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ANTIMICROBIAL ACTIVITY AGAINST SELECTED BACTERIA ALONGWITH PHYTOCHEMICAL AND PHYSICOCHEMICAL DETERMINATIONS FOR CURCUMA EXTRACT

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ABSTRACT

Curcuma longa rhizomes are widely used by the traditional medical practitioners and researchers for curing and developing medicines for various diseases. This research study shows that what quantity of curcuma rhizomes can we extract with different solvents and co-solvents. Ash value determination of curcuma rhizomes with calculation of safety parameter for different values like extraction value, physicochemical and phytochemical parameters. Various tests for phytochemical study were performed as per the authenticated procedure and techniques in a qualified laboratory environment. There are multiple techniques like maceration, percolation, soxhlation etc. But I used Soxhlet extraction of curcuma powder. The solvents were n-hexane, ethyl acetate, acetone and alcohol. The extraction value, ash value, oil contents were determined and a comparative study with help of charts and plots created with the researched values for better understandings. Antimicrobial activity of Curcuma extract was determined for gram-positive and gram-negative bacteria. Curcuma showed good inhibition zone against selected bacteria by agar well diffusion method. This research study will be helpful for development of new medicines and formulations.

Keywords: *Curcuma Longa, Physicochemical, Phytochemical, Ash Value, Extraction.*

Introduction

Natural products have demonstrated their ability to generate new drug leads, nutraceuticals, and agrochemicals. The most prevalent sources of biological active chemicals include plants, bacteria, fungi, and marine natural products[1]. India has one of the world's most diverse plant-based cultural contexts. Natural products and related medications are utilised to treat 87 percent of all classified human ailments, including their use as anticancer, anticoagulant, antiparasitic, and immunosuppressive agents, according to a review of medicinal indications by source of components[2]. Herbs have been used by people all over the world for ages. They are possibly the oldest kind of "evidence-based medicine." Humans have used herbal medicines to treat a wide range of ailments[3]. Herbal medicines are among the world's oldest treatments. The use of plants for health treatment is as old as mankind's existence on this planet. According to the definition of medicinal herbs, "crude medications of herbal origin are used for the treatment of sickness, frequently of chronic character, or to maintain a state of enhanced health." Despite the significant developments in modern medicine over the last few decades, medicinal plants have played an important part in global health [4]. When it comes to the market potential for herbal plants, it is predicted to grow at a pace of 20% each year. In India, sales of medicinal plants have surged by about 25% in the last ten years. Natural products drug sales account for 30% of all global sales. The skin has been referred to as the body's biggest organ. It serves several roles, and any damage to its layers makes organisms more vulnerable to further biological and physical risks, resulting in wounds. A wound is a bodily injury that demands the removal of the skin, causing damage to the underlying tissue [5,23]. An herbal drug with its organoleptic characteristics, macroscopic, microscopic, and chemical constituents/bioactivity can all be used to standardise it. Standardization of herbal medications is based on bioactive phytoconstituents [6,24].

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Turmeric is a traditional spice made from the rhizomes of Curcuma longa, a ginger family member (Zingiberaceae). It's also known as India's "Golden Spice." Turmeric has been used in India for ages for medical purposes. In traditional medicine, it has been used as a domestic remedy for biliary disorders, anorexia, cough, diabetic sores, hepatic disorders, rheumatism, and sinusitis. Turmeric and its compounds, namely curcumin and essential oils, have a wide range of biological effects in addition to their usage as a spice and colour. Curcuma has Anti-inflammatory, antioxidant, anti-carcinogenic, anti-mutagenic, anticoagulant, antifertility, anti-diabetic, anti-immunomodulatory, antibacterial, antifungal, antiprotozoal, antiviral, anti-fibrotic, anti-venom, anti-microbial, antiulcer, hypotensive, and hypercholesteraemic properties [7-9].

