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EXTRACTION AND TLC, OF GINGEROL AND CHECK THE ANTIMICROBIAL ACTIVITY.

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1.ABSTRACT:

Ginger (Zingiber officinale) is one of the most well-known spices with antimicrobial activity. However, different extraction methods of ginger will result in different antimicrobial properties due to the various substances extracted. This study aimed to compare antimicrobial activity between ethanolic extract and essential oil of ginger against food-borne bacteria grown in 2.1% Mueller Hinton agar. The current mode of treatment based on synthetic drugs is expensive and also causes genetic and metabolic alterations. However, safe and sound mode of treatment is needed to control the diseases development and progression. In this regards, medicinal plant and its constituents play an important role in diseases management via modulation of biological activities.

1.1 KEYWORDS:

Ginger, anti-tumour activity, anti-microbial activity, ginger ethanolic extract; food-borne bacteria.

2.INTRODUCTION:

Ginger (Zingiber officinale Roscoe) has been used widely as a food spice and an herbal medicine. In particular, its gingerol-related components have been reported to possess antimicrobial and antifungal properties, as well as several pharmaceutical properties. However, the effective ginger constituents that inhibit the growth of oral bacteria associated with periodontitis in the human oral cavity have not been elucidated.

A common screen for plant antimicrobial compounds consists of separating plant extracts by paper or thin-layer chromatography (PC or TLC), exposing the chromatograms to microbial suspensions (*e.g.* fungi or bacteria in broth or agar), allowing time for the microbes to grow in a humid environment, and visualizing zones with no microbial growth. The effectiveness of this screening method, known as bioautography, depends on both the quality of the chromatographic separation and the care taken with microbial culture conditions. This paper describes standard protocols for TLC and contact bioautography with a novel application to amino acid-fermenting bacteria. The extract is separated on flexible (aluminum-backed) silica TLC plates, and bands are

visualized under ultraviolet (UV) light. Zones are cut out and incubated face down onto agar inoculated with the test microorganism. Inhibitory bands are visualized by staining the agar plates with tetrazolium red. The method is applied to the separation of red clover (*Trifolium pratense* cv. Kenland) phenolic compounds and their screening for activity against *Clostridium stickhandling*, a hyper ammonia-producing bacterium (HAB) that is native to the bovine rumen. The TLC methods apply to many types of plant extracts and other bacterial species (aerobic or anaerobic), as well as fungi, can be used as test organisms if culture conditions are modified to fit the growth requirements of the species

2.1 PROPERTIES OF GINGER:

1. Potency: spicy

2. Taste: bitter

3. Properties: light, adhesive and thick

In 13th century, ginger culinary properties were discovered and soon it was widespread across the globe including Europe and was indicated for several diseases including travel sickness and flatulence. It is cultivated from Asia to Africa and used everywhere as a cooking spice. It is also useful in case of chills. In India, it is widely consumed in dose of 8-10 g as a flavouring agent .[9],[10]

2.2 BIOCHEMISTRY OF GINGER :

Ginger standards have been well documented in USP (United State Pharmacopoeia) and National formulary. The chemistry of ginger is well documented with the respect to the oleoresin and volatile oil. There is stringent criteria for the usage of medical grade (should contain 1.5% or more volatile oil). The studies have identified more than 400 different compounds in ginger and major constituents are as follows:

1. Carbohydrates- about 70%

2. Lipid- about 8% which includes free fatty acids.

3. Volatile oils- about 3% consist mainly of the

sesquiterpenes, beta-bisabolene

4. In addition, raw fibres, vitamins and minerals are also present in ginger.

Ginger also contains amadaldehyde, paradole, gingerdiols, gingerdiacetates, gingerenones, 6- gingersulfonic acid, diterpense, gingerglycolipids A, B and C (Qian and Liu 1992; Huang et al., 1991; Pecoraro et al., 1998; Anonymous 1997; Frisch et al., 1995).

