Characterisation, Antifungal Potential Of Oil Derived From Aromatic And Medicinal Plant

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Abstract- *As drug-resistant fungal diseases continues to rise, currently used therapeutic antibiotics have become ineffective. In response to this, new approaches are needed to combat drug-resistant fungal diseases. Adjuvants, such as phytochemicals or essential oils, can be used with existing antibiotics to increase their potency and efficacy. The current study aimed to investigate if the essential oils derived from Aegle marmelos (L), Murraya koenigii, Callistemon viminalis, Hypericum oblongifolium plants could be used to create a natural antifungal treatment. Oils were derived from plants and characterization was done using FTIR,TLC,GC MS. The target of this study was the infections carried on by the opportunistic pathogen Candida albicans.*

Keywords- Aegle marmelos (L), Murraya koenigii, Callistemon viminalis, Hypericum oblongifolium, Antifungal activity.

I. INTRODUCTION

Fungal Infections as a Global Concern

The prevalence of fungal diseases is alarmingly rising, which poses a significant obstacle for medical practitioners to overcome (Kainz et al., 2020). This rise can be traced back to the increasing number of people who are immune compromised as a consequence of modifications in medical practice,such as the and subcutaneous fungal infections can be quickly detected and effectively treated. Systemic infections are frequently referred to as "opportunistic" infections because they typically arise when the usual host defense system is weakened (Patel et al., 2021).Systemic fungal infections can arise from an opportunistic pathogen that encroaches on a vulnerable host,or they can be linked implementation of more intensive chemotherapy and the administration of immunosuppressive medicines. Candidiasis,cryptococcosis,aspergillosis and histo plasmasis are the most widely seen forms of fungal infections (Cuietal.,2022).Each and every year,fungal infections are accountable for around one-sixth of amillion casualties (Nidhietal.,2020).Fungi are abroad category of organisms that encompass over 250,000 distinct species.It has been estimated that more than 300 of these species have the potential to cause disease in humans (Vaghefi et al., 2021). The severity of fungal diseases can range from mild to lethal,based on the patient' s immune response and the place of the infection with in the body.Fungal infections can be either superficial or subcutaneous affecting the skin, mucous membranes and keratinous tissues (Kaushik and Agarwal,2019). There are many types of skin disorders, but this group contains some of the most common ones that impact millions of people all over the world. Even while they rarely cause death, these conditions can severely impair an individual's overall well-beingand,inextreme cases, even spread to other people or become invasive (Hube et al., 2015). The majority of superficial native to the area. Systemic infections pose a serious health risk and are a leading cause of death worldwide.Systemic infections are difficult to identify since they are generally hard to diagnose and the causative agent is typically verified only at autopsy (Richardson and Lass-Flörl,2008). These infections pose a significant risktoone's life and area assssociated with a high death rate.Candidaalbicans, to a more aggressive pathogen that is native to the area. Aspergillus fumigates Cryptococcus neoformans and Histoplasma capsulatum are the most common species of fungi that are most likely to be responsible for opportunistic (Kim, 2016).

Candidiasis Infection

The term"Candidiasis"is used to describe a wide range of fungal infections caused by the genus Candida, which can affect people of any age and frequently appear in the context of modifiable risk factors (Rivera-Yañez et al., 2017). There are at least 15 different species of Candida that can cause disease in humans. However, just five pathogens—Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, and Candida krusei are responsible for the greater part of invasive infections (Santos and Pereira, 2018). The prevalence of Candida spp. varies greatly from placeto place, from centerto center and from unit to unit. C. albicans is still the most common disease-causing Candida species in both adults and children (Chow et al., 2021). C. albicans is an invasive opportunistic microorganism that can

switch between a benign and a virulent state as shown in Figure 1.1.

II. MATERIALS AND METHODS

Collection and Identification of medicinal plants

Essential oils can be extracted from plants that have medicinal or aromatic characteristics, and their potential for producing essential oils was researched. One or more of the following parameters were applied in the selection process: Use of plants or plant parts in traditional medicine to cure diseases caused by microbes.