Classification

The plant curcuma is generally known as Haldi in common tongue. It is divided in various categories which is given below. [10]

Kingdom of Plantae	
Subkingdom	Viridaeplantae
Phylum	Tracheophyta Sinnott
Subphylum	Euphyllophytina
Class	Magnoliopsida, or monocotyledons
Order	Zingiberales
Family	Zingiberaceae
Subfamily	Zingiberoideae

The effectiveness of extraction, phytochemical analysis, and ash value determination methodologies on Curcuma longa were compared in this study based on % yield, curcuminoids content, and volatile components.

Fig. 1: Curcuma Fresh and Dried Rhizome

- **Plant Collection and Identification:** This is a very important process for any research study.
- **Plant Material:** Curcuma rhizomes were used for the study in my research work.
- **Plant Material Collection:** The Rhizomes of Curcuma (Family: Zingiberaceae) were gathered from DKNMU Medicinal Garden Newai, Tonk, Rajasthan, in the month of April in 2021.
- **Plant Material Authentication:** Both the plants were recognized and identified by Dr. Shailesh Sharma, Department of Pharmacy, Dr. K. N. Modi University, Newai, Rajasthan. A voucher specimen for Curcuma longa was submitted to Herbarium Department of Botany, Rajasthan University, Jaipur, Rajasthan with book and receipt no. respectively 343 and 34282, the authentication no. was provided for Curcuma longa was RUBL 21219.

Materials and Methods

Materials

Here I used many chemicals for different experimental confirmations, which were purchased from R. S. Enterprises, Jaipur, Rajasthan. Some useful requirements were porcelain dish, beakers of different size, test tubes, watch glasses, test tube holder, spatula, glass rod, Soxhlet apparatus, cotton, filter paper, Whatman paper no. 41, safety gloves, lighter etc.

For Extraction value I used Hexane, Ethyl acetate, acetone and alcohol.

For Physicochemical analysis I used Muffle furnace and laboratory chemicals purchased from R. S. Enterprises and issued from School of pharmaceutical studies, faculty of health sciences.

For phytochemical analysis different chemicals issued from School of pharmaceutical studies, faculty of health sciences.

Methods

- Determination of extraction value in different solvents.
- Determination of physicochemical parameters of Curcuma longa rhizomes.
- Determinations of phytochemicals for curcuma longa rhizomes.
- Anti-microbial activity determination.
- Determination of Extraction value in different solvents.

There are some different methods of extraction i.e., maceration, percolation, Decoction, Soxhlet Extraction, Reflux extraction, Supercritical fluid extraction, Ultrasound assisted extraction, microwave assisted extraction. In this work I used Soxhlet extraction method and the process of this is given here.

Soxhlet extraction is a conventional equipment usually used for the extraction of lipids and substances that aren't water-soluble. Soxhlet may even shop those substances, keeping their properties. The look at the use of the inventory liquor after the isolation of curcumin from oleoresin includes about 40% oil. The isolation and identity of the antibacterial fractions from the leftover turmeric oleoresin have been accomplished via way of means of Soxhlet extraction. An article referred for the soxhlation process related to this study it says, the first-rate extraction became acquired with 5% ethyl acetate in hexane, at the same time as the ar-turmerone, turmerone, and curlone have been diagnosed because the extensive compounds after fuel line chromatography analysis. The cloth confirmed antibacterial activity towards Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa [11]. 150 grams of curcuma rhizomes crushed for soxhlation and the obtained yield noted.

Images of Extraction Process

- **Determination of physicochemical parameters of Curcuma longa rhizomes.**

The physicochemical parameters were determined such as pH, moisture contents and different kind of ash determinations of values.

- **Moisture content**

10 g of Dried and powdered rhizomes were taken in a pre-weight 50 mL beaker and kept in an oven at 105 °C for 5 h. Removed beaker for oven and kept in a desiccator to cool at room temperature and weighed. Repeat the procedure till constant weight is obtained. Calculate the percentage of loss in weight of the sample (12).

