IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

COMAPARATIVE STUDY OF PHYTOCHEMICALS, ANTIOXIDANTS AND ANTIMICROBIAL ACTIVITY OF CURCUMA CAESIA ROXB. AND ITS MICROPROPOPOGATED RHYZOME.

PAYAL JETHAVA[1] , VRUNDA MEHTA[1], SIRIN DAL[2], DR. SAURABHKUMAR MEHTA[2]

DEPARTMENT OF APPLIED SCIENCE, PARUL UNIVERSITY ,VADODARA

ABSTRACT

The bioefficiency of plants and their derivatives has recently received renewed attention. The purpose of this study was to compare the Phytochemicals, antioxidant and antimicrobial activity *Of curcuma caesia* and micropropagated *curcuma caesia*. A variety of phytoconstituents, including flavonoids, terpenoids, alkaloids, proteins, carbohydrates, tannins, and glycosides, were found in the plant extracts taken from the rhizome by means of qualitative chemical analysis. Plant derived secondary metabolites make up a significant segment of natural product based pharmaceuticals especially from the herbal medicinal plants. Secondary metabolites screening done by using HPTLC at wavelength 366nm, different separation band on TLC plate were found. The antioxidant activity is performed with reducing power assay Which have reduction potential react with the potassium ferricyanide to form potassium ferrocyanide, which then react with ferric chloride to form ferric-ferrous complex that give absorbance at 700nm. Higher the concentration of the plant extract higher the antioxidant. *Curcuma caesia* have phenolic compounds which an act as antimicrobial agents. in present study it can measured by the disc diffusion method against the gram-positive bacteria, gram-negative bacteria and Candida albicans fungal.

INTRODUCTION

Curcuma caesia roxb. An important perennial plant belonging to the ginger family.[1] The zingiberaceae family includes over 1200 species in 53 genera[2]. One of them is *Curcuma Caesia*, also known as "Kali haldi".

Black turmeric is an upright rhizomatous plant with large leaves. The rhizome is bluish black and the leaf vein is dark brown.[3] Fresh rhizomes have a strong camphor smell and are used externally for sprains and bruises.[4] It is also used to make cosmetics. This plant is considered very auspicious and is often used in India for various magical treatments. *Curcuma caesia* powder is used by tribal women as a facial mask during engagements and marriages.[2]

Black turmeric (*Curcuma caesia*) is belonging to north, eastern and central part of India. It is a wild species in Bangladesh.[5] It is also rare in the Papicondalu hill of Eastern Godavari, the Root hill of the Himalayas and the forests of the Northern Hills of Sikkim.[6] In Madhya Pradesh, this plant is considered very lucky and it is said that the owner of it will never lack grains and food. In Arunachal Pradesh, the Adi tribe uses a extract of fresh rhizomes as an antidiarrheal. The Hamti family of the Lohit region used a paste of fresh rhizomes for concussions and scorpion stings.[7]

The black turmeric rhizome is of great economic usefulness because of its supposed medicinal purposes. The rhizome is used to treat hemorrhoids, leprosy[12], asthma, cancer[5], epilepsy[5,13], fever, wounds, vomiting, menstrual disorders, smooth muscle relaxant, anthelmintic, aphrodisiac, inflammation and gonorrhoea. The rhizome is widely used in traditional medicine and used worldwide as a condiment and spice.[8] *Curcuma caesia* has been reported to contain more phenolic compounds than *Curcuma amada*. [10] It contains curcuminoids, oils, flavonoids, phenolic compounds, various amino acids, proteins, and high alkaloid content,[11] which suggests that presence of the bioactive compounds has contributed to the benifical medical use of *Curcuma caesia* as a flavoring agent, and many important pharmaceuticals.[14]
Since almost all types of turmeric have antioxidant activity, pharmacological effects and future clinical use prospects have been studied.[15] black Turmeric is considered a powerhouse of active phytochemicals with multiple biological effects, including antibacterial, anti-inflammatory, antiviral, anti-aging, and anti-cancer activities.[16]