1. Presenceof aroma – producing compounds in The plant or plant components.
2. Plants that have been identified as being capable of producing essential oils Soils with a pH of slightly acidic, neutral, or basic (slightly alkaline) are optimal for growth, however, the plant is also able to thrive in extremely acidic and extremely alkaline environments.

The plant can survive at 120oFahrenheit (48.89oC) in the shade during the summer and live upto -20o Fahrenheit(-6.67oC) during the winter, as well as in regions of prolonged droughts. The geographic allocation is shown in Figure3.1.The details of the classification of the 4 plants used.

Extraction of essential oil from the leaves of the medicinal plants

The Clevenger apparatus (JSGW, JSGW/476) was used to extract the essential oils (EOs) from the leaves of the medicinal plants as shown in Figure 3.2. The fresh and young leaves of the plants were washed to eliminate the dust particles on their surfaces. With the help of a paper towel (semi-bleached, core size: 1-1/2 inches), the excess moisture was absorbed. In around bottom flask (JSGW,JSGW/1196/6), 200 grams of fresh leaves were mixed with 1000ml of double distilled water and then boiled at 40°C in the Clevenger apparatus. Essential oil (oil being lighter than water) was extracted for about 4 hours before being collected and separated from the hydrosolus in ganhydrous sodium sulphate (HiMedia, GRM419). The EO was stored in dark eppendorf and was refrigerated at 4°C for future use (Fagbemi et al., 2021).

Essential oil Yield percentage is calculated from Equation1 as given below: Where,VEO is the Volume of Essential oil (ml) and WLeaves is the fresh weight ofleaves (gms). These yields allowed determining antifungal properties, which is desirable as essential oils have a positive effect on humans. For instance, more than 300 unique EOs fall under the GRAS category for human use which demonstrates the great potential that these compounds have for therapeutic application in human beings(Yuetal.,2020).However, the use of Eos in conjunction with antifungal antibiotics will necessitate the conduct of sufficient clinical trials in order to rule out the possibility of the toxicity of such combinations, as well as the possibility of side effects and poisoning.

Characterization of the phyto compounds present in the essential oils of the selected medicinal plants

1 Thin-LayerChromatography(TLC)

Through the use of thin-layer chromatography, the various essential oils extracted from medicinal plants and their constituent chemicals were analyzed (TLC).TLC plates that had been pre-cast on an aluminum basis were utilized (Merck brand). Etching with a blade was used to create lanes that were 1cm wide on the TLC plate,and an origin line was drawn 1-2cm from the plate's base.To remove any trace of moisture from the dish, it was baked in an oven set to 80 degrees Celsius for one minute. Following drying in a hot air oven at 40oC, 5-10 µl of each essential oil was loaded into separate lanes and then transferred to a TLC chamber that contained mobile phase. Toluene and ethyl acetate were combined in the appropriate proportions to create the mobile phase ,which was 9.3:0.7.It was permitted for the sample store solve all the way up until the front of the solvent reached the top of the plate.
After removing the TLC plate, it was heated in a hot air oven to a temperature of 40oC. The TLC plate was developed by first spraying it with vanillin stain, then placing it in a hot air oven set to 50-60oC for two to three minutes, until colorful streaks emerged (Háznagy-Radnai et al., 2007).

2 Fourier transform infrared spectroscopy(FTIR)

The Agilent Cary 630 series FTIR spectrometer was utilized in order to carry out the FTIR analysis of the essential oils. On the mirror stage of the instrument was put anywhere from 5 to 10 µl of essential oil. The data were gathered at wave numbers ranging from 4000-650 cm-1 during collection. The spectra were analyzed using the Microlab PC software, and the various functional groups that were found in the sample were identified by comparing the peak values of the NIST library with the frequencies of the peaks in the spectra. Acetonitrile was used to clean both the lens and the stage mirror in order to eliminate any oil deposits (Li et al., 2013 settings.µg of fluconazole, 10 µg of amphotericin B, and 50 µl of dimethyl sulfoxide(DMSO).Every test was run three times

to ensure accuracy, and the findings were analyzed using a mean and standard deviation format (Nidhi etal.,2020).