- **Total ash:** 2 g of the sample was taken accurately in a previously ignited and tarred Silica dish. Spread the material evenly and ignite in a muffle furnace by gradually increasing the temperature to 600 degree C until it is white, indicating the absence of carbon. Cool the dish in desiccators before weighing it. If carbon-free ash cannot be produced in this method, chill the dish and wet the residue with roughly 2 mL of water or an Ammonium nitrate saturated solution. Dry on a water bath before igniting in the muffle furnace to achieve consistent weight. Cool the dish in desiccators for another 30 minutes before weighing. Calculate the percentage of total ash of air-dried material (12).

- **Insoluble acid ash**
  - **Add 25 mL of Hydrochloric Acid:** water (1: 5, v/v) to the dish containing the complete ash, cover with a watch glass, and slowly boil for 5 minutes. Rinse the watch glass with 5 mL of hot water and place it in the dish with the washings. Collect the insoluble debris on ash-free filter paper (Whatman No. 41) and rinse with hot water until the residue is acid-free. Return the insoluble matter-containing filter paper to the original dish, dry, and burn to constant weight. Cool the dish in desiccators for 30 minutes before weighing it. Determine the air-dried material's proportion of acid insoluble ash (12).
- **Water-soluble Extractive**

In a glass stoppered flask, 4 g of the sample was collected. Add 100 mL of distilled water and shake it occasionally for 6 hours before allowing it stand for 18 hours. Filter the solution and pipette 25 mL of the filtrate into a pre-weighed 100 mL beaker until dryness on a water bath. Put it in an air oven at 105 °C for 6 hours, then cool for 30 minutes in desiccators before weighing. Repeat the experiment twice and average the results (12).

- **Extractive Soluble**

In alcohol 4 g of the material was placed in a glass stoppered flask. Add 100 mL of distilled alcohol and mix occasionally for 6 hours before allowing it stand for 18 hours. Filter the solution and pipette off 25 mL of the filtrate into a pre-weighed 100 mL beaker, then evaporate to dryness on a water bath. Keep it in an air oven at 105 °C for 6 hours, then cool in desiccators for 30 minutes before weighing. Calculate the sample's proportion of alcohol extractable materials. Repeat the experiment again and obtain the average value (12).

Determinations of phytochemicals for curcuma longa rhizomes

The individual extract was subjected to qualitative phytochemical screening for the presence of some chemical constituents. Phytochemical tests were carried out by adopting the standards procedure from the articles referenced with number 1, 13, and 14.

- **Alkaloids:** A quantity (3 ml) of concentrated extract was taken into a test tube, and 1 ml of HCl was added. The mixture was heated gently for 20 minutes, cooled, and filtered. The filtrate was used for the following test. (13)