Rare and endangered medicinal plants can be preserved *ex situ* by inventing advanced biotechnology approaches to growing plant cells and tissues.[9] Traditional vegetative propagation of this rare species is inefficient due to its low reproductive rate and also takes a long time to mature. [7] Natural reproduction of *Curcuma caesia* is very slow and does not produce good results even under controlled conditions. Also, flowering is very rare, so seed formation rarely occurs. For these reasons, tissue culture has proven to be the best alternative to overcome these challenges.[9]

Oxidative stress caused by various free radicals or reactive oxygen species (ROS) plays an important role in the occurrence and development of chronic diseases such as cancer, vascular disease, hypertension, cardiac hypertrophy, neurogenetic diseases, diabetes and aging.[22] Antioxidants help reduce oxidative stress. Artificial antioxidants such as BHT and BHA are being studied as they are suspected to play a role in carcinogenesis.[23] Therefore, there is an urgent need for natural additives as potential antioxidants to play an important role in the prevention of various stress-related diseases.[24] Antioxidants found in vegetables, beverages and fruits are a health-promoting part of the human diet and are also responsible for the prevention and treatment of free radical-mediated diseases.[25]

MATERIALS AND METHODS

Collection of plants:

The plant of *curcuma caesia* obtained from the Etrade marketing private limited kochi, Kerala and tissue culture *curcuma caesia obtained from genewin* biotech, tamilnadu.

Cleaning of plant :
The plant needs to be properly cleaned after collecting. The plant washed with running tap water and cleaning is done by hands in order to get better outcomes.

Drying of plant :
Getting rid of the water content in plants so they may be stored is the fundamental goal of drying. After washing plants was shade dried until all the water molecules evaporated, cut into pieces, and shed dried plant materials was taken and grinded into coarse powder. The powdered sample were kept in a tidy and airtight glass container.

Extraction of plant material :
The powder of plant material put in a certain quantity of solvent and left for 24 hr in shaking condition. Filter the extract by using Whatmann no. 1 filter paper. solvent- methanol, ethyl acetate, water were used.

Qualitative analysis of phytochemicals :

Wagner's test for alkaloids:
Take 1 ml of curcuma caesia plant extract and 1ml of tissue cultured plant extract. Add few drops of wagner's reagent. Formation of brown/ reddish precipitates indicates the presence of alkaloids.

Xanthoproteic test for amino acid:
Take 1 ml of both plant extract. add 1ml Concentrated HNO3 and then added 2ml of 40% NaOH. Dark yellow or orange colour shows presence of amino acid.

Ferric chloride test for tannins:
Take 1 ml of both plant extract. Add few drops of ferric chloride solution. Formation of dark green or deep blue colour indicates the presence of tannins.

Salkowski test for terpenoids:
Take 1 ml of both plant extract. Add 2ml of chloroform then added 2 ml of H2SO4 side of the test tube. Brown colour show positive result

Molisch test for carbohydrates:
Take 1 ml of both plant extract. Add few drops of Molisch reagent. Add 1 ml of concentrated H2SO4. Colour change shows the presence of carbohydrates.

Lead acetate test for flavonoids:
1 ml of both plant extract. Added few drops of lead acetate .yellow colour precipitates shows presence of flavonoids.

Legal's test for glycosides :
Take 1 ml of both plant extract dissolve in pyridine , sodium nitroprusside is added then add 10% NaOH solution make alkaline. Pink colour shows presence of glycosides

Qualitative analysis by HPTLC

Preparation of crude extract:

Centrifuge at 4000 rpm for 5 minutes after dissolving 0.1 g of curcuma caesia power in methanol. For HPTLC analysis, take the supernatant.

Apparatus:

For the analysis, a camag HPTLC system with an automatic TLC sampler (ATS4), scanner 4, and integrated software win CATS version 3.0.20196.1 was employed. On a precoated HPTLC Linomate 5 Si 60F254 plate, HPTLC was carried out. A spray-on approach was used to apply samples to TLC plates.

