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In vitro and *in situ* efficacy of *Cymbopogon khasans* sobti, *Cyperus scariosus* r. Br. and their combination against two important dermatophytes

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Abstract: **Dermatomycoses is a serious subcutaneous complication of humans and other vertebrates caused by an important group of keratinophylic fungi namely *Microsporum gypseum* and *Trichophyton rubrum*. The aim of the present study was to investigate the efficacy of *Cymbopogon khasans* (CKEO), *Cyperus scariosus* essential oil (CSEO) and their combination (1:1) against induced dermatophytes. The CKEO, CSEO and their combination (1:1) completely inhibited the growth of *Microsporum gypseum* and *Trichophyton rubrum* at 0.1, 0.25 and 0.1 μL/mL, respectively. The inhibition of ergosterol suggests plasma membrane as the possible target of CKEO, CSEO and their combination. During *in situ* investigation, the CKEO, CSEO and their combination completely cures induced dermatomycoses in guinea pigs. The safety profile of CKEO, CSEO and their combination was tested through oral acute toxicity and was found to be 2312.20, 8269.60 and 2117.30 μL/kg body weight, respectively. Based on overall findings, it can be concluded that CKEO, CSEO and their combination offers a promising potential for the treatment of dermatomycoses.**

Index Terms*: *Cymbopogon khasans*, *Cyperus scariosus* essential oil, Dermatomycoses, *Microsporum gypseum*, *Trichophyton Rubrum

I. INTRODUCTION

Dermatomycoses is one of the prevalent subcutaneous infections of humans and other vertebrates caused by a group of keratinophylic fungi. The disease is more prevalent in body part having high content of keratinized tissues like skin, hoofs, hairs and nails and varies from mild to intense (Vander et al., 2003, Faway et al. 2018). Among different keratinophylic fungi, *Microsporum gypseum* and *Trichophyton rubrum* are the important causative agent of dermatophytic epidemiology in humans throughout the world (White et al., 2008). The infection is more severe in the people of tropical and subtropical belts, where favourable environmental conditions favour the colonization of these fungi (Falahati et al., 2005). Several topical remedies have been developed and introduced in the market to treat the dermatomycoses; however, resistance development due to their repeated use and adverse effect in form of nausea, abdominal pain and itching limits their application and has generated a clear need for novel curative approach (Sadhasivam et al., 2016). To overcome these problems, recently plant derived products are achieving attention as an alternative and complementary approach for controlling dermatophytic infection. The antimicrobial property of plant derived products has been well documented since the antiquity. Among different plant products, essential oils (EOs) extracted from different part of the plants are gaining momentum of concern, as they

are the composite blend of diverse volatile components that effectively reverts the probability of resistance development due to complex mode of action and may serve as safer substitutes to the presently employed drugs of synthetic origin (Oprean et al., 1998, Prasad et al., 2016).

Cymbopogon khasans Sobti and *Cyperus scariosus* R. Br. are the delicate aromatic grasses belonging to the family cyperaceae. It has been reported to possess diverse range of antimicrobial and antioxidant activities (Lahariya and Rao 1979, Nayak et al., 2003, Mishra et al., 2015). They are also used by local traditional practiceners to treat various ailments like diarrhoea, epilepsy, gonorrhoea, syphilis and liver disease (Gilani et al., 1994, Mishra et al., 2015). In spite of their excellent potentials against different pathogens, only few literatures are available on its antidermatophytic activities yet.

Therefore, the present work is designed to investigate the efficacy of chemically characterized *C. khasans* (CKEO), *C. scariosus* essential oil (CSEO) and their combination (1:1) against *M. gypseum* and *T. rubrum* was investigated. The probable mode of action of CKEO, CSEO and their combination was assessed by determining the ergosterol inhibition. Further, the ointments were used to test the curative property of CKEO, CSEO and their combination on guinea pigs. In addition, the safety profile of the test EOs and their combination were evaluated through acute toxicity in order to recommend its application as safe and novel substitutes to the synthetic antifungal drugs against dermatomycoses.