  - **The Wagner Test:** 1ml of the extract was treated with Wagner's reagent; the formation of a brown-reddish precipitate indicates the presence of alkaloids.
  - **Dragen Droff's Test:** 2 drops of Dragen Droff's reagent were added to 1 ml of the extract. The development of a creamy ppt was indicative of the presence of alkaloids.
  - **Hager's Test:** 1ml of the extract was treated with Hager's reagent, and the presence of alkaloids was confirmed by the yellow-coloured precipitate.
- **Saponin:** 5 mL of extract was combined with 20 mL of distilled water and agitated in a graduated cylinder for 15 minutes. The production of foam confirms the presence of Saponin.
- **Steroid:** 1 ml of extract was dissolved in 10 ml of chloroform and an equal volume of concentrated H2SO4 acid was added from the side of the test tube. The upper layer turned red, and the H2SO4 layer showed yellow with green fluorescence. This indicates the presence of steroids. (1, 14)
- **Tannin:** 4 ml of extract was treated with 4 ml of FeCl3. The formation of a green colour indicates the presence of condensed tannin. When 2 ml of aqueous extract is added to 2 ml of 2N HCl and NH3, the appearance of pink red turns blue violet, indicating the presence of anthocyanin 1, 13, and 14.
- For coumarin, 3 ml of 10% NaOH was added to 2 ml of aqueous extract. The formation of a yellow colour indicates coumarins.
- **Emodin's:** 2 ml of NH4OH and 3 ml of benzene were added to extract the appearance of a red colour, which indicates the presence of emodin's.
- **Proteins:** Xanthoproteic test: extract was treated with a few drops of concentrated HNO3; the formation of yellow indicates the presence of proteins. (1, 13)
- **Amino Acids:** Ninhydrin test: To the 2 ml of extract, 2 ml of ninhydrin reagent were added and boiled for a few minutes. The formation of a blue colour indicates the presence of an amino acid.
- **Flavonoids can be confirmed by the following tests**
  - **Alkaline reagent test:** extract was treated with a 10% NaOH solution. The formation of an intense yellow colour indicates the presence of flavonoid.
  - **NH4OH test:** 3 ml of extract were 10% NH4OH solution. The development of yellow fluorescence indicates a positive test.
  - **Mg turning test:** The extract was treated with Mg turning and then conc. HCl was added to this solution, followed by 5ml of 95 percent ethanol. The formation of a crimson red colour indicates flavonoid.
  - **Zn test:** 2 ml of extract was treated with Zn dust and conc. HCl; the presence of flavonoids is indicated by the development of a red colour (1,14).
- **Diterpenes:** Copper acetate test: Extracts were dissolved in water and treated with 10 drops of copper acetate solution; the formation of an emerald green colour indicates the presence of diterpenes.
- **Phytosterol:** Salkowski's test: The extract was treated with chloroform and filtered. The filtrate was treated with a few drops of concentrated H2SO4 and shaken. After allowing standing, the appearance of golden red indicates the positive test. (13)
- **Phenol:** Ferric chloride test: Test extracts were treated with 4 drops of alcoholic FeCl3 solution. The formation of a bluish black colour indicates the presence of phenol.
- The presence of phlobatannins was determined by the deposition of red ppt when aqueous extracts of each plant sample were boiled with 1% aqueous HCl.
- 5 ml of isoamyl alcohol added to 5 ml of aqueous extract. The upper layer appears red in colour, which indicates the presence of leucoanthocyanin.
- **Anthraquinone:** 5ml of extract was hydrolysed with dilute H2SO4, then added 1ml of benzene and 1ml of NH3, resulting in the formation of a rose-pink coloration that suggests anthraquinone.

- **Chalcones:** 2ml of NH4OH was added to 0.5 gm of ethanolic extract. The appearance of a red colour showed the presence of chalcones. (14)
- **Cardiac Glycosides: Cardiac Glycosides can be tested by the following tests.**
  - **Legal's Test:** To the extract, 1 ml of pyridine and a few drops of freshly prepared sodium nitroprusside solution were added. The appearance of a pink to red colour indicates the presence of glycosides.
  - **Keller-Killani Test:** Plant extract treated with 2 ml of glacial acetic acid containing a drop of FeCl3. A brown coloured ring indicates the presence of a positive test.
- **Carbohydrate:** Extracts were dissolved individually in 5 mL of distilled water and filtered. The filtrate was used for the following tests: 1, 13, and 14.
  - **Molisch's Test:** filtered samples were treated with 2 drops of alcoholic-naphthol solution. The formation of a violet ring at the junction indicates the presence of carbohydrate.
  - **Barfored's Test:** Take 1ml of test solution, add 1ml of Barfored's reagent in a test tube, then keep this test tube in a boiling water bath. A brick red coloured ppt is formed at the bottom, indicating carbohydrate
  - **Iodine Test:** 2 ml of extract were treated with 5 drops of iodine solution, giving a blue colour that indicates a positive test. The Fehling
  - **Fehling's Test:** 2ml of extract were hydrolysed with dilute HCl and neutralised with alkali and heated with Fehling's solutions A and B. The formation of red ppt indicates the presence of reducing sugar.
  - **Benedict's test:** filtrates were treated with Benedict's reagent and heated gently. An orange-red ppt indicates the presence of reducing sugar [22].

