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Isolation of Anti-oxidant and Anti Inflamatory Substances in Turmeric Tincture

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Abstract

Currently, numerous diseases are prevalent worldwide, and many chemical drugs have been developed to combat them. However, these drugs often cause side effects in patients. Cancer, in particular, remains a significant challenge globally, with many chemical treatments leading to adverse effects. Consequently, there is a growing interest in natural products for cancer treatment. Turmeric, a fragrant and carminative spice with mild diuretic properties, is one of nature's most potent medicines. The active compound in turmeric, curcumin, has been used in India for over 2,500 years, initially as a dye. Studies have shown turmeric possesses anti-diabetic, anti-inflammatory, and antioxidant properties. To facilitate its consumption, turmeric is often prepared as a tincture, preserving its beneficial properties. Turmeric extract is known to relieve stress, reduce inflammation, and improve blood circulation, and it has a natural ability to cleanse toxins from the body. It is effective in treating a range of illnesses, including cancer and Alzheimer's disease, and is used in India as an antibacterial ointment. Curcumin has demonstrated efficacy against infections caused by Staphylococcus aureus and is used to treat anemia, diabetes, digestive issues, IBS, poor circulation, and wounds, among other conditions. Turmeric is also an ingredient in cosmetics.

In conclusion, the methods and results of the microbial and chemical tests on turmeric tincture demonstrate its efficacy and safety as an antimicrobial and antifungal agent. Serial dilutions and extractions were carried out meticulously, ensuring precision and accuracy in the results. The total microbial count test, coliform test, yeast and mold test, and Salmonella and E. coli tests all showed no microbial growth, indicating the tincture's sterility and purity. Furthermore, the antibacterial and antifungal tests revealed significant zones of inhibition, confirming the tincture's potent antimicrobial properties. The UV-Vis spectroscopy analysis validated the presence of curcumin, a key bioactive compound, in the tincture at a concentration of 24.58 mg/ml. These findings collectively support the potential use of turmeric tincture as a reliable natural antimicrobial and antifungal product.

Keywords:- Turmeric, Tincture, Anti-Cancer properties, Curcumin.

1. Introduction

Approximately 90 % of the time, plants are utilized as the basis for medication in the traditional systems of Ayurveda, Siddha, and homeopathy. Plant sources are easily found in the environment, are safe, cost less money, and rarely have adverse effects. Understaning of chemicals. A finished, in-depth of research of the secondary metabolites of Indian medicinal plant species. India needed to intervene because they are in charge of the plants that have medicinal properties. Over millions of years, plants have evolved chemical defences to protect them from environmental hazards like UV radiation, reactive oxygen species, and microbial assaults. Consequently, phytochemicals are more physiologically active and less poisonous. Plant-based medication are in demand all round the world. They are extensively employed in a variety of fields, including human therapy, veterinary care, agriculture, and academic research. Plant-based medications are in demand all around the world. They are extensively employed in a variety of fields, including human therapy, veterinary care, agriculture, and academic research. Plants and plant parts are use for many purpose. Among them mostly plants are use for medicinal purpose. Plants which are used for medicinal purpose are

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Turmeric, Neem, Moringa, Papaya, Ginger, Cumin, Tulsi, Orange, Clove, Rose, Lemon, pepermint, etc. Apart from medicinal use these plants are also used as food. From years of knowledge of the usase of turmeric in medicine is constantly growing. Turmeric, a blooming plant, is one of the key components in curry powder is a popular food colouring from the ginger family. To treat a variety of medical conditions include digestive and liver issues, wound healing and skin disease therapy. As an anti-inflammatory, turmeric has long been utilized in traditional medicine. The active component of turmeric is curcumin, has been demonstrated to have a variety of medicinal effects.A prominent component of Ayurveda, Siddha medicine, traditional Chinese medicine, Unani, and the animistic rites of Austronesian peoples, Turmeric has a long history of USA a in Asia. It was used initially as a dye and then for its purported therapeutic properties. Along with Hinduism and Buddhism, the yellow dye travelled from India to Southeast Asia and is used to colour the robes of monks and priests. Before the arrival of the Europeans, turmeric was also discovered in Tahiti, Hawaii, and Ester Island. The Austronesian peoples spread and used turmeric in Oceania and Madagascar, as shown by Linguistic and Indirect evidence. Even though they have never met with Indians, people in Polynesia and Micronesia commonly utilize turmeric in food and colours[1] [2][3].

