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Formulations Containing Essential Oils and Evaluation of their Cytotoxicity & Invitro Anti-Inflammatory Activity

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KEYWORDS:

Ylang-Ylang oil;
Cytotoxicity;
Anti-inflammatory;
Essential oil;
Mosquito repellents.

ABSTRACT:

The main aim of this study is to develop the formulations by using the combinations of essential oils. Flax seed oil was used in the preparation of formulation with other volatile oils. Thyme oil, Lavender oil, Ylang-Ylang oil, and Nagarmotha oil used in the preparation. The formulation containing thyme oil and ylang-ylang oil showed the least cytotoxicity with IC50 value 47.2 ± 0.01922 µl/ml followed by the F1 formulation. The F2 formulation further evaluated for its enzyme inhibition assay and showed effective result in invitro anti-inflammatory activity.

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Introduction

Essential oils are the volatile components present in aromatic plants. They are the main ingredients of aromatherapy (Tiwari, et al., 2021). Essential oils were used in many medicinal preparations and responsible for many pharmacological actions like analgesic, anti-fungal, anti-bacterial, anti-inflammatory, anti-depressant, and many more. Volatile oils are effective in the formulations when used as the repellant (Mishra, et al., 2023). Like citronella oil had been used traditionally for its mosquito repellency activity (Banard, 2000; Mishra et al). The repellent activity of *Azadirachta indica* (Maliaceae) and *Zanthoxylum armatum* (Rutaceae) to repel mosquitoes has also been studied (Mishra et.al.,2023).

The main aim of this study is to develop the formulations using the combinations of different essential oil and evaluated for its cytotoxic and enzyme inhibition activity.

2. Material and Methods

2.1 Preparation of formulations by using essential oils

Essential oils of *Thymus vulgaris, Lavandula angustifolia, Cyperus rotundus, Canaga odorata*were used in the preparation of different emulgel formulations. Aloevera leaves were collected and after peeling the upper layer gel used to remove. The obtained gel of aloevera stabilized by heating for 4-5 minutes (Navale S et al., 2022).Theemulgel was prepared by the method described by Kumar D et al., 2022 with doing slight modification in it. The aloevera gel in the stabilized form was used in preparation. 30 gm of gel mixed with xanthine (100 gm) with continuous stirring. In another beaker 18 ml of flax seed oil added to the 2ml of essential oil. The formulations used to develop by using the different combinations of oils (Table 1). At 3000 rpm both the phases used to mix together for 20 minutes slowly. The emulgel of different combinations were prepared by repeating the same procedure (Kumar D et al., 2022).

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Table 1: Combinations of essential oil

S. No.	Formulations	Combinations
1.	F1	Lavender oil + Ylang-Ylang oil
2.	F2	Thyme oil + Ylang-Ylang oil
3.	F3	Thyme oil + Lavender oil
4.	F4	Nagarmotha oil + Ylang-Ylang oil

2.2 Cytotoxicity evaluation of the prepared formulations by MTT assay

Cytotoxicity of the formulations on HaCaT (Procured from NCCS Pune) cell line was determined by MTT Assay. The cells (10000 cells/well) were cultured in 96 well plate for 24 h in DMEM medium (Dulbecco's Modified Eagle Medium-AT149-1L) supplemented with 10% FBS (Fetal Bovine Serum - HIMEDIA-RM 10432) and 1% antibiotic solution at 37°C with 5% CO2. Next day cells were treated from different concentrations (0, 0.78, 1.56, 3.125, 6.25, 12.5, 25, and 50µg/ml).). After incubation for 24 hours, MTT Solution (Concentration as per mentioned in excel sheet) was added to cell culture and further incubated for 2 h. At the end of the experiment, culture supernatant was removed and cell layer matrix was dissolved in 100 µl Dimethyl Sulfoxide (DMSO –SRL-Cat no.- 67685) and read in an Elisa plate reader (iMark, Biorad, USA) at 540 nm and 660 nm. IC-50 Was calculated by using software Graph Pad Prism -6. Images were captured under inverted microscope (Olympus ek2) using Camera (AmScope digital camera 10 MP Aptima CMOS) (Morgan, 1998; Van-Meerlo, et al., 2011; Fotakis, et al., 2006; Tihauan, et al., 2020).