Conditions for chromatographic investigations:

The following circumstances were used for chromatographic studies. HPTLC Linomate aluminium sheet with silica 60F254 precoated as the stationary phase; toluene: glacial acetic acid as the mobile phase; volume of the mobile phase: 10 ml; chamber saturation time: 20 min; room temperature; migration time: 20 min; wavelength of detection: 366 nm; scanning speed: 100 mm/s; data resolution: 100 m/step; space between two bands: 21.4 mm.

Chromatographic separation:

A camag ATS4 automatic TLC sampler spotting device was used to spot each extract of 5.0 µl of tissue-cultured curcuma caesia and curcuma caesia solution onto the HPTLC Linomate plate at a distance of 10 mm from the bottom and 10 mm from the side. That plate belonged to TLC. grew in an ascending mode in a twin trough chamber that had been pre-saturated for 30 minutes with 10mL of an 8:2 v/v toluene : glacial acetic acid mobile phase. The plate was removed from the chamber, allowed to air dry, and scanned using a camag TLC scanner 4 in absorbance mode at 254 and 366 nm. The peak area's data was gathered using the camag Win CATS programme.

Antioxidant activity
Reducing power assay
The antioxidant-reducing ability of *Curcuma caesia* plant extract and micro-propagated *Curcuma caesia* plant extract was determined using a standard protocol. Take 0.1, 0.2, 0.3, 0.4 and 0.5 ml of plant extracts in test tube and make 1 ml with methanol.1.25 ml Potassium ferricyanide and 0.2 M phosphate buffer saline (pH 6.6) should be added. Following a 20-minute incubation period at 50 °C, 10% trichloroacetic acid was added. then for 10 minutes, centrifuged at 3000 rpm. In order to dissolve the supernatant, 1.5 ml of distilled water was added to 0.3 ml of (0.1%) iron chloride. All reagents other than plant extracts were used to generate the negative control. Ascorbic acid is utilised as the positive control. A UV-VIS spectrophotometer was used to assess the mixture's absorbance at 700 nm in comparison to the control sample. By graphing the absorbance against the appropriate extract concentration, the concentration of the Curcuma caesia extract with a 50% absorbance value was calculated.

Anti-bacterial activity :
Disc diffusion method
MHA (Mueller Hinton Agar) media pour into the sterile petri plate in aseptic condition. Spread the bacterial culture on the media surface. Place the disc containing the plant extract on the lawn of bacteria. Incubate for 24 hours' and observe the zone of inhibition.

Anti-fungal activity :
Disc diffusion method
The PDA (potato dextrose agar) medium is poured aseptically into sterile Petri dishes. The threshing is done with a sterile handle. The inoculation loop is first sterilized by flame. When the slices have cooled, dip them in the *Candida albicans* broth and put them on a plate. The zone of inhibition was recorded after incubation for 24 hours and 48 hours respectively.

RESULT AND DISCUSSION

Qualitative analysis of phytochemicals:

phytochemicals of *Curcuma caesia* plant:

TESTS:	Methanol	Ethyl acetate	Water
Alkaloids	+	+	+
Amino acid	+	+	-
Tannins	+	+	-
terpenoids	+	+	+
Carbohydrate	+	+	+
Flavonoids	+	-	-
Glycosides	+	+	-