II. MATERIALS AND METHODS

A. Materials

All the chemicals *viz*. acetone, *n*–heptane, anhydrous sodium sulphate, Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB) and antibiotics such as itraconazole and flucanozole were supplied by Sisco Research Laboratory (SRL), Pvt. Ltd. Mumbai, India.

B. Ethics Statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered. The study was carried out after approval of the guidelines laid by Animal Ethics Committee (AEC), Banaras Hindu University, Varanasi, India (AEC no.: I. Sc./AEC/2017-18/201). The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

C. Experimental animals

Guinea pigs (weight about 370–400 g) and white albino male mice (25–30 g) of the same age were obtained from Central Animal House (CAH), Banaras Hindu University (BHU), Varanasi, India and acclimatized under laboratory conditions (27±2 °C) in polypropylene cage and fed with rodent's feed.

D. Test dermatophytes

M. gypseum and *T. rubrum* were collected and isolated from selected place of the BHU and adjacent areas using Vanbreuseghem's hair bait technique. The identification of test dermatophytes was made on the basis of colony morphology on Petri plates containing SDA media using Atlas of clinical fungi by de Hoog et al (2000). The fungal strains were maintained on SDA slants at 4 °C.

E. Isolation of EOs and chemical characterization through GC-MS

The 500 grams fresh aerial parts of *C. khasans* and *C. scariosus* were harvested at the flowering stage from departmental garden of BHU campus. The species were identified by Prof. N. K. Dubey using Flora of BHU Campus (Dubey, 2004) and the voucher specimens were deposited in the herbarium of Department of Botany, Banaras Hindu University, Varanasi, India. Five hundred grams of fresh shoots of each plant was cleaned with running water and placed in Clevenger's apparatus for 4 h at 45 °C. The volatile fractions (EOs) obtained were then stored in glass vial and kept away from light at 4 ºC until analysis.

F. In vitro efficacy of CKEO, CSEO and their combination (1:1) against dermatophytes

The effectiveness of CKEO, CSEO and their combination against the *M. gypseum* and *T. rubrum* were determined in term of minimum inhibitory concentration (MIC) using poisoned food technique. Nine-fold dilution of CKEO, CSEO and their combination prepared in acetone (0.01%) were added to SDA medium in Petri plates to attain the desired concentration of 0.05, 0.1, 0.15, 0.2 and 0.25 μL/mL. The plates without EOs were served as control. Then, a 5 mm disc of each test dermatophyte was inoculated to Petri plates and kept in BOD (27±2 °C) for 15 days. In addition, the efficacy of two important antibiotics i.e., itraconazole and flucanozole were also tested at the concentration of 0.1 to 3.0 μL/mL and compared with CKEO, CSEO and their combination. The minimum dose of EOs and their combination completely inhibited the visible occurrence of test dermatophytes was considered as their MICs. For MFC, the discs from the abovementioned MIC experiments showing no growth were cut off and re–cultured onto SDA medium without CKEO, CSEO and their combination. The minimum concentration required for preventing the restoration of growth was measured as their MFCs.

G. Effect of CKEO, CSEO and their combination (1:1) on cellular ergosterol

The ergosterol content in *M. gypseum* and *T. rubrum* cells treated with different concentration of test EOs and their combination was determined following Tian et al. (2012) with some minor changes. For this purpose, briefly, a 5 mm disc of test dermatophytes were inoculated into conical flasks containing SDB medium to achieve the required concentration (0.05, 0.1, 0.15, 0.2 and 0.25 μL/mL) of CKEO, CSEO and their combination. The flask without CKEO, CSEO and their combination served as control. After 10 days of incubation, the mycelium from each conical flask was harvested, cleaned with sterile distilled water (DW) and wet weight of the mycelia were measured. Subsequently, 5 mL of solution containing 25% KOH was added to each tube having mycelium and vortex mixed for 1 min and incubated on water bath for 2 h at 75 °C. Ergosterol from each sample was then extracted by adding 2 mL DW and 5 mL *n*–heptane followed by vortex mixing for 1 min. After 30 min of incubation at room temperature, the upper transparent *n*–heptane containing ergosterol was analyzed by scanning between 230 and 300 nm using UV-visible spectrophotometry (Model 2600, Shimadzu, Japan). The amount of ergosterol was calculated the following formula (Kedia et al., 2014):

