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EVALUATION OF PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *CURCUMA LEUCORRHIZA* (*ZINGIBERACEAE*) ROXB. RHIZOME

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Keywords:

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ABSTRACT: The aim of the present study was to characterize the petroleum ether, chloroform, and ethanol extracts of rhizomes of *Curcuma leucorrhiza* Roxb and to evaluate their biological activities such as anti-oxidant, anti-bacterial, and anti-fungal activities. Sequential solvent extraction was performed by Soxhlet apparatus using petroleum ether, chloroform, and ethanol, and respective extracts were named as CL-P, CL-C and CL-E. Antioxidant activities of the extracts were evaluated by DPPH, ABTS, and lipid per oxidation assays. Antibacterial and antifungal activities were evaluated against Gram-negative bacteria *Escherichia coli* (MTCC 739), Gram-positive bacteria *Bacillus subtilis* (MTCC 121), Micrococcus luteus (MTCC 106), *Mycobacterium smegmatis* (MTCC 6), fungal plant pathogen Rhizoctonia solani (MTCC 4633) and fungal animal pathogen *Aspergillus niger* (MTCC 1344) using agar well diffusion method with some modifications. Phytochemical analysis showed that solvent extracts of *Curcuma leucorrhiza* rhizome contained polyphenol, phenols, quinones, flavonoids, terpenoids, carbohydrates, glycosides, cardiac glycosides, coumarins and alkaloids. All the three extracts used in the study displayed the ability to scavenge DPPH and ABTS radicals and to inhibit lipid peroxidation. The antioxidant efficacy of the three extracts followed the order of CL-E ~ CL-C > CL-P. With regard to anti-bacterial and anti-fungal activity, exacts, CL-E and CL-P were almost comparable to each other and better than CL-C. Specifically, CL-P extract showed maximum antibacterial activity against Micrococcus luteus and antifungal activity against *Aspergillus niger*. In conclusion, ethanol being a food grade extractant, can be considered in place of petroleum ether and chloroform to prepare the extract from *Curcuma leucorrhiza* rhizome with significant medicinal values.

INTRODUCTION: Phytochemicals are the plant-derived, non-nutritive and naturally synthesized bioactive compounds found in all parts of the plant body: bark, leaves, stems, roots, flowers, fruits, seeds, and so on [1, 2]. They are used by the plants to safeguard their cells from environmental hazards such as drought, pollution, stress, UV exposure and pathogenic attack [1, 4]. Solvent extracts of medicinal plants are considered a rich source of phytochemicals such as carotenoids, flavonoids, polyphenols, tannins, phenolic and terpenes, which can exhibit anti-

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oxidant activity quenching free radical produced in the body, antibacterial and antifungal activities among others [5, 7]. Accordingly, the extracts of medicinal herbs are traditionally consumed as Ayurveda medicine/supplement for health benefits and for the management of several kinds of chronic inflammatory and infectious disorders. Antioxidant activity of the extract, in particular, is considered to be useful in protecting cells from free radicals or reactive oxygen species (ROS)-mediated oxidative damages [8].

ROS comprises singlet oxygen, superoxide anion radical (O_2•−), hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), nitric oxide (NO) and peroxy nitrite anion (ONOO-) among others and are known to cause oxidation of bio-molecules such as proteins, nucleic acid and lipids leading to oxidative stress and in turn causes immune disorders, diabetes, cancer, inflammation, ageing, hypertension, neurodegenerative diseases and art herosclerosis [12, 13]. ROS are produced in the body during the normal metabolic process, such as oxidation of carbohydrates, fats, and proteins through both aerobic and anaerobic processes as well as during the exposure of exogenous pollutants and oxidants [9, 11].

Moreover, infectious diseases are also the major health issues of the world. Although antibiotics have been playing a key role in the management of such diseases [14, 15], the emergence of drug-resistant strains has been a big issue nowadays [16, 17]. Viruses have been mutating and transforming into virulent strains such as HIV, Ebola virus [18] and SARS-CoV-2 of COVID-19, *etc*., which has affected millions of people worldwide [19]. Clinical translation of new antibiotics usually takes a very long time to be commercially available [18]. In addition to that, synthetic drugs may also cause adverse side effects such as allergies, hypersensitivity, and immune-suppression etc. Therefore, it is very important to search for an effective and safer alternative herbal-based medicine for the treatment of various infectious diseases [20].