(+ = present , - = absent)

phytochemicals of Tissue cultured plant of *curcuma caesia*

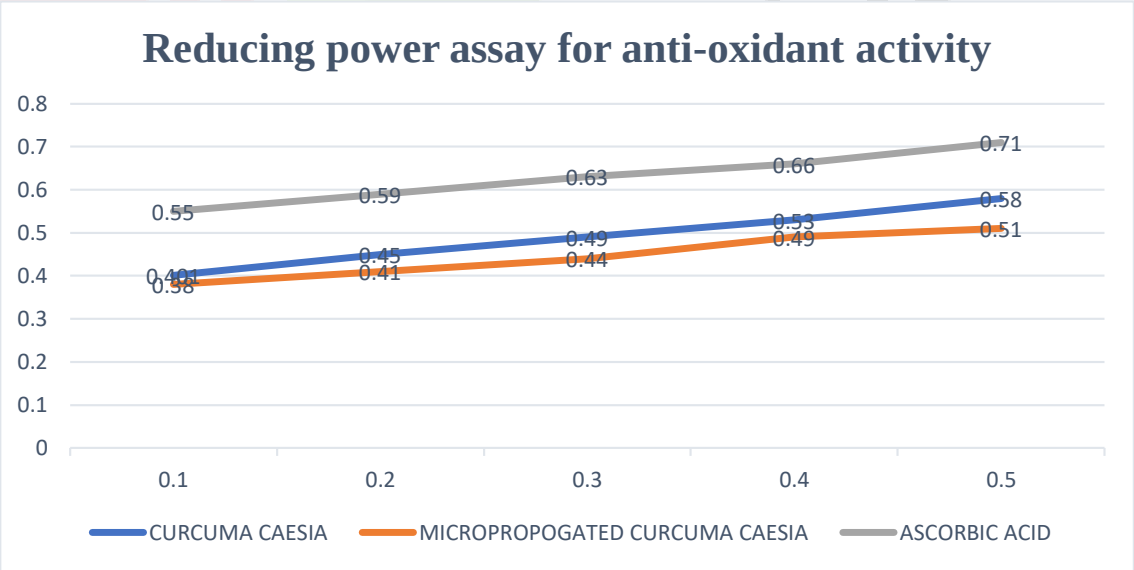
TESTS:	Methanol	Ethyl acetate	Water
Alkaloids	+	+	+
Amino acid	+	-	-
Tannins	-	-	-
terpenoids	+	+	-
Carbohydrate	+	+	+
Flavonoids	+	-	-
Glycosides	+	+	-

(+ = present , - = absent)

This phytochemical screening shows that methanolic extract give more result compared to ethyl acetate and water. In methanolic extract of *curcuma caesia* all the phytochemicals are present And in methanolic extract of tissue cultured *curcuma caesia* also all the phytochemical present except tannins.

Reducing power assay:

Ascorbic acid was used as a benchmark to test the reducing power of turmeric and micro-propagated turmeric extracts. From 1 to 5 g/ml of various concentrations were used to measure the reducing power. In a UV-Vis spectrophotometer, absorbance was measured. The lowering action may be seen to rise as the plant extract concentration increases. From the data, the plant extract was observed to have a maximum potency of 5 µg/ml. Therefore, the antioxidant properties were more effective with increasing concentrations of plant extracts. Turmeric has higher antioxidant properties than micro-propagated turmeric.



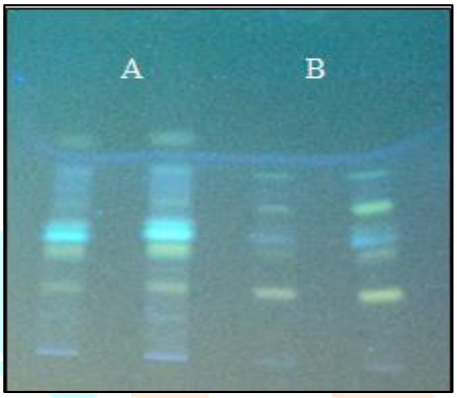
The comparative reducing power assay of *Curcuma caesia* and micro-propagated *Curcuma caesia* against the standard ascorbic acid.