2.3 CHEMICAL STRUCTURE AND ACTIVE CONSTITUENTS:

Numerous active ingredients are present in ginger including terpenes and oleoresin which called ginger oil. Ginger also constitutes volatile oils approximately 1% to 3% and non-volatile pungent components oleoresin [11]. The major identified components from terpene are sesquiterpene hydrocarbons and phenolic compounds which are gingerol and shogaol [12]and lipophilic rhizome extracts, yielded potentially active gingerols, which can be converted to shogaols, zingerone, and paradol [13]

GINGER PLANT

GINGER ROOT

Chemical of structure of active ingredients of Ginger.

2.4 Mechanism of action of ginger in diseases management:

Ginger, the rhizome of the Zingiber officinale, plays an important role in prevention of diseases (Table 1). But the exact mechanism of action in diseases management is not understood fully. It is thought that ginger act as anticancer due to various constituents such as vallinoids, viz. [6]-gingerol and [6]- paradol , shogaols, zingerone, and galanals A and B [10-12] and constituents show a therapeutics role in diseases control via modulation of various biological activities as describe as follwing:

1. Ginger and its constituents show antioxidant activity and prevent the damage of macromolecules, caused by the free radicals/oxidative stress.

2. Ginger and its constituents also show a vital role as anti-inflammatory processes. Ear-lier studies on in vitro investigations of ginger preparations and some isolated gingerol-related compounds showed that anti-inflammatory effects of ginger such as inhibition of COX [14]and inhibition of nuclear factor κB.

3. Ginger also acts as antitumor via modulation of genetic pathways such as activation tumour suppressor gene, modulation of apoptosis and inhibition of VEGF. Earlier study has shown that terpenoids, constituents of ginger induce apoptosis in endometrial cancer cells through the activation of p53. [15]

4. Ginger also shows antimicrobial and other biological activities due gingerol and paradol, shogaols and zingerone. An important finding showed that 10% ethanolic ginger extract was found to possess antimicrobial potential against pathogens .[16]

3. Literature Survey :

1) Dr. Gaikwad DD, Shinde Sachin K, Kawade Ashwini V, Dr. Jadhav SJ and Dr. Gadhave MV et al. (2017)

In this article the author had discussed the Many drugs commonly used today are herbal origin. About 25% of prescription drug in the US contains atleast one active ingredient derived from plant material. Herbal medicine is the oldest form of healthcare known to mankind.

2) Chinedu Imo* and Jivini Salvation Za'aku et.al. (2019)

Some chemical constituents of these medicinal plants have been reported in various literatures to contribute to the prevention and treatment of various diseases and ailments. In literatures, some of the documented properties of garlic and/or ginger include antioxidant, anti-inflammatory, rheumatologic, blood circulation and anti-cramp, anti-ulcer, anticholinergic, analgesic, antimicrobial, anti-stress, anti-cancer, immunity booster, anti-diabetic, regulation of blood pressure and treatment of cardi ovascular diseases.

3) M. N. Azian, A. N. Ilia Anisa, Y. Iwai et.al. (2014)

The mechanism for extraction bioactive compounds from plant matrix is essential for optimizing the extraction process. As a benchmark technique, a soxhlet extraction has been utilized for discussing the mechanism and compared with an accelerated water extraction.

4)Shania Foustine1, Andre1, Bastian Setiadi1, Leonny Yulita Hartiadi1, Agnes Anania Triavika Sahamastuti1*et al. (2019)

Ginger (Zingiber officinale) is one of the most well-known spices with antimicrobial activity. However, different extraction methods of ginger will result in different antimicrobial properties due to the various substances extracted. This study aimed to compare antimicrobial activity between ethanolic extract and essential oil of ginger against food-borne bacteria grown in 2.1% Mueller Hinton agar.

5)Keith Single tary, PhD et al. (2010)

Ginger (Zingiber officinale Roscoe) is a member of the Zingiberaceae family of plants. It has been a part of healing strategies in Asia, India, Europe, and the Middle East forcenturies for treatment of such disorders as arthritis,

stomach upset, asthma, diabetes, and menstrual irregularities, to name a few. There is scientific support that ginger may alleviate the symptoms of nausea and vomiting followingpregnancy, surgery, cancer therapy, or motion sickness and suggestive evidence that ginger reduces inflammation and pain.