Plants of the *Zingiberaceae* family are well-known useful natural resources that provide food, spices, perfume, medicines, dyes, and aesthetics [21]. Several reports have been published concerning the biological properties such as antimicrobial, antioxidant, anticancer activities of the extracts prepared from the plants of this family [22, 31]. The plant species of the *Zingiberaceae* family are distributed mainly in tropical and subtropical regions of Asia, Africa, Australia, and Americas [32].

India is a very rich and diverse region for *Zingiberaceae* wherein almost 22 genera, and about 170 species are known to be inhabited. Particularly, the North-East region of India has the highest numbers of inhabitation with 19 genera and about 88 species reported till date [33]. Geographically, the North-East region of India lies between 21°34′ N to 29°50′ N latitude and 87°32′ E to 97°52′ E longitudes, and comprises the states of Arunachal Pradesh, Mizoram, Nagaland, Sikkim, Tripura, Assam, Manipur, and Meghalaya. Of these, Manipur state lies under two of the global biodiversity "hotspots" *viz.* Himalayan Biodiversity Hotspot and Indo-Burma Biodiversity Hotspot is forming a unique biogeography province harbouring major flora and fauna recognized in the world [34, 36]. *Curcuma leucorhiza* Roxb is a plant of the *Zingiberaceae* family having medicinal properties and is found as a wild herb in Manipur.

The rhizome of the plant is used as a traditional medicine for the treatment of various diseases by indigenous people of Manipur since time immemorial. The plant is not only available in other parts of India but also in other countries like Bangladesh, Burma, Thailand, and China. There are not many research groups working on the characterization of the medicinal properties of this plant. Previously our group reported the preparation of petroleum ether, chloroform, and methanol extracts from the rhizome of these plants and their antioxidant, antimicrobial and antifungal activities [37].

Notably, in this study, the chloroform extract had shown the highest antioxidant activity in terms of scavenging of DPPH radical, and petroleum ether extract was most effective for anti-bacterial and anti-fungal activities. However, both petroleum ether and chloroform do not belong to generally regarded as safe (GRAS) chemical, and therefore extracts prepared using these solvents may not be considered safe for human consumption or application [38].

On the other hand, ethanol is considered as a food grade excipient and is widely used in the preparation of medicinal grade Ayurveda and/or homeopathy formulations [38]. Therefore, the present study was aimed to prepare ethanolic extract from rhizomes of *Curcuma leucorrhiza* Roxb and to compare its antioxidant, antibacterial, and antifungal properties with those prepared from petroleum ether and chloroform. Additionally, the present study not only used multiple assays (like DPPH, ABTS, lipid peroxidation and agar well diffusion) to measure biological activities but also made an attempt to character rise phytochemicals present in these extracts through qualitative assays.

MATERIALS AND METHODS:

Chemicals & Reagents: Petroleum ether (LR Grade), chloroform (EMPLURA), ethanol (99.8%), nutrient broth, potato dextrose agar medium, potato dextrose broth, and dimethyl sulfoxide (DMSO) were purchased from Hi-Media Laboratories, India. Methanol and carbon tetrachloride (CCl_4) was of UV spectroscopy grade purchased from local suppliers. 1, 1-Diphenyl-2-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), and soy lecithin were from Sigma-Aldrich chemicals. All other chemicals, including monosodium phosphate, disodium phosphate, potassium persulphate ($K_2S_2O_8$) and diammonium salt of 2, 2'-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) were of analytical grade. High purity nitrous oxide (N_2O) gas was used for the pursing of soy lecithin liposome. The irradiation of soy lecithin liposome was carried out using Cobalt-60 (60 °C) source available at Bhabha Atomic Research Centre, Mumbai, India.

Steady-state absorption studies were carried on a JASCO V-630 spectrophotometer. Nano pure water of conductivity ~ 0.06 μS cm^{-1} from Milli-Q system was used throughout the experiments.

Sample Collection: The rhizomes of Curcuma leucorrhiza were collected from Senapati district, Manipur, and identified by an expert (voucher specimen 000815, Department of Life Sciences, Manipur University, Imphal) [37, 39]. The rhizomes were washed with running tape water thoroughly, followed by distilled water. It was sliced into small pieces and air-dried in the shade at room temperature for about 30 days. The dried rhizomes were then finely ground into powder form using an electric grinder and stored in air-tight containers for further use.