1.1 Bioactive compound of turmeric

1.1.1 Chemical composition of Turmeric

According to a proximate analysis, turmeric comprises 6-13% moisture, 60-70 % carbohydrates, 6-8 % protein, 5-10% fat, 3-7% minerals (potassium, salt, calcium, iron, phosphours), and trace levels of vitamins. It also contains 6-8% carbohydrates. Steam distillation produces essential oils that make up 3-7% of the turmeric rhizome and are primarily made of terpenoids such sesquiterpenoids. Additionally, 3-5% of the mixiyure is made up of more than 50 structurally similar chemicals called curcuminoids. Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin are the three main ones the composition of turmeric generally varies depending on the kind of soil used for growth, with Indian turmeric being prize for its excellent quality and high curcumin concentration. Essential oils and curcuminoids are considered to be well-defined secondary metabolites produced by curcuma plants [4][5].

2. Materials and Method

2.1 Method of serial dilution

Dilutions are made serially In order to do this, a small, precisely measured sample must be diluted with a sizable amount of sterile distilled water or ordinary saline, also known as the diluent or dilution blank. Pipettes are used to obtain precise dilutions of a sample. Dilutions are typically made in multiples of ten for convenience's sake. The formula for a single dilution is as follows:

2.2Extraction of Compounds from Turmeric:-

For extraction of compounds from Turmeric Whole the tincture is made. And the process of making tincture is Collect the fresh Turmeric Whole from farm then wash the turmeric continuously by using water and during washing the whole take care that the whole does not damage. After washing the whole air dry the whole (do not put on direct sun light). When the whole are completely dry then chop the whole. After chopping the whole weight the whole and according to the weight of glycerine is weight by maintaining the ratio is as Company decide. Then transfer the chop and glycerine into air tight bottle. After that put the bottle in boiling water bath at 90° c for 5 hrs. After that put the bottle in boiling water bath at 80° c for 2 hrs till the tincture is completely prepared. Take care when the bottle is transfer from one place to another place cover the bottle. So the sunlight does not comes into contact with bottle (because if the sunlight comes into contact with tincture it may be chances to cross contamination). When the tincture is completely prepare filter it and remove the solid particals and in liquid portion the compounds are isolates. After complete filtration which liquid portion is obtained perform microbial tests by using it's as a sample[6], [7].

2.3 Total Microbial Count test

In order to construct a poured plate, the liquid test sample is poured into an empty petri dish together with a predetermind amount of molten agar culture medium, or in the case of other commodities, a predetermind amount of a beginning suspension. Other plates are prepared under the same condition using decimal dilutions of the test sample or the initial solution. The plate is incubated in aerobic conditions for 72 hours at 30 c. The number of microorganisms per gramme or per milliliter of the test sample is calculated using the number of colonies discovered on the plates with more than 300 colonies [8][9], [10].

2.3.1 Media preparation

Plate Count Agar (PCA) are used in to the TPC method. Heat the water if necessary to help the components or dehydrating media dissolved. After fully combining, let the mixture stand for a while. If necessary, change the PH (6.4) such that it is 7.0 0.2 at 25 c following sterilization. Pour the medium into the appropriate number of tubes, flask, or bottles(6.8). sterilize for 15 min at 121 c in an autoclave(6.1). if the medium needs to be used right away, let it cool in a water bath to between 44 and 47 c. if not, keep it in the dark at a temperature of 5 to 3 c for no more than three months, making sure that neither its composition nor its qualities are altered. Complete the following before starting the microbiological examination. The medium should be fully melted, then it should be cooled to 44 c to 47 c in a water

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bath before usage. Look at ISO 11133. Use the molten agar as soon as you can; don't keep it for more than four hours[11], [12]. Allow to set up as previously mentioned. The prepare plates should be placed upside down in the incubator at 30 °C for 72 °C for three hours. Keep the dishes separate from one another, the walls of the incubator, and its top. Do not stack the dishes more than six high.