CONCENTRATION	*CURCUMA CAESIA*	MICROPROPOGATED *CURCUMA CAESIA*	ASCORBIC ACID
0.1	0.401	0.38	0.55
0.2	0.2	0.41	0.59
0.3	0.3	0.44	0.63
0.4	0.4	0.49	0.66
0.5	0.5	0.51	0.71

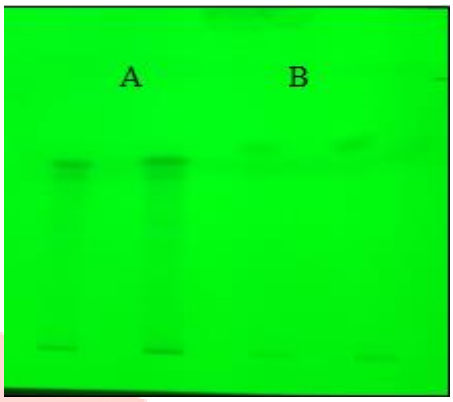
antioxidant activity of *Curcuma caesia* and micro-propagated *Curcuma caesia* against the standard antioxidant at 700 nm.

Qualitative analysis by HPTLC

By using uv chamber different chemical separation band on TLC plate are visualized under uv light 254nm and visible light 366 nm.



TLC plate under UV 366 nm



TLC plate under UV 254 nm

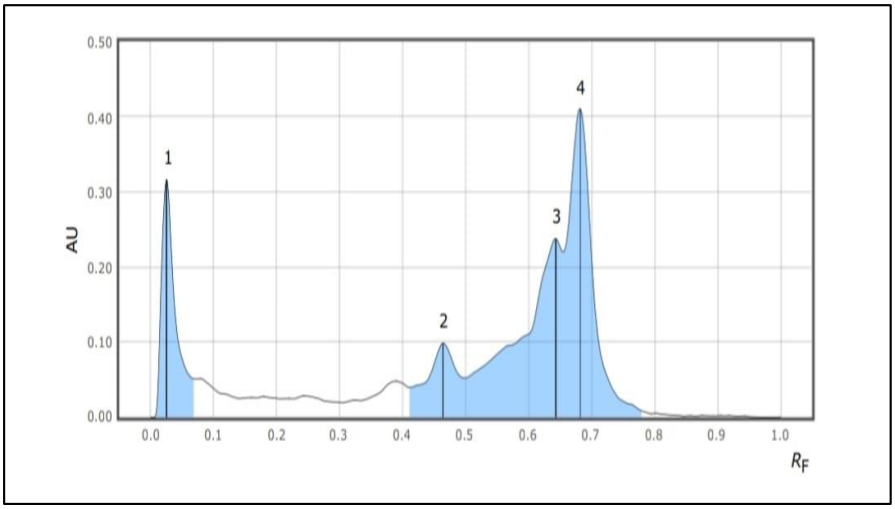
Qualitative analysis by HPTLC shows the different secondary metabolites are present in rhizome of curcuma caesia. It shows different Rf value at wavelength 254 nm and 366 nm. But due to absence of standard solution it is not possible to identify particular chemical present in sample.

Rf value of *Curcuma caesia* at 254 nm:

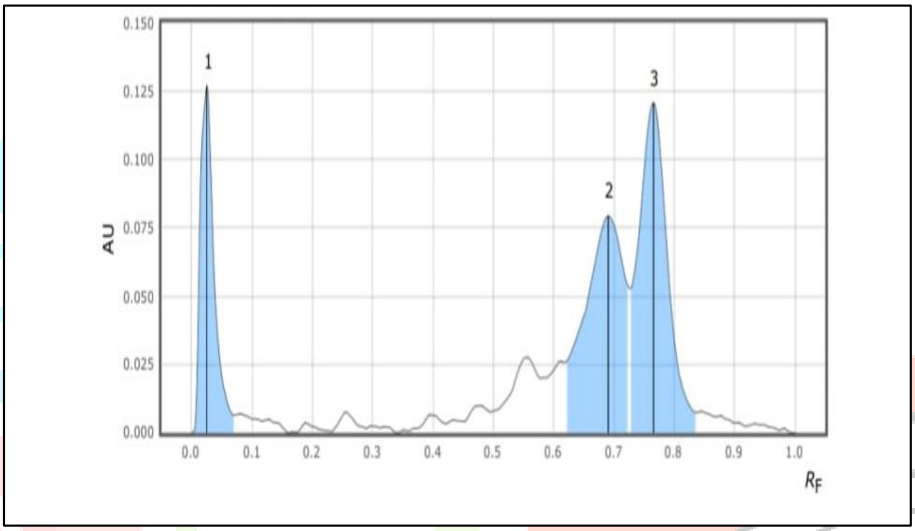
peak	solvent	Rf	Area %
1	methanol	0.025	16.49
2	methanol	0.464	10.76
3	methanol	0.643	36.20
4	methanol	0.682	36.55