Preparation of Extracts: The extracts were prepared by the sequential solvent extraction method as depicted in **Fig. 1**. For this, 115 g of the pulverized rhizome powder was taken in a Soxhlet apparatus and subjected to sequential extraction with 300 ml each of solvents starting from low polarity to high polarity (non-polar to polar) *viz.* petroleum ether, chloroform, and ethanol. The extracts of *Curcuma leucorrhiza* prepared from petroleum ether, chloroform, and ethanol extraction were named as CL-P, CL-C, and CL-E. The extracts collected were concentrated using a rotary evaporator under reduced pressure and further dried using petri dices at room temperature. Finally, dried extracts were collected in air-tight containers and then stored in a refrigerator at 4 °C for further analyses.

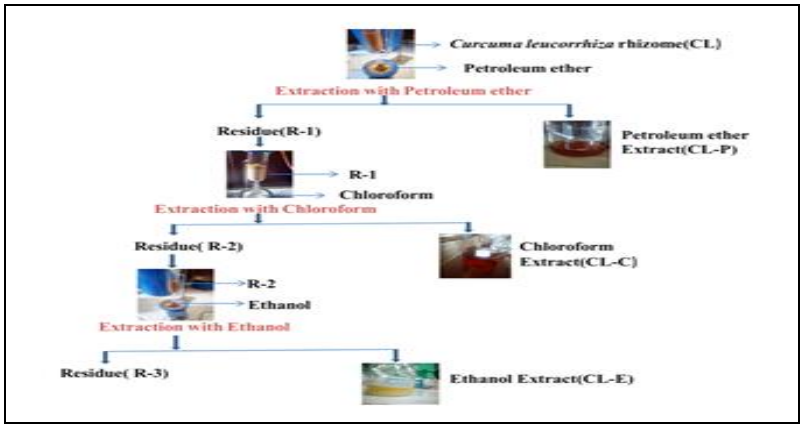

FIG. 1: SCHEMATIC SHOWS THE EXTRACTION PROCESS USING THREE DIFFERENT ORGANIC SOLVENTS *VIZ.* PETROLEUM ETHER, CHLOROFORM AND ETHANOL AND CORRESPONDING EXTRACTS WERE NAMED AS CL-P, CL-C AND CL-E RESPECTIVELY

Antioxidant Activity: The antioxidant properties of the different solvent extracts were studied through DPPH, ABTS and lipid peroxidation assays.

DPPH Assay: The different concentrations (0.1-7.0 mg/ml) of the extracts were mixed with fixed concentration (50 µM) of DPPH radical solution in methanol, incubated for 20 min in the dark and subjected to absorbance measurement at 518 nm on UV-visible spectrophotometer. The percent scavenging activity was calculated according to equation [1].

$$\% \text{ Scavenging activity} = A_{control} - A_{extract} \times 100 / A_{control}$$

Here, A control and A extract are the absorbances in the absence and in the presence of extracts, respectively. The % scavenging was plotted as a function of the increasing concentrations of the extracts (CL-P, CL-C and CL-E) to estimate IC_{50} values (concentration of extracts required to decrease the absorbance of DPPH radicals to half of initial value) [40, 41].

ABTS Assay: For this assay, ABTS radicals were prepared by an oxidizing aqueous solution of 7.4 mM ABTS ion by potassium persulfate (2.6 mM). The concentration of ABTS radical was estimated by recording its absorbance at 415 nm ($\varepsilon 415\text{ nm} = 3.6 \times 10^4\ M^{-1}cm^{-1}$). The reaction between ABTS radical and CL extracts was studied by monitoring the absorbance of ABTS radical at 734 nm [41, 42]. In brief, ABTS radical at a fixed concentration (120 µM) was allowed to react with different concentrations (5-200 µg/ml) of extracts (CL-P, CL-C, and CL-E) for 20 min in the dark at room temperature. The IC_{50} values were calculated from the percentage scavenging activity by using equation [1].

Lipid Peroxidation: The extent of lipid per oxidation was measured by estimating thiobarbituric acid reactive substances (TBARS) as reported in the literature [40, 41]. In brief, 3 ml of soy lecithin liposomal solution (1 mg/ml) in 5 mM pH 7 phosphate buffer with and without extracts (50 mg/ml) like CL-P, CL-C, and CL-E was saturated with N_2O and air in the ratio of 4:1 and then irradiated with 60 °C γ-radiation at a dose of 270 Gy. Due to the low aqueous solubility of extracts (CL-P/CL-E/CL-C), these were first dissolved in pure methanol and subsequently diluted with phosphate buffer. The methanol content was kept constant in control and test reactions. About 0.5 ml of the irradiated liposome solution was treated immediately with 2 ml of aqueous TBA reagent and heated at 80 ºC in a water bath for half an hour.