6) Ahmed I. Foudah 1 , Faiyaz Shakeel 2 , Hasan S. Yusufoglu 1, Samir A. Ross 3,4 and Prawez Alam 1,* et al. (2020)

Various analytical methodologies have been reported for the determination of 6-shogaol (6-SHO) and 6-gingerol (6-GIN) in ginger extracts and commercial formulations. However, green analytical methods for the determination of 6-SHO and 6-GIN, either alone or in combination, have not yet been reported in literature.

7) Najim A. Jabir Al-Awwadi et.al (2017)

Ginger has been known for its several scientific properties and valued for the last 2500 years in different parts of the globe. Ginger has rich phytochemistry and several health promoting perspectives. In ginger family, Zingiber officinalis is one of most widely used species and it is found in several foods and beverages. Ginger has been used commonly to treat diarrhea, stomach upset, indigestion and nausea. It also has anti-inflammatory and antioxidant properties.

8) Pankaj Pandotra, Rajni Sharma1, Prabhu Datt1, Manoj Kushwaha2, Ajai Prakash Gupta2, Suphla Gupta et al. 2013

Ginger, the rhizome of Zingiber officinale Roscoe (family Zingeberace) has been used as a spice. Ginger, the rhizome of Zingiber officinale Roscoe (family Zingeberace) has been used as a spice throughout the worldsince times immemorial. Ginger has been valued as a medicinal herb in several countries and has been reported to possess carminative, anti-emetic, anti-nauseate and anti-inflammatory properties.

4. Aim and Objective:

4.1 . Aim:

This work deals with the Anti-Microbial activity of gingerol.

4.2 . Objective:

- To extract gingerol from Ginger
- Ginger improved a marker for long-term blood sugar levels, as well as reduced markers for oxidized lipoproteins - major risk factors for heart disease.
- This research suggests that ginger may lower blood sugar and the risk of heart disease.
- assess antimicrobial activities of ginger extract to identified bacteria.

5. Plan of work:

Biological source, chemical constituents Gingerol.

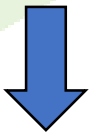
Properties and characterization of ginger.

Material and method for extraction of gingerol from ginger by soxhlet apparatus.

find out the RF value by using TLC or paper chromatography method.

Find out the antimicrobial activity of extract by using agar method.

Result and Discussion



Conclusion

References

6. GINGER :

6.1.Biological source: obtained from root of ginger.

6.2.Family: Zingiber officinalis.

6.3.Chemical constituents : vallinoids, viz. [6]-gingerol and [6]-paradol, shogaols, zingerone, and galanals A and B [10-12].

6.4.Uses: Ginger has been used commonly to treat diarrhea, stomach upset, indigestion and nausea.

6.5.Adulterants: The 'Spent ginger' is also uses for adulteration purpose. In this type, the aroma has beenextracted which yields low quality of ginger.

6.6.Properties and characteristics of gigerol :

Systematic name: (5S)-5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one

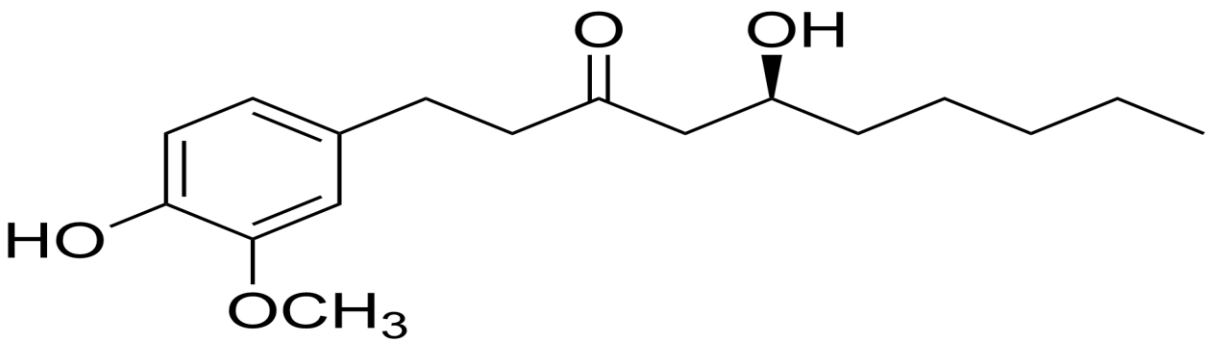
Other names: [6]-Gingerol; 6-Gingerol

Molecular formula: C17H26O4

Molecular weight: 294.38 g/mol

Melting point: 30 to 32 °C (86 to 90 °F; 303 to 305 K)