Per cent ergosterol + per cent 24(28) dehydroergosterol = (Absorbance at 282/290)/pellet weight,

Per cent 24(28) dehydroergosterol = (Absorbance at 230/518)/pellet weight,

Per cent ergosterol = (Per cent ergosterol + Per cent 24(28) dehydroergosterol) – Per cent 24(28) dehydroergosterol

Where, 290 and 518 = E values (per cent/cm) determined for crystalline ergosterol and dehydroergosterol, respectively. Pellet weight is the wet weight of mycelium in g.

H. In situ curative efficacy of CKEO, CSEO and their combination (1:1)

To test the efficacy of CKEO, CSEO and their combination on living system**,** the test guinea pigs were anaesthetized by ether and infected with the *M. gypseum* and *T. rubrum*. Prior to experiments, the hairs were removed with a single use sterile blade. Shaving makes them susceptible towards infection. Subsequently, the paste of inoculums prepared in sterilized honey was inoculated on the skin and abraded with sandpaper. The infected areas were covered with the sterilized bandages using transparent adhesive tape. After 24 h, the infection was confirmed (Anita and Misra 2012). The hair stubs collected from the upper edge of the infected regions were cultured in Petri plates containing SDA medium. Thereafter, the ointment of CKEO, CSEO and their combination were prepared by mixing with petroleum jelly. The treatments of test guinea pigs were started from the tenth day after infection and were continued till complete recovery. The infected areas were applied with 0.2 g ointment two times a day. In control sets, only petroleum jelly without the test EOs was applied. Effect of CKEO, CSEO and their combinations against test dermatophytes were evaluated by percent culture recovery test and was calculated by the following formula (Qureshi et al. 2011).

$$\% \text{ Culture recovery} = 100 \times \frac{\text{Total number of site positive for culture in each set}}{\text{Total number of site in each set}}$$

I. Safety profile assessment of CKEO, CSEO and their combination (1:1)

The safety profile of CKEO, CSEO and their combination were determined in term of 50 per cent lethal dose (LD_{50}) on male albino mice. LD_{50} is defined as the concentration of EOs and their combination required for causing 50 per cent death of the test organism population). To perform the experiment, different doses of CKEO, CSEO and their combination dissolved in 0.5 mL stock solution of 5% tween 80 was orally administered to mice (12 in each group). Two control sets (positive and negative) were kept parallel to the test EOs and their combination. In positive control the mice were administered with stock solution (0.5 mL) without EOs. The mice were observed for first 4 h and then at 24 h for any toxicity symptom. After 24 h, the number of killed mice was counted in each group. The percentage of mice that had died at different dose was calculated based on probit analysis and then LD_{50} was calculated following Finney, (1971) and Dwivedy et al. (2017).

J. Statistical analysis

All the experiments were performed in triplicate except *in situ* and safety profile and data analysis was done on mean ± SE (SPSS 16.0; IBM Corporation, IL, USA).

III. RESULTS AND DISCUSSION

A. Chemical characterization of EOs through GC-MS

GC-MS analysis of CKEO and CSEO have been presented in Table 1 and 2 respectively.