The solutions were cooled down to room temperature, centrifuged at 10000 rpm for 5 min, and the supernatant was subjected for measurement of absorbance at 532 nm. The concentration of TBARS was estimated by using equation [2].

$$[\text{TBARS}] = Abs_{532} / 1.56 \times 10^5 \times 0.5/1000 \text{ moles/mg liposome}$$

Here, 0.5 is the volume (ml) of irradiated liposome in the absence and presence of extract. Subsequently, the % inhibition of lipid peroxidation by the extracts was calculated by using equation [3].

$$\% \text{ Inhibition} = [\text{TBARS}]_{control} - [\text{TBARS}]_{extract} / [\text{TBARS}]_{control} \times 100$$

Here, [TBARS] control and [TBARS] extract are the concentrations in the absence and presence of extracts (CL-P, CL-C and CL-E respectively.

Antimicrobial and Antifungal Activities

Test Organisms: Bacterial test pathogens included Gram-negative bacteria *Escherichia coli* (MTCC 739), Gram-positive bacteria *Bacillus subtilis* (MTCC 121), *Micrococcus luteus* (MTCC 106) and *Mycobacterium smegmatis* (MTCC 6). *Escherichia coli*, *Bacillus subtilis*, and *Micrococcus luteus* were subcultured in nutrient broth at 37 ºC for 24 h. *Mycobacterium smegmatis* was grown in Middle brook 7H9 broth and incubated at 37 ºC for 24 hours. Fungal test pathogens included plant pathogen *Rhizoctonia solani* (MTCC 4633) and animal pathogen *Aspergillus niger* (MTCC 1344). The fungal pathogens were subcultured in potato dextrose broth at 37 ºC for 24 h. Ampicillin 25 µg/ml) and fluconazole 25 µg/ml was used as the positive controls for antibacterial and antifungal screening, respectively.

Biocidal Assays: The extracts (CL-P, CL-C, and CL-E) were screened for antibacterial and antifungal activities using agar well diffusion method 43 with some modifications. Crude extracts were dissolved in DMSO to obtain a solution of 25 µg/ml concentrations.

About 100 µl of the bacterial and fungal culture were spread onto nutrient agar and potato dextrose agar plates. The wells of diameter ~6 mm were made in each plate using a sterile cork borer. About 100 µl of the extract solution was inoculated in 6 mm wells, and the plates were incubated at 37 °C for 24 h. Clearing zone around the well indicated antibacterial/antifungal activity and was compared with the activity of the standards, ampicillin 25 µg/ml and fluconazole 25 µg/ml. The negative control well contained the same volume of DMSO.

Phytochemical Screening: The three crude extracts were used for the preliminary phyto-chemical screening to detect the presence of active chemical constituents, *viz*. quinones, flavonoids, terpenoids, steroids, carbohydrates, glycosides, cardiac glycosides, coumarins, and phenolic compounds according to standard procedures reported previously [44, 47]. The readout was performed by direct visual observation of the coloration profile of the reactions and/or the formation of precipitates.

Results:

Antioxidant Activity: Antioxidants are chemical compounds that prevent or delay the oxidation of substances even with very low concentration. It works either through scavenging of ROS or preventing their generation in the body [40, 42].

DPPH Scavenging: DPPH radical is a stable radical at room temperature. It is often used to estimate the antioxidant activity of polyphenolic as well as non-polyphenolic natural compounds, which reduce colour of DPPH radical to a colourless product.

Therefore, the change in absorbance at 518 nm is used to estimate the DPPH radical scavenging activity and also to quantify the relative antioxidant activity of different CL extracts (CL-P, CL-C, and CL-E). The results indicated that the presence of extracts in the reaction mixture led to a concentration (0.1-7.0 mg/ml) dependant increase in the scavenging of DPPH **Fig. 2**.

From the graph, the IC_{50} values estimated was 3.40 ± 0.20 µg/ml, 0.37 ± 0.05 µg/ml and 0.30 ± 0.04 µg/ml, for CL-P, CL-C and CL-E extracts, respectively (inset of **Fig. 2**). Interestingly, the IC_{50} values of CL-C and CL-E extracts are ~ 10 times higher as compared to that of CL-P extract, suggesting that CL extracts in polar solvents like chloroform and ethanol have more potent antioxidant activity than extract of a non-polar solvent such as petroleum ether. This is in line with our previous report [37], wherein chloroform extract showed the highest DPPH scavenging property (51.66%) followed by petroleum extract (43.33%) at an equi-concentration of treatment.