Rf value of tissue cultured *Curcuma caesia* at 254 nm:

peak	solvent	Rf	Area
1	methanol	0.025	21.10
2	methanol	0.690	37.03
3	methanol	0.765	41.87



Under uv 254 nm *curcuma caesia*



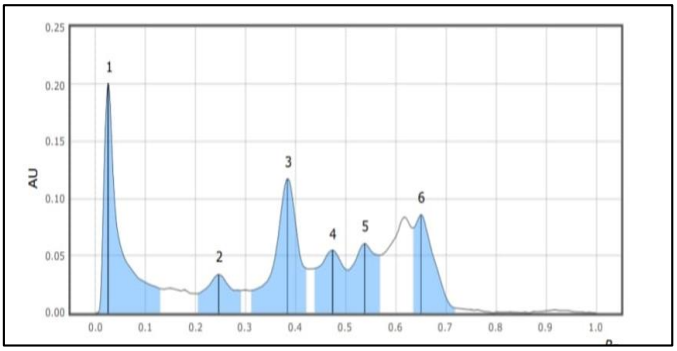
Under uv 254 nm tissue cultured *c. caesia*

Rf value of *Curcuma caesia* at 366 nm:

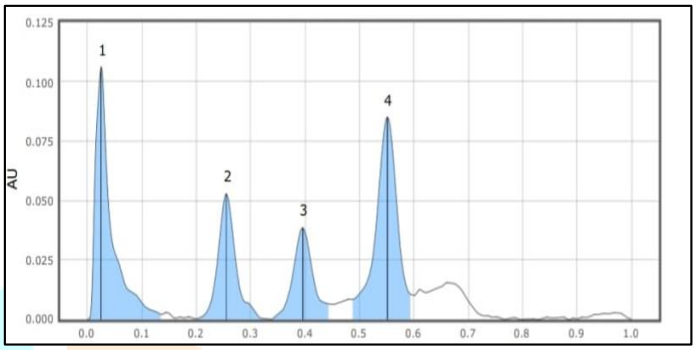
peak	solvent	Rf	Area
1	methanol	0.025	27.79
2	methanol	0.246	8.05
3	methanol	0.383	23.92
4	methanol	0.474	12.82
5	methanol	0.537	12.38
6	methanol	0.650	15.04

Rf value of tissue cultured *Curcuma caesia* at 366nm

peak	solvent	Rf	Area
1	methanol	0.025	32.22
2	methanol	0.256	17.76
3	methanol	0.396	14.69
4	methanol	0.551	35.33



Under UV 366 nm *c. caesia*

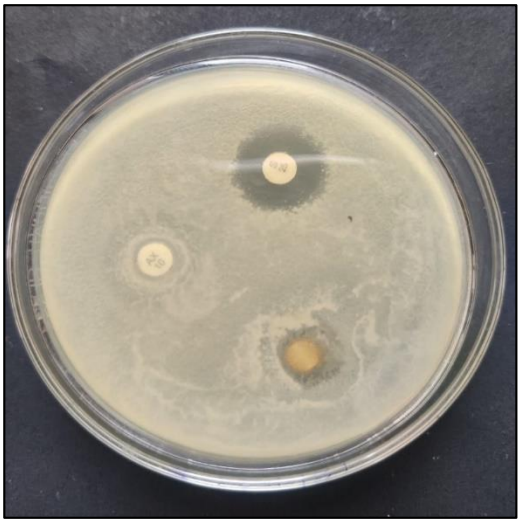


Under UV 366 nm tissue cultured *c. caesia*

Anti-bacterial activity :

