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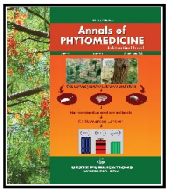

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Development and evaluation of eugenol-based gel formulation for analgesic and anti-inflammatory action

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Abstract

Eugenol is acknowledged as a potent anti-inflammatory and analgesic agent. Due to the opacity for the development of effective and affordable topical pharmaceuticals and lack of a multi-mechanistic approach, the present study is aimed to develop a eugenol-based topical formulation and explore the multi-mechanistic effect of eugenol-based gel formulation for analgesic and anti-inflammatory action. Pre-formulation and post formulation studies were conducted to evaluate the drug compatibility with certain bases. Furthermore, network pharmacology and gene ontology analysis were conducted to determine the multi-mechanistic role of eugenol in treating pain, inflammation and associated malfunctions. The results showed that among the four developed formulations, F3 formulation was found as the optimized formulation with the average drug content 1.584 ± 0.070 mg/g. Evaluation of post-formulation parameters such as pH, spreadability, viscosity and FT-IR showed the optimistic characteristic of the optimized formulation. Furthermore, in network pharmacology and gene ontology (GO) study, it can be demonstrated that the developed eugenol based formulation play multiple physiological actions such as regulation of angiogenesis, inflammatory response, dopaminergic synapse, response to reactive oxygen species, *etc*., *via* regulation of genes such as ALOX5, BDKRB2, CASP3, MAOA, SCN10A, SCN11A, UGT1A7, VR1 *etc*., It can be demonstrated that the developed formulation provides an effective pharmaceutical formulation with potent analgesic and anti-inflammatory property.

1. Introduction

Pain is generally characterized as the unpleasant sensory as well as emotional experience due to actual or potential tissue damage or can be described in the milieu of such damage. As per the International Association for the Study of Pain, inflammation is the immune response of tissue to any of the acute or chronic injury considered by accumulation of white blood cells and antibodies, swelling and fluid accumulation at the injured site. It causes serious morbidity and mortality of the tissues. Different types of cytokines interleukin tumor necrosis factor-alpha (TNF-α), interleukins (ILs) and mediators such as reactive oxygen, or nitrogen species and prostaglandins are unrestricted which trigger inflammation with progressive distortion of tissue (Kany *et al*., 2019; Moldoveanu *et al*., 2015; Neurath, 2014). In addition, the prostaglandins such as prostaglandin E2 (PGE2) are acknowledged as the major pain inducing inflammatory markers which is persuaded by cyclooxygenase 2 (COX-2) in the process of inflammation (Chen *et al*., 2021; Varia *et al*., 2021; Kimura and Kontani, 2009; Oh *et al*., 2018). It is also cited that pain is the common reason for accessing the indication of inflammation (Lai *et al*., 2019; Lee *et al*., 2018). Most of the cases, pain is acknowledged as the major cause of disability and because of that, it also influencing the health care system to find a sustainable and compatible solution for pain. Approximately 76.2 million, one in four Americans, have suffered from pain that lasts longer than 24 h and millions more suffer from acute pain(Kola-Mustapha *et al*., 2020).

The human skin acts as afence between the human body and the external environment. It is a protective organ with three layers: epidermis, dermis, and hypodermis. Lipids of the cornified layer are important in the selective absorption of compounds from the skin surface. Drug absorption occurs through the epidermis and through the skin appendages which facilitate the availability of the targeted drug in the blood where the primary route of drug absorption is through the epidermis.Topical analgesics are pain medications applied to the skin. They work in different ways for different conditions but are commonly used to treat musculoskeletal and some types of neuropathic pain (Balzani *et al*., 2021; Franz *et al*., 2019; Hennemann-Krause and Sredni, 2016). Oral analgesic and anti-inflammatory drugs are usually prescribed for the treatment of acute and chronic pain and inflammation. These agents often have adverse systemic effects which are sometimes severe. Topical analgesics and anti-inflammatory drugs provide the same relief with reduced adverse systemic effects (Kola-Mustapha *et al*., 2020)

In addition, quality-based assessment of pharmaceuticals is one of the concerning needs to validate them for their regulatory purpose. Several analytical techniques such as UV-visible spectroscopy, fourier transform infrared spectroscopy (FTIR), high-performance liquid

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chromatography (HPLC), gel filtration chromatography, high-Performance thin layer chromatography (HPTLC) (Venkatesham *et al*., 2021), capillary zone electrophoresis, liquid chromatography and mass spectroscopy (LC-MS), MS and nuclear magnetic resonance spectroscopy (NMR) methods (Fan *et al*., 2006; Harinantenaina *et al*., 2006; Yan *et al*., 2013) are available for the analysis of drug or active pharmaceutical ingredients in a mixture of drugs or even formulation and generates the scientific evidence concerning the authenticity of pharmaceuticals.