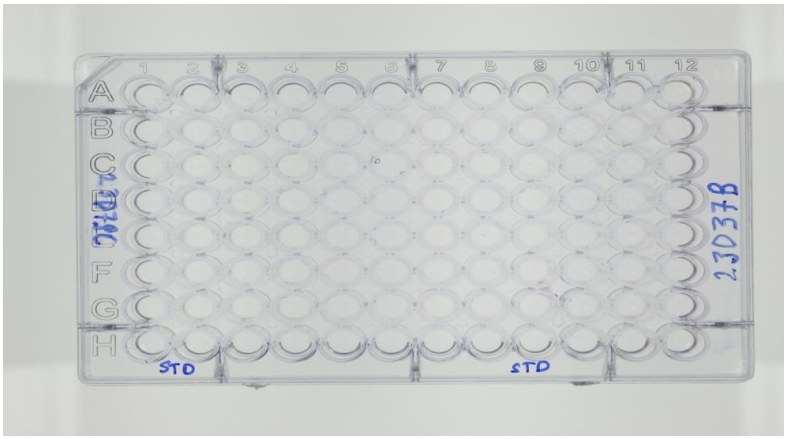

Fig: 1 MTT Assay plates

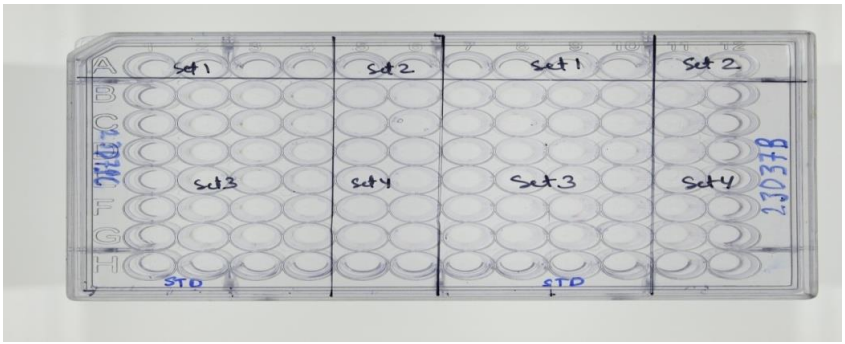

Fig 2: MTT assay plates labeling and preparation

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2.3 COX II Enzyme inhibition Assay of the formulations

Enzyme Inhibition Assay Sample dilutions in Buffer (Tris Cl buffer, 100mM, pH 8.0) were prepared. Reaction buffer (Enzyme in Tris/heme/phenol; 100mM/1µM/1µM buffer- Bovine Hemin Chloride – SRL-78372, Phenol – Fischer Scientific- 35953) were placed in defined well of a 96-well plate. The reaction was initiated by adding 5 µl substrate (Arachidonic acid, 10 mMSRL - 20975) and 5 µl TMPD solution (17mM- HiMedia GRM445-5G) and then plate was incubated at room temperature for 10 minutes and absorbance was taken at 595 nm using a micro plate reader (iMark, BioRad). Inhibitor, Celecoxib (25 µM final

Concentration- TCI-C2816) was used as a positive control (Kanj, et al., 2007).

3. Result and Discussion

Gel prepared by the above procedure was stable in the room temperature. F1 was light yellow in colour, F2 was in dark yellow, F3 formulation was in light yellow appearance, and F4 was in darker shade of yellow.

3.1 Results for cytotoxicity of the prepared formulations by MTT assay

Based on the results obtained from the MTT assay, it was observed that when the HaCaT cell line was exposed to different concentrations of the sample, cytotoxic activity was observed in samples F1 (IC50= 48.17 ± 0.038µl/ml), F2 (IC50= 47.2 ± 0.01922 µl/ml), F3 ((IC50= Above 50 µl/ml), F4 (IC50= Above 50 µl/ml (Table 2). Sample F3 and Sample F4 were found least effective. The IC50 is the concentration of an inhibitor/sample/ formulation at which the viable cells reduced by half.