Anti-microbial Activity Determination by Agar Well Diffusion Method

The antibacterial activity of turmeric extract was tested against different bacterial isolates using agar well diffusion method [18]. The culture plates were inoculated with 100µl of standardized inoculums (1.5x108 CFU/ml) of each bacterium (in triplicates) and spread with sterile swabs. Circles are 6 mm sizes were created with sterile circle maker or boarer into agar plates which has the inoculums of bacteria and the lower side portion was sealed with a little medium of molten agar. The extract was dissolved in water 20 % portion in 500ml of DMSO (it was 100mg in 500 ml). From this stock solution different concentrations i.e., 25µl, 50µl and 100µl volume was poured into the rounds of the culture plates. Standard antibiotic disc was used as a negative control. The plates prepared were left at room the temperature for 10 minutes allowing the diffusion of the extract into the agar. After incubation for one day and night (24 hrs) at 37ºC, the plates were observed. If antibacterial activity was present on the plates, it was indicated by an inhibition zone surrounding the circle containing the turmeric extract. The inhibition zone was measured and expressed in milimeters (mm). Consideration of antibacterial activity when it inhibits the microbial growth with zone of inhibition greater than 6 mm. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and 18 mm as very active [19]. The mean of ZOI and standard deviation (SD) of the diameter of inhibition zones were separately calculated.

Results

- **Determination of Extraction value in different solvents.**

Extraction yields and the weight of the extracts were obtained and used for the calculations of the percentage yield as carried out by Zaibunnisa et al. (15).

Table 2: Extraction with Different Solvents According Polarity

Extraction with different solvents according polarity			
Solvents for Extraction	**Weight of powdered Rhizome**	**Gram Weight of Extract**	**Gram % Yield**
n-hexane	20	0.30	1.5
Ethyl acetate	20	0.36	1.8
Acetone	20	0.65	3.25
Alcohol	20	2.67	13.25