For decades, computational approaches have been playing an immense role in drug design, synthesis, and evaluating the biological interaction of newly developed drug molecules. Network pharmacology is one of the computational approaches which explore the multi-mechanistic role of any therapeutically active drug molecule based on the multiple genomic interactions at a single time. Protein-protein interaction and protein-drug interaction explore the role of selected targets in related dysfunction and while protein-drug interaction reveals the ligation efficacy of a drug molecule based on the degree of ligation (Kamalakkannan *et al*., 2021; Gezici and Sekeroglu, 2021).

Taking all these facts into consideration, the study is associated to determine the analgesic and inflammatory activity of developed gel formulation as a topical drug delivery system, which can be used to alleviate joint pain, muscular cramps, *etc*. It would be an effective, economic and alternative therapy that can overcome the use of existing pharmaceuticals.

2. Materials and Methods

2.1 Chemical and reagents

n-octanol, potassium bromide eugenol, propylene glycol, methylparaben and carbopol were bought from the Sisco Research Laboratories Pvt. Ltd. (SRL), New Delhi, India, for the experimental studies. Double distilled water used in the study, was procured from the own institution.

2.2 Pre-formulation studies

2.2.1 Determination of the absorption maximum of eugenol in ethanol

The absorption maximum of eugenol was determined for validation analysis using the described protocol with some modification. In brief, the different concentrations (0.5, 01, 02, 04, 06, 08, 10 µg/ml) of eugenol were prepared and validation analysis was performed using the UV spectroscopical method at 240 nm. The measurement was taken in triplicate to achieve the specificity and accuracy of the developed method. The resulted outcomes were expressed statistically (Shantier *et al*., 2011). The validation analysis restricted to determine linearity, accuracy, precision, the limit of detection, the limit of quantification, specificity, *etc.*, were determined as per ICH guidelines.

2.2.2 Estimation of lipophilicity

The partition coefficient of the eugenol was determined using an n-octanol and water partition method using a standard protocol with some modification. The measured amount of eugenol was poured into a flask containing equal volumes of n-octanol and aqueous buffer solution. The flask was shaken with a uniform time interval for 10 min to achieve balance and afterward. The mixture was pooled into a separating funnel and kept the funnel undisturbed to get separate the mixture into two different layers. The layers were estimated spectrophotometrically (UV spectrophotometer, Shimadzu-1700, Japan) at 282 nm. The measurements were taken in triplicate (Di and Kerns, 2016).

2.2.3 FT-IR spectroscopy

The spectral analysis for eugenolwas accomplished using Win-IR, Bio-Rad FTS spectrophotometer. The sample was mixed with potassium bromide and later proceeds for spectroscopical analysis. The analysis was performed under the range of 4000 to 400 cm^{-1}(Hosseini *et al*., 2019).

2.2.4 ADME/TOX analysis

ADME (absorption, distribution, metabolism and excretion) and toxicological analysis was performed for eugenol through "Swiss ADME (http://www.swissadme.ch/index.php)" and ProTox-II (https://tox-new.charite.de/protox_II/index.php?site=home) as a tool. Topological polar surface area (TPSA), Consensus Log Po/w to determine the drug lipophilicity, Log K_p (skin permeation) as well as drug-likeness was projected as the standard constraints for considering the ADME response of eugenol (Daina *et al*., 2017).

2.3 Development of gel formulation

Methylparaben was accurately weighed and placed in a beaker contain hot water (75 ± 2ºC) and enthused to dissolve the content. An optimum amount of eugenol was mixed with propylene glycol, followed by the addition of methylparaben. Carbopol was added into the mixture and the volume of the mixture was made up to 100 ml using distilled water. The content was stirred continuously using a stirrer for 30 min. Triethanolamine was used to adjust the pH during the process. The process was continued till a transparent and clear gel was obtained (Kola-Mustapha *et al*., 2020). In this method, four different gel formulations were prepared based on the different composition presented in Table 1.