6.8. Chemical structure of gingerol :



7. Health Benefits of Gingerol:

7.1. Contains gingerol, which has powerful medicinal properties

Ginger has a very long history of use in various forms of traditional and alternative medicine. It's been used to aid digestion, reduce nausea, and help fight the flu and common cold, to name a few of its purposes. The unique fragrance and flavor of ginger come from its natural oils, the most important of which is gingerol. Gingerol is the main bioactive compound in ginger. It's responsible for much of ginger's medicinal properties.

7.2. Can treat many forms of nausea, especially morning sickness:

Ginger appears to be highly effective against nausea .

It may help relieve nausea and vomiting for people undergoing certain types of surgery. Ginger may also help chemotherapy-related nausea, but larger human studies are needed.

7.3. May help with weight loss:

Ginger may play a role in weight loss, according to studies conducted in humans and animals.

A 2019 literature review concluded that ginger supplementation significantly reduced body weight, the waist-hip ratio, and the hip ratio in people with overweight or obesity (10Trusted Source).

A 2016 study of 80 women with obesity found that ginger could also help reduce body mass index (BMI) and blood insulin levels. High blood insulin levels are associated with obesity.

7.4. May drastically lower blood sugars and improve heart disease risk factors

This area of research is relatively new, but ginger may have powerful anti-diabetic properties.

In a 2015 study of 41 participants with type 2 diabetes, 2 grams of ginger powder per day lowered fasting blood sugar by 12% (19Trusted Source).

It also dramatically improved hemoglobin A1c (HbA1c), a marker for long-term blood sugar levels. HbA1c was reduced by 10% over a period of 12 weeks.

7.5. Can help fight infections :

Gingerol can help lower the risk of infections.In fact, ginger extract can inhibit the growth of many different types of bacteria (44Trusted Source, 45Trusted Source).According to a 2

008 study, it's very effective against the oral bacteria linked to gingivitis and periodontitis. These are both inflammatory gum diseases

7.6. May improve brain function and protect against Alzheimer's disease :

Oxidative stress and chronic inflammation can accelerate the aging process.

They're believed to be among the key drivers of Alzheimer's disease and age-related cognitive decline.

Some animal studies suggest that the antioxidants and bioactive compounds in ginger can inhibit inflammatory responses that occur in the brain (39Trusted Source).

7.7. Contains a substance that may help prevent cancer:

Ginger has been studied as an alternative remedy for several forms of cancer.

The anti-cancer properties are attributed to gingerol, which is found in large amounts in raw ginger. Contains a substance that may help prevent cancer