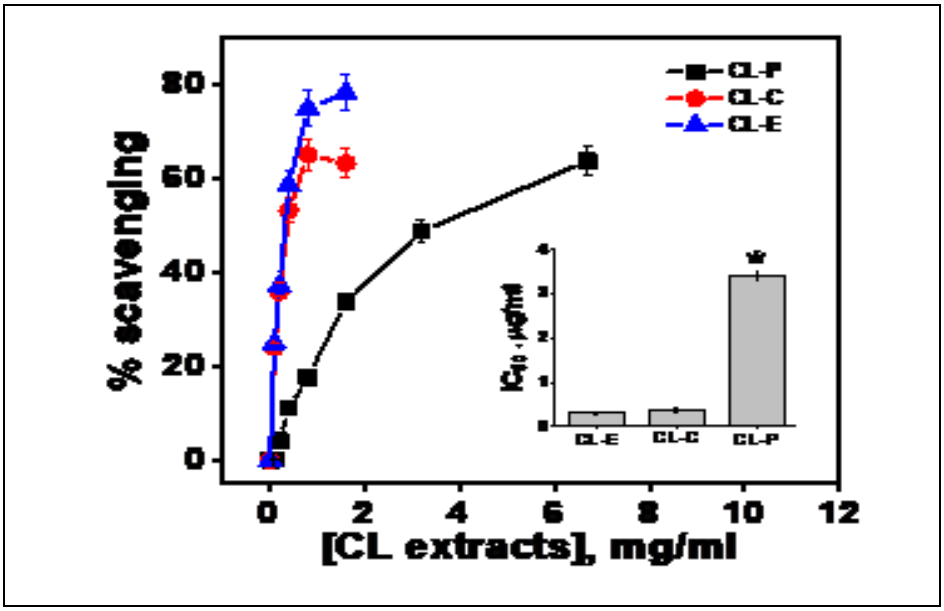

FIG. 2: PLOT OF PERCENT (%) SCAVENGING OF DPPH RADICAL VERSUS CONCENTRATION OF CL EXTRACTS (CL-P, CL-C AND CL-E) INSET SHOWS THE IC_{50} VALUES OF CL-P, CL-C AND CL-E EXTRACTS FOR DPPH SCAVENGING. Results are presented as Mean ± SD (n = 2). * Significant (<0.05) difference as compared to cl-e and cl-c by unpaired t-test

ABTS Scavenging: ABTS radical reaction is another important assay for the evaluation of antioxidant activity of polyphenolic as well as non-polyphenolic compounds. In the presence of a suitable electron donor, the absorbance of ABTS radical at 734 nm is decreased. Similarly, in the presence of the increasing concentrations (5-200 µg/ml) of CL extracts (CL-P, CL-C and CL-E), the absorbance of ABTS radical was decreased or its scavenging increased in a concentration dependant manner **Fig. 3**. The IC_{50} values estimated was 180.1 ± 6.2 µg/ml, 85.2 ± 3.8 µg/ml, and 62.4 ± 4.0 µg/ml for CL-P, CL-C and CL-E extracts, respectively (inset of **Fig. 3**. Again, CL-C and CL-E extracts exhibited ~2 and ~3 times higher radical scavenging activity than CL-P extract and is in agreement with those obtained from the scavenging of DPPH radical.