Table 1: Composition of eugenol gel formulations

Ingredients (g)	Composition of gel formulation			
	F1	F2	F3	F4
Methyl paraben	0.2	0.2	0.2	0.2
Eugenol (mg)	50	100	200	500
Propylene glycol	15	15	15	15
Triethanolamine	Qs	Qs	Qs	Qs
Carbopol	1	1	1	1
Water qs to	100	100	100	100

2.4 Post formulation studies

2.4.1 Physical evaluations

Organoleptic characteristic of the developed optimized formulation such as odor, color and texture of the developed formulation were determined.

2.4.2 Determination of pH

The pH value of the developed gel formulation was determined by calibrated digital pH meter. The observation carried out in triplicate (Kumar *et al*., 2020).

2.4.3 Spreadability

The spreadability test was performed based on spreading diameter of developed formulation between two horizontal plates (20 x 20 cm) as reported with slight modifications. 1 g of the sample was weighed accurately and kept carefully on the center of the horizontal glass slide. The second glass plate on top of the gel was placed and the time engaged for the gel to spread within 1 min was noted. The process was conducted in triplicate. The results were expressed as Mean ± SD (Kumar *et al*., 2020).

2.4.4 Determination of viscosity

Viscosity was estimated using Viscometer (NDJ-5S Digital Display, Rinch, China) at 25p C and 60 rpm using the Number 4 spindle. The analysis was carried out in triplicates and results were noted (Kumar *et al*., 2020).

2.4.5 FT-IR spectroscopy

The spectral analysis for the developed gel formulation was accomplished using Win-IR, Bio-Rad FTS spectrophotometer. The sample was mixed with potassium bromide and later the sample was placed for spectroscopical analysis. The analysis was performed under the range of 4000 to 400 cm^{-1} (Hosseini *et al*., 2019).

2.5 Network pharmacology analysis

Different targets or proteins or genomes were screened from Genecard (https://www.genecards.org/). The UniPort ID of each genes were recorded from the UniPort database (https://www.UniProt.org/uploadlists/) (Li *et al*., 2021). The ligation efficacy was predicted for each selected target. In this analysis, protein-protein interactions (PPI) network for estimation of genes interrelation and compound-proteins interactions network for compound interaction with gene were constructed using STRING and Cytoscape (version 3.8.2) software. The analysis covered maximum interaction exhibited by each gene and exposed as network proteins-proteins network and compound-proteins network (Li *et al*., 2021; Yi *et al*., 2018).

2.6 Gene ontology (GO) analysis

In gene ontology (GO) analysis, metascape gene analysis (metascape.org) software was used to evaluate multiple roles of each gene in physiological of the targeted disease. The generated bar graph was directly exported and saved for record purpose.

2.7 Statistical analysis

Results were expressed as Mean ± SD. One-way ANOVA with Tukey test was used to compare all the pairs of columns. The significance level was considered in terms of *p*-value (*) while the significance level was considered at $p<0.05$.

3. Results

3.1 Pre-formulation studies

3.1.1 Determination of the absorption maximum of eugenol in ethanol

Determination of absorption maxima and method validation analysis of eugenol on different concentrations ranging 2000-50 ng/ml was performed successfully using a UV spectrophotometer. The outcomes of the study revealed the absorption maxima of gallic acid at 240 nm (Shantier *et al*., 2011). The analysis showed that the developed method was found linear, robust, accurate and precise under the different concentrations ranging 2000-50 ng/ml. The calibration equation and regression coefficient for developed method of gallic acid was found as $y = 0.0004x + 0.0312$ and $R^2 = 0.9949$, respectively. The limit of detection (LOD) and the limit of quantitation (LOQ) for gallic acid were found as 10.471 ± 0.352 and 31.731 ± 0.859 ng/ concentration. The precision was determined as intra-day and inter-day precision percentage relative standard deviation (%RSD), the results showed intra-day and inter-day precision 0.261-1.753 and 0.297-3.142, respectively. The accuracy was determined in form of percentage drug recovery by 0, 50, 100, and 150% per cent spiking of the standard to the sample. The exhibited percentage recovery for eugenol was found in the range of 101.418-103.758%. The UV spectra and calibration curve has been represented in Figure 1.