Table 1. GC-MS analysis of CKEO

Sr. No.	Compounds	Retention time	Concentration %
1	Sulcatone	20.02	0.03
2	β-Myrcene	20.08	0.28
3	L-Limonene	20.24	0.11
4	β-Ocimene	20.36	0.69
5	Trans-Geraniol	20.84	0.07
6	Trans-3-caren-2-ol	21.22	0.20
7	Patchulane	21.99	0.77
8	Z-Citral	22.52	59.69
9	Geraniol formate	23.32	0.11
10	Neryl acetate	24.06	0.31
11	Linalyl acetate	27.65	34.99
12	Farnesol	31.80	0.44
13	Geranyl butyrate	37.18	0.34

Note: Ketone-1; Monoterpenes-2, 3, 4, 5, 6,8, 9, 10, 11 and 13; Sesquiterpene- 7 and 12

Table 2. GC-MS analysis of CSEO

Sr. No.	Compounds	Retention time	Concentration %
1	α- Copaene	26.85	0.39
2	α-Guaiene	26.85	0.78
3	Tetraacetyl-d-xylonic nitrile	27.17	0.94
4	Methanoazulene	28.50	0.92
5	DL-Limonene	29.18	23.02
6	Cyperene	29.65	67.99
7	Cis-Limonene oxide	29.99	0.53
8	7-epi-Silphiperfol-5-ene	30.22	0.72
9	Cis-Calamenene	33.43	0.52
10	Trans-Calamenene	33.44	0.42
11	α- Calacorene	34.35	0.18
12	Aristol-1	35.98	0.84
13	Valerenol	42.00	0.88

Monoterpenes- 5, 7; Sesquiterpene- 1, 2, 4, 6, 8, 9, 10, 11, 12, 13; Cyano compound- 3

B. In vitro efficacy of CKEO, CSEO and their combination (1:1) against dermatophytes

During antifungal screening it was found that the CKEO, CSEO and their combination exhibited pronounced antifungal activity and completely inhibited the visible growth of *M. gypseum* and *T. rubrum* at 0.1, 0.25 and 0.1 μL/mL, respectively. The MIC of CKEO, CSEO and their combination are presented in Table 3. Further, the test EOs and their combination showed more than ten times much better efficacy (0.1 to 0.25 μL/mL) that that of synthetic antifungal drug *viz*. itraconazole and fluconazole (2.5 and 3.0 μL/mL, respectively; data not shown). The MIC of the CKEO and their combination showed better antidermatophytic activity than the CSEO. The superior MIC of CKEO and combination over CSEO may probably due to the presence of phenolic group and synergistic effect between the components of CKEO and CSEO, respectively (Das et al. 2019). Generally, it has been attributed that the EOs with non-phenolic compound citral and phenolic components viz., eugenol and carvacrol show better antimicrobial efficacy as compared to the other groups (Lambert et al., 2001, Cox et al., 2001, Oussalah et al., 2007, Ng et al., 2019). The MFC of test EOs and their combination were found higher than respective MIC, showing fungistatic rather than fungicidal nature. As compared to antibacterial

drugs only restricted numbers of drugs are available in the market to treat fungal infections, and they are also nonacceptable to the consumers due to similar metabolic machinery of fungus with us. In this context we have tested the efficacy of EOs against test dermatophytes. Based on broad range antidermatophytic effect of EOs and their combination over synthetic antifungal drugs, it can be recommended as novel plant-based agents for the formulation of drug to treat dermatomycoses and mucormycosis. Mucormycosis is the broad term used to describe the infections caused by fungi mostly belonging to the order Mucorales. The mucormycosis is the current and ongoing problem in India, which has an overall mortality rate of around 50%, especially in immune-suppressed humans with covid-19 infections (Singh et al., 2021). Further, there are no accurate drugs available to this life-threatening disease. Presently, there are numerous reports available regarding the use of EOs as potential fungicides. For instance, Abdolahi et al. (2010) during investigating the antifungal activities of *Ocimum basilicum*, *Foeniculum vulgare*, *Satureja hortensis*, and *Thymus vulgaris* EOs against *Botrytis cinerea* and *Mucor piriformis* reported significant inhibitory effects of all tested EOs on the growth of both fungi. In another research, Ferdes et al. (2017) during demonstrating the antifungal efficacy of sage, rosemary, anise, quinoa and savory EOs observed remarkable inhibitory effect against *Aspergillus niger*, *A. oryza*, *Mucor pusillus* and *Fusarium oxysporum*. Hence, it is possible that the CKEO and CSEO as well as their 1:1 combination in addition to possessing efficient antidermatophytic activities could also be helpful in treating mucormycosis.