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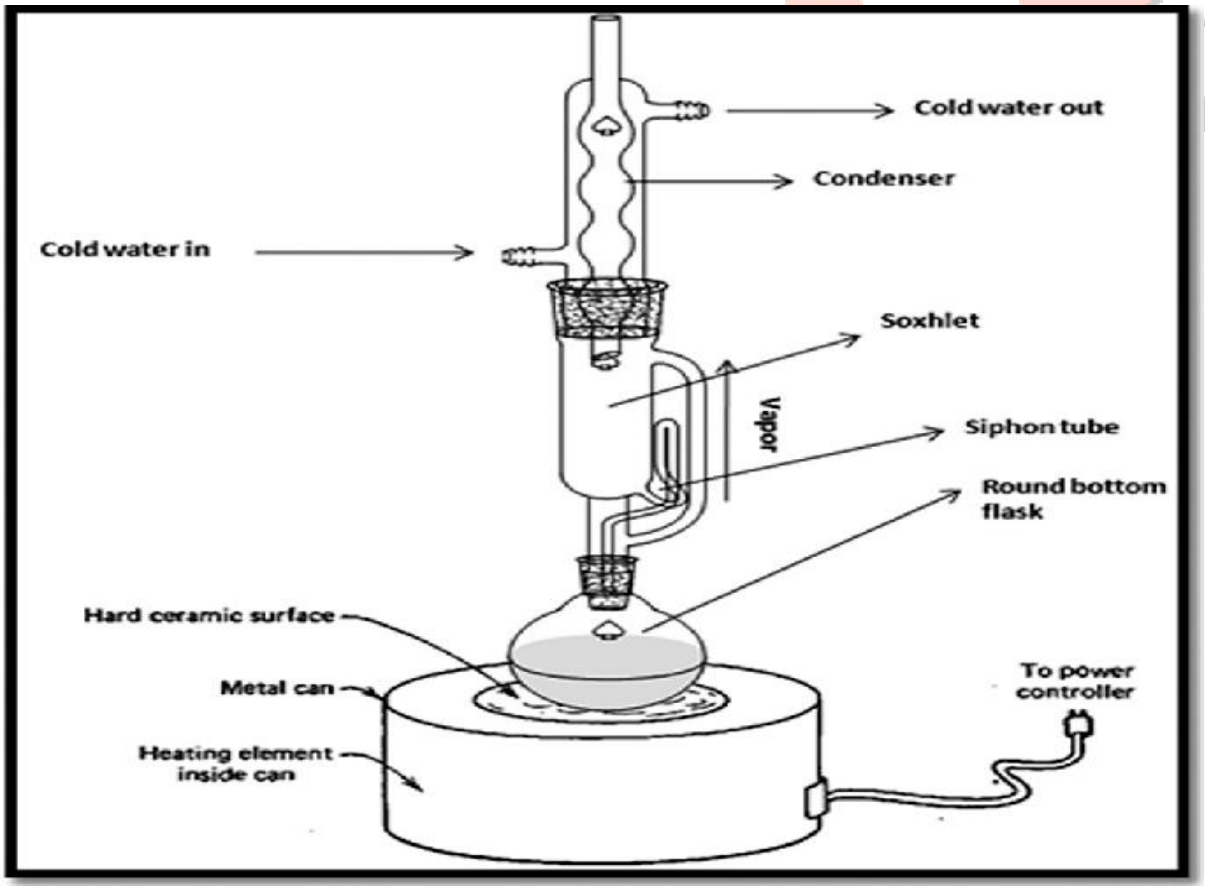
8. Apparatus used in a sepration of gingerol:



Principle:

Soxhlet extractor extracts the components using the condensed vapors of the solvent. The condensed vapors come in contact with the sample powder and the soluble part in the powder gets mixed with the solvent.

Working :

- First we turn on the heat and the metal plate gets heated.
- The RBF which contains our solvent starts boiling.
- The vapors from the RBF travel from RBF to the condenser via the distillation tube.
- The condenser condenses the vapors of solvent and those condensed vapors fall down to thimble.
- We put our sample powder inside the thimble. The powder has to be covered from the bottom with a cotton ball to avoid powder directly falling into the thimble. And also cover the powder from the top.
- So, when the condensed vapors fall into the thimble, the powder gets wet with the solvent and the components which are soluble in the solvent gets along with it.
- Siphon connects the thimble to RBF as we saw earlier. The solvent mixture starts filling thimble and siphon. A point reaches where the siphon starts overflowing under the influence of gravity.
- Since the Siphon directly connects RBF, the overflowed liquid falls back to RBF. This marks the first cycle.
- As I mentioned earlier, we can perform as many cycles as we want.
- One thing to mention is, we don't change the solvent for every cycle. And despite that, when the solvent vaporizes, the components from the sample do not get vaporized. So, each time we get 100% pure solvent vapors.

Uses :

We use the soxhlet apparatus in various ways. I have listed some of them below:

- Biofuel extraction from coffee beans
- Extraction of components having anti-cancer properties from fenugreek seed extract
- Extraction of caffeine from beverage plants using soxhlet extraction method
  Oil extraction from plants
- In the food industry for analyzing food components
- Determination of extractives (e.g., fixed oils in seeds)

9.MATERIALS AND CHEMICAL (EXPRIMENTALS):

MATERIALS:

9.1.Plant Materials:

Fresh rhizomes of ginger (Zingiber officinale Rosace) were collected from different places of North Western Himalayas of India. Each sample was crushed into small pieces and air-dried for 10 h. These accessions are also being maintained in the experimental fields of Indian Institute of Integrated Medicine, Jammu, India.Ginger powder were purchased from a local market of sinnar.