2.3.3 Counting of colonies

Once, tally the colonies on the plated the allotted incubation time has passed. If necessary, use the colony counting appartatus. Examine the dishes in low light. It's crucial to count pinpoint colonies, but the operator must be careful not to mistakenly identify undissolved or precipited debris in the dishes as pinpoint colonies. Carefully inspect suspicious things, using a greater magnification as necessary to identify colonies from foregin substance. If a spreading colony occupies less than one-quarter of the plate, it will be treated as a single colony. Spreading overgrowth count the colonies on the portion of the plate that is unaffected, then multiply that figure by the number of colonies on the total dishes. Throw away the count if spreading colonies have taken over more than 25 %.

2.4 Coliform test

A solid selective culture medium is employed, and two prepared poured plates are used, a specific amount of the test sample is used if the starting product is liquid, or a specific amount of an initial suspension is used if the starting product is another kind. Preparation of additional pairs of poured under the same conditions, using decimal dilutions of the test sample or the initial suspension. The plates are incubated for 24 hrs at 30 °C, 35 °C, 37 °C (as agreed). Counting the number of distinctive colonies that were found on the selected plates and converting that figure to the number of coliforms in a sample's milliliter or gramme. Pick up two sterile petri dishes. If the product is liquid, transfer 1ml within the test sampleor 1ml initially suspended to each plate using a sterile pipette.Take two clean petri dishes in the case of other items. Using a brand-new, sterile pipette, add 1 ml of the 1 ml of the first dilution (10), or test sample, if the product is a liquid, of the original suspension in the case of other products. Each petri dish should receive about 15 ml of the VRBL medium at 45 °C. Repeat the process outlined with the other dilutions, using a new, sterile pipette for each decimal dilution. The interval between the conclusion of the initial preparation and the exceed 15 min. As the petri dishes are set up on a cool, flat surface, carefully combine the inoculum with the medium. Additionally, prepare a control plate for sterility testing with 15 ml of the medium. Pour 4 ml of the VRBL medium onto the surface of the sample at 40 °C infected media when it has fully solidified. Allow to solidify as directed above. Invert the prepared plates and incubate them for 24 hrs in an incubator set to 30 °C, 35 °C, or 47 °C.

2.5 Yeast and Mould test

Preparation of poured plates with a certain amount of the test sample (if the original products is liquid) and a specific amount of the selected culture medium for other goods. Preparation of additional plates dilution of the test sample or the control sample in decimal first solution under the same circumstances. Plates must be incubated aerobically for 3, 4, or 5 days at 25 °C. determining the number of colonies found on plates that were diluted to a level that would yield an accurate result, then calculating the amount of yeast and mould per gramme or per milliliter of material. Chloramphenicol Yeast Glucose Agar (CYGA) used it Yeast and Mould method [13].

2.6 Salmonella test

Salmonella detection requires the completion of four steps in succession. Even while Salmonella can exist in very small levels, other Enterobacteriaceae or members of other families frequently coexist in much higher numbers. As a result, selective enrichment is required, and pre-enrichment is frequently required to enable the detection of damaged Salmonella. Rappaport broth composition are used in this method XLDA Agar (Xylose Lysine Desoxycholate Agar) are used in this method [14].

2.6.1 Procedure

If the test portion's indicated mass is less than 25 gm, pre-enrichment medium in the required quantity should be used to produce a 1/10 dilution in buffered peptone water. The initial suspension should be incubated for 18 to 2 hrs at 37 °C. In a tube containing 10 ml of RVS broth, add 0.1 ml of the resulting culture. Incubate the inoculated RVS broth at 41.5° ± 1° c for 24h ± 3 hrs. The surface of one large-size petri dish containing the first selective plating out medium (XLDA agar), which is set at 37 °C for the first plating-out medium, was inoculated using a loop after incubation for employing the culture produced in RVS broth so that well-isolated colonies will be obtained. Set the two plates as a control after 24+3 hours of incubation (don't inoculate any samples). For 24-48 hrs, incubate the plate inverted at 37 °C after sealing it.. After 24-48 hrs observe the colony and count all the colony by CFU /ml or CFU/gm in colony colony counter.