3 Gas chromatography mass Spectroscopy(GC-MS

The samples were prepared by dissolving the essential oil in n-hexane in a defined amount (1% v/v) and analysed in triplicate using GC-MS/MS (Thermo Fisher Scientific, USA) equipped with TriPlus RSH autosampler and TQS-Duo mass spectrometer having X caliber software(Version4.0)

The temperature programming for GC was started initially from 80º C for 1 minute and increased constantly to 240ºC with a ramping of 10º C/min, and finally held for 12 minutes. The injector temperature was maintained at 250ºC with a split flow of 50 ml/min having a split ratio of 71.4. Whereas for MS the transfer line and ion source temperatures were set at 250º C and 230ºC, respectively.

The carrier gas used was helium setata constant flow of 0.7ml/min.The injection volume of1µLwas taken to perform a qualitative analysis of samples. A column, TG- 5 MS (Thermo Scientific, USA) is chiral having dimensions of 40m×0.15mm×0.15µm was utilized to separate the individual components of essential oil. The mass spectrum was obtained at 1 scan/sec at 70 electron volt energy, mass range 45-450 amu, for MS analysis.

Evaluation of antifungal activity of the Eos of the selected medicinal plants

Microbial Strains and culture media

Candida albicans (MTCC277) and *Candida albicans* (ATCC90028)were the two fungal strains used for the research. The fungal stains were grown for 48 hours in YEPD broth at 30°C and then stored in the cold room at 4°C in the on YPD agar plates for further use.

Agar well diffusion of antibiotics and essential oil for the fungal strains

In a sterile petriplate measuring 100 mm in diameter, approximately 25 ml of liquid YPD agar was allowed to cool and then solidify. Using sterile cotton swabs, the culture of *C. albicans* with an Absorbance at 600 nm (<1.0) was spread out in a consistent manner throughout the surface of the YPD agar plates. After using the cork borer to create wells measuring 10ml in diameter in the agar, the samples were then placed into the wells. The following components were utilized:50µl of 10% (v/v in DMSO) essential oils, 10 In contrast to the one study of Mahomoodally et al. (2018)

Determination of Minimum Inhibitory Concentration (MIC) of antibiotic and essential oils for the fungal strains

MIC was determined according to the methodology of Hannan, 2000. In a 96- well micro titer plate (Thermo Fisher Scientific),essential oil (1:10 diluted in 99.9%DMSO (Loba Chemie, LU1661802) was evaluated, with the negative control (DMSO) and positive control (RPMI media inoculated with C. albicans culture), RPMI 1640 (HiMedia,M1972) containing Fluconazole (HiMedia, CMS8387) and Amphotericin B (HiMedia,CMS462)(10µg) as standard drugs. For C.albicans, microtiter plates were incubated for 48hours at 30 °C. The color shift was then examined visually after the addition of Resazurindye (HiMedia,RM125). The growth was observable as the color changed from purple initially to pink later. The MIC value was calculated at the minimum concentration at which the color changed (Balakumar et al., 2011).