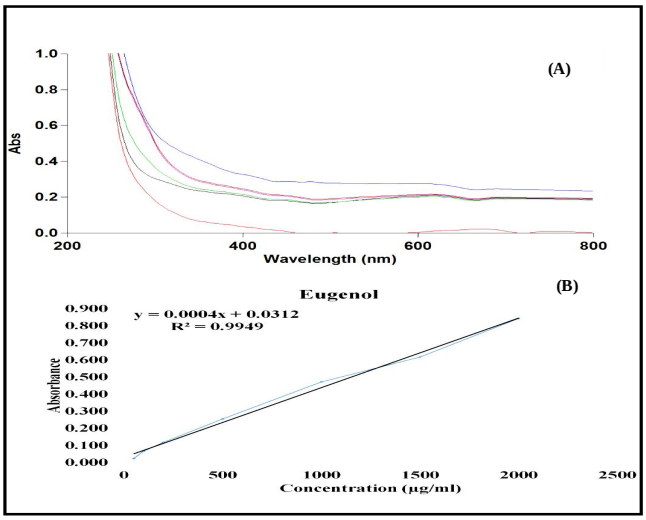

Figure 1: Determination of absorption maxima and validation analysis of eugenol.

3.1.2 Determination of lipophilicity

Lipophilicity of a drug determines the biological availability of the drug and is considered as the extent of the drug. Pre-formulation studies were conducted to determine the lipophilicity nature of eugenol. The hydrophilicity of eugenol was determined in distilled water, the outcome of the study showed that the log *p*-value of eugenol was found as 2.13 ± 0.011.

3.1.3 FT-IR spectroscopy

FT-IR spectroscopy analysis was done for the identification of the test compound based on different functional groups that appeared at different frequency ranges. The outcome of the study showed various signals of represent the purity of the eugenol. The signals found at 3525.3 represents the signal of a hydroxy group, 2931.7 represent the signal of $-CH_2/-CH_3$ vibrations, 1619.1 represents the signal of -C=C vibration, 1362.5 represents the signal of -C-O-C- group vibration, while signals appeared at 1079.32, 925.7 and 812.5 represent the signals of aromatic and aliphatic scissoring vibrations. FT-IR spectra of eugenol is depicted in Figure 4.

3.1.4 ADME/TOX analysis

ADME/TOX analysis of eugenol was performed successfully using the computational tool "SwissADME". 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, lipophilicity and the drug-likeness response of eugenol. The TPSA value for eugenol was found as 29.46. The models accelerate the prediction accuracy for the physicochemical properties through consensus log Po/w which was found as 2.25. A positive value for logP represents the lipophilicity of the molecule while a negative value represents the hydrophilicity of the compound. Furthermore, in our findings, high skin permeability, as the log Kp valuefor eugenolwas found as-5.69 with high BBB permeant affinity. In toxicity analysis, it has been revealed that eugenol falls in the predicted toxicity class: 4 while the predicted LD50: 1930 mg/kg. The ADME egg and radar plot has been summarized in Figure 2.

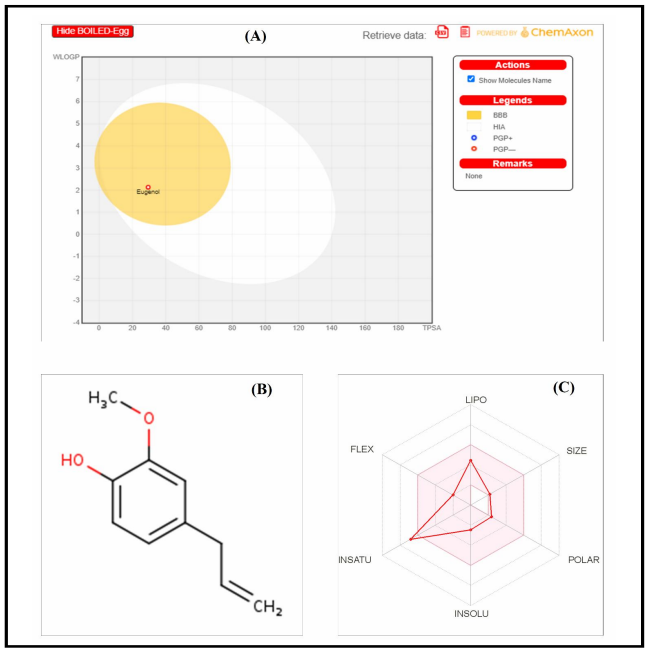

Figure 2: Swiss ADME analysis of eugenol, Figure (A) represents the boiled egg plot of eugenol, Figure (B) represents the chemical structure of eugenol, and Figure (C) represents the radar plot for lipophilicity and drug-likeness based on size, polar, insolubility index.