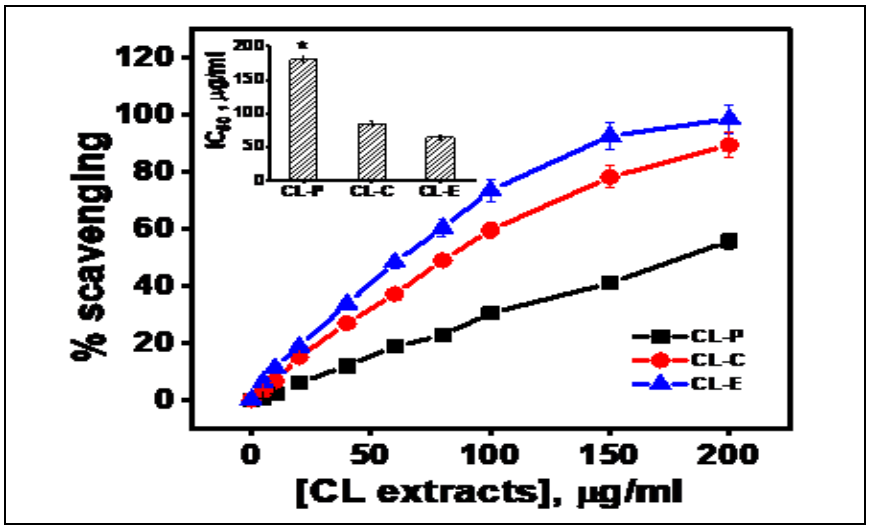

FIG. 3: PLOT OF PERCENT (%) SCAVENGING OF ABTS RADICAL VERSUS CONCENTRATION OF CL EXTRACTS (CL-P, CL-C AND CL-E) INSET SHOWS THE IC50 VALUES OF CL-P, CL-C AND CL-E EXTRACTS FOR ABTS SCAVENGING. Results are presented as mean ± sd (n = 2). *significant (<0.05) difference as compared to cl-e and cl-c by unpaired t-test

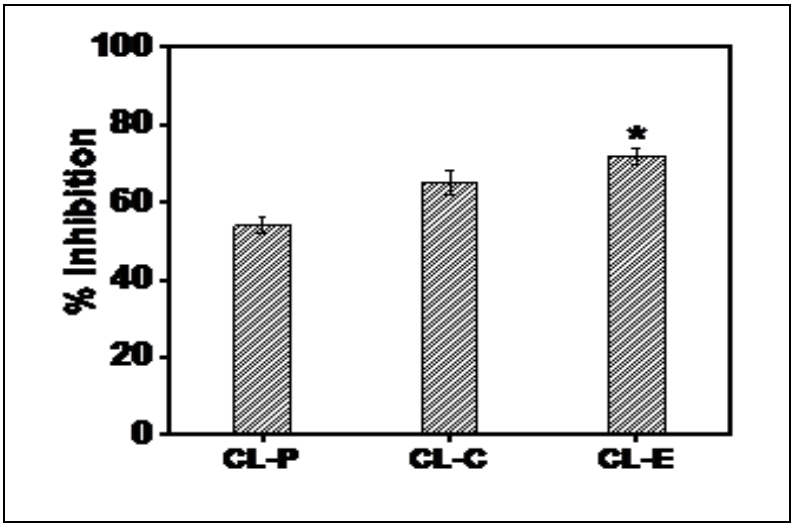

FIG. 4: THE PLOT OF PERCENTAGE (%) INHIBITION OF LIPID PEROXIDATION AT A FIXED CONCENTRATION (50 µG/ML) OF CL EXTRACTS (CL-P, CL-C, AND CL-E). Results are presented as Mean ± SD (n = 2). *significant (<0.05) difference as compared to cl-p and cl-c by unpaired t-test

Inhibition of Lipid Peroxidation: Lipid peroxidation is defined as the oxidative degradation of unsaturated lipids in the presence of ROS [40, 42]. Soy lecithin liposome is a well-established model of cellular membrane to study the ROS-mediated oxidative degradation of lipids or lipid peroxidation. The oxidative degradation of soy lecithin liposomes is induced by exposing it to 60 °C γ- radiation. The exposure of γ- radiation leads to radiolysis of water present in liposomal solution

forming •OH radicals. This in turn, attacks the lipid molecule of liposome forming the lipid peroxyl radical, which is degraded through a series of complex reactions to malondialdehyde as one of the degraded products and detected as TBARS.

The presence of antioxidant inhibits the process of lipid peroxidation or TBARS formation by scavenging •OH radicals. In the present study, the presence of a fixed concentration (50 µg/ml) of CL extracts (CL-P, CL-C and CL-E) significantly inhibited the formation of TBARS as compared to the radiation control (without extracts) group. The percentage inhibition of lipid peroxidation by CL-P, CL-C, and CL-E extracts was 54.0 ± 2.0%, 65.0 ± 3.0%, and 72.0 ± 2.0%, respectively **Fig. 4**.

Thus, CL-C and CL-E extracts exhibited significantly higher inhibition of lipid peroxidation as compared to CL-P extract agreeing on previous results on DPPH and ABTS scavenging activities.

Antibacterial and Antifungal Activities: The results of antibacterial assay are shown in **Table 1** and **Fig. 5**. All the three extracts CL-P, CL-C and CL-E showed inhibitory effects against all of the bacteria strains used in this study. The zone of inhibition was in the range from 10 mm to 18 mm. In this study, *Micrococcus luteus* showed the highest sensitivity against CL-P and CL-E with the zone of inhibition of ~17 mm whereas Escherichia coli showed the lowest sensitivity against CL-E, CL-C and CL-P.