III. RESULTS AND DISCUSSION

Extraction and yield of Essential Oils

The extraction of essential oils from leaves of 4 medicinal and aromatic plants was carried out using hydro distillation process. The extraction temperature was 100oC.The flask containing leaves mixed with water was heated for 30 minutes to reach this extraction temperature (100oC) and there fore acquire the distillation of the first essential oil droplet. The highest percentage yields of essential oils was obtained from A.marmelos leaves and Callistemon viminalis which were 5.12 ± 0.015% and 0.34±0.026 (v/w) and the lowest yields were of Murraya koenigii (0.10±0.020%) and Hypericumob longifolium (0.12±0.011%). also reported 0.18% (v/w) of essential oil from the leaves of *A.marmelos* from the island of Mauritius after 3 hrs of hydrodistillation.Ranaetal.,1997 showed the yield of essential oil from leaves of *A. marmelos* from Varanasi, India was between 0.26–0.33% (v/w) (Mahomoodally et al.,2018).Sales et al. reported 0.45% yield of *Callistemon viminalis*(Sales et al.,2017). Oyedeji et al. found 0.90% of the essential oil in leaves of *Callistemon viminalis* plants collected in South Africa (Oyedeji et al., 2009). Differences in yield is attributed to genotype, geographic location, plant sampling season, and environmental conditions (Muñoz-Bertomeu et al., 2007). Although, studies on oil yield are selective across the literature, the rear several instances utilizing different parts of the plants, including it roots, fruit pulp, and leaf extracts showing utility comparable to standard drugs, such as antibiotics among others (Moghaddam and Mehdizadeh, 2017).

These plants offer direct and indirect benefits to the rural residents of the villages around the area. Cattle feed, fruit, and timber for tool bits are all products of these plants (Easterling et al., 2007). There is a dearth of education on the extraction, distillation, and commercialization of essential oils.
1- Physical characterization and percent age yield of the selected essential oils

S.NO	BOTANICAL NAME	YIELD
1	Aele marmelos(L)	0.51±0.015
2	Murraya koenigii	0.10±0.020
3	Callistemon vimnalis	0.34±0.026
4	Hpericum oblongifolium	0.12±0.011

The market for essential oils derived from aromatic grasses is much more developed than that for oils extracted from tree leaves especially leaf oil which has only a small but dedicated niche in the health industry. Extraction of EOs could be a source of Additional revenue for the average family, increasing their yearly income by as much as 30-40% (Guha, 2006).

Characterization of phytocompounds present in essential oils with significant antifungal activity

1.Analysis of essential oils by Thin-Layer Chromatography (TLC)

TLC was performed in the manner indicated in section 3.4.1 in order to conduct an analysis of the phytochemical compounds that were found in the essential oils. In the EOs,there were seen to be distinct bands of a variety of co The Rf values of the distinct bands that were found in the various essential oils were analysed.

Table 2: Values of the retention factors(Rf) of phyto constituents in the essential oils of various medicinal and aromatic plants analysed by thin-layer chromatography.

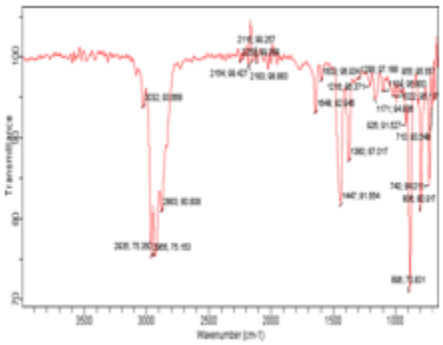
S. No.	Essential Oils	Rfvalues/Band position
1.	***A. marmelos***	0.18(#1),0.31(#2),0.62 (#3),0.81(#4), 0.90(#5)
2.	***C. viminalis***	0.22(#1),0.38(#2), 0.75(#3), 0.93(#4)
3.	***M. koenigii***	0.31(#1),0.56(#2), 0.75(#3), 0.87(#4)
4.	***H. oblongifolium***	0.18(#1),0.25(#2),0.31 (#3),0.42(#4),0.72(#5),0.91(#6)

Six distinct colored bands appeared in the essential oil of *A. marmelos* with Rf values of 0.31(#1), 0.42(#2), 0.57(#3), 0.67(#4), 0.73(#5) and 0.77(#6), respectively as Shown in Figure4.1, panelA. Similarly, the TLC plate loaded with essential oil of*C. Viminalis* showed four distinct coloured bands with Rf values of 0.28(#1),0.47(#2),0.52(#3) and 0.66 (#4)as shown in Figure4.1, panel B.