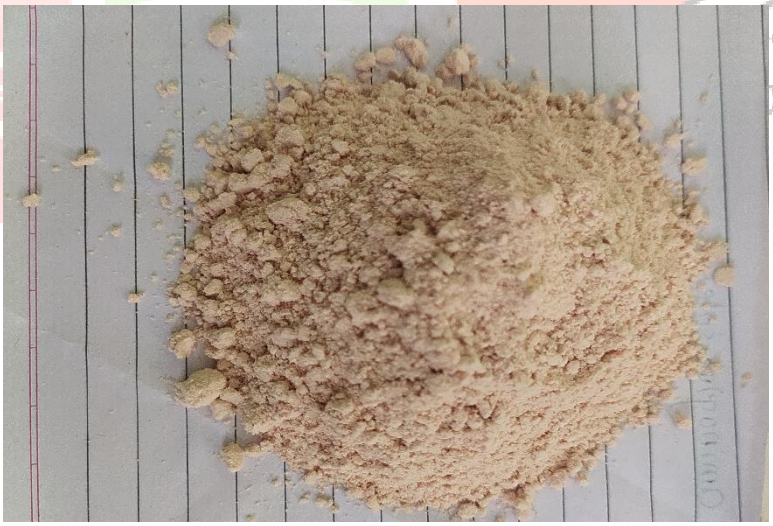
9.2.Chemicals:

Ethanol was obtained from Merck (Germany) , Hexane , Diethyl ether ,silica gel, methanol .

9.3. Isolation of gingerol from ginger :

Dry ginger was crushed to a coarse powder and extracted with95% ethanol by simple maceration process. Solvent was evaporated by distillation to obtain thick pasty mass. The thick pasty mass was suspended in water. The Ginger resin precipitates in water which was removed by filtration and the residue obtained was dried under vacuum.

10. Extraction of gingerol :



Dride ginger powder

Extraction of ginger

The initial concentrations of bioactive compounds presented in the ginger matrix were identified through the Soxhlet extraction. 20.0g of dried and ground ginger with <1.18mm of particle size were weighed and extracted with 200mL of ethanol (96% AR grade, QReC). The extraction temperature was constant at the normal boiling point of ethanol (78.1oC) and monitored using an infrared laser thermometer (AR300, China). The experiments were conducted for 12 h in triplicate and the standard deviations were calculated (<5%).[17]

After Extraction Ginger Find Gingerol

11.Analysis and characterization Techniques for gingerol:

Principle: TLC is an important analytical tool for the separation, identification and estimation of different classes of compound. This method is based on the principle of adsorption. When a mixture of compound dissolved in a mobile phase move through a column of stationary phase compound which has more affinity towards the stationary phase travels slower and the compound with lower affinity travels faster.[18]

11.1. TLC Method : [19],[20]

Preparation of plates:

Prepare a suspension of coating substance and spread a uniform layer of suspension, 0.25 to 0.30 mm thick, on flatglass plate of 20 cm long. Dried in air and heat at 100 to 1050for atleast 1 hr. store the plates protected from moisture, dry the plates at time of use if necessary.

$$Rf(retention\ factor) = \frac{distance\ traveled\ by\ the\ compound}{distance\ traveled\ by\ the\ solvent\ front}$$

Mobile phase:

Hexane: Diethyl ether (30:70)

Test solution:

Reflux 1 g of the coarsely powdered substance under examination with 25 ml of methanol for 15 minutes, cool and filter. Wash the residue with 10 ml of methanol. Combine all the filtrates and concentrate to 10 ml.

Reference solution :

Reflux 0.5 g of coarsely powdered sunthi RS with 5 ml methanol for 15 minutes, cool and filter. Apply to the plate 10 µl of each solution as bands 10 mm by 2 mm.

11.2.chemical test :

Goldbeater's skin test:

Add 2% hydrochloric acid to a small piece of goldbeater's skin, rinse it with distilled water and place in the solution to be tested for five minutes. Then give wash of distilled water and transfer to 1% ferrous sulphate solution. A brown or black colour on the skin indicates presence of tannins. (Goldbeater's skin is a membrane obtained from intestine of the ox and behaves similarly to an untanned hide.)