Table 2: 1C50 value of different formulations

S. No.	Sample code	IC50 Value
1.	F1	48.17 ± 0.038µl/ml
2.	F2	47.2 ± 0.01922 µl/ml
3.	F3	Above 50 µl/ml
4.	F4	Above 50 µl/ml

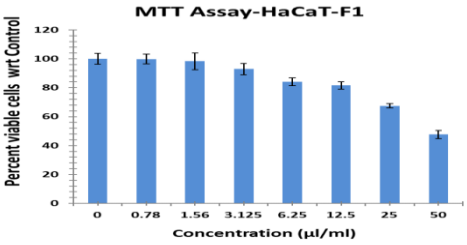

Fig 3: MTT Assay for (F1)

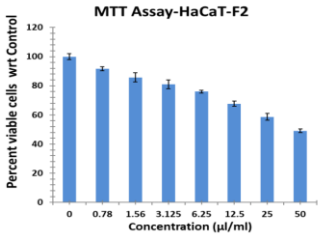

Fig 4: MTT assay for (F2)

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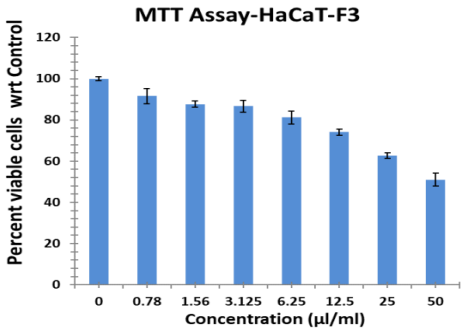

Fig 5: MTT assay for (F3)

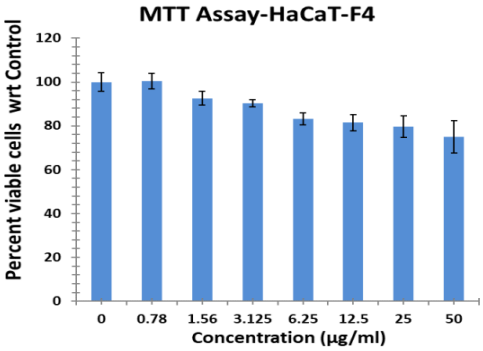

Fig 6: MTT assay for(F4)

3.2 Result of COX II Enzyme Inhibition Assay of the formulations

Based on the results obtained from the study, COX II enzyme inhibition activity was observed in sample F2 (IC50 = 0.9854 ± 0.13 µg/ml i.e., 50% inhibition at this concentration) (Table 3) with respect to standard Celecoxib (IC50 = 0.1735 ± 0.46 µg/ml).

Table 3: IC50 Value of F2 and Celecoxib

S. No.	Sample code	IC50 value
1.	Celecoxib	0.1735 ± 0.46 µg/ml
2.	F2	0.9854 ± 0.13µg/ml

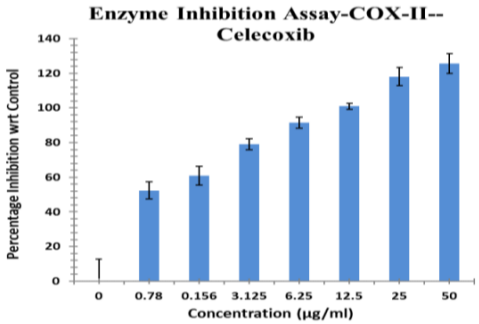

Fig 7: Enzyme Inhibition Assay-COX-II (Celecoxib)

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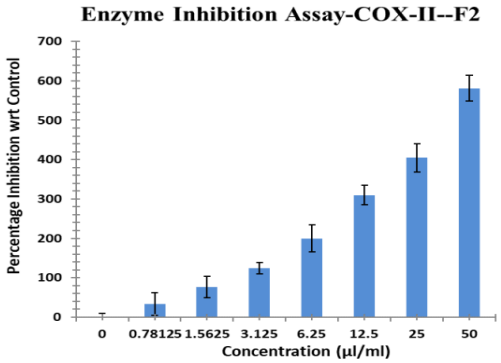

Fig 8: Enzyme Inhibition Assay-COX-II (F2)

4. Conclusion

Essential oils used in many preparations and have commercial importance. The prepared formulations showed the prominent results in cytotoxic evaluation. F2 formulation which contained ylang-ylang oil and thyme oil in combinations shows the best result in the cytotoxicity evaluation with least IC50 value followed by F1. The best formulation further evaluated for its COX-II enzyme inhibition activity. The data concluded that the formulations containing essential oils used in topical preparations and can be used for various purposes like mosquito repellents.

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Conflict of Interest: None

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