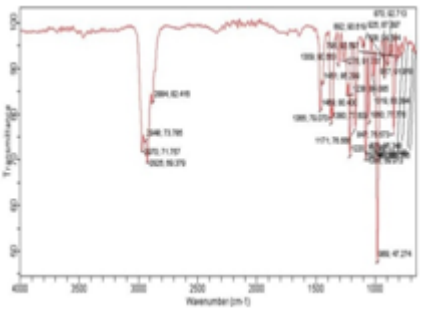
2 Analysis of the functional groups of essential oils by Fourier transform infrared red spectroscopy (FTIR)

The FTIR analysis mentioned in section 3.4.2 was carried out to determine functional groups in the 4 essential oils. The spectra of essential oils are presented in Figure 4.2 (A-D) and theTable4.3(A-D) provides an overview of the functional groups. A scan be seen in figure 4.7, distinct peaks were found in each of the 4 EOs, which suggests that a variety of functional groups are present. In essential oil of *Aegle marmelos*, the wave number range of the peaks observed include 740,806,896,926,1380,1447,1648, 2883 and 2935cm^{-1},which indicate the presence of C-H(Aromatics),C=C(Alkenes), C-X(Fluorides),andC-C(Alkanes),C=O(Aldehydes)andCOOH(Carboxylicacids) functional groups as shown in Table 4.3 (A) and Figure 4.2 (A).

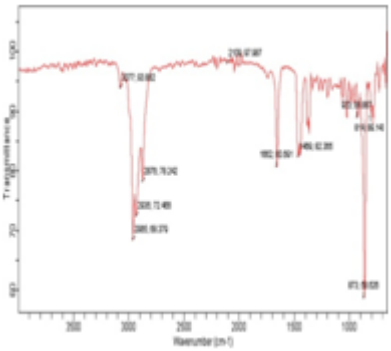
Aeglemarmelos



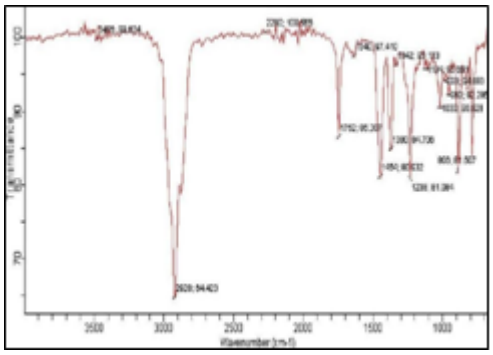
Callistemon viminalis



Murraya koenigii



Hypericum oblongifolium



3 Analysis of the phytocompounds present in selected essential oils from medicinal plants by Gas Chromatography Mass Spectroscopy (GC-MS)

Due to the volatile nature of essential oils,GC-MS analysis was performed on the EOs that were chosen in order to identify the phytocompounds that were present in them (3.4.3). GC-MS/MS helped identify compounds in EOs that play a major role in the antifungal activity and synergistic effect with antibiotics.Figure4.3(A-D) displays the GC-MS spectra of essential oils that were isolated from *Aegle marmelos*, *Callistemon viminalis*, *Murraya koenigii* and *Hypericum oblongifolium*. The retention time was used as a base for making predictions regarding the compounds of essential oils, as detailed in section 3.4.3. The anticipated compound's abundance was calculated using the percentage area of the peak that corresponded to it play a major role in the antifungal activity and synergistic effect with antibiotics.Figure4.3(A-D) displays the GC-MS spectra of essential oils that were isolated from *Aegle marmelos*, *Callistemon viminalis*, *Murraya koenigii* and *Hypericum oblongifolium*. The retention time was used as a base for making predictions regarding the compounds of essential oils, as detailed in section 3.4.3. The anticipated compound's abundance was calculated using the percentage area of the peak that corresponded to it.