3.2 Development of gel formulation

Different gel formulations were prepared based on the composition of the drug used in the formulation and to evaluate the effective formulation with no substantial interaction of the drug molecule to the base used for the final development of the formulation. The effectivity of the different formulations was determined based on the content uniformity and content (w/w) found in the final formulation. UV spectrometer was used to determine the content of the drug in the final developed formulations. The measurements were assessed in triplicate to determine the accuracy, precision and robustness of the method. The outcome of the study showed that formulation 4 (F4) was found with a uniform drug content. The average content of the drug for different formulation (F1-F4) was found as 0.258 ± 0.032, 0.8955 ± 0.079, 1.584 ± 0.070, 1.6455 ± 0.150 mg/g of the sample, respectively. The study is based on the drug contents which proportionate the biological effectivity of the formulation. The outcome of the study has been summarized in the Figure 3.

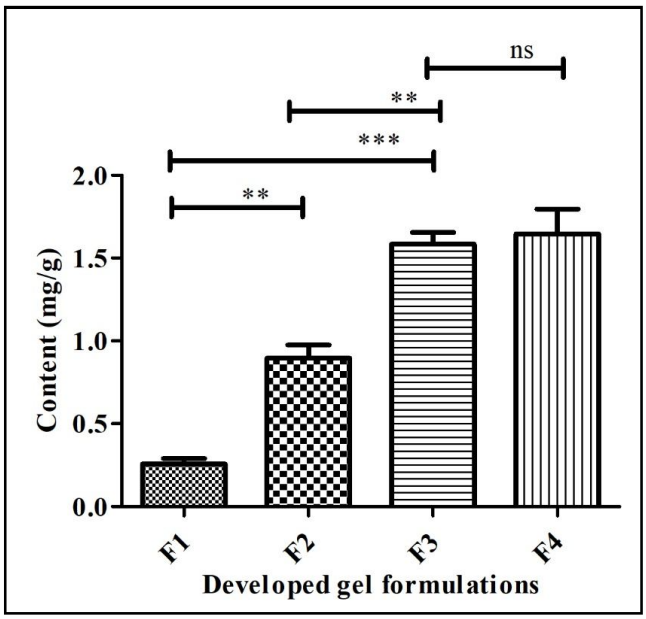

Figure 3: Content (w/w) found of eugenol in different developed formylation.

3.3 Post formulation studies

Organoleptic parameters such as odor, color and texture of the developed formulation were evaluated. The observations revealed that the developed formulation exhibited a pleasant aromatic odor, creamish color with a smooth texture. The pH value of the developed formulation was found as 5.313 ± 0.023. The spreadability of the developed formulation was targeted to determine the smooth texture and targeted site covered at the minimum dose. The affinity of the developed formulation was determined based on the glass coverslip spreadability method. The outcome of the study showed that the spreadability of the developed formulation was found as 121.42 ± 1.665 g.cm/s. The therapeutic efficacy of the developed topical gel formulation depends on their spread index so that it may cover the maximum area and can exhibit the maximum drug extant to the bloodstream. Furthermore, it is reported that the gel spreading supports the uniformity of the gel to the skin, so that the developed gel formulation must have a good spreadability and imply the ideal standard quality in topical application. Furthermore, it is significant factor in patient compliance respect of the therapeutic application.

Furthermore, the consistency of the substance is an imperative factor for such formulation deals for analgesic as well as anti-inflammatory topical formulations due to being applied to the thin layers of the skin. The viscosity of gel formulation is also essential to determine the drug permeability. The viscosity of the developed formulation was determined and the outcomes of the study showed 1792. ± 8.619 Pas. Furthermore, the compatibility of the eugenol was determined with the bases used in the formulation and the analysis was performed through the FT-IR method. The outcome of the study showed that principle adsorption base peaks at 3518.7 represents the signal of a hydroxy group, 2931.7 represents the signal of $–CH_2$/ $–CH_3$ vibrations, 1619.1 represents the signal of -C=C vibration, 1362.5 represents the signal of -C-O-C- group vibration, while signals appeared at1079.32, 925.7 and 812.5 represent the signals of aromatic

and aliphatic scissoring vibrations. The outcome of the study was comparatively matched with the FT-IR results of eugenol and reported literature which strongly supports the outcome of our study. The spectra of the analysis have been depicted in Figure 4.