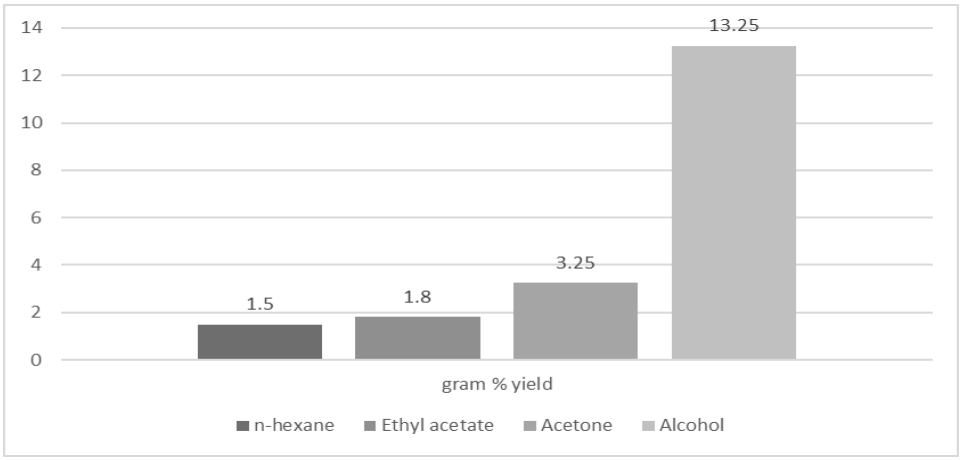


Chart 1: Extractive % Yield with Different Solvents

Determination of Physicochemical Parameters of Curcuma Longa Rhizomes

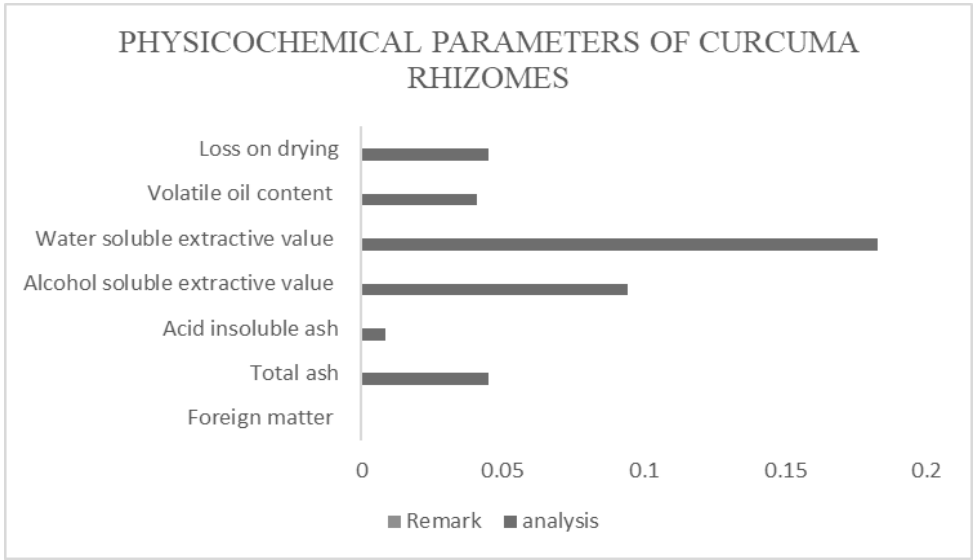
The rhizomes of Curcuma longa were physiochemically standardized in term of determination of extractive value and ash value. Powder of rhizomes showed 4.25% total ash, 0.8% acid-insoluble ash content which is found under the limit as mentioned by Ayurvedic Pharmacopoeia of India (API).

Table 3: Physicochemical Parameters of Curcuma Rhizomes

Physicochemical Parameters of Curcuma Rhizomes			
S.No.	**Parameters**	**analysis**	**Remark**
1	Foreign matter	Nil	NMT 2.0%
2	Total ash	4.30%	NMT 9%
3	Acid insoluble ash	0.85%	NMT 1%
4	Alcohol soluble extractive value	9.10%	NLT 8%
5	Water soluble extractive value	16.90%	NLT 12%
6	Volatile oil content	3.90%	NLT 4%
7	Loss on drying	4.5%	NMT 5%

This indicated negligible amount of foreign matter and less amount of siliceous matter was present in the rhizome of plant [16,25].

Chart 2: Comparative Chart of Physicochemical Parameters of Curcuma Rhizomes



Alcohol soluble extractive was found 9.10% indicated the presence of polar constituents and non-polar secondary metabolites present in the plant while water-soluble extractive was found 16.90% indicated the presence of sugar, acids and inorganic compounds. The percentage of total ash, acid-insoluble ash, alcohol-soluble extractive and water-soluble extractive were found under the permissible limit of API (16).

Determinations of Phytochemicals for Curcuma Longa Rhizomes

As there many phytoconstituents were present in different solvent extractions. It is now considered as a valuable source of unique natural products for development of medicines against various diseases (10).

Table 3: Phytochemicals for Curcuma Longa Rhizomes

Determination of phytoconstituents in curcuma rhizomes				
Sr. No.	**Phytochemical**	**Test Name**	**Curcuma Extract**	
			Acetone	**Ethanol**
1	Alkaloids	Wagner's	P	P
		Dragendroff's	P	P
		Hager's	P	P
2	Saponin		A	P
3	Tannin		P	P
4	Anthocyanin		A	P
5	Emodin's		P	P
6	Flavonoids	10% NaOH	A	A
		10% NH4OH	P	A
		Mg test	P	P
7	Diterpenes		P	P
8	Phytosterol		A	A
9	Phenol		A	A
10	Phlobatannin		P	P
11	Leucoanthocyanin		P	P
12	Anthroquinone		A	P
13	Chalcones		P	P
14	Cardiac Glycosides	Legal's test	P	P
		Kellar-Killiani test	A	P
15	Carbohydrates	Molisch's	P	P
		Barfoed	P	P
		Iodine	A	P
		Fehling	P	P

As different tests were performed for the confirmation of various phytoconstituents. I found alkaloids, saponin, tannin, anthocyanin, emodin's, flavonoids, diterpenes, phytosterol, phenol, phlobatannins, anthraquinone, cardiac glycosides, carbohydrates, leucoanthocyanin and chalcones were present in the different extracts (acetone and alcohol) of curcuma rhizomes. These are very useful for various kind of medicinal activities.