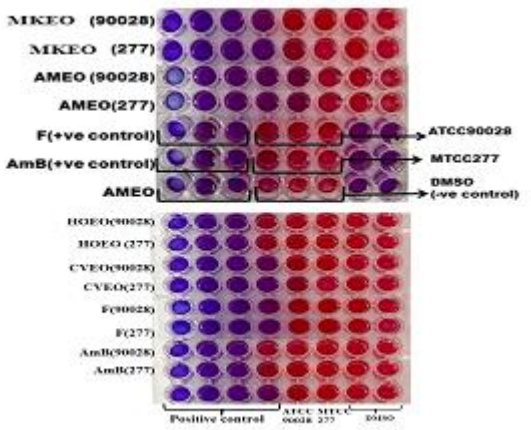
3.Study of the antifungal potency of essential oils extracted from selected medicinal plants

Agar well diffusion of conventional antibiotics and essential oils against *Candida* strains

The anticandida activity of the essential oils isolated from 4 plants in this study was evaluated using the agar well diffusion assay described in Section 3.5.2. *Candida albicans* ATCC90028 and MTCC277 were used in the experiment,both of which were isolated and characterized in distinct ways. Almost all the essential oils put to the test inhibited *C. albicans* growth, leading to a zone of clearance around each essential oil- treated well as demonstrated in Figure4.4. The development of *Candida albicans* was inhibited by the majority of the essential oils that were tested,resulting in the formation of a zone of clearance around each well that contained essential oil. The result was distinct to the essential oil that was being evaluated, while the solvent as a Whole (the control) was unable to establish any zone of clearance. All the 4 essential oils that were put through the testing procedure showed antifungal efficacy against *Candida albicans* (ATCC 90028) and *Candida albicans* (MTCC 277).

Determination of MIC (Minimum Inhibitory Concentration) and MFC (Minimum Fungicidal Concentration) of antibiotics and essential oils against *C. albicans* strains (ATCC90028 and MTCC277)

In order to quantify the growth inhibition activity of essential oils that exhibited potent antifungal activity in the well diffusion experiment (4.3.1), the MIC and MFC of the essential oils were measured in accordance with the procedures outlined in section.The essential oils displayed an inhibitory effect on the growth of C. albicans strains to varied degrees, as evidenced by the change in colour ofthe indicatordye, as can be seen inFigure4.6.Calculations of theMICandMFCwere performed for each EO, and the results are given in Table 4.6. The minimum inhibitory concentration (MIC) and maximum fungicidal concentration (MFC) values for both C. albicans strains were the same for all of the tested EOs as well as the clinical antibiotics. The minimum inhibitory concentration (MIC) value acts as a guide for the antifungal potential of a drug; the lower the MIC, the stronger the antifungal potential.



The results of this study reveal that there are variations in the MIC of the essential oils that were evaluated. These variations in MIC could be the result of differences in the environment of the place, the plant section that was used ,and the strains of C.albicans that were employed. There is no information available regarding the extraction of essential oil from H. oblongifolium or its possible antifungal activity.

Analysis of the Agar well diffusion of α-Phellandrene and Eucalyptol for the Candida strains.

The agar well diffusion experiment that is described in Section3.5.2 was utilized in this investigation to examine the anti-candida activity of the major phytocompounds which were α-Phellandrene in A. marmelos EO and Eucalyptol in C. viminalis. Candida albicans (ATCC 90028) and Candida albicans (MTCC 277) were the two strains of Candi examine the anti-candida activity of the major phytocompounds which were α-Phellandrene in *A. marmelos* EO and Eucalyptol in *C. viminalis*. *Candida albicans* (ATCC 90028) and *Candida albicans* (MTCC 277) were the two strains of Candida albicans that were used in the experiment.

Determination of MIC of α-Phellandrene and Eucalyptol for the fungal strains

The procedures described in section 3.7.2 were followed while conducting the tests necessary to determine the MIC of the phytocompounds(α-Phellandreneand Eucalyptol). The phytocompounds had an inhibitory impact on the growth of *C. albicans* strains to various extents, as shown by the change in colour of the indicator dye, which is depicted . For each phytocompounds, calculations of the MIC and MFC were done, and the findings are presented in the minimum inhibitory concentration (MIC) values for both strains of *Candida albicans* were similar across all of the essential oils that were evaluated in addition to the clinical antibiotics. The minimum inhibitory concentration (MIC) value of a medicine serves as a guide for determining the drug's potential to fight fungal infections; the lower the MIC,the greater the drug's antifungal potency.