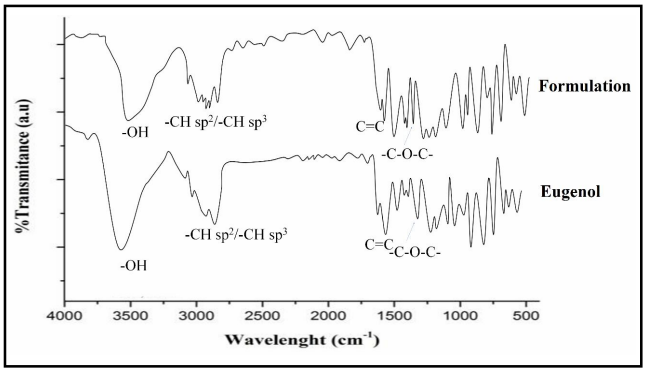

Figure 4: FT-IR spectroscopy of developed formulation and eugenol.

3.4 Network pharmacology analysis

Network pharmacological studies were conducted to determine the multifunctional role of eugenol against the inflammation and removal of the toxins which are generally entrapped in the synovial joints and causes pain (Li *et al*., 2021). In this study, the ligation efficacy of each selected gene was predicted for the targeted ligand as eugenol. PPI and CPI networks were constructed using the STRING platform (https://string-db.org/) and Cytoscape (version 3.8.2) software and interpreted based on ligation efficacy or interaction among protein-protein, protein-compound. The analysis covered all the nearly functional interactions among the expressed proteins-proteins and compound-proteins (Li *et al*., 2021; Yi *et al*., 2018). The outcome of the study showed that the selected genes possessed a strong interaction with each other. The PPI network showed that number of edges: 116, number of nodes: 36, average node degree: 6.44, expected number of edges: 11, avg. local clustering coefficient: 0.647, PPI enrichment p-value: < 1.0e-16. The PPI interaction showed that each gene possessed a strong interaction with each other, while some of the genes such as CCL3, ALOX5, MAOA, SPICE1, *etc*., have less interaction with other genes. The PPI network of the selected genes has been represented in Figure 5.

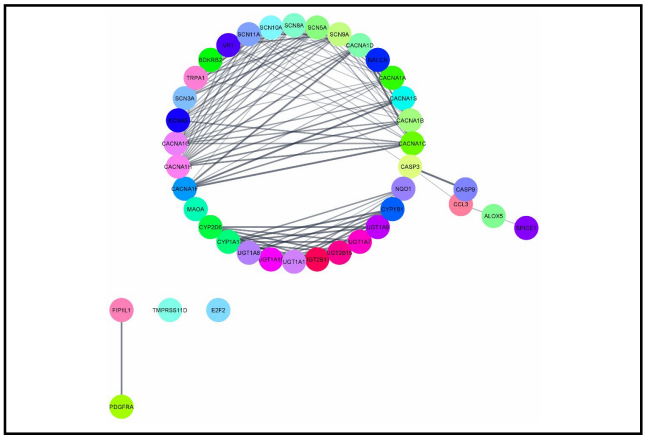

Figure 5: Protein-protein interaction (PPI) network showing the enriched gene interaction with the black edges (line) and the genes which possessed less interaction, found with no edges or out of the network.

In CPI network analysis, it has been found that a total of 37 genes (Table 2) were found to have strong interaction with eugenol. The majority of the genes found in the network are namely ALOX5 BDKRB2, CACNA1A, CASP3, CYP1A1, MAOA, SCN10A, SCN11A, UGT1A7, VR1, *etc*., and suggested that eugenol possessed the anti-inflammatory activity, regulates toxins excretion, reduce muscle cramps, *etc*. The CPI network is shown in Figure 6.

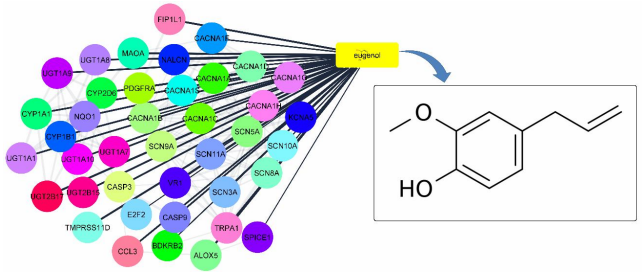

Figure 6: Compound and protein interaction network of eugenol showing the enriched interaction with the selected genes.