(b) Ferric chloride test:

Treat the extract with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

(d) Gelatin test:

To the test solution, add 1% gelatin solution containing 10% sodium chloride. Precipitate is

Formed.

(e) Test for catechin:

Dip a matchstick in the test solution, dry it and lastly moisten with concentrated hydrochloric acid. Then warm the stick near flame. The colour of the wood changes to pink due to phloroglucinol. (Phloroglucinol is formed when catechins are treated with acids).

11.3.Antimicrobial Activity :

The antimicrobial activity of ginger extract against various human pathogens was determined by agar diffusion method. Four bacterial cultures were used, named Staphylococcus aureus, Escherichia coli, Bacillus subtilis Streptococcus faecalis.

Apparatus :

Sterile saline tubes (5ml), 70% IPA, sterile test tubes, sterile petri-plates, 250ml conical flasks, Borer, Micropipette, sterile tips, sterile inoculating loop, autoclave, shaker, oven (32-35°C), laminar air flow hood, incubator, weighing balance and Vernier caliper were used for the assay.

Culture media:

From cultures of E. Coli, Bacillus subtilis, Staphylococcus aureus and Streptococcus faecalis, slants were made and incubated at 32°C for 24-48 hrs. Loops from each slant culture were transferred separately in 5 ml sterile saline solution tube to prepare suspension of each culture. These suspensions were transferred separately to 150 ml sterileSCDA when cooled to a temperature of 40-45°C. Each conical flask having SCDA and culture suspension was shaken to allow uniform distribution of microbial cells in medium. After shaking each SCDA medium with culturesuspension was poured into four plates, labeled and allowed to solidify. After solidification of the medium in plates, the wells were cut in each plate using sterile borer.0.1 ml of 10% (w/v) ginger extract to be tested was poured into different wells and the plates were incubated at 32°C for 24 hrs. After incubation the plates were observed for the presence of of inhibition was measured with the help of vernier caliper.

12.RESULTS AND DISCUSSION: ;

12.1.THIN LAYRE CROMATOGRAPHY :

Gingerol is analysed for retention factor. TLC plate showed result illustrated in figure 10 of TLC chromatogram. Clear spot observed from ethanolic extract when visualized by eye, however under UV lamp in long wavelength 365nm the spotcolour were fluorescent blue.

Rf Values for ethanolic extract of Zingiber officinale by 0.99

Solution	Solvent Front Height (cm)	No. of spots	Spot height(cm)	Rf Value
Reference Solution	5.5	1	5.4	0.98
Test Solution	6.2	1	6.0	0.97

12.2chemical test :

(a) Goldbeater's skin test:

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(f) Test for chlorogenic acid :

Treat the test solution with aqueous ammonia and expose to air gradually, green colour is developed.

12.3.Antimicrobial activity:

The findings of the present study revealed that Zingiber officinale contain potent antimicrobial property against tested microbes. The antimicrobial activity of the ginger extracts (chloroform extract) was initially evaluated by agar diffusion method using four strains of pathogenic bacteria Escherichia Coli, Bacillus subtilis, Staphylococcus aureus, Streptococcus faecalis and two stains of fungi Candida albicans and Aspergillus Niger. These extracts exhibited antimicrobial activity.

13.CONCLUSION :

Gingerol is one of the most abundant naturally occurring phenolic compound found in ginger It can be used as, treat diarrhea, stomach upset, indigestion and nausea. It is some used for weight loss, as a pesticide in plants.In this study, main importance was given on the Isolation and Characterization of gingerol from variety of ginger powder . There are several evidences from literatures on the medicinal ginger. properties of ginger In conclusion, the mechanisms of extraction compounds from ginger matrix are different extraction methods.

Our Study mainly focused on :

- Extraction of gingerol from ginger Powder by using suitable method.
- Identification of extracted gingerol was done by using different techniques such as Chemical tests, Thin layer Chromatography, and the results obtained in each case were compared with the result of a standard commercial gingerol.
- All the results obtained are perfectly matched with the result of standard gingerol , So we can conclude our isolated compounds are gingerol .
- All results are obtained in our Lab condition of our Institute.

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