Conc. (µL/mL)	Percent inhibition of growth					
	CKEO		CSEO		Combination (1:1)	
	M. gypseum	*T. rubrum*	*M. gypseum*	*T. rubrum*	*M. gypseum*	*T. rubrum*
CNT	2.13±0.03	2.16±0.13	3.26±0.12	3.14±1.05	2.60±0.11	2.70±0.11
0.05	1.33±0.13	1.23±0.08	2.33±0.12	2.26±0.08	0.86±0.12	1.03±0.23
0.1	0.00±0.00	0.00±0.00	1.70±0.10	1.56±0.12	0.00±0.00	0.00±0.00
0.15	0.00±0.00	0.00±0.00	0.96±0.06	1.00±0.15	0.00±0.00	0.00±0.00
0.2	0.00±0.00	0.00±0.00	0.23±0.08	0.53±0.13	0.00±0.00	0.00±0.00
0.25	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Note: Values are mean (n = 3) ± standard error

C. Effect of CKEO, CSEO and their combination (1:1) on cellular ergosterol

The percent reduction of ergosterol with respect to mycelial wet weight receiving different concentration of CKEO, CSEO and their combination were presented in Table 4, 5, and 6, respectively. At MIC each EO and their combination completely inhibited ergosterol and fungal growth which indicates that the inhibition of ergosterol may be responsible for inhibition of fungal growth. The knowledge in relation to the mode of action of EOs is a crucial step in determining the usefulness in practical. Therefore, in order to assess the target of CKEO, CSEO and their combination on fungal plasma membrane, we have evaluated the effect of different concentration of test EOs and their combination on cellular ergosterol. Ergosterol is an inimitable biomarker and suitable target for most of the synthetic group of antifungal drugs and specific to fungal cell membrane responsible for maintaining the integrity and fluidity of the cells under varied environmental conditions (Rodriguez et al., 1985; Kelly et al., 1995; Hu et al., 2018). Recently, many workers investigated the mode of action of EOs and proposed that the leakage of important cellular contents, collapse in membrane potential and ATP pools due to disruption of plasma membrane regulated by ergosterol inhibition might be the possible cause of inhibitory effect of EOs (Burt, 2004; Bakkali et al., 2008). All these findings suggest plasma membrane as target site for antifungal action of EOs and their combination and our results further confirmed this point of view.

Table 4. Effect of different concentrations of CKEO on wet mycelial weight and ergosterol content of *M. gypse*

Conc. (µL/mL)	CKEO against *M. gypseum*		CKEO against *T. rubrum*	
	MWW(g)	% Reduction	MWW(g)	% Reduction
CNT	1.059±0.61	0.00±0.00	1.141±0.65	0.00±0.00
50	0.983±0.56	75.033±1.48	0.893±0.51	62.53±0.96
100	0.623±0.35	100±0.00	0.556±0.32	100±0.00
150	0.312±0.18	100±0.00	0.312±0.18	100±0.00
200	0.00±0.00	100±0.00	0.000±0.00	100±0.00
250	0.00±0.00	100±0.00	0.008±0.00	100±0.00

Note: MWW=mean wet weight; Values are mean (n = 3) ± standard error

Table 5. Effect of different concentrations of CSEO on wet mycelia weight and ergosterol content of *M. gypseum* and *T. rubrum*