Table 2: List of the selected genes

Sl. No.	Gene	Protein name	Uniport ID
1.	ALOX5	Polyunsaturated fatty acid 5-lipoxygenase	P09917
2.	BDKRB2	B2 bradykinin receptor	P30411
3.	CACNA1A	Voltage-dependent P/Q-type calcium channel subunit alpha-1A	O00555
4.	CACNA1B	Voltage-dependent N-type calcium channel subunit alpha-1B	Q00975
5.	CACNA1C	Voltage-dependent L-type calcium channel subunit alpha-1C	Q13936
6.	CACNA1F	Voltage-dependent L-type calcium channel subunit alpha-1F	O60840
7.	CACNA1H	Voltage-dependent T-type calcium channel subunit alpha-1H	O95180
8.	CACNA1S	Voltage-dependent L-type calcium channel subunit alpha-1S	Q13698
9.	CACNAIG	Voltage-dependent T-type calcium channel subunit alpha-1G	O43497
10.	CASP3	Caspase-3	P42574
11.	CASP9	Caspase-9	P55211
12.	CCL3	C-C motif chemokine 3	P10147
13.	CYP1A1	Cytochrome P450 1A1	P04798
14.	CYP1B1	Cytochrome P450 1B1	Q16678
15.	CYP2D6	Cytochrome P450 2D6	P10635
16.	E2F2	Transcription factor E2F2	Q14209

17.	FIP1L1	Pre-m RNA 3'-end-processing factor FIP1	Q6UN15
18.	KCNA5	Potassium voltage-gated channel subfamily A member 5	P22460
19.	MAOA	Amine oxidase [flavin-containing] A	P21397
20.	NALCN	Sodium leak channel non-selective protein	Q8IZF0
21.	PDGFRA	Platelet-derived growth factor receptor alpha	P16234
22.	SCN10A	Sodium channel protein type 10 subunit alpha	Q9Y5Y9
23.	SCN11A	Sodium channel protein type 11 subunit alpha	Q9UI33
24.	SCN3A	Sodium channel protein type 3 subunit alpha	Q9NY46
25.	SCN5A	Sodium channel protein type 5 subunit alpha	Q14524
26.	SCN8A	Sodium channel protein type 8 subunit alpha	Q9UQD0
27.	SCN9A	Sodium channel protein type 9 subunit alpha	Q15858
28.	SPICE1	Spindle and centriole-associated protein 1	Q8N0Z3
29.	TMPRSS11D	Transmembrane protease serine 11D	O60235
30.	TRPA1	Transient receptor potential cation channel subfamily A member 1	O75762
31.	UGT1A1	UDP-glucuronosyl transferase 1A1	P22309
32.	UGT1A10	UDP-glucuronosyl transferase 1A10	Q9HAW8
33.	UGT1A7	UDP-glucuronosyl transferase 1A7	Q9HAW7
34.	UGT1A8	UDP-glucuronosyl transferase 1A8	Q9HAW9
35.	UGT2B15	UDP-glucuronosyl transferase 2B15	P54855
36.	UGT2B17	UDP-glucuronosyl transferase 2B17	O75795
37.	VR1	Transient receptor potential cation channel subfamily V member 1	Q8NER1

3.5 Gene ontology (GO) analysis

Gene ontology (GO) analysis using the metascape gene analysis (metascape.org) tool was performed to determine the multiple physiological function of each gene in the regulation of pain, inflammation and associated malfunction. The analysis was performed through the metascape gene analytical tool (each gene was collected of *Homo sapiens* species). The outcome of the study showed that the selected genes play an essential role in the regulation of the sensory signals of pain and associated malfunction *via* regulation of angiogenesis, inflammatory response, dopaminergic synapse, response to reactive oxygen species and response to inorganic substance, calcium ion transport, and serotonergic synapse. It is reported that eugenol plays an essential role in the regulation of pain, inflammation and associated malfunction functions. The bar chart of GO analysis is depicted in Figure 7.

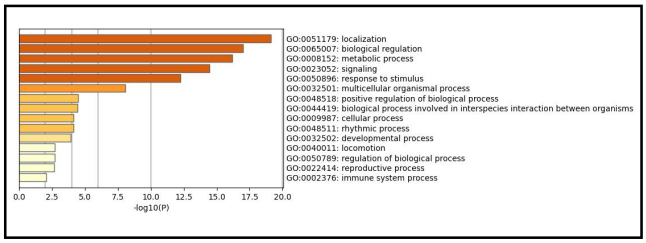

Figure 7: Gene ontology (GO) analysis showed that the selected genes were found to be exhibited multiple pathophysiological roles in alleviating inflammation, pain, or associated ailments.