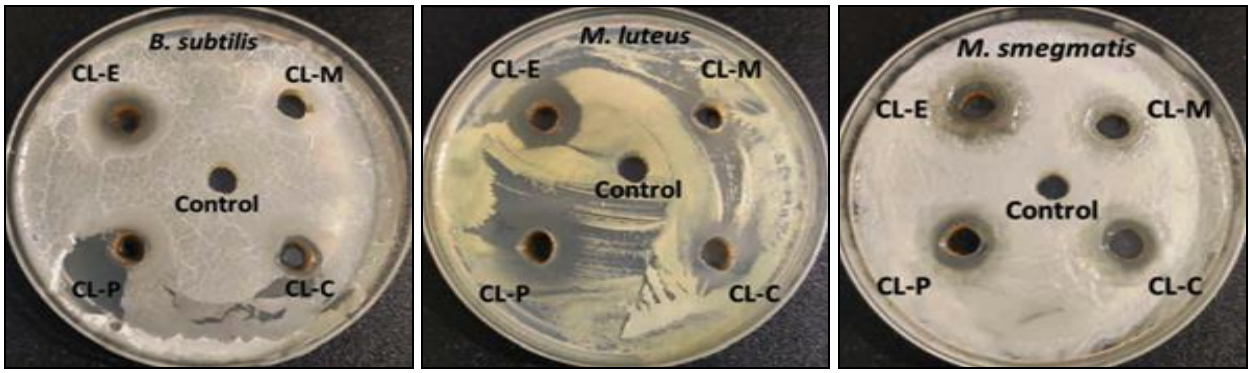

FIG. 5: REPRESENTATIVE AGAR PLATES SHOWING ZONE OF INHIBITION (ANTIBACTERIAL ACTIVITY) OF DIFFERENT CL EXTRACTS (CL-P, CL-C AND CL-E) AGAINST *BACILLUS SUBTILIS*, *MICROCOCCUS LUTEUS* AND *MYCOBACTERIUM SMEGMATIS*

TABLE 1: ANTIBACTERIAL ACTIVITIES OF CL-E, CL-C AND CL-P EXTRACTS THROUGH AGAR WELL DIFFUSION METHOD

S. no	Crude Extracts	Concentration (µg/ml)	Diameter of zone of inhibition (in mm)			
			Bacillus subtilis	*Micrococcus luteus*	*Escherichia coli*	*Mycobacterium smegmatis*
1	CL-E	25	10.5 ± 0.5	16.5 ± 0.5	10 ± 0	11 ± 1
3	CL-C	25	10.5 ± 0.5	14.5 ± 0.5	10 ± 0	10 ± 0
4	CL-P	25	11.5 ± 0.5	17 ± 1	10 ± 0	13.5 ± 0.5
5	Ampicillin	25	25 ± 0	30.5 ± 0.5	28 ± 1	24 ± 0

The values are presented as Mean ± SD (n = 2)

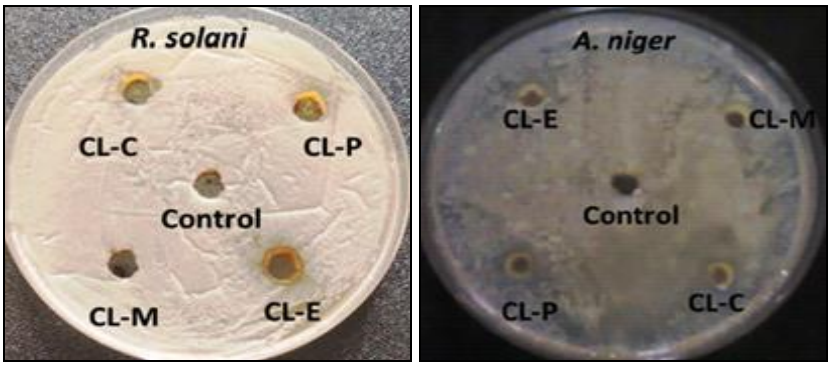

FIG. 6: REPRESENTATIVE AGAR PLATES SHOWING ZONE OF INHIBITION (ANTIBACTERIAL ACTIVITY) OF DIFFERENT CL EXTRACTS (CL-P, CL-C AND CL-E) AGAINST *RHIZOCTONIA SOLANI* AND *ASPERGILLUS NIGER*

Further, the results of the antifungal assay are shown in **Table 2** and **Fig. 6**. Of the 3 extracts screened, CL-E and CL-P extracts showed antifungal activities against *Rhizoctonia solani* and *Aspergillus niger*, respectively, but CL-C failed to show any antifungal activity against the fungus strains used in this study. Previous study 37 has shown that PE extract is effective against *Aspergillus niger* which is agreed by the present study too.