IV. CONCLUSION

Because there are fewer new antibiotics being developed and there is a rise in the number of Fungal pathogens that are resistant to antibiotics, the need is to come up with alternate ways to treat infections that are brought on by fungi that are resistant to drugs.In order to make existing antibiotics more effective, Eos with their active phytochemicals can be blended with standard antibiotics, making the medicine more effective and reduces side effects of antibiotics as well. Thus, acting as a keystep in refining drug design, making the process more sustainable. This is in line with the Sustainable Development Goals or specifically SDG3, which is to ensure that people of all ages are living healthy lives and promoting their own well-being.The purpose of the current research was to investigate the essential oils (EOs) that are extracted from medicinal and aromatic plants that are native to the North Western Himalayan region in order to establish an efficient treatment for human fungal illnesses. Four plants were identified for production of essential oils. Out of the four essential oils, A.marmelos (0.51±0.015%) showed the best results in terms of antifungal lactivity and C. viminalis (0.34±0.026%) showed the second best results. Characterizations revealed that the primary phytocompound of A.marmelos and C. viminalis were α- Phellandrene and Eucalyptol respectively. Zone of inhibition (mm) obtained by agar well diffusion method or Eos showed superior activity for AMEO (24±0.5against C. albicans ATCC90028 and 22±0.30 against C. albicans MTCC277) and CVEO(22±1) against ATCC90028 and 20±**0.6** against MTCC277).

REFERENCES

[1] Aguilar-Pérez KM, Avilés-Castrillo JI, Medina DI, Parra-Saldivar R, Iqbal HM. Insight into nanoliposomes as smart nanocarriers for greening the twenty-first century biomedical settings.Front. Bioeng. Biotechnol 2020;8:579-536.

[2] Ahmad K, Athar F. Phytochemistry and pharmacology of Callistemon viminalis (Myrtaceae): A Review. Nat Prod J 2017;7:166–75.

[3] Ali M, Arfan M, Ahmad H, Zaman K, Khan F, Amarowicz R. Comparative antioxidant and antimicrobial activities of phenolic compounds extracted from five Hypericum species. Food Technol Biotechnol 2011;49:205.

[4] AmjadMS, Arshad M, SaboorA,PageS,

ChaudhariSK.Ethnobotanical profiling of the medicinal flora of Kotli, Azad Jammu and Kashmir, Pakistan: Empirical reflections on multinomiallogit specifications.AsianPacJTropMed2017;10:503– 14.

[5] AyazM,UllahFarhat,SadiqA,UllahFarman,OvaisM,AhmedJ,etal.Synergistic interactions of phytochemicals with antimicrobial agents: Potential strategy to counteract drug resistance. Chem Biol Interact 2019;308:294–303.

[6] Balakumar S, Rajan S, Thirunalasundari T, Jeeva S. Antifungal activity of Aegle marmelos (L.) Correa (Rutaceae) leaf extract on dermatophytes. Asian Pac J Trop Biomed 2011;1:309–12.

[7] BaligaMS,BhatHP,PereiraMM,MathiasN,VenkateshP.Radioprotectiveeffects of Aegle marmelos (L.) Correa (Bael): a concise review. J. Altern. Complement. Med 2012;16:1109-16.

[8] Bhattacharya R, Rolta R, Dev K, Sourirajan A. Synergistic potential of essential oils with antibiotics to combat fungal pathogens: Present status and future perspectives. Phyther Res 2021; 35:6089-100

[9] Bouhdid S, Skali SN, Idaomar M, Zhiri A, Baudoux D, Amensour M, et al. Antibacterial and antioxidant activities of Origanum compactum essential oil. African J Biotechnol 2008;7-10.

[10] BrudWS.Industrialusesofessentialoils.Handb.Essent.Oils, CRCPress;2020, p. 1029–40.