4. Discussion

UV spectrophotometry is one of the robust and precise analytical techniques for qualitative and quantitative analysis of drugs or herbal constituents. Several studies have been reported based on qualitative and quantitative evaluation of drugs using UV spectrophotometry as an analytical method (Cazedey and Salgado, 2014; Ferraz *et al.*, 2015; Levin *et al.*, 2019). In a study conducted by Hameed his team evaluated phytoconstituents such as eugenol in the developed formulation based on clove oil-encapsulated nanofiber for wound-healing properties and reported that the developed nanofiber formulation exhibited a potential effect in wound healing (Hameed *et al.*, 2021).

Lipophilicity of a drug represents its bioequivalence as well as the extant reaching to the therapeutic site. More lipophilic nature of the drug represents its high bioequivalence and metabolism and excretion from the body. In our study, lipophilicity of the drug was determined and found that eugenol exhibits a high lipophilic nature. The outcome of the study was matched with the previous study conducted by Makuch *et al.*, which reported the log P value of eugenol was found as 2.20 ± 0.001 (Makuch *et al.*, 2020). Pre-formulation and post-formulation analysis was conducted to determine the purity and compatibility of the drug molecule and the outcomes of the study were matched with the reported literature which strongly supports the outcome of our study (Makuch *et al.*, 2020)

TPSA act as a useful descriptor in many models and rules to estimate ADME properties, especially concerning absorption and brain access (Daina and Zoete, 2016). The TPSA value for eugenol was found as 29.46. 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 (Mannhold *et al.*, 2009). The models accelerate the prediction accuracy for the physicochemical properties through consensus log Po/w which was found as 2.25. A positive value for log P represents the lipophilicity of the molecule while a negative value represents the hydrophilicity of the compound. Similarly, a model provided by Potts and Guy represents that skin permeability coefficient (Kp) and correlated it

with the molecular size and lipophilicity of the molecule. The more negative the log Kp (with Kp in cm/s), the less skin permeant the molecule (Daina *et al*., 2017). Our findings suggest that several metabolites possess high skin permeability, as the log Kp value for eugenol was found as –5.69.

The blood-brain barrier (BBB) permeant affinity of the molecules depends on consensus log Po/w and TPSA represent lipophilicity and apparent polarity. In case, if the egg-shaped molecular classification plot covers the yolk, it means that the molecule exhibits physicochemical space for highly probable BBB permeation, while it remains within the range of the white which represents the physicochemical space for highly probable HIA absorption (Daina *et al*., 2017). The outcomes of our study suggest that eugenol has a high BBB permeant affinity. In toxicity analysis, it has been revealed that eugenol falls in the predicted toxicity class: 4 while the predicted LD50: 1930 mg/kg.

It is reported that sodium voltage-gated channel alpha and beta subunit play an essential role in confirmed a significant role in voltage-gated sodium channels (Na^+; VGSCs) in regulation of neuronal excitability in both the conditions such as normal and pathological pain states. The targeted gene in this pathway, namely; SCN1A-SCN5A and SCN8A-SCN11A genes (Hameed, 2019). Monoamine neurotransmitters such as serotonin (5-HT), monoamine oxidase A, dopamine (DA), and norepinephrine (NE), have been studied for their involvement in chronic pain and depression (Sheng *et al*., 2017). Furthermore, it has been reported that eugenol enthused the steady-state inactivation arcs of both Na(+) currents to a hyperpolarizing route and reduces the maximal Na(+) current. Thus, eugenol results to inhibit Na(+) currents through its interaction with both resting and inactivated Na(+) channels. The inactivation recovery from both Na(+) currents is considered as one of the mechanisms by which eugenol exerts analgesia (Cho *et al*., 2008). Furthermore, it has been confirmed that the enzyme such as multiple UGT enzymes such as UGT1A1, UGT1A10, UGT1A7, UGT1A8, UGT2B15 seem to be involved in the hepatic catalysis of NSAID glucuronidation (Kuehl *et al*., 2005).

Furthermore, in GO analysis, it was revealed that the selected targets play an essential role in the regulation of the sensory signals of pain and associated malfunction *via* regulation of angiogenesis, inflammatory response, dopaminergic synapse, response to reactive oxygen species and response to inorganic substance, calcium ion transport, and serotonergic synapse. It is reported that eugenol plays an essential role in the regulation of pain, inflammation and associated malfunction functions.

5. Conclusion

The study concludes that eugenol concentration (100 µg/100 g, w/w) was successfully incorporated into the carbopol and provided an effective gel formulation. The developed eugenol gel formulation exhibited good physiochemical compatibility. Network pharmacology analysis showed that eugenol could be a promising alternative as an analgesic and anti-inflammatory agent for topical or transdermal treatment.

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Conflict of interest

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

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