2.7 Biochemical Confirmation

2.7.1 Urea agar

On the agar slant surface, streak. Examine at regular intervals for 24-28 hrs while incubating at 37 °C. if the reaction is successful, urea splitting releases ammonia, which causes the colour of the phenol to change from red to rose pink and then to deep cerise. The response is frequently noticeable 2 to 4 hrs later.

2.7.2 Methyl Red test

Inoculate GPB with the test culture and incubate the broth at 37 °C for 48-72 hrs. After incubation, add about 2 drops of methyl red indicator to the medium.

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Appositive reaction is indicated by red color and negative color by yellow color

2.7.3 Indol test

Inoculate one loopful in a nutrient broth and incubate at 37 °C for 24 hrs. Add 0.5 ml of KSovac's reagent, shake the tube gently, the apperence of red color indicates the presence of indole.

2.8 *E.coli* test

2.8.1 Procedure

Pre-Enrichment: Take 1ml of sample to be tested and into 10ml of Mac Conkey broth. After addition, incubate the broth at 37 °C for 24 hrs.

Secondary Enrichment: After the completion of incubation period, take one loopful of MacConkey broth to streak it on MacConkey agar and EMB agar. After the streaking incubated the plates at 37 °C for 24 hrs. Observe the result after 24 hrs and show for the colonies. Set two plates as a control [15][10].

2.9 Anti-Bacterial Test

Take a pure culture of E.coli and prepare a suspension of E.coli in sterile distilled water. Prepare Plate Count Agar (PCA) medium and autoclave this medium. After autoclave completion pour the medium in sterile petriplate. Allow the medium to solidify after that take E.coli culture from suspension and spread on the medium. After spreading of culture make a well in the medium by using cup borer. Then add turmeric tincture in this well. Allow the tincture to diffuse completely in medium. Than, put the plate for incubation at 37 °C for 24-48 hrs.After incubation observe the plate for zone of inhibition [16].

2.10 Anti-Fungal test

Take a pure culture of Aspergillus and prepare a suspension of Aspergillus in sterile distilled water. Prepare Chloramphenicol Yeast Agar (CYGA) medium and autoclave this medium. After autoclave completion pour the medium in sterile petriplate. Allow the medium to solidify after that take Aspergillus culture from suspension and spread on the medium. After spreading of culture make a well in the medium by using cup borer. Then add turmeric tincture in this well. Allow the tincture to diffuse completely in medium. Then put the plate for incubation at 25 °C ± 2 °C for 72 hrs. After incubation observe the plate for zone of inhibition [17].

2.11 Conformation Test for the Anti-oxidant compounds are Obtain in Tincture

UV – Vis Spectroscopy is used to check the presence of compound in Tincture.

2.11.1 UV – Vis Development for Concentration

Since there is no published UV-Visible method for the quick estimation of this extract, a Straight forward UV spectroscopic method was developed. Turmeric extract calibration curve was created in methanol at a maximum wavelength of 460 nm.

2.11.2 Conformation of Compounds

For the calibration curve's preparation, methanol was chosen. To get a concentration of 1000 ppm, 100 mg of the crude extract were dissolved in 100 ml of methanol. This solution was then used as a stock solution. To achieve various concentrations, this stock solution underwent further dilution. The UV-Vis spectrophotometer was used to scan the resulting solutions for λ maximums in the 400-800 nm wavelength range.

2.11.3 Calibration Curve of compounds

Turmeric tincture extract was diluted with methanol to achieve a final concentration of 20-100 mg/ml after being pipetted out into a series of 10 ml volumetric flasks in a methanol stock solution. At 460 nm, the absorbance of the resulting solution was measured[18], [19].

3.0 Results and Discussion:

3.1 Results

1) For Total microbial count test:-

After incubation there is no growth observe in plate count agar (PCA) plate which is spreaded by different dilution of sample.

