeability, charge and physicochemical properties via mimicking the decrease of negative surface charge,

hydrophobicity changes, and occurrence of local rupture or pore formation in the cell membranes with subsequent escape of essential intracellular constituents (Borges *et al*., 2013). In a study, it was reported that gallic acid-grafted-chitosan inhibits food borne pathogens through a membrane damage mechanism. The experimental findings showed MICs of gallic acid-g-chitosans under ranged from 16 to 64 μg/ml against gram-positive bacterial strains and ranged from 128 to 512 μg/ml against gram-negative bacterial strains. Moreover, it was demonstrated that gallic acid-g-chitosan increased the release of intracellular components, increased the 1-N-phenylanphthylamine (NPN) uptake and the release of α-galactosidase *via* increasing the permeability of outer membrane and inner membrane in the bacterial strains (Lee and Je, 2013).

DPPH is an unstable dark purple color probe extensively used to determine the scavenging potential of herbal and their derived products. It is also used to determine the scavenging potential of synthetic compound as antioxidant effect (Dar *et al*., 2014; Khadam *et al*., 2019). DPPH reacts with the hydrogen atoms of the molecules and turn into the yellow color which represents the antioxidant effect of the compounds. The yellow color is directly proportional to the antioxidant activity of the compounds. Several studies reported that gallic acid is a strong antioxidant compounds which significantly scavenge DPPH free radicals and turn the resulting solution yellow (Kedare and Singh, 2011). Moreover, in a study conducted by Feng-Lin Hsu and his team reported that gallic acid is a strong candidate against the DPPH free radicals which significantly potentiate the antioxidant activity. The IC_{50} value of the gallic acid was found as 7.59 μM (Hsu *et al*., 2012). Furthermore, gallic acid significantly ameliorates the antioxidant enzymes level (SOD, CAT, GPx, *etc*.) and reduces the oxidative stress against the toxicity induced by the drug or environmental induced toxicity.

ADME computational analysis was performed for gallic acid through the "SwissADME and the parameters such as TPSA, consensus LogPo/w, ESOL Log S values, GI absorption, BBB permeant and log Kp (cm/s) (skin permeation) were predicted to determine the ADME response of gallic acid. The outcomes of the study showed that each identified parameter in favor of gallic acid found significantly active for better pharmacokinetic assessment of gallic acid. TPSA for gallic acid was found as 97.99 which represent its concerning absorption and brain access (Daina and Zoete, 2016). A high positive value for logP represents lipophilicity of the molecule. Potts and Guy provide the model to predict the skin permeability as skin permeability coefficient (Kp). It was found that Kp linearly correlated with lipophilicity and molecular size of the molecule. The more negative the log Kp (with Kp in cm/s), revealed the less skin permeant of the molecule (Potts and Guy, 1992). Our findings suggested gallic acid possess high skin permeability as its log Kp value was found as -6.84. Moreover, in blood brain barrier (BBB) permeant affinity, both compartments of boiled egg plot are not reciprocally exclusive by the molecule and remains outside grey region represents the molecules implying low absorption and partial brain penetration (Daina *et al*., 2017).

In network pharmacology analysis, the compound-protein interaction (CPI), interaction of gallic acid was determined based to the targets, the results revealed that gallic acid found to be interacted with 21 genes such as CAT, MPO, TYR, AKTI, GATA3, CDH11, MMP9, MMP2, JUN, CASP7, CASP3, *etc*. The constructed network has been shown in Figure.

Catalase (CAT) is an antioxidant enzyme which protect cells from the toxic effects of hydrogen peroxide and reduce the oxidative stress and inflammation induced by oxidative stress (Ighodaro and Akinloye, 2018; Ruottinen *et al*., 2020).

Trans-acting T-cell-specific transcription factor GATA-3 (GATA3) act as the transcriptional activator and binds to the enhancer of the T-cell receptor alpha and delta genes and process following immune and inflammatory responses (Wan, 2014).

Component of inhibitor of nuclear factor kappa B kinase complex (CHUK) is a serine kinase which act as the inhibitor of NF-κB signaling pathway. It is activated by multiple stimuli such as bacterial or viral products, inflammatory cytokines, DNA damages or other cellular stresses (Moody *et al*., 2020; Su *et al*., 2021). Caspase-7 (CASP7) involved in the activation cascade of caspases which is responsible for apoptosis execution (Lamkanfi and Kanneganti, 2010). Cleaves and activates sterol regulatory element binding proteins (SREBPs) are functionally conserved in fungi, while the capability to respond to sterols is conserved, fungal SREBPs are hypoxic transcription factors required for adaptation to a low-oxygen environment which required for virulence and resistance to antifungal drugs for antifungal therapy (Bien and Espenshade, 2010). UDP-glucuronosyltransferase 2B17 (UGT2B17) possess major role in the conjugation and consequent elimination of potentially toxic xenobiotics and endogenous compounds. Hence, the present generated scientific evidences validate the developed formulation as a potent antibacterial agent or acting efficiently against the bacterial induced inflammation and oxidative stress.

5. Conclusion

The study demonstrated that the developed formulation (F5) was found the best formulation based on optimization of drug content uniformity and exhibited potential antibacterial potential against the survivability of the *E. coli* and *S. aureus* bacterial strains. Network pharmacology analysis showed multiple targets association with the gallic acid which specially found to be active against inflammation, oxidative stress and several biological stimulus. Furthermore, gene ontology (GO) analysis showed multi-mechanistic and therapeutic response of gallic acid in regulation of inflammation and oxidative stress induced by several biological stimulus.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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