TABLE 2: ANTIFUNGAL ACTIVITIES OF CL-E, CL-C AND CL-P EXTRACTS THROUGH AGAR WELL DIFFUSION METHOD

S. no	Crude Extracts /Compounds	Concentration (µg/ml)	Diameter of zone of inhibition(in mm)	
			Rhizoctonia solani	*Aspergillus niger*
1	CL-E	25	10 ±0	-
3	CL-C	25	-	-
4	CL-P	25	-	10.5 ±0.5
5	Fluconazole	25	20.5 ±0.75	17.5 ±0.5

The values are presented as Mean ± SD (n = 2)

Phytochemical Screening: Table 3 provides results of preliminary phytochemical screening of the extracts (CL-P, CL-C, and CL-E). Of the thirteen different classes of phytochemicals evaluated in the extracts, tannins, saponin, and steroids were not present in any of the three extracts. Of the three extracts, CL-C appeared to be the richest source of phytochemicals containing phenols, quinones, alkaloids, flavonoids, terpenoids, glycosides, coumarins, and carbohydrates. On the other hand, CL-P extract contained polyphenol, phenols, terpenoids, cardiac glycosides, coumarins and alkaloids. Similarly, CL-E extract contained polyphenol, phenols, quinones, flavonoids glycosides, cardiac glycosides, and coumarins.

TABLE 3: QUALITATIVE ANALYSIS OF THE PRESENCE OF PHYTOCHEMICALS IN DIFFERENT SOLVENT EXTRACTS OF *CURCUMA LEUCORRHIZA* RHIZOME. HERE, -VE = ABSENT, +VE = PRESENT

Phytoconstituents	Extracts obtained through the following solvents		
	Petroleum ether	Chloroform	Ethanol
Polyphenol	+ve	-ve	+ve
Tannins	-ve	-ve	-ve
Phenols	+ve	+ve	+ve
Quinones	-ve	+ve	+ve
Flavonoid	-ve	+ve	+ve
Terpenoids	+ve	+ve	-ve
Saponin	-ve	-ve	-ve
Steroids	-ve	-ve	-ve
Carbohydrates (Molisch's Test)	-ve	+ve	-ve
Glycosides	-ve	+ve	+ve
Cardiac Glycosides	+ve	+ve	+ve
Coumarins	+ve	+ve	+ve
Alkaloids	+ve	+ve	-ve

DISCUSSION: It was evident from the above results that the extracts of *Curcuma leucorrhiza* rhizome contained different classes of phytochemicals. The antioxidant activity on different rhizome extracts followed the order of CL-E ~ CL-C > CL-P. All the three extracts used in this study could inhibit the growth of all the bacterial strains, whereas CL-E and CL-P extracts were effective against selected fungal strains. Phytochemicals like polyphenols, flavonoids, and glycosides are well documented for contributing to the antioxidant activity of extracts [38, 42, 44, 48]. It can be noted that CL–P extract did not contain flavanoids as well as glycosides, and this could be one of the reasons for its lower antioxidant activity as compared to CL-C and CL-E. Further, both CL-C and CL-E were comparable in terms of antioxidant activity, and this is attributed to the presence of phytochemicals like flavonoids and glycosides. The higher antimicrobial and antifungal activities of CL-P are attributed to the presence of non-polar secondary metabolites like terpenoids and alkaloids. The differential biological activity of CL extracts (CL-P, CL-C, and CL-E) is attributed

to the relative abundance of the various class of phytochemicals present is these extracts, which expected to be different due to varying degree of solubility of the active constituents within different solvents *viz*., petroleum ether, chloroform, and ethanol. Accordingly, our future interest is to perform quantitative characterization of all the three extracts, CL-P, CL-C, and CL-E in order to identify the active ingredients. Nevertheless, ethanolic extract (CL-E) of Curcuma leucorrhiza rhizome showing antioxidant, antimicrobial, and antifungal activities gain a lot of significance in terms of the preparation of food-grade formulation for medicinal application.

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CONFLICTS OF INTEREST: None

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