Antibacterial Activity of Curcuma Extract

The turmeric extract powder was active against E. coli mentioned in [20] who studied antimicrobial activity of turmeric reported that it was effective against E. coli, Pseudomonas aeruginosa and Staphylococcus aureus and suggested that the activity is due to the presence of curcuminoid and phenolic compound. The antimicrobial property of turmeric has been attributed to the presence of essential oil, an alkaloid, curcumin and other curcuminoids, turmeric oil, turmerol and veleric acid [21].

Table 5: Anti-Microbial Activity of Turmeric Extract against Different Bacterial Strains

Sr. No.	**Bacteria's**	**Concentration**		
		25µl	**50µl**	**100µl**
1	Pseudomonas aeruginosa	10	11	13
2	E. coli	7	9	15
3	Staphylococcus. aureus	11	12	14

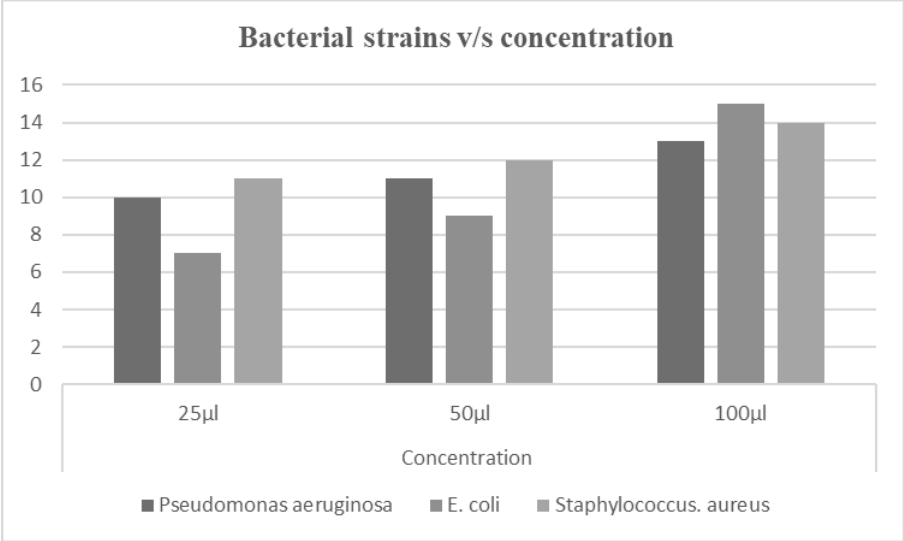


Chart 3: Bacterial Inhibition Zone in Various Concentrations

In this study it was observed that the turmeric extract possesses a good antibacterial activity against pathogenic microbes Pseudomonas aeruginosa, E. coli and S. aureus. At a dose level of 100µl the turmeric extract was able to inhibit the growth of all the bacteria tested. This indicates that the traditional remedy turmeric medicine and herbal products can be used as antibacterial preparation.

Conclusion

From various methods of extraction Soxhlet was selected as it has capability to extract out curcumin and its active ingredients like curcuminoids. Powdered rhizomes of plant were extracted by Soxhlet extraction method using different solvents and I found best extract weight and percentage value of curcuma powder in ethanol. Then determination of physicochemical parameters of curcuma rhizomes were carried out by determining different values and all values found within the standard limits. After that phytoconstituents tested and most of the phytoconstituents found present in the extract of acetone and alcohol like alkaloids, saponin, tannin, anthocyanin, emodin's, flavonoids, diterpenes, phytosterol, phenol, phlobatannins, anthraquinone, cardiac glycosides, carbohydrates, leucoanthocyanin and chalcones. These constituents are helpful in the exploration of the different activities and formulation of new pharmaceutical products.

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