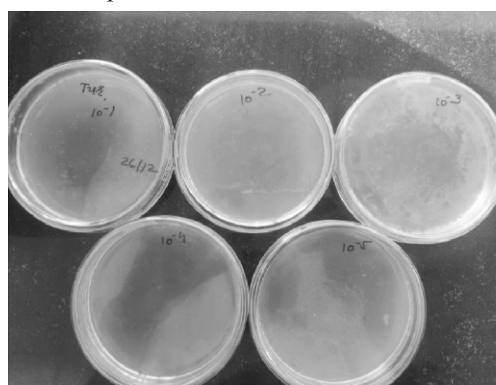

Figure 1 PCA Plates

2) For Coliform Test:-

After incubation there is no microbial growth observe in the crystal Violet neutral red Bile Lactose (VRBL) plate which is spreaded by Turmeric tincture.

Figure 2 VRBL Plates

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3) For Yeast and Mould Test :-

After incubation there is no growth observe in Chloramphenicol Yeast Glucose Agar (CYGA) plate which are spreaded by different dilutions of sample.

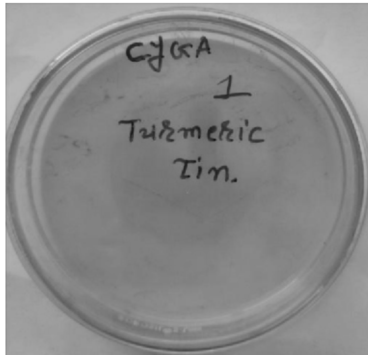

Figure 3 CYGA Plates -1

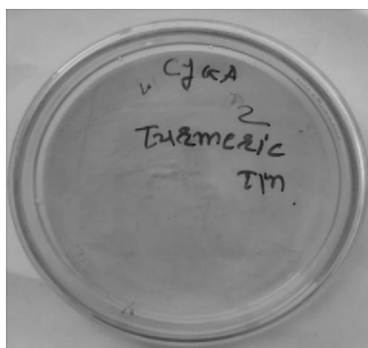

Figure 4 CYGA Plates -2

4) For Salmonella Test :-

After incubation there is no turbidity observed in Rappaport broth which means that Salmonella absent in the sample so doesn't require to prepare Xylose Lysine Deoxycholate Agar (XLDA) plate which is used to isolate the Salmonella.

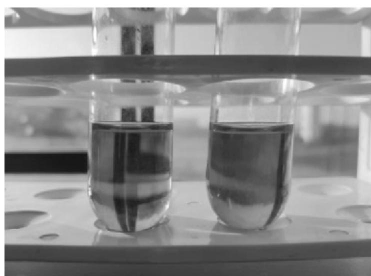

Figure 5 Rappaport broth

Figure 6 Rappaport broth streak on XLDA plate

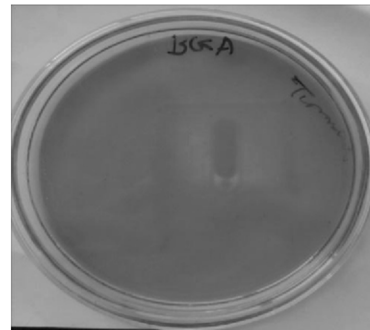

Figure 7 Rappaport broth streak on BGA plate

5) For E.coli Test :-

After incubation there is no turbidity observe in MacConkey broth which means that E.coli absent in the sample so no need to require MacConkey agar plate.

Figure 8 MacConkey broth

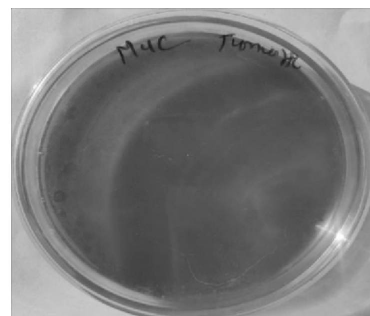

Figure 9 MacConkey broth streak on MacConkey plate

Figure 10 MacConkey broth streak in EMB plate

6) For Anti-Bacterial Test :-

After incubation of PCA plate zone of inhibition is observed around the well which is filled withTurmeric tincture. Which means that tincture is inhibit the growth of bacteria.