Conc. (µl/ml)	CSEO against *M. gypseum*		CSEO against *T. rubrum*	
	MWW(g)	% Reduction	MWW(g)	% Reduction
CNT	1.541±0.88	0.00±0.00	1.321±0.76	0.00±0.00
50	1.193±0.68	11.95±0.37	1.156±0.66	12.013±2.09
100	0.680±0.39	47.70±0.58	0.813±0.46	55.043±1.38
150	0.409±0.23	95.24±1.12	0.486±0.28	87.556±1.37
200	0.180±0.10	100±0.00	0.260±0.15	100±0.00
250	0.061±0.03	100±0.00	0.088±0.05	100±0.00

Note: MWW= mean wet weight; Values are mean (n = 3) ± standard error

Table 6. Effect of different concentrations of Combination (1:1) on wet mycelia weight and ergosterol content of *M. gypseum* and *T. rubrum*

Conc. (µl/ml)	Combination (1:1) against *M. gypseum*		Combination (1:1) against *T. rubrum*	
	MWW(g)	% Reduction	MWW(g)	% Reduction
CNT	0.949±0.54	0.00±0.00	1.133±0.06	0.00±0.00
50	0.690±0.39	46.28±1.40	0.886±0.51	61.02±0.39
100	0.423±0.24	100±0.00	0.593±0.34	100±0.00
150	0.00±0.00	100±0.00	0.00±0.00	100±0.00
200	0.00±0.04	100±0.00	0.00±0.00	100±0.00
50	0.00±0.00	100±0.00	0.00±0.00	100±0.00

Note: MWW=mean wet weight; Values are given as mean (n=3) ± standard error

D. Effectiveness of CKEO, CSEO and their combination (1:1) in cure of induced dermatomycoses

In situ confirmation of antidermatophytic efficacy of CKEO, CSEO and their combination (1:1) against *M. gypseum* and *T. rubrum* was performed on guinea pig model. Fig. 1; Table 7 and 8 showed that the ointments of CKEO, CSEO and their combination represented curative property against induced dermatomycoses. Both CKEO ointments completely cured the induced dermatomycoses caused by *M. gypseum* and *T. rubrum*, respectively by 0–30th days of treatments in comparison to control groups. The combination (1:1) completely cured the dermatomycoses at 30th days, showing better efficacy over individual EO, which was confirmed by per cent culture recovery (images not shown). During *in situ* investigation, the absence of mycelial growth of *M. gypseum* and *T. rubrum* after 20 and 30 days of CKEO, CSEO and combination treatments during culture recovery tests clearly indicates the chemotherapeutic potential of test EOs and their combination. Similar finding was also reported by Tiwari et al (2003). Guinea pig was used as test model in this experiment due to their excellent susceptibility towards *M. gypseum* and *T. rubrum* as well as other dermatophytes (Nandi Bose, 1976). The clinical features attributed by this model are clearly comparable to those observed in case of humans or another mammalian system (Ghannoum et al., 2009). Based on overall findings, it can be concluded that the test EOs and their combinations have the potential to treat dermatomycoses and may be utilized for future application in preparation of novel drugs against dermatophytes.