The antimicrobial activity of the gram-positive bacteria was found to be comparatively less then the gram-negative bacteria against the methanolic plant extract. *Curcuma caesia* contains more antimicrobial compound then the micro-propagated *Curcuma caesia*. the disc diffusion method was used to determine the inhibitory effect. The zone of inhibition of *E. coli* contains the *Curcuma caesia* plant extract (8 mm) and micro-propagated *curcuma caesia* less activity (4 mm). *Curcuma caesia* and micro-propagated *Curcuma caesia* plant extract shows very less antibacterial activity. *Curcuma caesia* plant extract and micro-propagated plant extract shows negative result against the gram-positive bacteria *S. aureus.*

Antibacterial activity of methanol extract of *Curcuma caesia* against gram negative bacteria *(E. coli)*



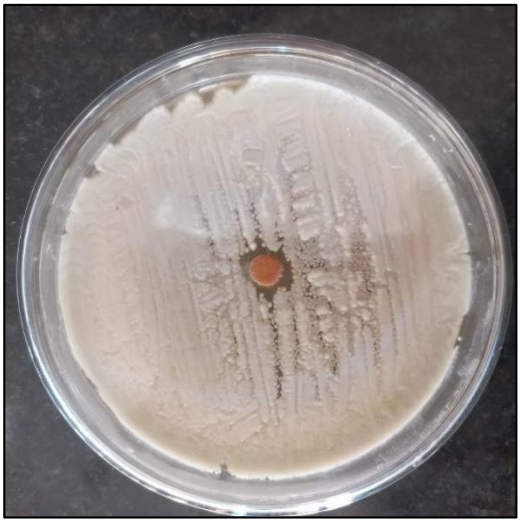
Antibacterial activity of methanol extract of micro-propagated *Curcuma caesia* against gram negative bacteria *(E. coli)*

Antibacterial activity of methanol extract of *Curcuma caesia* against gram positive bacteria *(S. aureus)*

Antibacterial activity of methanol extract of micro-propagated *Curcuma caesia* against gram positive bacteria *(S. aureus)*

Anti-fungal activity :

The anti-fungal activity of the micro-propagated *curcuma caesia roxb.* is less Then the *curcuma caesia roxb.* plant extract against the *candida albicans* fungal culture. The disc diffusion method was used to determine the inhibitory effect. The zone of inhibition of *Curcuma caesia roxb.* plant extract is 6 mm and micro-propagated plant extract is shows 2 mm.



Antifungal activity of the *Curcuma caesia* plant extract against the *Candida albicans*.

Antifungal activity of the micro-propagated *Curcuma caesia* plant extract against the *Candida albicans*.

CONCLUSION:

The result of phytochemical analysis shows that the methanol is best solvent for *c. caesia* rhizome and in both rhizome almost all phytochemical were found. Secondary metabolites screening by HPTLC shows that more peak, Rf and area was found almost same in both rhizome *c. caesia* and tissue cultured *c. caesia*. From the previous study can concluded that terpenoids are more present in *c. caesia*. According different region different terpenoids are present in rhizome. It contains various compounds like phenolic acid and flavonoids which have multiple biological activity including antioxidant and antibacterial activity. Various synthetic antioxidant such as butylated hydroxytoluene, butylated hydroxy anisol, ascorbic acid have been used as antioxidant in food for years but their safety for long has been questioned. Thus the natural antioxidants are more safe for the use. Methanolic extract of *Curcuma caesia* roxb. contains effective antioxidant as ascorbic acid. The phenolic compounds also act as antimicrobial compound which can serve lesser side effect then the synthetic antimicrobials. The present study was elevated that the endangered species are used as biological agents and conserved with micropropagation technique.