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Figure 11 Antibacterial activity of Turmeric tincture

7) For Anti-Fungal Test :-

After incubation of CYGA plate zone of inhibition is observed around the well which is filled with Turmeric tincture. Which means that tincture is inhibit the growth of Fungi.

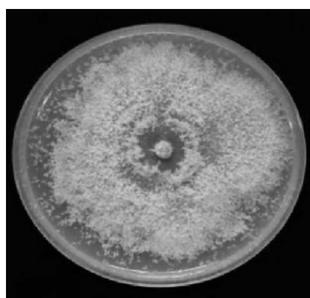

Figure 12 Antifungal activity of Turmeric tincture

8) For UV Method Development for Concentration;-

Standard solution Reading

Concentration(mg/ml)	Absorbance
0.0	0
20	0.25
40	0.50
60	0.65
80	0.75
100	1.0

Table 1 U.V Absorbance Reading

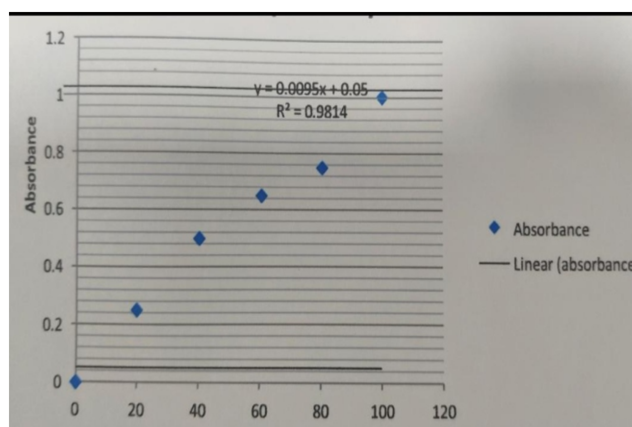

Figure 13 U.V Absorbance Graph

Calculation :-

According to Standard Graph,

If the Absorbance is 0.25 the concentration of compounds in the sample is 20 mg/ml Now the Absorbance of tincture is 0.3073 then the concentration of compound is =? So here , 0.3073 × 20 ÷0.25

= 24.58 mg/ml

From the above calculation, it can be observed that the given tincture contains curcumin at a concentration of 24. 58 mh/ml.

3.2 Discussion:

From above studies we come to know that the Turmeric tincture having some compounds which having anti-cancer properties. And these compounds are decrease DNA damage and increase apoptosis of cancer cell. Also while assay for tincture was performed successfully at

Geo- Fresh Organic. After incubation the growth of well-developed, red colonies, with or without black centres on XLD agar, indicates the presence of Salmonella which indicate sample is contaminated. If colonies of the types mentioned are absent or the confirmatory identification test returns a negative result, the product complies with the test. During the investigation of tincture TMC, YEM, Salmonella test, Coliform test, E.coli test are perform to check the presence / absence of microorganisms. But in the results of these test observe that in tincture sample the organisms are not found. Also for checking the Anti-microbial activity of tincture also perform Anti-Bacterial and Anti-Fungal test. And in the results of these tests observe the zone of inhibition which means that the Turmeric tincture having Anti-microbial activity. Which means that there is no need to require add any preservatives in tincture because tincture already having antimicrobial property. Also when Perform UV – Vis spectroscopy to find out the compounds are obtained in the tincture or not. So during that it will be found that the in Turmeric tincture 29.92 mg/ml compounds obtained. So from the above results it will be said that the Turmeric tincture is used for the treatment of Anti-oxidant, relieves stress, inflammation & smoothen blood circulation. Natural ability to cleanse toxins from the body. Curcumin exhibits significant anti-inflammatory properties, as evidenced by various experimental and pharmacological studies[6][20]. This efficacy is achieved through the modulation of multiple signaling pathways. Specifically, curcumin down-regulates the activity of cyclooxygenase-2 (COX-2), mitogen-activated protein kinases (MAPKs), and Janus kinases (JAKs)[21][22], while also inhibiting the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukins IL-1, IL-2, IL-6, IL-8, IL-12, as well as 5′-adenosine monophosphate-activated protein kinase (AMPK)[22][23].

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