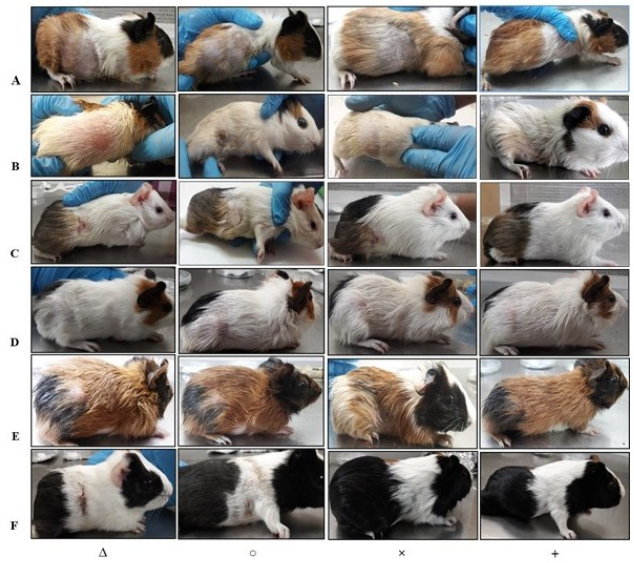

Fig.1. Efficacy of CKEO, CSEO and their combination (1:1) ointments in cure of induced dermatomycoses caused by *M. gypseum* and *T. rubrum*. (A–B) represents the treatment efficacy of CKEO, (C–D) denotes the treatment efficacy of CSEO, and (E–F) presents the treatment efficacy of their combination (1:1) against *M. gypseum* and *T. rubrum*, respectively after 0th day (Δ), 10th day (○), 20th day (×) and 30th day (+) of infection

Table 7. Effect of ointment of CKEO, CSEO and their combination in cure of induced dermatomycoses by *M. gypseum* on guinea pigs

Treatment (days)	Percent culture recovery			
	Control	CKEO at MIC	CSEO at MIC	Combination (1:1) at MIC
5	100	100±0.00	100.00±0.00	100±0.00
10	100	100±0.00	100.00±0.00	100±0.00
15	100	87.50±0.35	81.08±0.50	75.00±0.29
20	100	62.51±0.10	56.11±0.12	50.00±0.33
25	100	37.50±0.20	31.28±0.20	0.00±0.00
30	100	0.00±0.00	0.00±0.00	0.00±0.00

Note: MIC = Minimum inhibitory concentration; Values are given as mean (n=3) ± standard error

Table 8. Effect of ointment of CKEO, CSEO and their combination in cure of dermatomycoses by *T. rubrum* on guinea pigs

Treatment (days)	Percent culture recovery			
	Control	CKEO at MIC	CSEO at MIC	Combination (1:1) at MIC
5	100	100±0.00	100.00±0.00	100±0.00
10	100	100±0.00	100.00±0.00	100±0.00
15	100	75.50±0.35	71.08±0.50	67.00±0.29
20	100	68.51±0.10	62.11±0.12	39.00±0.33
25	100	28.50±0.20	24.28±0.20	0.00±0.00
30	100	0.00±0.00	0.00±0.00	0.00±0.00

Note: MIC =Minimum inhibitory concentration; Values are given as mean (n=3) ± standard error

E. Safety profile assessment of EOs and their combination (1:1)

The LD_{50} value for CKEO, CSEO and their combination were found to be 2312.20 μL, 8269.60 μL and 2117.30 μL/Kg of body weight, respectively. The LD_{50} of test EOs were found higher than some of the previously reported synthetic compounds such as pyrethrum (350-500 mg/kg), carvone (1640 mg/kg), bavistin (1500 mg/kg), lindane (59-562 mg/kg) and malathion (1522-1945 mg/kg) (Kumar et al. 2010). The high LD_{50} value of CKEO, CSEO and their combination indicates their safety profile and non-toxic nature to mammalian system. Acute toxicity of EOs is an important parameter to determine the safety limit and deciding the dose for future application in antifungal drug preparation. Although their LD_{50} values were found in favourable range for utilization of these essential oils and their combinations for formulation but at the same time it is also recommended to carry out more study.

CONCLUSIONS

Our results demonstrate that the CKEO, CSEO and their combination (1:1) exhibited potential efficacy against *M. gypseum* and *T. rubrum* under both *in vitro* and *in situ* condition. Decrease in ergosterol content in fungi treated with different concentration of test EOs and their combination revealed their probable mode of action. Further, the test EOs and their combination were found to be effective in treating induced dermatomycoses caused by *M. gypseum* and *T. rubrum* during *in situ* investigation. Higher LD_{50} value of CKEO, CSEO and their combination, suggests its favourable safety profile. Based on effectiveness of EOs and their combination during *in vitro* and *in situ* trials, they can be recommended as novel plant-based formulation for the treatment of dermatomycoses.

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DISCLOSURE STATEMENT

The authors report no conflict of interest.

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Graphical Abstract: